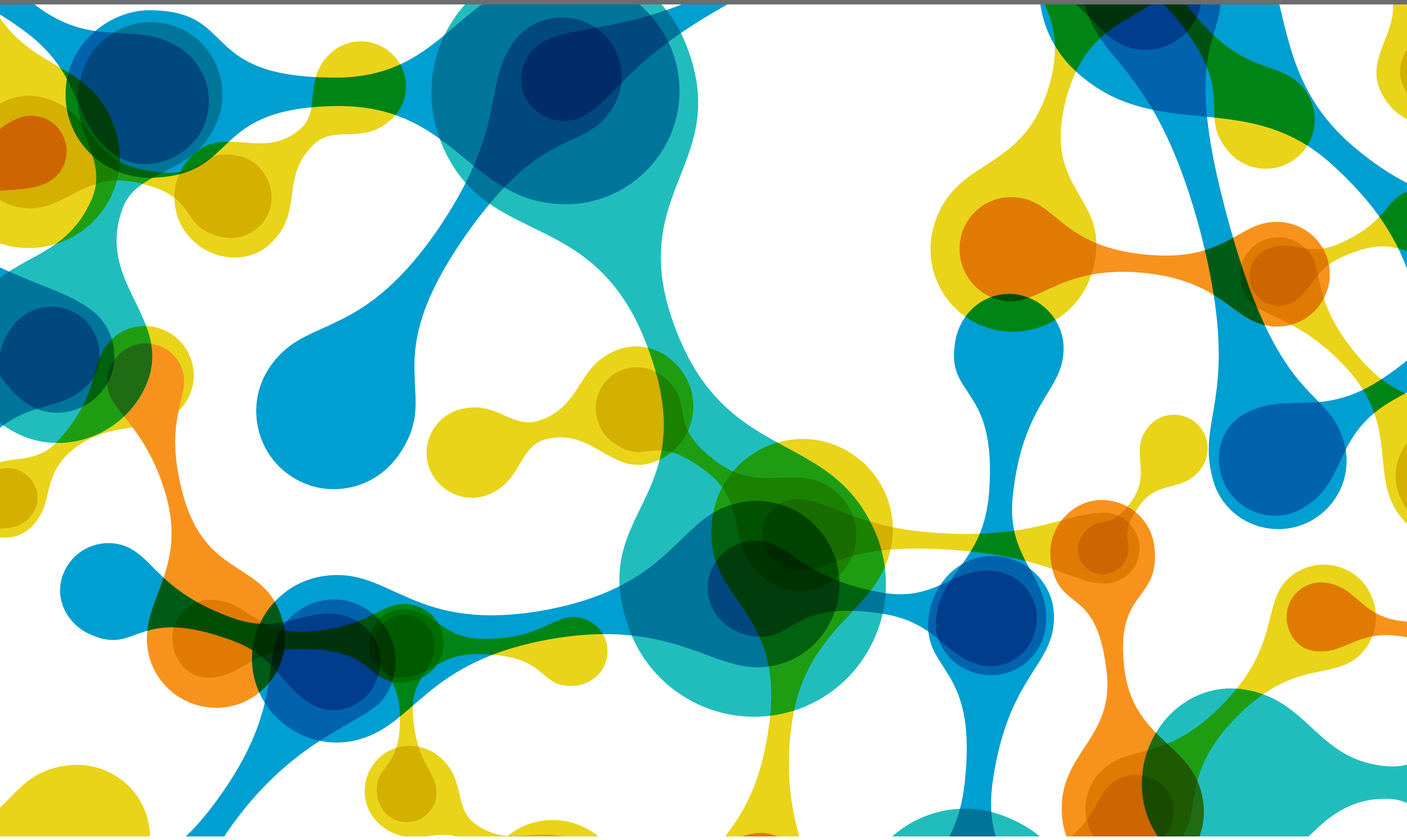
CEPHS*IN*ACTION: TOWARDS FUTURE CHALLENGES FOR CEPHALOPOD SCIENCE

EDITED BY: Lindy Holden-Dye, Giovanna Ponte, Graziano Fiorito,
A. Louise Allcock, Ryuta Nakajima, Erica A. G. Vidal and
Tarla Rai Peterson

PUBLISHED IN: Frontiers in Physiology and Frontiers in Communication



cost
EUROPEAN COOPERATION
IN SCIENCE & TECHNOLOGY

COST is supported by
the EU Framework Programme
Horizon 2020

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88963-437-8
DOI 10.3389/978-2-88963-437-8

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

CEPHS*IN*ACTION: TOWARDS FUTURE CHALLENGES FOR CEPHALOPOD SCIENCE

Topic Editors:
Lindy Holden-Dye, University of Southampton, United Kingdom
Giovanna Ponte, Association for Cephalopod Research 'CephRes', Italy; Stazione Zoologica Anton Dohrn, Italy
Graziano Fiorito, Stazione Zoologica Anton Dohrn, Italy
A. Louise Allcock, National University of Ireland Galway, Ireland
Ryuta Nakajima, University of Minnesota Duluth, United States
Erica A. G. Vidal, Federal University of Paraná, Brazil
Tarla Rai Peterson, The University of Texas at El Paso, United States

Image: Lindy Holden-Dye, Giovanna Ponte, Graziano Fiorito, A. Louise Allcock, Ryuta Nakajima, Erica A. G. Vidal and Tarla Rai Peterson Editors of CephsInAction Research Topic.

The last five years have been extremely challenging, but also very innovative for cephalopod science, and the outstanding tradition of biological contribution with cephalopod molluscs as key players in science and human activities and interests has continued. This Research Topic is one of several dedicated to cephalopod molluscs (e.g., Hanke and Osorio, 2018; Ponte et al., 2018) hosted by Frontiers over the last few years, not to mention other papers published separately. Highlighting of cephalopod science is important because it has much to offer not only the life science community, but also more broadly the public perception of science and its understanding and relationship with scientific endeavour and cephalopods as living organisms and part of our everyday life (at least for most of us). This contribution illustrates the key needs that need to be overcome by the cephalopod research community, i.e. rapid and effective mechanisms for exchange of knowledge and resources, sharing of laboratory protocols, videos, tissues, samples and data-sets, innovative approaches and initiatives in public engagement. The cuttlefish comic included is an excellent example of a type of media that can be used to expand scientific knowledge to the public and human relationship with live animals.

There are strategic challenges in convincing globally distributed policy makers and funders of the relevance of cephalopods in scientific advances, and also in the regulatory aspects, since cephalopods are the only invertebrates whose use is regulated in Europe in a research context and this increases the need for integrated oversight and direction in terms of ethics and animal welfare (e.g., Jacquet et al., 2019a; 2019b; Ponte et al., 2019). This Research Topic also aligns with the interests of the cephalopod community in stimulating public interest in cephalopods extending to a broader audience that could include chefs and gourmets, and fishers and scientists aiming to develop sustainable food resources.

"CephsInAction: Towards Future Challenges for Cephalopod Science" Research Topic includes 14 papers from about 40 authors representing ten different countries, thus overlapping with the original parties that contributed to the COST FA1301 that, together with CephRes, promoted and supported this editorial initiative.

Hanke, F. D., and Osorio, D. C. (2018). Editorial: vision in cephalopods. *Front. Physiol*. 9:18. doi: 10.3389/fphys.2018.00018 PMID:29403394

Jacquet, J., Franks, B., and Godfrey-Smith, P. (2019a). The octopus mind and the argument against farming it. *Anim. Sent*. 4:19.

Jacquet, J., Franks, B., Godfrey-Smith, P., and Sánchez-Suárez, W. (2019b). The case against octopus farming. *Issues Sci. Technol*. 35, 37–44.

Ponte, G., Almansa, E., and Andrews, P. L. R. (2018). The digestive tract of cephalopods: at the interface between physiology and ecology. *Front. Physiol*. 9:1409. doi: 10.3389/fphys.2018.01409 PMID:30356773

Ponte, G., Andrews, P., Galligioni, V., Pereira, J., and Fiorito, G. (2019). "Cephalopod welfare, biological and regulatory aspects: an EU experience," in *The Welfare of Invertebrate Animals*, eds C. Carere and J. Mather (Cham: Springer International Publishing), 209–228. doi: 10.1007/978-3-030-13947-6_9

Citation: Holden-Dye, L., Ponte, G., Fiorito, G., Allcock, A. L., Nakajima, R., Vidal, E. A. G., Peterson, T. R., eds. (2020). Cephs*In*Action: Towards Future Challenges for Cephalopod Science. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88963-437-8

COST
EUROPEAN COOPERATION
IN SCIENCE & TECHNOLOGY

COST is supported by
the EU Framework Programme
Horizon 2020

This publication is based upon work from COST Action FA1301 "CephsInAction", supported by COST (European Cooperation in Science and Technology).

COST (European Cooperation in Science and Technology) is a funding agency for research and innovation networks. Our Actions help connect research initiatives across Europe and enable scientists to grow their ideas by sharing them with their peers. This boosts their research, career and innovation.

www.cost.eu

Table of Contents

frontiers in Physiology

EDITORIAL
published: 25 July 2019
doi: 10.3389/fphys.2019.00980

Editorial: Cephs*In*Action: Towards Future Challenges for Cephalopod Science

Lindy Holden-Dye*[1], *Giovanna Ponte*[2,3], *A. Louise Allcock*[4], *Erica A. G. Vidal*[5], *Ryuta Nakajima*[6], *Tarla Rai Peterson*[7] *and Graziano Fiorito*[3]

[1] School of Biological Sciences, University of Southampton, Southampton, United Kingdom, [2] Association for Cephalopod Research 'CephRes', Naples, Italy, [3] Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Naples, Italy, [4] Ryan Institute, National University of Ireland Galway, Galway, Ireland, [5] Centro de Estudos do Mar, Universidade Federal do Paraná (UFPR), Pontal do Paraná, Brazil, [6] University of Minnesota Duluth, Duluth, MN, United States, [7] The University of Texas at El Paso, El Paso, TX, United States

Keywords: cephalopods, invertebrates, welfare, outreach, communication, neuroscience, physiology

Editorial on the Research Topic

Cephs*In*Action: Towards Future Challenges for Cephalopod Science

OPEN ACCESS

Edited and reviewed by:
Sylvia Anton,
Institut National de la
Recherche Agronomique
(INRA), France

****Correspondence:***
Graziano Fiorito
graziano.fiorito@szn.it

Specialty section:
This article was submitted to
Invertebrate Physiology,
a section of the journal
Frontiers in Physiology

Received: *16 June 2019*
Accepted: *11 July 2019*
Published: *25 July 2019*

Citation:
Holden-Dye L, Ponte G, Allcock AL,
Vidal EAG, Nakajima R, Peterson TR
and Fiorito G (2019) Editorial:
CephsInAction: Towards Future
Challenges for Cephalopod Science.
Front. Physiol. 10:980.
doi: 10.3389/fphys.2019.00980

The collection of papers included in this Research Topic represents the outcome of one of the activities of the COST Action FA1301—"A network for improvement of cephalopod welfare and husbandry in research, aquaculture, and fisheries" (Cephs*In*Action)—that operated for 4 years from 2013 to 2017. The idea of a "Cephs*In*Action" Research Topic entitled "Towards Future Challenges for Cephalopod Science" emerged at one of the last meetings of the COST Action FA1301: Cephs*In*Action and CIAC Meeting "Cephalopod Science from Biology to Welfare[1]" (Hellenic Centre for Marine Research, Heraklion, Crete, Greece, 28–31 March 2017), and from some editorial initiatives discussed at that time. This Research Topic (RT) is just one example of several RTs, and indeed other separate papers, dedicated to cephalopod molluscs (Hanke and Osorio, 2018; Ponte et al., 2018)[2] that have been hosted by Frontiers over the last few years.

This highlighting of cephalopod science is important as it has much to offer not only the life sciences community, but also more broadly the public perception of science and its understanding and relationship with scientific endeavor. To make this contribution, there are logistical challenges facing the cephalopod research community that need to be overcome. Importantly, given that cephalopod science is a relatively small, globally distributed research community, there is a need for rapid and effective mechanisms for exchange of knowledge and resources, encompassing everything from the sharing of laboratory protocols, videos, tissues and samples, and data-sets to innovation in public engagement. This also presents strategic challenges in convincing globally distributed policy makers and funders of the relevance of cephalopods in scientific advances. There are regulatory aspects too, as cephalopods are the only invertebrates whose use is regulated in Europe in a research context (e.g., Fiorito et al., 2014, 2015; Di Cristina et al., 2015), which increases the need for an integrated oversight and direction in terms of ethics and animal welfare (Ponte et al., 2019). This links to the important recognition that cephalopods are not "simple" laboratory

---

[1] http://www.cephsinaction.org/activities/meetings/year2016/cephsinactioncretaquarium/

[2] see also: https://www.frontiersin.org/research-topics/10233/vision-in-cephalopods-part-ii; https://www.frontiersin.org/research-topics/9997/cephalopod-research-across-scales---molecules-to-ecosystems

animals and that we need to understand their physiology and behavior by intersecting studies in their natural environment with those in standardized settings such as the lab-bench. Only by a better understanding of the normal range of behaviors of distinct species of cephalopods can their welfare be improved. There is the need for better phylogenetic resolution and for more accurate field data to facilitate this.

This Research Topic also aligns with the interests of the cephalopod community in stimulating public interest in cephalopods and their artistic interpretation. This extends to a broader audience that could include chefs and gourmets[3], fishers and scientists aiming to develop sustainable food resources. School children's natural fascination with cephalopods (e.g., Sperduti et al., 2012) can excite their interest in scientific discovery and encourage them to engage in conversations about the scientific process and what it means. The importance of such conversations cannot be underestimated in a world in which the public needs to be scientifically literate, as they must be equipped to make important socio-economical and political decisions facing the current condition of the world, whilst being confronted with 'fake news', 'alternative truths', and when expert opinion is often derided. There is great potential for innovative schemes for public engagement that springs from the natural wonderment the cephalopods incite.

The last five years have been extremely challenging, but also very innovative for cephalopod science and have continued the outstanding tradition of biological contribution with cephalopod molluscs as key players (e.g., Keynes, 1989; De Sio, 2011; Albertin et al., 2012; Garrett and Rosenthal, 2012; Huffard, 2013; Liscovitch-Brauer et al., 2017; Marini et al., 2017; Sanchez et al., 2018).

This Research Topic includes 13 papers from about 40 authors representing ten different countries, thus overlapping with the original parties that contributed to the COST FA1301. Three papers present original data and 10 others are reviews and perspective articles on various topics, as examples of the interest that humans have for these fascinating marine molluscs. This Research Topic offers a journey that spans cephalopod gastronomy (Mouritsen and Styrbæk) offering a glance at the interest among chefs and gastroscientists to explore these organisms as a counterpoint to other seafood, a look at new protein sources to replace meat from land-animal production, and a test of texture and flavor properties of cuttlefish, squid and octopus and how these provide the ground for a variety of culinary transformations. "CephsInAction: Towards Future Challenges for Cephalopod Science" also offers an "OctopusEye,": a "refracted spectatorship" perspective and conceptual analysis of the film "The Love Life of the Octopus (Les Amours de la pieuvre) (1965)" (Hayward). But cephalopods are also at the boundaries between "science, art and engineering" (Nakajima et al.). They are among the most enthusiastically visited animals in public aquaria, providing a way of communicating science and conservation (Marchio), and offer various "Critical Challenges Ahead" (O'Brien et al.) as envisioned by three junior "researchers who have recently embarked on careers in cephalopod biology" and that provide their suggestions on a variety of topics spanning from genetics, to welfare, behavior, cognition, and neurobiology.

This volume includes studies on the effects of maternal and embryonic stress on the behavior of offspring (*Sepia officinalis*, O'Brien et al.), presenting evidence for age-related differences in defensive behaviors in the sepiolid *Euprymna* (Seehafer et al.), or the development of swimming abilities of paralarvae of *Doryteuthis opalescens* (Vidal et al.). A preliminary analysis of the expression of protocadherins in *Octopus vulgaris* is also included providing the ground for future analysis of the way these genes may drive neural wiring during development and in the cases of biological and neural plasticity in the adult (Styfhals et al.).

Finally, several reviews are included in this Research Topic, (i) examining parasites that cephalopod host, (ii) raising the possibility of the existence of stem cells in cephalopod brains, (iii) considering possible cases of functional and convergent evolution of neural-systems, when compared with vertebrates, and (iv) overviewing the extraordinary and historically well-studied biological cases of tissue and neural regeneration (Deryckere and Seuntjens; Imperadore and Fiorito; Roumbedakis et al.; Shigeno et al.).

In sum, all contributions reflect a broad, interdisciplinary active and vital scientific community.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

This editorial and the e-book are based upon collaboration under the COST Action FA1301 Cephs*In*Action that also supported the meeting and partially funded the open access costs of the papers included in this RT. The Association for Cephalopod Research CephRes supported this work. We are deeply thankful to the CretAquarium (Heraklion) for help for the organization of the meeting, and in particular to Dr. Panagiotis Grigoriou and Dr. Aspasia Sterioti. We are also grateful to Dr. C. Balestrieri (CephRes) for her scientific and managerial assistance. The studies included herein are considered a contribution to the COST (European COoperation on Science and Technology) Action FA1301.

---

[3] See for example the Atlantic Area Interreg Project "Cephs and Chefs" https://www.cephsandchefs.com/

REFERENCES

Albertin, C. B., Bonnaud, L., Brown, C. T., Crookes-Goodson, W. J., da Fonseca, R. R., Di Cristo, C., et al. (2012). Cephalopod genomics: a plan of strategies and organization. *Stand. Genomic Sci.* 7, 175–188. doi: 10.4056/sigs.3136559

De Sio, F. (2011). Leviathan and the soft animal: Medical humanism and the invertebrate models for higher nervous functions, 1950s−90s. *Med. Hist.* 55, 369–374. doi: 10.1017/S0025727300005421

Di Cristina, G., Andrews, P., Ponte, G., Galligioni, V., and Fiorito, G. (2015). The impact of Directive 2010/63/EU on cephalopod research. *Invert. Neurosci.* 15, 8. doi: 10.1007/s10158-015-0183-y

Fiorito, G., Affuso, A., Anderson, D. B., Basil, J., Bonnaud, L., Botta, G., et al. (2014). Cephalopods in neuroscience: regulations, Research and the 3Rs. *Invert. Neurosci.* 14, 13–36. doi: 10.1007/s10158-013-0165-x

Fiorito, G., Affuso, A., Basil, J., Cole, A., de Girolamo, P., D'Angelo, L., et al. (2015). Guidelines for the care and welfare of cephalopods in research - A consensus based on an initiative by CephRes, FELASA and the Boyd Group. *Lab. Anim.* 49, 1–90. doi: 10.1177/0023677215580006

Garrett, S., and Rosenthal, J. J. C. (2012). RNA editing underlies temperature adaptation in K^+ channels from polar octopuses. *Science* 335, 848. doi: 10.1126/science.1212795

Hanke, F. D., and Osorio, D. C. (2018). Editorial: Vision in Cephalopods. *Front. Physiol.* 9:18. doi: 10.3389/fphys.2018.00018

Huffard, C. L. (2013). Cephalopod neurobiology: an introduction for biologists working in other model systems. *Invert. Neurosci.* 13, 11–18. doi: 10.1007/s10158-013-0147-z

Keynes, R. D. (1989). The role of giant axons in studies of the nerve impulse. *Bioessays* 10, 90–93.

Liscovitch-Brauer, N., Alon, S., Porath, H. T., Elstein, B., Unger, R., Ziv, T., et al. (2017). Trade-off between transcriptome plasticity and genome evolution in cephalopods. *Cell* 169, 191–202.e111. doi: 10.1016/j.cell.2017.03.025.

Marini, G., De Sio, F., Ponte, G., and Fiorito, G. (2017). "Behavioral analysis of learning and memory in cephalopods," in *Learning and Memory: A Comprehensive Reference, 2nd Edn,* ed J. H. Byrne (Amsterdam: Academic Press; Elsevier), 441–462.

Ponte, G., Almansa, E., and Andrews, P. L. R. (2018). Editorial: the digestive tract of cephalopods: at the interface between physiology and ecology. *Front. Physiol.* 9:1409. doi: 10.3389/fphys.2018.01409

Ponte, G., Andrews, P., Galligioni, V., Pereira, J., and Fiorito, G. (2019). "Cephalopod welfare, biological and regulatory aspects: an EU experience," in *The Welfare of Invertebrate Animals,* eds C. Carere and J. A. Mather (Basel: Springer Nature Switzerland AG), 209–228.

Sanchez, G., Setiamarga, D. H. E., Tuanapaya, S., Tongtherm, K., Winkelmann, I. E., Schmidbaur, H., et al. (2018). Genus-level phylogeny of cephalopods using molecular markers: current status and problematic areas. *PeerJ* 6:e4331. doi: 10.7717/peerj.4331

Sperduti, A., Crivellaro, F., Rossi, P. F., and Bondioli, L. (2012). "Do octopuses have a brain?" Knowledge, perceptions and attitudes towards neuroscience at school. *PLOS ONE* 7:e47943. doi: 10.1371/journal.pone.0047943

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Physiology

REVIEW
published: 06 June 2018
doi: 10.3389/fphys.2018.00700

The Current State of Cephalopod Science and Perspectives on the Most Critical Challenges Ahead From Three Early-Career Researchers

Caitlin E. O'Brien[1,2]*, *Katina Roumbedakis*[2,3] *and Inger E. Winkelmann*[4]

[1] Normandie Univ., UNICAEN, Rennes 1 Univ., UR1, CNRS, UMR 6552 ETHOS, Caen, France, [2] Association for Cephalopod Research – CephRes, Naples, Italy, [3] Dipartimento di Scienze e Tecnologie, Università degli Studi del Sannio, Benevento, Italy, [4] Section for Evolutionary Genomics, Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark

Edited by:
Lindy Holden-Dye,
University of Southampton,
United Kingdom

Reviewed by:
Ana Turchetti-Maia,
Tel Aviv University, Israel
Peter John Shand Smith,
University of Southampton,
United Kingdom

***Correspondence:**
Caitlin E. O'Brien
c_obrien@cephalopodresearch.org

Specialty section:
This article was submitted to
Invertebrate Physiology,
a section of the journal
Frontiers in Physiology

Received: 02 March 2018
Accepted: 18 May 2018
Published: 06 June 2018

Citation:
O'Brien CE, Roumbedakis K and Winkelmann IE (2018) The Current State of Cephalopod Science and Perspectives on the Most Critical Challenges Ahead From Three Early-Career Researchers. Front. Physiol. 9:700. doi: 10.3389/fphys.2018.00700

Here, three researchers who have recently embarked on careers in cephalopod biology discuss the current state of the field and offer their hopes for the future. Seven major topics are explored: genetics, aquaculture, climate change, welfare, behavior, cognition, and neurobiology. Recent developments in each of these fields are reviewed and the potential of emerging technologies to address specific gaps in knowledge about cephalopods are discussed. Throughout, the authors highlight specific challenges that merit particular focus in the near-term. This review and prospectus is also intended to suggest some concrete near-term goals to cephalopod researchers and inspire those working outside the field to consider the revelatory potential of these remarkable creatures.

Keywords: aquaculture, behavior, cephalopod, cognition, climate change, genetics, neurobiology, welfare

GENERAL INTRODUCTION

Cephalopods have long haunted the human imagination as monsters, inspiring mythology dating back to ancient Greek culture (e.g., the Hydra from the labors of Hercules, see Cousteau and Diolé, 1973, p. 72–73, 75; the Gordon Medusa in Wilk, 2000), to legends of sea monsters in Nordic culture and among sailors throughout the middle ages (Salvador and Tomotani, 2014), to the science fiction of the modern world (e.g., Sphere: Crichton, 1988; 20,000 Leagues Under the Sea: Verne, 1988), where they – or creatures strongly resembling them – often lurk in outer space as alien creatures from other worlds (as in the motion pictures *Arrival*[1] and *Life*[2], to mention some). And while they were once reviled as "stupid" by Aristotle (1910), and dangerous, as in *Toilers of the Sea* (Hugo, 2002), this unique molluscan taxon has now come to be admired by both scientists, artists and the general public alike (Nakajima et al., 2018). Their growing popularity is reflected in the choice of many aquariums to house them as star attractions, despite the sometimes formidable challenges associated with keeping them. They are also depicted fondly in contemporary culture from computer generated animations in blockbuster films (e.g., *Pirates of the Caribbean; At World's*

---

[1] 2016, Paramount Pictures.

[2] 2017, Columbia Pictures.

End[3], *Finding Dory*[4]), to clothing, jewelery and artwork, to the surfeit of online videos[5] featuring cephalopods. Few other invertebrates garner this degree of recognition or status.

Cephalopods have also come to be respected for their various contributions to scientific research. During the first half of the 20th century (white bars in **Figure 1**), they played a pivotal role in our understanding of the neuron, thanks to the relative accessibility of the giant axon in squid (Keynes, 2005). This was followed by a period of intense investigation of the cephalopodan nervous system and learning abilities, led by John Z. Young and his fellows, including B. B. Boycott and M. J. Wells among others, from the 1950s to 1970s (see light gray bars in **Figure 1**). Progress slowed from the 1970s to 1990 (see dark gray bars in **Figure 1**), due mainly to a lack of appropriate investigative tools to address outstanding questions (see Bitterman, 1975; see also Young, 1985). Thankfully, the end of the 20th century to the present day has seen a steadily growing body of work concerned with various other aspects of cephalopod biology, including genetics, welfare and the effects of climate change (see black bars in **Figure 1**, and detailed subject-by-subject breakdown in **Figure 2**).

Today, some of the most unique characteristics of cephalopods are also inspiring various technological developments, including adaptive camouflage based on cephalopod skin that can display a variety of patterns (Wang Q. et al., 2014; Yu et al., 2014) or spontaneously match its surroundings (Pikul et al., 2017), suction cups for wound repair (Choi et al., 2016), propulsion and buoyancy systems for Autonomous Underwater Vehicles (AUV, Song et al., 2016), distributed cognitive control systems for artificial intelligence (Íñiguez, 2017) and the design of soft robots (Laschi et al., 2012; Renda et al., 2012).

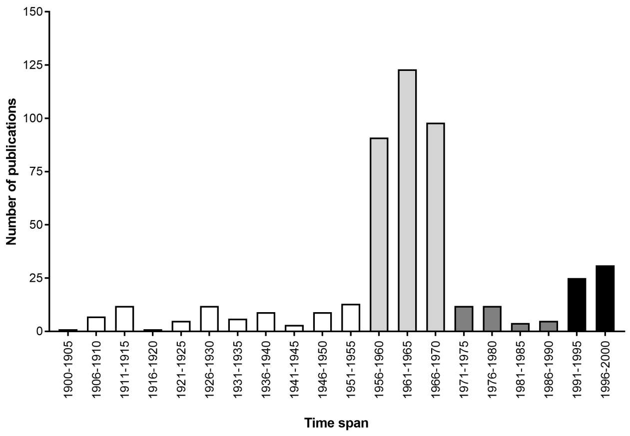


FIGURE 1 | Total number of publications on cephalopods per quinquennium that appeared in a genus-name search of the Zoological Record during the 20th century (adapted from Borrelli and Fiorito, 2008). Bar colors highlight different paces of research (see text for details).

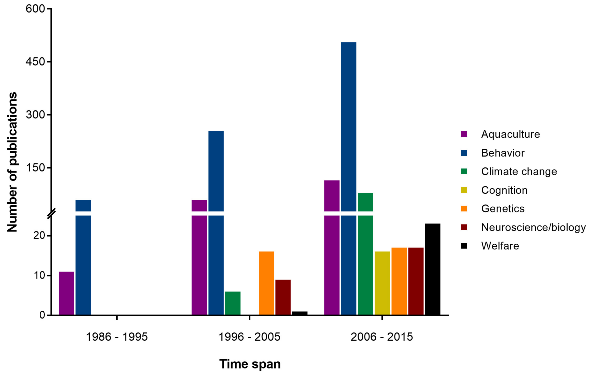


FIGURE 2 | The number of publications per decade between 1986 and 2015 as derived from a search on the Clarivate Web of Knowledge Core Collection (WoS) with "cephalopod" and the research topics addressed in this manuscript used as keywords, i.e., "aquaculture," "behavior," "climate change," "cognition," "genetics," "neuroscience/biology," and "welfare." Note that total numbers differ between **Figures 1**, **2** due to variations in indexing of the two databases and differences in search criteria.

Despite their great popularity and scientific relevance, detailed information on the biology, ecology, and physiology exists for about 8% (60 species) of the 800 or so known extant species of cephalopods (Jereb and Roper, 2005, 2010; Norman et al., 2014). Much more work is needed if we are to take advantage of all the scientific, technological and cultural inspiration that cephalopods have to offer. In order to stimulate further progress, we here focus on the potential of emerging technologies and of growing interest in cephalopods to address gaps in knowledge in seven particular subfields. We highlight some recent examples of progress in the fields of cephalopod genetics, aquaculture, climate change, welfare, behavior, cognition and neurobiology, and suggest challenges meriting particular focus in the near future (summarized in **Table 1**). The authors are three researchers who recently completed Ph.Ds in cephalopod biology, and who are thus particularly well-positioned (and motivated) to speculate about the future of the field. This manuscript follows from a series of keynote lectures ("Cephalopod Research; Visions of the Future") delivered during the CephsInAction and CIAC Meeting: Cephalopod Science from Biology to Welfare, held at the CRETAquarium (Crete, Greece, March 28–30, 2017). Hereafter, we first review the current state of cephalopod genetics (an especially fertile area of potential growth) and discuss some of the many ways omic technology can be applied to cephalopod research, including aquaculture. Next, we explore three topics related to cephalopod-human interactions: aquaculture, climate change and anthropogenic impact and welfare of animals in captivity. Finally, we discuss research concerning cephalopod behavior, cognition, and neurobiology, three distinctive biological innovations that occurred during the evolution of this lineage.

---

[3] 2007, Walt Disney Pictures.

[4] 2016, Walt Disney Pictures.

[5] For examples, see YouTube for videos depicting Paul the "psychic" octopus, who "predicted" the results of all of Germany's World Cup football games in 2010 (https://www.youtube.com/watch?v=kFvrAdyFUJ8, accessed May 3, 2018) or the clip from 2015 of an octopus jumping out of an Australian tide pool to capture a crab (https://www.youtube.com/watch?v=Ar5WJrQik2o, accessed May 3, 2018).

CEPHALOPODS AND GENETICS

Current Affairs: Ongoing and Recent Developments in Cephalopod Genetics

The incorporation of genetic tools in cephalopod research has progressed at a relatively slow pace in comparison with other taxonomic groups, as was recently noted by Xavier et al. (2015), and has faced many challenges, such as large and highly repetitive genomes. However, the tide is changing and even in the short time since this previous review by Xavier et al. (2015), there have been several important developments. Generally, DNA sequencing prices have continued to drop per bp sequenced, and the output capacity of commercial platforms has continued to increase, to the point where we can find ourselves inundated with data. Indeed, it is predicted that we will soon be dealing with a field where sufficient data storage and bioinformatic processing resources will be of much greater concern than generation of sequence data itself (Stephens et al., 2015).

TABLE 1 | Summary of the most pressing future tasks ahead in cephalopod research as viewed by the authors.

Genetics
- Improved phylogenies
- Refinement of eDNA technology
- Genome assembly

Aquaculture
- Sustainable food sources
- Control of reproduction in captivity
- Improved healthcare

Climate Change
- Determination of thermal tolerances
- Investigation of compound effects
- Particular vigilance for ELS and polar species

Welfare
- Validated anesthetics and analgesics
- Non-invasive health and welfare assessment
- Environmental enrichment

Behavior
- Field data and naturalistic experiments
- Investigation of inter-individual differences
- Ecotoxicology

Cognition
- Use as comparative model
- More precise lineage history
- More information from paleontological record

Neurobiology
- Primary neuronal cell culture
- Non-invasive neurological assays
- Brain atlases

General
- Open access platform
- Citizen science
- Cephalopod-specific initiatives

The section on "Aquaculture" was compiled in part from Vidal et al. (2014), Villanueva et al. (2014) and Xavier et al. (2015). The section on "Welfare" was compiled in part from Andrews et al. (2013) and Fiorito et al. (2015).

Several cephalopod genome projects are in the works, and have been for some years, but the completion and publication of these has been delayed by the overwhelming complexity of cephalopod genomes. Currently running projects include several of those cephalopod species selected by the CephSeq Consortium (Albertin et al., 2012), such as the pygmy squid (*Idiosepius paradoxus*), the bobtail squid (*Euprymna scolopes*), the blue-ringed octopus (*Hapalochlaena maculosa*) and the deep-sea giant squid (*Architeuthis dux*). This initial choice of species[6] was based on the potential practical use of the animals in a laboratory setting, as well as on particularly interesting and unique biological traits.

As a result of these efforts, a huge milestone was recently reached, when the first cephalopod genome – that of the California two-spot octopus (*Octopus bimaculoides*) – was finally completed and published (Albertin et al., 2015), making front page news in the journal Nature. The main findings were both surprising and fascinating. There was no apparent evidence of a whole genome duplication, which had been previously thought to explain the large genome size and pervasive repeats. The octopus genome was found, instead, to be broadly similar to those of other invertebrates, apart from an immense expansion of two specific gene families, which were previously known to be expanded in vertebrate genomes only. The first of these is the Protocadherins (a type of cell-adhesion proteins), which are particularly important for neuronal development. The second is the C2H2 class of zinc finger transcription factors (small protein structures, which typically function as interaction modules between DNA, RNA, proteins, or other small, useful molecules within a cell), hundreds of which were unique to the octopus. Moreover, these transcription factors were found to be selectively expressed in exactly the kinds of tissues that are special to the cephalopods, such as their suckers, nervous system and color-changing skin. Overall, what this first cephalopod genome revealed is that the expansion and diversification of these two gene families may have played a pivotal role in the evolution of those neural and morphological traits that make cephalopods so exceptional.

Mind the Gaps: The Problem of Assembling Cephalopod Genomes

As mentioned above, the cephalopods have presented a particular challenge to researchers in terms of assembling their nuclear genomes, in part due to their large size, but especially due to the rampant repetitive regions (strings of the same DNA sequence over and over again) scattered across them (Albertin et al., 2012). The reason this has presented such a problem is to do with the underlying technology of the sequencing platforms which have thus far been commercially available for use. Popular sequencing platforms, like the Illumina HiSeq, require the genomic DNA to be broken into short fragments of a few hundred base-pairs, so that they can be read by the sequencer. These short reads are then assembled, often by the billions, a bit like a large jigsaw puzzle, to re-create the original genomic sequence. This is accomplished

---

[6] https://www.cephseq.org/pioneer-species-list, accessed March 1, 2018

with powerful, highly specialized bioinformatic software, such as Meraculous (Chapman et al., 2011).

It is well known that these short-read technologies have limitations for "*de novo*" genome assembly, that is putting together a genome from scratch without prior knowledge or references, when it comes to repetitive regions. The problem arises when the length of the reads from the sequencer is shorter than those repetitive genomic regions that are to be assembled. Picture a gigantic puzzle, made up of tiny square pieces, where many regions of the image are exact copies of each other. How would you work out which copy each piece originally belonged to? It's an impossible task, and the result has been that *de novo* assemblies of repeat-rich genomes, which have been sequenced with short-read technologies, come out with many gaps and missing parts (Alkan et al., 2010). That is, if they can be assembled at all.

These kinds of problems, however, are (hopefully) about to become a thing of the past. Emerging long-read technologies, such as Pacific Biosciences (PacBio) (Rhoads and Au, 2015), and the Oxford Nanopore (Jain et al., 2016) series, are already available to researchers. These new sequencing technologies can currently produce reads that in some cases are more than a hundred thousand base pairs long, thus overcoming most issues with assembling repetitive regions (Pennisi, 2017). It is still early days, and both of these platforms remain relatively expensive to use and they suffer from higher error rates than the Illumina short-read technology (currently, roughly 15%, compared to only 1% for the Illumina), but this is bound to change, just as it did for the platforms that came before them, and perhaps just as rapidly. Together with improved software algorithms and other clever innovations (a couple of examples are given in Korbel and Lee, 2013; Kitzman, 2016), these developments have led to a recent flood of high-quality plant and animal genomes. This is important, because genome quality makes a big difference for the quality of science it is possible for researchers to do, and it will not be long before the trend includes cephalopods too.

Seeing the Forest for the Trees: The Importance of Improved Phylogenies

There will be many scientific benefits of this influx of high quality genomes to various fields of cephalopod research. The first of these will be the procurement of more accurate phylogenies. Due to the evolutionary history of the modern coleoid cephalopods, with a rapid radiation of the many different groups happening over a hundred million years ago, combined with their characteristic soft bodies leaving very few fossils, it has been difficult to accurately reconstruct their deep-level relationships to each other using phylogenetic analyses. Mitochondrial genome (Strugnell et al., 2017) and nuclear transcriptome (a genomic approach of sequencing all protein coding genes via their transcribed RNA, Lindgren and Anderson, 2017; Tanner et al., 2017) studies have already made progress toward solving this issue. In doing so, they overturned several previous notions about cephalopod relationships, such as the assumed monophyly of the squids, but with different published datasets also recovering slightly different phylogenetic trees. A couple of factors that have been found to influence the topology are marker coverage, that is how much of the genome is available for comparison in all of the sequenced species, and taxon sampling, that is how broadly and densely species were sampled across the true phylogeny (Lindgren and Anderson, 2017). High quality genomes from a growing number of cephalopod species will help to amend these problems, and, hopefully, finally provide a resolved picture of their evolutionary history.

The availability of accurate phylogenetic trees is crucial for studies of comparative evolutionary biology, as they allow independently observed traits to be mapped onto them, revealing the evolutionary histories of these traits and helping researchers to distinguish between functional similarity and relatedness. Some traits may be shared because several species share an ancestor who carried that trait, while other traits may be shared between species due to convergent evolution – the independent invention of the same functional trait more than once. This ability to unravel the history of morphological or behavioral trait evolution, and to classify traits as ancestral or derived, is highly relevant, for instance, to the study of cephalopod neurobiology and cognition, as well as other cephalopod specializations such as evolution of the ink sac, vision, acquisition of symbionts and toxin production.

Plastic Fantastic: A New Model for Fundamental Research on Genome Plasticity

Yet another intriguing specialization of the coleoid cephalopods, which we have only just begun to discover as we probe their genomes, is prolific RNA editing. While only a handful of a human's roughly 20,000 genes yield edited RNA transcripts (Pinto et al., 2014), a recent study found that more than half of translated gene transcripts in coleoids are edited, making it the rule rather than the exception (Liscovitch-Brauer et al., 2017). This pattern was not found in their distant relatives, the nautiloids, or in other molluscs. Moreover, most of these edits (65%) were found to change the amino acid sequence in the resulting protein, and are thus meaningful to the development of the animals. In neural tissue of *O. bimaculoides* specifically, 11–13% of edits change the amino acid, compared with less than a percent in mammals. Interestingly, many of these changes were made in the Protocadherins, that same gene family found to be massively expanded in the octopus genome. This implies that the behaviorally complex coleoid cephalopods have invented another ingenious way to quickly change and diversify the expression of their genome, especially in the genes important for their neural development. The extensive RNA editing to diversify their neural proteome does, however, appear to come at the cost of limiting their genomic DNA sequence flexibility and evolution. The flanking regions of the genes, which are important for the editing enzymes to perform their task, and which make up more than a quarter of the entire exome (the protein coding parts of the genome), are highly conserved, and seem to be evolving more slowly than in other animals.

This phenomenon is unparalleled in any studied vertebrate. It is yet another example of cephalopods taking a, sometimes strikingly, different evolutionary route to solve a similar problem, just as they have done for complex eye development (Nilsson, 1996), multidimensional vision (Temple et al., 2012; Stubbs and Stubbs, 2016), and fast action potential velocity in their giant axons versus our own myelin-insulated axons (Hartline and Colman, 2007). For this reason, coleoid cephalopods are likely to become the future model for studying RNA editing and genome plasticity, just as they became the first model for the experimental study of neuronal function after the discovery of their giant axon.

Fast Forward Selection: The Potential of Genomic Tools for Cephalopod Aquaculture

Another important development, which will be relevant for staking out future directions in genetic work, is a recently revived interest in the culture of cephalopods for experimental purposes, for ornamental aquarium trade, and for commercial food production. Currently, only small-scale culture is possible, and just for a small handful of species (Vidal et al., 2014; Xavier et al., 2015), but the intensity of research into husbandry techniques is increasing, and is likely to result in significant improvements over the coming years. This means that researchers may very soon unlock the potential of cephalopods to be kept and studied as an experimental laboratory model organism, much like mice, only with many traits that are extraordinarily similar to those of vertebrates, yet with an independent evolutionary history. It will also be important for industrial-scale aquaculture, as the world's wild cephalopod stocks are under increasing pressure as a fisheries resource (Rodhouse et al., 2014).

When it comes to keeping cephalopods as cultured animals, just learning how to farm the wild-type cephalopods, as they occur naturally, is unlikely to be enough for efficient results. Just as it has been the case for domesticated animals and plants in the past, it is likely to be in the future; humans have proven highly skilled at selecting and shaping our chosen creatures. Over the centuries, humans have domesticated animals for meat, dairy and company (Wang G.D. et al., 2014), plants for food, decoration and raw materials (Meyer and Purugganan, 2013), as well as microorganisms for food fermentation (Douglas and Klaenhammer, 2010). Unlike these past events, however, contemporary domestication of animals and plants comes with an unprecedented set of tools for breeders, such as genomics. With genomic information and information about the heritability of phenotypic traits, suitable technologies for marker-assisted selection, genome selection, and genome editing can be developed for applications in aquaculture.

High quality genome resources make genotyping of individual animals easy, and eventually cheap, for breeders. Genomic selection has already been a huge success in dairy cattle breeding, which since 2009 has not used progeny testing as a standard for evaluating young bulls, instead relying purely on genomic information (Boichard et al., 2016). The techniques first became common practice at a large scale in the most common breeds, but as prices dropped and efficiency rose, they quickly became a useful tool for less common cattle breeds as well.

Traits of interest for genomic characterisation, heritability assessment and selection in cephalopods for aquaculture include, but are not limited to, feeding preferences, environmental stress and crowding tolerance, disease resistance, size, growth rate and timing of sexual maturation. Much progress has already been made in aquaculture genomics for dozens of fish and shellfish species, including various stages of genome reference sequences, the development of genetic linkage maps, single nucleotide polymorphism (SNP) chip arrays and transcriptome databases (Abdelrahman et al., 2017). With the expansion of available genome resources, which is sure to follow further drops in sequencing prices and implementation of new sequencing technologies, and the substantial economic interest in large scale culture of cephalopods for consumption, this group of animals is sure to follow.

Given the era in which cephalopod domestication will happen, aquaculturists will not be limited only to the genomic technologies available to breed developers today. New and exciting possibilities lay ahead, such as the constantly, and rapidly, improving DNA base-editing technology (and also RNA, see Cox et al., 2017) derived from the molecular scalpel CRISPR-Cas9 (Gaudelli et al., 2017). CRISPR (Clustered Regular Interspaced Short Palindromic Repeats) is part of a rudimentary microbial adaptive immune system, which was only discovered in the 1990's and first understood in 2005 (Mojica et al., 2005). There is debate about which research group broke first ground in harnessing the system for genome editing around 2012, but while that debate continues, researchers across the globe are testing and refining the technology at a dizzying pace.

The development of such precise and easily programmable gene editors has enormous implications for quickly engineering high-performance aquaculture breeds. A case in which successful gene-editing has already been performed to attain a highly desired phenotype for a commonly cultured animal is that of the double-muscled pig. Double-muscled animals, such as the cattle breed Belgian Blue, are animals with a massive increase in skeletal muscle mass, which are known to occur naturally among cattle at very low rates (Fiems, 2012). Carriers of the gene cannot be identified by their phenotype, but since the mutation causing the phenotype was discovered (a so-called 'knock-out' mutation causing a non-functional myostatin gene), it became instantly possible, first of all, to identify carriers by genotyping. Recently, it also led to the engineering of other species with a similar phenotype, by using gene-editing to knock out that same gene. The first double-muscled pigs (Cyranoski, 2015) were reported to have been engineered by researchers using TALEN (Transcription Activator-Like Effector Nucleases) gene editing, a slightly older technique that has been in use since 2011 and was crowned a Nature Method of the Year that same year (Anon, 2011). The same result could have been accomplished, as it was for the Belgian Blue, with traditional breeding methods, but that did take almost 200 years. Likewise, with sufficient understanding of the genomics of cephalopods, it may be possible to further increase the speed with which we can adapt the animals to aquaculture, by using gene-editing to copy any desired, genetically determined, traits between species or breeds.

Free-Floating Data: Using Environmental DNA for Next Generation Ecology

Finally, another development in genetics, which holds great potential for cephalopod research, lies in the realm of population ecology and biodiversity monitoring. It is the emerging technology for environmental DNA (eDNA) detection and analysis. With eDNA analysis, DNA is isolated directly from an environmental sample, such as soil or water, without first isolating any type of organism. The technology for working with eDNA has its roots in the field of soil microbiology, where it was initially used to detect DNA from microbial life in sediments, but it has since been successfully adapted for eDNA from a wide range of sources, including sea water (Thomsen et al., 2012). Although detection of animals from eDNA sampling has thus far focused mainly on targeted sets of species or genera, it is anticipated that studies of eDNA will increasingly focus on meta-genomic surveys of entire ecosystems to investigate spatial and temporal patterns of biodiversity (Thomsen and Willerslev, 2015). This could be a tremendously useful technology to apply to the study of cephalopod populations, especially pelagic, because so little is currently known. This is mostly due to the difficulty of finding and observing the animals, and of collecting direct tissue samples. Shallow-water cephalopod species and those that are either commercially important for fisheries or easy to raise in captivity are the most studied and best understood. However, roughly 45% of all known cephalopod species are non-commercially important open-ocean or deep-sea squids and octopods (Sweeney and Roper, 1998). Application of eDNA methods have been shown to work for detecting and monitoring not only common species, but also those that are endangered, invasive, or elusive (Bohmann et al., 2014), and it could therefore be an especially potent tool for presence/absence monitoring of pelagic cephalopod species. Eventually, further development and application of these tools will open up an entirely new avenue for the study of population and community dynamics of these cephalopods.

Because detectable eDNA does not persist in the marine environment for long (Thomsen et al., 2012), the results are in real time, and the methods can be applied on any time-scale, from the monitoring of daily migration patterns to whole population range shifts in response to variations in climate. It can also be adapted from the collection of free floating DNA, using nets with different mesh sizes, to target single cells or planktonic organisms of a specific size, such as elusive cephalopod paralarvae. Furthermore, it may not remain restricted only to presence/absence or relative abundance estimates. Remote population genetic analyses may also be possible, as was recently demonstrated for an aggregation of whale sharks (*Rhincodon typus*) in the Arabian Gulf (Sigsgaard et al., 2016; Creer and Seymour, 2017). The authors collected sea water samples totalling 30 L and, incredibly, used them to estimate the genetic diversity of the whale shark population currently occupying the area.

Another exciting technological advance in the realm of eDNA is the so-called Environmental Sample Processor (ESP) developed at MBARI. It is a robotic device, which filters seawater down to 4000 m depth and applies a variety of molecular assays to the water samples, including quantitative PCR (qPCR), to identify specific target organisms and genes *in situ* (Ussler et al., 2013). This means it is not even necessary to collect water samples manually. Instead, the ESP can be placed or buoyed at a survey site, and programmed to test for the presence of defined species or genera at specific time intervals and transmit the results to researchers remotely. For now, the ESP is built with qPCR capabilities, but there is no reason this should not be upgraded to a type of small high-throughput sequencing technology in the future, thereby expanding its capabilities from a restricted focus on the target species (or genus), to a tool which can perform remote surveys of biodiversity and composition of entire biological communities.

Lastly, the impact of eDNA methods may even be felt by researchers working on aquaculture and breeding of cultured cephalopods as well. One of the problems when working with small and vulnerable larvae of cultured organisms is that of collecting sufficient DNA for genotyping without causing lethal injury to the larvae. This problem has recently been solved by genotyping of free-swimming, early fish larvae in a non-lethal and non-invasive way, by collecting and characterizing their eDNA (Espinoza et al., 2017), in a way that could quickly be adapted for cephalopods.

What treasures the future of genetics holds.

HUMAN IMPACTS ON AND INTERACTIONS WITH CEPHALOPODS

Culturing Consensus: Best Practices for Cephalopod Husbandry

Cephalopods are cultured for a variety of reasons, including human consumption, public display and restocking (Iglesias et al., 2014; Nabhitabhata and Segawa, 2014; Vidal et al., 2014). The potential use of aquacultural by-products, including pharmaceutical compounds (Koueta et al., 2014) is seen as another potential benefit. Certain species are particularly well-suited to aquaculture due to their rapid growth, short life cycles and market value (Lee et al., 1994; Pierce and Portela, 2014). Culture techniques have been developed for some of the species consumed for food, displayed in aquaria or used for scientific purposes, and information regarding capture methods, basic requirements (e.g., water quality, tank systems, hatching conditions, etc.) and diets (e.g., natural and artificial) for these species have been published (Boletzky and Hanlon, 1983; Iglesias et al., 2014; Vidal et al., 2014). There is, however, a consensus that two major obstacles limit large-scale and sustainable cephalopod culture in general: a lack of knowledge regarding optimal nutritional requirements and difficulties associated with successful reproduction in captivity (for details see **Table 1**).

The ultimate goal of cephalopod aquaculturists is the development of sustainable artificial diets, preferably based on non-marine ingredients (less expensive than marine equivalents), or derived from the discarded by-products of other fisheries (Vidal et al., 2014; Villanueva et al., 2014; Xavier et al., 2015). Cephalopods are short-lived and thus fast-growing animals, due to highly efficient ingestion, digestion and assimilation of proteins. They are also active swimmers and predators, consequently exhibiting a high metabolic rate and considerable demand of food (Boyle and Rodhouse, 2008). Understanding the process by which proteins and other nutrients are digested and assimilated can help to better design diets (e.g., Martínez et al., 2014).

A better understanding of digestive physiology and the feeding habits of each life stage is also needed in order to properly tailor diets to the requirements of specific cephalopod species. Formulated diets should be visually attractive and have proper texture and palatability, as well as appropriate digestibility (Villanueva et al., 2014). This tends to be especially difficult for the early planktonic life stages and juveniles of some species (e.g., *Octopus vulgaris*), which are active visual predators with high metabolic activity and sophisticated predatory behaviors (for review see Nande et al., 2017). On the other hand, it may be possible to facilitate changes in prey preferences during rearing, and train cephalopods to feed on artificial diets due to the remarkable behavioral plasticity of many cephalopod species (Vidal et al., 2014; Villanueva et al., 2014).

Knowledge about the processes and timing of digestion in candidate species for aquaculture is also lacking. A better understanding of how external factors (e.g., temperature, light cycle) can influence these processes, as well as their relation with circadian rhythms, is also needed. Due to differences in environmental temperature, species from different geographical regions (e.g., *Octopus maya* and *Octopus mimus*) will vary in digestive dynamics, the temporality of digestion and in their efficiency and patterns in absorbing and assimilating nutrients (Linares et al., 2015; Gallardo et al., 2017). It is also possible that similar differences occur between disparate populations of species with wide geographical distribution (e.g., *Sepia officinali*s, *Sepioteuthis lessoniana*). Additionally, understanding the physiological regulation of appetite, food intake (feeding frequency and amount of food ingested) and digestion will enable the design of feeding protocols and timetables that can maximize growth and survival rates. Such investigations will greatly optimize the growth and survival of these species at each life stage and improve our ability to properly maintain these animals in captivity, likely increasing both productivity and welfare at the same time (Sykes et al., 2017a).

Techniques for the management of cephalopod reproduction must also be improved in order to enhance aquacultural yields and overcome production bottlenecks. Several specific advances are necessary according to recent reviews (Vidal et al., 2014; Villanueva et al., 2014; Xavier et al., 2015): (i) the development of protocols for accelerating and/or retarding sexual maturation and spawning, thus allowing the control of reproduction under laboratory conditions; (ii) a better understanding of the influence of natural variables (e.g., temperature, photoperiod) on sexual maturation, reproductive performance, spawning, embryonic development and hatching success; (iii) the development of methods (hormonal or otherwise) to induce reproductive maturation; (iv) the improvement of broodstock conditioning and a better understanding of maternal effects on hatching quality and offspring competence; (v) greater knowledge of the role of chemical messaging, olfaction and sex pheromones in reproduction and its associated behavior.

Additionally, cephalopods can be subject to maternal effects, due to differences in embryo provisioning, egg placement, maternal care (for octopods and some squid) and stress-induced changes in behavior (Bloor et al., 2013; Juárez et al., 2016; O'Brien et al., 2017), and a better understanding of these may lead to improvements in hatching success and offspring fitness. Likewise, the potential for paternal effects on offspring should also be investigated, as this is known to be an influential factor in other animal groups (for review, see Rando, 2012) but, to our knowledge, has not yet been investigated in cephalopods. Finally, reproduction in cephalopods is further complicated by the existence of polyandry (e.g., Naud et al., 2005; Squires et al., 2012, 2014; Morse et al., 2018), sperm competition (e.g., Hanlon et al., 1999; Wada et al., 2005) and multiple male mating strategies that exist in several species of cephalopods (Hanlon et al., 2005; Iwata et al., 2011). A better understanding of these dynamics could potentially enable higher fertilization rates and reduce the number of injuries related to male-male competition for females.

The majority of the information available about cephalopod brooding behavior, reproduction and their physiological bases has been obtained under laboratory conditions. Unfortunately, certain information can only be derived from fieldwork. For example, the observation that wild female octopuses often repeatedly open and close the entrance to their den in order to facilitate the release of hatchlings (Cosgrove, 1993; Garci et al., 2016), gives cephalopod keepers insights with which they can improve environmental enrichment for captive brooding cephalopods. Providing materials that allow brooding octopuses to perform this behavior in captivity (e.g., by using natural benthic debris rather than plastic or any other artificial material for shelter/den) could improve reproductive outcomes, reducing maternal stress and improving welfare. More field observations and studies (e.g., direct observation of mating, egg-laying and brooding in the wild, larva counts from plankton tows, etc.) would greatly augment our current knowledge and lead to improved reproductive yields as well as better animal welfare.

In addition to improving management of nutrition and reproduction, cephalopod researchers should strive to establish a set of standardized husbandry techniques for commonly cultured species. As with diet, the culture of the same species may require different standards in tropical and temperate regions, and so region-specific guidelines may be required for certain species (e.g., *Sepia officinalis*, whose range extends from the Northern Atlantic and English Channel to the Mediterranean Sea). Particular attention should be given to the development of adequate artificial incubation techniques for small-egged species, such as *O. vulgaris*, which produce small, delicate planktonic paralarvae (Vidal et al., 2014; Villanueva et al., 2014). In the wild, planktonic paralarvae naturally experience very high mortality rates, and in the laboratory, survival rates are reduced further due to a lack of appropriate food sources and standardized culture systems, as well as due to trauma caused by contact with tank walls (Iglesias et al., 2007; Vidal et al., 2014). In general, research that focuses on facilitating life stage and phase transitions will further aquacultural aims, since these are the most critical and vulnerable periods of the life cycle (Vidal et al., 2014). As discussed previously, genetic manipulation may one day provide a means of controlling cephalopod reproductive capacity and success. Genetic selection/manipulation and biased genomic assays targeting potential genes of interest (e.g., those related to broodstock features, control of sexual maturation, growth, immunology and pathology) are potential methods to be employed (Vidal et al., 2014; Xavier et al., 2015). For example, genetic selection might be used to help sustain cultured populations through multiple generations by selecting for traits that improve success during challenging portions of the life cycle (e.g., reproduction, larval settlement) that currently limit aquacultural production (Vidal et al., 2014).

A better understanding of the functioning of the cephalopod immune system, along with its potential pathologies, infections, parasites and diseases, is critical to optimizing aquacultural output and animal welfare. It is well known that poor culture practices in commercial fish farms tend to compromise animal well-being and to encourage the outbreak of disease (Huntingford et al., 2006; Ashley, 2007). The conditions associated with intensive aquaculture (e.g., confinement, overpopulation and stress) tend to facilitate the incubation and transmission of parasites and disease. Parasites and pathogens normally found in wild populations, may, in many cases, also be responsible for diseases in captivity (Lafferty et al., 2015). Thus, knowledge of the pathogenic agents in wild populations of commonly cultured cephalopod species may aid in the prevention of disease outbreaks and the early diagnosis of health problems when they do occur, preventing or minimizing economical losses. The standardization of techniques for the collection, identification and documentation of parasites and pathogens would greatly facilitate this process and allow important information to be shared more easily.

Cephalopod aquaculture may also be advanced using techniques employed with other animal groups, but not yet tested in cephalopods. For instance, in some commercial fish farms, probiotics (microorganisms introduced to a host for its beneficial qualities) are used to promote growth, improve water quality, prevent disease, increase stress tolerance, enhance immune responses, and serve as a supplemental source of nutrients and digestive enzymes (Balcázar et al., 2006; Cruz et al., 2012; Michael et al., 2014). In cephalopods, however, the potential use of probiotics remains completely unexplored. Future work should focus on the identification of the intestinal biota of wild healthy cephalopod species and the identification of potential probiotic strains.

Finally, because most cephalopod aquaculture is focused on a small number of benthic, shallow-water species, almost no information is available for offshore, pelagic and deep-sea cephalopods (Vidal et al., 2014; Xavier et al., 2015). Given recent interest in the aquarium display of such creatures (e.g., the vampire squid), special attention should be given to the refinement of capture and transport methods for these species and to understanding their nutritional, behavioral and environmental requirements. Such knowledge will improve welfare and boost husbandry success, as well as facilitate the uniformity of experiments conducted on these species in disparate locations.

Forecasting the Future: Cephalopod Research and Climate Change

The effects of global climate change in marine environments include ocean warming, acidification and changes in dissolved oxygen availability. The consequences of these changes on marine organisms are of growing concern. Ocean warming is likely the most relevant of these changes to cephalopods: it may increase growth rates (if enough food and oxygen are available), consequently accelerating their life cycles (Doubleday et al., 2016) and increasing population turnover (Pecl and Jackson, 2008). Moreover, higher temperatures can shorten the length of embryonic development and increase the likelihood of premature hatching, both of which may cause serious biological impairments during crucial early life stages (Repolho et al., 2014; Caamal-Monsreal et al., 2016; Uriarte et al., 2016).

Thermal windows (the temperature range within which an animal performs optimally) differ between life stages in a given species as well as between species (Pörtner and Farrell, 2008). Establishing thermal windows and tolerances (especially critical thermal maxima, CTMax) for important species should be a priority since these biological limits have implications for the reproductive success and survival of juveniles. In particular, studies evaluating the thermal sensitivity and tolerance of embryos and early life stages are essential to better understanding how these animals will respond to a warming environment, since these are believed to be the most vulnerable stages within the life cycle (Rosa et al., 2012). Published aquacultural guidelines may also need to be periodically updated as species adapt to changing conditions. In particular, cephalopod populations residing in the Arctic and Antarctic may be more susceptible to climate change than populations in other regions due to the heightened environmental sensitivity and volatility of the polar regions (e.g., changes in temperature as well as changes in salinity from melting sea ice) and thus should be monitored especially vigilantly (Xavier et al., 2018).

Ocean deoxygenation and eutrophication, phenomena primarily attributed to the effects of ocean warming, also have implications for cephalopods. Marine hypoxia events have been found to alter the depth distribution of certain squids, as seen in *Dosidicus gigas* (Seibel, 2015). The effects of environmental deoxygenation can also be mediated by thermal tolerance to further affect cephalopod physiology: They can experience thermally induced oxygen limitation due to a reduction of the oxygen binding properties of haemocyanin (which is highly temperature-dependent), limiting survival time and eventually causing premature death (Melzner et al., 2007). In addition, physical abnormalities, such as defects in external yolk sac morphology, reduced embryonic size, as well as mantle, eye and arm deformities (potentially caused by a combination of temperature variation and hypoxic conditions during embryonic development) can occur in newly hatched specimens, as observed, for example, in *Sepioteuthis australis* (Gowland et al., 2002; Steer et al., 2002).

Similarly, ocean acidification could have deleterious effects on cephalopods, such as degrading the hard parts of their anatomy, e.g., cuttlebones (Gutowska et al., 2010; Kaplan et al., 2013), statoliths (Kaplan et al., 2013), and the external shells of nautiluses and argonauts (Wolfe et al., 2012), in addition to altering development time and hatching rate (Kaplan et al., 2013; Xavier et al., 2015). Global changes in oceanic currents may also affect the planktonic paralarvae of cephalopods, and the consequences of this may be positive, negative or both depending on the species (Xavier et al., 2015). Potential positive effects include the colonization of new areas and consequent expansion of species range (Zeidberg and Robison, 2007; Golikov et al., 2013), while potential negative effects include changes in food availability and impacts to the transport of early life stages (Pierce et al., 2010).

Although some information regarding the effects of isolated aspects of global climate change on cephalopods exists in the literature, the impact of combined effects (i.e., ocean warming plus acidification and marine hypoxia, etc.) are, to date, poorly known. Furthermore, questions about cephalopod tolerance and adaptability in the face of changing environments abound. One recent study suggests that the plasticity inherent to cephalopods may allow them to adapt more rapidly than other animal groups: coleoid cephalopods exhibit unprecedented levels of post-transcriptional modification to RNA, allowing the diversification of proteomes beyond the genomic blueprint (Liscovitch-Brauer et al., 2017). This ability may enable them to handle the effects of global climate change more rapidly and adeptly than other animal groups, contributing to increases in cephalopod populations that have been observed around the globe (Doubleday et al., 2016). Nevertheless, while cephalopods may benefit in some ways from a changing ocean environment (Doubleday et al., 2016), population dynamics are difficult to predict and human activities may yet have unpredictable deleterious effects. We must remain vigilant for these.

Improving Welfare: An Ethical Approach to Cephalopod Research

In the last decade, cephalopod welfare has gained much attention. This is due, in large part, to their addition to the list of animals regulated for use in scientific procedures within the European Union (European Parliament and Council of the European Union, 2010; Andrews et al., 2013; Smith et al., 2013; Di Cristina et al., 2015). Directive 2010/63/EU stipulates that all surgical and investigative procedures applied to vertebrates and now also cephalopods for research purposes should be carried out in such a way as to minimize pain, suffering, distress and lasting harm (PSDLH). In accordance with this principle, experimental procedures should be carried out under anesthesia and analgesia whenever possible and when sacrifice is necessary, animals must be killed humanely. Moreover, cephalopods used for scientific purposes must be maintained under conditions which meet basic health and welfare standards, and have their well-being monitored regularly. Here, we discuss the challenges that remain obstacles to fulfilling these mandates.

Around 20 substances and/or combinations of anesthetic agents have been tested in a few cephalopod species with some apparent success (for review, see Gleadall, 2013; Fiorito et al., 2015), but knowledge of their mechanisms of action is very limited. Moreover, descriptions of cephalopod behavior during anesthetic induction and recovery (e.g., Andrews and Tansey, 1981; Gonçalves et al., 2012; Gleadall, 2013; Butler-Struben et al., 2018) or of the physiological effects of putative anesthetic agents on the animals (Pugliese et al., 2016; Butler-Struben et al., 2018) are relatively few. Variations in the effectiveness of anesthetics in relation to cephalopod age, sex, life stage, body weight, physiological condition and health status, remain largely unexplored, as do the interactions of anesthetics with various parameters, such as temperature, salinity, pH and oxygen level. All of these factors are critical for the humane treatment of animals in experimental contexts, and also for husbandry, which may require anesthesia during handling and surgical procedures.

The information available for analgesia in cephalopods is even more limited than for anesthetics (Andrews et al., 2013; Fiorito et al., 2015). Although ketoprofen and butorphanol have been proposed as analgesics for cephalopods, the dosing guidelines are based on studies performed on fish and amphibians (Gunkel and Lewbart, 2008) and, to date, there are no specific studies testing these substances in cephalopods to the best of our knowledge. Tests of potential analgesic agents and evaluation of their effectiveness are urgently required. This would be facilitated by the development of pain scales, such as those proposed for mammals (e.g., Mouse Grimace Scale, Miller and Leach, 2015). In addition, tests of analgesic self-administration for pain relief, such as those utilizing facultative oral administration in mammals (e.g., Colpaert et al., 1980, 2001), could be used to evaluate a substance's efficacy in cephalopods.

Protocols for the humane killing of cephalopods also require refinement. Although recommendations of methods have been published (Fiorito et al., 2015), no specific guidelines are provided by Directive 2010/63/EU. The suitability of the methods currently in use needs to be validated and alternative methods should be tested. Future studies should also focus on evaluating the level and nature of any suffering caused by these methods. Apart from pain assessment, a standardized way to assess of responsiveness to stimuli (i.e., consciousness) should be developed so that current and proposed methods of humane killing can be evaluated objectively.

Determining how to properly assess health and welfare in cephalopods is a critical issue to address in the near future but developing species-specific guidelines for welfare assessment and ethical treatment is not an easy task. One potential model for cephalopod welfare assessment is a scored model, based on animals' physiology (e.g., respiration, osmotic balance, nutrition) and behavior (e.g., feeding, rest, sexual behavior), such as the one designed for the Atlantic salmon *Salmo salar* by Stien et al. (2013). Another potential technique is the use of cognitive assays, such as preference tests, to assess animals' status (Brydges and Braithwaite, 2008). Some efforts in this vein have been made in recent years. A list of potential indicators for health and welfare in cephalopods, utilizing overall appearance, behavior and clinical indicators, including a graded severity scale, has recently been published (for details see Table 5 in Fiorito et al., 2015). In addition, an attempt to develop a framework for monitoring and assessing cephalopod welfare a "Cephalopod Welfare Index" is currently underway[7] under the aegis of the COST Action FA1301.

The development of non- or minimally invasive methods to assess the health of cephalopods is needed. For instance, ultrasonography is considered to be a suitable tool to determine sex and the maturation status of the gonads, and to assess the body condition of living animals. In *O. vulgaris*, ultrasonography has also been used to observe mantle contractions during locomotion and respiration (Tateno, 1993), the central nervous system (Grimaldi et al., 2007), the arms (Margheri et al., 2011) and the digestive tract (Ponte et al., 2017). In *S. officinalis*, ultrasound has been used to analyze cardiovascular activity (King et al., 2005), as well as cardiac and ventilatory rates in response to sudden visual stimuli (King and Adamo, 2006). While the potential of ultrasound imaging as a non-invasive method for assessing health in cephalopods is clear, further refinement is required, including the establishment of standardized protocols to assess normal (and abnormal) physiological conditions (e.g., assessment of cardiovascular and respiratory function, reproductive status, parasite infection).

In addition to ultrasound, other non- or minimally invasive methods have recently begun to be explored. For instance, a series of techniques, including behavioral responses to prey, the rate of food intake, fluctuations in body weight, oro-anal transit times, defecation frequencies, fecal appearance and composition, endoscopic assays, and needle biopsy (which may require ultrasound guidance) have been suggested as methods to assess the digestive health of cephalopods (Ponte et al., 2017). Another group of researchers have recently tested methods for *in vivo* sex determination of adult cuttlefish (*S. officinalis*) using an endoscope (Sykes et al., 2017b). Additionally, they suggest the use of subcutaneous elastomer implants for marking individuals and of mucus swabs from the inside of the mantle cavity to obtain DNA samples as minimally invasive techniques to be utilized with cephalopods. The extension of these techniques to other species, and the development of other non-invasive approaches may contribute to better *in vivo* assessment of cephalopod health status and assist in future efforts to improve cephalopod welfare in captivity.

The conception of welfare encompasses not only animal maintenance and basic health care, but animals' "psychological" well-being as well. In addition to having their basic physiological needs met and not suffering from discomfort, pain or stress, cephalopods used as experimental subjects or kept in public aquaria should be free to express their natural behavior (Mellor, 2016). As such, "enrichment" of housing conditions for captive cephalopods (e.g., providing shelters, intellectual stimulation, a varied environment) is a topic of great interest (Anderson and Wood, 2001; Williams et al., 2009; Baumans and Van Loo, 2013). As with many vertebrates, an enriched environment can positively influence cephalopod behavior as shown in cuttlefishes (Poirier et al., 2004, 2005; Yasumuro and Ikeda, 2016) octopuses (Beigel and Boal, 2006; Yasumuro and Ikeda, 2011), as well as memory formation and animal growth (Dickel et al., 2000). Future studies should test ways of presenting food that stimulate natural foraging behavior and yet are compatible with the ethical treatment of prey species, and identify tank materials and substrates that enable the expression of natural behaviors such as camouflage, hiding and exploration. Of course, environmental enrichment must also always be balanced against the need for good environmental hygiene and the ability to assess the status of the animals (Fiorito et al., 2015).

CEPHALOPODAN INNOVATIONS; BEHAVIORAL PLASTICITY, ADVANCED COGNITION AND SOPHISTICATED NEUROBIOLOGY

Some of the phenotypic features that make cephalopods such atypical invertebrates and so compelling to scientists and casual observers alike include their behavioral plasticity and advanced cognition, supported by sophisticated underlying neural organization. The past decade has seen the publication of a number of excellent reviews and books dealing with these topics singly or in conjunction with each other. For a superb and thorough overview of cephalopod behavior, refer to the recently updated eponymous book by Hanlon and Messenger (2018), as well as reviews by Huffard (2013), Marini et al. (2017), Mather and Dickel (2017) and Villanueva et al. (2017). Body patterning, for the purposes of both signaling and camouflage have been reviewed recently (Borrelli et al., 2006; Tublitz et al., 2006; Mäthger et al., 2009) as for learning and memory capabilities (Borrelli and Fiorito, 2008; Amodio and Fiorito, 2013; Dickel et al., 2013; Mather and Kuba, 2013; Darmaillacq et al., 2014; Tricarico et al., 2014; Zarrella et al., 2015; Mather and Dickel, 2017), while the evolution of cognition in this group is explored in several others (Grasso and Basil, 2009; Godfrey-Smith, 2013, 2016; Vitti, 2013). The mid-20th century brain ablation experiments by J. Z. Young and colleagues are comprehensively surveyed by Sanders (1975), while Marini et al. (2017) offer a briefer and modern synopsis of this work.

Young (1985) summarized early investigations of the visual and equilibrium systems and extraocular photoreceptors, while more recent reviews of sensory systems, particularly vision, are available in a number of works (Budelmann, 1995, 1996; Budelmann et al., 1997; Hanlon and Shashar, 2003; Alves et al., 2008; Nilsson et al., 2012; Dröscher, 2016; Levy and Hochner, 2017; Hanke and Osorio, 2018). Recent developments in the cephalopod neurosciences has been largely based on the initiative of Dr. B. Hochner and colleagues, including study of the cellular, molecular and synaptic mechanisms of the cephalopodan nervous system (e.g., Hochner et al., 2006; Hochner, 2010, 2012, 2013; Zullo and Hochner, 2011; Brown and Piscopo, 2013; Hochner and Shomrat, 2013; Shomrat et al., 2015; Zarrella et al., 2015; Turchetti-Maia et al., 2017).

---

[7] www.cephsinaction.org/working-groups/working-group-4/#Database

Because these topics have recently been addressed so extensively, we have opted to focus this section primarily on what we view as pressing near-term challenges and highlight some particularly promising potential methods with which we might investigate them.

Into the Wild: Studying Cephalopod Behavior

The current understanding of cephalopod behavior is limited by the fact that it has mainly been studied in laboratory settings. Unfortunately, without the ecological context of the natural environment, the survival value *sensu* Tinbergen (1963) of particular behaviors often cannot be perceived, leading to misinterpretations of evolutionary or ecological fitness. Thus, in order to improve understanding of cephalopod behavior, more field observations and field experiments are needed. While there are obvious difficulties to field work, the insight gained will be well-worth the effort. A recent study by Schnell et al. (2015) is a good illustration of this: via controlled laboratory experiments, the authors found that the white lateral stripe displayed by female *Sepia apama* signals non-receptivity for mating (they are less likely to mate when showing it). However, observations of natural behavior in the field showed that males largely ignored this and tried to mate anyway. This combination of laboratory tests with natural observations allowed observers to deduce the intended meaning of an intraspecific signal, but also provided contextual data about its relevance and efficacy in actual mating situations. And where experiments in the field are not possible, we encourage researchers to consider conducting their experiments in the field or in semi-natural conditions (such as a mesocosm), which have the advantage of promoting natural behaviors while also allowing for more experimental control.

The effort to increase the canon of field data will be aided by the pace of technological development and decreasing costs of data acquisition tools. Various types of tagging have been utilized successfully in recent years to answer questions about geographic range, migration and diving habits (Fuentes et al., 2006; Gilly et al., 2006; Replinger and Wood, 2007; Bazzino et al., 2010; Barry et al., 2011; Sims et al., 2011; Wearmouth et al., 2013; Sykes et al., 2017b). Remote monitoring through videography and photography is another increasingly accessible option thanks to the profusion of low-cost cameras that have come on the market in recent years. In particular, the use of cameras mounted onto cephalopods, in or near their dens or on their predators or prey are enabling researchers to study previously inaccessible behavior. For instance, Rosen et al. (2015) were able to use cameras mounted on Humboldt squid to document and analyze two distinct body patterns ("flashing" and "flickering") *in situ* and to infer their likely purpose as intraspecific signal and dynamic camouflage, respectively. Similarly, remotely-operated underwater vehicles (ROVs), AUVs and submersibles are also becoming more affordable, and have greatly expanded knowledge of deep-sea cephalopod behavior, such as providing evidence as to the purpose of the bizarre asymmetric eyes of cockeyed squids (Thomas et al., 2017) characterizing arm autotomy in a mesopelagic squid (Bush, 2012), and even capturing footage of the elusive giant squid[8].

In addition to embracing the benefits of this evolving technology, the cephalopod research community should consider sharing this raw video footage and data to an open access repository. Such a repository would allow students and researchers lacking funds, facilities or animals to perform their own analyses and contribute to the body of knowledge. This would be in line with a recent suggestion by other authors (Xavier et al., 2015) who have urged a community-wide shift in focus from data acquisition to data analysis. More importantly, the sharing and reuse of raw data and footage would improve welfare by reducing the total number of animals manipulated for experiments (Fiorito et al., 2014).

Regardless of whether research takes place in the laboratory, mesocosm or the field, greater efforts at standardization across experiments is needed. Due to the sensitivity and advanced perceptive abilities of cephalopods, even minor methodological differences can skew results and lead to divergent conclusions. For example, the standard method of measuring learning and memory in cuttlefish is the "Prawn-in-the-Tube" (PIT) procedure (Messenger, 1973) which has been used for decades by a number of research groups. While this standardized method theoretically allows direct comparisons to be made between experiments conducted in different times and places, the discovery that cuttlefish and other cephalopods are able to perceive differences in the polarization of light has led to the realization that the seemingly irrelevant choice of tube material (i.e., glass versus plastic—each of which alters the properties of light in different ways) could potentially affect results (Cartron et al., 2013). One technique to increase standardization across experiments and research groups is the creation and use of standardized video stimuli (e.g., approach of a predator, prey item or conspecific) from a set of such videos for use in behavioral experiments. Such a system has already been used by one group (Pronk et al., 2010) to study the reactions of octopus over time and between individuals. If such video clips were shared to a common open-access platform as suggested above, experiments could be replicated at different times and by different labs in a standardized fashion using commercially available audiovisual playback equipment.

In addition to standardizing and replicating experiments and observations within the same species, the cephalopod research community should also strive to duplicate across multiple species. Having corresponding data on closely related animals allows comparisons to be made and conclusions to be drawn about the entire lineage by giving a sense of what behaviors are evolutionary conserved from earlier shared ancestors and which represent novel adaptations to the particular environment of that species. In the family Hominidae for example, social differences between such congeners as apes, chimps and bonobos allow assessment of the factors driving behavioral evolution (e.g., Stanford, 1998; Malone et al., 2012). Similar comparisons between such commonly studied cephalopod species as *Sepia officinalis*, *Loligo vulgaris* and/or other squids, and *Octopus*

---

[8] https://www.youtube.com/watch?v=lzrzw4FpoKU, accessed March 1, 2018

vulgaris would be a good place to start, although the eventual goal should be to assess behavior across a wide variety of species, including the non-coleoid cephalopod *Nautilus* spp. (see for example, Crook and Basil, 2008), which can serve as an ancestral reference point.

Cephalopod research would also benefit greatly from the formal investigation of inter-individual differences and behavioral plasticity in this group. Anecdotal observations by aquarists and researchers give the distinct impression that individual animals have distinct "personalities." Indeed, in *S. officinalis*, certain behaviors were expressed predictably and consistently over time, although the expression of other behaviors differed between testing situations (see for example findings in Carere et al., 2015). Further research into this subject may indicate different tactics and interpretations need to be applied at the population level, such as distinguishing between "personality types" when calculating group means. Ultimately, plasticity may explain some of cephalopod's extraordinary evolutionary success, including their evolutionary persistence through three mass extinctions and recent increases in population despite (or perhaps because of) the effects of global climate change as discussed by Doubleday et al. (2016). Behavioral plasticity may buffer cephalopods against the rapid changes in environmental conditions that the world is currently experiencing (e.g., bleached coral reefs, invasive species, changing temperature regimes), and this hypothesis will be put to the test in coming years.

Another anthropogenic environmental impact that is increasingly relevant is how cephalopod behavior is affected by environmental pollutants. As neurologically complex organisms often residing in nearshore environments polluted by pharmaceutical residues, pesticides, and other chemicals, the cephalopod nervous system can potentially be affected. Indeed, the selective serotonin re-uptake inhibiter (SSRI) Fluoxetine, a pharmaceutical product found in high concentrations near heavily populated coastal areas across the globe, has been shown to affect young *S. officinalis* in different ways depending on age and dose (Di Poi et al., 2013; Bidel et al., 2016b). Moreover, in one case, differences could not be identified with standard behavioral tests but only by combining assays (Bidel et al., 2016b), demonstrating that the effects of such pollutants can be subtle and not immediately apparent. Considering the rapid pace of anthropologically induced environmental change, it is important that to get a behavioral "baseline" of vulnerable species as quickly as possible, since such information can be used to guide future environmental and fishing regulations that will mitigate the effects of these pollutants and climatic shifts.

Alien Intelligence? The Evolution of Advanced Cognition in Cephalopods

Cephalopods demonstrate unexpectedly advanced cognitive abilities and should play a much larger role in scientific discussions about cognitive evolution. A number of cephalopodan features, have experienced convergent evolution with vertebrates, allowing cephalopods to serve as a phylogenetically distant reference point from which to examine the universal selective pressures driving the evolution of organ systems and other traits. For instance, both the vertebrate and cephalopod eye have evolved to function similarly, but via alternative physiological means (review in Fernald, 2000). This demonstrates that despite vast differences in ancestry and underlying physiology, selection can sometimes arrive at the same evolutionary solution to an ecological challenge – in this case, the need to gather highly accurate and detailed visual information from the environment. In a similar manner, cephalopods have enormous potential to reveal the general evolutionary principals driving cognition. By making direct comparisons between cephalopods and "cognitively advanced" vertebrates, such as mammals and birds, the evolutionary pressures driving cognitive evolution, as well as the physiological prerequisites for such advances, can be inferred with less bias from shared ancestry. For instance, the existence of such cognitive abilities as learning and memory in relatively non-social cephalopods demonstrates that sociality is not necessarily a prerequisite for cognitive evolution, and calls the social intelligence hypothesis – the idea that the need to navigate complex intraspecific social interactions may have been the primary driver of cognitive evolution in primates, cetaceans and birds – into question (see Holekamp, 2007). It is also worth mentioning that in a similar manner, cephalopods can also be used as a non-vertebrate model with which to study the nature of animal consciousness (Mather, 2008; Edelman and Seth, 2009; Mather, 2011).

Complex nervous systems and cognition come at a high metabolic cost for organisms (Godfrey-Smith, 2013), and in cephalopods, the size of the brain limits the amount of food that can be ingested per swallow and puts animals at risk of brain injury (Huffard, 2013). Thus, there must be strong selective pressure or pressures (survival value, *sensu* Tinbergen, 1963) driving its evolution in the face of these disadvantages. Cross-phyla comparisons to identify circumstances common to organisms that share this feature are currently underway, and promise fruitful insights in the very near future. Initial comparisons with birds and mammals suggest that a variable environment is an indispensable driver of advanced cognition, since that is a factor common to all three groups (Vitti, 2013), but more investigation is necessary before any concrete conclusions can be drawn. Other potential selective pressures driving cognitive development in this group can be addressed through a better understanding of the timing of evolutionary history in general. For instance, Packard (1972) suggested that cognitive evolution was driven by the rise of and competition with bony fishes, while more recently, other authors (Grasso and Basil, 2009) argue that cephalopodan cognitive development actually occurred long before the advent of bony fishes in response to competition with the first jawed fishes and with other cephalopods. A more comprehensive and precise timeline of evolutionary events during the Paleozoic and Mesozoic will obviously aid in resolving this question.

Like external selective pressures, the proximate mechanistic factors (causation, *sensu* Tinbergen, 1963) that enabled such an impressive degree of cognitive evolution in this group also require investigation. It has been suggested recently that the loss of the hard external shell (Mather, 2011) and the advent of sophisticated vision (Vitti, 2013) were key innovations supporting cognition. However, neural gigantism of the molluscan lineage (Gillette, 1991), than may account for exceptional cerebralization in cephalopods, which increases the transmission efficiency of the molluscan nervous system despite the absence of the vertebrate myelin-sheath gaps, is another factor to consider. The cognitive abilities and behavioral plasticity of cephalopods may also be related to recently discovered dynamic-editing of RNA (Liscovitch-Brauer et al., 2017). Some authors even go so far as to suggest that cephalopod cognition is of alien origin, the result of genes introduced by extraterrestrial viruses that arrived on earth via meteorite 270 million years ago (Steele et al., 2018). To address these hypotheses, a more complete and accurate history of the cephalopod lineage is needed, including more accurate phylogenies as well as more precise timeline of the advent of certain physiological changes and innovations (e.g., shell loss, encephalization). A good first step in this effort would be a more comprehensive survey of the learning abilities of the "living fossil" *Nautilus* (Basil et al., 2011), the extant cephalopod most similar to the putative ancestral condition from which coleoids evolved. Comparisons of the coleoids (150 million years old) with their smaller-brained, less-encephalized *Nautilus* relatives (400 million years old) would allow deduction of the role of various senses and neural structures in the cognitive abilities of cephalopods. The *Nautilus* has only 13 lobes compared to the 40 identified in octopus, and, importantly, lacks a vertical lobe—the structure thought to be the seat of higher cognitive processes in coleoids. Recent experiments with *Nautilus* have demonstrated that they possess more advanced cognitive abilities than traditionally thought, including rapid learning, biphasic memory and advanced olfactory spatial navigation skills (Crook and Basil, 2008; Crook et al., 2009; Basil et al., 2011). This contradicts traditional interpretations of nautilus' cognition, and suggests that either a prototype vertical lobe system is present in the *Nautilus* (perhaps the plexiform layer and suboesophageal nerve cords), or that the vertical lobe is not as critical to advanced cognition in coleoids as currently thought (sensu Basil et al., 2011).

Inquiries into the cognitive evolution of cephalopods would also be greatly facilitated by increasing the amount of genomic and paleontological data available. For example, comparison of gene expression in the eyes of nautilus, squid, other molluscs and humans has enabled the identification of at least three types of genetic innovations that occurred during evolution of the cephalopod eye, including the duplication and subsequent re-purposing of some genes (Yoshida et al., 2015). Sutton et al. (2016) conducted a phylogenetic analysis on a morphological dataset constructed from both extinct (fossil) and extant specimens, and were able to confirm many of the putative relationships between coleoid groups, but found a few to be para- or polyphyletic. The recent sequencing of the entire *O. bimaculoides* genome has revealed that unlike other molluscs, this species (and probably other octopus species) has experienced expansion of some of the same gene families involved in vertebrate neuronal development (Albertin et al., 2015). Finally, another study used data from 180 genes across 26 species to test hypotheses about divergence times and were able to date the origin of specific groups, including vampire squids, dumbo octopuses, incirrate octopuses and decabrachians (Tanner et al., 2017).

While the recent boom in genetic data has led to some neglect of more traditional paleontological and morphological methods (Xavier et al., 2015), new imaging and phylogenetic techniques are being used to extract more information from existing fossil specimens. For example, UV light has been used to reveal structures not normally visible in a fossilized belemnite (*Acanthoteuthis speciosus*), including cranial cartilage, vague imprints of the statocysts and the first-ever evidence of a belemnitid radula (Klug et al., 2016). Though a fossil record for most soft-bodied cephalopods is lacking, a few specimens do exist. Recently, researchers were able to reconstruct soft body parts in three dimensions (including the eyes and some suckers) from a fossilized octopus using synchrotron microtomography (Kruta et al., 2016). The presence of suckers in this specimen forced researchers to re-evaluate the advent of this structure, which was thought to be a more recent development. Other possible tools include isotope analysis of fossil material and X-ray tomography, a method which allows the internal investigation of fossils and which can reveal preserved soft tissues. Synthesis and integration of information gained from more "traditional" paleontological and phylogenetic methods with data gleaned from modern "omic" tools promises to be a fruitful path forward for the study of cephalopod cognition.

Action Potential: The Future of Cephalopod Neurobiology

The work conducted by J. Z. Young and colleagues mid-twentieth century continues to serve as the foundation of our understanding of the cephalopod brain and nervous system, and how they control behavior. A lag in progress followed this work (see closing paragraph of Young, 1985), punctuated by a few exploratory experiments (e.g., Bullock and Budelmann, 1991; Williamson and Budelmann, 1991), but interest and improved techniques enabled an uptick in progress starting in the early 2000s. In particular, new neurophysiological approaches were developed in the labs of Drs. B. Hochner (Hebrew University, Israel) and G. Fiorito (Stazione Zoologica Anton Dohrn, Italy) that fueled a resurgence in the study of cephalopod neurophysiology. Electrophysiological recordings from brain-slice preparations in these labs have demonstrated the existence of a long term potentiation similar to that of vertebrates (Hochner et al., 2003) which is considered the cellular analog of long-term memory. A combination of behavioral and electrophysiological approaches have provided insights in the mechanisms involved in short and long-term memory in cephalopods (Shomrat et al., 2008). Comparisons of slice preparations of cuttlefish and octopus show that the vertical lobe of both species although similarly organized express synaptic plasticity in different layers and 'modes' (Shomrat et al., 2011), suggesting multiple independent evolutions of this computational system in coleoids. The next step in these electrophysiological efforts will be to adapt the recently developed wireless *in vivo* neural recording techniques (e.g., Hasegawa et al., 2007) to cephalopods, so that brain activity can be monitored as they move freely and perform natural behaviors.

Non-electrophysiological methods have also been recently used to gain insight into the cephalopod nervous system. For instance, anatomical and histological comparisons between the hatchlings of six different coleiod species showed that the sizes and shapes of the visual and nervous systems of various species demonstrate plasticity according to their respective ecological niche (Wild et al., 2015). This information could be useful in situations where the origin of a specimen is unknown—measurement of the relative size of various neural structures might yield clues about its ecological niche, much the same way as tooth shape suggests diet in vertebrates. Another group compared the expression of four genes encoding transcription factors important for nervous system development in squid to that of other bilaterians. They found that the roles of these genes have been largely conserved across these widely divergent groups, and thus represent a shared legacy with other bilaterians (Wollesen et al., 2014).

Further progress in the field depends on the continuing development and adaptation of new neurobiological methods and techniques, and advances in neuroimaging hold particular promise for the study of cephalopod brains. Recently, Bidel et al. (2016a) adapted and validated a method to quantify dopamine, serotonin, norepinephrine and their metabolites simultaneously in brains of cuttlefish using high performance liquid chromatography electro-chemical detection. Array tomography and calcium imaging are two methods which might soon be possible with cephalopods. In array tomography, tissues are stabilized by a glass substrate that allows samples to be stained with multiple markers so that both brain structure and 20 or more neurotransmitters can be viewed simultaneously in three dimensions (Micheva and Smith, 2007). By contrast, neuronal calcium imaging has the advantage that it can be used on animals that are awake and moving (Grienberger and Konnerth, 2012).

As the study of cephalopod neurobiology progresses, it is critical to make every effort to avoid unnecessary pain, suffering, distress and lasting harm (PSDLH) to the animals. This will be greatly facilitated by determining whether or not cephalopods are capable of experiencing pain and suffering, and to validate our standards of anesthesia for this taxon, investigations that are only just beginning (Crook et al., 2013; Alupay et al., 2014; Di Cristina et al., 2015; Butler-Struben et al., 2018). Such work is especially important given recent legislative changes (see above) and our growing knowledge of their sensory and cognitive sophistication. Luckily, technological advances and cost-reductions have made some non-invasive methods available. One example is primary neuronal cell culture, in which neurons are disassociated from the octopus brain and used to establish cell lines that can be cultured and studied *ex vivo* (Maselli et al., 2018), reducing the need for experimentation on live animals. Likewise, ultrasound machines have been used to study brain size in octopus and arm morphology (Grimaldi et al., 2007; Margheri et al., 2011), while non-destructive X-ray microtomography has been used to map the brain of bobtail squid (Kerbl et al., 2013).

As we utilize these methods to glean new data, this and existing information should be digitized and shared as suggested by Xavier et al. (2015), both to facilitate further scientific progress and avoid the unnecessary or redundant use of animals. In particular, the development of online, shared digital brain atlases such as those that exist for rodents (e.g., the Allen Brain Atlas) is within reach and urgently needed for commonly studied cephalopod species such as like *S. officinalis* and *Octopus vulgaris*. Non-digital atlases, already exist for the squids *Sepioteuthis lessoniana* and *I. paradoxus* (Shigeno et al., 2001; Yamamoto et al., 2003), and should be expanded and digitized. Such efforts should include not only physiological structures and gene expression but also extend to mapping the "connectomes" (all of the connections that exist in the nervous system) of the cephalopod brain.

Some important research topics that have been pursued in the last two decades with the various methods described and proposed above are the motor control of posture and limbs, especially regarding the parallel processing necessary to control 8 or 10 appendages of coleoids (e.g., Sumbre et al., 2001, 2005, 2006; Zullo et al., 2009; Levy and Hochner, 2017), as well as neural control of body patterning (Wardill et al., 2012; Rosen and Gilly, 2017) and texture (Gonzalez-Bellido et al., 2018). Finally, the existence and role of sleep in cephalopods, which undergo periods of behavioral and physiological quiescence that strongly resembles sleep in vertebrates (Mather, 2008; Meisel et al., 2011; Frank et al., 2012) is in our view a fascinating area of inquiry that could give insight into the phylogenetic origins and biological reasons for sleep in animals.

FINAL THOUGHTS

In addition to focusing on research and investigation, cephalopod researchers should also be on the lookout for new creative ways to disseminate knowledge and to further augment public awareness and interest. Some novel forms of public outreach that have been used recently include an interactive museum exhibit which encourages visitors to participate in their own neuroscientific data analysis ("Surprising Minds" at the Brighton Sea Life Centre, United Kingdom[9]), a graphic novella illustrating the results of a scientific study ("Cuttlefish Brawl" by Shanna Baker and Mark Garrison[10]) and a virtual reality game allowing visitors to see through the eyes of a cuttlefish ("Eye Sea" by Darmaillacq and Bellanger, 2016[11]). More traditional mediums are important too, of course, and a slew of recent books targeting the non-scientific public (e.g., Williams, 2011; Montgomery, 2015; Godfrey-Smith, 2016; Staaf, 2017) have been published in the last decade.

---

[9] http://www.everymind.online/SurprisingMinds/, accessed March 1, 2018

[10] https://www.hakaimagazine.com/videos-visuals/cuttlefish-brawl/, accessed March 1, 2018

[11] http://recherche.unicaen.fr/ressources/outils/eye-sea-825922.kjsp, accessed March 1, 2018

The public fascination with cephalopods should also be leveraged to promote conservation efforts and to encourage marine research and exploration. Interest could also be channeled in non-traditional ways, such as citizen science via crowd-sourced data collection and analysis. Dozens to hundreds of photographs and videos of cephalopods are shared to social media every year. There is no reason why such media cannot be put to scientific use by posting them to an open access online repository. Aquarists, divers and fishermen should be encouraged to share observations, photographs, videos and data with the cephalopod research community. We could also harness public aid in analyzing large data sets through crowd-sourced analysis, such as the manual assessment of cuttlefish body pattern components or for measuring the size of brain structures from digitized histological thin sections. Public participation is already utilized by marine scientists to collect data (e.g., tag-and-release tracking programs), as well as in analyzing large data sets online (e.g., NASA's hunt for exoplanets, "Backyard Worlds: Planet 9"[12], Seabirdwatch[13]). However, it is important to bear in mind that while an animal's popularity may be harnessed for worthy causes, fame is not without its pitfalls—such as potential overfishing by the hobby aquarium industry, as for the plight of clownfish after the release of *Finding Nemo* (Yan, 2016) or ornamental shell trade (e.g., Nijman and Lee, 2016). Human advocates for cephalopods must work to avoid such exploitation.

Another goal the cephalopod research community should work toward is the development of a shared, open-access platform for data sharing. With a rapidly changing climate and growing food demands, the continued generation and dissemination of data that can guide fisheries and environmental practices is ever more important in order to mitigate human impact. Moreover, it is likely that there are many aspiring cephalopod researchers who may not have access to animals or suitable equipment to conduct their own experiments (e.g., at land-locked academic institutions for instance), but could make use of shared data or media. Shared open-access tools and data can also help pursue cephalopod research in a way which minimizes pain, suffering and lasting harm, by reducing the total number of animals that need to be manipulated and by promoting best-practices. In addition, researchers working in countries where cephalopod research is not currently regulated by animal welfare legislation (i.e., outside of the European Union) or with invertebrate groups that are not currently regulated but will likely be in the future (e.g., bees, decapods), could refer to this platform in developing their own welfare practices. Finally, researchers could use this platform to share information with each other regarding the health and maintenance of animals in their care, and publicize their own research findings. At least two such platforms are currently being developed by the research community: one for the cataloging of cephalopod diseases and parasites for the purpose of improving cephalopod welfare and another for sharing data and media.

Finally, we also feel that it is important to encourage other aspiring cephalopod scientists. Each of the authors was drawn to study cephalopods due to their deep fascination with these animals. Surely other young prospective scientists share this passion, and deserve a productive outlet. The creation of M.Sc. or Ph.D. programs in cephalopod research would be a good first step. Involving early-career researchers in the activities and decisions of the cephalopod scientific community (e.g., conferences, workshops, courses, establishment of welfare guidelines) would also foster and support their development. For those already established in the field, we encourage participation in short courses, training schools and workshops related to cephalopods. Over the past four years (October 2013–September, 2017) the cephalopod community in Europe was able to stage a number of classes, training schools, international meetings and short-term research projects through the support of a COST Action. These have contributed greatly to the standardization of techniques across the field and facilitated networking between labs throughout Europe and beyond. Hopefully, such international exchange will continue, and cephalopod researchers will continue to reach across international borders in order to build interdisciplinary teams that combine different areas of expertise in order to address the challenges discussed here (summarized in **Table 1**).

AUTHOR CONTRIBUTIONS

The authors contributed equally to this manuscript, with each writing 3,000–4,000 words. KR composed the sections aquaculture, welfare, and climate change. IW composed the section on genetics. CO composed the General Introduction and Final Thoughts section as well as the sections on cognition, behavior and neuroscience/biology. All authors read and agreed on the final version.

FUNDING

This work was supported by the COST Action FA1301 "A network for improvement of cephalopod welfare and husbandry in research, aquaculture and fisheries" (http://www.cephsinaction.org/).

ACKNOWLEDGMENTS

We are indebted to a number of people including Professors Jochen Pflueger, Paul Flecknell, Ludovic Dickel, Felix Mark, Sylvia Anton, and Ansgar Büschges. Dr. Graziano Fiorito and Professor Lindy Holden-Dye provided invaluable mentorship and guidance. IW would like to thank her advisor, Prof. M. Thomas P. Gilbert. CO would like to thank the Fyssen Foundation as well as Drs. Alex Schnell, Flavie Bidel, and Anne Quiedville for sharing their perspectives as fellow young researchers. KR would like to thank CO for the valuable suggestions and English editing of her section. Alessia Iannuzzi assisted with the composition of **Figure 1**.

---

[12] https://www.zooniverse.org/projects/marckuchner/backyard-worlds-planet-9, accessed March 1, 2018

[13] https://www.zooniverse.org/projects/penguintom79/seabirdwatch, accessed March 1, 2018

REFERENCES

Abdelrahman, H., ElHady, M., Alcivar-Warren, A., Allen, S., Al-Tobasei, R., Bao, L., et al. (2017). Aquaculture genomics, genetics and breeding in the United States: current status, challenges, and priorities for future research. *BMC Genomics* 18:191. doi: 10.1186/s12864-017-3557-1

Albertin, C. B., Bonnaud, L., Brown, C. T., Crookes-Goodson, W. J., da Fonseca, R. R., Di Cristo, C., et al. (2012). Cephalopod genomics: a plan of strategies and organization. *Stand. Genomic Sci.* 7, 175–188. doi: 10.4056/sigs.3136559

Albertin, C. B., Simakov, O., Mitros, T., Wang, Z. Y., Pungor, J. R., Edsinger-Gonzales, E., et al. (2015). The octopus genome and the evolution of cephalopod neural and morphological novelties. *Nature* 524, 220–224. doi: 10.1038/nature14668

Alkan, C., Sajjadian, S., and Eichler, E. E. (2010). Limitations of next-generation genome sequence assembly. *Nat. Methods* 8, 61–65. doi: 10.1038/nmeth.1527

Alupay, J. S., Hadjisolomou, S. P., and Crook, R. J. (2014). Arm injury produces long-term behavioral and neural hypersensitivity in octopus. *Neurosci. Lett.* 558, 137–142. doi: 10.1016/j.neulet.2013.11.002

Alves, C., Boal, J. G., and Dickel, L. (2008). Short-distance navigation in cephalopods: a review and synthesis. *Cogn. Process.* 9, 239–247. doi: 10.1007/s10339-007-0192-9

Amodio, P., and Fiorito, G. (2013). "Observational and other types of learning in Octopus," in *Handbook of Behavioral Neuroscience*, eds R. Menzel and P. R. Benjamin (New York, NY: Elsevier), 293–302.

Anderson, R. C., and Wood, J. B. (2001). Enrichment for giant pacific octopuses: happy as a clam? *J. Appl. Anim. Welf. Sci.* 4, 157–168. doi: 10.1207/S15327604JAWS0402_10

Andrews, P., and Tansey, E. (1981). The effects of some anaesthetic agents in *Octopus vulgaris*. *Comp. Biochem. Physiol. C Comp. Pharmacol.* 70, 241–247. doi: 10.1016/0306-4492(81)90057-5

Andrews, P. L. R., Darmaillacq, A.-S., Dennison, N., Gleadall, I. G., Hawkins, P., Messenger, J. B., et al. (2013). The identification and management of pain, suffering and distress in cephalopods, including anaesthesia, analgesia and humane killing. *J. Exp. Mar. Biol. Ecol.* 447, 46–64. doi: 10.1016/j.jembe.2013.02.010

Anon (2011). Method of the Year 2011. *Nat. Methods* 9:1. doi: 10.1038/nmeth.1852

Aristotle (1910). *Historia Animalium, English Wenthworth Thompson*, trans. D'Arcy (Oxford: Clarendon Press). doi: 10.5962/bhl.title.147382

Ashley, P. J. (2007). Fish welfare: current issues in aquaculture. *Appl. Anim. Behav. Sci.* 104, 199–235. doi: 10.1016/j.applanim.2006.09.001

Balcázar, J. L., De Blas, I., Ruiz-Zarzuela, I., Cunningham, D., Vendrell, D., and Muzquiz, J. L. (2006). The role of probiotics in aquaculture. *Vet. Microbiol.* 114, 173–186. doi: 10.1016/j.vetmic.2006.01.009

Barry, P. D., Tamone, S. L., and Tallmon, D. A. (2011). A comparison of tagging methodology for North Pacific giant octopus *Enteroctopus dofleini*. *Fish. Res.* 109, 370–372. doi: 10.1016/j.fishres.2011.02.011

Basil, J., Barord, G., Crook, R., Derman, R., Hui Ju, C., Travis, L., et al. (2011). A synthetic approach to the study of learning and memory in chambered nautilus L. (Cephalopoda, Nautiloidea). *Vie et Milieu* 61, 231–242. doi: 10.1242/jeb.018531

Baumans, V., and Van Loo, P. (2013). How to improve housing conditions of laboratory animals: the possibilities of environmental refinement. *Vet. J.* 195, 24–32. doi: 10.1016/j.tvjl.2012.09.023

Bazzino, G., Gilly, W. F., Markaida, U., Salinas-Zavala, C. A., and Ramos-Castillejos, J. (2010). Horizontal movements, vertical-habitat utilization and diet of the jumbo squid (*Dosidicus gigas*) in the Pacific Ocean off Baja California Sur, Mexico. *Prog. Oceanogr.* 86, 59–71. doi: 10.1016/j.pocean.2010.04.017

Beigel, M., and Boal, J. (2006). The effect of habitat enrichment on the mudflat octopus. *Shape Enrich.* 15, 3–6.

Bidel, F., Corvaisier, S., Jozet-Alves, C., Pottier, I., Dauphin, F., Naud, N., et al. (2016a). An HPLC-ECD method for monoamines and metabolites quantification in cuttlefish (cephalopod) brain tissue. *Biomed. Chromatogr.* 30, 1175–1183. doi: 10.1002/bmc.3663

Bidel, F., Di Poi, C., Imarazene, B., Koueta, N., Budzinski, H., Van Delft, P., et al. (2016b). Pre-hatching fluoxetine-induced neurochemical, neurodevelopmental, and immunological changes in newly hatched cuttlefish. *Environ. Sci. Pollut. Res.* 23, 5030–5045. doi: 10.1007/s11356-015-4591-7

Bitterman, M. E. (1975). "Critical commentary," in *Invertebrate Learning*, eds W. C. Corning and J. A. Dyal (New York, NY: Plenum), 139–145. doi: 10.1007/978-1-4684-3012-7_4

Bloor, I. S., Attrill, M. J., and Jackson, E. L. (2013). A review of the factors influencing spawning, early life stage survival and recruitment variability in the common cuttlefish (*Sepia officinalis*). *Adv. Mar. Biol.* 65, 1–65. doi: 10.1016/B978-0-12-410498-3.00001-X

Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., et al. (2014). Environmental DNA for wildlife biology and biodiversity monitoring. *Trends Ecol. Evol.* 29, 358–367. doi: 10.1016/j.tree.2014.04.003

Boichard, D., Ducrocq, V., Croiseau, P., and Fritz, S. (2016). Genomic selection in domestic animals: principles, applications and perspectives. *C. R. Biol.* 339, 274–277. doi: 10.1016/j.crvi.2016.04.007

Boletzky, S. V., and Hanlon, R. T. (1983). A review of the laboratory maintenance, rearing and culture of cephalopod molluscs. *Mem. Natl. Mus. Vic.* 44, 147–187. doi: 10.24199/j.mmv.1983.44.11

Borrelli, L., and Fiorito, G. (2008). "Behavioral analysis of learning and memory in cephalopods," in *Learning and Memory: A Comprehensive Reference*, ed. J. J. Byrne (Oxford: Academic Press), 605–627. doi: 10.1016/B978-012370509-9.00069-3

Borrelli, L., Gherardi, F., and Fiorito, G. (2006). "A catalogue of body patterning," in *Cephalopoda Stazione Zoologica*, ed. A. Dohrn (Naples: Firenze University Press).

Boyle, P., and Rodhouse, P. (2008). *Cephalopods: Ecology and Fisheries*. Hoboken, NJ: John Wiley & Sons.

Brown, E. R., and Piscopo, S. (2013). Synaptic plasticity in cephalopods; more than just learning and memory? *Invertebr. Neurosci.* 13, 35–44. doi: 10.1007/s10158-013-0150-4

Brydges, N. M., and Braithwaite, V. A. (2008). Measuring animal welfare: what can cognition contribute? *Annu. Rev. Biomed. Sci.* 10, T91–T103. doi: 10.5016/1806-8774.2008.v10pT91

Budelmann, B. (1995). Cephalopod sense organs, nerves and the brain: adaptations for high performance and life style. *Mar. Freshw. Behav. Physiol.* 25, 13–33. doi: 10.1080/10236249409378905

Budelmann, B. U. (1996). Active marine predators: the sensory world of cephalopods. *Mar. Freshw. Behav. Physiol.* 27, 59–75. doi: 10.1080/10236249609378955

Budelmann, B. U., Schipp, R., and Boletzky, S. V. (1997). "Cephalopoda," in *Microscopic Anatomy of Invertebrates*, eds F. W. Harrison and A. J. Kohn (New York, NY: Wiley-Liss, Inc), 119–414.

Bullock, T. H., and Budelmann, B. U. (1991). Sensory evoked potentials in unanesthetized unrestrained cuttlefish: a new preparation for brain physiology in cephalopods. *J. Comp. Physiol. A* 168, 141–150. doi: 10.1007/BF00217112

Bush, S. L. (2012). Economy of arm autotomy in the mesopelagic squid *Octopoteuthis deletron*. *Mar. Ecol. Prog. Ser.* 458, 133–140. doi: 10.3354/meps09714

Butler-Struben, H. M., Brophy, S. M., Johnson, N. A., and Crook, R. J. (2018). *In vivo* recording of neural and behavioral correlates of anesthesia induction, reversal, and euthanasia in cephalopod molluscs. *Front. Physiol.* 9:109. doi: 10.3389/fphys.2018.00109

Caamal-Monsreal, C., Uriarte, I., Farias, A., Díaz, F., Sánchez, A., Re, D., et al. (2016). Effects of temperature on embryo development and metabolism of *O. maya*. *Aquaculture* 451, 156–162. doi: 10.1016/j.aquaculture.2015.09.011

Carere, C., Grignani, G., Bonanni, R., Della Gala, M., Carlini, A., Angeletti, D., et al. (2015). Consistent individual differences in the behavioural responsiveness of adult male cuttlefish (*Sepia officinalis*). *Appl. Anim. Behav. Sci.* 167, 89–95. doi: 10.1016/j.applanim.2015.03.005

Cartron, L., Josef, N., Lerner, A., McCusker, S. D., Darmaillacq, A.-S., Dickel, L., et al. (2013). Polarization vision can improve object detection in turbid waters by cuttlefish. *J. Exp. Mar. Biol. Ecol.* 447, 80–85. doi: 10.1016/j.jembe.2013.02.013

Chapman, J. A., Ho, I., Sunkara, S., Luo, S., Schroth, G. P., and Rokhsar, D. S. (2011). Meraculous: de novo genome assembly with short paired-end reads. *PLoS One* 6:e23501. doi: 10.1371/journal.pone.0023501

Choi, M. K., Park, O. K., Choi, C., Qiao, S., Ghaffari, R., Kim, J., et al. (2016). Cephalopod-inspired miniaturized suction cups for smart medical skin. *Adv. Healthc. Mater.* 5, 80–87. doi: 10.1002/adhm.201500285

Colpaert, F., Tarayre, J., Alliaga, M., Slot, L. B., Attal, N., and Koek, W. (2001). Opiate self-administration as a measure of chronic nociceptive pain in arthritic rats. *Pain* 91, 33–45. doi: 10.1016/S0304-3959(00)00413-9

Colpaert, F. C., De Witte, P., Maroli, A. N., Awouters, F., Niemegeers, C. J., and Janssen, P. A. (1980). Self-administration of the analgesic suprofen in arthritic rats: evidence of *Mycobacterium butyricum*-induced arthritis as an experimental model of chronic pain. *Life Sci.* 27, 921–928. doi: 10.1016/0024-3205(80)90101-0

Cosgrove, J. (1993). In situ observations of nesting female Octopus dofleini (Wülker, 1910). *J. Cephalopod Biol.* 2, 33–45.

Cousteau, J. Y., and Diolé, P. (1973). *Octopus and Squid, the Soft Intelligence*. New York, NY: Doubleday & Co.

Cox, D. B. T., Gootenberg, J. S., Abudayyeh, O. O., Franklin, B., Kellner, M. J., Joung, J., et al. (2017). RNA editing with CRISPR-Cas13. *Science* 358, 1019–1027. doi: 10.1126/science.aaq0180

Creer, S., and Seymour, M. (2017). Marine ecology: genetics from a drop in the ocean. *Nat. Ecol. Evol.* 1, 1–2. doi: 10.1038/s41559-016-0037

Crichton, M. (1988). *Sphere: A Novel*. New York, NY: Random House.

Crook, R., and Basil, J. (2008). A biphasic memory curve in the chambered nautilus, *Nautilus pompilius* L.(Cephalopoda: Nautiloidea). *J. Exp. Biol.* 211, 1992–1998. doi: 10.1242/jeb.018531

Crook, R. J., Hanlon, R. T., and Basil, J. A. (2009). Memory of visual and topographical features suggests spatial learning in nautilus (*Nautilus pompilius* L.). *J. Comp. Psychol.* 123, 264–274. doi: 10.1037/a0015921

Crook, R. J., Hanlon, R. T., and Walters, E. T. (2013). Squid have nociceptors that display widespread long-term sensitization and spontaneous activity after bodily injury. *J. Neurosci.* 33, 10021–10026. doi: 10.1523/JNEUROSCI.0646-13.2013

Cruz, P. M., Ibáñez, A. L., Monroy Hermosillo, O. A., and Ramírez Saad, H. C. (2012). Use of probiotics in aquaculture. *ISRN Microbiol.* 2012:916845. doi: 10.5402/2012/916845

Cyranoski, D. (2015). Super-muscly pigs created by small genetic tweak. *Nature* 523, 13–14. doi: 10.1038/523013a

Darmaillacq, A.-S., Dickel, L., and Mather, J. (2014). *Cephalopod cognition*. Cambridge: Cambridge University Press. doi: 10.1017/CBO9781139058964

Di Cristina, G., Andrews, P., Ponte, G., Galligioni, V., and Fiorito, G. (2015). The impact of Directive 2010/63/EU on cephalopod research. *Invert. Neurosci.* 15:8. doi: 10.1007/s10158-015-0183-y

Di Poi, C., Darmaillacq, A.-S., Dickel, L., Boulouard, M., and Bellanger, C. (2013). Effects of perinatal exposure to waterborne fluoxetine on memory processing in the cuttlefish *Sepia officinalis*. *Aquat. Toxicol.* 132, 84–91. doi: 10.1016/j.aquatox.2013.02.004

Dickel, L., Boal, J. G., and Budelmann, B. U. (2000). The effect of early experience on learning and memory in cuttlefish. *Dev. Psychobiol.* 36, 101–110. doi: 10.1002/(SICI)1098-2302(200003)36:2<101::AID-DEV2>3.0.CO;2-L

Dickel, L., Darmaillacq, A.-S., Jozet-Alves, C., and Bellanger, C. (2013). Learning, memory, and brain plasticity in cuttlefish (*Sepia officinalis*). *Handb. Behav. Neurosci.* 22, 318–333. doi: 10.1016/B978-0-12-415823-8.00025-3

Doubleday, Z. A., Prowse, T. A., Arkhipkin, A., Pierce, G. J., Semmens, J., Steer, M., et al. (2016). Global proliferation of cephalopods. *Curr. Biol.* 26, R406–R407. doi: 10.1016/j.cub.2016.04.002

Douglas, G. L., and Klaenhammer, T. R. (2010). Genomic evolution of domesticated microorganisms. *Annu. Rev. Food Sci. Technol.* 1, 397–414. doi: 10.1146/annurev.food.102308.124134

Dröscher, A. (2016). Pioneering studies on cephalopod's eye and vision at the Stazione Zoologica Anton Dohrn (1883-1977). *Front. Physiol.* 7:618. doi: 10.3389/fphys.2016.00618

Edelman, D. B., and Seth, A. K. (2009). Animal consciousness: a synthetic approach. *Trends Neurosci.* 32, 476–484. doi: 10.1016/j.tins.2009.05.008

Espinoza, G. J., Poland, J. M., and Alvarado Bremer, J. R. (2017). Genotyping live fish larvae: non-lethal and noninvasive DNA isolation from 3–5 day old hatchlings. *BioTechniques* 63, 1–6. doi: 10.2144/000114598

European Parliament, and Council of the European Union (2010). *Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes*. Strasbourg: Concil of Europe.

Fernald, R. D. (2000). Evolution of eyes. *Curr. Opin. Neurobiol.* 10, 444–450. doi: 10.1016/S0959-4388(00)00114-8

Fiems, L. O. (2012). Double muscling in cattle: genes, husbandry, carcasses and meat. *Animals* 2, 472–506. doi: 10.3390/ani2030472

Fiorito, G., Affuso, A., Anderson, D. B., Basil, J., Bonnaud, L., Botta, G., et al. (2014). Cephalopods in neuroscience: regulations, Research and the 3Rs. *Invert. Neurosci.* 14, 13–36. doi: 10.1007/s10158-013-0165-x

Fiorito, G., Affuso, A., Basil, J., Cole, A., De Girolamo, P., D'Angelo, L., et al. (2015). Guidelines for the care and welfare of cephalopods in research - a consensus based on an initiative by CephRes, FELASA and the Boyd Group. *Lab. Anim.* 49(2 Suppl.), 1–90. doi: 10.1177/0023677215580006

Frank, M. G., Waldrop, R. H., Dumoulin, M., Aton, S., and Boal, J. G. (2012). A preliminary analysis of sleep-like states in the cuttlefish *Sepia officinalis*. *PLoS One* 7:e38125. doi: 10.1371/journal.pone.0038125

Fuentes, L., Otero, J. J., Sánchez, F. J., and Iglesias, J. (2006). Application of different external tagging methods to *Octopus vulgaris* Cuvier, 1797, with special reference to T-bar anchor tags and Petersen disks. *Bol. Inst. Esp. Oceanogr.* 22, 3–11.

Gallardo, P., Olivares, A., Martínez-Yáñez, R., Caamal-Monsreal, C., Domingues, P. M., Mascaró, M., et al. (2017). Digestive physiology of *Octopus maya* and *O. mimus:* temporality of digestion and assimilation processes. *Front. Physiol.* 8:355. doi: 10.3389/fphys.2017.00355

Garci, M. E., Hernández-Urcera, J., Gilcoto, M., Fernández-Gago, R., González, Á. F., and Guerra, Á. (2016). From brooding to hatching: new insights from a female *Octopus vulgaris* in the wild. *J. Mar. Biol. Assoc. United Kingdom* 96, 1341–1346. doi: 10.1017/s0025315415001800

Gaudelli, N. M., Komor, A. C., Rees, H. A., Packer, M. S., Badran, A. H., Bryson, D. I., et al. (2017). Programmable base editing of AT to GC in genomic DNA without DNA cleavage. *Nature* 551, 464–471. doi: 10.1038/nature24644

Gillette, R. (1991). On the significance of neuronal giantism in gastropods. *Biol. Bull.* 180, 234–240. doi: 10.2307/1542393

Gilly, W. F., Markaida, U., Baxter, C. H., Block, B. A., Boustany, A., Zeidberg, L., et al. (2006). Vertical and horizontal migrations by the jumbo squid *Dosidicus gigas* revealed by electronic tagging. *Mar. Ecol. Prog. Ser.* 324, 1–17. doi: 10.3354/meps324001

Gleadall, I. G. (2013). The effects of prospective anaesthetic substances on cephalopods: summary of original data and a brief review of studies over the last two decades. *J. Exp. Mar. Biol. Ecol.* 447, 23–30. doi: 10.1016/j.jembe.2013.02.008

Godfrey-Smith, P. (2013). Cephalopods and the evolution of the mind. *Pac. Conserv. Biol.* 19, 4–9. doi: 10.1016/B978-0-12-380858-5.00008-3

Godfrey-Smith, P. (2016). *Other Minds: The Octopus, the Sea, and the Deep Origins of Consciousness*. New York, NY: Farrar, Straus and Giroux.

Golikov, A. V., Sabirov, R. M., Lubin, P. A., and Jørgensen, L. L. (2013). Changes in distribution and range structure of Arctic cephalopods due to climatic changes of the last decades. *Biodiversity* 14, 28–35. doi: 10.1080/14888386.2012.702301

Gonçalves, R. A., Aragão, C., Frias, P. A., and Sykes, A. V. (2012). The use of different anaesthetics as welfare promoters during short-term human manipulation of European cuttlefish (*Sepia officinalis*) juveniles. *Aquaculture* 370, 130–135. doi: 10.1016/j.aquaculture.2012.10.014

Gonzalez-Bellido, P. T., Scaros, A. T., Hanlon, R. T., and Wardill, T. J. (2018). Neural control of dynamic 3-dimensional skin papillae for cuttlefish camouflage. *iScience* 1, 24–34. doi: 10.1007/s00359-009-0430-y

Gowland, F., Moltschaniwskyj, N., and Steer, M. (2002). Description and quantification of developmental abnormalities in a natural *Sepioteuthis australis* spawning population (Mollusca: Cephalopoda). *Mar. Ecol. Prog. Ser.* 243, 133–141. doi: 10.3354/meps243133

Grasso, F. W., and Basil, J. A. (2009). The evolution of flexible behavioral repertoires in cephalopod molluscs. *Brain Behav. Evol.* 74, 231–245. doi: 10.1159/000258669

Grienberger, C., and Konnerth, A. (2012). Imaging calcium in neurons. *Neuron* 73, 862–885. doi: 10.1016/j.neuron.2012.02.011

Grimaldi, A. M., Agnisola, C., and Fiorito, G. (2007). Using ultrasound to estimate brain size in the cephalopod *Octopus vulgaris* Cuvier in vivo. *Brain Res.* 1183, 66–73. doi: 10.1016/j.brainres.2007.09.032

Gunkel, C., and Lewbart, G. A. (2008). "Anesthesia and analgesia of invertebrates," in *Anesthesia and Analgesia in Laboratory Animals*, 2nd Edn, eds R. Fish, P. Danneman, M. Brown, and A. Karas (New York, NY: Elsevier), 535–545. doi: 10.1016/B978-012373898-1.50026-7

Gutowska, M. A., Melzner, F., Pörtner, H. O., and Meier, S. (2010). Cuttlebone calcification increases during exposure to elevated seawater pCO2 in the cephalopod *Sepia officinalis*. *Mar. Biol.* 157, 1653–1663. doi: 10.1007/s00227-010-1438-0

Hanke, F. D., and Osorio, D. C. (2018). Vision in cephalopods. *Front. Physiol.* 9:18. doi: 10.3389/fphys.2018.00018

Hanlon, R., Ament, S., and Gabr, H. (1999). Behavioral aspects of sperm competition in cuttlefish, *Sepia officinalis* (Sepioidea: Cephalopoda). *Mar. Biol.* 134, 719–728. doi: 10.1007/s002270050588

Hanlon, R. T., and Messenger, J. B. (2018). *Cephalopod Behaviour*. Cambridge: Cambridge University Press. doi: 10.1017/9780511843600

Hanlon, R. T., Naud, M.-J., Shaw, P. W., and Havenhand, J. N. (2005). Behavioural ecology: transient sexual mimicry leads to fertilization. *Nature* 433:212. doi: 10.1038/433212a

Hanlon, R. T., and Shashar, N. (2003). "Aspects of the sensory ecology of cephalopods," in *Sensory Processing in Aquatic Environments*, eds S. P. Collin and N. J. Marshall (New York, NY: Springer), 266–282. doi: 10.1007/978-0-387-22628-6_14

Hartline, D. K., and Colman, D. R. (2007). Rapid conduction and the evolution of giant axons and myelinated fibers. *Curr. Biol.* 17, R29–R35. doi: 10.1016/j.cub.2006.11.042

Hasegawa, A., Hisatomi, O., Yamamoto, S., Ono, E., and Tokunaga, F. (2007). Stathmin expression during newt retina regeneration. *Exp. Eye Res.* 85, 518–527. doi: 10.1016/j.exer.2007.07.003

Hochner, B. (2010). Functional and comparative assessments of the octopus learning and memory system. *Front. Biosci.* 2:764–771.

Hochner, B. (2012). An embodied view of octopus neurobiology. *Curr. Biol.* 22, R887–R892. doi: 10.1016/j.cub.2012.09.001

Hochner, B. (2013). How nervous systems evolve in relation to their embodiment: what we can learn from octopuses and other molluscs. *Brain Behav. Evol.* 82, 19–30. doi: 10.1159/000353419

Hochner, B., Brown, E. R., Langella, M., Shomrat, T., and Fiorito, G. (2003). A learning and memory area in the octopus brain manifests a vertebrate-like long-term potentiation. *J. Neurophysiol.* 90, 3547–3554. doi: 10.1152/jn.00645.2003

Hochner, B., and Shomrat, T. (2013). "The neurophysiological basis of learning and memory in advanced invertebrates: the octopus and the cuttlefish," in *Handbook of Behavioral Neuroscience*, eds R. Menzel and P. R. Benjamin (New York, NY: Elsevier), 303–317. doi: 10.1016/B978-0-12-415823-8.00024-1

Hochner, B., Shomrat, T., and Fiorito, G. (2006). The octopus: a model for a comparative analysis of the evolution of learning and memory mechanisms. *Biol. Bull.* 210, 308–317. doi: 10.2307/4134567

Holekamp, K. E. (2007). Questioning the social intelligence hypothesis. *Trends Cogn. Sci.* 11, 65–69. doi: 10.1016/j.tics.2006.11.003

Huffard, C. (2013). Cephalopod neurobiology: an introduction for biologists working in other model systems. *Invert. Neurosci.* 13, 11–18. doi: 10.1007/s10158-013-0147-z

Hugo, V. (2002). *The Toilers of the Sea*. New York, NY: Modern Library.

Huntingford, F. A., Adams, C., Braithwaite, V., Kadri, S., Pottinger, T., Sandøe, P., et al. (2006). Current issues in fish welfare. *J. Fish Biol.* 68, 332–372. doi: 10.1111/j.0022-1112.2006.001046.x

Iglesias, J., Fuentes, L., and Villanueva, R. (2014). *Cephalopod Culture*. Berlin: Springer Science & Business Media. doi: 10.1007/978-94-017-8648-5

Iglesias, J., Sánchez, F. J., Bersano, J., Carrasco, J. F., Dhont, J., Fuentes, L., et al. (2007). Rearing of *Octopus vulgaris* paralarvae: present status, bottlenecks and trends. *Aquaculture* 266, 1–15. doi: 10.1016/j.aquaculture.2007.02.019

Íñiguez, A. (2017). "The octopus as a model for artificial intelligence-a multi-agent robotic case study," in *Proceedings of the 9th International Conference on Agents and Artificial Intelligence*, Porto. doi: 10.5220/0006125404390444

Iwata, Y., Shaw, P., Fujiwara, E., Shiba, K., Kakiuchi, Y., and Hirohashi, N. (2011). Why small males have big sperm: dimorphic squid sperm linked to alternative mating behaviours. *BMC Evol. Biol.* 11:236. doi: 10.1186/1471-2148-11-236

Jain, M., Olsen, H. E., Paten, B., and Akeson, M. (2016). The Oxford Nanopore MinION: delivery of nanopore sequencing to the genomics community. *Genome Biol.* 17:239.

Jereb, P., and Roper, C. (2005). *Chambered Nautiluses and Sepioids (Nautilidae, Sepiidae, Sepiolidae, Sepiadariidae, Idiosepiidae and Spirulidae)*. Rome: FAO.

Jereb, P., and Roper, C. (eds). (2010). "Cephalopods of the world. an annotated and illustrated catalogue of cephalopod species known to date," in *FAO Species Catalogue for Fishery Purposes*, Vol. 2, (Rome: FAO),

Juárez, O. E., Hau, V., Caamal-Monsreal, C., Galindo-Sánchez, C. E., Díaz, F., Re, D., et al. (2016). Effect of maternal temperature stress before spawning over the energetic balance of *Octopus maya* juveniles exposed to a gradual temperature change. *J. Exp. Mar. Biol. Ecol.* 474, 39–45. doi: 10.1016/j.jembe.2015.10.002

Kaplan, M. B., Mooney, T. A., McCorkle, D. C., and Cohen, A. L. (2013). Adverse effects of ocean acidification on early development of squid (*Doryteuthis pealeii*). *PLoS One* 8:e63714. doi: 10.1371/journal.pone.0063714

Kerbl, A., Handschuh, S., Nödl, M.-T., Metscher, B., Walzl, M., and Wanninger, A. (2013). Micro-CT in cephalopod research: investigating the internal anatomy of a Sepiolid squid using a non-destructive technique with special focus on the Ganglionic system. *J. Exp. Mar. Biol. Ecol.* 447, 140–148. doi: 10.1016/j.jembe.2013.02.022

Keynes, R. (2005). JZ and the discovery of squid giant nerve fibres. *J. Exp. Biol.* 208, 179–180. doi: 10.1242/jeb.01405

King, A. J., and Adamo, S. A. (2006). The ventilatory, cardiac and behavioural responses of resting cuttlefish (*Sepia officinalis* L.) to sudden visual stimuli. *J. Exp. Biol.* 209, 1101–1111. doi: 10.1242/jeb.02116

King, A. J., Henderson, S. M., Schmidt, M. H., Cole, A. G., and Adamo, S. A. (2005). Using ultrasound to understand vascular and mantle contributions to venous return in the cephalopod *Sepia officinalis* L. *J. Exp. Biol.* 208, 2071–2082. doi: 10.1242/jeb.01575

Kitzman, J. O. (2016). Haplotypes drop by drop. *Nat. Biotechnol.* 34, 296–298. doi: 10.1038/nbt.3500

Klug, C., Schweigert, G., Fuchs, D., Kruta, I., and Tischlinger, H. (2016). Adaptations to squid-style high-speed swimming in Jurassic belemnitids. *Biol. Lett.* 12:20150877. doi: 10.1098/rsbl.2015.0877

Korbel, J. O., and Lee, C. (2013). Genome assembly and haplotyping with Hi-C. *Nat. Biotechnol.* 31, 1099–1101. doi: 10.1038/nbt.2764

Koueta, N., Viala, H., and Le Bihan, E. (2014). "Applications, uses and by-products from cephalopods," in *Cephalopod Culture*, eds J. Iglesias, L. Fuentes, and R. Villanueva (Dordrecht: Springer), 131–147.

Kruta, I., Rouget, I., Charbonnier, S., Bardin, J., Fernandez, V., Germain, D., et al. (2016). Proteroctopus ribeti in coleoid evolution. *Paleontology* 59, 767–773. doi: 10.1111/pala.12265

Lafferty, K. D., Harvell, C. D., Conrad, J. M., Friedman, C. S., Kent, M. L., Kuris, A. M., et al. (2015). Infectious diseases affect marine fisheries and aquaculture economics. *Annu. Rev. Mar. Sci.* 7, 471–496. doi: 10.1146/annurev-marine-010814-015646

Laschi, C., Cianchetti, M., Mazzolai, B., Margheri, L., Follador, M., and Dario, P. (2012). Soft robot arm inspired by the octopus. *Adv. Robot.* 26, 709–727. doi: 10.1163/156855312X626343

Lee, P. G., Turk, P. E., Yang, W. T., and Hanlon, R. T. (1994). Biological characteristics and biomedical applications of the squid *Sepioteuthis lessoniana* cultured through multiple generations. *Biol. Bull.* 186, 328–341. doi: 10.2307/1542279

Levy, G., and Hochner, B. (2017). Embodied organization of *Octopus vulgaris* morphology, vision, and locomotion. *Front. Physiol.* 8:164. doi: 10.3389/fphys.2017.00164

Linares, M., Caamal-Monsreal, C., Olivares, A., Sánchez, A., Rodríguez, S., Zúñiga, O., et al. (2015). Timing of digestion, absorption and assimilation in octopus species from tropical (*Octopus maya*) and subtropical-temperate (*O. mimus)* ecosystems. *Aquat. Biol.* 24, 127–140. doi: 10.3354/ab00642

Lindgren, A. R., and Anderson, F. E. (2017). Assessing the utility of transcriptome data for inferring phylogenetic relationships among coleoid cephalopods. *Mol. Phylogenet. Evol.* 118, 330–342. doi: 10.1016/j.ympev.2017.10.004

Liscovitch-Brauer, N., Alon, S., Porath, H. T., Elstein, B., Unger, R., Ziv, T., et al. (2017). Trade-off between transcriptome plasticity and genome evolution in cephalopods. *Cell* 169, 191–202.e11. doi: 10.1016/j.cell.2017.03.025

Malone, N., Fuentes, A., and White, F. J. (2012). Variation in the social systems of extant hominoids: comparative insight into the social behavior of early hominins. *Int. J. Primatol.* 33, 1251–1277. doi: 10.1007/s10764-012-9617-0

Margheri, L., Ponte, G., Mazzolai, B., Laschi, C., and Fiorito, G. (2011). Non-invasive study of *Octopus vulgaris* arm morphology using ultrasound (vol. 214, pg 3727, 2011). *J. Exp. Biol.* 214, 4065–4065. doi: 10.1242/jeb.067454

Marini, G., De Sio, F., Ponte, G., and Fiorito, G. (2017). "Behavioral analysis of learning and memory in cephalopods," in *Learning Theory and Behavior*, ed. R. Menzel, *Learning and Memory: A Comprehensive Reference*, Vol. 1, 2nd Edn, ed. J. H. Byrne (Oxford: Academic Press), 441–462. doi: 10.1016/B978-0-12-809324-5.21024-9

Martínez, R., Gallardo, P., Pascual, C., Navarro, J., Sánchez, A., Caamal-Monsreal, C., et al. (2014). Growth, survival and physiological condition of *Octopus maya* when fed a successful formulated diet. *Aquaculture* 426, 310–317. doi: 10.1016/j.aquaculture.2014.02.005

Maselli, V., Xu, F., Syed, N. I., Polese, G., and Di Cosmo, A. (2018). A novel approach to primary cell culture for *Octopus vulgaris* neurons. *Front. Physiol.* 9:220. doi: 10.3389/fphys.2018.00220

Mather, J. A. (2008). Cephalopod consciousness: behavioural evidence. *Conscious. Cogn.* 17, 37–48. doi: 10.1016/j.concog.2006.11.006

Mather, J. A. (2011). Consciousness in cephalopods? *J. Cosmol.* 14. Available at: http://journalofcosmology.com/Consciousness113.html

Mather, J. A., and Dickel, L. (2017). Cephalopod complex cognition. *Curr. Opin. Behav. Sci.* 16, 131–137. doi: 10.1016/j.cobeha.2017.06.008

Mather, J. A., and Kuba, M. J. (2013). The cephalopod specialties: complex nervous system, learning, and cognition. *Can. J. Zool.* 91, 431–449. doi: 10.1139/cjz-2013-0009

Mäthger, L. M., Denton, E. J., Marshall, N. J., and Hanlon, R. T. (2009). Mechanisms and behavioural functions of structural coloration in cephalopods. *J. R. Soc. Interface* 6(Suppl. 2), S149–S163. doi: 10.1098/rsif.2008.0366.focus

Meisel, D. V., Byrne, R. A., Mather, J., and Kuba, M. J. (2011). Behavioral sleep in *Octopus vulgaris*. *Vie et Milieu* 61, 185–190.

Mellor, D. J. (2016). Updating animal welfare thinking: moving beyond the "Five Freedoms" towards "a Life Worth Living". *Animals* 6:21. doi: 10.3390/ani6030021

Melzner, F., Mark, F. C., and Pörtner, H. O. (2007). Role of blood-oxygen transport in thermal tolerance of the cuttlefish, *Sepia officinalis*. *Integr. Comp. Biol.* 47, 645–655. doi: 10.1093/icb/icm074

Messenger, J. B. (1973). Learning in the cuttlefish, Sepia. *Anim. Behav.* 21, 801–826. doi: 10.1016/S0003-3472(73)80107-1

Meyer, R. S., and Purugganan, M. D. (2013). Evolution of crop species: genetics of domestication and diversification. *Nat. Rev. Genet.* 14, 840–852. doi: 10.1038/nrg3605

Michael, E. T., Amos, S. O., and Hussaini, L. T. (2014). A review on probiotics application in aquaculture. *Fish. Aquac. J.* 5:111. doi: 10.4172/2150-3508.10000111

Micheva, K. D., and Smith, S. J. (2007). Array tomography: a new tool for imaging the molecular architecture and ultrastructure of neural circuits. *Neuron* 55, 25–36. doi: 10.1016/j.neuron.2007.06.014

Miller, A. L., and Leach, M. C. (2015). The mouse grimace scale: a clinically useful tool? *PLoS One* 10:e0136000. doi: 10.1371/journal.pone.0136000

Mojica, F. J., Díez-Villaseñor, C., García-Martínez, J., and Soria, E. (2005). Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. *J. Mol. Evol.* 60, 174–182. doi: 10.1007/s00239-004-0046-3

Montgomery, S. (2015). *The Soul of an Octopus: A Surprising Exploration into the Wonder of Consciousness*. New York, NY: Simon and Schuster.

Morse, P., Huffard, C. L., Meekan, M. G., Mccormick, M. I., and Zenger, K. R. (2018). Mating behaviour and postcopulatory fertilization patterns in the southern blue-ringed octopus, *Hapalochlaena maculosa*. *Anim. Behav.* 136, 41–51. doi: 10.1016/j.anbehav.2017.12.004

Nabhitabhata, J., and Segawa, S. (2014). "Aquaculture to restocking," in *Cephalopod Culture*, eds J. Iglesias, L. Fuentes, and R. Villanueva (New York, NY: Springer), 113–130. doi: 10.1007/978-94-017-8648-5_7

Nakajima, R., Shigeno, S., Zullo, L., De Sio, F., and Schmidt, M. R. (2018). Cephalopods between science, art, and engineering: a contemporary synthesis. *Front. Commun.* 3:20. doi: 10.3389/fcomm.2018.00020

Nande, M., Presa, P., Roura, Á., Andrews, P. L., and Pérez, M. (2017). Prey capture, ingestion, and digestion dynamics of *Octopus vulgaris* paralarvae fed live zooplankton. *Front. Physiol.* 8:573. doi: 10.3389/fphys.2017.00573

Naud, M.-J., Shaw, P. W., Hanlon, R. T., and Havenhand, J. N. (2005). Evidence for biased use of sperm sources in wild female giant cuttlefish (*Sepia apama*). *Proc. R. Soc. Lond. B Biol. Sci.* 272, 1047–1051. doi: 10.1098/rspb.2004.3031

Nijman, V., and Lee, P. B. (2016). Trade in nautilus and other large marine molluscs as ornaments and decorations in Bali, Indonesia. *Raffles Bull. Zool.* 64, 368–373.

Nilsson, D. E. (1996). Eye ancestry: old genes for new eyes. *Curr. Biol.* 6, 39–42. doi: 10.1016/S0960-9822(02)00417-7

Nilsson, D.-E., Warrant, E. J., Johnsen, S., Hanlon, R., and Shashar, N. (2012). A unique advantage for giant eyes in giant squid. *Curr. Biol.* 22, 683–688. doi: 10.1016/j.cub.2012.02.031

Norman, M., Finn, J., Hochberg, F., Jereb, P., and Roper, C. (2014). "Cephalopods of the world. *An annotated and illustrated catalogue of cephalopod species known to date. Volume* 3. Octopods and vampire squids," in *FAO Species Catalogue for Fishery Purposes*, eds P. Jereb, C. F. E. Roper, M. D. Norman, and J. K. Finn (Rome: FAO), 370.

O'Brien, C. E., Jozet-Alves, C., Mezrai, N., Bellanger, C., Darmaillacq, A.-S., and Dickel, L. (2017). Maternal and embryonic stress influence offspring behavior in the cuttlefish *Sepia officinalis*. *Front. Physiol.* 8:981. doi: 10.3389/fphys.2017.00981

Packard, A. (1972). Cephalopods and fish: the limits of convergence. *Biol. Rev.* 47, 241–307. doi: 10.1111/j.1469-185X.1972.tb00975.x

Pecl, G. T., and Jackson, G. D. (2008). The potential impacts of climate change on inshore squid: biology, ecology and fisheries. *Rev. Fish Biol. Fish.* 18, 373–385. doi: 10.1007/s11160-007-9077-3

Pennisi, E. (2017). New technologies boost genome quality. *Science* 357, 10–11. doi: 10.1126/science.357.6346.10

Pierce, G. J., Allcock, L., Bruno, I., Bustamante, P., Gonzalez, A., Guerra, A., et al. (2010). *Cephalopod Biology and Fisheries in Europe*. New Delhi: ICES.

Pierce, G. J., and Portela, J. (2014). "Fisheries production and market demand," in *Cephalopod Culture*, eds J. Iglesias, L. Fuentes, and R. Villanueva (Dordrecht: Springer), 41–58. doi: 10.1007/978-94-017-8648-5_3

Pikul, J. H., Li, S., Bai, H., Hanlon, R. T., Cohen, I., and Shepherd, R. F. (2017). Stretchable surfaces with programmable 3D texture morphing for synthetic camouflaging skins. *Science* 358, 210–214. doi: 10.1126/science.aan5627

Pinto, Y., Cohen, H. Y., and Levanon, E. Y. (2014). Mammalian conserved ADAR targets comprise only a small fragment of the human editosome. *Genome Biol.* 15:R5. doi: 10.1186/gb-2014-15-1-r5

Poirier, R., Chichery, R., and Dickel, L. (2004). Effects of rearing conditions on sand digging efficiency in juvenile cuttlefish. *Behav. Process.* 67, 273–279. doi: 10.1016/j.beproc.2004.04.006

Poirier, R., Chichery, R., and Dickel, L. (2005). Early experience and postembryonic maturation of body patterns in cuttlefish (*Sepia officinalis*). *J. Comp. Psychol.* 119, 230–237. doi: 10.1037/0735-7036.119.2.230

Ponte, G., Sykes, A. V., Cooke, G. M., Almansa, E., and Andrews, P. L. (2017). The digestive tract of cephalopods: toward non-invasive in vivo monitoring of its physiology. *Front. Physiol.* 8:403. doi: 10.3389/fphys.2017.00403

Pörtner, H. O., and Farrell, A. P. (2008). Physiology and climate change. *Science* 322, 690–692. doi: 10.1126/science.1163156

Pronk, R., Wilson, D. R., and Harcourt, R. (2010). Video playback demonstrates episodic personality in the gloomy octopus. *J. Exp. Biol.* 213, 1035–1041. doi: 10.1242/jeb.040675

Pugliese, C., Mazza, R., Andrews, P. L., Cerra, M. C., Fiorito, G., and Gattuso, A. (2016). Effect of different formulations of magnesium chloride used as anesthetic agents on the performance of the isolated heart of *Octopus vulgaris*. *Front. Physiol.* 7:610. doi: 10.3389/fphys.2016.00610

Rando, O. J. (2012). Daddy issues: paternal effects on phenotype. *Cell* 151, 702–708. doi: 10.1016/j.cell.2012.10.020

Renda, F., Cianchetti, M., Giorelli, M., Arienti, A., and Laschi, C. (2012). A 3D steady-state model of a tendon-driven continuum soft manipulator inspired by the octopus arm. *Bioinspir. Biomim.* 7:025006. doi: 10.1088/1748-3182/7/2/025006

Replinger, S. E., and Wood, J. B. (2007). A preliminary investigation of the use of subcutaneous tagging in Caribbean reef squid *Sepioteuthis sepioidea* (Cephalopoda : Loliginidae). *Fish. Res.* 84, 308–313. doi: 10.1016/j.fishres.2006.11.028

Repolho, T., Baptista, M., Pimentel, M. S., Dionísio, G., Trübenbach, K., Lopes, V. M., et al. (2014). Developmental and physiological challenges of octopus (*Octopus vulgaris*) early life stages under ocean warming. *J. Comp. Physiol. B* 184, 55–64. doi: 10.1007/s00360-013-0783-y

Rhoads, A., and Au, K. F. (2015). PacBio sequencing and its applications. *Genomics Proteomics Bioinformatics* 13, 278–289. doi: 10.1016/j.gpb.2015.08.002

Rodhouse, P. G., Pierce, G. J., Nichols, O. C., Sauer, W. H., Arkhipkin, A. I., Laptikhovsky, V. V., et al. (2014). *Environmental Effects on Cephalopod Population Dynamics: Implications for Management of Fisheries*. New York, NY: Elsevier Ltd. doi: 10.1016/B978-0-12-800287-2.00002-0

Rosa, R., Pimentel, M. S., Boavida-Portugal, J., Teixeira, T., Trübenbach, K., and Diniz, M. (2012). Ocean warming enhances malformations, premature hatching, metabolic suppression and oxidative stress in the early life stages of a keystone squid. *PLoS One* 7:e38282. doi: 10.1371/journal.pone.0038282

Rosen, H., Gilly, W., Bell, L., Abernathy, K., and Marshall, G. (2015). Chromogenic behaviors of the Humboldt squid (*Dosidicus gigas*) studied in situ with an animal-borne video package. *J. Exp. Biol.* 218, 265–275. doi: 10.1242/jeb.114157

Rosen, H. E., and Gilly, W. F. (2017). Myogenic activity and serotonergic inhibition in the chromatophore network of the squid *Dosidicus gigas* (family Ommastrephidae) and *Doryteuthis opalescens* (family Loliginidae). *J. Exp. Biol.* 220, 4669–4680. doi: 10.1242/jeb.164160

Salvador, R. B., and Tomotani, B. M. (2014). The Kraken: when myth encounters science. *Hist. Ciênc. Saúde Manguinhos* 21, 971–994. doi: 10.1590/S0104-59702014000300010

Sanders, G. D. (1975). "The cephalopods," in *Invertebrate Learning. Cephalopods and Echinoderms*, eds W. C. Corning, J. A. Dyal, and A. O. D. Willows (New York, N.Y: Plenum Press), 1–101.

Schnell, A. K., Smith, C. L., Hanlon, R. T., and Harcourt, R. T. (2015). Female receptivity, mating history, and familiarity influence the mating behavior of cuttlefish. *Behav. Ecol. Sociobiol.* 69, 283–292. doi: 10.1007/s00265-014-1841-5

Seibel, B. A. (2015). Environmental physiology of the jumbo squid, *Dosidicus gigas* (d'Orbigny, 1835)(Cephalopoda: Ommastrephidae): implications for changing climate. *Am. Malacol. Bull.* 33, 161–173. doi: 10.4003/006.033.0113

Shigeno, S., Tsuchiya, K., and Segawa, S. (2001). Embryonic and paralarval development of the central nervous system of the loliginid squid *Sepioteuthis lessoniana*. *J. Comp. Neurol.* 437, 449–475. doi: 10.1002/cne.1295

Shomrat, T., Graindorge, N., Bellanger, C., Fiorito, G., Loewenstein, Y., and Hochner, B. (2011). Alternative sites of synaptic plasticity in two homologous "fan-out fan-in" learning and memory networks. *Curr. Biol.* 21, 1773–1782. doi: 10.1016/j.cub.2011.09.011

Shomrat, T., Turchetti-Maia, A., Stern-Mentch, N., Basil, J., and Hochner, B. (2015). The vertical lobe of cephalopods: an attractive brain structure for understanding the evolution of advanced learning and memory systems. *J. Comp. Physiol. A* 201, 947–956. doi: 10.1007/s00359-015-1023-6

Shomrat, T., Zarrella, I., Fiorito, G., and Hochner, B. (2008). The octopus vertical lobe modulates short-term learning rate and uses LTP to acquire long-term memory. *Curr. Biol.* 18, 337–342. doi: 10.1016/j.cub.2008.01.056

Sigsgaard, E. E., Nielsen, I. B., Bach, S. S., Lorenzen, E. D., Robinson, D. P., Knudsen, S. W., et al. (2016). Population characteristics of a large whale shark aggregation inferred from seawater environmental DNA. *Nat. Ecol. Evol.* 1, 0004–0005. doi: 10.1038/s41559-016-0004

Sims, D. W., Humphries, N. E., McHugh, M., and Wearmouth, V. J. (2011). Electronic tagging of cuttlefish *Sepia officinalis* and octopus *Eledone cirrhosa* to study commonalities in behaviour patterns and comparisons with marine fish. *J. Shellfish Res.* 30, 1020–1021.

Smith, J. A., Andrews, P. L. R., Hawkins, P., Louhimies, S., Ponte, G., and Dickel, L. (2013). Cephalopod research and EU Directive 2010/63/EU: requirements, impacts and ethical review. *J. Exp. Mar. Biol. Ecol.* 447, 31–45. doi: 10.1016/j.jembe.2013.02.009

Song, Z., Mazzola, C., Schwartz, E., Chen, R., Finlaw, J., Krieg, M., et al. (2016). A compact autonomous underwater vehicle with cephalopod-inspired propulsion. *Mar. Technol. Soc. J.* 50, 88–101. doi: 10.4031/MTSJ.50.5.9

Squires, Z. E., Wong, B. B., Norman, M. D., and Stuart-Fox, D. (2012). Multiple fitness benefits of polyandry in a cephalopod. *PLoS One* 7:e37074. doi: 10.1371/journal.pone.0037074

Squires, Z. E., Wong, B. B., Norman, M. D., and Stuart-Fox, D. (2014). Multiple paternity but no evidence of biased sperm use in female dumpling squid *Euprymna tasmanica*. *Mar. Ecol. Prog. Ser.* 511, 93–103. doi: 10.3354/meps10898

Staaf, D. (2017). *Squid Empire: The Rise and Fall of the Cephalopods*. Lebanon, NH: University Press of New England.

Stanford, C. B. (1998). The social behavior of chimpanzees and bonobos: empirical evidence and shifting assumptions. *Curr. Anthropol.* 39, 399–420. doi: 10.1086/204757

Steele, E. J., Al-Mufti, S., Augustyn, K. A., Chandrajith, R., Coghlan, J. P., Coulson, S., et al. (2018). Cause of Cambrian explosion-terrestrial or cosmic? *Prog. Biophy. Mol. Biol.* [Epub ahead of print]. doi: 10.1016/j.pbiomolbio.2018.03.004

Steer, M., Moltschaniwskyj, N., and Gowland, F. (2002). Temporal variability in embryonic development and mortality in the southern calamary *Sepioteuthis australis*: a field assessment. *Mar. Ecol. Prog. Ser.* 243, 143–150. doi: 10.3354/meps243143

Stephens, Z. D., Lee, S. Y., Faghri, F., Campbell, R. H., Zhai, C., Efron, M. J., et al. (2015). Big data: astronomical or genomical? *PLoS Biol.* 13:e1002195. doi: 10.1371/journal.pbio.1002195

Stien, L. H., Bracke, M., Folkedal, O., Nilsson, J., Oppedal, F., Torgersen, T., et al. (2013). Salmon Welfare Index Model (SWIM 1.0): a semantic model for overall welfare assessment of caged Atlantic salmon: review of the selected welfare indicators and model presentation. *Rev. Aquac.* 5, 33–57. doi: 10.1111/j.1753-5131.2012.01083.x

Strugnell, J. M., Hall, N. E., Vecchione, M., Fuchs, D., and Allcock, A. L. (2017). Whole mitochondrial genome of the Ram's Horn Squid shines light on the phylogenetic position of the monotypic order Spirulida (Haeckel, 1896). *Mol. Phylogenet. Evol.* 109, 296–301. doi: 10.1016/j.ympev.2017.01.011

Stubbs, A. L., and Stubbs, C. W. (2016). Spectral discrimination in color blind animals via chromatic aberration and pupil shape. *Proc. Natl. Acad. Sci. U.S.A.* 113, 8206–8211. doi: 10.1073/pnas.1524578113

Sumbre, G., Fiorito, G., Flash, T., and Hochner, B. (2005). Neurobiology: motor control of flexible octopus arms. *Nature* 433, 595–596. doi: 10.1038/433595a

Sumbre, G., Fiorito, G., Flash, T., and Hochner, B. (2006). Octopuses use a human-like strategy to control precise point-to-point arm movements. *Curr. Biol.* 16, 767–772. doi: 10.1016/j.cub.2006.02.069

Sumbre, G., Gutfreund, Y., Fiorito, G., Flash, T., and Hochner, B. (2001). Control of octopus arm extension by a peripheral motor program. *Science* 293, 1845–1848. doi: 10.1126/science.1060976

Sutton, M., Perales-Raya, C., and Gilbert, I. (2016). A phylogeny of fossil and living neocoleoid cephalopods. *Cladistics* 32, 297–307. doi: 10.1111/cla.12131

Sweeney, M. J., and Roper, C. F. E. (1998). "Classification, type localities, and type repositories of recent Cephalopoda," in *Smithsonian Contributions to Zoology Systematics and Biogeography of Cephalopods*, eds N. A. Voss, M. Vecchione, R. B. Toll, and M. J. Sweeney (Washington, DC: Smithsonian Institution Press), 561–599.

Sykes, A. V., Almansa, E., Cooke, G. M., Ponte, G., and Andrews, P. L. (2017a). The digestive tract of cephalopods: a neglected topic of relevance to animal welfare in the laboratory and aquaculture. *Front. Physiol.* 8:492. doi: 10.3389/fphys.2017.00492

Sykes, A. V., Alves, A., Capaz, J. C., Madeira, C., Couto, A. T., Gonçalves, R. A., et al. (2017b). Refining tools for studying cuttlefish (*Sepia officinalis*) reproduction in captivity: *In vivo* sexual determination, tagging and DNA collection. *Aquaculture* 479, 13–16. doi: 10.1016/j.aquaculture.2017.05.021

Tanner, A. R., Fuchs, D., Winkelmann, I. E., Gilbert, M. T. P., Pankey, M. S., Ribeiro, Â. M., et al. (2017). Molecular clocks indicate turnover and diversification of modern coleoid cephalopods during the Mesozoic Marine Revolution. *Proc. Biol. Sci.* 284:20162818. doi: 10.1098/rspb.2016.2818

Tateno, S. (1993). "Non-invasive analysis of mantle movements in *Octopus vulgaris*," in *Recent Advances in Fisheries Biology*, eds T. Okutani, R. K. O'Dor, and T. Kubodera (Tokyo: Tokai University Press), 559–569.

Temple, S. E., Pignatelli, V., Cook, T., How, M. J., Chiou, T. H., Roberts, N. W., et al. (2012). High-resolution polarisation vision in a cuttlefish. *Curr. Biol.* 22, R121–R122. doi: 10.1016/j.cub.2012.01.010

Thomas, K. N., Robison, B. H., and Johnsen, S. (2017). Two eyes for two purposes: in situ evidence for asymmetric vision in the cockeyed squids *Histioteuthis* *heteropsis* and *Stigmatoteuthis dofleini*. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 372:20160069. doi: 10.1098/rstb.2016.0069

Thomsen, P. F., Kielgast, J., Iversen, L. L., Møller, P. R., Rasmussen, M., and Willerslev, E. (2012). Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PLoS One* 7:e41732. doi: 10.1371/journal.pone.0041732

Thomsen, P. F., and Willerslev, E. (2015). Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. *Biol. Conserv.* 183, 4–18. doi: 10.1016/j.biocon.2014.11.019

Tinbergen, N. (1963). On aims and methods of ethology. *Z. Tierpsychol.* 20, 410–433. doi: 10.1111/j.1439-0310.1963.tb01161.x

Tricarico, E., Amodio, P., Ponte, G., and Fiorito, G. (2014). "Cognition and recognition in the cephalopod mollusc *Octopus vulgaris*: coordinating interaction with environment and conspecifics," in *Biocommunication of Animals*, ed. G. Witzany (Dordrecht: Springer), 337–349.

Tublitz, N. J., Gaston, M. R., and Loi, P. K. (2006). Neural regulation of a complex behavior: body patterning in cephalopod molluscs. *Integr. Comp. Biol.* 46, 880–889. doi: 10.1093/icb/icl020

Turchetti-Maia, A., Shomrat, T., and Hochner, B. (2017). "The vertical lobe of cephalopods—a brain structure ideal for exploring the mechanisms of complex forms of learning and memory," in *The Oxford Handbook of Invertebrate Neurobiology*, ed. J. H. Byrne (New York, NY: Oxford University Press).

Uriarte, I., Martínez-Montaño, E., Espinoza, V., Rosas, C., Hernandez, J., and Farías, A. (2016). Effect of temperature increase on the embryonic development of Patagonian red octopus *Enteroctopus megalocyathus* in controlled culture. *Aquac. Res.* 47, 2582–2593. doi: 10.1111/are.12707

Ussler, W. III, Preston, C., Tavormina, P., Pargett, D., Jensen, S., Roman, B., et al. (2013). Autonomous application of quantitative PCR in the deep sea: in situ surveys of aerobic methanotrophs using the deep-sea environmental sample processor. *Environ. Sci. Technol.* 47, 9339–9346. doi: 10.1021/es4023199

Verne, J. (1988). *20,000 Leagues Under the Sea*. Hertfordshire: Wordsworth Editions Ltd.

Vidal, E. A., Villanueva, R., Andrade, J. P., Gleadall, I. G., Iglesias, J., Koueta, N., et al. (2014). Cephalopod culture: current status of main biological models and research priorities. *Adv. Mar. Biol.* 67, 1–98. doi: 10.1016/B978-0-12-800287-2.00001-9

Villanueva, R., Perricone, V., and Fiorito, G. (2017). Cephalopods as predators: a short journey among behavioral flexibilities, adaptions, and feeding habits. *Front. Physiol.* 8:598. doi: 10.3389/fphys.2017.00598

Villanueva, R., Sykes, A. V., Vidal, E. A., Rosas, C., Nabhitabhata, J., Fuentes, L., et al. (2014). "Current status and future challenges in cephalopod culture," in *Cephalopod Culture*, eds J. Iglesias, L. Fuentes, and R. Villanueva (Dordrecht: Springer), 479–489.

Vitti, J. J. (2013). Cephalopod cognition in an evolutionary context: implications for ethology. *Biosemiotics* 6, 393–401. doi: 10.1007/s12304-013-9175-7

Wada, T., Takegaki, T., Mori, T., and Natsukari, Y. (2005). Sperm displacement behavior of the cuttlefish *Sepia esculenta* (Cephalopoda: Sepiidae). *J. Ethol.* 23, 85–92. doi: 10.1007/s10164-005-0146-6

Wang, G.-D., Xie, H.-B., Peng, M.-S., Irwin, D., and Zhang, Y.-P. (2014). Domestication genomics: evidence from animals. *Annu. Rev. Anim. Biosci.* 2, 65–84. doi: 10.1146/annurev-animal-022513-114129

Wang, Q., Gossweiler, G. R., Craig, S. L., and Zhao, X. (2014). Cephalopod-inspired design of electro-mechano-chemically responsive elastomers for on-demand fluorescent patterning. *Nat. Commun.* 5:4899. doi: 10.1038/ncomms5899

Wardill, T. J., Gonzalez-Bellido, P. T., Crook, R. J., and Hanlon, R. T. (2012). Neural control of tuneable skin iridescence in squid. *Proc. R. Soc. B* 279, 4243–4252. doi: 10.1098/rspb.2012.1374

Wearmouth, V. J., Durkin, O. C., Bloor, I. S., McHugh, M. J., Rundle, J., and Sims, D. W. (2013). A method for long-term electronic tagging and tracking of juvenile and adult European common cuttlefish *Sepia officinalis*. *J. Exp. Mar. Biol. Ecol.* 447, 149–155. doi: 10.1016/j.jembe.2013.02.023

Wild, E., Wollesen, T., Haszprunar, G., and Heb, M. (2015). Comparative 3D microanatomy and histology of the eyes and central nervous systems in coleoid cephalopod hatchlings. *Org. Divers. Evol.* 15, 37–64. doi: 10.1007/s13127-014-0184-4

Wilk, S. R. (2000). *Medusa: Solving the Mystery of the Gorgon*. Oxford: Oxford University Press.

Williams, T., Readman, G., and Owen, S. (2009). Key issues concerning environmental enrichment for laboratory-held fish species. *Lab. Anim.* 43, 107–120. doi: 10.1258/la.2007.007023

Williams, W. (2011). *Kraken: The Curious, Exciting, and Slightly Disturbing Science of Squid*. New York, NY: Abrams.

Williamson, R., and Budelmann, B. U. (1991). Convergent inputs to *Octopus* oculomotor neurons demonstrated in a brain slice preparation. *Neurosci. Lett.* 121, 215–218. doi: 10.1016/0304-3940(91)90688-P

Wolfe, K., Smith, A. M., Trimby, P., and Byrne, M. (2012). Vulnerability of the paper nautilus (*Argonauta nodosa*) shell to a climate-change ocean: potential for extinction by dissolution. *Biol. Bull.* 223, 236–244. doi: 10.1086/BBLv223n2p236

Wollesen, T., McDougall, C., Degnan, B. M., and Wanninger, A. (2014). POU genes are expressed during the formation of individual ganglia of the cephalopod central nervous system. *EvoDevo* 5:41. doi: 10.1186/2041-9139-5-41

Xavier, J. C., Allcock, A. L., Cherel, Y., Lipinski, M. R., Pierce, G. J., Rodhouse, P. G. K., et al. (2015). Future challenges in cephalopod research. *J. Mar. Biol. Assoc. United Kingdom* 95, 999–1015. doi: 10.1017/S0025315414000782

Xavier, J. C., Cherel, Y., Allcock, L., Rosa, R., Sabirov, R. M., Blicher, M. E., et al. (2018). A review on the biodiversity, distribution and trophic role of cephalopods in the Arctic and Antarctic marine ecosystems under a changing ocean. *Mar. Biol.* 165:93. doi: 10.1007/s00227-018-3352-9

Yamamoto, M., Shimazaki, Y., and Shigeno, S. (2003). Atlas of the embryonic brain in the pygmy squid, Idiosepius paradoxus. *Zool. Sci.* 20, 163–179. doi: 10.2108/zsj.20.163

Yan, G. (2016). Saving Nemo–Reducing mortality rates of wild-caught ornamental fish. *SPC Live Reef Fish Inform. Bull.* 21, 3–7.

Yasumuro, H., and Ikeda, Y. (2011). Effects of environmental enrichment on the behavior of the tropical octopus *Callistoctopus aspilosomatis*. *Mar. Freshw. Behav. Physiol.* 44, 143–157. doi: 10.1080/10236244.2011.598643

Yasumuro, H., and Ikeda, Y. (2016). Environmental enrichment accelerates the ontogeny of cryptic behavior in pharaoh cuttlefish (*Sepia pharaonis*). *Zool. Sci.* 33, 255–265. doi: 10.2108/zs150197

Yoshida, M. A., Ogura, A., Ikeo, K., Shigeno, S., Moritaki, T., Winters, G. C., et al. (2015). Molecular evidence for convergence and parallelism in evolution of complex brains of cephalopod molluscs: insights from visual systems. *Integr. Comp. Biol.* 55, 1070–1083. doi: 10.1093/icb/icv049

Young, J. Z. (1985). Cephalopods and neuroscience. *Biol. Bull.* 168, 153–158. doi: 10.2307/1541328

Yu, C., Li, Y., Zhang, X., Huang, X., Malyarchuk, V., Wang, S., et al. (2014). Adaptive optoelectronic camouflage systems with designs inspired by cephalopod skins. *Proc. Natl. Acad. Sci.* 111, 12998–13003. doi: 10.1073/pnas.1410494111

Zarrella, I., Ponte, G., Baldascino, E., and Fiorito, G. (2015). Learning and memory in *Octopus vulgaris*: a case of biological plasticity. *Curr. Opin. Neurobiol.* 35, 74–79. doi: 10.1016/j.conb.2015.06.012

Zeidberg, L. D., and Robison, B. H. (2007). Invasive range expansion by the Humboldt squid, *Dosidicus gigas*, in the eastern North Pacific. *Proc. Natl. Acad. Sci. U.S.A.* 104, 12948–12950. doi: 10.1073/pnas.0702043104

Zullo, L., and Hochner, B. (2011). A new perspective on the organization of an invertebrate brain. *Commun. Integr. Biol.* 4, 26–29. doi: 10.4161/cib.4.1.13804

Zullo, L., Sumbre, G., Agnisola, C., Flash, T., and Hochner, B. (2009). Nonsomatotopic organization of the higher motor centers in octopus. *Curr. Biol.* 19, 1632–1636. doi: 10.1016/j.cub.2009.07.067

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer PS and handling Editor declared their shared affiliation.

frontiers in Communication

REVIEW
published: 13 June 2018
doi: 10.3389/fcomm.2018.00020

Cephalopods Between Science, Art, and Engineering: A Contemporary Synthesis

Ryuta Nakajima[1]*, *Shuichi Shigeno*[2†], *Letizia Zullo*[3], *Fabio De Sio*[4] *and* *Markus R. Schmidt*[5]*

[1] Department of Art and Design, University of Minnesota Duluth, Duluth, MN, United States, [2] Stazione Zoologica Anton Dohrn, Naples, Italy, [3] Center for Synaptic Neuroscience and Technology (NSYN), Istituto Italiano di Tecnologia, Genova, Italy, [4] Department of the History, Philosophy and Ethics of Medicine, Centre for Health and Society, Medical Faculty, Heinrich Heine Universität Düsseldorf, Düsseldorf, Germany, [5] Biofaction KG, Vienna, Austria

OPEN ACCESS

Edited by:
Tarla Rai Peterson,
The University of Texas at El Paso,
United States

Reviewed by:
Karen M. Taylor,
University of Alaska Fairbanks,
United States
Emily Plec,
Western Oregon University,
United States

***Correspondence:**
Ryuta Nakajima
rnakajim@d.umn.edu
Markus R. Schmidt
schmidt@biofaction.com

†Present Address:
Shuichi Shigeno,
Department of Neurobiology,
University of Chicago, Chicago, IL,
United States

Specialty section:
This article was submitted to
Science and Environmental
Communication,
a section of the journal
Frontiers in Communication

Received: 27 February 2018
Accepted: 02 May 2018
Published: 13 June 2018

Citation:
Nakajima R, Shigeno S, Zullo L, De
Sio F and Schmidt MR (2018)
Cephalopods Between Science, Art,
and Engineering: A Contemporary
Synthesis. Front. Commun. 3:20.
doi: 10.3389/fcomm.2018.00020

Cephalopods are outstanding animals. For centuries, they have provided a rich source of inspiration to many aspects of human cultures, from art, history, media, and spiritual beliefs to the most exquisite scientific curiosity. Given their high esthetical value and "mysteriously" rich behavioral repertoire they have functioned as boundary objects (or subjects) connecting seemingly distinct thematic fields. Interesting aspects of their being span from the rapid camouflaging ability inspiring contemporary art practices, to their soft and fully muscular body that curiously enough inspired both gastronomy and (soft) robotics. The areas influenced by cephalopods include ancient mythology, art, behavioral science, neuroscience, genomics, camouflage technology, and bespoken robotics. Although these might seem far related fields, in this manuscript we want to show how the increasing scientific and popular interest in this heterogeneous class of animals have indeed prompted a high level of integration between scientific, artistic, and sub-popular culture. We will present an overview of the birth and life of cephalopod investigations from the traditional study of ethology, neuroscience, and biodiversity to the more recent and emerging field of genomics, material industry, and soft robotics. Within this framework, we will attempt to capture the current interest and progress in cephalopod scientific research that lately met both the public interest and the "liberal arts" curiosity.

Keywords: cephalopod, interdisciplinary, culture, art, science, communication

INTRODUCTION

Cephalopods are the molluscan class including octopus, squid, cuttlefish, and nautilus. There are over 800 species found in the oceans around the world ranging from shallow tropical water to deep sea at more than 5,000 m (Hanlon and Messenger, 2018). Their body size can vary from mere 1 cm to over 18 m in its total length and their brain to body mass ratio can be higher than that of some vertebrates (Packard, 1972). They can rapidly change their body pattern and shape to avoid predation and for inter, and intraspecific communication. Many can glow in the dark using bioluminescent ink to create their body double, cross-dress to deceive rivals during the mating season, move through the water column using jet Propulsion, etc. (Nixon and Young, 2003). The list of cephalopods' unique abilities and features continues on and on, not to mention that they also provide the essential protein source for many marine animals and humans alike (section Modern Cephalopod Science). Sperm whales are estimated to consume equal biomass of squid each year as the total annual catch of the world fishing industries (Vidal, 2014). Each one of these diverse abilities and attributes of cephalopods has fascinated people from diverse range of fields and disciplines for centuries.

In recent years, with the help of social media and Internet providing access to specialized information and growing interest in interdisciplinary academic collaboration fields, there has been increasing attention to cephalopods not only as model animals but also as a boundary object/subject connecting fields together. As examples, we show the organization of art exhibitions in conjunction with scientific conferences, the establishment of side-by-side collaboration between cephalopod behavioral scientist and art schools and military departments on topics such as camouflage technology, to end with sports fishermen working with scientists in a citizen science project. Although these fields are still in their infancy of interdisciplinary collaboration, the slow but assertive new developments in cephalopod research and culture have certainly began to transform the traditional paradigm of the cephalopod research. In this study, we attempt to capture this moment of transformation by revisiting the scientific development as well as to list and analyze some of the significant progress in the respective fields. In this way, we wish to capture the energy that will drive cephalopod research and culture in the twenty-first century.

HISTORY

Scientific Development

Messenger (1988) wrote that animals could be studied for two reasons: because of their inherent beauty or because they provide especially suitable conditions for tackling one particular problem of general interest. This issue was addressed in 1929 by the Nobel laureate August Krogh (1929) who formulated the following principle: "for such a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied" (later known as Krogh's Principle—see Krebs, 1975).

Cephalopods have played both roles in the history of biology and medicine; they provided answers and (more often) questions that have kept generations of researchers busy. Systematic observation of cephalopod structure and behavior can be traced back to Aristotle's The History of Animals, Book IV (ca. 350 BC) and, after a rather long eclipse, this knowledge was further developed during the Renaissance (Schmitt, 1965). In the early nineteenth century, cephalopods had a prominent place in the famous Cuvier-Geoffroy debate on comparative anatomy. This landmark confrontation in the history of zoology was spurred by Geoffroy's comparison of the cephalopods' internal structure to that of a vertebrate, the body of which is bent so that the pelvis touches the head (see Flourens, 1865; Packard, 1972; Appel, 1987).

By the end of the nineteenth century, the development of aquaria and marine stations, places where these animals could be kept alive for long, started the age of cephalopods as experimental animals. Inevitably, the period in which this development took place, and its specific, dominant "scientific atmosphere" have strongly influenced the way researchers have looked at them. Most cephalopods were subjected, thanks to the new infrastructure, to the professional gaze of the most diverse experimenters, psychologists, physiologists, zoologists, and, later, biophysicists. Whereas a few efforts were made (most notably by Jatta, Lo Bianco and Naef at the Naples Zoological Station) to expand our knowledge of these animals in their natural environment, it was the use-value of cephalopods in the laboratory that first defined them—notably, as "Guinea pigs of the sea" (Grimpe, 1928). This identity, duly separated from that of mythical monsters or allegorical representations that characterized their earlier relations with humans (see, e.g., Hugo, 1866; Lee, 1875), stuck for quite some time. Early in the twentieth century, cephalopods started to leave fairy-tales to massively colonize laboratory manuals for physiologists and physicians (Grimpe being a case in point, but see also von Uexküll, 1905). Octopuses, in particular, became a much sought-after preparation for the study of the effect of poisons, thanks to their excellent capacity for acclimatization, resilience to surgical interventions in comparison with sepia and squids and the long survival of their organs after extirpation. The study of regeneration of nerve and tissue also greatly profited from the "contribution" of cuttlefish and octopuses (Sereni and Young, 1932), as did the physiology of vision (Dröscher, 2016). In 1913, Wilhelm Fröhlich reportedly obtained the first electroretinograms ever from the eyes of *Eledone* and *Octopus*, which proved to be particularly suitable to the task thanks to their relative structural simplicity and the easy access of the recording locations. On the other hand, cephalopods turned out to be an excellent source of questions also in this domain: the debate on color vision in cephalopods spanned more than half a century (Messenger et al., 1973) before the necessary consensus was reached on their color-blindness (but see Gagnon et al., 2016; Stubbs and Stubbs, 2016). Physiologists and psychologists were also fascinated by the camouflage abilities especially of cuttlefish and the chances the chromatophore system afforded to study reflex responses following visual input. The reason behind can be found in the extremely fast response of the chromatophore organs following an eye-directed visual stimuli. This is due to the existence of a direct input connection between specific brain centers (the chromatophore lobes) and the chromatophores located overall the animal body.

Then, in the late 1930s, the Age of the Squid began. Their so-called giant axon—a syncytium, and as such an exception to the strict Cajalian rule of anatomical independence of nerve cells (Young, 1938)—was famously re-discovered by the zoologist John Zachary Young in 1936. The Marine Stations of Plymouth and the Woods Hole (Massachusetts, US) became hothouses for the introduction and development of this model, which was soon adopted by axonologists worldwide. In particular, the giant axon became the experimental model of the Cambridge biophysical school thanks to the work of Alan Hodgkin, Andrew Huxley (Hodgkin and Huxley, 1939), and Bernhard Katz, all of whom it helped to win the Nobel Prize. As Hodgkin later mused: "It is arguable that the introduction of the squid giant nerve fiber [...] did more for axonology than any other single advance in technique during the last 40 years. Indeed a distinguished neurophysiologist remarked recently at a congress dinner (not, I thought, with the utmost tact) 'It's the squid they really ought to give the Nobel Prize to" (Hodgkin, 1975, p. 16).

Young was also responsible for another significant turn in cephalopod laboratory history. After the war, and, initially, with the sole help of his assistant Brian B. Boycott, he started at the Naples Zoological Station an ambitious research program on the comparative study of memory. The idea was to set up a comparative study of the neural correlates of learning and memory in different classes. *Octopus vulgaris* is abundant in Naples and—as Young knew from his previous collaboration with the physician and physiologist Enrico Sereni—is a suitable animal for the laboratory experiments. Among its virtues were an enormous appetite (essential for behavioral experiments), a reportedly excellent learning ability, exploratory and aggressive behavior and a non-compact, "compartmentalized" brain. Moreover, it lacked any hard component, which made it easily accessible for the kind of research Young had in mind. The idea, following the standard defined by Karl Lashley in his work with mice, was to teach the animals a task (discrimination, or even problem-solving), then cut portions of their higher ganglia (purportedly controlling the more complex functions) and repeat the learning paradigm, with the hope of thus establishing correlations among the missing parts and the lost abilities.

It took Boycott 3 years to fine-tune the experimental system, and basically to single-handedly discover the needs, potentials, and problems of a laboratory octopus. By 1950, however, the system was in place, complete with a learning paradigm (a discrimination task: crab alone vs. crab + lead plate and shock), the preliminary functional anatomy of the higher ganglia and a vague but promising theoretical framework, based on vertebrate research (Boycott, 1954). The first full experimental report was published only in 1955 (Boycott and Young, 1955) but was encouraging. The octopus not only had kept all of its promises, (it learned fast, and a tentative association between learning and specific ganglia above the esophagus was presented)—it also had provided a few surprises. Most notably, Boycott and Young reported of a probable bi-partition of the memory storage, with two distinct (putative) neuronal circuits for long- and short-term memories, which at the time resonated incredibly well with the first studies of Brenda Milner on subjects. Boycott and Young were also the first to define the concept of a "memory system" (Buckner, 2007), a dedicated "neuronal net" devoted to the storage of memories. So did *Octopus vulgaris* begin a new career, that of "model of the brain" (De Sio, 2011). The early successes attracted new collaborators, who in turn helped to improve and diversify the learning paradigm and added to the growing complexity of the anatomical picture. Meanwhile, since the early 1950s Young was falling more and more under the influences of cybernetics. The comparative project became the octopus project, and the octopus progressively became a mechanical model—a living computer containing, rather than being characterized by, a memory.

The natural end-point of such a development was the attempt at building a learning machine based on what the researchers had learned about the performances and structure of the animal. In 1953, an electrical engineer by the name of Wilfrid Kenelm Taylor was hired and started the design and construction of a "feature detector" simplified retina made out of nine photocells, randomly connected to a whole wall full of electrical synapses, mimicking a part of an optic lobe. By 1956 the machine was in operation, or perhaps one should rather say it was undergoing education. It was, in fact, a wholly analog device, extremely plastic and fast-learning, but it required long training sessions, in which it was "shown" different pictures of human faces. In a relatively short time, it proved able to tell a female from a male face. It was also possible to have it "forget" things, by re-setting the electrical neurons, and then "re-learn" them. This machine later came to be subsumed under the wider category of "perceptron," or neural networks and is rather part of the pre-history of artificial intelligence (AI) than of its history proper. The analog phase of AI research was in fact very short, with heavy, expensive and cumbersome machines being soon superseded by more efficient and economic purpose-developed software being run on all-purpose computers. It would take more than three decades for this avenue to be re-opened. At that time, however, the cephalopod research was about to take a different, more promising avenue: that of robotics and biomimicry, which has today reached its first staggering results (see section Fisheries, Conservation and Biodiversity and Camouflage Technology). The Octopus-perceptron was dismantled in the late 1960s and consigned to oblivion, but it had served its function. Despite the promises to his patrons (developing a learning computer) what Young had in mind was a comparative study of animal and machine learning, in which not only the animal could provide a blueprint for the machine, but the machine could also help in the interpretation of the structure-function nexus in the octopus. It was from this unlikely resonance that Young's famous model of the "mnemon" or memory cell, was born—the first selective model for memory formation (Young, 1964; Edelman, 1987; Changeux, 2006).

MODERN CEPHALOPOD SCIENCE

In the 1980's, cephalopod science took a big step. Cephalopods, which have rapid growth rates are abundant in the sea, were considered to be a vital source of protein to feed the increasing world population (Vidal, 2014). In order to create a more accurate stock assessment of cephalopods in the world ocean, seven scientists were gathered to investigate life cycle, population distribution, and species identification in 1983. This meeting, then, became the first the Cephalopod International Advisory Council (CIAC) meeting in 1985. Since then, CIAC meetings have grown to over 250 participants from diverse scientific fields including robotics, AI, neuroscience, behavior, and more (Fiorito et al., 2014). This meeting enlightened the multidimensionality of cephalopod research and provided a valuable platform to create a synergy of multiple fields. Among these, the most current and immediate frontier seems to lie in neuroscience, behavioral biology, and conservation. The rich and flexible behavioral repertoire supported by the well-developed brain, muscular structure, and circulatory systems drives and offers multivalent research opportunities to be explored.

Neuroscience and Cellular Biology

Cephalopods have proven excellent experimental models for a number of general problems in physiology, cell biology, and neuroscience (Abbott et al., 1995; Fiorito et al., 2014), including synaptic transmission (Bullock, 1948; Katz and Miledi, 1970; Armstrong and Bezanilla, 1973) and neural control of behavior (Nixon and Young, 2003). They have evolved a specialty in cognition among invertebrates and even more interestingly a "different" type of brain centers and decentralized decision making areas at many levels of their body including the arms (Hochner et al., 2006; Zullo and Hochner, 2011; Mather and Dickel, 2017). Despite the utterly unique brain organization, Edelman et al. (2005), for example, emphasized that the brains composed of about 170 million neurons, complex sensory receptors, and motor control systems are the obviously comparable situation to its vertebrate counterparts. As a representative case, the cephalopod brain analogous to the reentrant loops of the thalamo-cortical system will be a landmark as a conscious system of the mammalian and birds.

Furthermore, the possibility that they might have personality has been suggested by a series of investigations where the general behavior of animals held in captivity and their reaction to the environmental threats have been carefully observed. The neural basis of personality is largely unknown, but these studies have nicely shown that animals respond to the threats differently (for example interacting, hiding, or escaping the stimulus) following what seems to be an exclusive "personal" variability (Mather and Anderson, 1993, 1999; Mather and Carere, 2012). In addition to this, cephalopods seem to have a simple form of consciousness adapted to their behavioral abilities such as environment navigation, requiring a form of self-awareness possibly similar to that vertebrates and insects, motor control of highly flexible arms and, eventually, to their potential social interaction (**Figure 1**). In light of this, a test such as the mirror self-recognition test (MSR) has been conducted with cephalopods to measure their ability to visual self-recognize (Ikeda, 2009; **Figure 1**). Taken together, this makes them interesting animals with big brains in comparison and contrast to vertebrates for the study of evolution (Mather and Kuba, 2013).

Behavioral Ecology and Biology

Beyond the traditional knowledge of cephalopod neuroscience and behaviors as summarized in Wells (1978), Mangold (1989), Abbott et al. (1995), Hanlon and Messenger (2018), and Borrelli et al. (2006), recent cephalopod behavioral studies continue to provide many interesting discoveries. Researchers have found new cues for cephalopod novelty or intelligent behaviors, including new body chromatophore coloration, light sensing skin, observational learning, human-like arm use, mimicking, developmental cognition, sociality, and possible tool use and play (Hochner et al., 2006; Zullo and Hochner, 2011; Darmaillacq et al., 2012; Mather and Dickel, 2017 for reviews). These behaviors are supported by a complex and well-developed sensory system that possibly integrates a variety of information coming from different sources such as visual system, motor system, etc. (Budelmann, 1995; Zullo et al., 2009; Hanke and Osorio, 2018).

Moreover, we have to consider that cephalopods are worldwide-distributed animals and can occupy almost all kind of marine habitats, an aspect that is reflected in the incredible number and diversification of existent species. Given this ecosystem diversity, any researcher studying cephalopods can face a wide variety of problems connected to the animal collection and lifestyle along with having access to a number of different, and fascinating, scientific questions.

As an example, exploring the behavioral ecology of deep-sea species has long been challenging, but Kubodera and his team became the first to capture photos of the live giant squid and to observe its active pre-capture behaviors (Kubodera and Mori, 2005). The mysterious vampire squid, *Vampyroteuthis infernalis* lives in extreme deep sea conditions and, unlike most cephalopods, it has been shown to have multiple reproductive cycles (Hoving et al., 2015). Furthermore, a deep-sea incirrate octopus has been shown to breed big eggs almost for 4 years, the longest in any known animal (Barratt et al., 2007; Robison et al., 2014). Another interesting aspect of mesopelagic and deep-sea species that live across a broad range of depths ∼1,200 m is their adaptability to aphotic (lightless) depths and a new strategy of visual adaptation has been recently revealed (Chung and Marshall, 2017).

Fisheries, Conservation, and Biodiversity

Differences in the behavioral ecology of cephalopods are well represented also in other fields such as that of fisheries. Cephalopod fishery science has been continuing to focus on life history, population dynamics, and stock assessment of commercially important species in the ever-changing state of world oceans. Since Malcolm Clarke estimated that sperm whales consume 100 million tons of squid per year, this task has been challenging to accomplish due to oceanic nature of the target species. In light of this, currently, an ecosystem approach to fisheries combining biological information, taxonomy, biogeography, systematics, annual sampling, and oceanographic data has become the desired method for stock assessment and monitoring (Rodhouse et al., 2014). In addition to interdisciplinary data analysis, various tagging methods have been used to track migration pattern, movement and distribution in species such as Caribbean Reef squid, Humboldt Squid, Short finned squid, Arrow squid, Chokka squid, Japanese flying squid, and more. Furthermore, creating economically viable aquaculture for the Common octopus, *Octopus vulgaris* has also been a significant goal of fishery science, ecology, and conservation (Iglesias et al., 2004; Navarro et al., 2014).

The extensive commercial fishery for cephalopods started in 1950. According to FAO, total annual world catch of cephalopods was 750,000 tons in 1961 and has increased to 4 million tons in 2013. Although the total number is growing due to jumbo flying squid, *Dosidicus gigas*, harvest in the East Pacific, cuttlefishes are showing steady to slight decrease (FAO, 2016). More significantly, the annual catch of commercially important species as Japanese flying squid, *Todarodes pacificus* has plummeted from

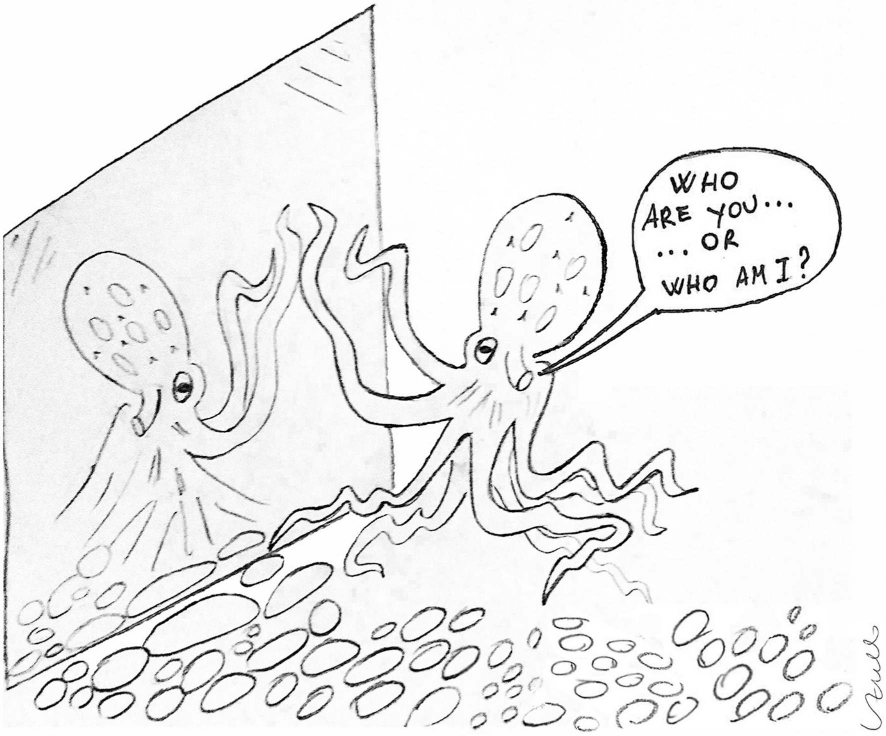


FIGURE 1 | What consciousness in Cephalopods? Sketch representing the Gallup's mirror test technique applied to an octopus to assess its visual self-recognition abilities (drawing by L. Zullo).

444,000 tons in 1996 to 67,800 tons in 2016 (MAFF, 2017). With increasing threats due to ocean acidification and climate changes related to human activities, monitoring marine food chains is now an urgent task. Cephalopods exhibit rapid growth, short lifespans, and strong life-history plasticity, allowing them to adapt quickly to changing environments. Also, related to these fields, cephalopod fisheries and aquaculture have traditional importance as a food source, and recent advancements were reviewed in Iglesias et al. (2014) and Vidal (2014). Hence, studying cephalopod ecology, biodiversity and conservation occupy a unique position in marine science (Boyle and Rodhouse, 2008; Doubleday et al., 2016).

There has been a rapid technological advancement of molecular tools and powerful next-generation sequencers to perform species identification. DNA barcoding, mitochondrial and 16S rRNA sequences, for example, offered rapid species assignment and provided significant potential for species identification and biodiversity (Dai et al., 2012). Analysis of environmental DNA is also a powerful tool to estimate large-scale biomass and ecological niche from a limited seawater sample by using a highly sensitive quantified PCR technology (Mauvisseau et al., 2017). Also, natural history studies of cephalopods have been based on the molecular methodological tools available. After the first publication of a cephalopod DNA in 1983 (Walker and Doolittle, 1983), understanding of the cephalopod phylogeny and classification has advanced through the comprehensive approaches of mitochondrial and nuclear genomics, and transcriptomic multi-gene sequence analyses (Allcock et al., 2015; Lindgren and Anderson, 2017; Uribe and Zardoya, 2017).

In addition to the technical advancement of molecular tools and sequencers, the rapid growth of information technologies and related infrastructures has changed the methodology employed in cephalopod studies over the past 20 years. Advanced research vessels, submersibles, physical sensors, acoustic transmitters, and observing systems are now mutually combined with computer networks to investigate deep-sea cephalopods (Hoving et al., 2014). For example, monitoring seasonal habitat changes of deep-sea benthic cephalopods, a novel Internet Operation Vesicle, a benthic crawler was used. This vesicle was connected to the NEPTUNE cabled infrastructure operated by Ocean Networks Canada (Doya et al., 2017).

The worldwide global networks are now dramatically changing our communication tools through the website and multi-institute administered databases, i.e., the World Register of Marine Species (WoRMS) has provided the most authoritative data since its launch in 2007. Not only the specialists in the fields, scuba divers, nature enthusiasts, sports fishermen, and many others can now mutually share the cephalopod information via social network systems such as Facebook, WhatsApp, Instagram, Line, Twitter, Flickr, and Youtube. As users of social network systems and their daily activities have blurred the boundary between social media and the traditional web, the phenomenon has become a great influence over scientific communities. Indeed, scientists can significantly benefit from the prompt availability of a massive amount of data in the form of images, videos and data records of cephalopods in the wild.

Genomics and Molecular Biology

Twenty years ago, our ability to study cephalopod genes and proteins homologous to other model animals were limited, but recent advances in high-throughput techniques including next-generation sequencing, as stated above, enable us to profile molecular data from a number of species rapidly (Albertin et al., 2012; Liscovitch-Brauer et al., 2017). Indeed, molecular data in cephalopod transcriptomes indicate that key neurotransmitters and regulatory genes are present similarly in the tissues of vertebrates, insects, worms, and other marine invertebrates. There is a minor variation, but no obvious evidence exists for systematic expansion of neurotransmission gene families between octopus and vertebrate genomes (Albertin et al., 2015). These similarities are interpreted as evidence for evolutionary conservation inherited from a common ancestor. For some examples, studies of DNA-binding homeobox domain Pax6 (Tomarev et al., 1997) and Hox genes (Lee et al., 2003), each of which showed evolutionary conservation with those of vertebrates and insects for the evolution of eyes or whole body. By analyzing such evolutionary conserved developmental control genes, biologists are now challenged to understand how cephalopod brain and body are organized.

In a paper on the octopus draft genome, Albertin et al. (2015) provided evidence that the genome size of *Octopus bimaculoides* was comparable to the 3 billion base pair human genome and there was no evidence for whole genome duplication as in vertebrates. As notable cephalopod novelties, they found dramatic diversification of kinds of genes including the C2H2 superfamily of zinc finger transcription factors, protocadherins, interleukin 17-like genes, RNA editing, and elevated transposon expression in the neural tissues that may produce a genome rearrangement as seen in Hox gene complement unusually splitting into nine clusters in the octopus. Interestingly, Garrett and Rosenthal (2012) found that transcribed messenger RNAs of K+ channel gene are extensively edited in Antarctic and Arctic octopuses compared to those of tropical species, creating the functional diversity of ion channels to accelerate gating kinetics greatly. Namely, they showed that adenosine-to-inosine RNA editing can respond to the cold-water environment. Furthermore, Yoshida et al. (2014) showed splicing variants of developmental genes that display unique features in cephalopod eye evolution, and Liscovitch-Brauer et al. (2017) discovered a cephalopod specific novelty, exhibiting extensive RNA editing of squids, cuttlefish, and octopuses. Cephalopods may have transcriptome plasticity via RNA editing in evolution beyond genome.

Cephalopods genomic complexity has to be seen not only as a strategy developed to allow survival in different habitats and various lifestyle. As highlighted in the previous section genome together with body shape and environment co-evolved to generate animals that are unique also in their nervous system and the control architecture of body and behaviors. It is indeed the flexibility that they demonstrate at several levels of their biological organization that in the last decades fed another far-related discipline, that of engineering and in particular of soft robotics.

Soft Robotics

Soft robotics is a booming field that has attracted significant research interest in the last decade, because of the potential of soft robots to better interact with real-world environments. When, in March 2014, the scientific journal "Soft Robotics" was launched, the editor commented: "By building soft materials into the fundamental design of machines, or by building them completely from soft materials, we add a new dimension for design and create an untapped resource for entirely new types of machinery" (Trimmer, 2014).

Soft robots are devoid of rigid components and have several mechanical advantages over classically structured robots such as the ability to squeeze, stretch, and stiff (Laschi et al., 2016). They can operate in unstructured environments and, due to their inherent and modifiable compliance, they can perform operations across a wide variety of substrates and environments (Wang et al., 2015). Soft-robotics stands on "a completely different way of building robots," and instead of vertebrate, the octopus (and generally cephalopods) can be used as a natural template to learn from. Cephalopods are explicitly and repeatedly mentioned as a natural template for soft robotics. Indeed, cephalopods are currently an important source of inspiration for many bio-roboticists and material scientists due to the interesting characteristics of softness, robustness, environmental adaptability and control mechanisms of their body (**Figure 2**). Rus and Tolley (2015), for example, wrote in their review paper on soft robotics: "Cephalopods, for example, achieve amazing feats of manipulation and locomotion without a skeleton [...]. Inspired by nature, engineers have begun to explore the design and control of soft-bodied robots composed of compliant materials."

Cephalopod limbs are muscle hydrostats; they are almost entirely composed of muscle and connective tissue used both for force production and as structural support. They can bend in any direction and change the stiffness at any point of the entire arm length. Hence, they offer a valuable model to study two fundamental properties (and challenges) in soft-robotics that are: (1) the modulation of stiffness and (2) the position control (**Figure 3**). In one word, they are "hyper-redundant" structure whose control and coordination present a dramatic computational complexity. To date, several aspects of their behavioral repertoire have been addressed both for computational modeling and Artificial Intelligence (AI) and for the development of robotic prototypes (Guglielmino et al., 2012, 2013; Nakajima et al., 2015). Among others, propulsion swimming, single and multiple arm manipulation, crawling and exploratory behaviors and sucker attachment have been studied in more detail.

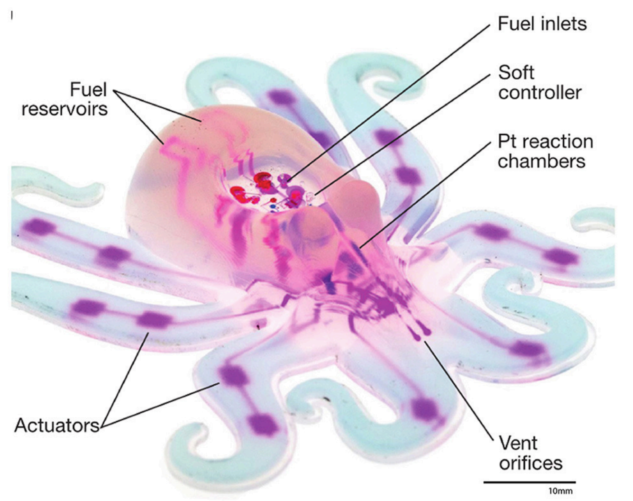


FIGURE 2 | The design of the so-called octobot is taken from the octopus. It is a fully soft, autonomous robot that is controlled via the embedded microfluidic soft controller and powered by monopropellant decomposition. Scale bar, 10 mm, Source: Wehner et al. (2016). The figure is reproduced with the permission of the copyright holder [Springer Nature].

Recently developed soft-robots prototypes have a wide variety of functions and application spanning from robotic gripper for minimally invasive surgery, to soft-robot underwater exploration and even soft manipulators for assistive human care (Calisti et al., 2015; Krieg et al., 2015; Shen, 2016). The last application takes advantages of the high compliance and dexterity of soft manipulators, which ensures safe human-robot interaction (Ansari et al., 2017). Given the complexity of soft-robotics, results have been possible only through the cross-integration of diverse expertise coming from neuroscientists, engineers, material scientists, and computational biologists, thus setting the bases for a "melting pot" between biology and soft-robotics engineering. Altogether, these investigations have highlighted the potential for soft machines as well as the drawbacks of the available technologies and the limitation in the current knowledge of cephalopods intelligence and motor control strategies. In particular, it has clearly emerged the need for new materials that embed together softness and robustness and of new control strategies for these deformable materials.

Most importantly, they provided a proof of concept of the existence and modes of operation of an "embodied intelligence." This term was originally conceived to describe autonomous robots to explain how their efficiency derives from the interaction between the controller (the brain or actuation system), the mechanical system (the body artifact) and the testing environment (Pfeifer et al., 2007). Whilst deriving from a robotic field, this terminology has been lately adopted by biologists to underpin the existence of a self-contained intelligence within each animal body. In this case, embodied intelligence stands for the co-evolution of animal body/nervous system and environment as a result of natural selection (Hochner, 2013).

FIGURE 3 | The Lighthouse: prototype of an artificial bio-inspired arm developed during the EU OCTOPUS Integrating project, 231608 (photograph by Massimo Brega).

Octopus and more generally (shell-less) coleoid cephalopods are living examples of this concept.

Camouflage Technology

Cephalopods have been a source of inspiration in the robotic field also for another important behavioral capability, their amazing capacity of modifying their appearance and their body pattern in response to a variety of different stimuli (Osorio, 2014; How et al., 2017). This aspect has drawn the attention of engineers and material scientists aiming at developing biomimetic artificial skin able not only to match its background but also to fast adapt to a changing environment, all this, without losing flexibility. Few interesting prototypes based on electroluminescent material have been developed taking inspiration from cephalopods skin. For example, researchers from Cornell University have recently produced a synthetic skin able to emit light while undergoing large stretching and surface area modifications. In the latest version, this stretchable surface has been provided with the ability to change "on demand" both color and texture thus transforming from 2D to 3D shape just like it happens in cephalopod skin following environmental stimuli or communication needs (Larson et al., 2016; Pikul et al., 2017).

Although these prototypes are remarkable in their ability to change their appearance, we believe we are still far from reaching an active cephalopod-like camouflage system. This should not surprise as camouflage is a feature of immense complexity and, despite many decades of investigation, we are just at the start of our comprehension of the biology behind cephalopod visual-spatial perception and accustomization to the surrounding environment. To make things even simpler, recent investigations started disclosing the existence of independent mechanisms of control of the skin pigmentation based on solely "skin perception" of environmental illumination (Kingston et al., 2015; Ramirez and Oakley, 2015). These studies showed that the primary elements of pigmentation in cephalopods, the chromatophore organs, can be light-activated in a manner completely independent of the central nervous system. Interestingly enough this process seems to be based on a common and conserved molecular mechanism of light photo-transduction between the eye and the skin. But, despite the mechanisms underlying the formation of pattern and texture the unique and exquisite endpoint of the chromatophore marvelous machinery is the generation of ordered images and eventually the arousal of "beauty." We can definitely state that these animals carry a high aesthetic value, and this has been caught early by our ancient predecessors.

ART

Historical Representations of Cephalopods

Cephalopods are present in the art of many a coastal culture around the world. During New-Place Phase II (LM IB) to Post-Palace Phase II (LM IIIA) of late Minoan civilization (c. 1550–1100 BCE), representations of Cephalopods in pots, coins, thumbs, pendants, etc., are ubiquitous (**Figure 4**, octopus pendant and a large octopus pot). Such Minoan representation of cephalopods is roughly categorized into two different styles separated by the era, Marine Design style of around 1500 BC and Palace Style of between 1450 BCE and Mycenaean invasion (Betancourt, 1985). At first glance, Marine style octopus vessels seem formally and technically primitive with simplified and abstracted features such as googly eyes over an exaggerated mantle, very long and extended arms. These abstracted features create somewhat of a comical impression and familiarity of infantile art. However, with careful examination, one would realize that these images are not a product of nonchalant and causal relationship to the animals rather they are a product of careful and deliberate observation of both form and behavior of the animals. For instance, An octopus represented on a large vessel depicts regenerated arms, a biological attribute used as a symbol of regeneration and rebirth. In another example, a hectocotylus, a specialized arm to transfer spermatophores to the reproductive tract of a female's mantle cavity has been described in a small gold pendant top as a symbol of fertility. These careful observations of morphology and behavior, seem to have supported the symbolic use of octopus in Abstract Design style where octopus design was not a mere representation of an animal but a codified cultural signifier.

While there are many different manifestations of cephalopod motifs, there is one distinctive feature that is shared among many, the gaze. These octopus designs look straight at the viewer with two large eyes simultaneously recognizing both viewers and its existence. This exchange builds psychological dimension that includes both a sense of mutual recognition and self-awareness. The Ancient Greek artisans have successfully represented this rather abstract and enigmatic character of cephalopods and our metaphysical relationship with them.

In addition to the historical representation of cephalopods through arts and crafts across the cultures from Roman mosaic to Katsushika Hokusai's woodcut print, there are many notable accomplishments in scientific illustrations that sparked people's imagination. Cephalopoda is the volume 18 of Wissenschaftliche Ergebnisse der Deutschen Tiefsee-Expedition auf dem Dampfer "Valdivia" 1898–1899 written by Carl Chun. The book contains over 100 color and black and white plates visually describing many deep-sea cephalopods including the first image of the vampire squid, Vampyroteuthis infernalis. The illustrations provided in the book are not only scientifically accurate descriptions of taxonomically essential details but also show aesthetic and formal considerations. In contrast to Chun, the work of Ernst Haeckel is biologically less accurate but highly designed with an acute sense of formal concerns such as form, line, and color. Haeckel with much fascination for symmetry organized composition and design based on the overall page layout with overtly elongated tentacles and perfectly composed arms of octopuses with symmetry and counter symmetry. In addition to the 2-dimensional biological illustrations, glass models by Leopold and Rudolf Blaschka are products of extraordinary craftsmanship and care. Using transparent properties of the glass, Blaschkas were able to represent more than 700 different species including squid and octopus, which are collected at institutions around the world. Although these late nineteenth to early twentieth century scientific artifacts do not share the level of conceptual and philosophical foundation of their contemporary artists, the level of craftsmanship and aesthetic considerations produced a vital foundation in cephalopod visualization culture.

Japanese had a different take on octopuses representation. In 1814, Hokusai, a Japanese woodcut printer produced one of the most famous Shunga (erotic prints) titled The Dream of the Fisherman's Wife. Unlike his more mainstream series such as Thirty-six Views of Mount Fuji, this is an illegal and underground operation depicting an Ama diver sexually entangled with two octopuses. This rather odd sexual fantasy has made such a profound impact on the Japanese psyche that such notable contemporary Japanese artists as Makoto Aida, Masami Teraoka, Namada, Yuji Moriguchi have made homages to it. In addition to these fine art homages, the print has also affected Japanese pornographic anime. Toshio Maeda was the first to introduce in 1987 this type of representation to avoid the strict censorship law in Japan which banned all representation of genitalia. Maeda used tentacles to replace both male genitalia and bondage expression. Urotsukidoji, which was produced initially as a two-volume

FIGURE 4 | A gold octopus pendant is showing a hectocotylus **(A)**, and a large octopus ceramic pot is describing two regenerated arms **(B)**. Collections of Heraklion Archaeological Museum, Crete Greece (photographs by R. Nakajima).

comic book, has expanded into 15 video series, two featured movies, video games, and special feature books. Since then the release of the original series, "Shokushu (tentacle)" has become one of the dominant genres in Japanese pornographic and non-pornographic anime alike.

Cephalopods in Contemporary Art

Cephalopods remain popular motifs in contemporary art and continue to draw public attention. Takashi Murakami's solo exhibition entitled *The Octopus Eats Its Own Leg* at the Museum of Contemporary Art Chicago, which featured over 50 sculptures and large-scale paintings, has broken attendance record in the MCA's 50-year history. More than 193,000 people attending the exhibition came to see Murakami's "Superflat" Japanese pop anime, subculture combined with traditional imageries, including many Octopus-inspired characters. The exhibition at Qatar Museum "What About the Art?" curated by Chinese artist Cai Guo-Qiang featured work by Huang Yong Ping's six-ton giant "sea monster" hanging from the museum ceiling wrapping its arms on the large 20-foot Column overpowering the audiences walking underneath. A Japanese painter, Yutaka Mukoyama who paints various marine animals, has been creating incredibly detailed photorealistic oil paintings of squids, which are stunning and mesmerizing. All of these are just fragments of cephalopod inspired art that is produced in the recent years that can be found in the ever expansive artistic realm from artworks in high profile art museums and galleries to the street of San Francisco bringing and nurturing people's curiosity and interests toward cephalopods (**Figure 5**).

In addition to plastic art such as paintings, drawings sculptures and other traditional mediums, cephalopod motifs have been used in many diverse modes of representation, from an underwater site-specific installation of an 80-foot Kraken sank to the British Virgin Island Seafloor (BVI ART Reef, http://www.divethebviartreef.com) to a rideable large-scale kinetic squid sculpture at Les Machines de l'Île de Nantes (http://www.lesmachines-nantes.fr/en/). While many cephalopods themed artworks have been produced since the time of the ancient Greeks, there are three notable artworks that may represent possible future direction in Cephalopod art as synergy of art and science, Insane in the Chromatophores by Backyard Brains, Chromatophores simulation system (**Figure 6**) by Todd Anderson and Octopus Brainstorming: Empathy by Victoria Vesna and Mark Cohen. Insane in the Chromatophores was produced in collaboration with Dr. Roger Hanlon's laboratory at Woods Hole Marine Biological Laboratory. This project connects iPhone music to living tissue of a Longfin Inshore Squid (*Doryteuthis pealeii*) by electrodes. As a result, electric signals of the music contract radial muscle fiber surrounding chromatophores allowing a rhythmical change in the visual appearance of the squid synchronized with the music. In Anderson's Chromatophores simulation system, is an interactive digital simulator of chromatophore movement and change in colors. Anderson's simulator detects the movement of spectators and moves color dots based on an algorithm of cephalopod chromatophores. Finally, Octopus Brainstorming: Empathy is a performance/installation that allows viewers to visually detect performer's thoughts through octopus-like contraptions worn by them. Although these three projects are technically and conceptually different, all of these projects focus on multiple characteristics of cephalopods and represent an innovative fusion of cell biology, neuroscience, computer programing, video, sculpture, performance, and more, thereby expanding the share notion on artistic and creative practice and scientific investigations alike.

The synergy between science and art has been a part of artistic practice, perhaps, since Leonardo Da Vinci's extensive notes on art, biology, and engineering. Other examples may include the use of camera obscura by Johannes Vermeer, hyper-detailed animal illustration paintings of Jakuchu Ito, highly aestheticized biological illustrations of Ernst Haeckel. In 1960's the rise of American Avant-garde movement that explored many avenues of non-traditional art making has drastically opened the door for more conscious and deliberate use of scientific practices in art. Artists such as Harold Cohen (http://www.aaronshome.com/) who pioneered in integrating AI "AARON" system to study the process of painting, Helen Mayer and Newton Harrison (http://theharrisonstudio.net/) who

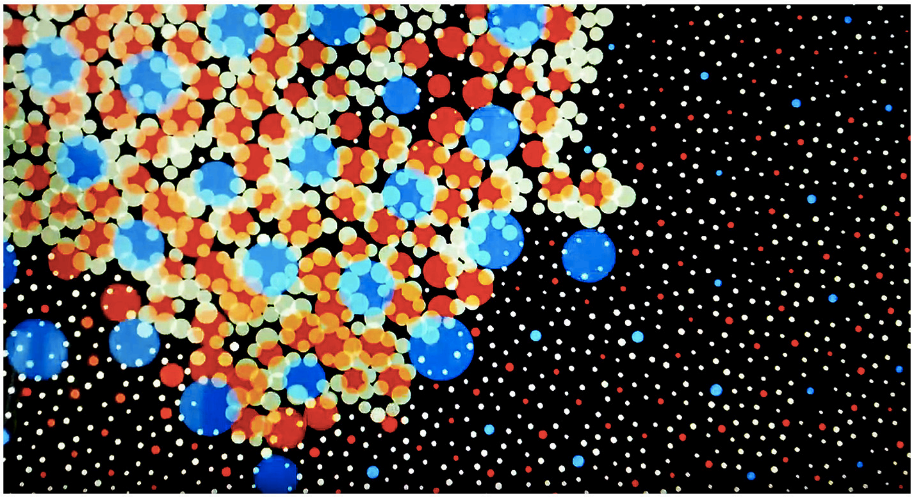


FIGURE 5 | This is a still image of an interactive video installation "Chromatophore simulator" by Todd Anderson. The image is provided at courtesy of the artist.

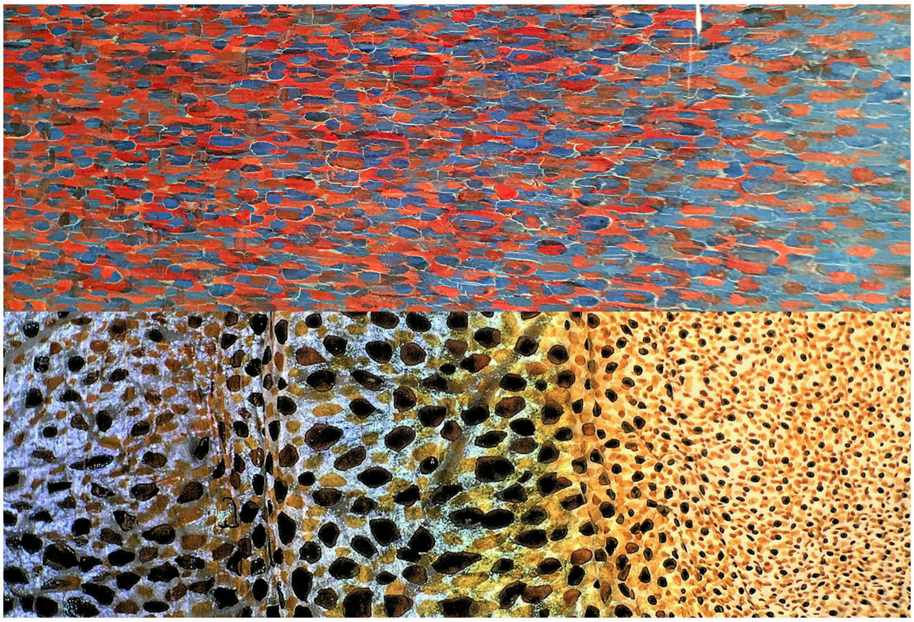


FIGURE 6 | Image comparison of detail close up of painted frame of Georges Seurat's painting View of Le Crotoy from Upstream (1889), oil on canvas, collection of Detroit Institute of Arts **(Top)** and octopus skin **(Bottom)** showing the dense distribution of chromatophores and iridophores. Note: (1) A momentary flash of an anesthetized octopus multi-color skin that was taken with a next-generation KEYENCE digital VHX900F microscope and a 20 Å∼ greater depth-of-field VH-Z00R lens under a multi-angle observation. Photographed by S. Shigeno. (2) The detail of Seurat's painting frame was photographed by R. Nakajima at Detroit Institute of Arts in 2017.

have combined environmental science, agriculture, activism and art, Nam June Paik (https://americanart.si.edu/artist/nam-june-paik-3670) who explored video texture, have all helped firm down the foundation for the interdisciplinary approaches to art making that evolved into current New Media Arts including Biology Inspired Art practices. These multiplicities not only gather independent fields together but also generate interests and discovery in an unexpected category of audiences and help expand the possibility of each area. As more traditional creative modes such as paintings, sculptures, ceramics, prints, photographs will continue to be active, these contemporary interdisciplinary approach truly removes many boundaries not only between arts and sciences, but also help create deeper mutual understanding between the two areas that provides a tangible platform for intellectual exchange expanding imagination, creativity, and vision (**Figure 7**).

FIGURE 7 | A cephalopod themed art exhibition *Cephalopod Interface* was organized at the Okinawa Prefectural Art Museum in conjunction with an interdisciplinary cephalopod symposium held at the same location (photographs by R. Nakajima).

Cephalopods in Popular Culture and Media

High art is not the only place for representations of cephalopods. They can be found in various corners of popular culture including movies, animes, illustrations, toys, video game characters, and more. In 1981, Nintendo released Octopus on a line up of their Game Watch that sold estimate of 250,000 to 1 million copies worldwide. In 2015, Nintendo released Splatoon, which sold 4.87 million copies in just 2 years, and is till now the top-selling video game designed for home console. Cephalopod figures and toys are sold at most aquariums and seaside resort gift shops to be collected (**Figure 8**). In movies, the five versions of 20,000 Leagues Under the Sea (1907, 1916, 1954, 1985, and 1997), might be one of the most extensive series with a giant cephalopod, which has been adapted from a novel by Jules Verne. More recent films such as Finding Dory (2016), Pirates of the Caribbean, At World's End (2007), SHARKTOPUS (2010), Mega Shark vs. Giant Octopus (2009), and Leviathan (2016) also feature cephalopod as a dominant element in their narrative and many others allude to it.

The original Star Wars (1977) invested 6 min sequence where Luke Skywalker, Han Solo, Princess Leia, and Chewbacca are trapped inside a garbage chamber battling with Dianoga, an octopus-like monster. The sequence starts with a statement by Han, "I am beginning to like her (Princess Leia)" to Luke who is his rival over the princess and the sequence ends with the first embrace between Han and Leia. George Lucas cleverly sets up this intergalactic romance by locking up two knights and a prince in a dungeon with a giant octopus. While Luke busies himself with his drone friend C3PO, Han continues to fondle Leia as trash compressor push them closer to each other. This, one of the most memorable and cinematic love scenes of the first Star Wars trilogy, references the legacy of the myth of Kraken as an embodiment of sublime forces of nature but also carefully fuse the story of Saint George and the Dragon with it in the narrative structure.

FIGURE 8 | A part of cephalopod figure collection of Dr. Yasunori Sakurai at his home office showing incredible variations in cephalopod character designs (photographs by R. Nakajima).

One other contemporary example of the legacy between myth and public interest can be found in the following happening that in 2010 took the appearance of a modern fairytale. During the FIFA world cup Germany 2010, a common octopus, *Octopus vulgaris* exhibited at Sea Life Center in Oberhausen, Germany was stealing the show as an animal oracle. During the tournament, Paul the octopus predicted the winning results of 10 out of 12 matches. Scientifically, an octopus selecting or not selecting one over the other is an interesting issue in terms of pattern recognition and color discrimination. However, here the interest is more metaphysical. Despite all religious differences in the world, people were mesmerized by the fact that this little sea creature was exercising its' "supernatural power" that is beyond natural human capability. The Octopus with the name of one of the most important patrons Saint Paul is prophesying the outcome of an important sports event that impacts the social, economic, cultural, and emotional well-being for millions of people. Paul, with much higher success rate than the best bookies in the world, was transformed from an invertebrate to a prophet. This happening together with the international impact and debate arising from it, has been wisely narrated in the much enjoyable documentary "The Life and Times of Paul the Psychic Octopus" (2012) by Philippe (2012).

The concept that the uncertainty of nature can only be accessible and comprehensible by counter parting it with its own natural force is very similar to that of ancient Greek or Chinese oracle and other Paganistic and shamanistic practices. The only difference here is that without any shared religious and social foundation, the media frenzy has recontextualized a marine invertebrate into an autonomous being with a superior consciousness that is directly communicating its own thought with its own logic. By stepping on the podium of predictor animals, the octopus was the prophet and not an instrument of ritual that required an interpreter and many have accepted it even with slight hesitation. Through news media, music, dance, movies, photographs, illustrations, Internet, articles, and all the other traditional and modern information dissemination methods, the octopus spoke and we listened. This seeming absence of mediator between the octopus and the people made an ordinary hunting behavior into a modern Totemism creating a temporary yet significant universality.

Cephalopod science has been active in the mass media increasing public interest in cephalopods. In 2012, a group of scientists led by NHK (Japan Broadcasting Corporations), and Discovery Channel successfully filmed a giant squid, *Architeuthis dux* in its natural environment. Combining social network systems and the major mass media sources, the news reached millions of viewers worldwide offering a sense of natural wonder, a joy of discovery and entertainment. The special exhibition "Deep-Sea" organized in conjunction with the release of the footage at The National Museum of Nature and Science Tokyo became the most visited exhibition in Japan in 2013 well exceeding Raphael, El Greco and J.W Turner bringing over 600,000 visitors in 86 days. Not as catchy as the news of giant squid, media coverage of the first complete sequencing of octopus genome published in 2015 was unique. The story was covered in 136 news articles following the initial press release by Okinawa Institute of Science and Technology Graduate University 64 out of 136 articles alluded to the idea of intelligence and/or octopus being an alien promoting the idea of octopus as an intelligent being comparable to human. By bringing Kraken to reality or by promoting the existence of extraterrestrial being and its potential intelligence, cephalopods science, and the media seems to be able to draw and trigger public attention. This unique characteristic of cephalopod helps build a useful information dissemination platform that brings public closer to nature, science, and culture promoting interdisciplinary and multivalent understanding.

CURRENT CHALLENGES

With ever-expanding horizons of cephalopod research brought by increasing numbers of direct and indirect associations to cephalopods and their characteristics, the relational dynamics between different fields is facing new challenges and opportunities. The current academic atmosphere is still carrying many aspects of later twentieth century necessity to subdivide academia into the smallest possible units without many lateral interactions with other disciplines (see Fiorito et al., 2014 for example). Due to this structural and philosophical division, interdisciplinary is still in its infancy. It is not so easy to find the time, place and occasion to have in-depth discussions with others. Moreover, the difference of financial standing between science and humanities is so large that it might represent a difficulty for researchers in art and scientists in forming an equal partnership. The development of an interdisciplinary mindset, in particular within the scientific community, might overcome some of these practical limitations. This might account for, as an example, support and easier access to cephalopod aquaculture facilities and marine stations to non-scientific investigators where they could meet live cephalopods and encounter investigators from various disciplines.

This aspect is particularly important if we consider that there are only very few laboratories in the world that regularly house cephalopod species that can extensively serve both science and art community. Cephalopod aquaculture is extremely time-consuming and costly, and only a few species are currently cultured on a small-scale due to several bottlenecks in their culturing system (Iglesias et al., 2014). Limiting factors are represented by, to mention a few, their low reproduction performance and fecundity in captivity (for some species), the broodstock management, the need for optimization of hatching efficiency and the absence of appropriate diets for each life stage and the consequent occurrence of cannibalism or massive death, etc.... These traits are unfortunately accompanied by the elevated cost of maintenance of the reproductive and spawning tanks in term of infrastructure, manpower, space, and even running costs. Taken together the biological and economic aspects represent a relevant obstacle for the expansion of cephalopod aquaculture over the world especially when the outcome does not directly represent a profitable end-product as it is the case in cephalopod open-sea aquaculture.

To maximize the potential of the interdisciplinary, it is imperative to create a stronger lateral relationship between the fields. By combining art and science, it will present a certain solution to raise awareness about cephalopod science. The urge for scientists to communicate and to engage with the public about their research, and the relevance and social implication of their findings both increase. Also, to explore further understanding and stimulate inspiration, it will become increasingly important to bring art and science together. Considering the current trends in cephalopod research, it may become a useful model for other fields to achieve its full creative, emotional and intellectual potential.

CONCLUSION AND PERSPECTIVE

Cephalopods, especially the octopus, have fascinated humanity since the ancient Minoan, Greek and old Chinese high cultures. With their large eyes and seemingly smart behavior, capable of learning and planning into the future, cephalopods trigger a strong fascination in scientists and lay people alike. Their complex brain and behavioral repertoire have evolved independently of all vertebrates including humans and show how evolution can independently lead to comparable central nervous systems. In this review, we found that such unique cephalopod features have provided a strong influence on scientists and engineers. Cephalopods are animals endowed with a well-developed brain that controls highly flexible appendages. This unique control strategy has been used by bio-roboticists as templates for the design of a new type of adaptable machines able to dynamically interact with a changing environment and "learn" from it just like animals do in the natural world.

The similarities and differences between cephalopods and humans are a rich source of wonder, fascination, and inspiration. Nowhere else has this sentiment been better captures than in Flusser and Bec's (1987) book "Vampyroteuthis infernalis," that is a part scientific treaty, part spoof, part philosophical discourse, and part fable. Vampyroteuthis infernalis, the vampire squid, lives in the ocean's abyss, a dark, cold space far away from the habitats that humans populate. Flusser and Bec explore the real and philosophical ocean that separates Vampirotheutis from humans and eventually attempt to explore the metaphysical foundation that encompasses both the vampire squid and humans. They write, "The abyss that separates us [from the vampire squid] is incomparably smaller than that which separates us from extraterrestrial life as imagined by science fiction and astrobiology," thus proclaiming the cephalopod as the ultimate biological, technological, philosophical, and spiritual challenge that planet Earth has to offer. The quest for such an encompassing metaphysics stands in stark contrast to the fact that the main role cephalopods had in human history, is an involuntary contribution as tasty seafood.

A first step toward the acknowledgment of cephalopods was set by the European Union Directive 2010/63/EU, coming into force on 1 January 2013 across all Europe and regulating the research of any of the about 700 extant species of "live cephalopods." The Directive establishes measures for the "protection of animals used for scientific or educational purposes." Cephalopods are the sole invertebrate taxon included in this Directive following Canada in 1991, New Zealand in 1999, Australia in 2004, Switzerland 2008, and Norway in 2009. This marked a paradigm shift for invertebrates in EU, by covering the use of an entire class of Mollusks, namely "live cephalopods" (i.e., hatched juveniles and adults) in the legislation covering experimental procedures likely to cause pain, suffering, distress, or lasting harm. This means that, under the Directive and transposed national laws, cephalopods have the same legal status as vertebrates, in relation to their experimental use in research and testing.

The EU funded COST Action FA 1301 "A network for improvement of cephalopod welfare husbandry in research, aquaculture and fisheries (CephsInAction)" (2013–2017) was the first international network initiative to advance the understanding, methods, and dissemination of cephalopod research (see: http://www.cephsinaction.org/). CephsInAction was supported by scientists from 19 European countries, Israel, Australia, and the US, and could well be the starting point for a future and more intense interdisciplinary debate about cephalopods and how they could propel our understanding of evolution, natural and artificial intelligence, emotions, consciousness, and future technological innovations.

What is the goal of cephalopod research in the twenty-first century? As the squid giant axon provided a generalized model that pioneered modern neuronal studies in any animals including humans, we may expect to reach a complete molecular understanding of cephalopod cells, organs, and behaviors via post-genome approaches. This would, in turn, allow exploring a universal molecular basis of emotion, pain, sleep, and even consciousness. We may find a healthy stock of deep-sea cephalopod that would enlighten us on the evolutionary development of the extreme environment adaptability strategies. We would be able to create a viable aqua culturing method, and we may even create a deep neural network-driving soft robot that communicates to us by changing its body pattern. Are these far-fetched ideas that only belong to science fiction movies? The answer is No. These are the ideas that many scientists are currently working on, however independent and fragmented they might appear. Through this investigation, we found in many instances the possibilities and high potentials to interweave many existing thoughts, disciplines, practices and fields that already possess shared goals. In conclusion, the complexity and varieties provided by cephalopods and their biological properties, cultural symbolism, and history, appear to function as an ideal boundary object that offers great potential to accelerate development of truly innovative interdisciplinary platforms for science, art, and engineering alike.

AUTHOR CONTRIBUTIONS

Each author worked on their area of speciality. RN worked on Art and media. FD worked on the history. SS and LZ worked collaboratively on modern cephalopod science section. RN and MS worked on the introduction and conclusion sections. All of us worked on editing the entire text together.

FUNDING INFORMATION

SS has been supported by the Stazione Zoologica Anton Dohrn Fellowship (BEOM Department, Stazione Zoologica 548 Anton Dohrn). The initial panel discussion was supported by COST Action FA 1301 and CephsInAction.

REFERENCES

Abbott, N. J., Williamson, R., and Maddock, L. (1995). *Cephalopod Neurobiology: Neuroscience Studies in Squid, Octopus and Cuttlefish.* Oxford: Oxford University Press.

Albertin, C. B., Bonnaud, L., Brown, C. T., Crookes-Goodson, W. J., da Fonseca, R. R., Di Cristo, C., et al. (2012). Cephalopod genomics: a plan of strategies and organization. *Stand. Genomic Sci.* 7, 175–188. doi: 10.4056/sigs.3136559

Albertin, C. B., Simakov, O., Mitros, T., Wang, Y. Z., Pungor, J. R., Edsinger-Gonzales, E., et al. (2015). The octopus genome and the evolution of cephalopod neural and morphological novelties. *Nature* 524, 220–224. doi: 10.1038/nature14668

Allcock, A. L., Lindgren, A., and Strugnell, J. M. (2015). The contribution of molecular data to our understanding of cephalopod evolution and systematics: a review. *J. Nat. Hist.* 49, 1373–1421. doi: 10.1080/00222933.2013.825342

Ansari, Y., Manti, M., Falotico, E., Mollard, Y., Cianchetti, M., and Laschi, C. (2017). Towards the development of a soft manipulator as an assistive robot for personal care of elderly people. *Int. J. Adv. Robot. Syst.* 14:1729881416687132. doi: 10.1177/1729881416687132

Appel, T. A. (1987). *The Cuvier-Geoffrey Debate. French Biology in the Decades before Darwin*. Oxford: Oxford University Press.

Armstrong, C. M., and Bezanilla, F. (1973). Currents related to movement of the gating particles of the sodium channels. *Nature* 242, 459–461. doi: 10.1038/242459a0

Barratt, M., Johnson, M. P., and Allcock, A. L. (2007). Fecundity and reproductive strategies in deep-sea incirrate octopuses (Cephalopoda: Octopoda). *Mar. Biol.* 150, 387–398. doi: 10.1007/s00227-006-0365-6

Betancourt, P. P. (1985). *The History of Minoan pottery*. New York, NY: Princeton University Press.

Borrelli, L., Gherardi, F., and Fiorito, G. (2006). *A Catalogue of Body Patterning in Cephalopoda*. Firenze: Firenze University Press.

Boycott, B. B. (1954). Learning in *Octopus vulgaris* and other cephalopods. *Pubbl. della Staz. Zool. Napoli.* 25, 67–93.

Boycott, B. B., and Young, J. Z. (1955). A memory system in *Octopus vulgaris* Lamarck. *Proc. R. Soc. Lond. B.* 143, 449–480. doi: 10.1098/rspb.1955.0024

Boyle, P., and Rodhouse, P. (2008). *Cephalopods: Ecology and Fisheries*. New York, NY: John Wiley and Sons.

Buckner, R. L. (2007). "Memory systems: an incentive, not an endpoint," in *Science of Memory: Concepts*, eds H. L. Roediger, Y. D. Dudai, and S. M. Fitzpatrick (Oxford: Oxford University Press), 359–364.

Budelmann, B. U. (1995). Cephalopod sense organs, nerves and the brain: adaptations for high performance and life style. *Mar. Freshw. Behav. Physiol.* 25, 13–33. doi: 10.1080/10236249409378905

Bullock, T. H. (1948). Properties of a single synapse in the stellate ganglion of squid. *J. Neurophysiol.* 11, 343–364. doi: 10.1152/jn.1948.11.4.343

Calisti, M., Corucci, F., Arienti, A., and Laschi, C. (2015). Dynamics of underwater legged locomotion: modeling and experiments on an octopus-inspired robot. *Bioinspir Biomim.* 10:046012. doi: 10.1088/1748-3190/10/4/046012

Changeux, J. P. (2006). *The Neuronal Man*. Oxford: Oxford University Press.

Chung, W.-S., and Marshall, N. J. (2017). Complex visual adaptations in squid for specific tasks in different environments. *Front. Physiol.* 8:105. doi: 10.3389/fphys.2017.00105

Dai, L., Zheng, X., Kong, L., and Li, Q. (2012). DNA barcoding analysis of Coleoidea (Mollusca: Cephalopoda) from Chinese waters. *Mol. Ecol. Resour.* 12, 437–447. doi: 10.1111/j.1755-0998.2012.03118.x

Darmaillacq, A. S., Dickel, L., and Mather, J. A. (2012). *Cephalopod Cognition*. Cambridge: Cambridge University Press.

De Sio, F. (2011). Leviathan and the soft animal: medical humanism and the invertebrate models for higher nervous functions, 1950s—90s. *Med. Hist.* 55, 369–374. doi: 10.1017/S0025727300005421

Doubleday, Z. A., Prowse, T. A., Arkhipkin, A., Pierce, G. J., Semmens, J., Steer, M., et al. (2016). Global proliferation of cephalopods. *Cur. Biol.* 26, R406–R407. doi: 10.1016/j.cub.2016.04.002

Doya, C., Chatzievangelou, D., Bahamon, N., Purser, A., Leo, F. C. D., Juniper, S. K., et al. (2017). Seasonal monitoring of deep-sea megabenthos in Barkley Canyon cold seep by internet operated vehicle (IOV). *PLoS ONE* 12:e0176917doi: 10.1371/journal.pone.0176917

Dröscher, A. (2016). Pioneering studies on cephalopod's eye and vision at the Stazione Zoologica Anton Dohrn (1883-1977). *Front. Physiol.* 7:618. doi: 10.3389/fphys.2016.00618

Edelman, D. B., Baars, B. J., and Seth, A. K. (2005). Identifying hallmarks of consciousness in non-mammalian species. *Consci. Cognit.* 14, 169–187. doi: 10.1016/j.concog.2004.09.001

Edelman, G. M. (1987). *Neural Darwinism: The Theory of Neuronal Group Selection*. New York, NY: Basic books.

FAO Fisheries and Aquaculture Department (2016). *The State of World Fisheries and Aquaculture 2010*. Rome: Food and Agriculture Organization of the United Nations.

Fiorito, G., Affuso, A., Anderson, D. B., Basil, J., Bonnaud, L., Botta, G., et al. (2014). Cephalopods in neuroscience: regulations, research and the 3Rs. *Invert Neurosci.* 14, 13–36. doi: 10.1007/s10158-013-0165-x

Flourens, P. (1865). *Psychologie Comparée*. Paris: Garnier frères.

Flusser, V., and Bec, L. (1987). *Vampyroteuthis Infernalis. Eine Abhandlung samt Befund des Institut Scientifique de Recherche Paranaturaliste.* Göttingen: Immatrix Publications.

Gagnon, Y. L., Osorio, D. C., Wardill, T. J., Marshall, N. J., Chung, W. S., and Temple, S. E. (2016). Can chromatic aberration enable color vision in natural environments? *Proc. Natl. Acad. Sci. U.S.A.* 113, 6908–6909. doi: 10.1073/pnas.1612239113

Garrett, S., and Rosenthal, J. J. C. (2012). RNA editing underlies temperature adaptation in K+ channels from polar octopuses. *Science* 17, 848–851. doi: 10.1126/science.1212795

Grimpe, G. (1928). Pflege, Behandlung und Zucht der Cephalopoden fur zoologische und physiologische Zweeke. Abderhalden Handbuch der biologischen Arbeitsmethoden, Abt. IX. *Teil* 5, 331–402

Guglielmino, E., Godage, I., Zullo, L., and Caldwell, D. G. (2013). "A pragmatic bioinspired approach to the design of octopus-inspired arms," in *Proceedings of the IEEE/RSJ International Conference on Intelligent Robots and Systems (IROS 2013)* (Tokyo), 4577–4582.

Guglielmino, E., Zullo, L., Cianchetti, M., Follador, M., Branson, D., and Caldwell, D. G. (2012). "The application of embodiment theory to the design and control of an octopus-like robotic arm," in *Proceedings IEEE International Conference on Robotics and Automation (ICRA)* (Saint Paul, MN), 5277–5282.

Hanke, F. D., and Osorio, D. C. (2018). Vision in Cephalopods. *Front. Physiol.* 9:18. doi: 10.3389/978-2-88945-430-3

Hanlon, R. T., and Messenger, J. B. (2018). *Cephalopod Behaviour. 2nd Edn.* Cambridge: Cambridge University Press.

Hochner, B. (2013). How nervous systems evolve in relation to their embodiment: what we can learn from octopuses and other molluscs. *Brain Behav. Evol.* 82, 19–30. doi: 10.1159/000353419

Hochner, B., Shomrat, T., and Fiorito, G. (2006). The octopus: a model for a comparative analysis of the evolution of learning and memory mechanisms. *Biol. Bull.* 210, 308–317. doi: 10.2307/4134567

Hodgkin, A. L., and Huxley, A. F. (1939). Action potentials recorded from inside a nerve fibre. *Nature* 144, 710–711. doi: 10.1038/144710a0

Hodgkin, A. O. M. (1975). Address of the president sir Alan Hodgkin, OM at the Anniversary Meeting, 30 November 1974. *Proc. R. Soc. Lond. B* 188, 103–119. doi: 10.1098/rspb.1975.0006

Hoving, H. J. T., Laptikhovsky, V. V., and Robison, B. H. (2015). Vampire squid reproductive strategy is unique among coleoid cephalopods. *Curr. Biol. 25*, R322–R323. doi: 10.1016/j.cub.2015.02.018

Hoving, H. J. T., Perez, J. A., Bolstad, K. S., Braid, H. E., Evans, A. B., Fuchs, D., et al. (2014). The study of deep-Sea cephalopods. *Adv. Mar. Biol.* 67, 235–359. doi: 10.1016/B978-0-12-800287-2.00003-2

How, M. J., Norman, M. D., Finn, J., Chung, W. S., and Marshall, N. J. (2017). Dynamic skin patterns in cephalopods. *Front. Physiol.* 8:393. doi: 10.3389/fphys.2017.00393

Hugo, V. (1866). *Les Travailleurs de la Mer*. Paris: Librairie internationale.

Iglesias, J., Fuentes, L., and Villanueva, R. (2014). *Cephalopod Culture*. New York, NY: Springer.

Iglesias, J., Otero, J. J., Moxica, C., Fuentes, L., and Sánchez, F. J. (2004). The completed life cycle of the octopus (*Octopus vulgaris*, Cuvier) under culture conditions: paralarval rearing using Artemia and zoeae, and first data on juvenile growth up to 8 months of age. *Aquac. Int.* 12, 481–487. doi: 10.1023/B:AQUI.0000042142.88449.bc

Ikeda, Y. (2009). A perspective on the study of cognition and sociality of cephalopod mollusks, a group of intelligent marine invertebrates. *Jpn. Psychol. Res.* 51, 146–153. doi: 10.1111/j.1468-5884.2009.00401.x

Katz, B., and Miledi, R. (1970). Further study of the role of calcium in synaptic transmission. *J. Physiol.* 207, 789–801. doi: 10.1113/jphysiol.1970.sp009095

Kingston, A. C., Kuzirian, A. M., Hanlon, R. T., and Cronin, T. W. (2015). Visual phototransduction components in cephalopod chromatophores suggest dermal photoreception. *J. Exp. Biol.* 218, 1596–1602. doi: 10.1242/jeb.117945

Krebs, H. A. (1975). The August Krogh principle: "For many problems there is an animal on which it can be most conveniently studied". *J. Exp. Zool.* 194, 221–226. doi: 10.1002/jez.1401940115

Krieg, M., Sledge, I., and Mohseni, K. (2015). Design considerations for an underwater soft-robot inspired from marine invertebrates. *Bioinspir. Biomim.* 10:065004. doi: 10.1088/1748-3190/10/6/065004

Krogh, A. (1929). *The progress of physiology. Science* 70, 200–204.

Kubodera, T., and Mori, K. (2005). First-ever observations of a live giant squid in the wild. *Proc. R. Soc. B* 272, 2583–2586. doi: 10.1098/rspb.2005.3158

Larson, C., Peele, B., Li, S., Robinson, S., Totaro, M., Beccai, L., et al. (2016). Highly stretchable electroluminescent skin for optical signaling and tactile sensing. *Science* 351, 1071–1074. doi: 10.1126/science.aac5082

Laschi, C., Mazzolai, B., and Cianchetti, M. (2016). Soft robotics: technologies and systems pushing the boundaries of robot abilities. *Sci. Robotics* 1:3690. doi: 10.1126/scirobotics.aah3690

Lee, H. (1875). *The Octopus: or, the "Devil-Fish" of Fiction and of Fact*. London: Chapman and Hall.

Lee, P. N., Callaerts, P., De Couet, H. G., and Martindale, M. Q. (2003). Cephalopod *Hox* genes and the origin of morphological novelties. *Nature* 424, 1061–1065. doi: 10.1038/nature01872

Lindgren, A. R., and Anderson, F. E. (2017). Assessing the utility of transcriptome data for inferring phylogenetic relationships among coleoid cephalopods. *Mol. Phylogenet. Evol.* 118, 330–342. doi: 10.1016/j.ympev.2017.10.004

Liscovitch-Brauer, N., Alon, S., Porath, H. T., Elstein, B., Unger, R., Ziv, T., et al. (2017). Trade-off between transcriptome plasticity and genome evolution in cephalopods. *Cell* 169, 191–202. doi: 10.1016/j.cell.2017.03.025

MAFF (2017). *FY2016 Trends in Fisheries FY2017 Fisheries Policy White Paper on Fisheries: Summary (PDF)*. Available online at: http://www.maff.go.jp/e/data/publish/

Mangold, K. (1989). *Céphalopodes. Traité de Zoologie. Anatomie, Systématique, Biologie, Tome 5, Fascicule 4*. Paris: Masson.

Mather, J. A., and Anderson, R. C. (1993). Personalities of octopuses (*Octopus rubescens*) *J. Comp. Psychol.* 107, 336–340. doi: 10.1037/0735-7036.107.3.336

Mather, J. A., and Anderson, R. C. (1999). Exploration, play, and habituation in octopuses (*Octopus dofleini*) *J. Comp. Psychol.* 113, 333–338. doi: 10.1037/0735-7036.113.3.333

Mather, J. A., and Carere, C. (2012). Editorial: why (and how) personalities in invertebrates? *Curr. Zool.* 58:566. doi: 10.1093/czoolo/58.4.566

Mather, J. A., and Dickel, L. (2017). Cephalopod complex cognition. *Cur. Opin. Behav. Sci.* 16, 131–137. doi: 10.1016/j.cobeha.2017.06.008

Mather, J. A., and Kuba, M. (2013). The cephalopod specialties: complex nervous system, learning, and cognition. *Can. J. Zool.* 91, 431–449 doi: 10.1139/cjz-2013-0009

Mauvisseau, Q., Parrondo, M., Fernández, M. P., García, L., Martínez, J. L., García-Vázqueza, E., et al. (2017). On the way for detecting and quantifying elusive species in the sea: the *Octopus vulgaris* case study. *Fish. Res.* 191, 41–48. doi: 10.1016/j.fishres.2017.02.023

Messenger, J. B. (1988). Why octopuses? Have we learned anything from studying their brains? *Sci. Prog.* 72, 297–320.

Messenger, J. B., Wilson, A. P., and Hedge, A. (1973). Some evidence for colour-blindness in Octopus. *J. Exp. Biol.* 59, 77–94.

Nakajima, K., Hauser, H., Li, T., and Pfeifer, R. (2015). Information processing via physical soft body. *Sci. Rep.* 5:10487. doi: 10.1038/srep10487

Navarro, J., Monroig, Ó., and Sykes, A. (2014). "Chapter: 5. Nutrition as a key factor for cephalopod aquaculture," in *Cephalopod Culture*, eds J. Iglesias, L. Fuentes, and R. Villanueva (Dordrecht: Springer), 77–95.

Nixon, M., and Young, J. Z. (2003). *The Brain and Lives of Cephalopods*. Oxford: Oxford University Press.

Osorio, D. (2014). Cephalopod behaviour: skin flicks. *Curr. Biol.* 24, 684–685. doi: 10.1016/j.cub.2014.06.066

Packard, A. (1972). Cephalopods and fish: the limits of convergence. *Biol. Rev*. 47, 241–307. doi: 10.1111/j.1469-185X.1972.tb00975.x

Pfeifer, R., Lungarella, M., and Iida, F. (2007). Self-organization, embodiment, and biologically inspired robotics. *Science* 318, 1088–1093. doi: 10.1126/science.1145803

Philippe, A. O. (2012). *The Life and Times of Paul the Psychic Octopus*. Exhibit A Pictures.

Pikul, J. H., Li, S., Bai, H., Hanlon, R. T., Cohen, I., and Shepherd, R. F. (2017). Stretchable surfaces with programmable 3D texture morphing for synthetic camouflaging skins. Science 6360, 210–214. doi: 10.1126/science.aan5627

Ramirez, M. D., and Oakley, T. H. (2015). Eye-independent, light-activated chromatophore expansion (LACE) and expression of phototransduction genes in the skin of *Octopus bimaculoides*. *J. Exp. Biol.* 218, 1513–1520. doi: 10.1242/jeb.110908

Robison, B., Seibel, B., and Drazen, J. (2014). Deep-sea octopus (*Graneledone boreopacifica*) conducts the longest-known egg-brooding period of any animal. *PLoS ONE* 9:e103437. doi: 10.1371/journal.pone.0103437

Rodhouse, P. G., Pierce, G. J., Nichols, O. C., Sauer, W. H., Arkhipkin, A. I., Laptikhovsky, V. V., et al. (2014). Environmental effects on cephalopod population dynamics: implications for management of fisheries. in *Adv. Mar. Biol.* 67, 99–233. doi: 10.1016/B978-0-12-800287-2.00002-0

Rus, D., and Tolley, M. T. (2015). Design, fabrication and control of soft robots. *Nature* 521, 467–475. doi: 10.1038/nature14543

Schmitt, C. B. (1965). Aristotle as a cuttlefish: the origin and development of a Renaissance image. *Stud. Renaiss.* 12, 60–72. doi: 10.2307/2857069

Sereni, E., and Young, J. Z. (1932). Nervous degeneration and regeneration in cephalopods. *Pubbl. Staz. Zool. Napoli*, 12, 173–208.

Shen, H. (2016). Meet the soft, cuddly robots of the future. *Nature* 530, 24–26. doi: 10.1038/530024a

Stubbs, A. L., and Stubbs, C. V. T. (2016). Color vision via chromatic blur and pupil shape. *Proc. Nat. Acad. Sci. U.S.A.* 113, 8206–8211. doi: 10.1073/pnas.1524578113

Tomarev, S. I., Callaerts, P., Kos, L., Zinovieva, R., Halder, G., Gehring, W., et al. (1997). Squid *Pax-6* and eye development. *Proc. Natl. Acad. Sci. U.S.A*. 94, 2421–2426. doi: 10.1073/pnas.94.6.2421

Trimmer, B. (2014). A journal of soft robotics: why now? *Soft Robot.* 1, 1–4. doi: 10.1089/soro.2013.0003

Uribe, J. E., and Zardoya, R. (2017). Revisiting the phylogeny of Cephalopoda using complete mitochondrial genomes. *J. Moll. St*. 83, 133–144. doi: 10.1093/mollus/eyw052

Vidal, E. (2014). *Advances in Cephalopod Science: Biology, Ecology, Cultivation and Fisheries. Vol. 67*, Boston, MA: Academic Press.

von Uexküll, J., (1905). *Leitfaden in das Studium der Experimentellen Biologie der Wassertiere*. Wiesbaden: J. F. Bergmann.

Walker, W. F., and Doolittle, W. F. (1983). 5S ribosomal-RNA sequences from four marine-invertebrates and implications for base-pairing models of metazoan sequences. *Nucleic Acids Res*. 11, 5159–5164. doi: 10.1093/nar/11.15.5159

Wang, Z., Chen, M. Z., and Yi, J. (2015). Soft robotics for engineers. *HKIE Trans.* 22, 88–97. doi: 10.1080/1023697X.2015.1038321

Wehner, M., Truby, R. L., Fitzgerald, D. J., Mosadegh, B., Whitesides, G. M., Lewis, J. A., et al. (2016). An integrated design and fabrication strategy for entirely soft, autonomous robots. *Nature* 536:451. doi: 10.1038/nature19100

Wells, M. J. (1978). *Octopus. Physiology and Behaviour of an Advanced Invertebrate.* London: Chapman and Hall.

Yoshida, M., Yura, K., and Ogura, A. (2014). Cephalopod eye evolution was modulated by the acquisition of Pax-6 splicing variants. *Sci. Rep*. 4:4256. doi: 10.1038/srep04256

Young, J. Z. (1938). The functioning of the giant nerve fibres of the squid. *J. Exp. Biol*. 15, 170–185.

Young, J. Z. (1964). *A Model of the Brain*. Oxford: Oxford University Press.

Zullo, L., and Hochner, B. (2011). A new perspective on the organization of an invertebrate brain. *Comm. Integ. Biol*. 4, 26–29. doi: 10.4161/cib.13804

Zullo, L., Sumbre, G., Agnisola, C., Flash, T., and Hochner, B. (2009). Nonsomatotopic organization of the higher motor centers in *Octopus*. *Cur. Biol*. 19, 1632–1636. doi: 10.1016/j.cub.2009.07.067

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Communication

PERSPECTIVE
published: 20 April 2018
doi: 10.3389/fcomm.2018.00017

The Art of Aquarium Keeping Communicates Science and Conservation

Elizabeth A. Marchio*

Department of Recreation, Park and Tourism Sciences, Texas A&M University, College Station, TX, United States

In technology-driven societies, scientists, and educators alike flounder in making science interesting and applicable. Communicating science, defined as communicating scientific facts as well as teaching and using the scientific process, can also be done informally though leisure activities. In this qualitative study, I examined the leisure activity of aquarium keeping and its ability to communicate relative aquatic facts and processes. This study examined aquarium keepers across the United States via interviews, participant observation, and an ongoing analysis of aquarium hobby literature. Thus, this study indicates (1) caring for a home aquarium communicates science latently, (2) over time, latent science communication becomes activated, and (3) long-term aquarium keeping leads to a personal response in science, as well as conservation. In addition, artistic expression and innovation intersect with scientific knowledge and application to create beautiful, biodiverse, ecosystems. Through the process of successfully maintaining an aquarium, continued participation leads to a proficiency in applicable scientific facts, a better understanding of scientific processes, and an improved conservation ethic for aquatic resources. Further, this intersectionality motivates participation by providing new challenges and various forms of satisfaction. The human dimensions of the aquarium hobby and the values of aquarists are important to understand for many purposes, most notably because it encompasses an enormous sample of the American population and is extremely lucrative to those along most of the supply chain. Aquarium keeping is not only a hobby, but because of the relationship between science and art, it can communicate, as well as spark conservation efforts in serious aquarists.

Keywords: art, aquarium, fish, science as leisure, captive ecosystem, science, conservation, science communication

Edited by:
Tarla Rai Peterson,
University of Texas at El Paso,
United States

Reviewed by:
Samantha Senda-Cook,
Creighton University, United States
Tracy Marafiote,
State University of New York at
Fredonia, United States
Arren Mendezona Allegretti,
Santa Clara University, United States

****Correspondence:***
Elizabeth A. Marchio
elizabeth.marchio@gmail.com

Specialty section:
This article was submitted to
Science and Environmental
Communication,
a section of the journal
Frontiers in Communication

Received: *11 January 2018*
Accepted: *03 April 2018*
Published: *20 April 2018*

Citation:
Marchio EA (2018) The Art of
Aquarium Keeping Communicates
Science and Conservation.
Front. Commun. 3:17.
doi: 10.3389/fcomm.2018.00017

INTRODUCTION

To communicate science, including conservation science, one must elicit a personal response toward science (Burns et al., 2003). A "personal response" includes an awareness, enjoyment, interest, opinion forming experiences, and understanding. These outcomes can be achieved through means defined as formal (e.g., school) or informal (e.g., citizen science; Dickinson and Bonney, 2012). Informal scientific communication includes passive means, often bordering on entertainment. This line, between scientific communication and scientific entertainment, is dense with diverse recreation and leisure activities. One such activity is the hobby of home aquarium keeping.

Aquarium keeping is a global industry worth between 15 and 30 billion U.S. dollars (Hoff, 1996; Wood, 2001; Cato and Brown, 2003; Tlusty et al., 2013). In 2017 there were 12.5 million U.S. households keeping freshwater aquaria and 2.5 million keeping saltwater (American Pet Products Association, 2017) encompassing 139.3 million individual freshwater, 18.8 million saltwater organisms, and approximately 10% of the American population (American Pet Products Association, 2017). Because of these participation levels, home aquaria has huge outreach potential. It is important to understand the past, current, and future effects of the hobby on the aquarists who keep these systems.

Through interviews, content analyses, and participant observation, I found a modern and historical link between art and science in aquarium keepers. Further, this link seems stable, with the aesthetic value of aquatic organisms as one of the key motivators for hobby participation. In addition, the process of keeping a successful aquarium exposes the aquarium keeper to a vast array of scientific information, principles, processes, and methods. I argue the keeping of an aquarium may be motivated, and that motivation maintained, through an aesthetic interest, but the side effect of aquarium keeping is a personal response toward aquatic organisms. Results indicate this response affects scientific *and* conservation awareness, enjoyment, interest, opinion forming experiences, and understanding. This study indicates an aquarium helps link aquarists to an increasingly damaged aquatic world.

Introduction to Home Aquarium Keeping

Home aquarium keeping is an unlikely source of scientific communication due to its highly consumptive past and, to an extent, present. This consumption not only includes the removal of live aquarium specimens for the aquarium hobby, but also encompasses other related practices that detrimentally affect their populations and habitats. This includes the collection of live plants and coral, invertebrates such as shrimp and crabs, as well as abiotic structure (e.g., "*live rock*"). Additionally, the aquarium industry is historically tied to the curio trade (Wood, 2001; Rhyne et al., 2009; Townsend, 2011; Dee et al., 2014; Fujita et al., 2014), likely exacerbating the negative connotation surrounding the hobby.

A vast array of literature exists on the aquarium hobby, eliciting over 8,000 Google Scholar hits; however, it is primarily focused on understanding the aquarium industry's effects on the environment at both ends of its supply chain. For example, there are numerous studies on the impacts of removing aquatic organisms from the wild (Parks et al., 2003; Jones et al., 2008), the impacts of aquaculture (Tlusty, 2002; Parks et al., 2003; Rhyne and Tlusty, 2012; Bush and Marschke, 2017; Duggan and Pullan, 2017; Lorenzen et al., 2017), and the capture and care of organisms (Wood, 2001; Jones et al., 2008; Bell et al., 2009; Militz et al., 2016).

Once ornamental organisms are collected other factors are studied. This includes diseases associated with aquatic organisms (Whittington and Chong, 2007; Lawson et al., 2015), invasive species release (Padilla and Williams, 2004; Delaney et al., 2008; Howeth et al., 2016; Bandaranayake and Chandrasekara, 2017; Magalhães et al., 2017; Selwyn et al., 2017; Tuckett et al., 2017) and use for popular aquarium species for breeding and scientific research (Hoff, 1996; Moe, 2003; Moorhead and Zeng, 2010; Olivotto et al., 2011; Domínguez and Botella, 2014; Tehrani et al., 2014). Another, far less common focus for research includes the human dimensions of the aquarium trade and hobby. Instead of studying the organisms, the stakeholders are analyzed. Here, the aquarium hobby literature is increasing (e.g., Maceda-Veiga et al., 2014), but generally overlooks the aquarium hobbyist as a population of research interest.

LINKAGES BETWEEN ART AND SCIENCE COMMUNICATION

A captive ecosystem is a malleable canvas reliant upon science. James Shirley Hibberd, a nineteenth century part-time naturalist, documented the link between aquaria, art, and science in this quote: "The aquarium has become established as a triumph of art acting as the handmaid of science" Hibberd (1860). This link between art and science is evident in modern successful aquarium keeping; beautiful and creative ecosystems are created with a detailed understanding of the science working behind the scenes (**Figure 1**).

Behind the scenes, scientific facts, processes, and methods are slowly learned and mastered. For example, the goal of every aquarist is to keep aquatic organisms alive in captivity. Arguably, the major hurdle for all new aquarists is applying their knowledge of the nitrogen cycle (Paletta, 2002; Delbeek and Sprung, 2005; Fenner, 2008). During the nitrogen cycle, a new aquatic ecosystems must be *"cycled."* During this time, aquarists test their scientific knowledge, and patience, by monitoring the water chemistry and nutrient input as their *"beneficial bacteria colonizes."* Then, once this occurs, they can slowly add new organisms to their system (Paletta, 2002; Delbeek and Sprung, 2005; Fenner, 2008). Aquariums allow non-scientific people a place to observe aquatic organisms, and master relative scientific facts and ecological processes via a hands-on learning experience.

Aquaria have long acted as both formal and informal modes of educating the public; "Nor is it only for amusement that such parlor oceans [home aquaria] and lakes [ponds] are prepared and stocked; they are invaluable as a means of instruction" (Sowerby, 1857). Aquaria used as instructive devices are found in schools today (Rutherford, 2015a,b; Quality Marine, 2017). The link between science and aquarium care is not lost on teachers who aim to please students who ask, "When will I ever need to know or use scientific facts and knowledge?" Formal instruction with an aquarium communicates science, while informal learning through home aquarium care elicits a more personal response due to taking responsibility for those organisms.

Science communication suffers from several issues, with one being a failure to go where the people are, not where they want them to be. For example, instead of studying how to get more participants into places of science communication (e.g., museums), I suggest we go to participants at their hobbies to explore the informal educational potential of those activities. Previous research has shown hobbies act as early careers, with a

FIGURE 1 | A challenging and unconventional miniature 1.5-gallon reef aquarium can be grown in a glass vase. Home aquaria offers the home aquarist a chance to get close to oceanic organisms, appreciate their natural beauty, and even propagate them for sale and trade. Photo reproduced with the permission of the copyright holder [Matt Pederson].

potential to lead to professionalism (Stebbins, 1979, 1980, 1992). In his extensive body of work, Stebbins explores the different participation levels within hobbies (i.e., amateur, professional), focused on describing the different types of leisure (e.g., serious leisure; Stebbins, 1979, 1982). Results indicate an additional leisure type, science as leisure, where science and conservation may be communicated thorough leisure activities.

METHODOLOGICAL FRAMEWORK

Previous personal experience in aquarium keeping, from 2000 to 2015, indicated an emergent theory linking aquarium keeping and science communication. In fact, I attribute obtaining my Master's Degree in fish phylogenetics to my past history as an aquarium keeper (Marchio, 2015). More specifically, I chose ethnographic methodologies to immerse myself in the social context of the aquarium hobby (Glaser and Strauss, 1967; Spradley, 1980; Agar, 1996; Charmaz, 2006). This method allows aquarists to help me interpret situations and events. By contextualizing the topic, I can further explore the importance of science to aquarium keeping as a sensitizing concept (Bowen, 2006). The framework for this study draws from previous experience, participant observation, semi-structured interviews, and analyses of aquarium hobby content. Due to the lack of hobbyist-specific peer-reviewed research, grounded theory allowed me to discover and explore the interconnectivity between leisure and science.

Previous experience and participant observations were keys to this study. While many interviewees were open and willing to talk about the hobby, participants spoke more candidly outside a formal setting and much of the data comes from informal conversations. For example, at a *"frag swap"* I participated by shopping and *"swapping"* live coral fragments alongside other aquarists. There, I could be a "professional stranger" (Agar, 1996), listening to aquarists ask questions and comment on things important to them. Moreover, I attended the Marine Aquarium Conference of North America (MACNA) over 3 years (2015–2017) where a large vendor and conference room let me blend in and participate authentically.

Additionally, participant observation helped to triangulate the emergent data and themes (Spradley, 1980). This consisted of maintaining both salt- and freshwater aquaria in my own home. I documented the majority of my experience in a notebook. The notebook consisted mostly of water quality measurements and observations of the inhabitants and the system. Both systems were set up for at least 1 year.

To triangulate these data, I conducted twelve interviews. The interview protocol was semi-structured, providing a guide for the conversation. This allowed participants to go toward the most important topics to them, allowing emergence of theory. I carried out interviews in accordance with the recommendations of the Texas A&M University Institutional Review Board with informed verbal consent from all subjects. Aquarists interviewed were mainly saltwater aquarium keepers but some kept both saltwater and freshwater aquaria concurrently. A few maintained only freshwater at the time of the interview. It is important to note aquarium keepers often switch styles of involvement but usually begin their hobby with a freshwater bowl or aquarium.

I choose interviewees that represented different stages of participation—from novice to advanced. In the leisure sciences, studies have shown there are different stages and styles of involvement in a leisure activity, and these can reflect different values, motivations, and experiences (Bryan, 1977, 1979; Chipman, 1986; Chipman and Helfrich, 1988; Scott et al., 1999; Scott and Shafer, 2001; Waight and Bath, 2014). Aquarium keeping seems no different.

Novice aquarists were challenging to interview; they felt they had little information to offer which often lead to a decline for a conversation. Serious aquarists were the opposite. To supplement, online forum data were easy to obtain with new aquarium keepers seeming most comfortable talking behind a computer screen. Additionally, this study includes content from online forums, aquarium books and literature, and content of several aquarium conferences. Online forums included www.reefcentral.com and www.nano-reef.com.

In addition, personal correspondence with aquarists online allowed for constant comparison and on-demand participant validation (Bowen, 2006). This is important since my previous experience could bias analyses (Agar, 1996). Additionally, I was able to connect with several important people, or "gatekeepers," in the hobby. This includes two editors of major aquarium magazines, speakers at the national MACNA, speakers at freshwater aquarium club meetings, professional researchers in aquaculture, scientists from the California Academy of Sciences, aquarium club members across the United States, aquarium technicians and local fish store, or *"LFS,"* workers, as well as other leaders in the hobby. Using these connections, I made a network of individuals that I ask to comment on my conclusions of the hobby. Online discussions aided in receiving critical reviews.

Lastly, the results below follow an internal documentation system used to describe the data. Data from participant observation and content analyses are "Field notes" while interview quotations have a pseudonym attributed. Terms italicized throughout are community-applied terminology (Ritchie et al., 2014, p. 193). The Texas A&M University Institutional Review Board, IRB2017-0405 D, approved all protocol. All interview data and contact information are stored in accordance with the IRB protocol.

RESULTS

In this study, I discovered caring for a home aquarium (1) is largely motivated by aesthetic reasons, but (2) communicates science latently, over time, (3) and long-term aquarium keeping leads to a personal response in science, as well as conservation. Reviewed below, these findings are first situated within the context of the hobby—one that relies heavily on aesthetics.

Aesthetic Value of the Hobby

The following data exemplify the importance of aesthetics in aquarium keeping. Aesthetic value not only comes from the organisms themselves (i.e., color, movement), but also from the ecosystem as a whole including the display of the system itself. For example, serious aquarium keepers maintain large systems housed within a wall of their home:

> *"In my opinion, all the technical equipment should be unobtrusive, hidden and quiet (at least if not used to make sound), and simply should work. There was no choice, then; the new system would have to be an in-wall design." (TOTM Aug 2006)*

> *"For purely aesthetic purposes, the aquarium also needed to have no visible pumps and equipment." (Field notes)*

These quotes show the importance of a sleek, professional look with life-sustaining equipment hidden from view. Many regard this as reminiscent of a photograph hanging on a wall. In fact, some aquarists identify aquaria as "moving pictures," akin to colorful, moving photographs. Previous experience in the aquarium hobby also supports these data; many new aquarists pick organisms based on color and completely disregard compatibility. In fact, unless explicitly told, new aquarists mainly learn to keep aquatic organisms though trial and error. Supporting these points are the following quotes:

> *"And a lot of people… it's a picture on a wall. I'm gonna set it up and I'm gonna forget about it. Well, it doesn't work like that." (Mike, 40+ years in the hobby)*

> *"I find the aquarium to be an amazing medium of expression… You can paint a beautiful picture with your wood, rocks, plants, and fish. In addition to that, your work of art is constantly moving, evolving, growing, and changing. I see this comparable to the transition from still art on paper and canvas to the modern art of film and cinematography. Aquascaping is a living, breathing, work of art." (John, 5 years in the hobby)*

> *"I'm most interested in movement" (Leslie, Less than one year in the hobby).*

> *"I selected these particular fish based on their different color, shape, and patterns." (Beginner Forums)*

> *"A variety of corals that offer just about every color and shape imaginable." (TOTM May 2002)*

> *"I am new to the hobby and just bought a 75 g tank. I was hoping to get some advice on some cool looking reef safe fish" (Beginner Forums)*

> *"I think I am drawn toward [stony coral] species because of the seemly endless variations of colors and shapes." (TOTM Sept 2015)*

> *"The colors and growth of each coral is what has flourished my love for them." (TOTM Feb 2010).*

> *"When setting up the tank, I was more concerned with color than growth. My experience showed me that corals will grow with patience, but selecting a few choice corals and placing them in optimal places really can make a difference." (TOTM Feb 2010).*

These data reflect the aesthetic values involved in aquarium keeping; they also connote scientific communication. For example, different colored fish and invertebrates usually belonging to different species. Essentially, the new aquarist is most interested in biodiversity, reflected in words such as "variety," "endless variations" sometimes specifying this as "color," "shape," "movement," or "pattern". One of the hardest things for new aquarists is identification of species and compatibility within and across species. Similarly, this includes behavior and growth.

Latent to Active Scientific Communication

By focusing on an individual organism's attributes, a non-scientific aquarist is introduced to scientific concepts such as "species" and "biodiversity." In fact, driven to increase various forms of color and movement, many aquarists unwittingly strive to keep a biodiverse tank. Further, within the confines of a captive ecosystem there are stocking limitations and compatibility issues that push aquarists to obtain more information on each organism they keep.

> *"In the years that followed I became more and more accustomed to the tank's requirements, and more information on stony corals became available to me." (TOTM Aug 2006)*

Some aquarists identify joining an aquarium club or online forum as a turning point in their hobby; within that community, they can obtain accessible, increasingly specific knowledge and share their own. Here, the forum ReefCentral.com elicited such a response:

> *"While browsing on the internet I came across Reef Central. This was the turning moment for me. With the wealth of information that is freely shared among the great reefers throughout the world and with a little tweaking on my system I was able to keep fishes, corals (mainly LPS[1] and Softies[2]) and anemones alive." (TOTM May 2014)*

> *"There is no end to learning, trying to optimize the environment for the animals while still having fun looking at them and sharing the knowledge." (TOTM Aug 2006)*

These data support time in the hobby, including repetitive trial and error, leads to an increased knowledge of biology, ecology, animal physiology, zoology, and other natural science fields. Moreover, with specific organismal knowledge some aquarists begin to specialize in the organisms they can keep, or simply prefer. This continues an aquarist's informal scientific education and can spur specialization in one area or species, shown here:

> *"Due to the shallow depth of the tank and demanding requirements of the Acropora, I found myself forced to make a choice and decided to dedicate the tank entirely to SPS[3]." (TOTM Oct 2013)*

In the data above, science communication is informal and thus sensitive to the true motivations of the hobbyist—through aesthetics. Additionally, as time progresses, aquarists strive to keep challenging themselves and the sensitivity to aesthetics gives way to other motivation. For example, an aquarist may change from fresh- to saltwater or back again, follow a specific artistic style (e.g., Iwagumi aquascaping style, Dutch Synthetic Reefing, Jungle Style; **Figure 2**), engineer their ecosystem by bypassing all-in-one aquarium kits and doing it themselves (i.e., "*DIY.*"), or they may recreate a wild ecosystem in exacting detail (i.e., "*biotope*"). Increased attention is paid to the organisms as well as the "*aquascape*" (Figure 2; Amano, 1996; Veganbrian., 2012; Brenner, 2017). This further embeds non-science oriented aquarists to the biology and ecology of aquatic organisms and these organisms are not limited to fish.

Aquarists also challenge themselves to replicate wild ecosystems in exacting detail, a system called a "*biotope.*" In a biotope, only organisms that are found together in the wild are housed together in the same captive system (Stawikowski, 1993). Creating a biotope requires a large amount of research to accurately replicate natural conditions and house the appropriate organisms and aquarium décor (Stawikowski, 1993). A rather general biotope, a "*miniature reef,*" is often set up to display the oceanic biotope, or a "*slice of the ocean,*" in their home (**Figure 1**; Pederson, 2018).

FIGURE 2 | Houston's Hiep Hong's "Jungle Style" 54 gallon aquarium, *Aqueous Reflection*, placed 116th at the 2017 International Aquatic Plants Layout Contest (IAPLC) (Brenner, 2017). He used specific species and his knowledge of them to create a living piece of art. Photo reproduced with the permission of the copyright holder [Hiep Hong].

Another aquarium keeping style similar to a biotope is a "species-only" system. A species only system is one that houses only one species of interest. Usually species-only aquaria house challenging or dangerous species such as electric eel, seahorse, mantis shrimp, or octopus. One of the most sought after organisms to challenge the skill of an aquarist are cephalopods; for example:

> *"[I] have the smaller Dwarf Octopus.... Life span can be short but wouldn't trade the experience with this one. Arrived unannounced at local pet shop, unable to resist." (Field notes)*

> *"I have an A. aculeatus in one of my office tanks... and I spent almost a year prepping the tank for his arrival, with the understanding that I'd likely only have a few months with him." (Field notes)*

> *"About 10 years ago I got a bimac and within a few day it laid eggs. Stopped eating and died a few weeks later. Second one was the most amazing pet I've ever had. I had him for 8 months." (Field notes)*

Octopus are intriguing animals and aquarists appreciate them for their engrossing behavior, amazing ability to change color, shape, and movement, and their ephemeral nature. These attributes makes them one of the pinnacle species for the home aquarist and one that truly communicates to people who may not be as interested in aquarium fish, plants, or coral.

Personal Response Increases Conservation Ethic

Research on wildlife-oriented recreation (e.g., fishing) show participants may shift their focus from a consumption orientation to one that is conservation orientated toward

---

[1] LPS is an antonym for Large Polyp Scleractinian coral species such as *Acanthastrea* and *Favia* species.

[2] Softies is a term used for soft corals such as *Sarcophyton* and *Lobophytum* species.

[3] SPS is an antonym for Small Polyp Scleractinian coral species such as *Acropora* and *Montipora* species.

the wildlife in which they interact (Bryan, 1977, 1979; Oh and Ditton, 2008; Oh et al., 2013). For example, in a study across all angler types, anglers were initially motivated to participate for the consumption of the organisms (i.e., the removal of them from the wild). As the participant continuously interacts with the environment and the organisms, a conservation ethic emerges. A personal response was elicited by continued participation in a leisure activity and lead to an increase in conservation orientation (Bryan, 1977, 1979; Oh and Ditton, 2008; Oh et al., 2013).

Data collected for this study indicate aquarium hobbyists act similarly over time. New aquarists, and those with a casual orientation, are unaware of conservation implications stemming from the trade and undesirable behavior of aquarists. Undesirable behavior includes purchasing species that grow too large for captivity, purchasing animals without researching their requirements first, overstocking an aquarium, etc. Forums on the internet have regularly occurring conversations, and passionate debates, on these subjects. This is because long-term aquarists understand the importance of imparting a conservation ethic to new aquarists (Borneman, 2001; Tullock, 2001; Paletta, 2002; Delbeek and Sprung, 2005; Fenner, 2008). It seems it is up to the aquarium community to "*police*" the consumption and behavior of other aquarists, shown here:

> "*I think it's important that people reading the thread hear at least one person mention the fact that these are not animals that should be sought out. I hear they had a Wonderpus on the most recent episode of "Tanked!" and we all know that's not going to help anything.*" (Field notes)

> "*…I have to come out and say I am an advocate of tangs, however not a member of the tang police. In this sense I am interested in what is best for the fish.*" (Field notes)

According to Burns et al. personal significance of science and conservation facts is influenced by cultural, social, and political conditions in which they are produced and/or promoted (2003). Further, it is critical to involve all aquarists in scientific communication in order to contextualize and frame their interactions with the captive ecosystem and its inhabitants. Aquarium social groups, such as clubs and conferences, are ideal places to improve a science and conservation ethic.

Examples of an Embedded Scientific and Conservation Ethic in Aquarists

Perhaps due to the integration of professional scientists in the aquarium hobby (i.e., social and cultural conditions), some aquarists decide to follow advice from scientists or attempt to use the scientific method. An aquarist's understanding of the scientific method varies but a scientific ethic remains present in some aquarium keepers. For example:

> "*I tend to lean towards applying techniques to my reef by using proven data provided by the scientific method." (Field notes)*

> "*I'm just curious really of the science/method on it and what kind of factors affect it working vs them killing each other off" (Field notes)*

Due to aquarium keeping's massive participation levels and available biodiversity, some aquarists specialize in certain species. Specialization lead to the creation of species-specific clubs and societies within the hobby. Using money from club members and other donations, these clubs fund research on their species of interest. For example, the American Cichlid Association offers the Guy Jordan Research Fund ($600–1500), which specifically targets cichlid research. Other U.S. clubs have similar funds (Marine Aquarium Society of North America, 2016; American Cichlid Association, 2018; American Livebearer Association, 2018; Ohio Cichlid Association, 2018). Moreover, the Marine Aquarium Societies of North America has made it a point to incorporate science in their mission by offering publication funding for scientists (Dr. Junda Lin Memorial Fund), two $4,000 scholarships for undergraduate and graduate students, and a scientific poster presentation at their annual conference.

Again, seemingly motivated by passion for specific species, clubs and social groups also have "*species maintenance programs*" run by the aquarium community. These programs identify species in need and put captive individuals of that species in the hands of interested, and capable, home aquarists. Species maintenance programs are possible through the sharing of technical information about the species, often written up by aquarists as species reports. These have enough detail for aquaculture and laboratory use. Dr. Paul V. Loiselle, whose 50 years of experience as an aquarist turned into a scientific career studying fish in the Family *Cichlidae*, inspired programs such as CARES Preservation Program:

> *"The purpose of the CARES Preservation Program is to create a base stock of conservation priority species through encouraging hobbyists worldwide to devote tank space to one or more species at risk and distribute offspring to fellow qualified hobbyists, while forming an information network where possible between aquarists, scientists, and conservationists."*

Lastly, conservation is not only limited to species. Serious aquarists can become attached to the places their aquatic pet's live, or an *ex-situ* attachment to geographic place. This attachment motivates aquarists to take international trips to see, and potentially collect, organisms in their wild habitat. In conclusion, aquarium keeping has the potential to bond caregiver, organism, and wild ecosystem.

DISCUSSION

Understanding the human dimensions of the aquarium hobby is increasingly important. Due to previous mismanagement (i.e., Hawai'i; Tissot and Hallacher, 2003) as well as lack of any management whatsoever (e.g., the Philippines and Indonesia; Lunn and Moreau, 2004) wild ornamental fisheries are under intense scrutiny. In 2017, the state of Hawai'i closed its marine ornamental aquarium collection (McAvoy, 2017) and Fiji followed suit directly after on December 28, 2017 (Lacanivalu, 2018). Closing the aquarium trade to wild collection of organisms may be dutiful in the face of climate change; however, the positive short- and long-term effects of aquarium keeping on the home aquarist will be altered.

In this study, I discovered caring for a home aquarium is largely motivated by aesthetic reasons. In addition, aquarium keeping latently communicates science and long-term aquarium keeping may lead to a personal response in science and conservation. Aquarium keeping depends on the aquarist to be responsible for the organisms under their care. That is the point of the hobby. As one aquarist puts it:

> *"Little did I know what started off as a free tank and a couple clown fish would have developed into a lifelong passion." (TOTM Oct 2013)*

Finally, this research indicates ichthyologists and natural scientists may be encouraged to study science and conservation through latent scientific activities such as aquarium keeping (Maceda-Veiga et al., 2014; Marchio, 2015). These results indicate aquarium keeping may offer people the ability to informally increase their science and conservation ethic in a way that is creative, challenging, and always changing.

IMPLICATIONS AND FUTURE DIRECTIONS

Due to the consumptive nature of aquarium keeping and its reliance on wild caught organisms to maintain and invigorate the hobby, it is necessary to explain where an increase in science and conservation orientation breaks down. As shown above, serious aquarists may become more science and conservation oriented as they participate in the hobby, but not all follow this path. In fact, they may decide to do things that are not scientific nor conservationally aligned. Motivated by other factors, some aquarists are willing to break the law to keep threatened or endangered species. For example, in 2006 when one person turned in another for keeping what they thought, or what may have been an illegal coral species, the marine aquarium community debated. This conversation is not uncommon; here it took part on www.reefcentral.com, one of the post popular reef aquarium websites and community forum. While many had conservation-oriented views such as this:

> *"I think that the problem with posting illegal corals is that it may promote the collection of them." (Field notes)*
>
> *"Illegal coral collection is all of "our business," coral reefs are for all of us to enjoy and are all of our responsibility. I am not going to let someone else take a rare and illegal coral from the ocean for his personal enjoyment, resulting in the fact that I and everyone else will never be able to publically enjoy the benefits of that coral through a recreational dive or the general economic an recreational benefits that coral provides by being in the wild." (Field notes)*

Other aquarists feel much differently and do not appreciate other aquarists policing the actions of fellow hobbyists, even at the potential detriment of their hobby. Within the same online thread as the quotes above, fellow hobbyists dissented with the actions of the whistleblower rather than the illegal activity:

> *"I can understand why one should not endorse the collection of illegal corals. However, I think we can all agree that collection of Carib[4] corals for the aquarium industry will never mean the Atlantic reef's destruction. It is not [Dr. Eric] Borneman's place to play reef police by covertly turning someone in." (Field notes)*
>
> *"Still [Dr. Eric] Borneman actually narced out Bomber for his Carib coral. My opinion of Borneman has definately taken a hit.... You just don't go dropping the dime on fellow reefers. I mean it is not like Bomber poaches coral for a living. Not cool at all." (Field notes)*

Conservation issues seem more prolific in the saltwater aquarium community. The freshwater hobby is overall less expensive, older, more specialized, and it is currently not as reliant on wild caught organisms as it was in the past. These attributes must have meaning since there are 10 million more freshwater aquarium keepers (American Pet Products Association, 2017) with a seemingly higher conservation ethic. Further, antecedents to participation in the hobby should be studied; for example, brand new aquarists may already interested in science and conservation. Future research is required to unpack these major points.

In conclusion, it is important to understand the human dimensions of aquarium keepers across the United States, and globally, to continue understanding the human effect of home aquarium keeping. With 10% of the U.S. population *already invested* in keeping an aquarium, developing and facilitating scientific and conservation communication may aid in increasing a science and conservation ethic.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

ACKNOWLEDGMENTS

Thank you to my dissertation committee and chair, Dr. David Scott, three reviewers, and my six amazing undergraduates who helped compile and decipher data: Eli Kain, Stephen Blair, Minna Wong, Madison McClintock, Aaron Drotts, Samantha Huntley, and Emily How. Lastly, I traveled to interviews and conferences for data collection via funding from the Department of Recreation, Park, and Tourism Sciences at Texas A&M University and was accepted with open arms by the aquarium community.

---

[4] In 2006, Caribbean corals had limitations on take for the aquarium hobby.

REFERENCES

Agar, M. (1996). *The Professional Stranger: An Informal Introduction to Ethnography 2nd Edn*. San Diego, CA: Academic Press.

Amano, T. (1996). *Nature Aquarium World*. Neptune City, NJ: T.F.H. Publications.

American Cichlid Association (2018). *Guy Jordan Research Fund*. Available online at: http://cichlid.org (Accessed October 24, 2016).

American Livebearer Association (2018). *Vern-Parish-Fund*. Available online at: http://www.livebearers.org (Accessed October 24, 2016).

American Pet Products Association (2017). *Pet Industry Market Size: Ownership Statistics*. Stamford, CT.

Bandaranayake, C. H., and Chandrasekara, W. U. (2017). Are fancy guppies (*Poecilia reticulata*; Peters, 1859) invasive? *Sri Lanka J. Aquat. Sci.* 22:117. doi: 10.4038/sljas.v22i2.7536

Bell, J. D., Clua, E., Hair, C. A., Galzin, R., and Doherty, P. J. (2009). The capture and culture of post-larval fish and invertebrates for the marine ornamental trade. *Rev. Fish. Sci.* 17, 223–240. doi: 10.1080/10641260802528541

Borneman, E. (2001). *Aquarium Corals: Selection, Husbandry, and Natural History*. Neptune City, NJ: Microcosm Ltd.

Bowen, G. A. (2006). Grounded theory and sensitizing concepts. *Int. J. Qual. Methods* 5, 1–9. doi: 10.1177/160940690600500304

Brenner, W. A. (2017). *A Fish Tank Worth a Thousand Words*: *Hiep Hong's Award-Winning Aquascapes are Worthy of the Greatest Tanks*. Austin, TX: The Austin Chronicle.

Bryan, H. (1977). Leisure value systems and recreational specialization: the case of trout fishermen. *J. Leis. Res.* 9, 174–187. doi: 10.1017/CBO9781107415324.004

Bryan, H. (1979). *Conflict in the Great Outdoors: Towards Understanding and Managing for Diverse Sportsmen Preferences*. Tuscaloosa, AL: University of Alabama Press.

Burns, T. W., O'Connor, D. J., and Stocklmayer, S. M. (2003). Science communication: a contemporary definition. *Public Underst. Sci.* 12, 183–202. doi: 10.1177/09636625030122004

Bush, S. R., and Marschke, M. (2017). "Social and political ecology of fisheries and aquaculture in Southeast Asia," in *Handbook of Environment in Southeast Asia,* ed P. Hirsch (London: Routledge), 224–238.

Cato, J. C., and Brown, C. L. (2003). *Marine Ornamental Species: Collection, Culture and Conservation*. Ames, IA: Iowa State Press.

Charmaz, K. (2006). *Constructing Grounded Theory: A Practical Guide Through Qualitative Analyses*. London: Sage Publications, Inc.

Chipman, B. D. (1986). *Recreational Specialization as an Indicator of Attitudes and Management Preferences of Virginia River Anglers*. Blacksburg, VA: Virginia Polytechnic Institute and State University.

Chipman, B. D., and Helfrich, L. A. (1988). Recreational specializations and motivations of virginia river anglers. *North Am. J. Fish. Manag.* 8, 390–398. doi: 10.1577/1548-8675(1988)008<0390:RSAMOV>2.3.CO;2

Dee, L. E., Horii, S. S., and Thornhill, D. J. (2014). Conservation and management of ornamental coral reef wildlife: successes, shortcomings, and future directions. *Biol. Conserv*. 169, 225–237. doi: 10.1016/j.biocon.2013.11.025

Delaney, D. G., Sperling, C. D., Adams, C. S., and Leung, B. (2008). Marine invasive species: validation of citizen science and implications for national monitoring networks. *Biol. Invasions* 10, 117–128. doi: 10.1007/s10530-007-9114-0

Delbeek, J. C., and Sprung, J. (2005). *The Reef Aquarium, Vol. 3: Science, Art, and Technology*. Miami Gardens, FL: Ricordea Publishing.

Dickinson, J. L., and Bonney, R. (2012). *Citizen Science: Public Participation in Environmental Research*. Ithica, NY: Cornell University Press.

Domínguez, L. M., and Botella, Á. S. (2014). An overview of marine ornamental fish breeding as a potential support to the aquarium trade and to the conservation of natural fish populations. *Int. J. Sus. Dev. Plan.* 9, 608–632. doi: 10.2495/SDP-V9-N4-608-632

Duggan, I. C., and Pullan, S. G. (2017). Do freshwater aquaculture facilities provide an invasion risk for zooplankton hitchhikers? *Biol. Invas.* 19, 307–314. doi: 10.1007/s10530-016-1280-5

Fenner, R. M. (2008). *The Conscientious Marine Aquarist: A commonsense Handbook for Successful Saltwater Hobbyists 2nd Edn*. Neptune City, NJ: T.F.H. Publications.

Fujita, R., Thornhill, D. J., Karr, K., Cooper, C. H., and Dee, L. E. (2014). Assessing and managing data-limited ornamental fisheries in coral reefs. *Fish Fish*. 15, 661–675. doi: 10.1111/faf.12040

Glaser, B. G., and Strauss, A. L. (1967). *The Discovery of Grounded Theory: Strategies for Qualitative Research*. New Brunswick, NJ: Aldine Transaction.

Hibberd, J. S. (1860). *The Book of the Aquarium*. London: Groombridge and Sons.

Hoff, F. H. (1996). *Conditioning, Spawning and Rearing of Fish with Emphasis on Marine Clownfish*. Dade City, FL: Aquaculture Consultants Inc.

Howeth, J. G., Gantz, C. A., Angermeier, P. L., Frimpong, E. A., Hoff, M. H., Keller, R. P., et al. (2016). Predicting invasiveness of species in trade: climate match, trophic guild and fecundity influence establishment and impact of non-native freshwater fishes. *Divers. Distrib*. 22, 148–160. doi: 10.1111/ddi.12391

Jones, A. M., Gardner, S., and Sinclair, W. (2008). Losing "nemo": bleaching and collection appear to reduce inshore populations of anemonefishes. *J. Fish Biol*. 73, 753–761. doi: 10.1111/j.1095-8649.2008.01969.x

Lacanivalu, L. (2018). *Harvest, Export of Live Coral Now Banned*. Sun (Fiji) News Limited.

Lawson, B., Petrovan, S. O., and Cunningham, A. A. (2015). Citizen science and wildlife disease surveillance. *EcoHealth* 12, 693–702. doi: 10.1007/s10393-015-1054-z

Lorenzen, K., Ainsworth, C., Baker, S., Barbieri, L., Camp, E., Dotson, J., et al. (2017). "Climate change impacts on florida's fisheries and aquaculture sectors and options for adaptation," in *Florida's Climate: Changes, Variations, & Impacts* (Tallahassee, FL: Florida Climate Institute).

Lunn, K. E., and Moreau, M.-A. (2004). Unmonitored trade in marine ornamental fishes: the case of Indonesia's banggai cardinalfish (*Pterapogon kauderni*). *Coral Reefs* 23, 344–351. doi: 10.1007/s00338-004-0393-y

Maceda-Veiga, A., Domínguez-Domínguez, O., Escribano-Alacid, J., and Lyons, J. (2014). The aquarium hobby: can sinners become saints in freshwater fish conservation? *Fish Fish*. 17, 1–15. doi: 10.1111/faf.12097

Magalhães, A. L. B., Orsi, M. L., Pelicice, F. M., Azevedo-Santos, V. M., Vitule, J. R. S., Lima-Junio, D. P., et al. (2017). Small size today, aquarium dumping tomorrow: sales of juvenile non-native large fish as an important threat in brazil. *Neotrop. Ichthyol*. 15:e170033. doi: 10.1590/1982-0224-20170033

Marchio, E. A. (2015). My metamorphosis. *Science* 347, 206–206. doi: 10.1126/science.347.6218.206

Marine Aquarium Society of North America (2016). *Student Scholarship Program*. Available online at: http://masna.org/masna-programs/scholarship-program/ (Accessed October 24, 2016).

McAvoy, A. (2017). *Hawaii Stops Issuing Aquarium Fish Collection Permits*. U.S. News and World Report.

Militz, T. A., Kinch, J., Foale, S., and Southgate, P. C. (2016). Fish rejections in the marine aquarium trade: an initial case study raises concern for village-based fisheries. *PLoS ONE* 11:e0151624. doi: 10.1371/journal.pone.0151624

Moe, M. A. (2003). "Culture of marine ornamentals: for love, for money, and for science," in *Marine Ornamental Species : Collection, Culture and Conservation*, eds J. C. Cato and C. L. Brown (Ames, IA: John Wiley & Sons), 423.

Moorhead, J. A., and Zeng, C. (2010). Development of captive breeding techniques for marine ornamental fish: a review. *Rev. Fish. Sci*. 18, 315–343. doi: 10.1080/10641262.2010.516035

Oh, C.-O., and Ditton, R. B. (2008). Using recreation specialization to understand conservation support. *J. Leis. Res*. 40, 556–573. doi: 10.1080/00222216.2008.11950152

Oh, C.-O., Sutton, S. G., and Sorice, M. G. (2013). Assessing the role of recreation specialization in fishing site substitution. *Leis. Sci*. 35, 256–272. doi: 10.1080/01490400.2013.780534

Ohio Cichlid Association (2018). *Jim Smith Fund*. Available online at: http://www.ohiocichlid.com/jim-smith-fund/about-the-jim-smith-fund (Accessed October 24, 2016).

Olivotto, I., Planas, M., Simões, N., Holt, G. J., Avella, M. A., and Calado, R. (2011). Advances in breeding and rearing marine ornamentals. *J. World Aquac. Soc*. 42, 135–166. doi: 10.1111/j.1749-7345.2011.00453.x

Padilla, D. K., and Williams, S. L. (2004). Beyond ballast water: aquarium and ornamental trades as sources of invasive species in aquatic ecosystems. *Front. Ecol. Environ.* 2, 131–138. doi: 10.1890/1540-9295(2004)002[0131:BBWAAO]2.0.CO;2

Paletta, M. S. (2002). *The New Marine Aquarium: STEP-by-STEP SETUP and Stocking Guide*. Neptune City, NJ: T.F.H. Publications.

Parks, J. E., Pomeroy, R. S., and Balboa, C. M. (2003). "Marine ornamental species: collection, culture and conservation," in *Marine Ornamental Species*, eds J. C. Cato and C. L. Brown (Ames, IA: Blackwell Publishing Company), 183–206.

Pederson, M. (2018). *Pico Reef, Vase Reef, Micro-Reef Jar Resources*. Shelburne, VT. Available online at: https://www.reef2rainforest.com/pico-reef-resources/

Quality Marine (2017). *Classroom Clowns*. Available online at: https://www.reef2rainforest.com/2017/05/08/saddleback-clowns-bred-by-elementary-students-reach-aquarium-market/ (accessed January 11, 2018).

Rhyne, A., Rotjan, R., Bruckner, A., and Tlusty, M. (2009). Crawling to collapse: ecologically unsound ornamental invertebrate fisheries. *PLoS ONE* 4:e8413. doi: 10.1371/journal.pone.0008413

Rhyne, A. L., and Tlusty, M. F. (2012). Trends in the marine aquarium trade: the influence of global economics and technology. *Aquaculture* 5, 99–102.

Ritchie, J., Lewis, J., McNaughton Nicholls, C., and Ormston, R. (eds.). (2014). *Qualitative Research Practice: A Guide for Social Science Students and Researchers, 2nd Edn*. London: SAGE.

Rutherford, B. (2015a). *Reef Aquarium Keeping Helps Students Develop Scientific Skills*. Shelburne, VT: Microcosm Publishing.

Rutherford, B. (2015b). *Tanks in Classrooms: Setting Up an Educational Aquarium*. Shelburne, VT: Microcosm Publishing.

Scott, D., Baker, S. M., and Kim, C. (1999). Motivations and commitments among participants in the great Texas birding classic. *Hum. Dimens. Wildl.* 4, 50–67. doi: 10.1080/10871209909359144

Scott, D., and Shafer, C. S. (2001). Recreational specialization: a critical look at the construct. *J. Leis. Res*. 33, 319–343. doi: 10.1080/00222216.2001.11949944

Selwyn, J. D., Johnson, J. E., Downey-Wall, A. M., Bynum, A. M., Hamner, R. M., Hogan, J. D., et al. (2017). Simulations indicate that scores of lionfish (*Pterois volitans*) colonized the Atlantic ocean. *PeerJ* 5, 1–29. doi: 10.7717/peerj.3996

Sowerby, G. B. (1857). *Popular History of the Aquarium of Marine and Freshwater Animals and Plants*. London: Lovell Reeve.

Spradley, J. P. (1980). *Participant Observation*. Orlando, FL: Harcourt Brace College Publishers.

Stawikowski, R. (1993). *The Biotope Aquarium: An Authentic Imitation of Nature in Your Home*. Neptune City, NJ: T.F.H. Publications.

Stebbins, R. A. (1979). *Amateurs : On the Margin between Work and Leisure*. Beverly Hills, CA: Sage.

Stebbins, R. A. (1980). "Amateur" and "Hobbyist" as concepts for the study of leisure problems. *Soc. Probl*. 27, 413–417. doi: 10.2307/800168

Stebbins, R. A. (1982). Serious leisure: a conceptual statement. *Pacific Sociol. Rev*. 25, 251–272. doi: 10.2307/1388726

Stebbins, R. A. (1992). *Amateurs, Professionals and Serious Leisure*. Buffalo, NY: McGill-Queen's University Press.

Tehrani, J. M., Dorche, E. E., Goli, S. A. H., and Akbary, P. (2014). Enrichment of artemia (leach) nauplii with canola oil: effect on heros severus (Heckel) larvae performance and environmental stress. *Adv. Microbiol*. 4, 1242–1249. doi: 10.4236/aim.2014.416134

Tissot, B. N., and Hallacher, L. E. (2003). Effects of aquarium collectors on coral reef fishes in Kona, Hawaii. *Conserv. Biol*. 17, 1759–1768. doi: 10.1111/j.1523-1739.2003.00379.x

Tlusty, M. (2002). The benefits and risks of aquacultural production for the aquarium trade. *Aquaculture* 205, 203–219. doi: 10.1016/S0044-8486(01)00683-4

Tlusty, M. F., Rhyne, A. L., Kaufman, L., Hutchins, M., Reid, G. M., Andrews, C., et al. (2013). Opportunities for public aquariums to increase the sustainability of the aquatic animal trade. *Zoo. Biol*. 32, 1–12. doi: 10.1002/zoo.21019

Townsend, D. (2011). Sustainability, equity and welfare: a review of the tropical marine ornamental fish trade. *SPC Live Reef Fish Inf. Bull*. 20, 2–12.

Tuckett, Q. M., Ritch, J. L., Lawson, K. M., and Hill, J. E. (2017). Landscape-scale survey of non-native fishes near ornamental aquaculture facilities in Florida, U.S.A. *Biol. Inv*. 19, 223–237. doi: 10.1007/s10530-016-1275-2

Tullock, J. H. (2001). *Natural Reef Aquariums: Simplified Approaches to Creating Living Saltwater Microcosms*. Neptune City, NJ: T.F.H. Publications.

Veganbrian. (2012). *Tips and Tricks on Creating Amazing Aquascapes.* Available online at: http://www.reefcentral.com/forums/showthread.php?t=2217633andhighlight=art (Accessed October 30, 2017).

Waight, C. F., and Bath, A. J. (2014). Recreation specialization among atv users and its relationship to environmental attitudes and management preferences on the island of newfoundland. *Leis. Sci*. 36. 161–182. doi: 10.1080/01490400.2013.862887

Whittington, R. J., and Chong, R. (2007). Global trade in ornamental fish from an Australian perspective: the case for revised import risk analysis and management strategies. *Prev. Vet. Med*. 81, 92–116. doi: 10.1016/j.prevetmed.2007.04.007

Wood, E. (2001). *Collection of Coral Reef Fish for Aquaria: Global Trade, Conservation Issues, and Management Strategies*. Marine Conservation Society.

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Communication

CONCEPTUAL ANALYSIS
published: 19 February 2019
doi: 10.3389/fcomm.2018.00050

OctoEyes

*Eva Hayward**

Gender & Women's Studies, University of Arizona, Tucson, AZ, United States

Examining Jean Painlevé's and Geneviève Hamon's film *The Love Life of the Octopus* (Les Amours de la pieuvre) (1965), this essay offers a theory of refracted spectatorship. Refraction, here, describes the sexual nature of the eye/camera, and also how this refractory sight mis/sees animals, particularly octopuses.

Keywords: refraction, cinema, surrealism, sexuality, octopus

Edited by:
Tarla Rai Peterson,
The University of Texas at El Paso,
United States

Reviewed by:
Tema Milstein,
University of New Mexico,
United States
Jen Schneider,
Boise State University, United States

***Correspondence:**
Eva Hayward
evah@email.arizona.edu

Specialty section:
This article was submitted to
Science and Environmental
Communication,
a section of the journal
Frontiers in Communication

Received: *07 February 2018*
Accepted: *15 October 2018*
Published: *19 February 2019*

Citation:
Hayward E (2019) OctoEyes.
Front. Commun. 3:50.
doi: 10.3389/fcomm.2018.00050

> I think that if you asked any zoologist to select the single most startling feature in the whole animal kingdom, the chances are he would say, not the human eye, which by any account is an organ amazing beyond belief, not the squid-octopus eye, but the fact that these two eyes, man's and squid's, are alike in almost every detail.
>
> Berrill (1983)

> The visual life of the octopus, from Hokusai to Jean Painlevé and William Burroughs has been figured by a sense of illicit, perverse, and transgressive sexuality; and an irreducible visuality of the outside. And just as Oedipus is not one but many (he is the one, singled out, but marked and traversed nonetheless by a potent multiplicity), the octopus may be an exemplary figure for a multiple visuality, a multiplicity of visualities signaled by its eyes and legs.
>
> Lippit (2005)

> Reality is an active verb, and the nouns all seem to be gerunds with more appendages than an octopus. Through their reaching into each other, through their "prehensions" or graspings, beings constitute each other and themselves. Beings do not preexist their relations. "Prehensions" have consequences. The world is a knot in motion.
>
> Haraway (2003)

"REALITY IS AN ACTIVE VERB, AND THE NOUNS all seem to be gerunds with more appendages than an octopus," writes Donna Haraway. She continues, "Beings constitute each other and themselves through their reaching into each other, through their 'prehensions' or graspings" (Haraway, 2003, p. 6). Working from the process philosophy of Alfred North Whitehead, Haraway sees reality as a transaction between dynamic and continuously changing elements, such that the elements exist only in the dynamism; that is, the processual dynamism *is* existence. Reaching between—the transaction—is the engine of being—that amorous aim, yearning, and desiring that constitutes "reaching into each other" (Haraway, 2003, p. 6). For Haraway, this reaching is infectious, is a transfection: love is a "potent transfection," she writes (Haraway, 2003, p. 1). Unlike Karen Barad's "intra-action" (which follows Haraway's antimeria), Haraway's "graspings" include sexuality as central to materializations, mattering, and matter itself (Barad, 1999). Reality is not just bumptious inter- and intra-action between physical components—not just atoms in lively relationship—but also the refracting and refractory forces of longing, loss, and dis/pleasure. It is for this reason that Akira Mizuta Lippit wonders if Haraway's octopus is an "oectopus": "near and far, loved and reviled, loving and perverted, emotional and hysterical; each paradox marked visually by a unique legacy forming on the body an octo-paradoxy" (Lippit, 2005, p. 11). Oectopus, for Lippit, is related to Oedipus. He writes, "Oedipus is also a figure that falls outside the configuration for which he is named, marking him in a species close to but distinctly apart from the human. More or less and more and less human" (Lippit, 2005, p. 11). Like the octopus (Greek from okto "eight" + pous "foot"), Oedipus is foot-ful, having been left with a limp as an infant, and is asked a footed riddle by the Sphinx: "What goes on four feet in the morning, two feet at noon, and three feet in the evening?" Lippit suggests, "Oedipus is a kind of octopus, an organism defined by its eyes and legs" (Lippit, 2005, p. 12). Remember, Oedipus makes his eyes "dark" by dashing them out; the paradox of the octopus eyes, as is their resemblance to human eyes. "The visual life of the octopus, from Hokusai to Painlevé and William Burroughs has been figured by a sense of illicit, perverse, and transgressive sexuality; and irreducible visuality of the outside" (Lippit, 2005, p. 13).

Haraway is certainly not an obvious figure for thinking psychoanalytic ideas[1], but Lippit, working with William Burroughs, puts her octopoidal philosophy in conversation with the visuality and expressivity of octopuses, how its emotional life is "revealed on the surface of its skin" (Lippit, 2005, p. 10). Lippit writes, "In Burroughs's aquarium, the visuality of the octopus is an economy that moves from inside to out, from outside to in, defined by a unique form of exteriority, ecstasy" (Lippit, 2005, p. 10). As much as Haraway's octopus is its reaching, its prehension, for Burroughs the octopus "opens a field of outside visuality, what Burroughs calls a 'mutant' visuality" (Lippit, 2005, p. 10). With Haraway and Burroughs, the arm-y grasping of the octopus is always visual, is always simultaneous with visuality (a reminder of Freud, 1995 own discussion of the eye having a sexual function; Haraway and Burroughs, 1995). Arm-y eyes, "octoeyes" are also oectopoidal visions, as Lippit would have it. Which is to say, octoeyes is a visuality that reaches, is mobile, is verb—they do not simply touch as "fingeryeyes" aim to do (Hayward, 2010). But importantly, this visual reaching is sexual, is always refracted by fantasy, longing, desire, and even love. This does not necessarily mean that the reaching gets ahold, even as that hold is aimed for—this is what is meant by refracted. Refractions are intimacies built through loss, through partiality.

To think about octoeyes—refractory visuality—I turn to Jean Painlevé's (and Geneviève Hamon's) surrealist documentary film, *The Love Life of the Octopus* [*Les Amours de la pieuvre*] (Painlevé, 1965). This film, I argue in the following, offers a refractive zoom or magnified nearness with filmed octopuses that does not produce immediacy (the promise of natural history films), but rather a sexual visuality or octoeyes—a look that reaches but never touches—through imaging and imagining octopuses[2]. More importantly, and a point I can only begin to hint at here—and informed by Jean Laplanche's thoughts on animals (Laplanche, 2015)—the animal—or more specifically here, the octopus—is a function for the administration of sexuality; in the effort to name, define, describe, classify the octopus, we aim to slough off our own sexuality onto the organism. The octopus, then, confronts us with our sexuality displaced with all the exciting and unsettling responses that that entails. With this insight, and somewhat paradoxically, I propose that because the octopus is displaced sexuality and sexuality remains the unbound open of subjectivity, then perhaps the organism called octopus is most present, most at work. We can now see the full implications of the Oedipal in Lippit's oectopus. This is to say, the organism called octopus is absent—the organism is hidden by the sexual function it is made to serve (i.e., Haraway, Burroughs, and Painlevé)—but continues to be active in the refracted and partial space that the film (filmmaker, spectator) cannot see or image. The sensual imag(in)ing of the octopus—what absents the organism—is also the refracted space in which the organism presses back through the workings of sexuality.

"OCTOPUS... CREATURE OF HORROR"

The film opens with an extreme close-up of a portion of a black and white photograph. The camera's framing of the photograph is motionless and abstract. The lines of the image are organic, curved into living form. The title of the film, *The Love Life of the Octopus*, is the only guide for reading the image. It promises a prurient look into the sex lives of octopuses, but, as guidance, the title offers no direct reference for the image. The camera is too close to the photograph to establish a standard of distance. The enlargement distorts the edges of the image, blurring the resolution of the photograph into a grisaille. Doubled and distorted, the image troubles the positivist, indexical nature of the photograph as an unmediated copy of reality. And yet, the magnified image is marked by an investigative look—a look that is as inquisitive as it is fractured and incomplete. From this vantage, analytical reserve is abandoned in favor of a more sensuous view. It is a look that extends the eye's ability into an altogether unfamiliar dimensionality.

The first sound, a voiceover (with subtitles), reorients me: "Eight tentacles[3]... two thousand suckers." The direct address in French is coarse, masculine, and descriptive—it names and defines the indistinctness. Flooding the image with taxonomic precision, the camera pulls back from its tight focus on its ambiguous visual field, reestablishing a familiar scale by showing the whole photograph of a man holding a large *Octopus*

---

[1] In her "Cyborg Manifesto," Haraway famously positioned her cyborg against "the Oedipal project" and "was not generated in the history of sexuality," worrying that psychoanalysis relies upon a "myth of original unity, fullness, bliss and terror, represented by the phallic mother from whom all humans must separate." However, a more nuanced accounting of psychoanalysis that included Sigmund Freud's "body ego," Jean Laplanche's "implantation," or Jacque Lacan "*Spatlung*" (split subject) would consider how psychoanalysis has studied the *fantasy* of holism and unity that is always already foreclosed. Similarly, Haraway's "material semiotic"—which itself supposes re-unification (unity) of the real and its representation—is already considered in Freud's account of fantasy. He insists that fantasy is not mere illusion and reality is not an absolute—for Freud, psychical life is what produces our experience of reality, its certainty. We might call this, if not precisely Harawayian or Freudian, a materialsemiotic accounting of the world, but one, for Freud, can only feel unifying, but can never produce unity. This is all to say, there may be more sympathies between Haraway's approach and psychoanalytic theory than is suggested in her cyborg manifesto, and even more so in her "Companion Species Manifesto." In her later manifesto, love, longing, and desire substantively matter in Haraway's "significant otherness," indeed shape and reshape the transfecting force between her and Cayenne.

[2] Teresa de Lauretis theorizes the relationship between imagining and imaging. Cinematic form—from camera to editing and mise-en-scene—what de Lauretis means by imagining, is the condition through which imagining—fantasy, politics, and spectatorship—is made possible. Imag(in)ing are inextricable.

[3] Hamon and Painlevé have used the common misnomer "les tentacules" (tentacles) rather than "les bras" (arms). Octopuses, unlike their other cephalopod kin—squid and cuttlefish—do not have tentacles; the octopus's eight appendages are "arms." However, in many early accounts of octopuses there is some interchange between the names. For a history of octopod nomenclature see Lane (1960).

vulgaris. The narrative seems established: this is an expository documentary about the natural history and biology of the genus *Octopus*, and about encounters with octopuses. The motion picture camera, the photographic image, and the voiceover appear evidentiary, converging on the octopus to produce a rhetorical argument. Yet as the intriguingly abstract, magnified opening shots put forward, the film modulates between classic documentary style while undermining expository form through perceptual demands in the form of the extreme close-up.

The close-up, the magnified view proposes a different visual engagement—too close but without totality, intimate but without assuring the spectator of their omniscient orientation. Cinematic grammars that position the spectator as all-knowing and everywhere-present is refused for a refracted position—one that uses extreme closeness to refuse order and its control, which is to say, without the final visual hold or grasp. Paradoxically, the intimacy of this perspective is also its refusal, its foreclosure. This visual disorientation is supplemented in relation to the filmed octopus. In the opening image, the octopus is the location of refraction. What is refraction and magnification for this film is also its imagining of octopuses. The representation of the octopus is itself refracted. In doing so, this surreal documentary elaborates Lippit's oectopus. The octopus is the scene of visual loss, a lack constituted not through narrative (e.g., Metz, 1986) alone, but through vision itself. Prehensile and abjectly visual—as Haraway and Burroughs, respectively, describe—this imaged octopus is refractory (including the sexuality the term denotes).

Following the opening shots of a photograph, we see wet life. An octopus comes, arm over arm, from the ocean. It dares the dangers of the exposed intertidal shoreline, pouring through knots of seaweed that cover balanoid and laminaria zones. Its supple body shines, endlessly malleable—this is both Haraway's and Burroughs's octopus. Far from the water, nearly two thousand suckers use their sense of touch (which is bound with smell through chemoreceptors) to manipulate pebbles and shells, looking for shelter among the rocks and crevices. The complex musculature allows its suckers, requiring the wettable surfaces of low tide, to attach, contract and pull.

As marvelous as the landed octopus seems, a collage of sounds—echoes, vibrations, distortions, and whorls—ascend; they are radical sound more than music. Each tone is idiosyncratic and eerie, generating oddness and strangeness, contesting the relationship between sound and music. The experimental electronic composer, an important figure in the *musique concrete* movement, Pierre Henry scored the film. His work demonstrates a deep concern for the not unmusical properties of noise. These electronic sounds are disassociated from their source: manipulated, re-arranged, and recontextualized. Manipulating pitch, timbre, and loudness, the sound generates erratic, polyphonous rhythms that work on the body—accents and beats have no fidelity to the image. Here, Henry's noise-music functions as a form of sound refraction and disorientation; the octopus takes on an alien form through tones, pings, chirps and scrawling, twinkling, surging. The un-octopoidal sounds, the torqueing of sound through music (and *vice versa*)—articulated murmurs, odd phrasing, electric bubbling—the spectator is both invited *into* and alienated *from* the film. Accompanying these uncanny noises is a narration full of chilling affect. The narrator says: "Octopus... Cephalopod," "Creature of horror," "Completely spineless, devoid of shell;" "Changes in coloring reflect its environment as well as its emotions;" "[It] boasts folds serving in the guise of eyelids." The hand-held camera pulls back, giving the spectator an establishing shot, a panoramic view of the shore.

Through the meshing of electronic sound, natural history documentation, and poetic narration, *The Love Life of the Octopus* blurs fiction (surreal) and non-fiction (science/documentary). The ambivalence of octopoidal unfamiliarity coupled with scientific investigation within a playful narrative creates fissure and connection; paradoxically, radical differences are maintained through intimate conjunction. The image confuses the real and the imagined, the animate and the inanimate; the image is a literal *deségrégation* of the symbolic by referential elements. Binds and disentanglements are created and temporarily preserved, and then they are undone, even destroyed. The combinatory and ruinous relationships are palpable. Odd sounds trouble and recode sober descriptions of octopus biology; the music/sound of the film is contrapuntal to reasoned tone of the non-diegetic speech. The hand-held image is kinesthetic and mobile, suggesting its currency in the real. But the real is bound to monstrous and fantastic acousmatic adjectives and descriptors. The referent (the octopus) itself exceeds the assumption of exclusive aquatic inhabitation, crawling along slick rocks on dry land**.** The octopus's ability to become terrestrial is not uncommon, but the popular knowledge of octopus physiology disavows a more liminal existence, between water and air. At every resolution, the film is an example of excessive contradiction, the point where the real of the documentary is saturated and oozing with surreality and sexuality, where absence and decadence interpenetrate in a way that brooks no reconciliation.

SEXUAL DIFFERENCE, A TECHNOLOGY OF SPECIES

The film cuts to two octopuses: one tentatively approaches; the other is bright orange and alert. Noise-music tells the ear that it is immersed; I hear bubbling that sounds shallow and all surrounding. The narrator says, "The male must put his special arm [hectocotylus, or reproductive appendage] into the female's respiratory opening." Two octopuses fill the screen.

Their skins surge color: red, blue, green, brown, black, and white. As Burroughs would have it, affect is translated into an exterior visuality of chromatic expressivity. The narration: "The male turns white with fright as the female approaches." The narration continues: "[For reproduction] the male inserts his special arm, third right from the head, into the female's mantle cavity." While *Octopus vulgaris* is gonochoric (having two sexes), there is little sexual dimorphism, making it difficult (for non-octopuses) to distinguish sexes. And yet, the project of species is to sort sex, or better speciation is a technology of shoring up the logic of sex/gender (Haraway, 1989). To a certain degree, this film playfully problematizes the octopus as a sexual site, but not through the structure of identification. Ambiguity distorts a spectatorship predicated on sexual difference—anthropocentric, sexual difference is replaced by a blurring, a kind of instability at the level of species and sexes. However, the narration and narrative produce numerous tensions between sex differences through reversals: the male is represented as tentative and fearful; the female is engaged and imposing. The small size of this male makes him vulnerable to the larger female—she may choose to eat him rather than reproduce, suggesting death in sexuality. Numerous differences of this coupling alter expectations, even if those differences still rely on anthropocentric standards. But more importantly, these octopuses suggest difference between human sexuality and cephalopod sex and reproduction. Painlevé and Hamon quip: "There is no officially recommended position for achieving this." Although the narration, through this kind of tease, holds these behaviors up as mirrors, the film compels the spectator to consider the futility of superimposing sameness over difference. The narrative and narration suggest and refute anthropocentric cross-species identification. A friction is produced between the paradoxical tendencies of seeing octopuses (and animals in general) as pure alterity and as mirrors of us. The filmed octopuses are pressed into a sexual imaginary—predicated on sexual difference and primal scenes/fantasies—that fails to be completely human. Moreover, the sexual imaginary is exposed as a sensuous bestiary—are not the purrs, coos, licks, and infections of other organisms enmeshed in our earliest experiences? (Lingis, 2000).

A second focus supersedes the first narrative—structured around tropes of the natural history documentary—guiding me into a world of magnification, a central concern of this film (Nichols, 1991; Mitman, 1999; Bousé, 2000). Things are too close, then too distant, hardly ever just right. The film continues to move through various size and distance scales. The view is interpretable, but always pulls and pushes. Throughout this aquatic film we are confronted with magnification as a trans-medium movement that produces dialectic between the familiar and strange. The film's continual use of magnification brings into focus the optical apparatuses that produce these perceptions: the enlarged image of arms and suckers, the extreme-close-up of the octopus's beak-mouth, and the abstraction of the first shot of the film. Not central, not familiar: while eyes might strain to stretch into those eight arms, into those dazzling colors, we are made aware of the apparatuses and species differences that define the image, prohibiting easy identification and body borrowing.

"NEAR AND FAR, LOVED AND REVILED, LOVING AND PERVERTED...."

Akira Mizuta Lippit interprets Haraway's octopus as an "oectopus." He states, "near and far, loved and reviled, loving and perverted, emotional and hysterical; each paradox marked visually by a unique legacy forming on the body an octo-paradoxy" (Lippit, 2005, p. 11). The Oedipus of Lippit's oectopus is a visual riddle, a blinding as story of repression, a riddle of desire. Lippit writes, "I am infected by another, by significant otherness" (Lippit, 2005, p. 9). Haraway deploys the colloquialism of "significant other" (as in my lover, my partner) into an insistence on difference—an ethical imperative that runs through much of Haraway's work. Lippit evokes the Oedipal scene—another interpretive that insists on difference—as what fuels Haraway's "reality takes shape in a grasp" (Lippit, 2005, p. 9). As such, "A hysterical invertebrate, an invert, pervert, *oectopus*" (Lippit, 2005, p. 10). Oectopus is the kissing cousin of Oedipus:

> It [oectopus] embodies in a phantasmatic body (a body that consists entirely of eyes and legs; a body that is less without organs than simply *without*) a speculative and spectacular visuality rendered by perversion and irreducible exteriority. It is a figure of deviant visuality, a scene from the outside but also of the outside that glares back in the full splendor of a perverse and impossible visuality: 'WHAT ARE YOU LOOKING AT' (Lippit, 2005, p. 13)?

Oectopus is visuality that is too exterior, impossible, a paradox. There is something of the oectopus in magnification. Epstein writes about magnification in film, "I am hypnotized. Now the tragedy is anatomical.... Muscular preambles ripple beneath the skin. Shadows shift, tremble, hesitate" (Epstein, 1993a, p. 235). Magnification indexes paradoxes: identification is abandoned for intimacy; scopic distance is replace with fetishistic nearness; and, the apparatus (primary cinematic identification, according to Christian Metz) over takes narrative (secondary cinematic identification). Magnification is political, the lens a political project. The film's recurring deployment of magnified images relies on light passing through multiple interfaces. It is important to note that these interfaces are not merely lenses that mediate between the light, the camera, and my eye. Interface then is not only about the inseparability of the observer and observed, but also about the ongoing relationship that produces its conditions of possibility. Interfaces, then, are always in process, always setting up zones of interaction bound in specific spatial-temporal configurations—interfaces are the ecotones of the eyes, "eyecotones." The magnified image requires a look that makes constant reference to the instrumentation of the image, binding the image to the apparatus. Haraway, in her essay "The Persistence of Vision," teaches us the importance of attending to this kind of binding—what she calls "webbing." She writes, "The 'eyes' made available in modern technological sciences shatter any idea of passive vision; these prosthetic devices show us that all eyes, including our own organic ones, are active perceptual systems, building in translations and specific ways of seeing, that is, ways of life" (Haraway, 1990, p. 190). Attention to the production of visuality foregrounds specificity and difference. We see the imprint of light—luminosity reflecting off the object and refracted into the chemistry of the photograph then further refracted into the emulsion of the film stock—through visible strata. The magnified view is disarticulated as impure and a composite of interpretations, technologies, and actions; magnification brings into question the relationships between subject and object, and knower and known.

However, the magnified image is not without visible kinds of boundaries. It gives, as Haraway writes, a "situated knowledge" (Haraway, 1990). Situatedness is a mode of embodiment—to be located within the production of space and place. That is to say, magnification materializes the workings of light, marking the presence of transparencies, exposing how the image is produced. But magnification is also always partial. Magnification brings into focus the entwinement of apparatuses and the failure of the possibility of apparent vision, the failure to provide the unmediated, distant, and whole story. Indeed, magnification troubles the fantasy of *true* visual access—and, as such, absence and partial truths are operative analogies for this trope. Magnified views are more akin to interferences or interactions than immediacies—the referent and representation are not distinct, static entities unto themselves; they are concretely and semiotically bound in active process. I see: *what's* seen is not simply inaccessible-to-the-naked-eye perspectives mediated by a lens, but rather folds of enacted perception, what Laura Marks describes as "mutually enfolded in material processes" (xxi) (Marks, 2002). The focus here is on what it is to inhabit, to *live in*, inorganic and organic mediation, not merely *looking at* independent objects in the world—although vision, visuality, and visibility are as central to the subjective dimensions of techno-bodily existence as they are to its objective dimensions.

Magnification it is not an innocent practice. Cartwright rightly suggests that magnification carries a history of surveillance. She is concerned that the microscope fragments, abstracts, and spectacularizes bodily images (Cartwright, 1995). The microscopic image promises a tantalizing peek into an inaccessible world, and colonization of the infinitesimal looms large. The magnified view through instrumentation has deep histories dating back at least to the sixteenth-century in Europe. Lacking in precision and clarity, many early magnifying tools—lenses, lace-making baubles, looking glasses, and others—were used for entertainment purposes. Technicians as beguiling recognized the magnifying lens, known for its distortions, perceptual confusions, and optical plays. The magnified image "subverted the norm of lucid, coherent, and stable bodies.... What appeared clear and distinct to the naked eye was exposed as chaotic or flawed under the microscope" (Stafford, 1996, p. 147). The colonial tendency to make "the unknown visible" was worried by interpretation, instrumentation, and the instability of the magnified object. The unaided eye could not see whatever was observed through the aggrandizing lens; the original could not be directly consulted (of course, eye and brain themselves yield a highly interpreted image and perception. The microscope adds only another layer to the "original" unaided perception). Magnification signaled a rupture in the natural order, a challenge to rationality. Initially, the incoherence of static forms produced a negation of positivism, a kind of profane illumination that required a solution. It wouldn't be until the latter part of the nineteenth-century that resolution and distortion were reworked for accuracy (Crary, 1992). Once relative lucidity and detail were secured, the compound microscope—with its multiple lens structure—was deployed as an analytical and diagnostic instrument to view the infinitesimal. However, the magnified image remains haunted by loss, just as it offers clarity. Visual displacement and dislocation vex empirical certainty regarding the enlargement—that which is magnified is always already imbued with blind spots or scotomas.

The close observer encounters the optics of the lens through which the illuminated minute is seen. "Close inspection" as a spatial practice, as a mode of encounter, defines magnification. By encounter, I mean the observer becomes embedded into the apparatus of "seeing"; that is, the observer's eye is "prosthetically augmented" (Cartwright, 1995, p. 84). That magnification constructs encounters, suggests that otherwise visually inaccessible domains become a space that the observer inhabits. This is not a metaphysical move as such (though it may have metaphysical implications). The observer visually enters into another scale in which there are no originals or copies. The relationship between the microscope and the observer is generative, even as it is refracted or bent. Their bodies—organic/inorganic or animate/inanimate—are fused in the production and refraction of magnified images. Magnification is the instantiation of broken and conjoined knowledge pathways through which the observer enters a scale not to her/his own measure.

Extreme close-ups as well as macro- and microscopic magnifications produce a discourse on space and perception: defamiliarization and then re-meeting on other terms. It is true that the magnified image field and observer are fused, but the question remains: is "Perception... unhinged from the sensory body..." (Cartwright, 1995, p. 82)? Perception, through magnification, is part of a relay of viewing that engages mediation. To view the image is to use cornea, light receptors (in retina), ocular nerve, and the neurobiology of the brain (and all those inorganic apparatuses such as lenses, projectors, film stocks, etc.). This kind of perception is always (and already) bound to the body—a direct address of the body by relays of "bodies." Magnification is not necessarily or obligatorily used to police the body, or bind it to an abstract set of data. A surveillance of the body through magnification can never be totalizing, nor free from the apparatus (and ideology of that apparatus) of its construction, never unhinged from matter of its making. Magnification is not a view from nowhere. Magnification makes apparent cinematic space, extending the observer into the space by yoking the apparatus to the extension. That is, the observer (me, for example) is compounded with the apparatus; the apparatus via magnification extends the optical reach of the observer. The magnified image becomes translated into bodily experience. Perspective and image no longer function as discrete units, but as interfaces in contact, engaged in a constant action of reciprocal mis/re-alignment and de(in)flection (Merleau-Ponty, 1968). The apparatus is doubly bound to the extension that it initially produces. The viewing eye is submerged into another scale, and yet we are clearly on the other side; we are inside and outside, within and nearby. This indeterminacy articulates the ambivalent nature of magnification.

In microscopy, the incident wave and the refracted wave make an angle of incidence and an angle of refraction. Take for example, a double-sided plano-convex lens positioned in front of an object: light passes through the lens—made of a pellucid medium with the measurement of a transparent medium's ability to bend light—and bends according to the curvature, distance, and thickness of the lens. The bending of the light by the lens alters its direction. The location of the light reflected off the object is shifted by the degree of the medium's index (the ability of light to move through a particular medium). The form of the lens—material and thickness—determines how the redirected light will converge on smaller and smaller portions of the object. This convergence is called a focal point: points on the axis of a lens to which incident light rays are united or from which they appear to be diverged. Consequently, these smaller portions are enlarged.

The privileging of magnification in the *Love Life of the Octopus* transforms my reflective tendency to project and identify with an image that is not me. Generally, cinematic reflectivity engages the screen as a mirror that reflects spectator's imaginary selves (though seldom their own body). A relay of looks stitches spectator into the formal and thematic space of moving images. The reflective cinematic image engages psychical processes of representation that invoke identification with the characters or events of the image, or the cinematic apparatus itself. In contrast to reflectivity, magnification records the promiscuousness of light, the overt bending and disarticulation of light that does not duplicate images, but transforms them. As in *The Love Life of the Octopus,* magnification bends me away from the reverie of reflectivity, making me fetishistically aware of the apparatus, of materiality, and also proximity of bodies: octopus to octopus, lens to octopus, me to octopus.

The film, then, cuts to a cinemacroscopic—a magnified view that shows the very small in great detail—close-up of a captured crab passing from octopus sucker to sucker on its way to the beak-like mouth—I hear sucks, slurps, and grinds. The screen is saturated with orange. The sensuous meal takes place in the lower left of the screen—not central, not familiar. The film cuts; the camera is immersed underwater. Illuminated bits: particles in the water with different refractive indexes float in front of camera. The camera then returns to a cinemacroscopic look at the beak-mouth, offering a magnified view. It is as if the octopus, offering closeness, a proximity that is unsettling, might incorporate the camera itself. Here, the objectifying possibilities of distance are violated; transcendence is out of the question and out of the frame.

The Love life of the Octopus stresses the carnal response and the sensuous affect of the resistant image (a refractory image) that mixes delight and dread, attraction and repulsion, visibility and obscurity (Milstein, 2012). The film sustains rather than occludes this refracted tension and refractory friction at its source. Refraction, then, as exemplified in this film, carries a sensuous address (a fleshly appropriation) and stimulated, corporeal experience (corporeal light? carnal light?). As sensuous illumination, then, what is called refracted light opens up and exposes the inhabited space of sense experience as a condition of possible (if fractured) embodiment. Radical entailments of incarnate light: refracted light involves the visible, audible, kinetic aspects of sensible experience to make sense visually, audibly, and haptically. *The Love Life of the Octopus*, in form and "content," manifests through magnification a living interchange, a fleshy dialectic, that renders light visible; that is to say, light is made verb, it conjugates perception and expression.

SURREAL SCIENCE

While more ink has been spilled on Painlevé's biography than Hamon's, he is also a relatively unknown figure in film history. References to his work are rare: Brunius (1949); Barnouw (1974), and more recently Burt (2004) are among the few who have offered Painlevé's work as something more than a footnote. His story starts, for our purposes, in 1921, when he entered the Sorbonne to pursue studies in medicine. After a short-lived career as a medical student, he turned his attention to zoological sciences. During his time at the Sorbonne, "Painlevé coauthored a paper on the color staining of glandular cells in chironomid larvae... and presented it to the Académie des sciences" (Berg, 2000). Chironomid larvae are often found living in the mantle cavity around the gills, gonads, and siphonal tissues of various species of mussels. Painlevé's technique allowed for greater visualization of the cellular structure of these symbionts. He further pursued his zoological interests during an internship at Roscoff, a marine biology station. In 1928, Painlevé presented his first film, *The Stickleback's Egg: From Fertilization to Hatching,* to the Académie des sciences. The response to his film was overwhelmingly negative. One botanist, infuriated, stormed out, declaring, "Cinema is not to be taken seriously." This reception was not unexpected. Cinema had yet to prove itself as something other than superfluous entertainment full of optical illusions and trickery. The relationship between fiction and documentary was still molten. The scientific community was not yet convinced that film could document without altering, distorting, or transforming the filmed organism and/or its biological processes.

Little to nothing has been written on Geneviève Hamon. The daughter of political radicals and an activist herself, Hamon and her contributions have almost disappeared from the history of film scholarship, even though Catherine Tchernigovtzeff, a friend and research colleague of Painlevé's, asked, "Would a single film have existed without Ginette's devotion?" (Berg, 2000, p. 11). She is part of that growing list of women artists and scientists who have been "discovered" because they are left out of history. In many of her collaborative film projects, her trace is left only in the final credits, while her ambition and work shaped the piece throughout. For example, she was instrumental in "operating equipment, designing sets, and caring for the animals" for many of the films that are singly accredited to Jean Painlevé (Berg, 2000, p. 10).

Though they were never devoted to the Surrealist movement with its emphasis on psychoanalysis, automatism, the unconscious, and dreamwork, their films engage a surrealist aesthetic that in James Clifford's words "values fragments, curious collections, unexpected juxtapositions that work to provoke the manifestation of extraordinary realities" (Clifford, 1988, p. 118). According to Robert Short, cinema was "hailed as the elective surrealist means of expression on account of its power to disturb by betraying the expectations of the 'everyday eye' and its power to inspire by imposing original visions" (Short, 2003, p. 6). The Surrealist film movement—small and short-lived—was interested in how cinema might function as a "threat to the eye, and more radically, to the two eyes of the spectator:

one eye being the organ of sight, and the second 'I,' the spectator's personal identity" (Short, 2003, p. 6). These threats were never meant to be "terminal blindings," but were deployed to create fissures in the familiar, to dislodge commonsense (Short, 2003, p. 6). Surrealists were concerned with the mixing of the sexual in the visual, of the unconscious in the real. Films such as Luis Buñuel and Salvador Dalí's *Un Chien andalou* (1929), Man Ray's *L'Etoile de mer* (1928), or Antonin Artaud and Germaine Dulac's *La Coquille et le clergyman* (1928) construct the conscious world as a site of conflicted forces. Nature is constructed as a generative force of decomposition—inertia and entropic/regression toward inanimate/death in sexuality. (Interestingly, most cephalopods, including octopuses, die after reproducing).

To visualize erotically infused death as embedded in dream-life, Surrealists called for a dynamic image, one that made everyday objects, as André Breton suggested, into the "marvelous real." "It was a matter of discovering it, not inventing it" (Breton, 1988, p. 14). A tension—evident in Hamon and Painlevé's work—was produced between familiarizing oneself with materiality while disrupting a comfortable regularity. Arguably, the practice is about resolution, about bringing into focus at varied scales what was naturalized and imagined to be commonplace. Surrealist film practices encouraged observer involvement, not through representation (what Antonin Artaud called the "abyss"), but rather through an appeal to a direct transplantation of the image "into the film spectator's ocular nerves and sensations" (Barber, 1980, p. 46–47). This line of reasoning brings together the observer's identification with the cinema, which is constituted as a specular and psychical process, and an embodiment that is not abstracted from the lived body, nor merely mediated through language. A *dépaysement*: Surrealists saw the cinematic image as a dense object that physically engaged the observer's body—a collision of observer and observed image. This collision was not just a fantasy—though certainly fantasy is at work here. The cinematic image was imagined as an apparatus of psychical and bodily production. Transformation of the literal matter of the body was more important than visual narrative or representation. Surrealist cinema, as did Surrealist art in general, enacted the uncanny collision of seemingly desperate elements: representation/referent, living/non-living, human/non-human, mobile/immobile, real/surreal (Read and Breton, 1971; Short, 1980; Alexandrian, 1985).

Through an idiosyncratic interplay of visual and laboratory experimental practices, Hamon and Painlevé captured the attention of the Surrealist movement (Berg, 2000). For example, their film *The Vampire* [*Le Vampire*] (1945) is a sanguinary satire on Nazism told through the natural history of the vampire bat, *Desmodus rotundus*. Hamon and Painlevé juxtaposed the radical jazz of Duke Ellington with sequences from Murnau's *Nosferatu* and from biological sciences to illustrate threat, the traveling of contagion, and political resistance[4]. *The Vampire* is an unsettling imagistic and sonic encounter loaded with symbolic meaning and political intent. But, at the moments in which the observer is presumed to understand the message, the film reorients the focus, revealing just "how monstrously different this other life form actually is" (Rugoff, 2000, p. 51). The film shows that even the act of symbolism seems perversely anthropocentric, a house of human-centered mirrors, directing attention away from the "marvelous" diversity produced by speciation. The film argues that the vampire bat is not an easy, viable allegory for anti-Semitism and its infectious consequences. The vampire bat is not just a screen for identification, mis-identification, or representational strategies. Rather, the film shows monstrous differences, how those differences emerge in cultural practices, and how through these emergences, bat and human come to co-constitute each other in ways that exceed analogy and anthropomorphism even as the film plays with these protocols.

Many of Hamon and Painlevé's films were experiments with underwater cinematography that were, in the words of Barnouw, "sometimes in speeded, sometimes in slowed motion, often hugely magnified, and always artfully lighted, producing astonishing studies in the surrealism of natural phenomena, with their bizarre shapes and movements"(73). While their films deploy surrealist techniques, their focus was not on art *per se* but on "documenting" natural history. They constructed underwater cameras and aquarium staging that allowed them to look into the unfamiliar worlds of common but strange marine organisms. For underwater filming, Painlevé enclosed a Sept camera in a waterproof box fitted with a glass plate for the camera's lens (Berg, 2000, p. 23). The invention of an underwater breathing apparatus composed of a demand valve with a high-pressure air tank (a modification of the then existing Rouquayoi-Denayrouse pump tank system, which allowed only a few minutes of untethered breathing) permitted them greater freedom without the restrictions of external air pumps. "For Painlevé, [Yves] Le Prieur's new diving apparatus seemed to offer an entrance into a kind of utopia of underwater living. Indeed, he dreamed of one day creating a studio—complete with film equipment, scientific apparatus, and technicians—entirely underwater"(Berg, 2000, p. 29). The underwater camera enabled the presentation of a surreal technoscientific look, allowing new visual extensions into the watery domain.

In 1934 Hamon's and Painlevé's film *The Seahorse* [*L'Hippocampe*], used these underwater viewing technologies to show how the female of *Periophthalmus* puts the nipple of her cloaca into a specialized pouch on the male's belly, transferring over 200 eggs into the male's body. With beautiful close-ups and dazzlingly lit images, the film works at many levels to trouble categories of many kinds. Hamon and Painlevé saw the seahorse as a hopeful challenge to conventional anthropocentric notions of sex, sexuality, and the labor of generation. The film takes and offers much pleasure in watching the male seahorse laboring and contracting on the bottom of the aquarium. The seahorse, Painlevé wrote, "was for me a splendid way of promoting the kindness and virtue of the father while at the same time underlining the necessity of the mother. In other words, I wanted to re-establish the balance between male and female" (Berg, 2000, p. 23). Dominant heterosexual, masculinist fantasies that define much Surrealist imagery were reworked to invoke a different kind of sexual economy, one not predicated upon essentialist, human-centered, sexual difference.

---

[4] There is a cephalopod appearance in this film. An octopus crawls/floats over a human skull. The narrator says, "The grace and terror of gestures...."

Hamon and Painlevé looked toward animal worlds for alternative ways to think about anthropocentrism and anthropomorphism. Extravagant sexual displays of animals offered opportunities to examine plays of similarity and difference, the well-known and the strange. Excessive, erotic, and exotic stories of hermaphroditism, asexual cloning, sexual dimorphisms, and courtships provided the stories for these filmmakers to construct potent and astonishing science-*as-fiction* worlds. It is possible to suggest from their films that Painlevé and Hamon imagined that animals might offer stories that could shape and reshape the observer's understanding of themselves. Through their use of alternative imaging technologies, Painlevé and Hamon produced films in which animals act upon observers, producing a breach in the old stories of human domination and animal victimization. Their films are not simply documents of these organisms, but rather accounts of encounter.

It would be inappropriate to describe the treatment of the organisms in their films as ideal. The octopuses in *The Love Life of the Octopus* were dissected, enclosed in aquariums, subjected to experiments, and otherwise under the constant fascinated gaze of their human captors. As remarkable as their film practices may be, Painlevé and Hamon participated in the persistent surveillance of animal physiology and behavior; their *oeuvre* is predicated on the dying, reproducing, and living bodies of animals[5]. It is not apparent from these films that Painlevé and Hamon had any hope of rewriting some of the more conventional human/animal relations—these animals were clearly *used*. Throughout most of the film the presence of people is erased. This absence reinforces some of the old habits of the nature documentary (the effacement of presence in the frame but its assertion through editing).

"I," OCTOPUS EYE

In this film, the octopus is asymmetrically bound, but the octopus presses back. Consider the moment early in the film when an octopus traverses the interacting interfaces of air and water, slithering across densities. The camera follows, but not through water. It looks through aquarium glass. This layer of glass further refracts (though not precisely magnifying) the image. However, the framing of the aquarium obscures the enclosure. The off-screen space masks the glass boundaries of the aquarium, giving the illusion of greater space, but the octopus clings to the aquarium glass, pressing its suckers to the screen. (Of course the editor, not the octopus decided upon the inclusion of this image.) By clinging to the glass wall, the octopus exposes it's staging, it's enclosure (as does the editor). I am not seeing an unmediated image of "wild" octopus behavior—like most natural history documentaries inform their observers that they are privy to. At every level of the film contiguity grounds all the apparatuses and actors. In this film, the octopus is not some abstract representation but rather an actor (en-actor) in "intra-acting" with the apparatuses: lens, aquarium, camera, film, screen, and myself.

In *The Love Life of the Octopus* the octopus is subject and object, a figure of action (agency?) and a captive. But how can we talk critically about the agentive octopods of this film? Agency/actorship needs to be qualified in terms of power and the asymmetries therein. In other words, the octopus does not choose to be filmed, there is no agreed upon contract. The octopus cannot speak back to the curiosity of us simians. The octopus does not possess an innate or static agency. This definition of power leaves the octopus with none. However, if we see agency in a relational sense "emerging as an effect generated and performed in configurations of different materials," then we can see the octopus with some agential power (Philo and Wilbert, 2004, p. 17). Agency is always relational, not the property of a fixed subject. Thus, one does not "have" agency. Rather, one is in an agential relation. So what can we say about the octopus?

I want to return to that earlier image of the octopus clinging to the aquarium glass. First, what about the aquarium, that transparency that further mediates light touching the organism, with all of its own refractive properties? The aquarium glass, though it forms distinctions between inside and outside, also foregrounds, like the microscope, space and mediation. In fact, the glass is metonymic of the microscope. Layers of glass—slide and cover slip—and a drop of liquid—sometimes water, sometimes oil at higher powers of magnification—allow me into inaccessible worlds. And as with the microscope, what one sees through the aquarium glass is distorted by refraction. Refraction, through water, relocates the image field on either side of the aquarium/water/air interface. I see, as does the octopus for that matter, the aquarium space indistinctly.

There are moments in the film when the aquarium's presence seems to be slightly masked. The framing of the image pushes the aquarium edge off screen. I am led to believe that I am beneath the surface. But, in a precise moment I see the smaller octopus give away its enclosure, its stage, and a direct address of sorts, reminding me where I am. The suction cups of the octopus cling and hold to the glass as it's twisting arms become daring but tentative, approaching the larger octopus. It is a gestural move. It is an unscripted action. The octopus does not transgress the boundaries of its aquarium. However, the octopus does press against the fourth wall of its stage, the wall toward me. Its suckers cling to the off-screen space, to the apparatus of its image. I am led to consider the framing and staged enclosure of the octopus in this scene. Through this action, dare we say "acting," the octopus manipulates the appearance of its own image. The unintended gesture shapes how the viewer understands the space in which the octopus is forced to perform. So, no, the octopus doesn't speak, but it does effect and affect the workings of cinema. The octopus engages its own image in the visual field.

---

[5] Painlevé's own perverse curiosity (and betrayal) is seen in the following statement. "In 1925, during an internship at Roscoff, I would bring an egg to this octopus at 11:00 every morning. She soon began to recognize me by my shirt. Whenever she saw me, she turned black; the three layers of her skin—blue, red, and green—would swell with pleasure. Then she went off to eat her egg. We got along very well. But then one day, out of perversity, I brought her a rotten egg. She turned totally white. In extreme fury, an octopus's cells contract and the white of the underlying dermis appears. With one of her tentacles, she threw the egg back at me over the aquarium's glass window. She never greeted me again. Instead, she'd retreat to the back of the aquarium and turn white. I realized then that she had memory. This mollusk was as intelligent as a human (Berg, 2000).

Sustaining this inquiry into octopus acting, obligatory filming strategies also foreground the octopus's influence on the image. This is to say, aquatic organisms, such as the octopus, necessarily highlight cinematic instrumentations by requiring specialized filming techniques. Images of the octopus bind its environment—laboratory aquarium or intertidal zone—to the camera. The camera must accommodate the biology of the octopus. In the lab/film studio, the camera is always situated in relation to the aquatic environment of the octopus. The camera sits outside the aquarium glass. Layers of refracted light—lens, glass, air, water—illuminate the octopus in the aquarium stage. Although the octopus does not actively negotiate it's framing, its physiology requires careful negotiation to secure a "good enough" image. Likewise, in the intertidal, the camera is limited by its ability to follow the octopus into tide-pools. Reflected and refracted light off the surface of the water hinders visibility. Yet, when the octopus is crawling through rockweed, the camera—a primarily terrestrial apparatus—is at home. In these moments of filming in air, the camera can have distance and the capability to pursue. The camera can hide in the structure of cinema. But when the octopus returns to the water, the camera is vulnerable to distortions. The aquatic camera, at best, records an image that foregrounds its own constructedness in the image field. And as viewers through the camera lens, we too are bound in these couplings, hooking us into the image field and the environment of the octopus. This is relational agency.

What else can we say about the role of the octopus in effecting its own image? Jonathan Burt writes, "This rupturing effect of the animal image is mainly exemplified by the manner in which our attention is constantly drawn beyond the image and, in that sense, beyond the aesthetic and semiotic framework of the film" (Burt, 2004, p. 12). We might consider this rupturing effect as a product of symbolism, a purely anthropocentric effect. However, what Burt is suggesting, and I think convincingly, is that while the animal is figured through an array of conceptual and political frameworks, the bodies of the animals configure these frameworks[6]. That is to say, the physiological differences of the animal in question shape its conceptualization in the cultural imagination. The octopus's body, behavior, and lifeway help produce its figuration in images. The octopus's many armed, shape shifting, color changing, and quick learning shape and reshape the construction of "the octopus" in language and culture.

Moreover, like the octopuses in Painlevé and Hamon's film, the image of the animal exceeds familiar representational strategies. The referent becomes irrevocable; the referent becomes "real." This does not mean that the referent can be recovered from the representation—this is not an argument about returning representations to whole referents. The image of the octopus foregrounds the broken light between the cinematic apparatus and the lived organism; they are certainly bound to each other, but only through incompleteness and partiality. More a light-scar than a trace, the animal image foregrounds the failure of the apparatus in truly "capturing" the organism. Idealization of human-octopus relations is mitigated by the fact that *The Love Life of the Octopus* constantly exploits the limitations of seeing (refraction) and plays on the disjunctions between what is seen/what is not seen, what is known/what is not known. The film is about concealing and providing—I am provided with representation at its limits.

Refracting (what Burt calls rupturing) representation, the image offers a partial experience of the organism beyond the apparatus. This is not to suggest that the octopus is merely metonymic of oceanic ecosystems, linking the organism back to that place called "nature." Rather, the semiotic functions of the film that rely on precise kinds of selecting and combining (paradigmatic and syntagmatic operations) turn away from the conventional framework of narrative continuity, foregrounding the photo-chemical register of the "real" organism. That, as Burt suggests, "the animal image can so readily point beyond its significance on the screen to questions about welfare suggest that the boundaries of film art... cannot easily delimit the meaning of the animal within its fictions" (Burt, 2004, p. 13). As it is now impossible to disentangle ecosystems, bodies, and technologies, so too is it impossible to separate out mediated aspects of human-animal relations. The breaches, wounds, and scars between representation/real, human/animal, and technology/bodies become part of the apparatus. The consequence of these relays of rupture/loss/connectivity suggests that, "We are looking from within nature, and not at nature" (Burt, 2004, p. 47).

This notion of fractured (or refracted) light is suggested in an earlier image, where we see an extreme close-up of an octopus's eye. Burt writes, "Film effectively turns the animal eye into a camera, a non-human recording device." He is interested in how animal films tend to offer close-ups of the animal eye. Looking through the history of cinema and its foundation in animal images, he suggests that these close-ups propose that the animal's eye is the closest to "the technology that produces it" (64). The octopus's eye, for our specific purposes, is metonymic of the lens rather than the camera. The lens with its refractory qualities, its variation in refractive indexes, more precisely describes the octopus's eye. The octopus eye is refractory, as is our own. Light is admitted through the pupil, passing across the lens where it is altered, focused, refracted, then on to the retina. The close-up of the octopus's eye links it to the refractory nature of *The Love Life of the Octopus* itself: scale changes and interfaces. The movement of light through the lens, its changeability and variability, is conflated with the octopus's own mobility. The octopus eye also foregrounds the limits and distortions of its lens. In other words, like the lens, the octopus eye does not allow for a knowing "look." All I can know for sure is that layers of mediation both separate

---

[6] Burt has also started asking similar kinds of questions about "animal" images. We disagree in important ways; he is interested in how the image of the "animal" dissolves mediation. He argues that the "animal" creates a more affected viewer, unable to see cinematic structures. I, on the other hand, cannot help but notice how Painlevé's and Hamon's film about octopuses produces greater awareness of the mediation, hailing a critical viewer into the environment of the image and its referents. We differ in scale and conclusion. While this is a significant difference, we have reached similar conclusions on the role of, in his terms, "animal agency." I don't use that term, fearing its tendency toward subjectivity, preferring "actor" with its connections with Bruno Latour (Actor Network Theory) and James Clifford's intriguing account of Sea Otters as historical actors in Clifford (1997). We both want a more active non-human animal.

and bind the octopus's eye to mine. The octopus's eye, again like the lens, is a pathway through which we encounter the octopus in the visual field.

FROM EGG TO EYE

Depth, as a cinematic technique that foregrounds the background, is utterly collapsed in these magnified views. The developing oblong egg that fills the screen in the final third of the film seems two-dimensional, squashed into mere surface. The microscopic image is not deep, although fathoms of potential bio-technic information reside therein. What the image lacks in depth of field, however, it provides in depth of the observational space: the microscopic image foregrounds the space between the image and myself. These moments point to how relations are mediated by a spectrum of spatial settings and processes. If the film suggests a refractory space, then it offers an alternative to how *Octopus vulgaris* is represented.

I experience something other than "the impression... that animals are merely passive surfaces on to which human groups inscribe imaginings and orderings of all kinds" (Philo and Wilbert, 2004, p. 17). The octopus images are determined for observers and octopuses; the octopuses have no *say* in how they are represented, and observers receive a particular coding of the images. But the refracted space is a particularly slippery site of legibility. Might the refracted image be a metaphor for the familiar/strange quality of the octopus? Perhaps, the mediation and the partiality of magnification parallel the position of the octopus. I am confronted with *focused* difference, a recognition that cannot be easily repressed. Perhaps refraction can assist in this work, bending the mirrored image of self-back into the body in space, re-imaging the intra-active relationship between viewer, technology, and octopus in terms exceeding identification and representation. The lens is focused on making the unfamiliar visible, making the familiar strange. In this shifting focus the play of sexuality begins: interest, cohabitation, discomfort, distortion, and magnification of focus from self-location in relation to the cephalopod. *The Love Life of the Octopus* is about how sexuality—as suggested by refraction/refractory—is both a projection onto the animal, but as Lippit's oectopus suggests, as well as space in which the organism we call octopus may show up. Consider how the film opens with a man/octopus image, but ends in the presumed world (womb) of the octopus—"man" is literally shifted off-screen through the duration of the film. Refraction is not a framework, but a pathway. But importantly, it is the optics of refraction—its partiality, its intimacy—that produces sexual site in which fantasy (e.g., Haraway, Burroughs, Lippit) layers with un-representable sexuality.

The Love Life of the Octopus holds up to us inarticulate bodies and behaviors as if to ask, "Can you match that?" Painlevé's and Hamon's film rejects conventional modes of identification, as if to say: "Identification isn't enough." Moments of play such as these highlight both difference and familiarity, inviting us to experience—but not to identify with—the octopus. The film addresses us as "not octopus," it does so by portraying an experience that feels immediately not one's own. The filmic space extends to us by soliciting, cajoling, and seducing, but only to offer a space of heterogeneity, differences, and fragmented coherences. Adopting a seemingly critical stance, the film wants the spectators to see themselves in relation to the octopus—to see our profound otherness while playing with familiarity. The refracted image—like the metaphor of the octopus's gaze—is a sexual provocation. The spectator sees parts of the magnified octopus, but also see the mediation of the image and the inherent lack of that mediation (this tension is also paralleled in the failure of human cross-species identification) and the ongoing nature of the encounter. The magnified image is invasive and surveilling, but it is also incomplete. "... [M]agnification acts on one's feelings more to transform than to confirm them..." (Epstein, 1993b, p. 239). The refracted image and the filmed octopuses offer a productive alternative to both radical alterity and ultimate knowability, but only through sexuality and its structuring of subjectivity. What ought to be foreclosure of organismal presence, spectatorial sexuality, is the most promising site for experiencing the force and activity of the organism. As such, there pulses a flow between distance and closeness—they are not produced as incommensurable spatial relations. The refracted image makes apparent the space between the spectator and the representation—the space folds, building toward both the octopus and myself. *The Love Life of the Octopus*, witnessed in title alone, is an erotic narrative; octopus, camera, filmmakers, and spectators are conjugated in the refracted space—each is a wet reach.

NOTE

A shorter version of this essay, "Enfolded Vision: Refracting the Love Life of the Octopus," first appeared in the journal *Octopus* (2005). In that essay, I offered a cinematic theory of refraction (a supplement to reflection and mirroring) that attended to the physics of refraction, particularly magnification, to propose a bodily and sensuous spectatorship.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

REFERENCES

Alexandrian, S. (1985). *Surrealist Art*. New York, NY: Thames and Hudson.

Barad, K. (1999). *"Agential realism: feminist interventions in understanding scientific practices," in* The Science Studies Reader, ed M. Biagioli (New York, NY: Routledge), 1–11.

Barber, L. (1980). *The Heyday of Natural History: 1820–1870*. New York, NY: Doubleday & Company.

Barnouw, E. (1974). *Documentary: A History of Non-Fiction Film*. Oxford: Oxford University Press.

Berg, B. (2000). "Contradictory forces: jean painleve, 1902-1989," in *Science Fiction: The Films of Jean Painlevé*, eds A. M. Bellows, M. McDougall, and B. Berg (Cambridge: MIT Press), 2–47.

Berrill, N. J. (1983). The pleasure and practice of biology. *Can. J. Zool.* 61, 947–951.

Bousé, D. (2000). *Wildlife Films*. Philadelphia, PA: University of Pennsylvania Press.

Breton, A. (1988). *Mad Love*. Trans. by M. A. Claws. Lincoln: Bison Books.

Brunius, J. B. (1949). "Experimental film in France," in *Experiment in Film,* ed R. Manvell (London: Grey Walls Press), 60–112.

Burroughs, W. (1995). "Octopus," in *Living With the Animals,* ed G. Indiana (Boston, MA: Faber and Faber), 1–8.

Burt, J. (2004). *Animals in Film*. London: Reaktion Press.

Cartwright, L. (1995). *Screening the Body: Tracing Medicine's Visual Culture*. Minneapolis, MN: University of Minnesota Press.

Clifford, J. (1988). *The Predicament of Culture: Twentieth-Century Ethnography, Literature, and Art*. Cambridge: Harvard University Press.

Clifford, J. (1997). *Routes: Travel and Translation in the Late Twentieth Century*. Cambridge: Harvard University Press.

Crary, J. (1992). *Techniques of the Observer: On Vision and Modernity in the 19th Century*. Cambridge: MIT Press.

Epstein, J. (1993a). "Magnification," in *French Film Theory and Criticism: A History/Anthology, 1907-1939, Vol 1*, ed R. Abel (Princeton: Princeton University Press), 235–241.

Epstein, J. (1993b). "The senses," in *French Film Theory and Criticism: A History/Anthology, 1907-1939. Vol. 1*, ed R. Abel (Princeton: Princeton University Press), 241–246.

Freud, S. (1995). *The Basic Writings of Sigmund Freud*. New York, NY: Modern Library.

Haraway, D. (1989). *Primate Visions: Gender, Race, and Nature in the World of Modern Science.* New York, NY: Routledge.

Haraway, D. (1990). *Simians, Cyborgs, and Women: The Reinvention of Nature*. New York, NY: Routledge Press.

Haraway, D. (2003). *The Companion Species Manifesto: Dogs, People, and Significant Otherness*. Chicago, IL: Prickly Paradigm Press.

Hayward, E. (2010). Fingeryeyes: impressions of cup corals. *Cult. Anthropol.* 25, 577–599. doi: 10.1111/j.1548-1360.2010.01070.x

Lane, F. W. (1960). *Kingdom of the Octopus: Life History of the Cephalopoda*. New York, NY: Sheridan House.

Laplanche, J. (2015). *The Temptation of Biology: Freud's Theories of Sexuality*. Trans. by D. Nicholoson-Smith. New York, NY: Unconscious in Translation.

Lingis, A. (2000). *Dangerous Emotions*. Berkeley, CA: University of California Press.

Lippit, A. M. (2005). Oectopus. *Octopus* 1, 9–13.

Marks, L. (2002). *Touch: Sensuous Theory and Multisensory Media*. Minneapolis, MN: University of Minnesota Press.

Merleau-Ponty, M. (1968). *The Visible and the Invisible*. Trans. by A. Lingis. Evanston, IL: Northwestern University Press.

Metz, C. (1986). *The Imaginary Signifier: Psychoanalysis and the Cinema*. Bloomington, IN: Indiana University Press.

Milstein, T. (2012). "Banging on the divide: Cultural reflection and refraction at the zoo," in *Perspectives on Human-Animal Interaction: Internatural Communication*, ed E. Plec (London: Routledge), 162–181.

Mitman, G. (1999). *Reel Nature: America's Romance with Wildlife on Film*. Cambridge: Harvard University Press.

Nichols, B. (1991). *Representing Reality: Issues and Concepts*. Bloomington, IN: Indiana UP.

Painlevé, J. (1965). *Love Life of the Octopus.*

Philo, C., and Wilbert, C. (2004). *Animal Spaces, Beastly Places*. New York, NY: Routledge Press.

Read, H. E., and Breton, A. (1971). *Surrealism*. London: Faber and Faber.

Rugoff, R. (2000). "Fluid mechanics" in *Science is Fiction: The Films of Jean Painlevé*, eds A. M. Bellows, M. McDougall, and B. Berg (Cambridge: MIT Press), 48–57.

Short, R. (1980). *Dada and Surrealism*. London: Octopus Books.

Short, R. (2003). *The Age of Gold: Surrealist Cinema*. London: Creation.

Stafford, B. M. (1996). *Artful Science: Enlightenment Entertainment and the Eclipse of Visual Education*. Cambridge: MIT Press.

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Communication

REVIEW
published: 29 August 2018
doi: 10.3389/fcomm.2018.00038

Cephalopod Gastronomy—A Promise for the Future

Ole G. Mouritsen[1]* **and Klavs Styrbæk**[2]

[1] Design and Consumer Behavior and Nordic Food Lab, Department of Food Science and Taste for Life, University of Copenhagen, Frederiksberg, Denmark, [2] STYRBÆKS, Odense, Denmark

Cephalopods, specifically Coleoidea (squid, octopus, and cuttlefish), have for millennia been used as marine food by humans across the world and across different food cultures. It is particularly the mantle, the arms, the ink, and part of the intestines such as the liver that have been used. In addition to being consumed in the fresh and raw states, the various world cuisines have prepared cephalopods by a wide range of culinary techniques, such as boiling and steaming, frying, grilling, marinating, smoking, drying, and fermenting. Cephalopods are generally good nutritional sources of proteins, minerals, omega-3 fatty acids, as well as micronutrients, and their fat content is low. Whereas being part of the common fare in, e.g., Southeast Asia and Southern Europe, cephalopods are seldom used in regional cuisines in, e.g., North America and Northern Europe although the local waters there often have abundant sources of specific species that are edible. There is, however, an increasing interest among chefs and gastroscientists to source local waters in a more diverse and sustainably fashion, including novel uses of cephalopods to counterbalance the dwindling fisheries of bonefish, and to identify new protein sources to replace meat from land-animal production. The focus of the chefs and gastroscientists is on texture and flavor properties of the different cephalopods being subject to a variety of culinary transformations. Combining these trends in gastronomic development with the observation that the global populations of cephalopods are on the rise holds an interesting promise for the future.

Keywords: cephalopods, food, gastronomy, gastrophysics, sustainability

OPEN ACCESS

Edited by:
Lindy Holden-Dye,
University of Southampton,
United Kingdom

Reviewed by:
Angelique Christine Paulk,
Massachusetts General Hospital,
Harvard Medical School,
United States
Andrea Tarallo,
Stazione Zoologica Anton Dohrn, Italy

***Correspondence:**
Ole G. Mouritsen
ole.mouritsen@food.ku.dk

Specialty section:
This article was submitted to Science and Environmental Communication, a section of the journal Frontiers in Communication

Received: *16 February 2018*
Accepted: *10 August 2018*
Published: *29 August 2018*

Citation:
Mouritsen OG and Styrbæk K (2018) Cephalopod Gastronomy—A Promise for the Future. Front. Commun. 3:38. doi: 10.3389/fcomm.2018.00038

INTRODUCTION

There is archeological evidence that humans have caught cephalopods for food in the Mediterranean region for at least the last 4,000 years. The Egyptians and later the Greek and the Romans lowered ceramic amphorae tied to a string to the bottom of the sea and waited for an octopus to use it as a den. Other techniques use traps with a bait. At Hawaii octopus were caught on hooks, and squid and cuttlefish have around the world for centuries been captured by nets, e.g., using light to attract squids at night. Cephalopods have been part of the daily fare in coastal areas around the world, in particular in Southeast Asia and in Southern Europe for millennia. In ancient Greece, both octopus, cuttlefish, and squid also entered in large formal banquets.

It is probably in the Far East, in particular in China and Japan, where there are the richest traditions for consuming cephalopods. The Japanese are likely to be the people who value cephalopod food the most, not least octopus. Today Japan is the country that consumes more octopus per capita than anywhere else in the world.

For obvious reasons, there is not much information available regarding how the ancient food cultures prepared the various kinds of cephalopods for food. The famous Roman gourmet and hedonist Marcus Gavius Apicius (25 BCE—37 CE) is credited for a cookbook De re coquinaria that is the oldest known and existing cookbook from the Antique. In Apicius' book there is a recipe for octopus with pepper, lovage, ginger, and the Roman fish sauce garum (Grocock and Grainger, 2006). There are only few surviving manuscripts with recipes from the Middle Ages. In a handwritten manuscript from the Fourteenth century (Schweid, 2014) an anonymous writer from the kingdom Aragon presents a Catalan recipe for octopus filled with its own arms together with spices, parsley, garlic, raisins, and onions, and prepared over open fire or in an oven.

Upon the invention of printing, more cookbooks were seeing the light of day and often recipes with cephalopods appear, mostly with octopus; e.g., from the Sixteenth century a Catalan recipe for baked octopus and an Italian one using boiled, roasted, and marinated octopus (Schweid, 2014). A well-known example is the famous *pulpo à la Gallega*, a national dish of Galicia, where there is an abundance of octopus in the waters. Dried octopus has also there for centuries been used as a commodity for trading with people inland.

Today the annual catch of cephalopods around the world amounts to about 4.8 million tons (FAO, 2014) and protein from cephalopods covers about 2% of humans' global consumption of protein. In this light, it is striking that compared to the dramatic flow of cookbooks, more than 25,000 every year, there are extremely few cookbooks devoted to cephalopods (Cronin, 1981; Schultz and Regardz, 1987; Mouritsen and Styrbæk, 2018). Similarly, although cephalopods are a traditional component of the cuisine in many parts of the world, few top chefs or gastronomic entrepreneurs have until recently taking an interest in elevating cephalopods to the Michelin-stars. Examples of cephalopod dishes are illustrated in **Figures 1–5**.

There are signs that this is about to change on two counts. On the one side, chefs in food cultures where there is no tradition for either fishing or consuming cephalopods are starting to explore and define a local cephalopod cuisine; on the other side chefs in countries where there is a long tradition for eating cephalopods are gaining an interest in re-inventing the use of these, e.g., by considering new types of preparations or using body parts that were rendered worthless in the traditional cuisine.

These changes hold a promise for an emerging, new cephalopod gastronomy. As this gastronomy evolves we may not only see more interesting and delicious food for the curious gastronomist and the foodies, but possibly also novel industrial products that will be appreciated by a larger part of the population around the world. To grasp the full implications of this possible development we shall in this paper put the gastronomic potentials of cephalopods in the perspective of marine food supplies to a growing world population, sustainability, and global climate changes. An important piece of information in this context is that whereas world fisheries of bonefish are under great pressure and many fish populations are dwindling (FAO, 2014), it appears that the global populations of all squid, cuttlefish, and octopus species important for human consumption are on the rise and have been so consistently for the last sixty years (Doubleday et al., 2016).

FIGURE 1 | Octopus salad, a classical South European dish made of boiled octopus arms (*Octopus vulgaris*), carrots, celeriac, garlic, and Italian parsley with olive oil, lemon juice, and oregano. Photo: permission by and courtesy of Kristoff Styrbæk.

WORLD CATCH AND CONSUMPTION OF CEPHALOPODS

Cephalopods are caught for human consumption around the world both on large industrial scale and by small, artisanal fishing communities. Since there are very few and mostly small experimental activities with aquaculture of cephalopods (Vaz-Pries et al., 2004; Iglesias et al., 2007), the catch is dominated by wild sources. Cephalopods are together with tuna, shrimp, and lobster considered to be the most valuable marine fisheries. FAO (2014) has estimated the total global catch to be 4.8 million tons annually but the figure may be unreliable since not all countries report their catch and it is mostly the large industrial fisheries that contribute to the report. Although some of the catch is used for bait in other fisheries, by far the largest part is used for human consumption.

Catch of species from the Teuthida order constitutes the most important products accounting for 3.6 million tons, followed by octopus and *Sepia*-like species. The commercially most important species are from the family of the flying squids (Ommastrephidae), in particular *Illex argentinus, Dosidicus gigas* and *Todarodes pacificus. Todarodes pacificus* alone accounts for half of the world's catch of cephalopods, and it has been estimated that this species is possibly the only one of the wild species that has enough potential to contribute significantly to the world supplies of protein. This would however require the development of more effective and sustainable fishing methods with less bycatch and waste. Japan is one of the major consumers of *Todarodes pacificus*, and most of it is used for sashimi.

Every year, 350,000 tons wild octopus are caught with a trade value of around 1.5 billion dollars. Most of it is caught in Asia, in particular in Chinese waters. There is also catch of octopus

FIGURE 2 | Semi-dried squid (*ika no ichiya-boshi*), a classical Japanese dish, made of *Loligo forbesii* with *ponzu* mayo and *shichimi*. Photo: permission by and courtesy of Jonas Drotner Mouritsen.

(*Octopus maya*) at the off Yucotán coast in Mexico, and the catch is mostly exported to Europe and Asia. The European catch of octopus is traditionally made in Portugal and Spain but is now down to 40,000 tons a year and it has halved over the last thirty years. The fishing of Atlantic octopus has moved to the waters near the coasts of Morocco and Mauretania.

A substantial part of the catch of cephalopods consists of unspecified species, and because of an active trading pattern of imports and exports, traceability and quality control is complicated. As an example, octopus products aimed for the sushi marked may come from third parties via Japan. Concerning the catch of *Sepia*-like species, China and Thailand are the main producers. Thailand, Spain, China, Argentina, and Peru are the world's largest exporter of Teuthis and *Sepia*-like species. The largest producers and exporters of octopus are Morocco, Mauretania, and China. Spain, Italy, and Japan are major world consumers of cephalopods, and the demand is increasing.

NUTRIENT COMPOSITION AND TASTE OF CEPHALOPODS

Nutrients in Cephalopods

The available data for nutrient composition of cephalopods suffer from a variation over the different data bases, reflecting that it is often not the same species that are reported about and that the actual cephalopods are derived from different locations and in different states of their life cycles. Still, there are some general trends which we shall briefly review here in order to better access the nutritional value of the different cephalopods (Ozogul et al., 2008). We compare the values for octopus, squid, and cuttlefish with corresponding values for one other simpler mollusk (blue mussel), one lean fish (cod), one fat fish (salmon), and one land animal (beef), cf. **Table 1**.

FIGURE 3 | A semi-classical Cambodian-inspired dish of marinated and grilled siphon and retractor muscles of *Loligo forbesii* on lemon grass. Photo: permission by and courtesy of Jonas Drotner Mouritsen.

Cephalopods have a water content of about 80% and are high in protein, about 16%, which is similar to bonefish and beef and a little higher that mussel. In contrast, cephalopods are low in fat (0.7–1.4%, squid being the most fatty), about the same as cod (0.7%), less than mussel (2.2%), and much less than salmon (13%) and beef (13%). Except for octopus, cephalopods have an overweight of unsaturated fatty acids, in particular super-unsaturated omega-3 DHA and EPA (Ozogul et al., 2008). Cholesterol is singled out by high levels in squid and in cuttlefish compared to octopus whose cholesterol levels compare with those of bonefish but are less than in beef.

The caloric energy content (about 80–90 cal/100 g) in all mollusks including cephalopods is much less than in fish and beef. Cephalopods are in contrast higher in carbohydrates than the other species, but none of the mentioned species contain any sugars or dietary fibers.

All cephalopods are good sources of calcium. Iron and sodium levels are high in octopus and cuttlefish compared to fish and beef. Concerning micronutrients (trace elements) recent studies (Storelli et al., 2010) have shown for species caught in the Mediterranean that the essential elements copper, zinc, selenium, and chromium are heterogeneously distributed in the different cephalopod species, with more selenium in squid and more copper and zinc in octopus. Chromium is equally distributed in the different species. Based on these studies it was concluded that consumption of cephalopods could make a significant

TABLE 1 | Contents of water, calories, macro and micro nutrients, fibers, and vitamins in three groups of cephalopods compared with the composition of blue mussel, salmon, and beef (grass-fed; ground, raw) (Ozogul et al., 2008; USDA, 2018).

Content/100 g	Octopus	Squid	Cuttlefish	Blue mussel	Cod	Salmon	Beef
Water (g)	82	78	81	81	73	65	67
Energy (kcal)	82	92	79	86	143	208	198
Protein (g)	15	16	16	12	17	20	19
Carbohydrate (g)	2	3	0.8	3.7	0	0	0
Fiber (g)	0	0	0	0	0	0	0
Sugar (g)	0	0	0	0	0	0	0
Fat (total) (g)	1.0	1.4	0.7	2.2	0.7	13	13
Fatty acids							
Saturated (g)	0.2	0.4	0.1	0.4	0.1	3	5.3
Mono-unsaturated (g)	0.2	0.1	0.08	0.5	0.1	4	4.0
Poly-unsaturated (g)	0.2	0.5	0.13	0.6	0.2	4	0.5
Cholesterol (mg)	48	233	112	28	43	55	63
Ca (μg)	53	32	90	26	16	9	12
Fe (μg)	5	0.7	6	4	0.4	0.3	2
Mg (μg)	30	33	30	34	32	27	19
P (μg)	186	221	387	197	203	240	175
K (μg)	350	246	354	320	413	363	289
Na (μg)	230	44	372	286	54	59	68
Zn (μg)	1.7	1.5	1.5	1.6	0.5	0.4	4.6
Vitamin C (mg)	5	5	5	8	1	4	0
Folate (μg)	16	5	16	42	7	26	6
Vitamin B_{12}(μg)	20	1.3	3	12	0.9	3	2
Vitamin A (IU)	150	33	375	160	40	193	0
Vitamin D (IU)	–	–	–	0	36	441	6
Vitamin E (mg)	1.2	1.2	–	0.6	0.64	3.5	0.35

contribution to the necessary daily intake of selenium, copper, and zinc.

When it comes to vitamins, it holds for all mollusks that they contain no vitamin D and very little vitamin K, in contrast to large amounts of vitamin D in fish, in particular fatty fish. It is noteworthy, that octopus has a high level of vitamin B_{12}.

Taste of Cephalopods

All seafood, including cephalopods, have different tastes depending on the species, where they have lived, and which part of the animal you eat. In particular, the texture-component of the taste experience varies vastly with a marked difference in mouthfeel between, e.g., octopus and mussels, and squid and bonefish. This variation is due to the fundamental difference in the muscular collagen structure in the different organisms. So even if the protein content are basically the same, the texture reflects dramatic differences in the motional behavior of the different species. Still, cephalopods have tastes that are similar to those of other mollusks and partly also bonefish when it comes to umami (Mouritsen and Styrbæk, 2014).

Cephalopods have as other organisms from salty waters, like bonefish, shellfish, and seaweeds, many different tastes and flavors, but they share a component of umami taste due to their high content of nucleic acids, like ATP, that enzymatically can be turned into free nucleotides such as inosinate and adenylate under the proper conditions after the animal has been killed under not too stressed conditions. In particular squid can contain high levels of adenylate, up to 184 mg/100 g (Yamaguchi and Ninomiya, 2000), which is about as much as scallops and six times as much as a sun-ripe tomato. Moreover cephalopods can also contain large amounts of free glutamate, up to 146 mg/100 g, that is comparable to scallops and corn. The simultaneous presence of both free nucleotides and free glutamate is the precondition for the very potent umami-synergy mechanism coming into play (Mouritsen and Khandelia, 2012).

Animals that live in salty water, including cephalopods, need in their cells to accumulate osmolytes that can counterbalance the osmotic pressure across the cell walls. Such substances include free amino acids and trimethylaminoxid (TMAO). Cephalopods use TMAO as osmolyte to a larger extent and sweet-tasting amino acids (e.g., glycine, alanine) to a lesser extent than, e.g., mussels. TMAO is tasteless and cephalopods therefore have a less sweet taste than other mollusks. When an organism dies its content of TMAO is by the organism's own active enzymes turned into trimethylamine (TMA) that has the unpleasant "fish odor." Cephalopods therefore more easily develop unpleasant flavors than fish if not kept at very low temperatures that render the enzymes less active.

The preferred taste of prepared cephalopods is very dependent on the food culture. Japanese prefer a mild flavor as close to the

FIGURE 4 | A novel dish of tartare made of *Sepia officinalis* with pistachio, lime, and avocado. Photo: permission by and courtesy of Jonas Drotner Mouritsen.

FIGURE 5 | Squid "fettucine:" a novel dish made with squid (*Loligo forbesii*), lobster, cherry tomatoes, lime juice, *ponzu*, salmon roe, lemon spheres, and black dried lime. Photo: permission by and courtesy of Jonas Drotner Mouritsen.

cephalopods natural flavor as possible and they will therefore only add very subdued and subtle flavors, e.g., from marinating liquids. In other places in Southeast Asia such as Vietnam, Thailand, and China, eaters prefer cephalopods with more spicy, powerful, and fishy flavors.

It is generally true that the taste and flavor of fresh cephalopods are reasonably mild and quite easily blend in with other flavors. Therefore, in gastronomic uses of cephalopods one needs to be aware of the danger of suppressing the subtle flavors of the cephalopods by stronger tasting ingredients, such that one is left with the texture as the only surviving characteristics of the cephalopod used.

Safety Issues

Sharing the same water and often the same feed the cephalopods can suffer from the same bacterial and parasitic diseases as bonefish, e.g., anisakis (Abollo et al., 2001), and the same measures must be taken when dealing with cephalopods a food as with other types of seafood. The formation of biogenic amines, e.g., histamin from the amino acid histidin, is a serious concern in seafood, particularly during storage (Kim et al., 2009; Hu et al., 2012). It turns out, that cephalopods are low in histidin and are therefore less prone to bacterial production of histamin.

Pollution by heavy metals and other toxins is an increasing problem in the marine environment and the different species are affected to different degrees. A major problem is accumulation of mercury and arsenic in fish and shellfish, and cadmium in squid. In principle, all cephalopods will accumulate heavy metals in their tissues, particularly the innards. However, since all cephalopods have very short life spans, typically less than three years, the problem of accumulation is much less than with longer-lived predators higher up in the food web, such as tuna and whales.

Recent measurements of non-essential, toxic elements (mercury, cadmium, lead, and arsenic) in the most commonly consumed species octopus, squid, and cuttlefish caught in the Mediterranean have shown (Storelli et al., 2006, 2010) that octopus are the most loaded and squid the least. Hepatopancreas contains the largest amounts of the toxins, except of mercury and arsenic that are equally distributed between innards and muscles. Regarding arsenic, cuttlefish accumulate the most. However, since arsenic is mostly found in organic form it is not considered to of any health concern. The combined evaluation of the health risk based on recommendations for the maximal weekly intake is that only cadmium can be a potential health hazard. e.g., a portion of 70 g octopus can contain 36% of the recommended weekly load of cadmium. In contrast, the contents of mercury and lead found in all three type of species is so low that is not considered a danger to health. The authors of the study conclude that in general there is no reason to discourage consumers from eating cephalopod meat (Storelli et al., 2006).

GASTROPHYSICS APPLIED TO CEPHALOPODS

Gastrophysics is a new and emerging, interdisciplinary field of science that can be defined as qualitative reflections and quantitative studies of all gastronomic aspects pertaining to food, including culinary precisions and transformations, preparation techniques, texture, and taste with focus on physical effects and physico-chemical characterization (Mouritsen and Risbo, 2013). The empirical basis of gastrophysics is gastronomy itself as well as food and food preparations of specific gastronomical value and potential. It is possible that gastrophysics in combination with neurogastronomy (Shepherd, 2012) can furnish the scientific underpinnings of gastronomy at large.

The usual starting point for a gastrophysical approach is a gastronomically-inspired question. In the context of cephalopod gastronomy, several obvious questions pose themselves pertaining to texture and taste. The most prominent question would be: how does one treat the muscular tissues of cephalopods, specifically the mantle and arms, and for squid also the tentacles and retractor muscles, to obtain a particular structure that leads to a desired texture and mouthfeel (Mouritsen and Styrbæk, 2017).

Cephalopod Muscular Structure: The Principle of a Muscular Hydrostat

The special construction of muscular structures in cephalopods has imparted them with a unique freedom in their motional patterns that compensates for their lack of skeletal structures. Octopus has lost shells completely, and cuttlefish and squid only have rudimentary inner shells in the form of a cuttlebone and a gladius, respectively, neither of which provide much skeletal support for muscular movements.

In the absence of any supportive internal or external skeletal support, cephalopods have solved the problem with mobility by building muscular tissues that work according to the principle of a muscular hydrostat (Kier and Smith, 1985; Hanlon and Messenger, 1996), that is a deformable system subject to constant pressure and volume. In order to exploit this principle the muscle fibers are strongly cross-bound and organized in three dimensions in contrast to unidirectional muscles in, e.g., bonefish and mammals.

Gastrophysics Applied to Cephalopod Muscular Structure

There is typically four times as much collagen in cephalopods compared to bonefish and it is much more cross-bound and hence much stronger. The level of cross-binding is a determining factor for the toughness of the tissues. The muscle fibers in cephalopods are longer and typically ten times thinner than in bonefish. This implies that the muscular structure appears more smooth than in bonefish. These facts together with the three-dimensional organization of the cephalopod muscles is the main reason why these organisms can use the principle of a muscular hydrostat to exhibit an artistic degree of mobility in all directions. If the muscle mass was a structure-less fluid hydrostat, this would not be possible.

The details of the organization of the muscular fibers in different cephalopods and in different parts of the cephalopods, i.e., arms, tentacles, and mantle, are different (Mizuta et al., 2003; Kier and Stella, 2007; Kier, 2016) and this difference is of uttermost importance for the use of the meat as food and how tender it will be. As an example, decapods like squid, because of their special ability to perform jet-repulsion swimming, do not have parallel muscles in the long direction of the mantle, but particularly strong muscles circularly organized around the mantle. This implies for fried squid dishes that cutting the conventional rings across the mantle is in fact the worst possible way regarding tenderization. Cutting along the long direction of the mantles cut more muscle fibers and lead to a more tender product. Knowledge about the physical structure of the muscles in cephalopods can therefore be a useful guide for gastronomy.

Tenderizing Cephalopod for Optimizing Texture

Many cephalopods, in particular octopus, are notoriously known to be challenging to prepare and have a reputation for becoming chewy and rubberlike, rather than tender, creamy, succulent, or crisp. The cure is to tenderize the cephalopods. Tenderization can involve heating, freezing, pressurizing, mechanical massaging, fermenting, and curing with salt, acids, and enzymes, as well as combinations of these approaches (Katsanidis, 2004, 2008; Mouritsen and Styrbæk, 2018).

It is generally recommended to freeze cephalopods before further preparation as food because it will do away with possible parasites. Some chefs also claim that it will tenderize the meat because ice-crystal formation will break-up the muscle fibers. This is possibly true in the case of octopus but less so in the case of mantles from decapods where there is only little effect of freezing on texture. The drawback of freezing cephalopod meat is that it will invariably loose some water.

Heat treatment is the most used procedure to tenderize cephalopods, e.g., by boiling in water or sous vide, baking, frying, and grilling. Since both temperature and duration of the treatment are in play at the same time it can be quite complicated because the collagen and the muscular protein in the tissue have different ways of responding to heat. Salt and acid will also affect the result of cooking octopus and how quickly the proteins in the meat denature. Acid, e.g., vinegar, tends on the one hand to stiffen the muscular proteins but will on the other hand promote the break-down of the collagen and lead to seeping out of gelatin into the cooking water, rendering the result drier. Salt is supposed to have the opposite effect but may not do anything good for the taste (McGee, 2008).

The mantle from decapods, like *Sepia officinalis* and *Loligo forbesii,* need very brief heating at low temperatures (50–60°C) to become more tender and succulent, and in many cases you are better off eating the mantle raw and only heat-treat the arms and tentacles very lightly.

Concerning tenderization by mechanical means, traditional Greek chefs recommend taking an octopus by one arm and bash it repeatedly against rocks, and Japanese chefs suggest to massage the octopus arms by your hands, adding grated *daikon* and salt. More modern techniques involve tumbling the raw octopus in saltwater for hours in tumbler. Other techniques imply scoring the outer surface of decapod mantles possibly in a crisscross pattern or simply to puree the meat in a blender or in a Paco-jet machine. The latter type of tenderization is used, e.g., to produce *surimi* of squid meat.

Similarly to meat from fish and land animals, the meat from cephalopods can be tenderized by the action of specific enzymes. A traditional approach proceeds using squid intestinal enzymes from the hepatopancreas that contains some very aggressive enzymes. The hepatopancreas of squid (e.g., *Todarodes pacificus*) is therefore employed to start fermentation in traditional Asian fish sauces. A fermentation medium with 10–30% salt and squid hepatopancreas is used to form a special marinade *shiokara* in Japanese or *chokkaru* in Korean. The Korean *chokkaru* often enters *kimchi*, and *shiokara* is used in Japan to prepare the traditional squid dish *ika no shiokara* that is shredded squid mantle fermented in its own intestinal enzymes. During the fermentation process a lot of free amino acids and peptides are formed that lead to strong umami and *kokumi* tastes. The high levels of salt prevent putrefaction over the very long fermentation periods that can last for months. Fresh and non-pasteurized ink from cuttlefish also contain enzymes that can facilitate some tenderization.

Enzymes from fruits, such as bromelain from pineapple juice, are also known to be able to tenderize squid muscles by loosening the collagen network (Ketnawa and Rawdkuen, 2011).

SOME USES OF CEPHALOPODS IN THE WORLD CUISINE

It is hardly surprising that different countries have different traditions with respect to using a given food commodity and ingredient in their cuisine, which is amply reflected in recipes and the general food culture. But it may be surprising, e.g., that whereas the Japanese cuisine to a large extent uses cephalopods as raw or very lightly treated food there is in Spain hardly any tradition for eating raw cephalopods or raw seafood of any kind for that matter. Italy places herself somewhere in between, both with a tradition of regional dishes of raw or lightly marinated seafood (*pesce crudo*), including cephalopods, and a tradition like the Spanish of frying and grilling cephalopods.

Raw or Almost Raw

Many types of decapods can be eaten in raw or nearly raw form provided they are completely fresh and do not contain nematodes in which case they need to have been frozen first to at least −20°C in 24 h and preferably 72 h. Octopus are seldom eaten raw because it is generally too tough (Schweid, 2014). As a curiosity, it can me mentioned that there is an Apulian specialty dish from around Bari called *vurp arrazzat*, meaning "curly octopus," made from small octopus that are eaten raw after an elaborate tenderization procedure. This procedure, that is applied only to small specimens of *Octopus vulgaris* (less than a kilo) or other small octopus species (e.g., *Eledone moschata*) involves first beating of the octopus against rocks, then beating it with a wooden ladle, then agitated washing in seawater, and finally cradling or rolling it so it curls up. During this procedure, the animals are first unnerved and the fibers become disrupted and extended, rendering the flesh soft, after which the texture contracts again and the meat turns extremely crunchy. They are eaten completely fresh with a squeeze of lemon.

An extreme case of eating raw octopus is the Korean dish *sannakji* that is raw and live tips of octopus arms cut off a life octopus. Needless to say, this is a brutal way of treating an animal and in addition, taking pieces of living and moving octopus arms into the mouth, can be dangerous since the suckers will attach to the mucus membranes and can lead to suffocation.

The Japanese cuisine is rich in seafood that is eaten raw, typically as sashimi and sushi, it be of fish, shellfish, and cephalopods. The best-known kind of sushi is hand-pressed *nigiri-zushi* that often is topped with a piece of raw *ika* made of the mantle of cuttlefish or squid (Mouritsen, 2009). Octopus (*tako*) for sushi are prepared as thin slices cut across cooked arms.

In Marinade and Sauce

There is only a short way from raw cephalopods to lightly marinated preparations, such as South American *ceviche* or Italian *pesce crudo* where pieces of cephalopod mantles and arms are marinated in salt, acid (vinegar or citrus-fruit juice), or sauces like soy sauce, *ponzu*, or *sanbaizu* that contains *dashi* with lots of umami taste. Other interesting marinades can be made of miso or sake lees (*sake kasu*) that serve both to tenderize the meat and to impart umami taste. When marinating in acidic media one should be aware of the fact that acids make the muscle proteins contract and can lead to a firmer texture in the short run and only tenderizing over longer times.

Squid and cuttlefish mantles cut in fine strips along the long direction of the mantle are particularly suited for marinating. These strips, called *ika-somen* in Japan, look like a kind of fettuccine pasta and are often served on top of a bowl of rice (*chirashi-zushi*) or in a soup broth.

Dried

A traditional way of preparing octopus in Greece and at other coasts around the Mediterranean, e.g., Southern Spain, proceeds by air-drying the whole octopus outside so it loses about half its weight. They are then grilled. This procedure renders the meat very crisp but also somewhat chewy. A similar technique is used in Japan for both octopus and squid, *saki-ika*, i.e., dried *ika* from *Todarodes pacificus*. Fully dried cephalopods can be shredded and are often used like a kind of snack similar to jerky, in which case they typically are flavored by soy sauce, *yuzu*, or various spices.

Part of the traditional Japanese breakfast consist of grilled dried fish or squid. Often the squid is only semi-dried (*ichiya-boshi*) over night. The drying implies that the squid retains some of its firmness and its taste compounds do not seep out during grilling.

According to more modern recipes, octopus can be made rather tender by drying the raw arms a couple of hours at 65°C, possibly first rolled in spices like curry, after which they can be grilled or smoked and used as a snack (Mouritsen and Styrbæk, 2018).

Grilled

Some of the most genuine culinary experiences one can have by visiting the coastal areas in Portugal and Spain is to enjoy a simple meal of freshly caught grilled squid, *calamares à la plancha* in Spain and *calamares grelhados* or *lulas grelhados* in Portugal.

The serving is complete with a few boiled potatoes, a little green lettuce, and a wedge of lemon.

It is mostly decapods that are grilled this way, and the grilling is done quickly and involves both mantle including fins, arms, and tentacles. For the smaller species and specimens the whole cephalopod are put on the grill, and for the small *Sepia* species (e.g., *Sepia elegans*) this can be an interesting challenge for the diner due to the ink. A traditional way of grilling and presenting a grilled whole squid is by first cutting it half way through and across the mantle so it during grilling opens like an accordion.

Fried

Frying breaded or battered squid and cuttlefish in oil is a very fast and common way to prepare cephalopods, but it is not without dangers since the meat can become tough if cooked too much, and the crumble can become too greasy if it is not sufficiently dry and crunchy. A dry crumble can be obtained by using *panko* that is a special kind of Japanese bread crumbles with a lot of small airy pores that repel the oil. Ill-prepared fried squid rings are probably the best way of scaring people from eating cephalopods.

Andalusia in the Southern part of Spain has a cuisine with a rich tradition for fried seafood, *pescaíto frito*, not least cephalopods which are often served as a kind of *tapas*. Local specialties include *puntillitas* (*calamares chiquititos*) and *calamaritos* (*chipirones*). *Puntillitas* are made from *Alloteuthis subulata,* and *calamaritos* are small squid of the species *Loligo vulgaris. Chocos fritos* is the popular name for the mantle of small *Sepia officinalis.* An Andalusian specialty is *huevos de choco* that is made of the nidamental glands from large *Sepia officinalis*. These glands produce gelation materials that harden the roe in the female *Sepia* and is considered a valued delicacy that require little preparation. They are lightly grilled or served in a marinade of olive oil, garlic, and parsley.

Deep-frying of raw squid and cuttlefish is a classical approach in the Chinese cuisine and it has the advantage that high heat can be applied for a very short time when one uses a wok. In the Cantonese cuisine one of the most classical dishes is salt and pepper squid (*jiāoyán yóuyú*); another one is deep-fried squid with sugar peas (*zajin chao xianyou*). When using the squid or cuttlefish mantles for these dishes they are usually cut out in squares and then scored which make them curl up in a characteristic coniferous cone-like shape that contributes aesthetically to the presentation of the dish.

Steamed and Boiled

World cuisines have a great many different recipes for preparing steamed and cooked-in-water octopus and there are almost as many recipes as there are chefs. The trouble is related to the difference in response to heat by muscle protein and collagen which in turn raises the question regarding the best combination of temperature and cooking time, as well as the effect of salt and acid. The complexity of the problem is possibly best reflected in the great variety in traditional recipes for preparing octopus. Some Spanish chefs say that octopus should be boiled in a copper vessel and Italian chefs may say that it is instrumental to place two corks on the boiling water. More scientific approaches to cooking octopus in water can be facilitated by a pressure cooker and the cooking time can then be at least halved in many cases. A systematic approach to this problem may proceed by using sous-vide techniques (Myhrvold, 2010).

Cooked octopus arms are used in a variety of warm and cold preparations and dishes. A classical dish in Southern Europe is cold octopus salad with slices or chunks of octopus arms together with various vegetables, beans, and herbs.

In Japan, octopus caught around the Awaji island in the Strait of Akashi between Honshu and Shikoku in the Easter part of Japan is considered to be the most flavorful. It is particularly highly valued in the summertime when the water contains large supplies of shrimp and crabs which are octopus' favorite food. There is a rich food culture around Awaji octopus (*tako*). The most famous preparation is *tako-yaki* that is a kind of dumplings with a dough wrapped around minced meat from octopus arms, possibly supplemented by the remains from *tempura* preparations. The dough contains ginger and spring onions and is baked in special molds. The dumplings are dipped in different sauces, and the traditional *Akashi-yaki* is pure *tako-yaki* dipped in *dashi*. *Akashi-yaki* is served all around the Osaka area where it is a popular kind of streetfood. Octopus is so popular there that July 2 is designated as a special *tako*-day.

The cooking water from boiling an *Octopus vulgaris* can be very flavorful and umami-rich and upon reduction lead to a sauce that is viscous due to gelatin released from in particular the gelatinous skin of the arms. This sauce can be used to glaze octopus arms or enter in a very delicious emulsion or cream that has a strong meaty flavor. One of the most classical preparations in Spain is the Galician dish *pulpo à la Gallega* that consist of slices of boiled octopus arms with paprika and olive oil. It is by tradition served warm on a wooden plate with boiled potatoes. In this dish the octopus arms have cooked and simmered for a long time so they are extremely tender and creamy and when done right they are not too dry.

Although less often than octopus, decapods are also in some cuisines cooked or steamed, e.g., using sous-vide techniques. Cooking is more important for the texture of the arms and tentacles than the mantle.

With Ink

Ink from squid and in particular from cuttlefish are used in several food cultures to color various dishes as well as pre-prepared foodstuff such as rice, pasta, bread, and cakes. It is also possible to prepare interesting looking snacks by coloring with *Sepia* ink. Fresh ink does not only color a dish but can also help to tenderize meat because of its content of active enzymes. A classical Spanish dish is squid prepared in its own ink, *calamar in su tinto*.

CONCLUSIONS AND OUTLOOK: FOOD FOR THE FUTURE

Projections for the world population predicts that in 2050 there will be 10 billion people in the world. This rapid growth in population will imply a dramatic increase in the competition about the natural resources and hence put focus on the sustainability of our food supplies in the context of economy as well as social, environmental, and political factors. It is becoming clear that we live in the Anthropocene era where humans are making a significant and possibly irreversibly footprint on the earth, its ecosystems, and its climate. This raises questions as how to distribute and exploit our available resources in a more sustainable fashion.

This is not least the case when it comes to the world's food supplies. Production of meat, in particular beef, takes a heavy toll on the consumption of water and energy leading to large effects on carbon dioxide emissions and climate. The production of protein from beef is approximately twenty times as costly as production of protein from insects and farmed chicken and salmon. It is at this point it becomes relevant to look for other, more sustainable protein sources, and the cephalopods bring themselves into focus.

The oceans are in some sense a poorly exploited and poorly managed natural food resource when it comes to fisheries. Several species are endangered and overfished, and at the same time environmental factors put limits to expansions of agriculture of fish and shellfish (Boyle and Rodhouse, 2005; SAPEA, 2017). If we are to use the marine food resources in a better and more sustainable fashion to feed a growing population with healthy and safe food we need to learn to consume marine food in a more diverse and insightful manner, including eating from lower trophic levels and limiting bycatch and waste. We must come to term with eating more seafood directly rather that piping it through land animals as feed, thereby loosing typically 90% of the nutrients in each trophic level.

This is where the cephalopods come in. Cephalopods are extremely effective to turn their food into musculature, they grow quickly, and they have fast generational shifts. The global volume of fisheries of cephalopods has increased in recent years (Jereb and Roper, 2005, 2010; Payne et al., 2006; Anderson et al., 2011; Pauly et al., 2013; Jereb et al., 2016). One challenge in this context is that they are notoriously difficult to put into aquaculture (Vaz-Pries et al., 2004) and no real successful commercial cultures have yet been established.

In 2015 the UN passed an act "Agenda for Sustainable Development" toward 2030 (UN, 2015), which also considers distribution and administration of fisheries and aquaculture with a focus on safety and human nutrition. The cephalopods are part of this agenda. However, it is difficult to make plans and control the catch of cephalopods because a major part of the fisheries take place in international waters, but also because our knowledge about cephalopod behavior in the wild is rather limited. The limited data makes predictions cumbersome and uncertain. One circumstance that adds to the complexity is the short life span of most cephalopods.

An important finding in recent years has put this whole complex into a new and very interesting light (Doubleday et al., 2016): research has shown the surprising result that there is a global rise in the cephalopod populations. By analyzing data for the populations of 35 different species from six different families (31% Octopoda, 52% Teuthida, and 17% Sepioidea) over a period of 6 years (1953–2013), the researchers have in all cases found clear signs of growing populations. The unique aspect about these observations is that this observation holds true for both species that are fished and species that are not fished and the survey moreover included cephalopods from all levels of the water column. The similarity in the growth pattern for the different species suggests that the growth does not simply reflect an increase in the catch due to improved and more intensified fishing methods. Moreover, the different species included in the survey both encompass species that move over thousands of kilometers in the oceans and species that move around only locally. The reason for the universal and global growth pattern must consequently be related to some other mechanism.

The interpretation of this striking observation has been (Doubleday et al., 2016) that the cephalopods have profited from the antropocenic climate changes. It is known that cephalopods react and adjust quickly to environmental changes, in particular water temperature (Rodhouse et al., 2014). Increasing water temperatures could have caused migration of some species toward more northern and colder waters. What that eventually will imply for the fish and cephalopods native to those waters is still too early to say.

It is also possible that the decrease in the populations of some bonefish that prey on cephalopods on the one side have given cephalopods an advantage. On the other side certain fish are also prey for the cephalopods. In any case, it is possible that the changing climate has pushed to the million-year old competition and ecological balance between cephalopods and bonefish (O'Dor and Webber, 1986). In Perm (299–252 million years ago) the bonefish teemed and entered a fierce competition with the then ruling cephalopods, leading to the occurrence of the Coleoidea, the cephalopods without outer shells, and the bonefish got the upper hand with now 30,000 different species against the only 800 remaining cephalopods. Maybe the balance is now tipping and their enormous reproductive power and their ability to adapt to environmental changes now give the cephalopods a renewed chance to rule the oceans.

Hence, there is a good reason to look to the cephalopods as an important food resource which we should pay more attention to. This raises several questions. One is related to using the wild populations in a more sustainable fashion. Another one is related to as how we can possibly design systems for cephalopod aquaculture (Iglesias et al., 2007). The answers to both these questions require more research and biotechnological development (Vidal, 2014). A third question pertains to ethical issues regarding the treatment and killing of cephalopods (Fiorito et al., 2015), not least octopus that clearly, although an invertebrate, is imparted with faculties that may render it both intelligent and possibly having consciousness (Fiorito and Scotto, 1992; Mather, 2008; Montgomery, 2015a,b; Godfrey-Smith, 2016) as now manifested in the Cambridge Declaration on Consciousness[1].

The last question and the answer to this question has been the topic of the present paper. The question can be simply rephrased as: do we want to eat cephalopods? In many food cultures around the world this is obviously a silly question since they have a rich tradition for using cephalopods in their food and cooking. It is less obvious in other cultures in which cephalopods may be as

---

[1] *Cambridge Declaration on Consciousness*. Available online at: http://fcmconference.org/img/CambridgeDeclarationOnConsciousness.pdf

weird a foodstuff as insects for those who are not accustomed to eating insects. Even though arguments can be presented that cephalopods are certainly very edible, nutritious, healthy, and can even be prepared as tasty food, it is not easy to change people's food preferences. It is well known from public campaigns that even if a certain diet is known to be healthy, nutritious, and sustainable, people are not going to eat it unless it is delicious. Therefore, a focus on cephalopod gastronomy is required to stimulate a motion in the direction of more people eating more cephalopod-based food.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

The work by the authors was supported by Nordea-fonden *via* a center grant to the national Danish center *Taste for Life*.

REFERENCES

Abollo, E., Gestal, C., and Pascual, S. (2001). Anisakis infestation in marine fish and cephalopods from Galician waters: an updated perspective. *Parasitol. Res.* 87, 492–499. doi: 10.1007/s004360100389

Anderson, S. C., Flemming, J. M., Watson, R., and Lotze, H. K. (2011). Rapid global expansion of invertebrate fisheries: trends, drivers, and ecosystem effects. *PLoS ONE* 6:e14735. doi: 10.1371/journal.pone.0014735

Boyle, P. R., and Rodhouse, P. G. (2005). *Cephalopods: Ecology and Fisheries*. Oxford: Blackwell Science.

Cronin, I. (1981). *The International Squid Cookbook*. New York, NY: Aris Books.

Doubleday, Z. A., Prowse, T. A. A., Arkhipkin, A., Pierce, G. J., Semmens, J., Steer, M., et al. (2016). Global proliferation of cephalopods. *Cur. Biol.* 26, R406–R407. doi: 10.1016/j.cub.2016.04.002

FAO (2014) *Fisheries and Aquaculture Department. The State of World Fisheries and Aquaculture*. Rome: FAO.

Fiorito, G., Affuso, A., Basil, J., Cole, A., de Girolamo, P., D'Angelo, L., et al. (2015). Guidelines for the care and welfare of cephalopods in research–a consensus based on an initiative by CephRes, FELASA, and the Boyd Group. *Lab. Anim.* 49, 1–90. doi: 10.1177/0023677215580006

Fiorito, G., and Scotto, P. (1992). Observational learning in *Octopus vulgaris*. *Science* 256, 545–547. doi: 10.1126/science.256.5056.545

Godfrey-Smith, P. (2016). *Other Minds: The Octopus, the Sea, and the Deep Origins of Consciousness*. New York, NY: Farrar, Straus, and Giroux.

Grocock, C., and Grainger, S. (2006). *Apicius*. Totnes: Prospect Books.

Hanlon, R. T., and Messenger, J. B. (1996). *Cephalopod Behavior*. Cambridge: Cambridge University Press.

Hu, Y., Huang, Z., Li, J., and Yang, H. (2012). Concentrations of biogenic amines in fish, squid and octopus and their changes during storage. *Food Chem.* 135, 2604–2611. doi: 10.1016/j.foodchem.2012.06.121

Iglesias, J., Sánchez, F. J., Bersano, J. G. F., Carrasco, J. F., Dhont, J., Fuentes, L. et al. (2007). Rearing of *Octopus vulgaris* paralarvae: present status, bottlenecks and trends. *Aquaculture* 266, 1–15. doi: 10.1016/j.aquaculture.2007.02.019

Jereb, P., and Roper, C. F. E. (eds.). (2005). *Cephalopods of the World. An Annotated and Illustrative Cataloguee of Cephalopod Species known to Date. Volume 1. Chambered Nautiluses and Sepioids (Nautilidae, Sepiidae, Sepiolidae, Sepiadariidae, Idiosepiidae and Spirulidae)*. FAO Species Catalogue for Fishery Purposes No. 4, Rome.

Jereb, P., and Roper, C. F. E. (eds.). (2010). *Cephalopods of the World. An Annotated and Illustrative Cataloguee of Cephalopod Species known to Date. Volume 2. Myopsid and Oegopsid Squids*. FAO Species Catalogue for Fishery Purposes No. 4, Rome.

Jereb, P., Roper, C. F. E., Norman, M. D., and Finn, J. K., (eds) (2016). *Cephalopods of the World. An Annotated and Illustrative Cataloguee of Cephalopod Species Known to Date. Volume 3. Octopods and Vampire Squids*. FAO Species Catalogue for Fishery Purposes No. 4, Rome.

Katsanidis, E. (2004). Impact of physical and chemical pretreatments on texture of octopus (*Eledone moschata*). *Food Sci.* 69, 264–267. doi: 10.1111/j.1365-2621.2004.tb13626.x

Katsanidis, E. (2008). Physical and chemical pre-treatment of octopuses aiming at tenderisation with reduced processing time and energy requirements. *J. Process. Energy Agric.* 12, 45–48.

Ketnawa, K., and Rawdkuen, S. (2011). Application of bromelain extract for muscle foods tenderization. *Food Nutr. Sci.* 2, 393–401. doi: 10.4236/fns.2011.25055

Kier, W. M. (2016). The Musculature of coleoid cephalopod arms and tentacles. *Front. Cell Dev. Biol.* 4:10. doi: 10.3389/fcell.2016.00010

Kier, W. M., and Smith, K. K. (1985). Tongues, tentacles and trunks. The biomechanics of movement in molecular hydrostats. Zoo. *J. Linnean Soc.* 83, 307–324. doi: 10.1111/j.1096-3642.1985.tb01178.x

Kier, W. M., and Stella, W. M. (2007). The arrangement and function of octopus arm musculature and connective tissue. *J. Morphol.* 268, 831–843. doi: 10.1002/jmor.10548

Kim, M.-K., Mah, J.-H., and Hwang, H.-J. (2009). Biogenic amine formation and bacterial contribution in fish, squid, and shellfish. *Food Chem.* 116, 87–95. doi: 10.1016/j.foodchem.2009.02.010

Mather, J. A. (2008). To boldly go where no mollusc has gone before: personality, play, thinking and consciousness in Cephalopods. *Amer. Maloc. Bull.* 324, 51–58. doi: 10.4003/0740-2783-24.1.51

McGee, H. (2008). To cook an octopus: forget the cork, add science. *New York Times*, p. F3.

Mizuta, S., Tanaka, T., and Yoshinaka, R. (2003). Comparison of collagen types of arm and mantle muscles of the common octopus (*Octopus vulgaris*). *Food Chem.* 81, 527–532. doi: 10.1016/S0308-8146(02)00486-7

Montgomery, S. (2015a). *The Octopus Scientists: Exploring the Mind of a Mollusk.* Boston, MA: Houghton Mifflin Harcourt.

Montgomery, S. (2015b). *The Soul of an Octopus: A Surprising Exploration Into the Wonder of Conciousness*. New York, NY: Atria Paperback.

Mouritsen, O. G. (2009). *Sushi: Food for the Eye, the Body, and the Soul.* New York NY: Springer.

Mouritsen, O. G., and Khandelia, H. (2012). Molecular mechanism of the allosteric enhancement of the umami taste sensation. *FEBS J.* 279, 3112–3120. doi: 10.1111/j.1742-4658.2012.08690.x

Mouritsen, O. G., and Risbo, J. (2013). The emerging science of gastrophysics. *Flavour* 2:3.

Mouritsen, O. G., and Styrbæk, K. (2014). *Umami: Unlocking the Secrets of the Fifth.* New York, NY: Columbia University Press.

Mouritsen, O. G., and Styrbæk, K. (2017). *Mouthfeel: How Texture Makes Taste*. New York, NY: Columbia University Press.

Mouritsen, O. G., and Styrbæk, K. (2018). *Blæksprutterne kommer: spis dem!*. Copenhagen: Gyldendal.

Myhrvold, N. (2010). *Modernist Cuisine: The Art and Science of Cooking. Vol. 3.* The Cooking Lab Publishing.

O'Dor, R. K., and Webber, D. M. (1986). The constraints on the celaphods: why squid aren't fish. *Can. J. Zool.* 64, 1591–1605.

Ozogul, Y., Duysak, O., Ozogul, F., Özkütük, A. S., and Türeli, C. (2008). Seasonal effects in the nutritional quality of the body structural tissue of cephalopods. *Food Chem.* 108, 847–852. doi: 10.1016/j.foodchem.2007.11.048

Pauly, D., Hilborn, R., and Branch, T. A. (2013). Fisheries: does catch reflect abundance? *Nature* 494, 303–306. doi: 10.1038/494303a

Payne, A. G., Agnew, D. J., and Pierce, G. J. (2006). Trends and assessment of cephalopods fisheries. *Fish. Res.* 78, 1–3. doi: 10.1016/j.fishres.2005.12.010

Rodhouse, P. G. K., Pierce, G. J., Nichols, O. C., Sauer, W. H. H., Arkhipkin, A. I., Laptikhovsky, V. V., et al. (2014). Environmental effects on cephalopod population dynamics: implications for management of fisheries. *Adv. Mar. Biol.* 67, 99–233. doi: 10.1016/B978-0-12-800287-2.00002-0

SAPEA (2017). *Science Advice for Policy by European Academies. Food From the Oceans: How can More Food and Biomass be Obtained From the Oceans in a Way that Does not Deprive Future Generations of their Benefits?* SAPEA: Berlin.

Schultz, J., and Regardz, B. (1987). *Calamari Cookbook: Exploring the World's Cuisines Through Squid.* Berkeley, CA: Celestialarts.

Schweid, R. (2014). *Octopus*. London: Reaction Books.

Shepherd, G. (2012). *Neurogastronomy*. New York, NY: Columbia University Press.

Storelli, M. M., Garofalo, R., Giungato, D., and Giacominelli-Stuffler, R. (2010). Intake of essential and non-essential elements from consumption of octopus, cuttlefish and squid. *Food Add. Cont. Part B* 3, 14–18. doi: 10.1080/19440040903552390

Storelli, M. M., Giacominelli-Stuffler, R., Storelli, A., and Marcotrigiano, G. O. (2006). Cadmium and mercury in cephalopod molluscs: estimated weekly intake. *Food Add. Cont.* 23, 25–30. doi: 10.1080/02652030500242023

UN (2015). *Transforming Our World: the 2030 Agenda for Sustainable Development*. Available Online at: https://sustainabledevelopment.un.org/post2015/transformingourworld

USDA (2018). *Food Composition Databases.* USDA. Available online at https://ndb.nal.usda.gov/ndb/

Vaz-Pries, P., Sixas, P., and Barbosa, A. (2004). Aquaculture potential of the common octopus (*Octopus vulgaris* Cuvier, 1797): a review. *Aquaculture* 238, 221–238. doi: 10.1016/j.aquaculture.2004.05.018

Vidal, E. A. G. (ed) (2014). "Advances in cephalopod science: biology, ecology, cultivation, and fisheries," in *Advances in Marine Biology*, *Vol.* 76. (Amsterdam: Elsevier), 452.

Yamaguchi, S., and Ninomiya, K. (2000). Umami and food palatability. *Amer. Soc. Nutr. Sci.* 130, 921S–926S. doi: 10.1093/jn/130.4.921S

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Physiology

PERSPECTIVE
published: 20 November 2018
doi: 10.3389/fphys.2018.01573

A Perspective Around Cephalopods and Their Parasites, and Suggestions on How to Increase Knowledge in the Field

Katina Roumbedakis[1,2]*, Marie Drábková[3,4], Tomáš Tyml[3,5] and Carlo di Cristo[1]

[1] Dipartimento di Scienze e Tecnologie, Università degli Studi del Sannio, Benevento, Italy, [2] Association for Cephalopod Research, Naples, Italy, [3] Department of Parasitology, Faculty of Science, University of South Bohemia, České Budějovice, Czechia, [4] Institute of Parasitology, Biology Centre Academy of Sciences of the Czech Republic, České Budějovice, Czechia, [5] Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czechia

Although interest in several areas of cephalopod research has emerged over the last decades (e.g., neurobiology, aquaculture, genetics, and welfare), especially following their 2010 inclusion in the EU Directive on the use of animals for experimental purposes, knowledge regarding the parasites of cephalopods is lacking. Cephalopods can be intermediate, paratenic, or definitive hosts to a range of parasites with a wide variety of life cycle strategies. Here, we briefly review the current knowledge in cephalopod parasitological research, summarizing the main parasite groups that affect these animals. We also emphasize some topics that, in our view, should be addressed in future research, including: (i) better understanding of life cycles and transmission pathways of common cephalopod parasites; (ii) improve knowledge of all phases of the life cycle (i.e., paralarvae, juveniles, adults and senescent animals) and on species from polar deep sea regions; (iii) exploration of the potential of using cephalopod-parasite specificity to assess population boundaries of both, hosts and parasites; (iv) risk evaluation of the potential of standard aquacultural practices to result in parasite outbreaks; (v) evaluation and description of the physiological and behavioral effects of parasites on their cephalopod hosts; (vi) standardization of the methods for accurate parasite sampling and identification; (vii) implementation of the latest molecular methods to facilitate and enable research in above mentioned areas; (viii) sharing of information and samples among researchers and aquaculturists. In our view, addressing these topics would allow us to better understand complex host-parasite interactions, yield insights into cephalopod life history, and help improve the rearing and welfare of these animals in captivity.

Keywords: Cephalopoda, parasites, pathogens, diseases, welfare

Edited by:
Erica A. G. Vidal,
Universidade Federal do Paraná,
Brazil

Reviewed by:
David B. Edelman,
Dartmouth College, United States
Simonetta Mattiucci,
Università degli Studi di Roma La Sapienza, Italy
Mirela Petric,
University of Split, Croatia

***Correspondence:**
Katina Roumbedakis
katina.roumbedakis@gmail.com

Specialty section:
This article was submitted to Invertebrate Physiology, a section of the journal Frontiers in Physiology

Received: *05 February 2018*
Accepted: *22 October 2018*
Published: *20 November 2018*

Citation:
Roumbedakis K, Drábková M, Tyml T and di Cristo C (2018) A Perspective Around Cephalopods and Their Parasites, and Suggestions on How to Increase Knowledge in the Field. Front. Physiol. 9:1573. doi: 10.3389/fphys.2018.01573

CEPHALOPODS AND THEIR PARASITES: A SHORT OVERVIEW

The incidence of a given parasite in a cephalopod species depends on the presence of a potential definitive host and intermediate host(s) (in parasites with complex life cycles, i.e., those that use multiple hosts to complete their life cycle), as well as on biotic and abiotic factors (González et al., 2003). Cephalopods can be definitive hosts for protists, dicyemids, monogeneans and crustaceans, as well as intermediate or paratenic hosts for digeneans, cestodes and nematodes (summarized in **Table 1**; for review see also Table 1–5, Hochberg, 1990). As intermediate or paratenic hosts, cephalopods can accumulate parasites throughout their lifespan, thus increasing the chance of predation by the next host and, consequently, the probability of parasite transmission. This is especially relevant for cestodes and anisakid nematodes, which use cephalopod hosts as important vectors for transporting them to other intermediate or to definitive hosts (e.g., Pascual et al., 1995; Abollo et al., 1998; Petrić et al., 2011).

In contrast to other molluscs, two characteristics of coleoid cephalopods (all living cephalopods besides *Nautilus* spp.) have crucial roles in their susceptibility to parasites and disease: (i) the loss of external shell, which enables the extensive neural and muscular development that allows high-speed locomotion; and (ii) the evolution of complex skin capable of sophisticated camouflage and signaling, but also prone to lesioning (Kinne, 1990). By shedding the rigid external shell of their ancestors, coleoids became more agile predators and adopted a more active lifestyle. This likely increased the frequency of parasite transmission since, predators readily accumulate multi-host parasites that are transmitted upward through the food web (e.g., digeneans, cestodes and nematodes). Some parasites can even alter the behavior or appearance of their intermediate hosts (e.g., modifying host phenotypes) in order to increase the likelihood that they will be predated on by their definitive hosts (Lafferty, 1999; Heil, 2016), mechanisms that have yet to be explored in cephalopod hosts. In addition to the increased likelihood of transmission, the fragility of coleoid cephalopods' skin may increase the ease with which opportunistic pathogens (i.e., infection by bacteria, kinetoplastids, dinoflagellates, fungi, labyrinthulids) can invade the body (reviewed by Kinne, 1990).

To date, the most complete review of potential pathogenic agents affecting cephalopods is in "Diseases of Marine Animals" (DoMA; Kinne, 1990; chapters concerning cephalopods: Hanlon and Forsythe, 1990a,b; Hochberg, 1990). In his summary, Hochberg (1990) reported parasites for about 130 cephalopods, which represents less than a quarter of the described species at that time. Later reviews provided complementary information regarding the main viral, bacterial, fungal, parasitic, chemical and mechanical parasitic agents affecting cephalopods (see Pascual et al., 1996; Castellanos-Martínez and Gestal, 2013; Sykes and Gestal, 2014).

In the following paragraphs, we briefly overview the current knowledge on the most common parasites found in cephalopods. About 230 parasitic species of a variety of taxa (e.g., Chromista, Protozoa, Diciemyda, Monogenea, Trematoda, Cestoda, Acanthocephala, Nematoda, Annelida and Crustacea) are reported in the literature to date (**Table 1** and **Figure 1A**). A map of the geographic distributions of cephalopod parasites is provided in **Figure 1B**. We emphasize that the data provided here likely over-represents tropical and temperate locations and coastal environments, since these areas are more easily and frequently sampled.

Aggregata spp.

Some of the most common parasites of cephalopods are the coccidians *Aggregata* spp. (Apicomplexa, Aggregatidae). To date, 10 species of *Aggregata* have been described parasitizing cephalopods (for review, see Gestal et al., 2010), although other (undescribed) species have also been reported (reviewed in Hochberg, 1990), so the actual diversity is likely higher. *Aggregata* spp. have complex heteroxenous life cycles, with crustaceans as intermediate hosts and cephalopods as definitive ones (Dobell, 1925; Hochberg, 1990). Most recent research (e.g., Castellanos-Martínez et al., 2013; Tedesco et al., 2017) has focused primarily on *Aggregata octopiana* and *Aggregata eberthi*, parasites of *Octopus vulgaris* and *Sepia officinalis*, respectively. This group is associated with histological and ultrastructural lesions in the digestive tract (mainly the caecum and intestine) of their cephalopod hosts (Gestal et al., 2002a), with infections of the gills, mantle, arms and mesentery also occasionally occurring (Pascual et al., 1996; Mladineo and Bočina, 2007; Tedesco et al., 2017). In addition, *Aggregata* infection can impair body growth due to "malabsorption syndrome" (Gestal et al., 2002b).

Ciliates and Dicyemids

In the renal tissue, cephalopods harbor two very unique parasitic groups, the apostome ciliates, *Chromidina* spp., and metazoans Dicyemida (= Rhombozoa). Five *Chromidina* spp. and over one hundred dicyemids have been described infecting cephalopods (Catalano, 2012; Souidenne et al., 2016). The exact impact on the hosts is still uncertain; for instance, in *O. vulgaris*, low levels of tissue abrasion caused by dicyemids could be observed by electron microscopy (Ridley, 1968), but no impact was detectable using light microscopy (Furuya et al., 2004). Consequently, these organisms may eventually come to be considered symbiotic rather than parasitic (Katayama et al., 1995; Furuya et al., 2004). Bacterial symbionts are also observed in cephalopods: for instance, the bacteria colonizing the pericardial appendage of *Nautilus* sp. (Pernice et al., 2007; Pernice and Boucher-Rodoni, 2012) as well as the well-established association between *Euprymna scolopes* and *Vibrio fischeri* (Ruby, 1999, for review see Gerdol et al., 2018). Further studies of such symbiosis can improve not only our understanding of these complexes associations in cephalopods, but also give insights on how bacterial symbiosis occurs in mammals (Gerdol et al., 2018).

Monogeneans

A few studies have reported monogenean parasites in cephalopods (see Sproston, 1946; Palombi, 1949; Dollfus, 1958; Bychowsky, 1961). The gyrodactylid *Isancistrum subulatae* has been found in the arms and tentacles while *Isancistrum loliginis* in the mantle cavity and gills of *Alloteuthis subulata* (Llewellyn, 1984). Identifying monogeneans in cephalopods is extremely difficult due to their delicateness, small size and the thick layer of mucus in cephalopod tissues (Llewellyn, 1984), and this could be the reason for their supposed rarity. In the future, potential sites of occurrence (e.g., arms/tentacles, mantle, funnel and gills) should be thoroughly examined for a better assessment of their true prevalence.

TABLE 1 | Parasitic taxa (approximately 230 parasites identified at species level) infecting cephalopods (sorted by order) reported in the literature to date.

	Protozoa	Chromista	Dicyemida	Monogenea	Digenea	Cestoda	Acanthocephala	Nematoda	Annelida	Crustacea
Nautilida										● (1)
Spirulida		● (1)								
Sepiida		● (7)	● (31)		⭘ (2)	⊙ (6)		○ (3)		● (5)
Myopsida		● (2)	● (5)	● (2)	⭘ (3)	⊙ (9)		○ (3)	● (4)	● (5)
Oegopsida	● (1)	● (11)			⭘ (2)	⊙ (18)	◎ (1)	○ (16)		● (4)
Octopoda		● (9)	● (59)		⭘ (3)	⊙ (2)			● (4)	● (13)
Vampyromorpha										

The role of the cephalopod host in the parasitic life cycle is indicated as: definitive ●; intermediate ○; definitive, intermediate or paratenic ⭘; intermediate or paratenic ⊙; probably accidental ◎. Number of parasite species identified for each cephalopod order is indicated in parenthesis. The current assessment reflects the original source material updated with current species information according to World Register of Marine Species (WoRMS; available at http://www.marinespecies.org/index.php).

Digeneans

The majority of information regarding digenean parasites of cephalopods is provided by Overstreet and Hochberg (1975) and Hochberg (1990), with some information added over the following decades (e.g., Shukhgalter and Nigmatullin, 2001; Nigmatullin et al., 2009), including digenean records in squid paralarvae (Vidal and Haimovici, 1999). Around 20 species have been reported from nearly 30 cephalopod hosts, usually with low prevalence of infection (Hochberg, 1990). Cephalopods do not seem to play a major role in digenean life cycles (Hochberg, 1990), though our knowledge is too limited to support this premise definitively.

Cestodes

Cephalopods are second and/or third intermediate or paratenic hosts for cestodes, acting as important vectors transporting them to other intermediate (e.g., cetaceans; Aznar et al., 2007) or definitive hosts (e.g., elasmobranchs and fishes; Hochberg, 1990). Several species have been reported in around 60 cephalopod hosts: larval and post-larval cestodes from the orders Trypanorhyncha and Tetraphyllidea are commonly found freely in cephalopod digestive tracts, usually the stomach, caecum and intestine (Hochberg, 1990). However, they can also be found in the buccal mass (in octopus; Roumbedakis, unpublished data) or encysted in the digestive tract, mesentery and mantle cavity (Hochberg, 1990). *Phyllobotrium* spp. is the most frequently reported species (Hochberg, 1990). A general life cycle for Phyllobothriidae has recently been suggested (Klotz et al., 2018): procercoid development occurs in crustaceans (first intermediate hosts), followed by plerocercoid development in bony fish, sea turtle or squid (second intermediate host). Marine mammals can harbor both plerocercoids and merocercoids, acting as third intermediate or paratenic hosts, and sharks serve as the definitive hosts, harboring the adult parasites.

Nematodes

Larval nematodes are commonly found encysted in the viscera and musculature of cephalopods (Hochberg, 1990; Gestal et al., 1999; Abollo et al., 2001), making infected animals aesthetically unattractive for human consumption (Smith and Wootten, 1984). *Anisakis* (Anisakidae) is one of the most abundant and frequent cephalopod parasites causing important pathological effects to their hosts, such as ulceration (Abollo et al., 2001), and even castration if encysted in the gonads (Abollo et al., 1998). Transmitted through food webs, these parasites have complex life cycles involving multiple hosts: planktonic or bentho-planktonic crustaceans are the first intermediate hosts; fish and squids act as second intermediate or paratenic hosts and marine mammals (mainly cetaceans) as definitive hosts (Mattiucci and D'Amelio, 2014; Mattiucci et al., 2018). To date, a number of cephalopods (*S. officinalis*, *Ancistroteuthis lichtensteinii*, *Histioteuthis bonnellii*, *Illex coindetii*, *Todarodes sagittatus*, *T. pacificus*, *Todaropsis angolenis*, *T. eblanae*, *Nototodarus sloanii*, *Dosidicus gigas*, and *Moroteuthis ingens*) are known to be parasitized by six of the nine *Anisakis* species (*A. simplex*, *A. berlandi*, *A. nascettii*, *A. pegreffii*, *A. physeteris,* and *A. typica*) currently described (for review see Tables 2–5, Mattiucci et al., 2018). Recent advances in anisakid biology and systematics are comprehensively summarized by Mattiucci et al. (2018). It is also worth noting that humans may also become accidental hosts if live larvae of *Anisakis* spp. are ingested through the consumption of raw or undercooked infected squid and cuttlefish. Additionally, even when ingested dead, *Anisakis* larvae can induce allergic reactions (Audicana et al., 2002; Mattiucci et al., 2013) or gastrointestinal problems (Audicana et al., 2002). Although rare, anisakiasis (the infection of a human by this parasite) is likely underdiagnosed and thus underestimated worldwide and may pose a greater threat to public health in the future (Bao et al., 2017; Mattiucci et al., 2018).

Crustaceans

Crustaceans, primarily copepods and isopods, usually parasitize the gills and mantle cavities of coleoid cephalopods (Pascual et al., 1996), but can also parasitize external surfaces, such as arms or head (Hochberg, 1990). Some attention was lately focused on tisbid copepods, parasites of deep-sea octopods. The details of the *Cholidya polypi* morphology and life cycle as well as a summary of Tisbidae infecting octopods are provided by Humes and Voight (1997), while a genus/species with an endoparasitic life stage infecting *Vulcanoctopus hydrothermalis* is described by López-González et al. (2000).

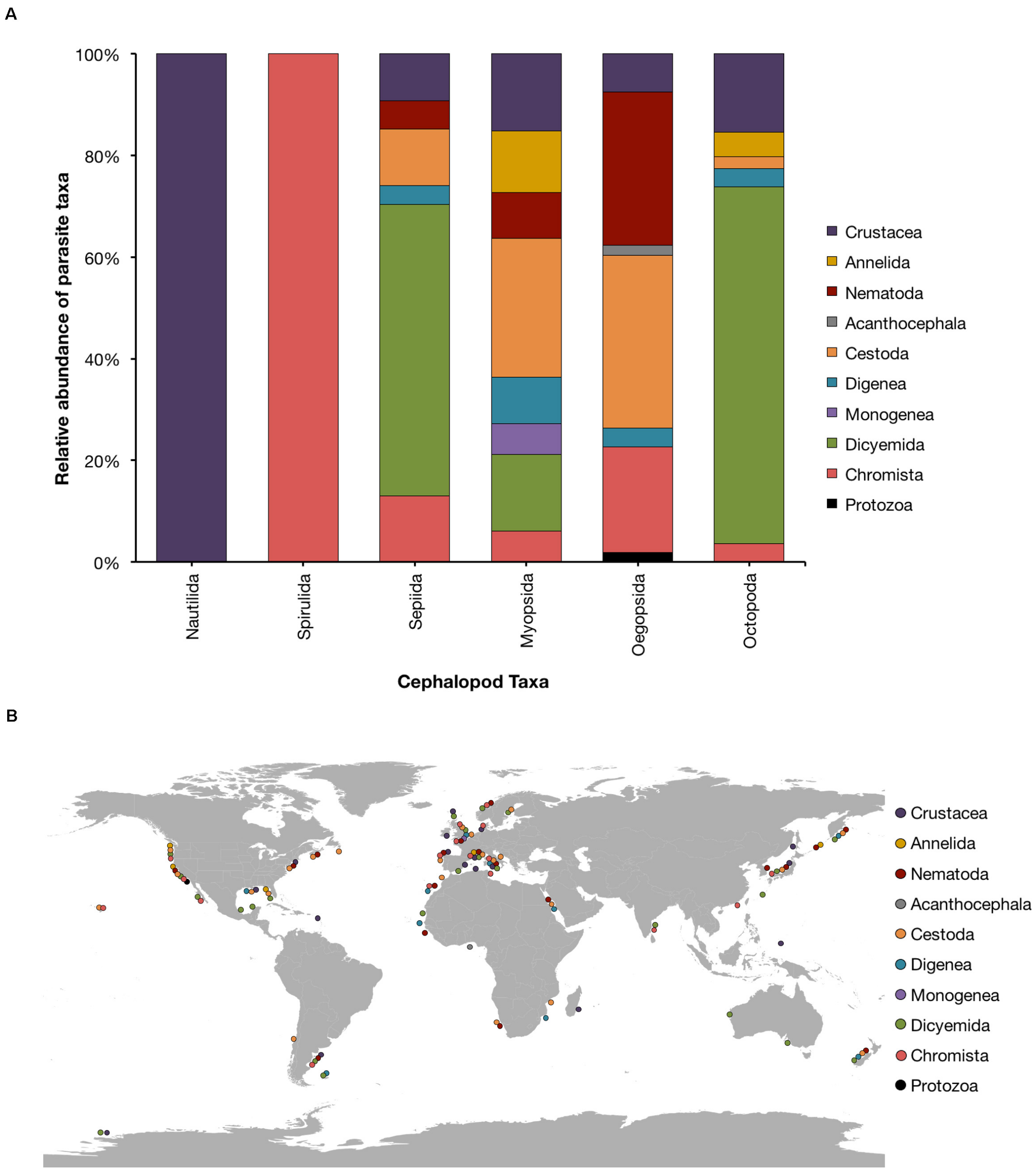


FIGURE 1 | (A) Relative abundance of parasitic taxa affecting cephalopods. **(B)** Place of capture of the cephalopod hosts. The current assessment reflects the original source material updated with current species information according to World Register of Marine Species (WoRMS; available at http://www.marinespecies.org/index.php).

CEPHALOPOD PARASITOLOGY: SUGGESTIONS FOR THE FUTURE

Despite an increase in the understanding of cephalopod parasitology during the last decades, there are still many gaps in current knowledge. Here, we briefly discuss what we believe to be the most critical issues/questions for basic and applied research that require attention.

Parasite Life Cycles and Transmission Pathways

The life cycles and transmission pathways of many cephalopod parasites are still unclear. For instance, the methods of dicyemid transmission are completely unknown (Catalano et al., 2013), and it has been estimated that less than 5% of the life cycle of marine helminths has been fully described (Poulin et al., 2016). In the case of helminths, accurate identification of these parasites by classical methods depends on the features of adult parasites, which normally occur in vertebrates. However, the adult stages of larval helminths are frequently unknown (Aznar et al., 2007), partially due to disparity in the number of parasitological studies of invertebrates compared to vertebrates (Poulin et al., 2016). Molecular tools combined with phylogenetics can help identify trophic interactions that lead to the transmission of parasites and to a better understanding of parasite life cycles (e.g., Randhawa and Brickle, 2011). Also, our understanding of interactions between diet, feeding behavior, parasitic disease, and transmission pathways of cephalopod parasites can be improved with similar combinations of traditional approaches and modern molecular methods (e.g., Petrić et al., 2011).

Poorly Explored Life-Stages and Species From Polar and Deep Sea Regions

Most of the cephalopod parasites have been described in shallow-water species. Emerging exploration of polar and deep-sea will likely expand our knowledge about the diversity of cephalopod parasites. Similarly, the current knowledge is largely restricted to juvenile and adult cephalopod hosts, with few parasites known for paralarvae/early juveniles (Vecchione, 1987; Vidal and Haimovici, 1999) and senescent animals (Pascual et al., 2010). The extension of these limits (geographical-, life-stage-, and habitat-wise-) may be the basis for new insights into host-parasite relationships, offering important insights about the parasite diversity and complexity.

Cephalopod Parasites as Biological Tags in Population Studies

Studies of parasite distribution and host specificity can provide information about host population structure, phylogeographic distribution, migration patterns and general biology. Insights into host specificity can also help predict the likelihood of a parasite successfully establishing itself and spreading in new populations, geographical regions and hosts (Poulin and Mouillot, 2003), a possibility which becomes increasingly important with accelerating global climate change.

Parasites are often utilized as "tags" for fisheries stock assessment, especially in small populations and limited timescales (MacKenzie, 1999; Mattiucci et al., 2015). *Anisakis* have been used as biological markers to identify sub-populations of pelagic and demersal fishes from the Mediterranean Sea (for review, see Mattiucci et al., 2015). In cephalopods, such studies are rare, mainly targeting squids (reviewed in Pascual and Hochberg, 1996; Catalano et al., 2014b). Although taxonomy within this clade is not yet well resolved (see Catalano, 2012 for review), dicyemids could serve the same purpose for certain benthic cephalopods, since they are closely bound to their hosts and differ across the hosts' geographical range (Catalano et al., 2014a). Another promising taxon is *Aggregata*, which, in the Mediterranean, is differentiated into three distinct clades, potentially reflecting population differentiation of its widespread host, *O. vulgaris* (Tedesco et al., 2017).

Possible Parasite Outbreaks in Cephalopod Aquaculture

Cephalopod parasites rarely cause mortality or serious damage to wild populations. However, synergic effects between different stressors associated with captivity may favor parasites and other pathogens, making parasite outbreaks more likely in aquaculture. Coincident with the development and proliferation of aquaculture, parasites and other pathogens have proliferated (e.g., Overstreet, 1973; Lom and Dyková, 1992), many causing serious economic and environmental problems. Although our knowledge of cephalopod parasites in captivity is limited, we can extrapolate (with some caution) from knowledge obtained from other, already well-established, marine organism cultures.

In fish culture for instance, high population density is known to favor rapid spread of infections, especially those caused by parasites with direct life cycles, such as monogeneans and caligid copepods (e.g., Thoney and Hargis, 1991; Johnson et al., 2004). Both groups have already been reported in cephalopods (e.g., Llewellyn, 1984; Pascual et al., 1996), and are thus worth monitoring particularly attentively in cephalopod aquaculture. High-density culture of hosts can also disrupt an otherwise stable parasite life-cycle scheme. For example, the myxosporeans *Enteromyxum* spp. normally alternate between two hosts (fish and annelid), but are known to be capable of direct fish-to-fish transmission in high-density conditions (Diamant, 1997). Likewise, another group of myxosporeans, *Kudoa* spp., which have been reported in wild octopus populations and are known to cause serious problems for marine fish aquaculture (Moran et al., 1999), has been suggested as a potential parasite in cephalopod culture (Yokoyama and Masuda, 2001). *Aggregata octopiana*, despite having a complex life cycle, can also impact octopus health during commercial ongrowing (Gestal et al., 2007).

In captivity, even apparently harmless symbionts, such as dicyemids and *Chromidina* spp., can become pathogens and inflict tissue damage to debilitated cephalopods (e.g., blocking the renal sacs ducts, Sykes and Gestal, 2014). At least three phylogenetically distant groups of potential eukaryotic pathogens that are capable of both a free-living and parasitic lifestyle (termed also saprophagic) can also be considered as potential pathogens of cephalopods: histophagous ciliates, known from cultured fish, crustaceans and bivalves (e.g., Cawthorn et al., 1996); amphizoic amoebae, known from cultured fish, crustaceans, bivalves and sea urchins (e.g., Dyková and Lom, 2004); and various fungal-like organisms known from cultured fish, crustaceans and molluscs (e.g., Derevnina et al., 2016). Since these pathogens are not limited by trade-offs regarding transmission or virulence because of their independent free-living stage (Kuris et al., 2014), they usually cause devastating economic impacts in aquaculture. Several 'fungus-like organisms' and histophagous ciliates have already been reported from cephalopods (Hanlon and Forsythe, 1990a; Tao et al., 2016) but, to date, no amphizoic amoebae have been identified.

Standardization of Parasite Sampling and Identification

Standardization of the sampling and identification methods used for cephalopods is required. Given the particular anatomy of the different cephalopod species, the publication of a guidelines, that could be used for example for parasitological and health status assessment of kept cephalopods or to determine their cause of death, would greatly facilitate research. For parasite identification, the use of classical methods (e.g., using taxonomic keys) can be extremely difficult for larval stages (Catalano et al., 2014b) or for species with high level of morphological plasticity (Poulin and Morand, 2000). In addition, some of the original parasite descriptions are not available in English (e.g., dicyemids, Nouvel, 1947, 1948; Van Beneden, 1876; Bogolepova-Dobrokhotova, 1953, 1960, 1962), are sometimes, incomplete (see Furuya, 2007), and often muddled by a variety of unresolved taxonomic and nomenclatural issues (e.g., nematodes, Smith and Wootten, 1978) which impair precise parasite identification.

The use of alternative approaches, such as search for additional morphological characters that complement classical parasite identification as suggested by Tedesco et al. (2017), the use of genetic and molecular techniques (e.g., Kopečná et al., 2006; Castellanos-Martínez et al., 2013; Souidenne et al., 2016; Tedesco et al., 2017), as well as combinations of multiple methods, is growing. Such approaches should help to better elucidate and re-evaluate the taxonomic status and host-parasite relationships, particularly where morphological plasticity might be of concern (Pascual et al., 2007). Moreover, it may clarify relationships within species complexes, such as that of *A. octopiana* infecting *O. vulgaris* in Mediterranean areas (Tedesco et al., 2017). Finally, taxonomic review of genera with morphological descriptions and molecular markers would aid research and improve assessment methods for cephalopod health and food safety in aquaculture.

The use of non- or minimally invasive methods for *in vivo* detection of cephalopod parasites should be explored in the near future. For instance, it has been suggested that *Aggregata* infection could be diagnosed through the presence of sporocysts in the feces of living animals or through inspection of the terminal intestine by gentle retraction of the ventral mantle or by endoscopy (Sykes et al., 2017). Detection of cephalopod parasite infection using ultrasound imaging or swabbing for parasite molecular/DNA sampling might also be possible. The development of these methods would facilitate early diagnosis, ultimately preventing disease outbreaks and improving animal welfare in captivity.

Cutting Edge Molecular Methods

Transcriptomics, genomics and proteomics ("omics") are relatively new tools for understanding direct host parasite relationship on a molecular level. By enabling the study of the microbiome and metagenome of different cephalopod organs in relation to parasitic infection, the consequent pathology and immune response of hosts can be better understood (see for example Castellanos-Martínez et al., 2014a,b). Additionally, low coverage genome re-sequencing or reduced representation sequencing (RADseq methods, Davey and Blaxter, 2010) provide a tool for probing the genomic structure of populations with an unprecedented level of clarity for both host and parasites. Ultimately, such genomic information coupled with environmental data results in a "seascape genomics" approach, which can reveal both local genetic adaptations as well as the broader dynamics of gene flow (Riginos et al., 2016).

Effect of Parasites in Cephalopod Physiology and Health

Host responses to parasites may involve a variety of physiological mechanisms (e.g., neural, endocrine, neuromodulatory and immune) that can interact and alter host behavior (see review in Thompson and Kavaliers, 1994). For example, in fishes, parasitism can cause conspicuous host behavior (e.g., impaired sensory and swimming performance, increased time at water surface, etc.), increasing predation risk (Lafferty and Morris, 1996). Parasites can also affect fish performance in terms of growth and reproduction, consequently impacting their health and welfare (Barber, 2007). Unfortunately, in cephalopods, the effects of parasitism are usually reported only at histopathological level, whereas physiological and behavioral effects are virtually unexplored. Experimental studies combining both behavioral and quantitative physiological indicators will help to better understand host-parasite systems and, hopefully, enable better assessment of cephalopod welfare. New technologies such as "omics" approaches and electron and florescent microscopy will certainly facilitate this research.

Resource Sharing

Although researchers have been able to build on previous research to some extent (e.g., through examination of collection of parasites and voucher specimens kept in museums, or gene mining in NCBI genetic database), there is much to be gained from employing a more open approach. The sharing of material through lab networks or open databases can reduce research effort and cost, maximize data use, and minimize the number of animals sampled. This is especially relevant for animals difficult to obtain, such as deep-sea cephalopods.

A database of cephalopod parasites and their cephalopod hosts available from the scientific literature, as already published for other species (e.g., Global Mammal Parasite Database, www.mammalparasites.org), possibly with extension of curated database of molecular barcodes, should be considered. In this regard, efforts are currently underway to publish a free online database of parasites and other pathogenic agents of cephalopods, the "Cephalopods' Pathogenic Agents Database (CephPAD)," which will include information on the affected tissue, anatomical-pathological findings, clinical presentation and mortality. An Atlas of Cephalopod Pathogens and Diseases is also in progress as follow-up to the activities of the COST Action FA1301. These initiatives will greatly facilitate the assessment of pathogenic agents and might facilitate early diagnosis of cephalopod pathogenic agents when they occur.

AUTHOR CONTRIBUTIONS

All authors contributed to the manuscript and approved the final version.

FUNDING

KR was supported through a scholarship from the Ministry of Foreign Affairs and International Cooperation, Italy (MAECI). MD and TT were supported by GACR (Nos. 15-08717S and 505/12/G112).

ACKNOWLEDGMENTS

This work is considered a contribution to the COST Action FA1301 "A network for improvement of cephalopod welfare and husbandry in research, aquaculture and fisheries" (http://www.cephsinaction.org/). We would like to thank Dr. Giovanna Ponte (Chair of the COST Action FA1301) and Dr. Graziano Fiorito (Stazione Zoologica Anton Dohrn, Naples, Italy) for support and advice. We also thank Professor Lindy Holden-Dye for suggesting this contribution following the 2017 Cephs*In*Action & CIAC Meeting, "Cephalopod Science from Biology to Welfare" (Heraklion, Crete, 2017). We are also grateful to Dr. Catlin E. O'Brien for English editing of the manuscript.

REFERENCES

Abollo, E., Gestal, C., López, A., González, A. F., Guerra, A., and Pascual, S. (1998). Squid as trophic bridges for parasite flow within marine ecosystems: the case of *Anisakis* simplex (Nematoda: Anisakidae), or when the wrong way can be right. *S. Afr. J. Mar. Sci.* 20, 223–232. doi: 10.2989/025776198784126575

Abollo, E., Gestal, C., and Pascual, S. (2001). *Anisakis* infestation in marine fish and cephalopods from Galician waters: an updated perspective. *Parasitol. Res.* 87, 492–499. doi: 10.1007/s004360100389

Audicana, M. T., Ansotegui, I. J., Corres, L. F., and Kennedy, M. W. (2002). *Anisakis* simplex: dangerous-dead and alive? *Trends Parasitol.* 18, 20–25. doi: 10.1016/S1471-4922(01)02152-3

Aznar, F. J., Agustí, C., Littlewood, D. T. J., Raga, J. A., and Olson, P. D. (2007). Insight into the role of cetaceans in the life cycle of the tetraphyllideans (Platyhelminthes: Cestoda). *Int. J. Parasitol.* 37, 243–255. doi: 10.1016/j.ijpara.2006.10.010

Bao, M., Pierce, G. J., Pascual, S., González-Muñoz, M., Mattiucci, S., Mladineo, I., et al. (2017). Assessing the risk of an emerging zoonosis of worldwide concern: anisakiasis. *Sci. Rep.* 7:43699. doi: 10.1038/srep43699

Barber, I. (2007). Parasites, behaviour and welfare in fish. *Appl. Anim. Behav. Sci.* 104, 251–264. doi: 10.1016/j.applanim.2006.09.005

Bogolepova-Dobrokhotova, I. I. (1953). *Materials on Dicyemids of Far-eastern Seas*. Ph.D. dissertation, Academiya Nauk and Zoologicheskii Institute, Leningrad.

Bogolepova-Dobrokhotova, I. I. (1960). Dicyemidae of the far-eastern seas. I. New species of the genus Dicyema. *Zool. Zhurnal* 39, 1293–1302.

Bogolepova-Dobrokhotova, I. I. (1962). Dicyemidae of the far-eastern seas. II. New species of the genus Dicyemennea. *Zool. Zhurnal* 41, 503–518.

Bychowsky, B. E. (1961). *Monogenetic Trematodes. Their Systematics and Phylogeny*. Washington, DC: American Institute of Biological Sciences.

Castellanos-Martínez, S., Arteta, D., Catarino, S., and Gestal, C. (2014a). De novo transcriptome sequencing of the *Octopus vulgaris* hemocytes using illumina RNA-Seq technology: response to the infection by the gastrointestinal parasite *Aggregata octopiana*. *PLoS One* 9:e107873. doi: 10.1371/journal.pone.0107873

Castellanos-Martínez, S., Diz, A. P., Álvarez-Chaver, P., and Gestal, C. (2014b). Proteomic characterization of the hemolymph of *Octopus vulgaris* infected by the protozoan parasite *Aggregata octopiana*. *J. Proteomics* 105, 151–163. doi: 10.1016/j.jprot.2013.12.008

Castellanos-Martínez, S., and Gestal, C. (2013). Pathogens and immune response of cephalopods. *J. Exp. Mar. Biol. Ecol.* 447, 14–22. doi: 10.1016/j.jembe.2013.02.007

Castellanos-Martínez, S., Pérez-Losada, M., and Gestal, C. (2013). Molecular phylogenetic analysis of the coccidian cephalopod parasites *Aggregata octopiana* and *Aggregata eberthi* (Apicomplexa: Aggregatidae) from the NE Atlantic coast using 18S rRNA sequences. *Eur. J. Protistol.* 49, 373–380. doi: 10.1016/j.ejop.2012.11.005

Catalano, S. R. (2012). A review of the families, genera and species of dicyemida van beneden, 1876. *Zootaxa* 32, 1–32. doi: 10.11646/zootaxa.3646.1.11

Catalano, S. R., Whittington, I. D., Donnellan, S. C., and Gillanders, B. M. (2013). Using the giant Australian cuttlefish (*Sepia apama*) mass breeding aggregation to explore the life cycle of dicyemid parasites. *Acta Parasitol.* 58, 599–602. doi: 10.2478/s11686-013-0186-y

Catalano, S. R., Whittington, I. D., Donnellan, S. C., and Gillanders, B. M. (2014a). Dicyemid fauna composition and infection patterns in relation to cephalopod host biology and ecology. *Folia Parasitol.* 61, 301–310. doi: 10.14411/fp.2014.034

Catalano, S. R., Whittington, I. D., Donnellan, S. C., and Gillanders, B. M. (2014b). Parasites as biological tags to assess host population structure: guidelines, recent genetic advances and comments on a holistic approach. *Int. J. Parasitol.* 3, 220–226. doi: 10.1016/j.ijppaw.2013.11.001

Cawthorn, R. J., Lynn, D. H., Despres, B., MacMillan, R., Maloney, R., Loughlin, M., et al. (1996). Description of Anophryoides haemophila n. sp. (Scuticociliatida: Orchitophryidae), a pathogen of American lobsters *Homarus americanus*. *Dis. Aquat. Org.* 24, 143–148. doi: 10.3354/dao024143

Davey, J. W., and Blaxter, M. L. (2010). RADSeq: next-generation population genetics. *Brief. Funct. Genomics* 9, 416–423. doi: 10.1093/bfgp/elq031

Derevnina, L., Petre, B., Kellner, R., Dagdas, Y. F., Sarowar, M. N., Giannakopoulou, A., et al. (2016). Emerging oomycete threats to plants and animals. *Philos. Trans. R. Soc. B Biol. Sci.* 371:20150459. doi: 10.1098/rstb.2015.0459

Diamant, A. (1997). Fish-to-fish transmission of a marine myxosporean. *Dis. Aquat. Org.* 30, 99–105. doi: 10.3354/dao030099

Dobell, C. C. (1925). The life history and chromosome cycle of *Aggregata eberthi* (Protozoa: Sporozoa: Coccidia). *Parasitology* 17, 1–136. doi: 10.1017/S0031182000082184

Dollfus, R. P. (1958). Copepodes, isopodes et helminths parasites de Cephalopodes de la Mediterranee et de l'Atlantique Europeen. *Faune Mar. Pyninees Orient.* 1, 61–72.

Dyková, I., and Lom, J. (2004). Advances in the knowledge of amphizoic amoebae infecting fish. *Folia Parasitol.* 51, 81–97. doi: 10.14411/fp.2004.014

Furuya, H. (2007). Redescription of two dicyemennea (Phylum: Dicyemida) From *Rossia Pacifica* (Mollusca: Cephalopoda: Decapoda). *J. Parasitol.* 93, 841–849. doi: 10.1645/GE-1003R.1

Furuya, H., Ota, M., Kimura, R., and Tsuneki, K. (2004). Renal organs of cephalopods: a habitat for dicyemids and chromidinids. *J. Morphol.* 262, 629–643. doi: 10.1002/jmor.10265

Gerdol, M., Gomez-Chiarri, M., Castillo, M. G., Figueras, A., Fiorito, G., Moreira, R., et al. (2018). "Immunity in molluscs: recognition and effector mechanisms, with a focus on bivalvia," in *Advances in Comparative Immunology*, ed. E. L. Cooper (Berlin: Springer).

Gestal, C., Abollo, E., and Pascual, S. (2002a). Observations on associated histopathology with *Aggregata octopiana* infection (Protista: Apicomplexa) in *Octopus vulgaris*. *Dis. Aquat. Org.* 50, 45–49. doi: 10.3354/dao050045

Gestal, C., De La Cadena, M. P., and Pascual, S. (2002b). Malabsorption syndrome observed in the common octopus *Octopus vulgaris* infected with *Aggregata* *octopiana* (Protista: Apicomplexa). *Dis. Aquat. Org.* 51, 61–65. doi: 10.3354/dao051061

Gestal, C., Guerra, A., and Pascual, S. (2007). *Aggregata octopiana* (Protista: Apicomplexa): a dangerous pathogen during commercial *Octopus vulgaris* ongrowing. *ICES J. Mar. Sci.* 64, 1743–1748. doi: 10.1093/icesjms/fsm154

Gestal, C., Pascual, S., Corral, L., and Azevedo, C. (1999). Ultrastructural aspects of the sporogony of *Aggregata octopiana* (Apicomplexa, Aggregatidae), a coccidian parasite of *Octopus vulgaris* (Mollusca, Cephalopoda) from NE Atlantic coast. *Eur. J. Protistol.* 35, 417–425. doi: 10.1016/s0932-4739(99)80051-0

Gestal, C., Pascual, S., and Hochberg, F. G. (2010). *Aggregata bathytherma* sp. nov. (Apicomplexa: Aggregatidae), a new coccidian parasite associated with a deep-sea hydrothermal vent octopus. *Dis. Aquat. Org.* 91, 237–242. doi: 10.3354/dao02264

González, A. F., Pascual, S., Gestal, C., Abollo, E., and Guerra, A. (2003). What makes a cephalopod a suitable host for parasite? The case of Galician waters. *Fish. Res.* 60, 177–183. doi: 10.1016/S0165-7836(02)00059-0

Hanlon, R. T., and Forsythe, J. W. (1990a). "Diseases of mollusca: cephalopoda: diseases caused by microorganisms," in *Diseases of Marine Animals, Vol. III, Cephalopoda to Urochordata*, ed. O. Kinne (Hamburg: Biologisches Anstalt Helgoland), 23–46.

Hanlon, R. T., and Forsythe, J. W. (1990b). "Diseases of mollusca: cephalopoda: structural abnormalitie and neoplasia," in *Diseases of Marine Animals, Vol. III, Cephalopoda to Urochordata*, ed. O. Kinne (Hamburg: Biologisches Anstalt Helgoland), 203–204.

Heil, M. (2016). Host manipulation by parasites: cases, patterns, and remaining doubts. *Front. Ecol. Evol.* 4:80. doi: 10.3389/fevo.2016.00080

Hochberg, F. G. (1990). "Diseases of mollusca: cephalopoda: diseases caused by protistans and metazoans," in *Diseases of Marine Animals, Vol. III, Cephalopoda to Urochordata*, ed. O. Kinne (Hamburg: Biologisches Anstalt Helgoland), 47–227.

Humes, A. G., and Voight, J. R. (1997). Cholidya polypi (copepoda: Harpacticoida: Tisbidae), a parasite of deep-sea octopuses in the north atlantic and northeastern pacific. *Ophelia* 46, 65–81. doi: 10.1080/00785326.1997.10432478

Johnson, S. C., Bravo, S., Nagasawa, K., Kabata, Z., Hwang, J., Ho, J., et al. (2004). A review of the impact of parasitic copepods on marine aquaculture. *Zool. Stud.* 43, 229–243.

Katayama, T., Wada, H., Furuya, H., Satoh, N., and Yamamoto, M. (1995). Phylogenetic position of the dicyemid Mesozoa inferred from 18S rDNA sequences. *Biol. Bull.* 189, 81–90. doi: 10.2307/1542458

Kinne, O. (1990). *Diseases of Marine Animals, Vol. III. Cephalopoda to Urochordata*. Hamburg: Biologisches Anstalt Helgoland.

Klotz, D., Hirzmann, J., Bauer, C., Schöne, J., Iseringhausen, M., Wohlsein, P., et al. (2018). Subcutaneous merocercoids of Clistobothrium sp. in two Cape fur seals (*Arctocephalus pusillus* pusillus). *Int. J. Parasitol. Parasites Wildl.* 7, 99–105. doi: 10.1016/j.ijppaw.2018.02.003

Kopečná, J., Jirku, M., Oborník, M., Tokarev, Y. S., Lukeš, J., and Modrý, D. (2006). Phylogenetic analysis of coccidian parasites from invertebrates: search for missing links. *Protist* 157, 173–183. doi: 10.1016/j.protis.2006.02.005

Kuris, A. M., Lafferty, K. D., and Sokolow, S. H. (2014). Sapronosis: a distinctive type of infectious agent. *Trends Parasitol.* 30, 386–393. doi: 10.1016/j.pt.2014.06.006

Lafferty, K. D. (1999). The evolution of trophic transmission. *Parasitol. Today* 15, 111–115. doi: 10.1016/S0169-4758(99)01397-6

Lafferty, K. D., and Morris, A. K. (1996). Altered behavior of parasitized killifish increases susceptibility to predation by bird final hosts. *Ecology* 77, 1390–1397. doi: 10.2307/2265536

Llewellyn, J. (1984). The biology of *Isancistrum subulatae* n.sp., a monogenean parasitic on the squid. *Alloteuthis subulata*, at Plymouth. *J. Mar. Biol. Assoc. U.K.* 64, 285–302. doi: 10.1017/S0025315400030009

Lom, J., and Dyková, I. (1992). *Protozoan Parasites of Fishes*. Amsterdam: Elsevier.

López-González, P. J., Bresciani, J., Huys, R., González, A. F., Guerra, A., and Pascual, S. (2000). Description of Genesis vulcanoctopusi gen. et sp. nov. (Copepoda: Tisbidae) parasitic on a hydrothermal vent octopod and a reinterpretation of the life cycle of cholidyinid harpacticoids. *Cah. Biol. Mar.* 41, 241–253.

MacKenzie, K. (1999). Parasites as biological tags in population studies of marine fish. *Qatar Univ. Sci. J.* 19, 117–127. doi: 10.1016/S1383-5769(98)80066-4

Mattiucci, S., Cimmaruta, R., Cipriani, P., Abaunza, P., Bellisario, B., and Nascetti, G. (2015). Integrating *Anisakis* spp. parasites data and host genetic structure in the frame of a holistic approach for stock identification of selected Mediterranean Sea fish species. *Parasitology* 142, 90–108. doi: 10.1017/S0031182014001103

Mattiucci, S., Cipriani, P., Levsen, A., Paoletti, M., and Nascetti, G. (2018). Molecular epidemiology of *Anisakis* and Anisakiasis: an ecological and evolutionary road map. *Adv. Parasitol.* 99, 93–263. doi: 10.1016/bs.apar.2017.12.001

Mattiucci, S., and D'Amelio, S. (2014). "Anisakiasis," in *Helminth Infections and Their Impact on Global Public Health*, ed. F. Bruschi (Vienna: Springer Vienna), 325–365. doi: 10.1007/978-3-7091-1782-8

Mattiucci, S., Fazii, P., De Rosa, A., Paoletti, M., Megna, A. S., Glielmo, A., et al. (2013). Anisakiasis and gastroallergic reactions associated with *Anisakis pegreffii* infection. Italy. *Emerg. Infect. Dis.* 19, 496–499. doi: 10.3201/eid1903.121017

Mladineo, I., and Bočina, I. (2007). Extraintestinal gamogony of *Aggregata octopiana* in the reared common octopus (*Octopus vulgaris*) (Cephalopoda: Octopodidae). *J. Inv. Pathol.* 96, 261–264. doi: 10.1016/j.jip.2007.05.010

Moran, J. D. W., Whitaker, D. J., and Kent, M. L. (1999). A review of the myxosporean genus Kudoa Meglitsch, 1947, and its impact on the international aquaculture industry and commercial fisheries. *Aquaculture* 172, 163–196. doi: 10.1016/S0044-8486(98)00437-2

Nigmatullin, C. M., Shchetinnikov, A. S., and Shukhgalter, O. A. (2009). On feeding and helminth fauna of neon flying squid *Ommastrephes bartramii* (Lesueur, 1821) (Cephalopoda: Ommastrephidae) in the southeastern Pacific. *Rev. Biol. Mar. Oceanogr.* 44, 227–235. doi: 10.4067/s0718-19572009000100023

Nouvel, H. (1947). Les dicyémides 1er partie: systématique, générations vermiformes, infusorigène et sexualité. *Arch. Biol.* 58, 59–214.

Nouvel, H. (1948). Les dicyemides 2em partie: infusoriforme, teratologie, specificite du parasitisime, affinites. *Arch. Biol.* 59, 147–223.

Overstreet, R. M. (1973). Parasites of some penaeid shrimps with emphasis on reared hosts. *Aquaculture* 2, 105–140. doi: 10.1016/0044-8486(73)90140-3

Overstreet, R. M., and Hochberg, F. G. (1975). Digenetic trematodes in cephalopods. *J. Mar. Biol. Assoc. U.K.* 55, 893–910. doi: 10.1017/S0025315400017781

Palombi, A. (1949). I trematodi d'ltalia, Parte I. Trematodi monogenetici. *Arch. Zool. Ital.* 34, 203–408.

Pascual, S., Gestal, C., Estévez, J. M., Rodríguez, H., Soto, M., Abollo, E., et al. (1996). Parasites in commercially-exploited cephalopods (Mollusca, Cephalopoda) in Spain: an updated perspective. *Aquaculture* 142, 1–10. doi: 10.1016/0044-8486(96)01254-9

Pascual, S., González, A., Arias, C., and Guerra, A. (1995). Helminth infection in the short-finned squid *Illex coindetii* (Cephalopoda, Ommastrephidae) off NW Spain. *Dis. Aquat. Org.* 23, 71–75. doi: 10.1645/GE-2562.1

Pascual, S., González, A., and Guerra, A. (2007). Parasites and cephalopod fisheries uncertainty: towards a waterfall understanding. *Rev. Fish Biol. Fish.* 17, 139–144. doi: 10.1007/s11160-006-9021-y

Pascual, S., González, A. F., and Guerra, A. (2010). Coccidiosis during octopus senescence: preparing for parasite outbreak. *Fish. Res.* 106, 160–162. doi: 10.1016/j.fishres.2010.05.013

Pascual, S., and Hochberg, F. G. (1996). Marine parasites as biological tags of cephalopod hosts. *Parasitol. Today* 12, 324–327. doi: 10.1016/0169-4758(96)40004-7

Pernice, M., and Boucher-Rodoni, R. (2012). Occurrence of a specific dual symbiosis in the excretory organ of geographically distant Nautiloids populations. *Environ. Microbiol. Rep.* 4, 504–511. doi: 10.1111/j.1758-2229.2012.00352.x

Pernice, M., Wetzel, S., Gros, O., Boucher-Rodoni, R., and Dubilier, N. (2007). Enigmatic dual symbiosis in the excretory organ of *Nautilus macromphalus* (Cephalopoda: Nautiloidea). *Proc. R. Soc. B Biol. Sci.* 274, 1143–1152. doi: 10.1098/rspb.2006.0353

Petrić, M., Mladineo, I., and Šifner, S. K. (2011). Insight into the short-finned squid *Illex coindetii* (Cephalopoda: Ommastrephidae) feeding ecology: is there a link between helminth parasites and food composition? *J. Parasitol.* 97, 55–62. doi: 10.1645/GE-2562.1

Poulin, R., Blasco-Costa, I., and Randhawa, H. S. (2016). Integrating parasitology and marine ecology: seven challenges towards greater synergy. *J. Sea Res.* 113, 3–10. doi: 10.1016/j.seares.2014.10.019

Poulin, R., and Morand, S. (2000). The diversity of parasites. *Q. Rev. Biol.* 75, 277–293. doi: 10.1086/393500

Poulin, R., and Mouillot, D. (2003). Parasite specialization from a phylogenetic perspective: a new index of host specificity. *Parasitology* 126, 473–480. doi: 10.1017/s0031182003002993

Randhawa, H. S., and Brickle, P. (2011). Larval parasite gene sequence data reveal cryptic trophic links in life cycles of porbeagle shark tapeworms. *Mar. Ecol. Prog. Ser.* 431, 215–222. doi: 10.3354/meps09108

Ridley, R. K. (1968). Electron microscopic studies on Dicyemid mesozoa. I. Vermiform Stages. *J. Parasitol.* 54, 975–998. doi: 10.2307/3277133

Riginos, C., Crandall, E. D., LIggins, L., Bongaerts, P., and Treml, E. A. (2016). Navigating the currents of seascape genomics: how spatial analyses can augment population genomic studies. *Curr. Zool.* 62, 581–601. doi: 10.1093/cz/zow067

Ruby, E. G. (1999). The euprymna scolopes-*Vibrio fischeri* symbiosis: a biomedical model for the study of bacterial colonization of animal tissue. *J. Mol. Microbiol. Biotechnol.* 1, 13–21.

Shukhgalter, O. A., and Nigmatullin, C. M. (2001). Parasitic helminths of jumbo squid *Dosidicus gigas* (Cephalopoda: Ommastrephidae) in open waters of the central east Pacific. *Fish. Res.* 54, 95–110. doi: 10.1016/s0165-7836(01)00374-5

Smith, J. W., and Wootten, R. (1978). *Anisakis* and anisakiasis. *Adv. Parasitol.* 16, 93–163. doi: 10.1016/S0065-308X(08)60573-4

Smith, J. W., and Wootten, R. (1984). "Anisakis larvae (herringworm) (nematoda) in fish. Parasitose des poissons par les larves du nématode *Anisakis*," in *Fiches d'identification des Maladies et Parasites des Poissons, Crustaces et Mollusques*, ed. C. J. Sinderman (Silver Spring, MD: NOAA National Marine Fisheries Service), 2–4.

Souidenne, D., Florent, I., Dellinger, M., Justine, J., Romdhane, M. S., Furuya, H., et al. (2016). Diversity of apostome ciliates, *Chromidina* spp. (Oligohymenophorea, Opalinopsidae), parasites of cephalopods of the Mediterranean Sea. *Parasite* 23:33. doi: 10.1051/parasite/2016033

Sproston, N. G. (1946). A synopsis of the monogenetic trematodes. *Trans. Zool. Soc. Lond.* 25, 185–600.

Sykes, A. V., Almansa, E., Cooke, G. M., Ponte, G., and Andrews, P. L. (2017). The digestive tract of cephalopods: a neglected topic of relevance to animal welfare in the laboratory and aquaculture. *Front. Physiol.* 8:492. doi: 10.3389/fphys.2017.00492

Sykes, A. V., and Gestal, C. (2014). "Welfare and diseases under culture conditions," in *Cephalopod Culture*, ed. R. Villanueva (Berlin: Springer), 479–489. doi: 10.1007/978-94-017-8648-5_6

Tao, Z., Liu, L., Chen, X., Zhou, S., and Wang, G. (2016). First isolation of *Miamiensis avidus* (Ciliophora: Scuticociliatida) associated with skin ulcers from reared pharaoh cuttlefish *Sepia pharaonis*. *Dis. Aquat. Org.* 122, 67–71. doi: 10.3354/dao03067

Tedesco, P., Gestal, C., Begić, K., Mladineo, I., Castellanos-Martínez, S., Catanese, G., et al. (2017). Morphological and molecular characterization of aggregata spp. Frenzel 1885 (Apicomplexa: Aggregatidae) in *Octopus vulgaris* Cuvier 1797 (Mollusca: Cephalopoda) from Central Mediterranean. *Protist* 168, 636–648. doi: 10.1016/j.protis.2017.08.002

Thompson, S. N., and Kavaliers, M. (1994). Physiological bases for parasite-induced alterations of host behaviour. *Parasitology* 109, S119–S138. doi: 10.1017/S0031182000085139

Thoney, D. A., and Hargis, W. J. Jr. (1991). Monogenea (Platyhelminthes) as hazards for fish in confinement. *Annu. Rev. Fish Dis.* 1, 133–153. doi: 10.1016/0959-8030(91)90027-H

Van Beneden, M. E. (1876). Recherches sur le Dicyemides, survivants actuels d'un embranchement des Mésozoaires. *Bull. Acad. R. Sci. Lett. Beaux Arts Belgique* 41, 1160–1205.

Vecchione, M. (1987). "Juvenile ecology," in *Cephalopod Life Cycles*, ed. P. R. Boyle (London: Academic Press), 61–84.

Vidal, E. A. G., and Haimovici, M. (1999). Digestive tract parasites in rhynchoteuthion squid paralarvae, particularly in *Illex argentinus* (Cephalopoda: Ommastrephidae). *Fish. Bull.* 97, 402–405.

Yokoyama, H., and Masuda, K. (2001). *Kudoa* sp. (Myxozoa) causing a post-mortem myoliquefaction of North-Pacific giant octopus *Paroctopus dofleini* (Cephalopoda: Octopodidae). *Bull. Eur. Assoc. Fish Pathol.* 21, 266–268.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 20 July 2018
doi: 10.3389/fphys.2018.00952

Cephalopod Brains: An Overview of Current Knowledge to Facilitate Comparison With Vertebrates

Shuichi Shigeno[1]†, Paul L. R. Andrews[1,2], Giovanna Ponte[1]* and Graziano Fiorito[1]*

[1] Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Naples, Italy, [2] Division of Biomedical Sciences, St. George's University of London, London, United Kingdom

OPEN ACCESS

Edited by:
Fernando Ariel Genta,
Fundação Oswaldo Cruz (Fiocruz),
Brazil

Reviewed by:
Daniel Osorio,
University of Sussex, United Kingdom
Euan Robert Brown,
Heriot-Watt University,
United Kingdom

***Correspondence:**
Shuichi Shigeno
sushigeno@gmail.com
Giovanna Ponte
giovanna.ponte@szn.it

†Present address:
Shuichi Shigeno,
Biosciences Division, Argonne
National Laboratory, Argonne, IL,
United States;
Department of Neurobiology,
The University of Chicago, Chicago,
IL, United States

Specialty section:
This article was submitted to
Invertebrate Physiology,
a section of the journal
Frontiers in Physiology

Received: *15 March 2018*
Accepted: *28 June 2018*
Published: *20 July 2018*

Citation:
Shigeno S, Andrews PLR, Ponte G and Fiorito G (2018) Cephalopod Brains: An Overview of Current Knowledge to Facilitate Comparison With Vertebrates. Front. Physiol. 9:952. doi: 10.3389/fphys.2018.00952

Cephalopod and vertebrate neural-systems are often highlighted as a traditional example of convergent evolution. Their large brains, relative to body size, and complexity of sensory-motor systems and behavioral repertoires offer opportunities for comparative analysis. Despite various attempts, questions on how cephalopod 'brains' evolved and to what extent it is possible to identify a vertebrate-equivalence, assuming it exists, remain unanswered. Here, we summarize recent molecular, anatomical and developmental data to explore certain features in the neural organization of cephalopods and vertebrates to investigate to what extent an evolutionary convergence is likely. Furthermore, and based on whole body and brain axes as defined in early-stage embryos using the expression patterns of homeodomain-containing transcription factors and axonal tractography, we describe a critical analysis of cephalopod neural systems showing similarities to the cerebral cortex, thalamus, basal ganglia, midbrain, cerebellum, hypothalamus, brain stem, and spinal cord of vertebrates. Our overall aim is to promote and facilitate further, hypothesis-driven, studies of cephalopod neural systems evolution.

Keywords: octopus, cephalopod, brain, evolution, neural networks

INTRODUCTION

Due to shared computational and functional constraints on the evolutionary development of complex neural systems, phyletically distant animals often exhibit 'phenotypic' similarity in their neural organization (Farris, 2008; Roth, 2013; Wolff and Strausfeld, 2016; Shigeno, 2017). However, the origin and evolution of neural systems across animal phyla remains uncertain (Moroz, 2009; Northcutt, 2012; Holland et al., 2013; Holland, 2016). For example, centralization of nervous systems has occurred on more than five occasions during evolution (e.g., molluscs, annelids, nematodes, arthropods and chordates; see discussion in Moroz, 2009), and the acquisition of behavioral 'capabilities' such as the need for foraging strategies, spatial-, social- and instrumental-learning are all considered major driving forces in the evolution of complex brains and "high intelligence" several times independently in the animal kingdom (Roth, 2015). New evidence supports the view that nervous systems are not monophyletic, suggesting widespread homoplasy in nervous systems (Moroz, 2009; Liebeskind et al., 2016).

Invertebrate nervous systems are extremely diversified spanning from diffuse nerve nets (e.g., cnidarians) to tetra-neury (molluscs), ventral cords (e.g., annelids, arthropods), nerve net-like in hemichordates, and do not resemble those of higher chordates that are organized around a dorsal "hollow tube" (see for example review in Moroz, 2009). To facilitate comparison and to favor the identification of "robust homology hypotheses" Richter et al. (2010) proposed a neuroanatomical terminology of invertebrate nervous systems. We will not necessarily follow the neuroanatomical terminology adopted by Richter et al. (2010) since we will prefer to refer to the classic terms as defined by Young and coworkers for cephalopod brains (Young, 1971; review in Nixon and Young, 2003).

In several protostomes, such as annelids and insects, the 'higher' centers (here considered as centers of associative and high-order sensory/motor neural-processing), such as the mushroom bodies, tend to congregate in anterior nervous territories, similar to the situation that occurs in the vertebrate pallium (Arendt, 2008; Loesel and Heuer, 2010; Tomer et al., 2010; Wiersma and Roach, 2011). In each of these taxa, 'higher' neural-centers are found in a few species, but absent in more 'basal' species of the group, suggesting that complex brains and higher centers evolve as a consequence of an independent specialization (Farris, 2008; Hejnol and Martindale, 2008; Moroz, 2009). An alternative explanation is that these species share molecular machinery with their deep ancestries, and that the 'loss' of higher centers in the basal species is the result of secondary simplification (Tomer et al., 2010). Neural structures such as the spinal cord, the hypothalamus, and basal ganglia have their 'equivalents' in annelids (Denes et al., 2007; Tessmar-Raible et al., 2007) and insects (Arendt and Nubler-Jung, 1999; Loesel et al., 2002; Strausfeld and Hirth, 2013) and are considered to share common molecular and structural profiles.

Molluscs allow an exploration of the potential evolutionary scenarios of nervous system evolution, due to the variety of different organizations (review in Kandel, 1979) of their acephalic ganglia, simple medullary cords, and centralized brains (Bullock, 1965a,b,c,d) that appear to be dissimilar to those of insects and vertebrates (Budelmann, 1995; Budelmann et al., 1997; Hochner, 2010). Molluscs also provide examples where some independent parallel events of centralization of nervous systems occur (Moroz, 2009).

Within the phylum Mollusca the coleoid cephalopod *Octopus vulgaris* has an exceptionally large brain that includes more than 30 differentiated lobes (Young, 1971), numerous cells (Young, 1963) possibly belonging to different cellular-types (Young, 1932; Bogoraze and Cazal, 1944; Young, 1972), highly organized neuropils and fasciculated tract bundles (Young, 1971; Hochner et al., 2006).

Here we review recent molecular, anatomical and developmental data to explore possible "homologies" of cephalopod neural structures with respect to vertebrate brains, a challenging task considering the more than 500 million years of independent evolution (see for example: Packard, 1972; Kröger et al., 2011; Roth, 2013). It is without doubt that many sensory-motor systems, locomotor abilities, and behaviors of cephalopods are traceable into vertebrate equivalents (e.g., Budelmann, 1995; Budelmann et al., 1997; Hochner and Glanzman, 2016; Villanueva et al., 2017). It is also true that the cephalopod brain is "truly molluscan" in its anatomical organization, making attempts to draw parallels between more than 30 lobes identified in its 'central' nervous system "and the brains of vertebrate species unrealistic" (Packard, 1972; see also Shigeno et al., 2015). However, some very "striking resemblances" occur (Packard, 1972): (i) the deep retina of fish and the surface of cephalopod optic lobe, (ii) the neural-architecture of the peduncle lobe in the octopus brain (which recalls the folia arrangements of the vertebrate cerebellum), (iii) the vertical lobe which is considered the analog of the mammalian limbic lobe (Young, 1991, 1995).

We summarize classic and modern views regarding neural-functional equivalencies between cephalopods and vertebrates, and highlight additional insights emerging from recent molecular and neurophysiological studies. Furthermore, we outline an embryological approach that allows identification of some features of relevance to the evolutionary paths leading to the neural centralization and differentiation of the cephalopod brain (see also: Focareta et al., 2014; Wollesen et al., 2014, 2015a; Shigeno et al., 2015; Buresi et al., 2016).

The 'Brain' of Cephalopods – An Outline and a Summary of Novelties

In the octopus, as far other cephalopod species, the 'brain' is assembled through a series of ganglia of molluscan origin to form lobes that are fused together into masses (for the common octopus see description in Young, 1971; see also an outline of the brain and its main connections in **Figure 1A**). These are connected to periphery by many nerve trunks regulating the arms, viscera and other part of the animal's body connecting with the sub-esophageal mass (SUB; lower structure in **Figure 1B**), and which in turn connects directly or indirectly to the lobes of the supra-esophageal mass (SEM; **Figure 1B**, top).

The major connectives, commissures, and matrix of interneurons have been analyzed extensively using the Golgi and Cajal reduced silver staining methods (Young, 1971; see also **Figures 1B,C**). In addition, horseradish peroxidase, cobalt, and carbocyanine dye tracing methods have provided further detail (e.g., Young, 1971; Saidel, 1982; Budelmann and Young, 1985; Plän, 1987; Robertson et al., 1993).

According to the classical view, the SEM, lying above the esophagus, is dorsal with respect to the body-axis, while the SUB, extending below the esophagus, is ventral. The foremost dorsal structure of the SEM, the vertical lobe (vtL in **Figures 1B,C**), is considered one of the most distinctive structures in the cephalopod brain. It comprises about 14% of the volume of the entire supra-esophageal mass in an adult octopus (Frösch, 1971; Maddock and Young, 1987), and has over 25 million nerve cells, more than half of all the cells located in the supraesophageal mass (Young, 1963). When considered with the nearby center, i.e., the superior-frontal lobe (sFL in **Figure 1B**), the 'vertical lobe system' is recognized as the largest learning and memory ('higher') center among all known invertebrate neural structures (Young, 1991; Shomrat et al., 2015; Marini et al., 2017; Turchetti-Maia et al., 2017).

The dorso-ventral orientation of the brain with respect to the body-axis, as described above (see also **Figure 1**) seems unconfirmed by developmental studies. The antero-posterior expression of *Hox* genes (a family of transcription factors responsible for defining axial identity in bilaterians, Pearson et al.,

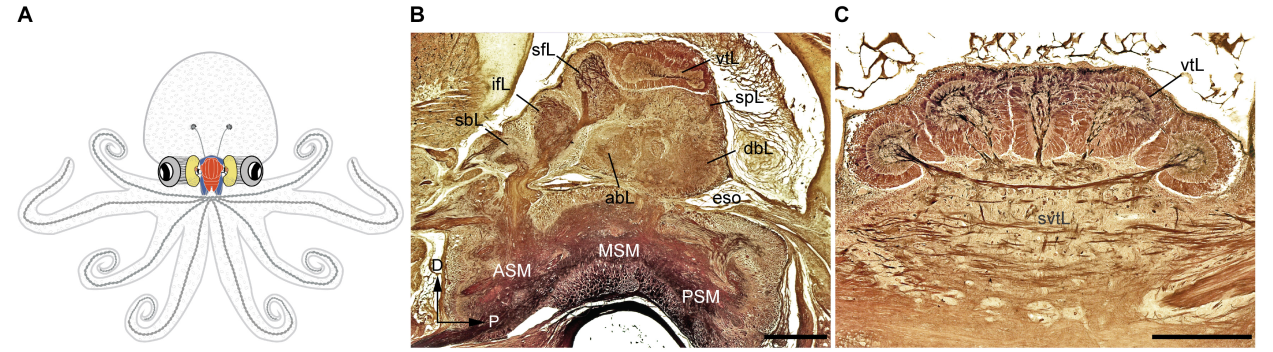


FIGURE 1 | The adult *Octopus vulgaris* brain. **(A)** Schematic outline of octopus body and the relative relationships to the main components of its nervous system. **(B)** A longitudinal section of the supra- and sub-esophageal mass of *O. vulgaris* (parasagittal plane). **(C)** A cross section of the vertical lobe (supra-esophageal mass), showing the five distinct gyri. The esophagus lies at the center between the supraesophageal and subesophageal mass. Sections of stained with the Cajal silver method. abL, anterior basal lobe; ASM, anterior subesophageal mass; dbL, dorsal basal lobe; eso, esophagus; ifL, inferior frontal lobe; MSM, middle subesophageal mass; PSM, posterior subesophageal mass; sbL, superior buccal lobe; sfL, superior frontal lobe; spL, subpedunculate lobe; svtL, subvertical lobe; vtL, vertical lobe. Scale bars: 500 μm.

2005) in structures such as the cephalopod brachial and buccal crown, funnel, and stellate ganglia are not predicted by *Hox* collinearity. Their expression along the axis does not appear to demonstrate the canonical nested domains characteristic of these transcription factors (see Lee et al., 2003). Furthermore, as defined by embryological orientation along the body axis (see for example: Shigeno et al., 2008, 2010; Buresi et al., 2016), the brain areas controlling arms and brachial centers are considered ventral, while those controlling the mantle and visceral organs appear dorsal: a remarkable shift.

Despite some initial interest, the phylogenetic origins of cephalopod neural centers remain largely unexplored (Young, 1977a; Nixon and Young, 2003; see also discussion in Grasso and Basil, 2009). The recent genomic sequencing of *O. bimaculoides* (Albertin et al., 2015) and the possible availability of other cephalopod genomes in the near future opens a new era. The analysis of *O. bimaculoides* genome revealed that the basic neuronal gene repertoire of cephalopods is shared with that of many other invertebrates. However, the octopus genome appears to be characterized by extensive expansion of transposons and other gene families, including an unusual (for invertebrates) expansion in the protocadherins and the C2H2 superfamily of zinc-finger transcription factors (Albertin et al., 2015). These genome level novelties are rendered more complex by the already well established extensive RNA editing, particularly in nervous system cells, which allows diversification of the proteins that the cells can produce (Garrett and Rosenthal, 2012a,b; Liscovitch-Brauer et al., 2017).

A short list of cephalopod novelties, excluding a discussion on the *Bauplan*, may include: (i) an extraordinarily large cadherin gene encoding over 70 extracellular cadherin domains found to be highly expressed in octopus suckers; (ii) gene families expansions (e.g., protocadherins, zinc finger proteins, interleukin-17 like genes, G-protein coupled receptors, chitinases and sialines); (iii) novel octopus-specific genes expressed in specialized structures such as skin and brain; (iv) Vascular Endothelial Growth Factor (VEGF) pathway, a possible prerequisite for the development of a closed vascular system; (v) octopressin/cephalotocin co-occurrence, never before reported in invertebrates; (vi) horizontal gene transfer as a possible origin of reflectin gene, allowing dynamic iridescence and structural color change in the skin, in cephalopod clades (Albertin et al., 2015; Guan et al., 2017; Wang and Ragsdale, 2017). These may originate by increase in genome complexity in the clade linked to polyploidy, differential arrangements of key genes (e.g., *Hox* appearing not clustered), exceptional RNA editing capacities, expansion of transposable elements (e.g., Packard and Albergoni, 1970; De Marianis et al., 1979; Lee et al., 2003; Albertin et al., 2015; Liscovitch-Brauer et al., 2017).

THE VERTEBRATE-LIKE NEURAL SYSTEMS IN CEPHALOPODS

It is without doubt that the most classic examples of vertebrate/mammalian-like comparison of cephalopod brain-functioning is provided by the work of Young (1961, 1964, 1965b, 1976a, 1991, 1995) and Hobbs and Young (1973).

The parallelism is seen in different structures and functional analogies; these differences encouraged later authors to consider cephalopod brains as unfamiliar structures, when compared to bird and mammalian brains, and as examples of analogous functions worth exploring as examples of phyletic boundaries of consciousness (Edelman and Seth, 2009).

Evolutionarily Conserved Axes as Defined by the Developmental Framework

Developmental approaches have been used to probe how the complex brain centers and body parts developed during the evolutionary history of cephalopods (**Figure 2**). Embryological studies suggest that all molluscan nervous systems share an early developmental stage in which three neurogenic domains

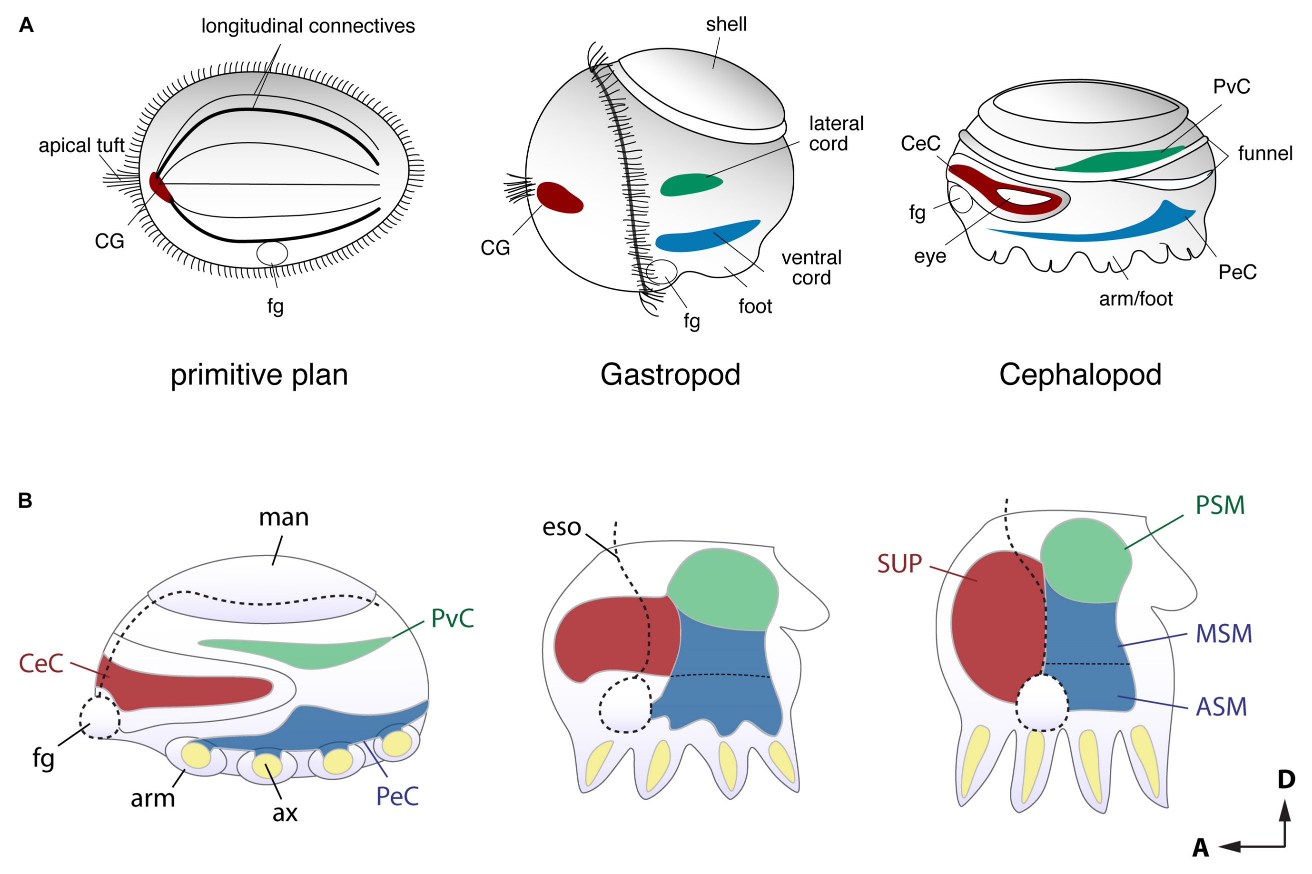


FIGURE 2 | Comparison of the early stage embryonic nervous systems in three invertebrates. **(A)** Acoelomorph or planula-like larva (left), a gastropod veliger larva (middle), and nautiloid embryo (right), defining the comparable topography of neural patterns (modified after permission of Tokai University Press following Shigeno et al., 2010). The cerebral-, ventral- and laterally situated neural cords are shaded in red, green and blue, respectively. Due to their diffuse nature, the homology of these nerve cords remains unclear, but the putative ancestral condition is shown for comparison. **(B)** Schematic drawing of embryonic brain development in *O. bimaculoides* (inspired to information contained in Shigeno et al., 2015), showing a transition from medullary cords to a centralized brain. The foregut or mouth (fg) initially lies at the anterior, but it moves to a more ventral position at the later stage. Reference to the A–P and D–V axes are provided. ASM, anterior sub-esophageal mass; ax, arm axial cord; CeC, cerebral cord; CG, cerebral ganglion; eso, esophagus; fg, foregut or mouth; man, mantle; MSM, middle subesophageal mass; PeC, pedal cord; PSM, posterior subesophageal mass; PvC, palliovisceral cord; SUP, supraesophageal mass.

of the ganglia or medullary cords at the cerebral, ventral, and lateral position are present (Naef, 1928; Haszprunar, 1992; Shigeno et al., 2010, 2015; **Figure 2A**). These neural cords correspond to the cerebral, pedal, and palliovisceral ganglia (or cords), respectively (Marquis, 1989; Shigeno et al., 2015; **Figure 2B**). Based on topographical criteria and the neural composition (exemplified by the form of neurons and organization of tracts, for example) these may be compared to analogous structures in vertebrates such as the mammalian spinal cord (**Figure 3**) and fore- and mid-brains (**Figure 4**).

In particular, the dorso-ventral (D-V) neural arrangement of the cephalopod subesophageal mass may allow comparison with the medio-ventral parts of the vertebrate spinal cord; the ventral peripheral layer of cells of the subesophageal mass (see dark red in **Figures 3B**, **4**) resembling the midline cells of the spinal cord, and most of the inputs (sensory) and outputs (motor) to/from the structures are conserved along their respective dorsal and ventral arrangements (**Figure 3B**).

Traditional terminology for the adult cephalopod brain distinguishes between the anterior and posterior parts of the subesophageal mass (Young, 1971). By contrast, the cephalopod embryological axis, as defined by Fioroni (1978), allows us to identify the antero-posterior (A–P) axis of the cephalopod body as corresponding to the D–V axis of vertebrates and thus allowing a comparison with the vertebrate spinal cord.

Developmental regulatory gene studies seem to support the cephalopod A–P/vertebrate D–V axis definition (see Lee et al., 2003). Recent molecular studies of various cephalopod species provide mixed evidence regarding the evolutionarily conserved nature of the axes. Tomarev et al. (1997) first found that a paired homeobox gene, *Pax-6*, is commonly expressed in the developing eyes and anterior cerebral fields of squid and vertebrate embryos. Along the A–P embryonic axis, the expression of the homeobox genes *otx*, *nkx2.1*, *hox*, and other homeodomain-containing genes, successfully distinguishes the developing brain fields (Lee et al., 2003; Buresi et al., 2012, 2016; Focareta et al., 2014). The *Pax2/5/8* expression domain has also been shown to mark a

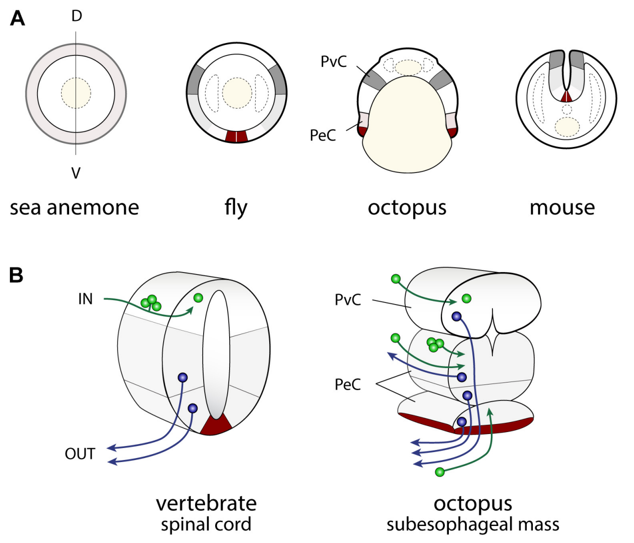


FIGURE 3 | Similarities in the developmental plans of the vertebrate spinal cord and cephalopod sub-esophageal mass. **(A)** Comparison of the neurogenic territories along the embryonic dorso-ventral axes. The color codes indicate the candidates of comparable territories. **(B)** Oblique cross-dissected views of the vertebrate spinal cord and of octopus sub-esophageal mass. Red areas indicate the midline cells in the spinal cord, and possible comparable parts of the ventral position of the sub-esophageal mass. A dorso-ventral segregation pattern of input sensory (green) or output motor neurons (blue) exists in the spinal cord, while no such segregation is obvious in the octopus sub-esophageal mass (see the text for further explanation). PeC, pedal cord; PvC, palliovisceral cord; D, dorsal; V, ventral.

boundary between the A–P neural territories (Wollesen et al., 2015b), similar to those in the midbrain-hindbrain boundary of vertebrate brains. Furthermore, in cuttlefish embryos the D–V or medio-lateral axis expression domains of *pax6-pax2/5/8-pax3/7* genes successfully detect the topographically equivalent genes in the developing spinal cords of vertebrates (Buresi et al., 2016; see also Navet et al., 2017).

A number of other molecular studies involving neurogenic and signaling molecule genes have suggested evolutionarily conserved domains as well as 'endemic' novelties in the developing cephalopod brain (Baratte et al., 2007; Farfán et al., 2009; Navet et al., 2009; Ogura et al., 2013; Wollesen et al., 2014, 2015b; Yoshida et al., 2014; Shigeno et al., 2015; Focareta and Cole, 2016; Koenig et al., 2016).

The Sensory and Motor Systems: The Spinal Cord and Hindbrain Analogy

The spinal cord is a principal sensory and motor center in vertebrate nervous systems (see **Figures 3**, **4**). The dorsal neurons receive inputs from the sensory receptors, and the ventral motor neurons regulate motor actions, such as rhythmic movements of body muscles (e.g., Cohen et al., 1988; Grillner and Wallén, 1999; see also: Ayali et al., 2015; Berg et al., 2015) that are modulated by these inputs.

In an attempt to provide a possible comparative overview of vertebrate neural structures such as the spinal cord and the hindbrain and their putative cephalopod analogs we will consider below a few examples based on neural organization including somatotopy, dorso-ventral segregation of sensory- and motor-neural systems, peripheral vs. central neural domains, and fast escape responses in cephalopods.

Somatotopic Organization?

In the spinal cord, and in their invertebrate analog as for example in insects (e.g., Packard, 1884; Arendt and Nubler-Jung, 1999; Svidersky and Plotnikova, 2002), neurons are organized in columns with intrasegmental interneurons arranged functionally, representing a kind of somatotopic map (e.g., Butler and Hodos, 2005; Kiehn, 2016; Mantziaris et al., 2017).

In cephalopods somatotopic maps are considered not to exist (Zullo et al., 2009). In the higher motor centers such as the basal lobes (supra-esophageal mass), electrical stimulation has failed to identify any kind of somatomotor map, suggesting that there may be none in the cephalopod brain (Zullo et al., 2009; but see Gutnick et al., 2011), thus suggesting that cephalopods evolved a 'unique' solution for motor control (Gutnick et al., 2011; Hochner, 2012, 2013).

However, a somatotopic map has been suggested to occur in the sub-esophageal mass (e.g., Boycott, 1961; Monsell, 1980; Saidel, 1981; Dubas et al., 1986; Gaston and Tublitz, 2004; Gaston and Tublitz, 2006). A multi-color neuro-tracing study of the central distribution and the resulting three-dimensional arrangement of fin chromatophore motoneurons in the cuttlefish (Gaston and Tublitz, 2006), provided preliminary possible topographic organization of fin chromatophore motoneurons. These data support previous findings by Boycott (1961) who proposed a type of 'somatotopy' when considering the neural representation (in the chromatophore lobes, SUB) of chromatophores in the skin of the animals, depending on the species.

It is clear that the identification of segregated sensory- and motor-maps in cephalopod brains will require further studies.

Dorso-Ventral Segregation of Sensory-Motor Neural Systems

Along the D–V axis (as depicted above for cephalopod brain), centers characterizing the sub-esophageal mass and controlling specific body parts are arranged in the same order as those body parts: the pallial cavity, then the viscera, collar, funnel, head, ocular system, oculomotor system, and finally arms (Young, 1976a; Budelmann and Young, 1985; Gaston and Tublitz, 2006; **Figure 4**). However, and based on the available knowledge, neuronal segregation of the ventral motor and dorsal sensory neurons has not been reported for cephalopod sub-esophageal mass, and an analogy with the vertebrate arrangement seems difficult.

Despite differences (**Figure 3B**), in the octopus the great majority of inputs are collated in the dorsal- and mid-parts of the supra-esophageal mass (pedal and palliovisceral cords in **Figure 3B**), and most of the outputs project from the palliovisceral cord (ventral, **Figure 3B**), thus challenging a possible analogy with vertebrates. It is also true that the putative motor nerves projecting to the arms, as for the sensory

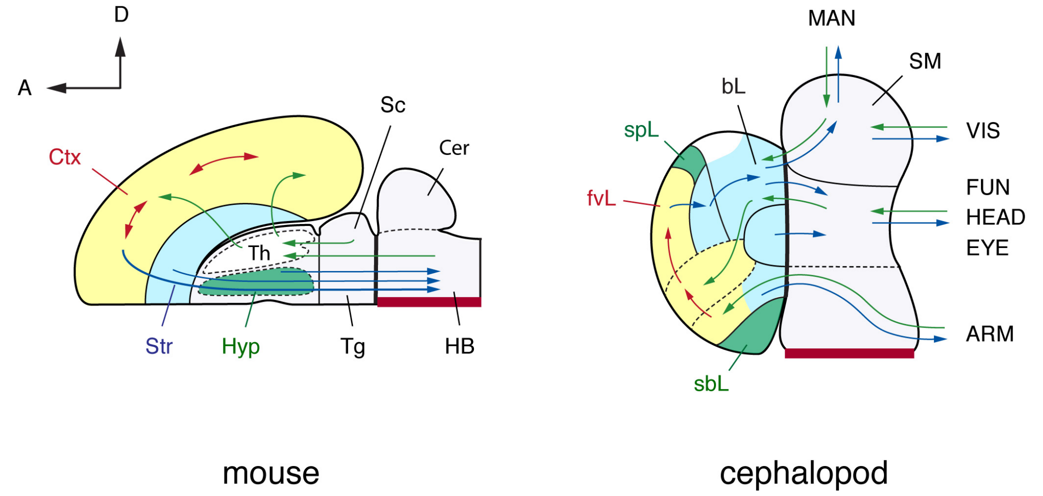


FIGURE 4 | A brain-wide, flat-map comparison of the mouse and octopus brain. Topographical similarities are highlighted using color-coding. Note similarities with the pallium and basal ganglia, and neurosecretory centers (hypothalamus). The hypothalamus and the octopus neurosecretory systems differ superficially in adult brains with the neurovenous tissues (Young, 1970), considered neurosecretory areas in cephalopod brain, such as the para-vertical and the sub-pedunculate that are situated more laterally together with the optic lobes (omitted for simplification in this figure). The sensory inputs and motor outputs indicate functionally equivalent centers. The maps are arranged along the embryonic A–P and D–V axes (outline of mouse brain inspired by the information included in: Rubenstein et al., 1994, 1998; Puelles and Rubenstein, 2003; Swanson, 2007). Cephalopod embryonic brains are initially cord-like, and the topographic position of adult brain centers is traced back to embryological position via successive histological observation (Marquis, 1989; Shigeno et al., 2001, 2015). The main driver pathways (see text for details) are selected following Young (1971) and Plän (1987). (mouse): Cer, cerebellum; Ctx, cerebral cortex; HB, hindbrain; Hyp, hypothalamus; Sc, superior colliculus; Str, striatum; Th, thalamus; Tg, tegmentum; (cephalopod): ARM, arm nerve cord; bL, basal lobe; fvL, frontal and vertical lobe; EYE, eyes; FUN, funnel; HEAD, head; MAN, mantle; SM, subesophageal mass; sbL, superior buccal lobe; spL, sub-pedunculate lobe; VIS, visceral organs.

information originating from the arms, usually come from both the ventral and dorsal sides of the SUB (Budelmann and Young, 1985). This is due to the arrangement of the anterior brachial lobes (SUB) with their intricate neuropil and connections, from where the four pairs of brachial nerves and the arm nerve cords originate (see description in: Young, 1971; Budelmann and Young, 1985; but see Lee et al., 2003).

Peripheral vs. Central Nervous System: The Case of the Arm Nerve Cord

Following Bullock (1965b), the foremost anterior part of the sub-esophageal mass accounts for "masses probably representing new ganglia associated with arms" (Bullock, 1965b, p. 1440) including the brachial ganglion (in the SUB) *sensu stricto,* the brachial nerves "to arms and suckers" and the interbrachial nerves (see also: Graziadei, 1971; Young, 1971). In the following pages, Bullock provided a description of the "complex nervous apparatus" characterizing arms and suckers as a "structure of the peripheral nervous system" (Bullock, 1965b; p. 1467, 1475–1479). It is interesting to note that Bogoraze and Cazal illustrated the central nervous system of the octopus including stellate ganglia and the related pallial nerves (see Figure 1 in Bogoraze and Cazal, 1944), a possible suggestion of an extended and distributed 'central nervous system.' It may be worth pointing out that the 'brain' is contained within the cranium (cf. skull) and that the 'brain + spinal cord' is in vertebrates considered as the central nervous system, as compared with the peripheral nervous system.

The overall arrangement of the arm nerve cord, medullary in the center with four small lateral cords, and its main features as "bilateral symmetry, segregation of tracts from synaptic regions, segmented outflow, and continuous medullary character of the axial cord" provides a strong analogy with "the vertebrate spinal cord; the similarity is increased on consideration of the physiological evidence of local, intersegmental, and superimposed higher mechanisms" (Bullock, 1965b; p. 1475).

The detailed descriptions provided by Graziadei, Young and coworkers (Graziadei, 1971; Young, 1971; Budelmann and Young, 1985; see also Margheri et al., 2011) are an example of a challenge for current neuroscience: to attribute a neural structure as complex as the arm nerve cord of octopods to the peripheral or to the central nervous system. Despite the typical invertebrate organization, we believe that the analogy with the vertebrate spinal cord is still largely unexplored, but likely.

Characterization of the acetylcholine synthetic enzyme choline acetyltransferase (ChAT) and serotonin in octopus arm nervous system supports this analogy. In the octopus arm two types of cholinergic nerves, cChAT-positive nerves from brain ganglia and pChAT-positive nerves intrinsic to the arm, have been identified (i.e., common type ChAT, cChAT; and peripheral type ChAT, pChAT; Sakaue et al., 2014). cChAT positive fibers appear in the arm ganglia and are likely related to brain efferents, appearing limited to fibers in octopus arm-nerve cord and in the cerebro-brachial tract. On the other hand, pChAT occurs in the intrinsic innervation of the octopus arm and is widely distributed in different nerve centers, probably associated with the sensory system (Sakaue et al., 2014). Similarly, two types of serotonin-like innervation have been shown in the arm: one type with fibers originating from the brain and innervating the periphery through the cerebro-brachial tract, and the other providing an intrinsic network to the cellular layer of the axial nerve cord (Bellier et al., 2017).

We reiterate here that the idea that the arm-nerve cord of cephalopods is not simply a neural structure belonging to the peripheral nervous system (as traditionally accounted, see for example Hochner, 2012), but a case of convergent evolution with functional and structural analogies existing between the vertebrate spinal cord and the octopus arm-nerve cord.

Neural Structures Controlling Fast Escape Responses in Cephalopods, a Case of Analogy With the Hindbrain?

Mauthner cells (Sillar, 2009) are one of the most historically notable motor systems for locomotory behavior reported in agnathans, teleost fish, and many amphibians. These are responsible for a rapid change in directionality and promote escape behavior (Fetcho, 1991; Korn and Faber, 2005). Mauthner neurons are characterized by a large neuronal cell, usually possessing a giant banana-shaped cell body located on either side of the midline in the brainstem with axon crossing to the contralateral spinal cord where they synapse with somato-motor neurons. The inputs to Mauthner neurons are primarily from receptors of the vestibular, auditory, and lateral line systems. In fish, for example, the neurons are not the same size in all species and this is considered to be linked to differences in taxa and possibly habitat (Zottoli, 1978).

We consider a similar neural system being present in cephalopods: the giant fiber system of squid (e.g., *Doryteuthis* or *Loligo*) and the magnocellular lobe of cuttlefish and octopus. The activation of giant axons induces the rapid escape behavior and vigorous jet propulsion (Otis and Gilly, 1990). Like the Mauthner neurons, the giant fiber system of squid is composed by a series of cells, some of them reaching over 250 μm in diameter (in *Doryteuthis pealeii*, see Young, 1939; see also Young, 1976b). These giant cells are multipolar with extensive dendritic arborization (Young, 1939, 1976b), resembling vertebrate neurons.

In squid, mantle contraction and jet propulsion are controlled by a giant fiber system consisting of two sets of three giant neurons organized in tandem (Young, 1939). According to J. Z. Young and later Authors, the "axons arising from the two first-order giant cells pass backward into the neuropil of the palliovisceral ganglion. Here they approach one another in the middle line, and are joined by the inter-axonic bridge [...]. The interest of this remarkable structure is that in the adult it consists not of a chiasma or crossing of two distinct fibers, but of a true protoplasmic bridge" (Young, 1939, p. 477). Such an organization allows synaptic inputs from either side of the brain to be integrated and propagated down the giant fiber system as a symmetrical event for synchronous contraction of both sides of the mantle musculature (Pozzo-Miller et al., 1998). After the chiasm, these giant axons branch and establish synapses (chemical and electrotonic-gap synaptic junctions) with several second-order giant axons in the neuropil of the palliovisceral lobe (SUB). From these cells, axons project from the central palliovisceral lobe (SUB) to the stellate ganglion in the mantle via the pallial nerve forming the presynaptic elements at the giant synapses (Young, 1939; Martin and Miledi, 1986). The axons of the giant system of cephalopods are thus part of an intricate network with other regions of the brain (Young, 1939; see also: Young, 1977a; Nixon and Young, 2003).

In the brain of *Sepia officinalis* and *O. vulgaris* the magnocellular lobe serve the same function (Young, 1971; Chichery and Chanelet, 1976). Interestingly, differences in cellular sizes among different species exists; however, their preserved functions (i.e., neural control and initiation of fast locomotion and escape responses) indicate another possible analogy when comparing vertebrates (e.g., fish) and cephalopods (Young, 1977a; Zottoli, 1978).

Further studies are required to provide data to support or contradict this working hypothesis.

The Neurosecretory System: An Analog to the Hypothalamus

Neurosecretion is pivotal for orchestrating essential body functions and metabolism and is considered a common metazoan phenomenon (Dorn, 1998; Hartenstein, 2006; Tessmar-Raible, 2007). Neurosecretory cells are characterized by large dense core vesicles that are not produced locally (at the synapse), but in the cell soma and have to travel along an axon (sometimes over a considerable distance) to reach their release site. In addition, neurosecretory centers are usually clustered in specific areas.

In vertebrates, the hypothalamus is located at the rostroventral region of the forebrain and among cellular-types are a set of neurosecretory cells (Butler and Hodos, 2005). The evolutionary origins of neurosecretory cells can probably be traced to a common bilaterian ancestor or pre-bilaterian animal such as a cnidarian (Hartenstein, 2006; De Velasco et al., 2007; Tessmar-Raible et al., 2007).

The neurosecretory centers of molluscan nervous systems tend to be distributed in the cerebral ganglia (gastropods). Alternatively the cells tend to be organized into distinct clusters in the preoral regions associated with the esophagus, or the stomatogastric nervous systems (e.g., Simpson et al., 1966; Kandel and Kupfermann, 1970). In cephalopods, neurosecretory cells are mainly found in the buccal (SEM), sub-pedunculate (SEM), and in part of dorsal basal lobes again in the supra-esophageal mass (Young, 1970). Surrounding the 'brain' there are several other 'potential' neurosecretory regions such as those present in the sub-buccal and sub-pedunculate areas and in the optic gland, and the neurovenous tissue of the vena cava (Bogoraze and Cazal, 1946; Young, 1970).

The optic glands and the sub-pedunculate lobe are considered to function as neurosecretory centers related to reproduction and are the candidates for pituitary-hypothalamus analogs in the cephalopod brain (Wells and Wells, 1969). We would expect to see an analogically equivalent area in the vertebrate brain, and indeed, studies have detected in the above-mentioned cephalopod brain centers a subset of neurons containing hypothalamus abundant molecules such as GnRH (Di Cosmo and Di Cristo, 1998; Iwakoshi-Ukena et al., 2004; Kanda et al., 2006; Shigeno and Ragsdale, 2015) and duplicated vasopressin orthologs, octopressin and cephalotocin (Kanda et al., 2003a,b, 2005;

Minakata, 2010; Shigeno and Ragsdale, 2015). Unfortunately, it is largely unknown how each neurosecretory tissue is derived from those of molluscan ancestors and what its relationship is to other higher brain centers.

In any case neurosecretion is a common control mechanism and cephalopods and vertebrates both show discrete groups of neurons in their 'brain' that secrete peptides with an action at a distant site via the blood. Note that we are not proposing that specialized neurosecretory areas are unique to cephalopods and vertebrates, as they are present in most animal species studied to date (Hartenstein, 2006; Tessmar-Raible, 2007; Williams et al., 2017).

Higher Sensory Centers: An Analog to the Thalamus

To the best of our knowledge, a cephalopod equivalent of the vertebrate thalamus has not been proposed. The thalamus is often referred to as a sensory relay center though which almost all sensory inputs run on their way to the cerebral cortex or pallium (Riss et al., 1972; Swanson, 2007). It is a gatekeeper to the cortex and is considered to have a role in 'pain' and 'consciousness' (Alkire et al., 2008; Schiff, 2008; Baliki and Apkarian, 2015; Rajneesh and Bolash, 2018). It is composed of a number of nuclei that usually have distinct sensory fields.

Using the above features as a basis for comparison we suggest that in the cephalopods dorsal basal- and sub-vertical lobes could be considered as candidates for analogs to the vertebrate thalamus.

The dorsal basal and sub-vertical lobes receive many input fibers from the entire body via direct and indirect pathways from the sub-esophageal mass (Young, 1971), suggesting that it is a relay center for the 'cortically located' frontal and vertical lobes in cephalopod brain. We counted between 11 and 15 main tracts originating and/or departing from (afferent and efferent) the two structures, i.e., dorsal basal- and sub-vertical lobes, based on the description available for *O. vulgaris* (Young, 1971); an estimation of the number of neural fibers composing these tracts is not available, or only possible for part of the dorsal basal following Plän (1987). The dorsal basal lobe also provides many outputs to the lower motor centers, suggesting it can also be categorized as a higher or intermediate motor centers (Boycott, 1961; Zullo et al., 2009). It is without doubt that the connectivity of these centers is very extensive, thus supporting our view of that they are relay centers analogous with the thalamus in vertebrates.

The inferior frontal lobe also appears to be another candidate. It is a major chemo-tactile sensory-motor center processing information originating from the suckers and arms, just as occurs in the olfactory cortex. It is involved in learning and memory recall being part of the so-called chemo-tactile memory system (Wells, 1959; Young, 1995). Also in this case, Young (1971) describes four afferent and seven efferent connections to/from the inferior frontal lobe, and considers it as the main part of the matrices involved in the chemo-tactile sensory-motor learning system (Young, 1991, 1995).

The above account is mainly based on *O. vulgaris*. In our view, a comparative analysis including information on main connections of homologous structures in the brain of other cephalopod species may provide further insight (for cephalopods – Decapodiformes, see: Young, 1974, 1976b, 1977b, 1979; Messenger, 1979; for a vertebrate based comparative overview see Butler, 2008).

Higher Motor Centers: Analogs to the Basal Ganglia

In vertebrates, the higher motor centers receive sensory inputs and modulate their output to the pattern generators, located in "lower" parts of the central nervous system, to orchestrate the actions of multiple appendages to regulate posture, orientation, breathing, autonomic control of the viscera, and also habit formation (Reiner et al., 1998; Yin and Knowlton, 2006). The basal ganglia and the dorsal striato-pallial complex along with the spinal cord, midbrain and cerebellum, are the major centers regulating the outputs of cascading projection neurons.

In different bilaterians the putative higher motor centers have been identified with different terminology (e.g., Young, 1971; Orrhage, 1995; Loesel et al., 2002; Strausfeld et al., 2006; Homberg, 2008; Beckers et al., 2011; Pfeiffer and Homberg, 2014): central complex (insects), arch-like bodies and midline neuropils (non-insect arthropods, annelids), cerebral commissures (other protostomes), basal lobe system (cephalopods). The homology of these structures among phyla remains uncertain, and each motor center has become independently specialized to the demands of each animal lineage, resulting in different body plans, locomotory systems, and life styles across these taxa.

Despite such specialization, it is possible that higher motor centers share a common origin that can be traced back to the cerebral or preoral commissural region of a bilaterian ancestor, since almost all bilaterian nervous systems, including primitive acoelomorphs, have several thick commissural pathways connecting paired cerebral ganglia with bilateral body parts (see description in Bullock, 1965a,b,c,d; see also Reichert and Simeone, 2001).

Just as in many vertebrate species, the higher motor centers of coleoid cephalopods are complex neural structures (Young, 1971, 1977b). The main motor output centers are the basal lobes in the supra-esophageal mass (Boycott, 1961). Based on neural connectivity and experiments testing function after lesion of specific areas of the cuttlefish 'brain,' the cephalopod anterior basal lobes have been proposed as being analogous to the vertebrate basal ganglia (Chichery and Chichery, 1987; Gleadall, 1990).

The anterior basal lobe and the vertebrate basal ganglia are both situated at the pre-oral and peri-esophageal regions at the base of the anterior brain, respectively (**Figure 4**; see also **Figure 1B**). Likewise, the major connectivity of the lobe and its functional structure are similarly hierarchical, progressing from motor pattern learning to central pattern controllers, initiators, generators, and motor neuron pools, and finally to behavior as is thought to occur in vertebrate brains (Stocco et al., 2010). Unfortunately, the physiological function of the basal lobes in cephalopods remains only vaguely known (Zullo et al., 2009) and so this hypothesis requires further testing.

It is noteworthy to mention that few studies maybe claimed in support of the existence (or not) of Central Pattern Generators (CPG) in cephalopods. We refer here to: (i) the excitable receptor units in the mantle of octopus by Gray (1960) and the neural control of breathing, that may provide indirect evidence for CPG; (ii) the tentacle strike of cuttlefish and squid, but with almost no data on neural control; (iii) the locomotor patterns involved in octopus crawling, with evidence that is difficult to interpret as CPGs *sensu stricto* (Levy et al., 2015; Levy and Hochner, 2017).

The Peduncle Lobe: Analog of the Cerebellum

The cerebellum is involved in controlling balance, proprioception, and ocular reflexes via fixation on a target object, planning bodily movements and also motor learning. It is highly interconnected with the optic tectum, thalamus, and midbrain (Swanson, 2007).

The cephalopod peduncle lobe is a candidate analog for the cerebellum (Messenger, 1967a,b; Hobbs and Young, 1973; Young, 1976a; Messenger, 1979; Camm et al., 1985). According to the ultrastructural characterization of the peduncle lobe of *O. vulgaris* made by Woodhams (1977), and based on evidence about the effects on locomotor responses of the animal after lesions to this lobe, Woodhams (1977) suggested a close functional and morphological analogy to a folium of the vertebrate cerebellum. The presence of a conspicuous and characteristic array of parallel fibers, originating from the spine cells, in the neuropil of the lobe and their "striking resemblance to those of vertebrate cerebellar granule cells," and "serial synaptic relays present along their length" support this conclusion (Woodhams, 1977, p. 329).

Like vertebrates, cephalopods have a hierarchical series of motor control centers that coordinates signals from the vestibular organs, eyes, and body (Young, 1976a). The fibers from the optic lobe run into the peduncle lobe along with those from the anterior basal and the magnocellular lobes, and then their outputs connect to the oculomotor center, i.e., the lateral pedal lobe in the SUB (Budelmann and Young, 1985) as is the case in vertebrates represented by the medulla-cerebellum-midbrain axis regulating vestibulo-ocular reflexes.

The Associative (or Auxiliary) Centers: Analogs of the Pallium/Cerebral Cortex

A number of studies have used an evolutionary perspective to postulate the ancestral form of the pallium/cerebral cortex in both vertebrates and invertebrates (e.g., annelid and insect 'brains'; see for example, Tomer et al., 2010; Strausfeld, 2012).

In some cephalopods, such as *S. officinalis* and *O. vulgaris,* experimental evidence for sleeping, decision-making, discrimination learning and lateralization of the brain suggests that cephalopods possess a higher level of cognitive ability (Mather, 1995, 2008; Edelman and Seth, 2009; Edelman, 2011; Marini et al., 2017) thus leading to the hypothesis that these cognitive features require in cephalopods the equivalent of a cerebral cortex as in mammals (Edelman et al., 2005; Edelman and Seth, 2009; Roth, 2013).

Through extensive experimentation using ablation of various brain areas followed by behavioral assays the higher centers, i.e., the frontal- and vertical lobe systems, have been shown to be involved in tactile and visual memory processing (Maldonado, 1963a,b, 1965; Young, 1971, 1991, 1995). These include (i) numerous uniquely distributed small-size interneurons, called amacrine cells (Young, 1971, 1979), (ii) the presence of parallel running fibers, and (iii) reverberating circuitry across different lobes (Young, 1991, 1995).

These areas are also characterized by synaptic long-term potentiation, neurotransmitter function, and heterogeneity of neurochemical identity (Hochner et al., 2003; Shomrat et al., 2008, 2010, 2011; Shigeno and Ragsdale, 2015; for review see: Shomrat et al., 2015; Turchetti-Maia et al., 2017). The reason for the deep homology between the vertebrate pallium and the cephalopod vertical lobe system – whether derived from a common ancestral plan or convergently evolved – remains uncertain, but the cephalopod vertical lobe is the best candidate for vertebrate pallium analog within the molluscan lineage (Young, 1991, 1995).

MODELS FOR ASSOCIATIVE NEURAL NETWORKS

If functional equivalents of the cerebral cortex evolved independently in both the cephalopod and vertebrate brains, what is the common structural and/or functional principle that drove this? Here, we summarize the most likely hypotheses.

The Paired Centers and Matrix Model

Young (1965b, 1991, 1995) studied the multi-level control of attack or retreat behavior resulting from the association of taste, touch, vision, and possible pain in response to the experience that animals have when interacting with objects or prey. According to Young, the 'paired cortical centers,' i.e., the inferior- and superior frontal-vertical lobe systems, determine the probability of a positive or negative response for pursuing a given 'food items'. The systems are composed of combined matrices of axons with intersecting axes where memory is stored. The 'classifying' neurons of lower centers send signals to higher cortical lobes or take a short-cut by directly dictating the proper response to output motor neurons. In the 'cortical' centers (e.g., the median frontal lobe) the response is modulated to increase the probability of attack, unless this action is vetoed by the vertical lobe. This constructs a hierarchical system of decision-making as suggested by the selective theory of the vertebrate higher sensory centers such as cerebral cortex or cerebellum (Eccles, 1977; Edelman, 1978).

Unfortunately, this model has not yielded a hypothesis as to how the neural connectivity patterns and cell types are equivalent to those of vertebrate cerebral cortex.

The Associative Learning Model

As reviewed by Marini et al. (2017), Young and coworkers were able to provide an associative learning model of the octopus brain (Boycott and Young, 1955; Maldonado, 1963c;

Young, 1964, 1965b, 1991; Maldonado, 1965) based on the existence of a series of matrices (see also above) that allow computation and that were considered analogous to the limbic lobe of higher vertebrates (Young, 1991, 1995; review in: Borrelli and Fiorito, 2008; Marini et al., 2017). In the octopus 'learning system,' the small interstitial neurons (amacrines) and their synapses play an important role in learning by means of their sensitizing effects on reward and punishment signals coming from outside. This model explains the short fluctuations in memory recall and long-term cumulative changes via Hebb's synaptic law, that frequent stimulation of certain synapses strengthen their signals and connectivity (Hebb, 1949). The associative learning of *O. vulgaris* has been also a 'model' in cybernetics (Clymer, 1973; Myers, 1992), and appears surprisingly similar to a more recent one, suggested for the learning system of insects (i.e., the mushroom bodies), particularly of the honeybee (Heisenberg, 2003). In the insects, the model posits on the assumption that sensory odor signals are spatio-temporally represented by synaptic sets of small intrinsic interneurons (Kenyon cells) in the neuropil of the mushroom body. The reward- or punishment-conditioned stimulus of these Kenyon cells strengthens synapses with their outputs.

Thus, octopus amacrine cells (**Figure 5**) and honeybee Keynon cells, as well as the octopus sub-vertical lobe and insect premotor centers, are candidates for being functionally equivalent analogs (Hochner, 2010). A partial cellular test of the system of functioning of the circuit underlying this 'model' has been achieved with the octopus (and cuttlefish) brain slice preparation (Hochner et al., 2003; Shomrat et al., 2008, 2011, 2015; Turchetti-Maia et al., 2017).

The Reverberating Circuitry Model (Young, 1991, 1995)

The similarity in connectivity between the cephalopod superior frontal-vertical lobe system and the vertebrate hippocampal formation, based on matrices and reverberating feedback network structure (Maldonado, 1963a, 1965; Young, 1991), is the basis of this model.

Cephalopod learning capacity is not localized in certain layers or 'grandmother cells' but is distributed within a highly redundant series of matrices with recurrent circuits. Young emphasized the similarity with the hippocampal complex but avoided any clear statement about its relationship to the cerebral cortex (Young, 1991, 1995). Indeed, the existence of long term potentiation in the cephalopod vertical lobe (Hochner et al., 2003; Shomrat et al., 2008, 2011) maybe the basis of long term memory as it is considered in the hippocampus of vertebrates with minor molecular differences (Hochner et al., 2003; Turchetti-Maia et al., 2017). However, the higher matrix system of cephalopods is also comparable to that of the mammalian cerebral cortex which also forms distinct cellular and matrix units (Young, 1995).

The Self-Organized Embodiment Model Without Somatotopy

The octopus higher motor centers are comparable to the motor cortex/pallium of vertebrates as a central control system, but

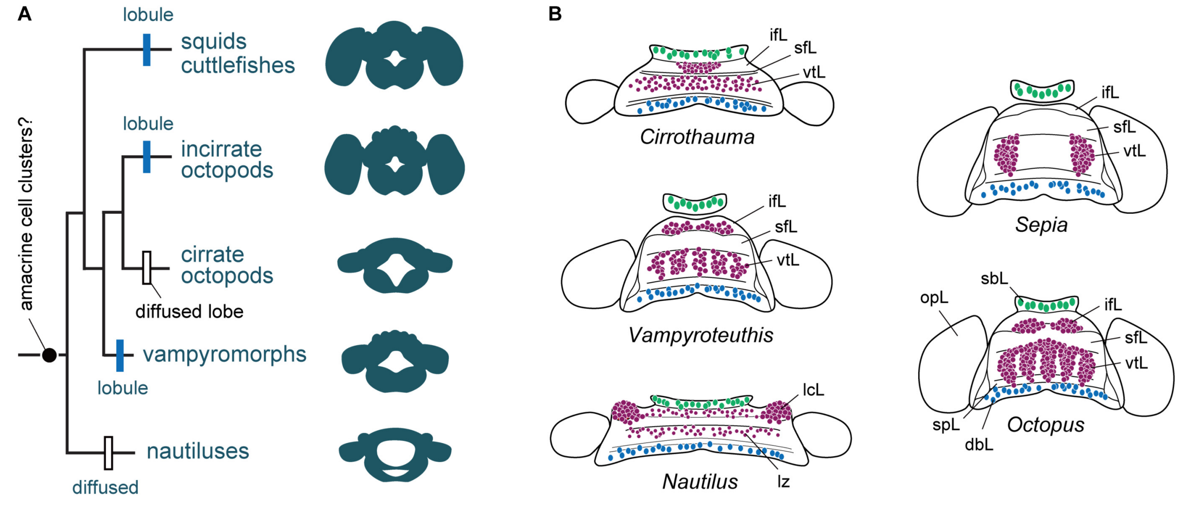


FIGURE 5 | The evolution of cortical territories represented by a zonation in cephalopod brain evolution. **(A)** Phylogram of the evolution of brain complexity and emergence (still controversial) and organization of the amacrine cells into clusters. Based on the information included in Lindgren et al. (2012), and data assembled from Young (1965a, 1977a), Nixon and Young (2003). The centers are primitively zonal or band-like (*Nautilus*) and they are enlarged, or centralized or reduced in more 'evolved' species such as cuttlefish and octopus. **(B)** Homology of cell types and appearance of amacrine cells or their equivalent cell types (purple) in different cephalopod species. Homology of cell types in *Nautilus* is also controversial when compared with other taxa, but the gross similarity of topographical distribution is apparent. Large cells (green) are commonly localized in the buccal lobe area, which are often serotonergic (Wollesen et al., 2012). Toward the posterior end of the dorsal basal lobe clusters of GABAergic cells (blue) have been identified in octopus (Cornwell et al., 1993; Ponte, 2012). Outline of supra-esophageal mass and optic lobes are exemplified as a view from top; the overall shape of the brains is simplified as that of later embryonic stage. dbL, dorsal basal lobe; ifL, inferior frontal lobe; lcL, lateral cerebral lobe; lz, laminated zone of cerebral cord; opL, optic lobe; sbL, superior buccal lobe; sfL, superior frontal lobe; spL, sub-pedunculate lobe; vtL, vertical lobe.

TABLE 1 | A list of the higher sensory, motor, and neurosecretory centers in the 'brains' of cephalopods and vertebrates.

Cephalopods	*Vertebrates*
Cerebral cord	Fore- and midbrain
Frontal-vertical lobe	Cerebral cortex (pallium) Hippocampus Amygdaloid complex
Dorsal basal lobe	Thalamus
Anterior basal lobe	Basal ganglia
Buc[1] and Spd[2] lobes	Hypothalamus
Optic lobe	Tectum
Magnocellular lobe	Tegmentum
Peduncle lobe	Cerebellum
Pedal cord	Hindbrain and spinal cord
Palliovisceral cord	Hindbrain and spinal cord

[1]Buc, buccal lobes, [2]Spd, subpedunculate lobe. Data assembled from Bullock (1965b), Butler and Hodos (2005), Hartenstein (2006). See text for details and exceptions (e.g., oculomotor centers).

they do not seem to be organized somatotopically (Zullo et al., 2009; Hochner, 2012, 2013). The lack of somatotopy in the higher motor centers of octopus may be explained by the non-biological concept of "self-organized embodiment" in robotics (Pfeifer et al., 2007; Cianchetti et al., 2012; Hochner, 2012; Laschi et al., 2012). The self-organized embodiment concept uses the dynamic interplay between the sensorimotor and a central controller to generate autonomous adaptive responses, and can explain very complex movements, such as the highly flexible motions of octopus arms.

Indeed, recent advances in artificial intelligence, including deep learning methods such as convolution networks (e.g., Mnih et al., 2015), show that neural-networks can be trained by and learn from numerically defined 'weights' provided to a whole network rather than from inputs due to local sensory representation. In support of this non-somatotopic idea, Grasso (2014) hypothesized that 'higher' neural centers of octopus have a role in time-series processing rather than acting as a spatial decoder. Reciprocal sensory information flow between the arms and 'higher' neural centers establishes a distributed memory trace in the Bayesian statistical sense. The reverberant circuits or recurrent matrices unique to the octopus frontal and vertical lobes produce signals lasting minutes to hours through Hebbian type learning. As a result, a brain-to-body spatial map or "Octo-munculus" (like the human "Homunculus") would be depicted as information processing systems distributed throughout each arm and a brachial center in the brain (Grasso, 2014).

CLOSING REMARKS

Cephalopods are not the only invertebrates that exhibit sophisticated behavioral repertoire, higher-order learning and cognitive abilities (e.g., Avarguès-Weber and Giurfa, 2013; Giurfa, 2013; Perry et al., 2013; Marini et al., 2017; Mather and Dickel, 2017; van Duijn, 2017).

Here we attempted to overview available knowledge to propose a brain-wide comparative 'model' between cephalopod neural-systems and the neural structures characterizing vertebrates. Such a comparison identifies the cephalopod cerebral cord as analogous to the vertebrate forebrain and midbrain, and the pedal and palliovisceral cords in the cephalopod brain as being comparable to their putative equivalent in vertebrates: the spinal cord and the hindbrain (**Table 1**).

The studies overviewed in this work have enabled us to draw functional analogies between cephalopod and vertebrate brains. Despite having fundamentally different anatomical organizations of adult brains, the embryologic patterns of longitudinal and transverse areas (orientation) along the A–P and D–V axes share similar topography in vertebrates and cephalopods. Surprisingly, the revised positional identities of the sub-esophageal centers (including brachial-, oculomotor-, funnel-, pallial- and visceral lobes) could account for much of the phylogenetic stability as well as novelties between the two taxa. Gene expression profiles controlling development support some of these proposed patterns, conserving the A–P and D–V axes of the brain and body regions as a whole (e.g., Albertin et al., 2015; Shigeno et al., 2015; Buresi et al., 2016; Navet et al., 2017).

Based on this developmental model, we have suggested that, unlike the vertebrate spinal cord, the octopus sub-esophageal system is arranged along the dorso-ventral body axis: the sensory-motor fibers run from the brachial, head, funnel, visceral mass, and the mantle. The basal lobes are placed, as in the basal ganglia, more anteriorly than the lower sensorimotor centers, and the associative centers (the frontal-vertical lobes) are at a more anterior-dorsal position as in the pallium or cerebral cortex of vertebrates. Our view establishes a topographical basis for a large-scale framework that encourages further discussion regarding analogs between the cerebral cortex, basal ganglia, and other vertebrate-like nervous systems in cephalopods.

AUTHOR CONTRIBUTIONS

SS conceived an earlier version of this manuscript. SS, PA, and GP provided an advanced draft. GF revised and finalized the manuscript. All authors discussed the content and commented the final manuscript. All authors read and approved the submitted manuscript.

FUNDING

SS was supported by a SZN fellowship (BEOM Department, Stazione Zoologica Anton Dohrn). RITMARE Flagship (MIUR and SZN) provided support to GP.

ACKNOWLEDGMENTS

We are in debt to Dr. Caitlin O'Brien and Professor Remo Sanges for discussion and critical reading of this manuscript. We would also like to thank Dr. Pamela Imperadore for her assistance.

We acknowledge permission from Tokai University Press to reproduce after modification one of the illustrations previously published by SS. This work benefited from networking activities carried out under the COST Action FA1301 and is considered a contribution to the COST (European Cooperation on Science and Technology) Action FA1301 "A network for improvement of cephalopod welfare and husbandry in research, aquaculture and fisheries (Cephs*In*Action)".

REFERENCES

Albertin, C. B., Simakov, O., Mitros, T., Wang, Z. Y., Pungor, J. R., Edsinger-Gonzales, E., et al. (2015). The octopus genome and the evolution of cephalopod neural and morphological novelties. *Nature* 524, 220–224. doi: 10.1038/nature14668

Alkire, M. T., Hudetz, A. G., and Tononi, G. (2008). Consciousness and anesthesia. *Science* 322, 876–880. doi: 10.1126/science.1149213

Arendt, D. (2008). The evolution of cell types in animals: emerging principles from molecular studies. *Nat. Rev. Genet.* 9, 868–882. doi: 10.1038/nrg2416

Arendt, D., and Nubler-Jung, K. (1999). Comparison of early nerve cord development in insects and vertebrates. *Development* 126, 2309–2325.

Avarguès-Weber, A., and Giurfa, M. (2013). Conceptual learning by miniature brains. *Proc. R. Soc. B* 280:20131907. doi: 10.1098/rspb.2013.1907

Ayali, A., Borgmann, A., Bueschges, A., Couzin-Fuchs, E., Daun-Gruhn, S., and Holmes, P. (2015). The comparative investigation of the stick insect and cockroach models in the study of insect locomotion. *Curr. Opin. Insect Sci.* 12, 1–10. doi: 10.1016/j.cois.2015.07.004

Baliki, M. N., and Apkarian, A. V. (2015). Nociception, pain, negative moods, and behavior selection. *Neuron* 87, 474–491. doi: 10.1016/j.neuron.2015.06.005

Baratte, S., Andouche, A., and Bonnaud, L. (2007). Engrailed in cephalopods: a key gene related to the emergence of morphological novelties. *Dev. Genes Evol.* 217, 353–362. doi: 10.1007/s00427-007-0147-2

Beckers, P., Faller, S., and Loesel, R. (2011). Lophotrochozoan neuroanatomy: an analysis of the brain and nervous system of *Lineus viridis* (Nemertea) using different staining techniques. *Front. Zool.* 8:17. doi: 10.1186/1742-9994-8-17

Bellier, J.-P., Xie, Y., Farouk, S. M., Sakaue, Y., Tooyama, I., and Kimura, H. (2017). Immunohistochemical and biochemical evidence for the presence of serotonin-containing neurons and nerve fibers in the octopus arm. *Brain Struct. Funct.* 222, 3043–3061. doi: 10.1007/s00429-017-1385-3

Berg, E. M., Hooper, S. L., Schmidt, J., and Büschges, A. (2015). A leg-local neural mechanism mediates the decision to search in stick insects. *Curr. Biol.* 25, 2012–2017. doi: 10.1016/j.cub.2015.06.017

Bogoraze, D., and Cazal, P. (1944). Recherches histologiques sur le système nerveux du poulpe. *Les neurones, le tissue interstitiel et les éléments neurocrines. Arch. Zool. Exp. Gén.* 83, 413–444.

Bogoraze, D., and Cazal, P. (1946). Remarques sur le système stomatogastrique du poulpe (*Octopus vulgaris* Lamarck): le complexe retro-buccal. *Arch. Zool. Exp. Gén.* 84, 115–131.

Borrelli, L., and Fiorito, G. (2008). "Behavioral analysis of learning and memory in cephalopods," in *Learning and Memory: A Comprehensive Reference*, ed. J. J. Byrne (Oxford: Academic Press), 605–627. doi: 10.1016/B978-012370509-9.00069-3

Boycott, B. (1961). The functional organization of the brain of the cuttlefish *Sepia officinalis*. *Proc. R. Soc. Lond. B Biol. Sci.* 153, 503–534. doi: 10.1098/rspb.1961.0015

Boycott, B. B., and Young, J. Z. (1955). A memory system in *Octopus vulgaris* Lamarck. *Proc. R. Soc. Lond. B Biol. Sci.* 143, 449–480. doi: 10.1098/rspb.1955.0024

Budelmann, B. (1995). "The cephalopod nervous system: what evolution has made of the molluscan design," in *The Nervous Systems of Invertebrates: An Evolutionary and Comparative Approach*, eds O. Breidbach and W. Kutsch (Basel: Springer), 115–138. doi: 10.1007/978-3-0348-9219-3_7

Budelmann, B. U., Schipp, R., and Boletzky, S. V. (1997). "Cephalopoda," in *Microscopic Anatomy of Invertebrates*, eds F. W. Harrison and A. J. Kohn (New York, NY: Wiley-Liss, Inc), 119–414.

Budelmann, B. U., and Young, J. Z. (1985). Central pathways of the nerves of the arms and mantle of *Octopus*. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 310, 109–122. doi: 10.1098/rstb.1985.0101

Bullock, T. H. (1965a). "Mollusca: Amphineura and Monoplacophora," in *Structure and Function in the Nervous Systems of Invertebrates*, eds T. H. Bullock and G. A. Horridge (San Francisco, CA: W.H. Freeman and Company), 1273–1281.

Bullock, T. H. (1965b). "Mollusca: Cephalopoda," in *Structure and Function in the Nervous Systems of Invertebrates*, eds T. H. Bullock and G. A. Horridge (San Francisco, CA: W.H. Freeman and Company), 1433–1515.

Bullock, T. H. (1965c). "Mollusca: Gastropoda," in *Structure and Function in the Nervous Systems of Invertebrates*, eds T. H. Bullock and G. A. Horridge (San Francisco, CA: W.H. Freeman and Company), 1283–1386.

Bullock, T. H. (1965d). "Mollusca: Pelecypoda and Scaphopoda," in *Structure and Function in the Nervous Systems of Invertebrates*, eds T. H. Bullock and G. A. Horridge (San Francisco, CA: W.H. Freeman and Company), 1387–1431.

Buresi, A., Andouche, A., Navet, S., Bassaglia, Y., Bonnaud-Ponticelli, L., and Baratte, S. (2016). Nervous system development in cephalopods: how egg yolk-richness modifies the topology of the mediolateral patterning system. *Dev. Biol.* 415, 143–156. doi: 10.1016/j.ydbio.2016.04.027

Buresi, A., Baratte, S., Da Silva, C., and Bonnaud, L. (2012). orthodenticle/otx ortholog expression in the anterior brain and eyes of *Sepia officinalis* (Mollusca, Cephalopoda). *Gene Expr. Patterns* 12, 109–116. doi: 10.1016/j.gep.2012.02.001

Butler, A. B. (2008). Evolution of the thalamus: a morphological and functional review. *Thalamus Relat. Syst.* 4, 35–58. doi: 10.1017/S1472928808000356

Butler, A. B., and Hodos, W. (2005). *Comparative Vertebrate Neuroanatomy: Evolution and Adaptation*. Hoboken, NJ: John Wiley & Sons. doi: 10.1002/0471733849

Camm, J. P., Messenger, J., and Tansey, E. (1985). New pathways to the "cerebellum" in *Octopus* studies by using a modified Fink-Heimer technique. *Cell Tissue Res.* 242, 649–656. doi: 10.1007/BF00225433

Chichery, M., and Chichery, R. (1987). The anterior basal lobe and control of prey-capture in the cuttlefish (*Sepia officinalis*). *Physiol. Behav.* 40, 329–336. doi: 10.1016/0031-9384(87)90055-2

Chichery, R., and Chanelet, J. (1976). Motor and behavioural responses obtained by stimulation with chronic electrodes of the optic lobe of *Sepia officinalis*. *Brain Res.* 105, 525–532. doi: 10.1016/0006-8993(76)90598-9

Cianchetti, M., Follador, M., Mazzolai, B., Dario, P., and Laschi, C. (2012). "Design and development of a soft robotic octopus arm exploiting embodied intelligence," in *Proceedings of the Robotics and Automation (ICRA), 2012 IEEE International Conference*, (Saint Paul, MN: IEEE), 5271–5276. doi: 10.1109/ICRA.2012.6224696

Clymer, J. C. (1973). *A Computer Simulation Model of Attack-Learning Behavior in the Octopus*. Ph.D. thesis, The University of Michigan, Ann Arbor, MI.

Cohen, A. H., Rossignol, S., and Grillner, S. (1988). *Neural Control of Rhythmic Movements in Vertebrates*. Hoboken, NJ: Wiley-Interscience.

Cornwell, C., Messenger, J., and Williamson, R. (1993). Distribution of GABA-like immunoreactivity in the octopus brain. *Brain Res.* 621, 353–357. doi: 10.1016/0006-8993(93)90127-9

De Marianis, B., Olmo, E., and Giuditta, A. (1979). Excess DNA in the nuclei of the subseophagel region of octopus brain. *J. Comp. Neurol.* 186, 293–300. doi: 10.1002/cne.901860211

De Velasco, B., Erclik, T., Shy, D., Sclafani, J., Lipshitz, H., McInnes, R., et al. (2007). Specification and development of the pars intercerebralis and pars lateralis, neuroendocrine command centers in the *Drosophila* brain. *Dev. Biol.* 302, 309–323. doi: 10.1016/j.ydbio.2006.09.035

Denes, A. S., Jékely, G., Steinmetz, P. R., Raible, F., Snyman, H., Prud'homme, B., et al. (2007). Molecular architecture of annelid nerve cord supports common origin of nervous system centralization in bilateria. *Cell* 129, 277–288. doi: 10.1016/j.cell.2007.02.040

Di Cosmo, A., and Di Cristo, C. (1998). Neuropeptidergic control of the optic gland of *Octopus vulgaris*: FMRF-amide and GnRH immunoreactivity. *J. Comp. Neurol.* 398, 1–12. doi: 10.1002/(SICI)1096-9861(19980817)398:1<1::AID-CNE1>3.0.CO;2-5

Dorn, A. (1998). "Comparative structural aspects of development in neuroendocrine systems," in *Microscopic Anatomy of Invertebrates*, eds F. W. Harrison and M. Locke (New York, NY: Wiley-Liss, Inc), 1059–1092.

Dubas, F., Hanlon, R. T., Ferguson, G. P., and Pinsker, H. M. (1986). Localization and stimulation of chromatophore motoneurones in the brain of the squid, *Lolliguncula brevis*. *J. Exp. Biol.* 121, 1–25.

Eccles, J. C. (1977). An instruction-selection theory of learning in the cerebellar cortex. *Brain Res.* 127, 327–352. doi: 10.1016/0006-8993(77)90550-9

Edelman, D. B. (2011). How octopuses see the world and other roads less traveled: necessity versus sufficiency and evolutionary convergence in the study of animal consciousness. *J. Shellfish Res.* 30:1001.

Edelman, D. B., Baars, B. J., and Seth, A. K. (2005). Identifying hallmarks of consciousness in non-mammalian species. *Conscious. Cogn.* 14, 169–187. doi: 10.1016/j.concog.2004.09.001

Edelman, D. B., and Seth, A. K. (2009). Animal consciousness: a synthetic approach. *Trends Neurosci.* 32, 476–484. doi: 10.1016/j.tins.2009.05.008

Edelman, G. M. (1978). "Group selection and phasic re-entrant signalling: a theory of higher brain function," in *The Mindful Brain*, eds G. M. Edelman and V. B. Mountcastle (Cambridge, MA: MIT Press), 55–100.

Farfán, C., Shigeno, S., Nödl, M. T., Couet, D., and Gert, H. (2009). Developmental expression of apterous/Lhx2/9 in the sepiolid squid *Euprymna scolopes* supports an ancestral role in neural development. *Evol. Dev.* 11, 354–362. doi: 10.1111/j.1525-142X.2009.00342.x

Farris, S. M. (2008). Evolutionary convergence of higher brain centers spanning the protostome-deuterostome boundary. *Brain Behav. Evol.* 72, 106–122. doi: 10.1159/000151471

Fetcho, J. R. (1991). Spinal network of the Mauthner cell (Part 1 of 2). *Brain Behav. Evol.* 37, 298–306. doi: 10.1159/000114367

Fioroni, P. (1978). *Morphogenese der Tiere. Erste Reihe: Deskriptive Morphogenese. Lieferung 2: G5 - I, Cephalopoda, Tintenfische*. Stuttgart: Gustav Fisher.

Focareta, L., and Cole, A. G. (2016). Analyses of Sox-B and Sox-E family genes in the cephalopod *Sepia officinalis*: revealing the conserved and the unusual. *PLoS One* 11:e0157821. doi: 10.1371/journal.pone.0157821

Focareta, L., Sesso, S., and Cole, A. G. (2014). Characterization of homeobox genes reveals sophisticated regionalization of the central nervous system in the European cuttlefish *Sepia officinalis*. *PLoS One* 9:e109627. doi: 10.1371/journal.pone.0109627

Frösch, D. (1971). Quantitative untersuchungen am zentralnervensystem der schlüpfstadien von zehn mediterranen cephalopodenarten. *Rev. Suisse Zool.* 78, 1069–1122.

Garrett, S., and Rosenthal, J. J. C. (2012a). RNA editing underlies temperature adaptation in K^+ channels from polar octopuses. *Science* 335, 848–851. doi: 10.1126/science.1212795

Garrett, S. C., and Rosenthal, J. J. (2012b). A role for A-to-I RNA editing in temperature adaptation. *Physiology* 27, 362–369. doi: 10.1152/physiol.00029.2012

Gaston, M. R., and Tublitz, N. J. (2004). Peripheral innervation patterns and central distribution of fin chromatophore motoneurons in the cuttlefish *Sepia officinalis. J. Exp. Biol.* 207, 3089–3098. doi: 10.1242/jeb.01145

Gaston, M. R., and Tublitz, N. J. (2006). Central distribution and three-dimensional arrangement of fin chromatophore motoneurons in the cuttlefish *Sepia officinalis*. *Invert. Neurosci.* 6, 81–93. doi: 10.1007/s10158-006-0021-3

Giurfa, M. (2013). Cognition with few neurons: higher-order learning in insects. *Trends Neurosci.* 36, 285–294. doi: 10.1016/j.tins.2012.12.011

Gleadall, I. (1990). Higher motor function in the brain of octopus: the anterior basal lobe and its analogies with the vertebrate basal ganglia. *Ann. Appl. Inf. Sci.* 16, 1–30.

Grasso, F. W. (2014). "The octopus with two brains: how are distributed and central representations integrated in the octopus central nervous system," in *Cephalopod Cognition*, eds A.-S. Darmaillacq, L. Dickel, and J. A. Mather (Cambridge: Cambridge University Press), 94–122.

Grasso, F. W., and Basil, J. A. (2009). The evolution of flexible behavioral repertoires in cephalopod Molluscs. *Brain Behav. Evol.* 74, 231–245. doi: 10.1159/000258669

Gray, J. A. B. (1960). Mechanically excitable receptor units in the mantle of the octopus and their connexions. *J. Physiol.* 153, 573–582. doi: 10.1113/jphysiol.1960.sp006556

Graziadei, P. (1971). "The nervous system of the arm," in *The Anatomy of the Nervous System of Octopus vulgaris*, ed. J. Z. Young (London: Oxford University Press), 45–61.

Grillner, S., and Wallén, P. (1999). On the cellular bases of vertebrate locomotion. *Prog. Brain Res.* 123, 297–309. doi: 10.1016/S0079-6123(08)62865-4

Guan, Z., Cai, T., Liu, Z., Dou, Y., Hu, X., Zhang, P., et al. (2017). Origin of the reflectin gene and hierarchical assembly of its protein. *Curr. Biol.* 27, 2833–2842.e6. doi: 10.1016/j.cub.2017.07.061

Gutnick, T., Byrne, R. A., Hochner, B., and Kuba, M. (2011). *Octopus vulgaris* uses visual information to determine the location of its arm. *Curr. Biol.* 21, 460–462. doi: 10.1016/j.cub.2011.01.052

Hartenstein, V. (2006). The neuroendocrine system of invertebrates: a developmental and evolutionary perspective. *J. Endocrinol.* 190, 555–570. doi: 10.1677/joe.1.06964

Haszprunar, G. (1992). The first Molluscs - small animals. *Boll. Zool.* 59, 1–16. doi: 10.1080/11250009209386641

Hebb, D. O. (1949). *The Organization of Behavior: A Neuropsychological Theory*. New York, NY: Wiley.

Heisenberg, M. (2003). Mushroom body memoir: from maps to models. *Nat. Rev. Neurosci.* 4, 266–275. doi: 10.1038/nrn1074

Hejnol, A., and Martindale, M. Q. (2008). Acoel development supports a simple planula-like urbilaterian. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 363, 1493–1501. doi: 10.1098/rstb.2007.2239

Hobbs, M., and Young, J. (1973). A cephalopod cerebellum. *Brain Res.* 55, 424–430. doi: 10.1016/0006-8993(73)90307-7

Hochner, B. (2010). Functional and comparative assessments of the octopus learning and memory system. *Front. Biosci.* 2:764–771.

Hochner, B. (2012). An embodied view of octopus neurobiology. *Curr. Biol.* 22, R887–R892. doi: 10.1016/j.cub.2012.09.001

Hochner, B. (2013). How nervous systems evolve in relation to their embodiment: what we can learn from octopuses and other molluscs. *Brain Behav. Evol.* 82, 19–30. doi: 10.1159/000353419

Hochner, B., Brown, E. R., Langella, M., Shomrat, T., and Fiorito, G. (2003). A learning and memory area in the octopus brain manifests a vertebrate-like long-term potentiation. *J. Neurophysiol.* 90, 3547–3554. doi: 10.1152/jn.00645.2003

Hochner, B., and Glanzman, D. L. (2016). Evolution of highly diverse forms of behavior in molluscs. *Curr. Biol.* 26, R965–R971. doi: 10.1016/j.cub.2016.08.047

Hochner, B., Shomrat, T., and Fiorito, G. (2006). The octopus: a model for a comparative analysis of the evolution of learning and memory mechanisms. *Biol. Bull.* 210, 308–317. doi: 10.2307/4134567

Holland, L. Z., Carvalho, J. E., Escriva, H., Laudet, V., Schubert, M., Shimeld, S. M., et al. (2013). Evolution of bilaterian central nervous systems: a single origin? *EvoDevo* 4:27. doi: 10.1186/2041-9139-4-27

Holland, N. D. (2016). Nervous systems and scenarios for the invertebrate-to-vertebrate transition. *Philos. Trans. R. Soc. B* 371:20150047. doi: 10.1098/rstb.2015.0047

Homberg, U. (2008). Evolution of the central complex in the arthropod brain with respect to the visual system. *Arthropod Struct. Dev.* 37, 347–362. doi: 10.1016/j.asd.2008.01.008

Iwakoshi-Ukena, E., Ukena, K., Takuwa-Kuroda, K., Kanda, A., Tsutsui, K., and Minakata, H. (2004). Expression and distribution of octopus gonadotropin-releasing hormone in the central nervous system and peripheral organs of the octopus (*Octopus vulgaris*) by in situ hybridization and immunohistochemistry. *J. Comp. Neurol.* 477, 310–323. doi: 10.1002/cne.20260

Kanda, A., Satake, H., Kawada, T., and Minakata, H. (2005). Novel evolutionary lineages of the invertebrate oxytocin/vasopressin superfamily peptides and their receptors in the common octopus (*Octopus vulgaris*). *Biochem. J.* 387, 85–91. doi: 10.1042/BJ20041230

Kanda, A., Takahashi, T., Satake, H., and Minakata, H. (2006). Molecular and functional characterization of a novel gonadotropin-releasing-hormone receptor isolated from the common octopus (*Octopus vulgaris*). *Biochem. J.* 395, 125–135. doi: 10.1042/BJ20051615

Kanda, A., Takuwa-Kuroda, K., Iwakoshi-Ukena, E., Furukawa, Y., Matsushima, O., and Minakata, H. (2003a). Cloning of *Octopus* cephalotocin receptor, a member of the oxytocin/vasopressin superfamily. *J. Endocrinol.* 179, 281–291.

Kanda, A., Takuwa-Kuroda, K., Iwakoshi-Ukena, E., and Minakata, H. (2003b). Single exon structures of the oxytocin/vasopressin superfamily peptides of octopus. *Biochem. Biophys. Res. Commun.* 309, 743–748.

Kandel, E. R. (1979). *Behavioral Biology of Aplysia. A Contribution to the Comparative Study of Opisthobranch Molluscs*. San Francisco, CA: W.H. Freeman and Company.

Kandel, E. R., and Kupfermann, I. (1970). The functional organization of invertebrate ganglia. *Annu. Rev. Physiol.* 32, 193–258. doi: 10.1146/annurev.ph.32.030170.001205

Kiehn, O. (2016). Decoding the organization of spinal circuits that control locomotion. *Nat. Rev. Neurosci.* 17, 224–238. doi: 10.1038/nrn.2016.9

Koenig, K. M., Sun, P., Meyer, E., and Gross, J. M. (2016). Eye development and photoreceptor differentiation in the cephalopod *Doryteuthis pealeii*. *Development* 143, 3168–3181. doi: 10.1242/dev.134254

Korn, H., and Faber, D. S. (2005). The Mauthner cell half a century later: a neurobiological model for decision-making? *Neuron* 47, 13–28. doi: 10.1016/j.neuron.2005.05.019

Kröger, B., Vinther, J., and Fuchs, D. (2011). Cephalopod origin and evolution: a congruent picture emerging from fossils, development and molecules. *BioEssays* 33, 602–613. doi: 10.1002/bies.201100001

Laschi, C., Cianchetti, M., Mazzolai, B., Margheri, L., Follador, M., and Dario, P. (2012). Soft robot arm inspired by the octopus. *Adv. Robot.* 26, 709–727. doi: 10.1163/156855312X626343

Lee, P. N., Callaerts, P., de Couet, H. G., and Martindale, M. Q. (2003). Cephalopod Hox genes and the origin of morphological novelties. *Nature* 424, 1061–1065. doi: 10.1038/nature01872

Levy, G., Flash, T., and Hochner, B. (2015). Arm coordination in octopus crawling involves unique motor control strategies. *Curr. Biol.* 25, 1195–1200. doi: 10.1016/j.cub.2015.02.064

Levy, G., and Hochner, B. (2017). Embodied organization of *Octopus vulgaris* morphology, vision, and locomotion. *Front. Physiol.* 8:164. doi: 10.3389/fphys.2017.00164

Liebeskind, B. J., Hillis, D. M., Zakon, H. H., and Hofmann, H. A. (2016). Complex homology and the evolution of nervous systems. *Trends Ecol. Evol.* 31, 127–135. doi: 10.1016/j.tree.2015.12.005

Lindgren, A. R., Pankey, M. S., Hochberg, F. G., and Oakley, T. H. (2012). A multi-gene phylogeny of Cephalopoda supports convergent morphological evolution in association with multiple habitat shifts in the marine environment. *BMC Evol. Biol.* 12:129. doi: 10.1186/1471-2148-12-129

Liscovitch-Brauer, N., Alon, S., Porath, H. T., Elstein, B., Unger, R., Ziv, T., et al. (2017). Trade-off between transcriptome plasticity and genome evolution in cephalopods. *Cell* 169, 191–202.e11. doi: 10.1016/j.cell.2017.03.025

Loesel, R., and Heuer, C. M. (2010). The mushroom bodies – prominent brain centres of arthropods and annelids with enigmatic evolutionary origin. *Acta Zool.* 91, 29–34. doi: 10.1111/j.1463-6395.2009.00422.x

Loesel, R., Nässel, D. R., and Strausfeld, N. J. (2002). Common design in a unique midline neuropil in the brains of arthropods. *Arthropod Struct. Dev.* 31, 77–91. doi: 10.1016/S1467-8039(02)00017-8

Maddock, L., and Young, J. Z. (1987). Quantitative differences among the brains of cephalopods. *J. Zool.* 212, 739–767. doi: 10.1111/j.1469-7998.1987.tb05967.x

Maldonado, H. (1963a). The general amplification function of the vertical lobe in *Octopus vulgaris*. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* 47, 215–229. doi: 10.1007/BF00298034

Maldonado, H. (1963b). The positive learning process in *Octopus vulgaris*. *Z. Vgl. Physiol.* 47, 191–214. doi: 10.1007/BF00303120

Maldonado, H. (1963c). The visual attack learning system in *Octopus vulgaris*. *J. Theor. Biol.* 5, 470–488. doi: 10.1016/0022-5193(63)90090-0

Maldonado, H. (1965). The positive and negative learning process in *Octopus vulgaris* Lamarck. Influence of the vertical and median superior frontal lobes. *Z. Vgl. Physiol.* 51, 185–203. doi: 10.1007/BF00299293

Mantziaris, C., Bockemühl, T., Holmes, P., Borgmann, A., Daun, S., and Büschges, A. (2017). Intra-and intersegmental influences among central pattern generating networks in the walking system of the stick insect. *J. Neurophysiol.* 118, 2296–2310. doi: 10.1152/jn.00321.2017

Margheri, L., Ponte, G., Mazzolai, B., Laschi, C., and Fiorito, G. (2011). Non-invasive study of *Octopus vulgaris* arm morphology using ultrasound. *J. Exp. Biol.* 214, 3727–3731. doi: 10.1242/jeb.057323

Marini, G., De Sio, F., Ponte, G., and Fiorito, G. (2017). "Behavioral analysis of learning and memory in cephalopods," in *Learning and Memory: A Comprehensive Reference*, 2nd Edn, ed. J. H. Byrne (Amsterdam: Elsevier), 441–462. doi: 10.1016/B978-0-12-809324-5.21024-9

Marquis, V. F. (1989). Die Embryonalentwicklung des Nervensystems von *Octopus vulgaris* Lam. (Cephalopoda, Octopoda), eine histologische Analyse. *Verh. Naturforsch. Ges.* 99, 23–76.

Martin, R., and Miledi, R. (1986). The form and dimensions of the giant synapse of squids. *Philos. Trans. R. Soc. Lond. B* 312, 355–377. doi: 10.1098/rstb.1986.0013

Mather, J. A. (1995). Cognition in cephalopods. *Adv. Stud. Behav.* 24, 317–353. doi: 10.1016/S0065-3454(08)60398-8

Mather, J. A. (2008). Cephalopod consciousness: behavioural evidence. *Conscious. Cogn.* 17, 37–48. doi: 10.1016/j.concog.2006.11.006

Mather, J. A., and Dickel, L. (2017). Cephalopod complex cognition. *Curr. Opin. Behav. Sci.* 16, 131–137. doi: 10.1016/j.cobeha.2017.06.008

Messenger, J. (1967a). The effects on locomotion of lesions to the visuo-motor system in octopus. *Proc. R. Soc. Lond. B Biol. Sci.* 167, 252–281. doi: 10.1098/rspb.1967.0026

Messenger, J. (1967b). The peduncle lobe: a visuo-motor centre in *octopus*. *Proc. R. Soc. Lond. B Biol. Sci.* 167, 225–251. doi: 10.1098/rspb.1967.0025

Messenger, J. (1979). The nervous system of *Loligo*: IV. The peduncle and olfactory lobes. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 285, 275–309. doi: 10.1098/rstb.1979.0007

Minakata, H. (2010). Oxytocin/vasopressin and gonadotropin-releasing hormone from cephalopods to vertebrates. *Ann. N. Y. Acad. Sci.* 1200, 33–42. doi: 10.1111/j.1749-6632.2010.05569.x

Mnih, V., Kavukcuoglu, K., Silver, D., Rusu, A. A., Veness, J., Bellemare, M. G., et al. (2015). Human-level control through deep reinforcement learning. *Nature* 518, 529–533. doi: 10.1038/nature14236

Monsell, E. M. (1980). Cobalt and horseradish peroxidase tracer studies in the stellate ganglion of octopus. *Brain Res.* 184, 1–9. doi: 10.1016/0006-8993(80)90583-1

Moroz, L. L. (2009). On the independent origins of complex brains and neurons. *Brain Behav. Evol.* 74, 177–190. doi: 10.1159/000258665

Myers, C. E. (1992). *Delay Learning in Artificial Neural Networks*. London: Chapman & Hall.

Naef, A. (1928). *Die Cephalopoden. Embryologie*. Berlin: R. Friedländer & Sohn, 357.

Navet, S., Andouche, A., Baratte, S., and Bonnaud, L. (2009). Shh and Pax6 have unconventional expression patterns in embryonic morphogenesis in *Sepia officinalis* (Cephalopoda). *Gene Expr. Patterns* 9, 461–467. doi: 10.1016/j.gep.2009.08.001

Navet, S., Buresi, A., Baratte, S., Andouche, A., Bonnaud-Ponticelli, L., and Bassaglia, Y. (2017). The Pax gene family: highlights from cephalopods. *PLoS One* 12:e0172719. doi: 10.1371/journal.pone.0172719

Nixon, M., and Young, J. Z. (2003). *The Brains and Lives of Cephalopods*. New York, NY: Oxford University Press.

Northcutt, R. G. (2012). Evolution of centralized nervous systems: two schools of evolutionary thought. *Proc. Natl. Acad. Sci. U.S.A.* 109(Suppl. 1), 10626–10633. doi: 10.1073/pnas.1201889109

Ogura, A., Yoshida, M.-A., Moritaki, T., Okuda, Y., Sese, J., Shimizu, K. K., et al. (2013). Loss of the six3/6 controlling pathways might have resulted in pinhole-eye evolution in *Nautilus*. *Sci. Rep.* 3:1432. doi: 10.1038/srep01432

Orrhage, L. (1995). On the innervation and homologues of the anterior end appendages of the Eunicea (Polychaeta), with a tentative outline of the fundamental constitution of the cephalic nervous system of the polychaetes. *Acta Zool.* 76, 229–248. doi: 10.1111/j.1463-6395.1995.tb00996.x

Otis, T. S., and Gilly, W. (1990). Jet-propelled escape in the squid *Loligo opalescens*: concerted control by giant and non-giant motor axon pathways. *Proc. Natl. Acad. Sci. U.S.A.* 87, 2911–2915. doi: 10.1073/pnas.87.8.2911

Packard, A. (1972). Cephalopods and fish: the limits of convergence. *Biol. Rev.* 47, 241–307. doi: 10.1111/j.1469-185X.1972.tb00975.x

Packard, A., and Albergoni, V. (1970). Relative growth, nucleic acid content and cell numbers of the brain in *Octopus vulgaris* (Lamarck). *J. Exp. Biol.* 52, 539–552.

Packard, A. S. (1884). Aspects of the body in vertebrates and arthropods. *Am. Nat.* 18, 855–861. doi: 10.1086/273754

Pearson, J. C., Lemons, D., and McGinnis, W. (2005). Modulating Hox gene functions during animal body patterning. *Nat. Rev. Genet.* 6, 893–904. doi: 10.1038/nrg1726

Perry, C. J., Barron, A. B., and Cheng, K. (2013). Invertebrate learning and cognition: relating phenomena to neural substrate. *Wiley Interdiscip. Rev. Cogn. Sci.* 4, 561–582. doi: 10.1002/wcs.1248

Pfeifer, R., Lungarella, M., and Iida, F. (2007). Self-organization, embodiment, and biologically inspired robotics. *Science* 318, 1088–1093. doi: 10.1126/science.1145803

Pfeiffer, K., and Homberg, U. (2014). Organization and functional roles of the central complex in the insect brain. *Annu. Rev. Entomol.* 59, 165–184. doi: 10.1146/annurev-ento-011613-162031

Plän, T. (1987). *Funktionelle Neuroanatomie Sensorisch/Motorischer Loben im Gehirn von Octopus vulgaris. Doktorgrades der Naturwissehschaften*. Doctoral dissertation, Universität Regensburg, Regensburg.

Ponte, G. (2012). *Distribution and Preliminary Functional Analysis of Some Modulators in the Cephalopod Mollusc Octopus vulgaris*. Ph.D. thesis, Università della Calabria, Rende.

Pozzo-Miller, L. D., Moreira, J. E., and Llinás, R. R. (1998). The first-order giant neurons of the giant fiber system in the squid: electrophysiological and ultrastructural observations. *J. Neurocytol.* 27, 419–429. doi: 10.1023/A:1006984410908

Puelles, L., and Rubenstein, J. L. (2003). Forebrain gene expression domains and the evolving prosomeric model. *Trends Neurosci.* 26, 469–476. doi: 10.1016/S0166-2236(03)00234-0

Rajneesh, K., and Bolash, R. (2018). "Pathways of pain perception and modulation," in *Fundamentals of Pain Medicine*, eds J. Cheng and R. W. Rosenquist (Cham: Springer International Publishing), 7–11. doi: 10.1007/978-3-319-64922-1_2

Reichert, H., and Simeone, A. (2001). Developmental genetic evidence for a monophyletic origin of the bilaterian brain. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 356, 1533–1544. doi: 10.1098/rstb.2001.0972

Reiner, A., Medina, L., and Veenman, C. L. (1998). Structural and functional evolution of the basal ganglia in vertebrates. *Brain Res. Rev.* 28, 235–285. doi: 10.1016/S0165-0173(98)00016-2

Richter, S., Loesel, R., Purschke, G., Schmidt-Rhaesa, A., Scholtz, G., Stach, T., et al. (2010). Invertebrate neurophylogeny: suggested terms and definitions for a neuroanatomical glossary. *Front. Zool.* 7:29. doi: 10.1186/1742-9994-7-29

Riss, W., Pedersen, R. A., Jakway, J. S., and Ware, C. B. (1972). Levels of Function and their representation in the vertebrate thalamus. *Brain Behav. Evol.* 6, 26–41. doi: 10.1159/000123695

Robertson, J. D., Schwartz, O. M., and Lee, P. (1993). Carbocyanine dye labeling reveals a new motor nucleus in octopus brain. *J. Comp. Neurol.* 328, 485–500. doi: 10.1002/cne.903280404

Roth, G. (2013). *The Long Evolution of Brains and Minds*. Dordrecht: Springer. doi: 10.1007/978-94-007-6259-6

Roth, G. (2015). Convergent evolution of complex brains and high intelligence. *Philos. Trans. R. Soc. B Biol. Sci.* 370:20150049. doi: 10.1098/rstb.2015.0049

Rubenstein, J. L., Martinez, S., Shimamura, K., and Puelles, L. (1994). The embryonic vertebrate forebrain: the prosomeric model. *Science* 266, 578–578. doi: 10.1126/science.7939711

Rubenstein, J. L., Shimamura, K., Martinez, S., and Puelles, L. (1998). Regionalization of the prosencephalic neural plate. *Annu. Rev. Neurosci.* 21, 445–477. doi: 10.1146/annurev.neuro.21.1.445

Saidel, W. M. (1981). Evidence for visual mapping in the peduncle lobe of octopus. *Neurosci. Lett.* 24, 7–11. doi: 10.1016/0304-3940(81)90350-5

Saidel, W. M. (1982). Connections of the octopus optic lobe: an HRP study. *J. Comp. Neurol.* 206, 346–358. doi: 10.1002/cne.902060403

Sakaue, Y., Bellier, J.-P., Kimura, S., D'Este, L., Takeuchi, Y., and Kimura, H. (2014). Immunohistochemical localization of two types of choline acetyltransferase in neurons and sensory cells of the octopus arm. *Brain Struct. Funct.* 219, 323–341. doi: 10.1007/s00429-012-0502-6

Schiff, N. D. (2008). Central thalamic contributions to arousal regulation and neurological disorders of consciousness. *Ann. N. Y. Acad. Sci.* 1129, 105–118. doi: 10.1196/annals.1417.029

Shigeno, S. (2017). "Brain evolution as an information flow designer: the ground architecture for biological and artificial general intelligence," in *Brain Evolution by Design. From Neural Origin to Cognitive Architecture*, eds S. Shigeno, Y. Murakami, and T. Nomura (Tokyo: Springer), 415–438.

Shigeno, S., Parnaik, R., Albertin, C. B., and Ragsdale, C. W. (2015). Evidence for a cordal, not ganglionic, pattern of cephalopod brain neurogenesis. *Zool. Lett.* 1:26. doi: 10.1186/s40851-015-0026-z

Shigeno, S., and Ragsdale, C. W. (2015). The gyri of the octopus vertical lobe have distinct neurochemical identities. *J. Comp. Neurol.* 523, 1297–1317. doi: 10.1002/cne.23755

Shigeno, S., Sasaki, T., Moritaki, T., Kasugai, T., Vecchione, M., and Agata, K. (2008). Evolution of the cephalopod head complex by assembly of multiple molluscan body parts: evidence from *Nautilus* embryonic development. *J. Morphol.* 269, 1–17. doi: 10.1002/jmor.10564

Shigeno, S., Sasaki, T., and Boletzky, S. V. (2010). "The origins of cephalopod body plans: a geometrical and developmental basis for the evolution of vertebrate-like organ systems," in *Cephalopods - Present and Past*, eds K. Tanabe, Y. Shigeta, T. Sasaki, and H. Hirano (Tokyo: Tokai University Press), 23–34.

Shigeno, S., Tsuchiya, K., and Segawa, S. (2001). Embryonic and paralarval development of the central nervous system of the loliginid squid *Sepioteuthis lessoniana*. *J. Comp. Neurol.* 437, 449–475. doi: 10.1002/cne.1295

Shomrat, T., Feinstein, N., Klein, M., and Hochner, B. (2010). Serotonin is a facilitatory neuromodulator of synaptic transmission and "reinforces" long-term potentiation induction in the vertical lobe of *Octopus vulgaris*. *Neuroscience* 169, 52–64. doi: 10.1016/j.neuroscience.2010.04.050

Shomrat, T., Graindorge, N., Bellanger, C., Fiorito, G., Loewenstein, Y., and Hochner, B. (2011). Alternative sites of synaptic plasticity in two homologous "fan-out fan-in" learning and memory networks. *Curr. Biol.* 21, 1773–1782. doi: 10.1016/j.cub.2011.09.011

Shomrat, T., Turchetti-Maia, A., Stern-Mentch, N., Basil, J., and Hochner, B. (2015). The vertical lobe of cephalopods: an attractive brain structure for understanding the evolution of advanced learning and memory systems. *J. Comp. Physiol. A* 201, 947–956. doi: 10.1007/s00359-015-1023-6

Shomrat, T., Zarrella, I., Fiorito, G., and Hochner, B. (2008). The octopus vertical lobe modulates short-term learning rate and uses LTP to acquire long-term memory. *Curr. Biol.* 18, 337–342. doi: 10.1016/j.cub.2008.01.056

Sillar, K. T. (2009). Mauthner cells. *Curr. Biol.* 19, R353–R355. doi: 10.1016/j.cub.2009.02.025

Simpson, L., Bern, H. A., and Nishioka, R. S. (1966). Survey of evidence for neurosecretion in gastropod Molluscs. *Am. Zool.* 6, 123–138. doi: 10.1093/icb/6.2.123

Stocco, A., Lebiere, C., and Anderson, J. R. (2010). Conditional routing of information to the cortex: a model of the basal ganglia's role in cognitive coordination. *Psychol. Rev.* 117, 541–574. doi: 10.1037/a0019077

Strausfeld, N. J. (2012). *Arthropod Brains: Evolution, Functional Elegance, and Historical Significance*. Cambridge, MA: Harvard University Press.

Strausfeld, N. J., and Hirth, F. (2013). Deep homology of arthropod central complex and vertebrate basal ganglia. *Science* 340, 157–161. doi: 10.1126/science.1231828

Strausfeld, N. J., Strausfeld, C. M., Stowe, S., Rowell, D., and Loesel, R. (2006). The organization and evolutionary implications of neuropils and their neurons in the brain of the onychophoran *Euperipatoides rowelli*. *Arthropod Struct. Dev.* 35, 169–196. doi: 10.1016/j.asd.2006.06.002

Svidersky, V., and Plotnikova, S. (2002). Insects and vertebrates: analogous structures in higher integrative centers of the brain. *J. Evol. Biochem. Physiol.* 38, 627–639. doi: 10.1023/A:1022073218825

Swanson, L. W. (2007). Quest for the basic plan of nervous system circuitry. *Brain Res. Rev.* 55, 356–372. doi: 10.1016/j.brainresrev.2006.12.006

Tessmar-Raible, K. (2007). The evolution of neurosecretory centers in bilaterian forebrains: insights from protostomes. *Semin. Cell Dev. Biol.* 18, 492–501. doi: 10.1016/j.semcdb.2007.04.007

Tessmar-Raible, K., Raible, F., Christodoulou, F., Guy, K., Rembold, M., Hausen, H., et al. (2007). Conserved sensory-neurosecretory cell types in annelid and fish forebrain: insights into hypothalamus evolution. *Cell* 129, 1389–1400. doi: 10.1016/j.cell.2007.04.041

Tomarev, S. I., Callaerts, P., Kos, L., Zinovieva, R., Halder, G., Gehring, W., et al. (1997). Squid Pax-6 and eye development. *Proc. Natl. Acad. Sci. U.S.A.* 94, 2421–2426. doi: 10.1073/pnas.94.6.2421

Tomer, R., Denes, A. S., Tessmar-Raible, K., and Arendt, D. (2010). Profiling by image registration reveals common origin of annelid mushroom bodies and vertebrate pallium. *Cell* 142, 800–809. doi: 10.1016/j.cell.2010.07.043

Turchetti-Maia, A., Shomrat, T., and Hochner, B. (2017). "The vertical lobe of cephalopods. a brain structure ideal for exploring the mechanisms of complex forms of learning and memory," in *The Oxford Handbook of Invertebrate Neurobiology*, ed. J. J. Byrne (Oxford: Oxford University Press), 1–27.

van Duijn, M. (2017). Phylogenetic origins of biological cognition: convergent patterns in the early evolution of learning. *Interface Focus* 7:20160158. doi: 10.1098/rsfs.2016.0158

Villanueva, R., Perricone, V., and Fiorito, G. (2017). Cephalopods as predators: a short journey among behavioral flexibilities, adaptions, and feeding habits. *Front. Physiol.* 8:598. doi: 10.3389/fphys.2017.00598

Wang, Z. Y., and Ragsdale, C. W. (2017). Cadherin genes and evolutionary novelties in the octopus. *Semin. Cell Dev. Biol.* 69, 151–157. doi: 10.1016/j.semcdb.2017.06.007

Wells, M., and Wells, J. (1969). Pituitary analogue in the octopus. *Nature* 222, 293–294. doi: 10.1038/222293a0

Wells, M. J. (1959). A touch-learning centre in octopus. *J. Exp. Biol.* 36, 590–612.

Wiersma, C. A. G., and Roach, J. L. M. (2011). "Principles in the organization of invertebrate sensory systems," in *Comprehensive Physiology Supplement 1: Handbook of Physiology, The Nervous System, Cellular Biology of Neurons* (Hoboken, NJ: Wiley-Blackwell), 1089–1135. doi: 10.1002/cphy.cp010128

Williams, E. A., Verasztó, C., Jasek, S., Conzelmann, M., Shahidi, R., Bauknecht, P., et al. (2017). Synaptic and peptidergic connectome of a neurosecretory center in the annelid brain. *eLife* 6:e26349. doi: 10.7554/eLife.26349

Wolff, G. H., and Strausfeld, N. J. (2016). Genealogical correspondence of a forebrain centre implies an executive brain in the protostome-deuterostome bilaterian ancestor. *Philos. Trans. R. Soc. B* 371:20150055. doi: 10.1098/rstb.2015.0055

Wollesen, T., McDougall, C., Degnan, B. M., and Wanninger, A. (2014). POU genes are expressed during the formation of individual ganglia of the cephalopod central nervous system. *EvoDevo* 5:41. doi: 10.1186/2041-9139-5-41

Wollesen, T., Monje, S. V. R., McDougall, C., Degnan, B. M., and Wanninger, A. (2015a). The ParaHox gene Gsx patterns the apical organ and central nervous system but not the foregut in scaphopod and cephalopod mollusks. *Evodevo* 6:41. doi: 10.1186/s13227-015-0037-z

Wollesen, T., Rodríguez Monje, S. V., Todt, C., Degnan, B. M., and Wanninger, A. (2015b). Ancestral role of Pax2/5/8 in molluscan brain and multimodal sensory system development. *BMC Evol. Biol.* 15:231. doi: 10.1186/s12862-015-0505-z

Wollesen, T., Sukhsangchan, C., Seixas, P., Nabhitabhata, J., and Wanninger, A. (2012). Analysis of neurotransmitter distribution in brain development of benthic and pelagic octopod cephalopods. *J. Morphol.* 273, 776–790. doi: 10.1002/jmor.20023

Woodhams, P. L. (1977). The ultrastructure of a cerebellar analogue in octopus. *J. Comp. Neurol.* 174, 329–345. doi: 10.1002/cne.901740209

Yin, H. H., and Knowlton, B. J. (2006). The role of the basal ganglia in habit formation. *Nat. Rev. Neurosci.* 7, 464–476. doi: 10.1038/nrn1919

Yoshida, M.-A., Yura, K., and Ogura, A. (2014). Cephalopod eye evolution was modulated by the acquisition of Pax-6 splicing variants. *Sci. Rep.* 4:4256. doi: 10.1038/srep04256

Young, J. Z. (1932). Memoirs: on the cytology of the neurons of cephalopods. *J. Cell Sci.* 75, 1–47.

Young, J. Z. (1939). Fused neurons and synaptic contacts in the giant nerve fibres of cephalopods. *Philos. Trans. R. Soc. Lond. B* 229, 465–503. doi: 10.1098/rstb.1939.0003

Young, J. Z. (1961). Learning and discrimination in the octopus. *Biol. Rev* 36, 32–96. doi: 10.1111/j.1469-185X.1961.tb01432.x

Young, J. Z. (1963). The number and sizes of nerve cells in Octopus. *Proc. Zool. Soc. Lond.* 140, 229–254. doi: 10.1111/j.1469-7998.1963.tb01862.x

Young, J. Z. (1964). *A Model of the Brain*. Oxford: Clarendon Press.

Young, J. Z. (1965a). The central nervous system of Nautilus. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 249, 1–25. doi: 10.1098/rstb.1965.0006

Young, J. Z. (1965b). The Croonian Lecture, 1965: the organization of a memory system. *Proc. R. Soc. Lond. B Biol. Sci.* 163, 285–320. doi: 10.1098/rspb.1965.0071

Young, J. Z. (1970). Neurovenous tissues in cephalopods. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 257, 309–321. doi: 10.1098/rstb.1970.0027

Young, J. Z. (1971). *The Anatomy of the Nervous System of Octopus vulgaris*. London: Oxford University Press.

Young, J. Z. (1972). The organization of a cephalopod ganglion. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 263, 409–429. doi: 10.1098/rstb.1972.0005

Young, J. Z. (1974). The central nervous system of Loligo I. The optic lobe. *Philos. Trans. R. Soc. Lond. B* 267, 263–302. doi: 10.1098/rstb.1974.0002

Young, J. Z. (1976a). The 'cerebellum' and the control of eye movements in cephalopods. *Nature* 264, 572–574. doi: 10.1038/264572a0

Young, J. Z. (1976b). The nervous system of Loligo. II. Suboesophageal centres. *Philos. Trans. R. Soc. B Biol. Sci.* 274, 101–167.

Young, J. Z. (1977a). Brain, behaviour and evolution of cephalopods. *Symp. Zool. Soc. Lond.* 38, 377–434.

Young, J. Z. (1977b). The nervous system of Loligo III. Higher motor centres: the basal supraoesophageal lobes. *Philos. Trans. R. Soc. Lond. B* 276, 351–398. doi: 10.1098/rstb.1977.0003

Young, J. Z. (1979). The nervous system of Loligo: V. The vertical lobe complex. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 285, 311–354. doi: 10.1098/rstb.1979.0008

Young, J. Z. (1991). Computation in the learning system of cephalopods. *Biol. Bull.* 180, 200–208. doi: 10.2307/1542389

Young, J. Z. (1995). "Multiple matrices in the memory system of Octopus," in *Cephalopod Neurobiology*, eds J. N. Abbott, R. Williamson, and L. Maddock (Oxford: Oxford University Press), 431–443.

Zottoli, S. J. (1978). Comparison of Mauthner cell size in teleosts. *J. Comp. Neurol.* 178, 741–757. doi: 10.1002/cne.901780409

Zullo, L., Sumbre, G., Agnisola, C., Flash, T., and Hochner, B. (2009). Nonsomatotopic organization of the higher motor centers in octopus. *Curr. Biol.* 19, 1632–1636. doi: 10.1016/j.cub.2009.07.067

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Physiology

REVIEW
published: 23 May 2018
doi: 10.3389/fphys.2018.00593

Cephalopod Tissue Regeneration: Consolidating Over a Century of Knowledge

Pamela Imperadore[1,2]* *and Graziano Fiorito*[2]

[1] *Association for Cephalopod Research - CephRes, Napoli, Italy,* [2] *Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Napoli, Italy*

Regeneration, a process consisting in regrowth of damaged structures and their functional recovery, is widespread in several phyla of the animal kingdom from lower invertebrates to mammals. Among the regeneration-competent species, the actual ability to restore the full form and function of the injured tissue varies greatly, from species being able to undergo whole-body and internal organ regeneration, to instances in which this ability is limited to a few tissues. Among invertebrates, cephalopod mollusks retain the ability to regenerate several structures (i.e., muscles, nerves, or entire appendages). Here we provide an overview of more than one-hundred studies carried out over the last 160 years of research. Despite the great effort, many aspects of tissue regeneration in cephalopods, including the associated molecular and cellular machinery, remain largely unexplored. Our approach is largely descriptive and aims to provide a reference to prior work thus to facilitate future research efforts. We believe such research may lead to important discoveries and approaches that can be applied to other animal taxa including higher vertebrates, as well as other research fields such as regenerative medicine.

Keywords: regeneration, wound healing, functional recovery, cephalopod, invertebrates

Edited by:
Fernando Ariel Genta,
Fundação Oswaldo Cruz (Fiocruz),
Brazil

Reviewed by:
David B. Edelman,
Dartmouth College, United States
Carlos Rosas,
Universidad Nacional Autónoma de
México, Mexico

***Correspondence:**
Pamela Imperadore
p_imperadore@
cephalopodresearch.org

Specialty section:
This article was submitted to
Invertebrate Physiology,
a section of the journal
Frontiers in Physiology

Received: *10 March 2018*
Accepted: *02 May 2018*
Published: *23 May 2018*

Citation:
Imperadore P and Fiorito G (2018)
Cephalopod Tissue Regeneration:
Consolidating Over a Century of
Knowledge. Front. Physiol. 9:593.
doi: 10.3389/fphys.2018.00593

INTRODUCTION

Johannes Japetus Steenstrup, a Danish zoologist (biography available in: Müller, 1976; Farley, 2001), was the first to report evidence for appendage regeneration in cephalopods. In his "*Hectocotyldannelsen hos Octopodslægterne Argonauta og Tremoctopus, oplyst ved Iagttagelse af lignende Dannelser hos Blæksprutterne i Almindelighed*" (Steenstrup, 1856), [which was translated into English one year later (1857)], Steenstrup provided a thorough description of how the hectocotylus is formed in species belonging to the *Argonauta* and *Tremoctopus* genera. The Author also provided a description of the ability of this arm to regenerate if lost during copulation.

These accounts appeared about 170 years after the first report of regenerative abilities in any animal (for review see Dinsmore, 1991).

By examining decades of scientific literature, we found accounts that provide evidence of regeneration occurring in a variety of cephalopod tissue types, including the appendages (arms and tentacles), as well as aspects of the peripheral and central nervous systems. It has been also observed in the fossil record (e.g., shell repair in Ammonoidea; Buckman, 1891; Keupp, 1976, 2000). Among many, Mathilde M. Lange was the first to both provide a detailed description of cephalopod tissue regeneration, and pioneered a new avenue of study through experimental lesioning of arms, tentacles, skin and nerves (Lange, 1920).

Similar experimental studies of regeneration events occurring after lesioning of peripheral nervous structures, such as the pallial- and the stellar nerves (which control skin patterning and breathing movements), were performed later (e.g., Sereni, 1929b; Sereni and Young, 1932; Sanders and Young, 1974). Collectively, this work has contributed to our understanding of the connectivity between the central and peripheral nervous systems of cephalopods.

AIMS, ORGANIZATION AND GENERAL OUTLINE OF THIS REVIEW

Cephalopods offer a valuable system with which to study regeneration phenomena and their underlying physiological mechanisms. Such research may lead to important discoveries and approaches that can be applied to other animal groups (including higher vertebrates) as well as other research fields, such as regenerative medicine.

Our review is based on a survey of the scientific literature initiated through an index provided by the Zoological Record (ZR)[1], including both library holdings (i.e., ZR-volume collection of the Stazione Zoologica Anton Dohrn) and ZR-modern e-databases, as well as a subsequent search for non-digitized references identified in these works.

An analysis of the indexed works in Zoological Record for the number of scientific publications from the last seventy years concerning "regeneration AND Mollusca" (excluding cephalopods) allowed us to identify about 50 published works out of a total of more than 13,000 references using "regeneration" as a topic.

By contrast, we identified around 120 works studying cephalopod regeneration (**Figure 1**) starting from Steenstrup's publication of 1857, as mentioned above. The figure shows a notable increase in the number of reports concerning the study of the regenerative phenomena in cephalopods over the last 50 years: this seemed especially pronounced over the last two decades (e.g., Rohrbach and Schmidtberg, 2006; Fossati et al., 2013, 2015; Tressler et al., 2014; Imperadore et al., 2017; Zullo et al., 2017).

Here, we summarize available knowledge of regeneration phenomena in cephalopod mollusks, providing an historical analysis of the studies carried out over the last 160 years on the regenerative abilities of the taxon.

Our approach is largely descriptive and aims to provide a convenient reference to prior work in order to facilitate future research efforts. The availability of new tools and approaches, as well as renewed interest in these complex invertebrates, may help in deciphering the molecular and cellular mechanisms involved in tissue regeneration, and could potentially inform our understanding of how the process can be dysregulated or inhibited in non-regenerating species.

The following pages offer a systematic overview of the findings described in a total of 119 works (**Table 1**) spanning the years 1856 to 2018, and a simplified outline of main discoveries (**Figure 2**). In addition to the tabularized overview of the regenerative process presented in **Table 1**, we also highlight first the events occurring in the early stages after damage (i.e., wound healing, both after skin injury and as first step of arm amputation), and second the ability of re-growing lost body parts, including regaining of function.

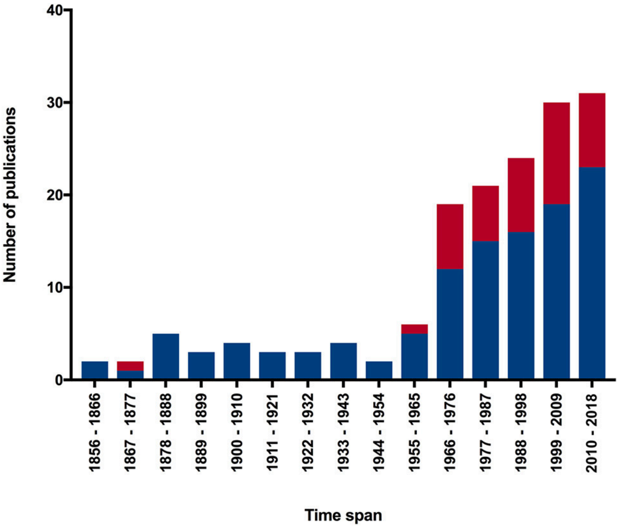


FIGURE 1 | Trend of the number of publications regarding cephalopod regeneration from the first study published in 1856 to present. Number of indexed scientific works deduced from a query to Zoological Record (http://wokinfo.com/products_tools/specialized/zr/) concerning "regeneration AND cephalopod*." The graph shows an enormous increase in published works in the last 50 years covering both living cephalopod tissue regeneration (blue) and fossil record shell repair (red). See text for further information.

WOUND HEALING

Skin, fin and arm damage occurs frequently in the course of a cephalopod lifespan as a result of such events as predator-prey interactions, agonistic and reproductive encounters, capture and transportation, and autotomy during predator evasion and autophagy (e.g., Hanlon et al., 1984; Budelmann, 1998; Florini et al., 2011; Bush, 2012). After injury, damaged structures can heal and recover their function, although wound repair appears delayed in fully mature animals, often leading to the failure of skin lesions to heal (O'Dor and Wells, 1978).

Cephalopod Skin and Wound Healing

The skin of cephalopods plays an important role in (i) concealment and communication and as (ii) a barrier that protects the animal body (review in e.g., Packard, 1988). Polglase and his colleagues were the first to describe the process of wound healing following injury to the skin of the mantle (*Eledone cirrhosa*, Polglase et al., 1983). Soon after surgery, octopuses (kept at 10–11°C) were seen holding and rubbing the wound with an arm tip. In the first 12 h following injury, in-folding of the epidermis close to the wound due to muscular contraction was observed (Polglase et al., 1983).

---

[1] http://wokinfo.com/products_tools/specialized/zr/; time-span 1945-2018; last accessed: February, 2018.

TABLE 1 | A tabular overview of the studies of regeneration abilities of cephalopod molluscs.

Main Topic Year of publication	General description	Species	Fossil record	Nautilus	Cuttlefish	Squid	Octopus	References
WOUND HEALING								
1983	Skin healing	*Eledone cirrhosa* (Lamarck, 1798)					✓	Polglase et al., 1983
1988	Wound healing in the arm	*Sepia officinalis* (Linnaeus, 1758)			✓			Féral, 1988
2006	Skin healing	*Sepia officinalis* (Linnaeus, 1758)			✓			Harms et al., 2006
2016	Wound healing in the arm	*Octopus vulgaris* (Cuvier, 1797)					✓	Shaw et al., 2016
ARM ABNORMALITIES								
1893	Double hectocotylus	*Eledone cirrhosa* (Lamarck, 1798)					✓	Appellof, 1893
1898	Sub-numerary arms (seven) in octopus	*Enteroctopus megalocyathus* (Gould, 1852)					✓	Lönnberg, 1898
1900	Extra arm in octopus; arm branching in octopus	*Octopus vulgaris* (Cuvier, 1797); *Eledone cirrhosa* (Lamarck, 1798); *Eledone moschata* (Lamarck, 1798)					✓	Parona, 1900
1907	Arm branching in octopus	*Octopus cephea* (Gray, 1849) **taxon inquirendum**					✓	Smith, 1907
1913	Arm branching	*Octopus vulgaris* (Cuvier, 1797); *Eledone cirrhosa* (Lamarck, 1798); *Eledone moschata* (Lamarck, 1798)					✓	Hanko, 1913
1929	Double hectocotylus	*Octopus rugosus* (Bosc, 1792) **taxon inquirendum**					✓	Robson, 1929
1937	Arm branching	*Sepia esculenta* (Hoyle, 1885)			✓			Okada, 1937
1960	Arm branching	*Octopus briareus* Robson, 1929					✓	Kumpf, 1960
1965	Specimens of Japanese octopus with several branched arms	N/A					✓	Okada, 1965a
1965	Arm branching "rules" in the Japanese octopus	N/A					✓	Okada, 1965b
1973	Double hectocotylus in octopus	*Octopus vulgaris* (Cuvier, 1797); *Octopus selene* (Voss, 1971)					✓	Palacio, 1973
1989	Sub-numerary arms (seven) in octopus	*Octopus* sp.						Gleadall, 1989
1991	Six-armed specimen (*Pteroctopus tetracirrhus*) 10-armed specimen (*Octopus briareus*)	*Pteroctopus tetracirrhus* (Delle Chiaje, 1830); *Octopus briareus* Robson, 1929					✓	Toll and Binger, 1991
2007	Double hectocotylus	*Octopus minor* (Sasaki, 1920)						Higashide et al., 2007
2013	Bilateral hectocotylization	*Enteroctopus dofleini* (Wülker, 1910)					✓	Brewer and Seitz, 2013
2014	Arm branching	*Octopus hubbsorum* (Berry, 1953)					✓	Alejo-Plata and Méndez, 2014
ARM AUTOTOMY								
1952	Arm autotomy; regeneration of lost structures is hypothesized	*Tremoctopus violaceus* (delle Chiaje, 1830)					✓	Portmann, 1952
1990	Automutilation syndrome in *Octopus dolfleini*, *O. bimaculoides*, and *O. maya*	*Enteroctopus dofleini* (Wülker, 1910); *Octopus bimaculoides* (Pickford & McConnaughey, 1949); *Octopus maya* (Voss & Solís, 1966)					✓	Reimschuessel and Stoskopf, 1990
1992	Arm autotomy	*Ameloctopus litoralis* Norman, 1992					✓	Norman, 1992
2001	Arm autotomy and regeneration; arm autotomy	*Abdopus capricornicus* (Norman & Finn, 2001) *Ameloctopus litoralis* Norman, 1992; *Octopus mutilans* (Taki, 1942)					✓	Norman and Finn, 2001

(Continued)

TABLE 1 | Continued

Main Topic Year of publication	General description	Species	Fossil record	Nautilus	Cuttlefish	Squid	Octopus	References
2012	Arm autotomy and regeneration	*Octopoteuthis deletron* Young, 1972				✓		Bush, 2012
ARM REGENERATION								
1856	Hectocotylus-formation in *Argonauta* and *Tremoctopus;* arm regeneration in *Octopus* sp.	N/A					✓	Steenstrup, 1856
1857	Hectocotylus-formation in *Argonauta* and *Tremoctopus;* arm regeneration in *Octopus* sp.	N/A					✓	Steenstrup, 1857
1881	Sucker, arm and tentacle regeneration in *Loligo pealei* and *Ommastrephes illecebrosus*	*Doryteuthis (Amerigo) pealeii* (Lesueur, 1821); *Illex illecebrosus* (LeSueur, 1821)				✓		Verrill, 1881
1881	Arm regeneration Shell aberration	*Octopus vulgaris* (Cuvier, 1797) *Sepia officinalis* (Linnaeus, 1758)			✓			Richiardi, 1881
1882	Arm regeneration in *Architeuthis harveyi*	*Architeuthis dux* Steenstrup, 1857				✓		Verrill, 1882
1901	Arm regeneration in *Octopus Defilippii*	*Macrotritopus defilippi* (Vérany, 1851)					✓	Riggenbach, 1901
1909	Arm autotomy and regeneration in *Octopus Defilippii*	*Macrotritopus defilippi* (Vérany, 1851)					✓	Lo Bianco, 1909
1916	Arm regeneration in *Polypus rugosus* and *Polypus tonganus*	*Octopus rugosus (*Bosc, 1792) **taxon inquirendum;** *Abdopus tonganus* (Hoyle, 1885)					✓	Massy, 1916
1920	Arm regeneration	*Octopus vulgaris* (Cuvier, 1797); *Eledone moschata* (Lamarck, 1798); *Sepia officinalis* (Linnaeus, 1758)			✓		✓	Lange, 1920
1929	Arm regeneration *Octopus (Octopus) tonganus*	*Abdopus tonganus* (Hoyle, 1885)					✓	Robson, 1929
1964	Arm regeneration, branchial gland and branchial heart healing	*Octopus vulgaris* (Cuvier, 1797)					✓	Taki, 1964
1977	Arm regeneration	*Sepia officinalis* (Linnaeus, 1758); *Sepiola atlantica* (d'Orbigny [in Férussac & d'Orbigny], 1839–1842); Loliginidae (Lesueur, 1821)			✓	✓		Féral, 1977
1978	Arm and hectocotylus regeneration	*Octopus vulgaris* (Cuvier, 1797)					✓	O'Dor and Wells, 1978)
1978	Arm regeneration	*Sepia officinalis* (Linnaeus, 1758)			✓			Féral, 1978
1979	Arm regeneration	*Sepia officinalis* (Linnaeus, 1758)			✓			Féral, 1979
1981	Tentacle and arm regeneration	*Ommastrephes bartramii* (Lesueur, 1821)				✓		Murata et al., 1981
1985	Arm and tentacle regeneration in *Sepia pharaonis* and *Loligo duvaucelii*	*Sepia pharaonis* Ehrenberg, 1831; *Uroteuthis (Photololigo) duvaucelii* (d'Orbigny [in Férussac & d'Orbigny], 1835)			✓	✓		Nair and Rao, 1985
1992	Arm regeneration in *Octopus digueti*	*Paroctopus digueti* (Perrier & Rochebrune, 1894)					✓	Voight, 1992
2001	Arm autotomy and regeneration in *Octopus (Abdopus) capricornicus* Arm autotomy in *Ameloctopus litoralis, Octopus mutilans*	*Abdopus capricornicus* (Norman & Finn, 2001); *Ameloctopus litoralis* Norman, 1992;*Octopus mutilans* (Taki, 1942)					✓	Norman and Finn, 2001
2003	Arm regeneration and arm-tip light organs regeneration	*Vampyroteuthis infernalis* (Chun, 1903)				✓		Robison et al., 2003
2006	Arm and tentacle regeneration	*Sepia officinalis* (Linnaeus, 1758)			✓			Rohrbach and Schmidtberg, 2006

(Continued)

TABLE 1 | Continued

Main Topic Year of publication	General description	Species	Fossil record	Nautilus	Cuttlefish	Squid	Octopus	References
2006	Arm regeneration	*Wunderpus photogenicus* (Hochberg, Norman & Finn, 2006)					✓	Hochberg et al., 2006
2011	Arm regeneration	*Octopus vulgaris* (Cuvier, 1797)					✓	Florini et al., 2011
2011	Arm regeneration	*Octopus vulgaris* (Cuvier, 1797)					✓	Fossati et al., 2011
2012	Arm autotomy and regeneration	*Octopoteuthis deletron* Young, 1972				✓		Bush, 2012
2013	Involvement of acetylcholinesterase in the arm regeneration	*Octopus vulgaris* (Cuvier, 1797)					✓	Fossati et al., 2013
2014	Arm regeneration	*Sepia officinalis* (Linnaeus, 1758); *Sepia pharaonis* (Ehrenberg, 1831)			✓			Tressler et al., 2014
2015	Acetylcholinesterase expression during adult arm regeneration and embryonic arm development	*Octopus vulgaris* (Cuvier, 1797)					✓	Fossati et al., 2015
2016	Arm regeneration in	*Octopoteuthis nielseni* (Robson, 1948)				✓		Young and Vecchione, 2016
2017	Arm loss and regeneration	*Abdopus* sp. (Norman & Finn, 2001)					✓	Wada, 2017
2018	Arm regeneration (micro-PET imaging)	*Octopus vulgaris* (Cuvier, 1797)					✓	Zullo et al., 2018
HECTOCOTYLUS REGENERATION								
1882	Tentacle regeneration in *Ommastrephes illecebrosus;* Hectocotylus regeneration in the family Philonexidae D'Orbigny.	*Illex illecebrosus* (LeSueur, 1821)					✓	Verrill, 1882
1887	Tentacle and hectocotylus regeneration in *Octopus fusiformis*, *Octopus inconspicuus*, *Octopus cuvieri*	*Octopus fusiformis* Brock, 1887 **nomen dubium;** *Octopus inconspicuus* Brock, 1887 **taxon inquirendum**; *Callistoctopus lechenaultii* (d'Orbigny [in Férussac & d'Orbigny], 1826)					✓	Brock, 1887
1940	Hectocotylus regeneration in castrated octopus	*Octopus vulgaris* (Cuvier, 1797)					✓	Callan, 1940
1944	Hectocotylus regeneration	*Octopus vulgaris* (Cuvier, 1797)					✓	Taki, 1944
1978	Arm and hectocotylus regeneration	*Octopus vulgaris* (Cuvier, 1797)					✓	O'Dor and Wells, 1978
1995	Hectocotylus regeneration	*Sepietta oweniana* (d'Orbigny [in Ferussac & d'Orbigny], 1839-1841); *Sepiola ligulata* (Naef, 1912)				✓		Bello, 1995
TENTACLES, ABNORMALITIES								
2008	Tentacle branching in *Moroteuthis ingens*	*Onykia ingens* (E. A. Smith, 1881)				✓		González and Guerra, 2008
TENTACLES, AUTOTOMY								
2012	Tentacle autotomy and regeneration	*Ommastrephes bartramii* (Lesueur, 1821)				✓		Kurosaka et al., 2012
TENTACLES, REGENERATION								
1881	Sucker, arm and tentacle regeneration in *Loligo pealeii* and *Ommastrephes illecebrosus*	*Doryteuthis (Amerigo) pealeii* (Lesueur, 1821); *Illex illecebrosus* (LeSueur, 1821)				✓		Verrill, 1881
1882	Tentacle regeneration in *Ommastrephes illecebrosus;* Hectocotylus regeneration in the family Philonexidae D'Orbigny.	*Illex illecebrosus* (LeSueur, 1821)				✓	✓	Verrill, 1882

(Continued)

TABLE 1 | Continued

Main Topic Year of publication	General description	Species	Fossil record	Nautilus	Cuttlefish	Squid	Octopus	References
1887	Tentacle and hectocotylus regeneration in *Octopus fusiformis*, *Octopus inconspicuus*, *Octopus cuvieri*	*Octopus fusiformis* Brock, 1887 **nomen dubium;** *Octopus inconspicuus* Brock, 1887 **taxon inquirendum**; *Callistoctopus lechenaultii* (d'Orbigny [in Férussac & d'Orbigny], 1826)					✓	Brock, 1887
1937	Tentacle regeneration	*Sepioteuthis lessoniana* (Férussac [in Lesson], 1831)				✓		Adam, 1937
1966	Tentacular stalk regeneration	*Liocranchia gardineri* (Robson, 1921) **taxon inquirendum**				✓		Clarke, 1966
1968	Tentacle regeneration	*Architeuthis dux* Steenstrup, 1857				✓		Aldrich and Aldrich, 1968
1981	Tentacle and arm regeneration	*Ommastrephes bartramii* (Lesueur, 1821)				✓		Murata et al., 1981
1985	Arm and tentacle regeneration in *Sepia pharaonis* and *Loligo duvaucelii*	*Sepia pharaonis* (Ehrenberg, 1831); *Uroteuthis (Photololigo) duvaucelii* (d'Orbigny [in Férussac & d'Orbigny], 1835)			✓	✓		Nair and Rao, 1985
1996	Tentacle regeneration	*Sepia officinalis* (Linnaeus, 1758)			✓			Hielscher et al., 1996
2006	Arm and tentacle regeneration	*Sepia officinalis* (Linnaeus, 1758)			✓			Rohrbach and Schmidtberg, 2006
2012	Tentacle autotomy and regeneration	*Ommastrephes bartramii* (Lesueur, 1821)				✓		Kurosaka et al., 2012
NERVE REGENERATION								
1932	Pallial and stellar nerve degeneration and regeneration in *E. moschata, E. cirrosa, O. vulgaris, O. macropus, S. officinalis, L. vulgaris, Loligo pealeii*	*Eledone moschata* (Lamarck, 1798); *Eledone cirrhosa* (Lamarck, 1798); *Octopus vulgaris* (Cuvier, 1797); *Octopus macropus* (Risso, 1826); *Sepia officinalis* (Linnaeus, 1758); *Loligo vulgaris* (Lamarck, 1798); *Doryteuthis (Amerigo) pealeii* (Lesueur, 1821)			✓	✓	✓	Sereni and Young, 1932
1932	Pallial and stellar nerve degeneration and regeneration	*Eledone moschata* (Lamarck, 1798); *Octopus vulgaris* (Cuvier, 1797); *Octopus macropus* (Risso, 1826); *Loligo vulgaris* (Lamarck, 1798); *Sepia officinalis* (Linnaeus, 1758)			✓	✓	✓	Young, 1932
1972	Pallial nerve and stellar nerve lesion, regeneration and degeneration. Effect of lesion on the stellate ganglion	*Octopus vulgaris* (Cuvier, 1797); *Sepia officinalis* (Linnaeus, 1758)			✓		✓	Young, 1972
1974	Recovery of function after pallial nerve cut or crush	*Octopus vulgaris* (Cuvier, 1797)					✓	Sanders and Young, 1974
2017	Pallial nerve degeneration and regeneration	*Octopus vulgaris* (Cuvier, 1797)					✓	Imperadore et al., 2017
2018	Pallial nerve regeneration (imaging through multiphoton microscopy)	*Octopus vulgaris* (Cuvier, 1797)					✓	Imperadore et al., 2018
SHELL, REPAIR AND REGENERATION								
1877	Shell repair in fossil records (Nautiloids) ê	N/A	✓					Barrande, 1877
1964	Shell repair in fossil records (Nautiloids)	N/A	✓					Gordon, 1964
1967	Shell repair in fossil records (Ammonoids)	N/A	✓					Guex, 1967

(Continued)

TABLE 1 | Continued

Main Topic Year of publication	General description	Species	Fossil record	Nautilus	Cuttlefish	Squid	Octopus	References
1972	Shell repair	*Nautilus pompilius* (Linnaeus, 1758)	✓	✓				Haven, 1972
1973	Shell repair in fossil records (Ammonoids)	N/A	✓					Hölder, 1973
1973	Shell repair in fossil records (Ammonoids)	N/A	✓					Saunders, 1973
1974	Shell repair	*Nautilus macromphalus* (G.B. Sowerby II, 1849)		✓				Meenakshi et al., 1974
1975	Shell repair in fossil records (Ammonoids)	N/A	✓					Lehmann, 1975
1976	Shell repair in fossil records (Ammonoids)	N/A	✓					Keupp, 1976
1977	Shell repair in fossil records (Ammonoids)	N/A	✓					Hölder, 1977
1977	Shell repair in fossil records (Ammonoids)	N/A	✓					Keupp, 1977
1978	Shell repair	*Nautilus pompilius* (Linnaeus, 1758)	✓	✓				Tucker and Mapes, 1978
1979	Shell repair in fossil records (Bactritoids)	N/A	✓					Mapes, 1979
1985	Shell, cirri, hood, buccal mass and appendages regeneration	*Nautilus pompilius* (Linnaeus, 1758)		✓				Arnold, 1985
1986	Shell repair in fossil records (Ammonoids)	N/A	✓					Landman and Waage, 1986
1988	Shell repair	*Nautilus pompilius* (Linnaeus, 1758)		✓				Tanabe et al., 1988
1989	Shell repair in fossil records (Ammonoids)	N/A	✓					Bond and Saunders, 1989
1991	Shell repair	*Nautilus* sp. (Linnaeus, 1758)		✓				Saunders et al., 1991
1991	Cuttlebone regeneration in *Sepia officinalis*	*Sepia officinalis* (Linnaeus, 1758)			✓			von Boletzky and Overath, 1991
1993	Shell repair	*Argonauta* sp. (Linnaeus, 1758)					✓	Trego, 1993
1993	Shell repair in fossil records (Ammonoids)	N/A	✓					Kakabadzé and Sharikadzé, 1993
1997	Shell repair in *Nautilus scrobiculatus* and in fossil records (Ammonoids)	*Allonautilus scrobiculatus* (Lightfoot, 1786)	✓	✓				Landman and Lane, 1997
1998	Shell repair in fossil records (Ammonoids)	N/A	✓					Keupp, 1998
2002	Shell repair in fossil records (Ammonoids)	N/A	✓					Morard, 2002
2002	Shell repair in fossil records (Ammonoids)	N/A	✓					Kröger, 2002b
2002	Shell repair in fossil records (Ammonoids)	N/A	✓					Kröger, 2002a
2003	Shell repair in *Nautilus sp.* and in fossil records (Ammonoids)	N/A	✓	✓				Mapes and Chaffin, 2003
2003	Cuttlebone repair in *Sepia orbignyana*	*Sepia orbignyana* Férussac [in d'Orbigny], 1826			✓			Bello and Paparella, 2003
2004	Shell repair in fossil records (Nautiloids)	N/A	✓					Kröger and Keupp, 2004
2004	Shell repair in fossil records (Nautiloids)	N/A	✓					Kröger, 2004

(Continued)

TABLE 1 | Continued

Main Topic Year of publication	General description	Species	Fossil record	Nautilus	Cuttlefish	Squid	Octopus	References
2005	Shell repair in fossil records (Belemnites)	N/A	✓					Mietchen et al., 2005
2006	Shell repair in fossil records (Ammonoids)	N/A	✓					Keupp, 2006
2007	Shell repair in fossil records (Ammonoids, Nautiloids, Bactritoids)	N/A	✓					Klug, 2007
2010	Shell repair	*Nautilus* sp. (Linnaeus, 1758)		✓				Saunders et al., 2010
2010	Shell repair in fossil records (Ammonoids)	N/A	✓					Zato, 2010
2011	Shell repair in fossil records (Ammonoids)	N/A	✓					Slotta et al., 2011
2011	Shell repair in fossil records (Endocerids)	N/A	✓					Kröger, 2011
2012	Shell repair	*Nautilus pompilius* (Linnaeus, 1758)		✓				Tsujino and Shigeta, 2012
2013	Shell repair	*Nautilus pompilius* (Linnaeus, 1758)		✓				Yomogida and Wani, 2013
2013	Shell repair in fossil records (Ammonoids)	N/A	✓					Odunze and Mapes, 2013
2015	Shell repair in fossil records (Ammonoids)	N/A	✓					Hoffmann and Keupp, 2015
OTHER								
1881	Sucker, arm and tentacle regeneration in *Loligo pealeii* and *Ommastrephes illecebrosus*	*Doryteuthis (Amerigo) pealeii* (Lesueur, 1821); *Illex illecebrosus* (LeSueur, 1821)				✓		Verrill, 1881
1933	Sucker regeneration	*Octopus vulgaris* (Cuvier, 1797)					✓	May, 1933
1964	Branchial gland and branchial heart healing	*Octopus vulgaris* (Cuvier, 1797)					✓	Taki, 1964
1981	Cornea regeneration in *Octopus dofleini* and *O. vulgaris*	*Enteroctopus dofleini* (Wülker, 1910); *Octopus vulgaris* (Cuvier, 1797)					✓	Dingerkus and Santoro, 1981
1985	Shell, cirri, hood, buccal mass and appendages regeneration	*Nautilus pompilius* (Linnaeus, 1758)		✓				Arnold, 1985
2000	Muscle repair in fossil records (Ammonoids)	N/A	✓					Keupp, 2000
2003	Arm regeneration and arm-tip light organs regeneration	*Vampyroteuthis infernalis* (Chun, 1903)					✓	Robison et al., 2003
2008	Jaw repair	*Nautilus belauensis* (Saunders, 1981); *Nautilus macromphalus* (G.B. Sowerby II, 1849); *Nautilus pompilius* (Linnaeus, 1758); *Allonautilus scrobiculatus* (Lightfoot, 1786)		✓				Kruta and Landman, 2008
2011	Chromatophore re-growth during fin regeneration	*Sepia officinalis* (Linnaeus, 1758)			✓			Yacob et al., 2011
2017	Muscle regenerative potential in cephalopods	N/A			✓	✓	✓	Zullo et al., 2017

A total of 119 studies are included in this list organized by topic (e.g., wound healing; arm abnormalities, autotomy and regeneration; hectocotylus regeneration; tentacle autotomy and regeneration; nerve regeneration; shell repair and regeneration) and chronological order. For each study, we provide a general description based on the topic and indicate the taxon (including fossil record) and the species that has been subject of the work. The taxonomy has been revised following WoRMS (World Register of Marine Species, http://www.marinespecies.org/index.php) whenever the case, and reported as in the original study (General Description) when the species differ from the currently accepted taxonomic nomenclature. In a few cases some species are indicated as nomen dubium (a species name is of uncertain taxonomic significance, no type and original description very vague) and taxon inquirendum (when the taxonomic validity is uncertain or disputed by different experts). In the table we do not include the work by Young on the anatomy of the nervous system of Octopus vulgaris, that provide description of regeneration occurring in the "central nervous system" in various regions of cephalopods' brain. N/A, not available.

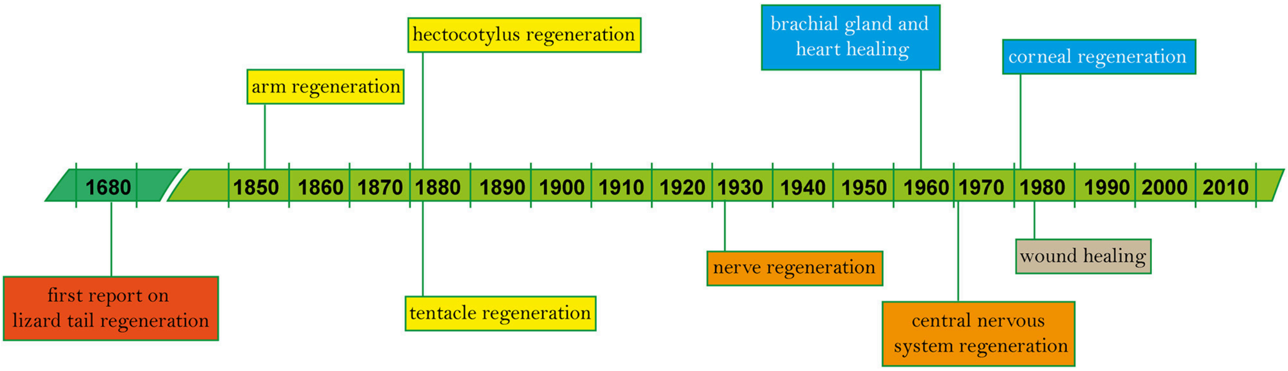


FIGURE 2 | Timeline of regeneration in cephalopods. Since the first known records on vertebrate regeneration (i.e., lizard tail) by Thevenot in 1686 and Perrault account, 2 years later (Dinsmore, 1991), main findings on tissue regeneration in cephalopods based on published works are highlighted in chronological order (see **Table 1** for the complete list).

Within an hour, the wound surface appears to be covered by dense amorphous eosinophilic material containing necrotic fibroblasts, which increase in number 3 h after lesioning. At this time, contraction of the adjacent skin continues, significantly reducing the size of the wound. About 5 h after injury, hemocytes proliferate at the site of the wound through diapedesis, accelerating at about 12 h post-lesion. This acceleration co-occurs with swelling of the central area of the wound, which is also exacerbated by migration of epidermal cells to the wound site (Polglase et al., 1983).

The following day, hemocytes penetrate deeper into the wound and transform from their classical round shape to a fusiform one. These cells eventually cover the entire wound, forming a dermal plug at about 30 h post-lesion, aided by inward migration of the epidermis surrounding the injury. This epidermal migration, which becomes extremely evident at 2 days post-lesion, is made possible by penetration of cells through the hemocyte plug (Polglase et al., 1983).

An increase in cellular organization is then observed at 3–4 days post injury. Notably, hemocytes assume the fibroblast cell type appearance. During this period, the size of the wound continues to shrink, with the closure usually completed after 5 days. Return to the normal morphology of the epidermis, however, was only achieved at least 50 days post-lesion, and slow, continuous contraction of the wound occurs at up to 150 days post-lesion (Polglase et al., 1983).

The existence of fatal ulcerative lesions in some laboratory-reared octopus species (Polglase, 1980; Hanlon et al., 1984) has led scientists to question the efficacy of the healing process in the presence of pathogens (Bullock et al., 1987). Bacterial infection appears to inhibit muscular contractions of the wound at early stages, as well as induce a greater response in hemocytes.

Normally, hemocytes are actively involved in the removal of necrotic tissue from the wound and in the formation of additional amorphous layers (usually one or two) that create supplementary barriers to protect healthy tissue. However, when pathogens are present, these blood cells often appear to be necrotic and to exhibit cytoplasmic granulation, especially when they are in close proximity to bacteria. The observed cell necrosis is thought to be induced by toxins produced by the pathogens. Even when bacterial activity at the wound site is no longer observable a few days after injury, epidermal migration can still be delayed, resulting in incomplete closure of the wound up to 7 days later.

Wound Healing of Appendages After Amputation

The aforementioned process of wound repair also characterizes the first phases of regeneration after arm damage (see for example studies in: Lange, 1920; Féral, 1977, 1978, 1979, 1988; Fiorito et al., 2014; Zullo et al., 2017) and determines the course of repair that follows (Féral, 1988). This process was first reported by Lange (1920) in several species, and then several years later by Féral (1978, 1979, 1988) in *Sepia officinalis*. More recently, Shaw et al. (2016) described the process as it occurs in *Octopus vulgaris*.

These studies have identified several variables that affect the speed of healing, including temperature, relative position of the injury (i.e., distal portion of the arm versus proximal), species, animal age, body size, and health status of an individual, among others.

Although several studies have demonstrated that the healing of a damaged arm requires at least 24 h, the timing is highly variable; some wounds may show little or no healing even after 30 h (Lange, 1920). Complete healing of an arm in *S. officinalis* requires about 5 days at temperatures between 14 and 19°C, and up to 2 weeks at 10°C (Féral, 1988). Interestingly Shaw et al. (2016) found that time of healing might also depend on "innate" differences in self-regenerative capacity. In comparing two sub-populations of *O. vulgaris*, one was found to heal significantly faster than the other. Six-hours after arm injury, the "fast" healers exhibited 80% coverage of their wound, while only 50–60% coverage was noted in the second group of animals.

Lange observed that immediately after a lesion to an arm, no bleeding occurs (1920). The edges of the wound, consisting mainly of dermal connective tissue, begin contracting around the lesion, as occurs in damage to skin on other parts of the body

(Polglase et al., 1983). Only the most external part of the wound is covered, leaving the central area of the injury exposed and the axial nerve protruding from the wound in the most severe cases. Transverse muscle degeneration is also evident soon after injury.

A few hours after lesion (ca. 5 or 6 h), blood enters and covers the wound, forming a blood clot which is later resorbed. Blood corpuscles also rush to the lesion and transform from the spherical circulating-type to a spindle shape. They also appear to undergo division, as the total number present at the wound site increases with time (Lange, 1920), although no mitosis is detected, suggesting that proliferation is occurring through direct or amitotic division (see below). These cells form cicatricial tissue, which creates an initial barrier to the external environment.

Later, the epithelium begins to regenerate through morphallaxis as old material rearranges itself, covering the cicatricial tissue, which is retained underneath. This structure, called the "primary blastema," is thought to be involved in supplying material for the regenerating connective tissue. Epithelial cells, after covering the entire wound, then change their shape from flat to cubic and initiate nuclear (and possibly amitotic) division (Lange, 1920).

In subsequent studies, Féral (1988) investigated the role of two types of fibrous material in the wound healing process of *S. officinalis*. A first type was identified as covering the nerve cord and muscles and forming a network between amoebocytes (i.e., hemocytes) in the scar tissue. A second type, made of collagen fibers, appears in the hypodermis. Agglutinated amoebocytes form scar tissue which is eventually infiltrated by collagen fibers that reinforce the scar and are probably produced by the blastema. A maximum amount of collagen is reported at 48 h after amputation, followed by a decrease to the initial levels at the end of the cicatrization phase. However, this process varies depending on temperature.

Almost a century after the first study by Lange, Shaw et al. (2016) investigated the process of regeneration in *O. vulgaris*. These authors suggest that muscle cells also contribute to the formation of the plug covering the wound, as well as the previously-described actions of hemocytes.

Along with the constructive processes initiated by hemocytes and muscle cells, destructive processes (i.e., cell death) of damaged tissues is also observed in the skin, muscles and nerve cells within the first 6 h after injury.

REGENERATION OF CEPHALOPOD BODY PARTS

Appendages

Cephalopod appendages (i.e., arms and tentacles) are extremely flexible muscular hydrostats lacking fluid-filled cavities (a hydrostatic skeleton is characteristic of many other invertebrates) and hard skeletal supports (review in: Kier and Smith, 1985; Kier, 2016). Each arm is composed of a nerve cord running along the central axis of the appendage, surrounded by three muscle bundles (transverse, longitudinal and oblique) each perpendicular to each other (see description in Margheri et al., 2011).

Arm damage seems to be a common occurrence among cephalopods in the wild (e.g., Steenstrup, 1856; Brock, 1887; Bush, 2006, 2012). For example, Florini et al. (2011) found that 51% of *O. vulgaris* collected from fishermen in the Bay of Naples (Italy) showed damage to one or more arms; Voight (1992) observed similar degrees of damage in 26% of *O. digueti* (from Cholla Bay, Mexico). In both species, dorsal arms appeared to be more affected than ventral ones. It is also notable that in *Abdopus* sp., where sneaker mating tactics are observed among small males, the frequency of arm loss in sneaker males was found to be 100%, compared to 25% in the males mate-guarding a female (population mean = 37%; Wada, 2017).

Although the ability of cephalopods to survive arm and tentacle loss has been known since antiquity (see accounts in *Historia Animalium*; Aristotle, 1910), the first paper formally describing arm regeneration in cephalopods dates back to the mid nineteenth century, when Steenstrup described the main structural features of the arms, including "sexual" appendages and their specialization (i.e., hectocotylus) in some cephalopod species, and focused in particular on the ability of octopods to regenerate arms lost during copulation, injured or bitten off by predators (Steenstrup, 1856, 1857). Streenstrup considered decapods (cuttlefish and squid) to be incapable of re-growing lost appendages, maintaining only a capacity for wound healing. This was confirmed in a later study (Brock, 1887).

Nevertheless, decapods are not completely lacking in regenerative ability; Verrill (1881) observed regenerating suckers in some species of squid (e.g., *Loligo pealei* and *Ommastrephes illecebrosus*; see **Table 1**).

The nineteenth century was characterized by the discovery of many new cephalopod species, a large proportion of which were found to possess regenerative abilities (Verrill, 1881; Brock, 1887; Riggenbach, 1901), including abnormalities such as "arm dichotomy," i.e., bifurcation (Appellof, 1893; Parona, 1900; Hanko, 1913). Most accounts were merely descriptive, lacking any experimental investigation.

At the beginning of the twentieth century, Lange initiated a detailed investigation of arm regeneration in three cephalopod species—*S. officinalis, O. vulgaris*, and *Eledone moschata*—employing both macroscopic observations and histological analysis (Lange, 1920). Her work was based on specimens kept at the Stazione Zoologica (Naples, Italy) as well as at Musee Oceanographie (Monaco) and inspired and guided by Carl Chun and Johann Georg Grimpe, who also provided guidance on the standardization of animal care (Grimpe, 1928). At that time at the Stazione Zoologica, the classical scientific illustration provided examples of the phenomenon originally described by Riggenbach (1901; see also **Figure 3**) that clearly inspired Lange's study.

Lange's work divided the process of arm regeneration into three stages: wound healing (previously described, see above), tissue degeneration and renewal. Her study also established that: (i) the whole process of arm regeneration occurs through morphallaxis, i.e., existing tissues are rearranged and then regenerated into new tissues (except for dermal connective tissue); (ii) cell proliferation seems to occur through amitotic

FIGURE 3 | Regenerating arm in octopus. A scientific drawing of (possibly) *Octopus vulgaris* showing the first right arm regenerating after a lesion that occurred in the most proximal part of the arm. The stump shows a big sucker and a regenerating tip. The third left arm shows the apparent effect of an injury, as it is much shorter and thinner than the other arms. Drawing is a gift to the Association for Cephalopod Research - CephRes from a private collection.

division, since mitosis was never observed; (iii) cuttlefish are indeed capable of regenerating lost appendages, contrary to Steenstrup's earlier assertion that decapods lack regenerative ability, but this is thought to occur via "compensatory regulation," i.e., development of a rudimentary buccal arm rather than actual regeneration of the lesioned arm; (iv) the arm tip, which Lange considered to be made of tissue at an undifferentiated embryonic stage, requires less time to regenerate and form the embryonic blastema than that required if the lesion is made at the base of an arm, where tissues are more differentiated (Lange, 1920).

Immediately after an arm lesion, muscles close to the wound begin degenerating, with the sarcoplasm breaking down and the spiral fibers apparently growing thicker. As degeneration advances, fibers begin losing their cylindrical shape becoming a "clotty mass." During this process, muscle nuclei change shape, becoming round and later fragmenting into two or three particles.

These fragments are then absorbed by the corpuscles that migrate from the blood clot to the muscles. Muscle regeneration is characterized by the appearance of large cells containing little protoplasm and one large nucleus. These are likely to be sarcoblasts originating from the area where muscles tissue has degenerated. In Lange's view, sarcoblasts are the only possible source of muscle fibers (Lange, 1920). Later, they move to the most distal part of the wound and collaborate with neuroblasts in the formation of the second blastema, increasing their number by mitosis.

Twelve to fourteen days are required for sarcoblasts to differentiate into muscle fibers, with the longitudinal ones being the first to begin this process close to the perimuscular connective-tissue membrane. Transverse muscles seem to require more time. Proliferation of the central muscle bundle dictates the production of sucker muscles, which also involves sarcoblasts, this time arranged in two parallel layers around the cavity of the forming sucker, and later developing into radiating and circular muscles.

Degeneration of the nerve cord also begins soon after lesioning and proceeds quite quickly, starting from the nuclei of the layer of ganglion cells. Waste from the nucleus usually disappears quickly, but some persists. Degeneration also involves glial cells whose nuclei shrink while fibers of the myelin cord swell, with degeneration being more marked and pronounced than in the ganglia layer and neuropil.

Around 10 h after surgery, the number of nuclei increases in the neuropil and in the myelin cord due to the migration of blood corpuscles and amitotic division of the glia nuclei. One or two days after lesioning, neuroblasts appear in the neuropil, later migrating to the distal part of the stump to form the second blastema. The source of these neuroblasts is thought to be either glial cells or small nerve cells (Lange, 1920).

Next, well-differentiated fibers of the myelin cords grow into the second blastema separating neuroblasts from sarcoblasts, producing neuropil fibers.

More time is required for neuroblasts to form ganglion cells, protoplasm, and fibers. An axial nerve requires 3 weeks to fully develop, while large ganglia probably appear very late. The axial nerve tends to occupy the majority of the regenerating stump, while in a normal arm, it occupies a quarter of the total volume.

Lange was not able to identify sucker ganglia or the four nerve cords in the muscles of the regenerating tissue, nor was any data on the regeneration of the vascular system available at the time of the study (Lange, 1920).

From a macroscopic point of view, Lange (1920) highlighted the involvement of the two suckers closest to the lesion. Soon after lesioning, they assume an abnormal position which helps in closing the wound. This position is retained for at least 2 or 3 days, and even up to some weeks before the suckers return to their initial location. When this occurs, a little knob is observed near the external part of the regenerated portion of the arm, while in the interior of the knob, a groove is formed. It is from this groove that suckers later regenerate, initially in a single row (all species), and later in paired rows (*O. vulgaris*), though one sucker remains unpaired. Though sucker re-innervation during arm regeneration was not observed by Lange (1920), May (1933) demonstrated through histological analysis that newly forming suckers attract nervous fibers from the central nervous axis, supporting Cajal's neurotropic theory.

The majority of reports regarding the regeneration of cephalopod appendages have been based on octopods, while published data on decapods remains scarce. Lange (1920) attributed this to both an overall lack of knowledge and the great difficulties associated with, rearing decapods compared to octopods (see accounts in, Lange, 1920; Sereni and Young, 1932; Taki, 1941), as well as a reduced frequency of arm and tentacle mutilation in squids and cuttlefishes (Lange, 1920; Adam, 1937). However, these and other assumptions by of Lange were questioned by Aldrich and Aldrich (1968) who investigated, again macroscopically, a specimen of the giant squid *Architeuthis dux* undergoing tentacle regeneration. They also discussed previous data on the frequency of regenerative phenomena occurring in decapods (at least in *Loligo pealei*, *Illex illecebrosus, A. dux,* and *Architeuthis harveyi*) which suggested that Lange underestimated the phenomenon (1920). While not completely refuting the hypothesis of "compensatory regulation," the authors go so far as to suggest that Lange's theory might have stemmed from a misinterpretation of arm or tentacle dimorphism (Aldrich and Aldrich, 1968).

It was only at the end of the 1970s, with improvements in breeding conditions for *S. officinalis,* that Jean-Pierre Féral was able to perform detailed studies of the process of arm regeneration in this species. Complete arm regeneration and functional recovery was achieved after 2–3 months (at 16°C) following experimental lesion to young cuttlefish. Regenerative capacity was dependent on age, physiological state and water temperature, with adults exhibiting diminished or no regenerative capacity after wound healing during late autumn or winter, particularly when seawater temperatures dropped below 14°C (Féral, 1978, 1979).

Féral identified six stages of regeneration by morphology (**Figure 4**) based on histological and cytological analyses (Féral, 1978; 1979). Those findings largely concur with Lange's observations of octopus arm regeneration. The six stages are summarized below:

> ***Stage 1*** *(from surgery to day-7)****:*** characterized by the protrusion of the central nervous axis and contraction of the wound's edge. A few hours after lesioning, one or two suckers adjacent to the lesion move forward; they assume their normal position only 2 or 3 days later. Five to seven days are required for the epidermis to completely cover the wound.
> ***Stage 2*** *(day 5 to 14)*: due to swelling of the scar at the level of the nervous axis, a bud-shaped hemisphere appears at the injury site.
> ***Stage 3*** *(day 10 to 21)*: characterized by the development of the regenerating tissue into a conical shape.
> ***Stage 4*** *(day 17 to 25):* rough suckers appear first on the ventral side of the stump closest to the lesion and then on the regenerating tissue.
> ***Stage 5*** *(day 25 to 35)*: chromatophores gradually appear within the regenerating tissue.
> ***Stage 6*** *(beyond day 30)*: The regenerated arm regains its functionality. It becomes thicker, the new suckers gain function, and chromatophores increase in number, growing larger and darker.

Based on the histological and cytological events occurring during arm regeneration in the cuttlefish, the following three phases were identified by Féral (1978, 1979); see **Figure 4**:

> **Phase I** (corresponding to Stage 1, above): characterized by wound healing, degeneration of nerve cords, muscles, and blood vessels, as well as cell de-differentiation.
> **Phase II** (corresponding to the end of Stage 1, Stage 2, and part of Stage 3; from day 5 to 20): represents the starting point of regeneration, during which blastema formation, cellular activation and growth of the regenerating tissue occurs.

The blastema is composed of de-differentiated cells which increase in number during the first 10 days, though no mitotic event is evident. At a certain point, cells activate and begin changing their appearance. Growth of the regenerating tissue starts at this point. Nervous fibers infiltrate the blastema and mitosis starts at the end of the second week.

The brachial artery penetrates the blastema along with the axial nerve cord. The epidermis appears to be multilayered, but by the end of the third week, it is again composed of a single layer.

> **Phase III** (corresponding to the end of Stage 3, and to stages 4, 5 and recovery of function: from day 20 to the third month): in this phase, the arm is observed to re-grow and cell differentiation occurs. Indeed, after the third week, mitotic events appear to wane and cells begin to differentiate in a concentric field around the nerve cord. The nervous system is the first tissue to differentiate: extending fibers of the cerebro-brachial tract penetrate into the blastema; later, the ganglionic layer formed by dividing neuroblasts appears. During this phase, putative glial cells support nerve fibers during regeneration. At around day 20, they proliferate and follow the axons' progress.

The axial nervous system, the brachial artery, and "epineuraux" (Féral, 1979) muscles differentiate jointly. Intrinsic longitudinal muscles become visible on the 20th day, together with the collagen that protects them from the outside environment. Later, extrinsic longitudinal muscles appear, followed by transverse muscles. Development of the longitudinal muscle bundles occurs through alignment of fusiform myoblasts along belts parallel to the nervous axis. Muscle cells differentiate in muscular fields of the stump. Myofilaments appear immediately and grow slowly between longitudinal muscles and nervous cord. At the beginning of the differentiation phase, while the transverse muscles are limited in thickness and built of myoblasts, the longitudinal muscle cells rely on the presence of myofilaments. During the second month post-lesion, the transverse muscle increase in thickness, with mitotic activity still visible (Féral, 1979).

During the third week, proliferation of the central fascicles induces the formation of sucker (or acetabular) muscles. During invagination of the sucker chambers, muscle cells first form one and then several parallel layers. These cells become the radial muscles and sphincters. Subsequently, acetabulo-branchial muscles also differentiate. Suckers innervation occurs only at later stages, when the suckers are completely formed (at around the 40th day), and they become functional only 3 months after injury.

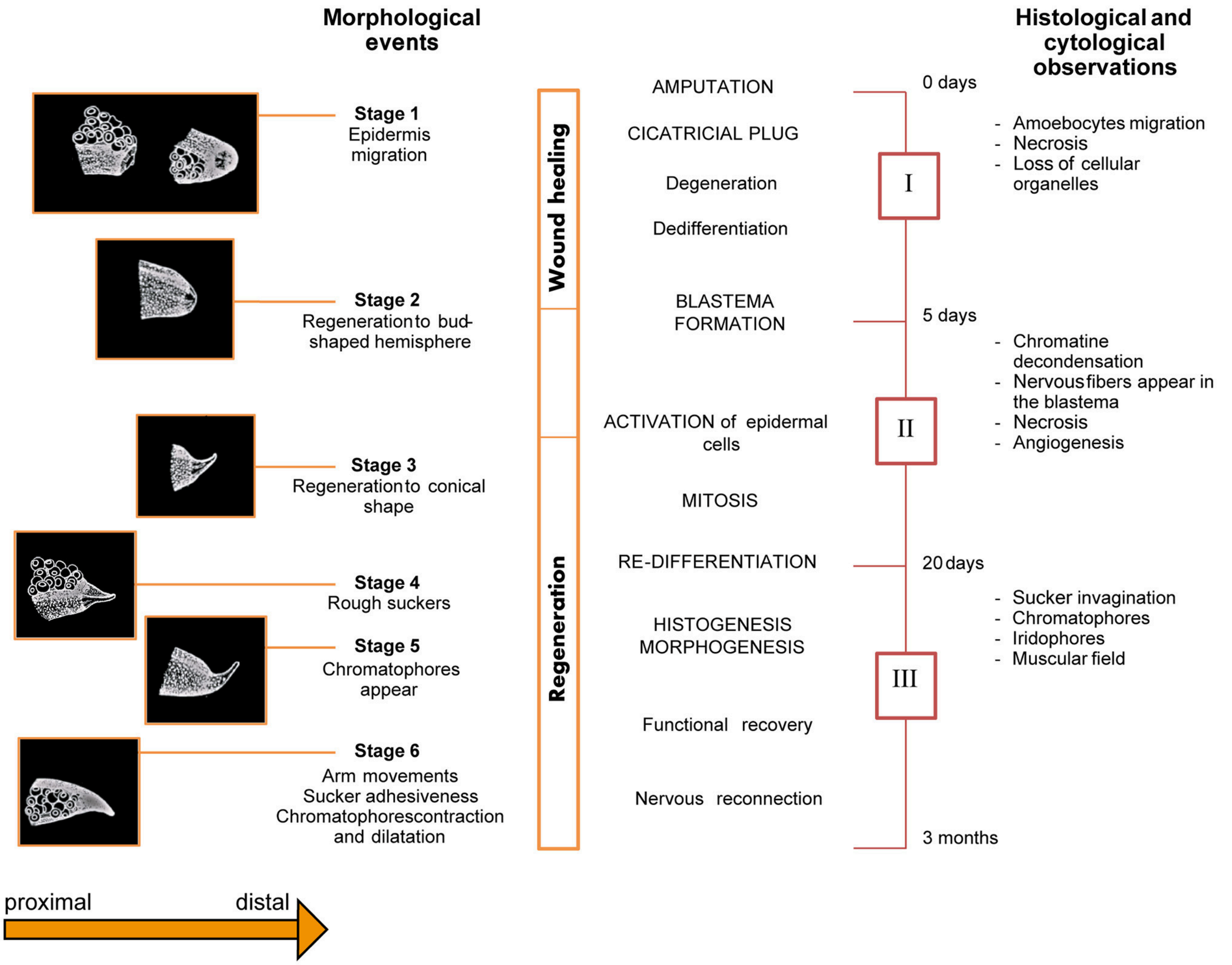


FIGURE 4 | Schematic overview of the series of events occurring during arm regeneration in the cuttlefish. Stages (Left) and phases (Right) of regeneration occurring after arm damage in *Sepia officinalis* are depicted as originally described by Féral. The diagram presents an overview of the morphological (six stages), histological and cytological (three phases) events occurring during arm regeneration. Adapted from Féral (1978, 1979) after permission of CBM - Cahiers de Biologie Marine.

Chromatophores are identifiable among the fibroblasts before they actually appear on the skin due to the presence of pigmented grains. At around day 20, the cells of the dermis differentiate, apparently originating from hemocytes. Iridophores appear some days later (day 25 to−27) and initially, they are positioned without a specific orientation. Later, they arrange themselves in parallel to each other.

The radial muscles of the chromatophores differentiate when the muscles form; however, their innervation occurs later. Indeed, fibers from the median nervous axis start growing at the end of the third week, even though the complete innervation of chromatophores and iridophores does not occur until between the second and third month after lesioning. The basal membrane of the epithelial cells appears at the moment of differentiation; it folds to form the initial structure of the suckers and then invaginates to form the suction and adherent chambers. This occurs along with the penetration of the brachial vein into the regenerating tissue (Féral, 1979).

Amoebocytes are the only cells that travel to the lesion from other parts of the body. However, when this migration stops, the number of cells forming the blastema continues to increase, despite the fact that no mitotic events can be observed. Instead, this appears to be due to local cellular reorganization. Within the lesion, damaged cells degenerate and are removed, while others de-differentiate, losing their particular features and becoming a source of regenerative cells.

After de-differentiation, muscle and nervous cells appear to be capable only of differentiating into the original cell type, whereas connective tissue cells may originate from either fibrocytes or amoebocytes (Féral, 1979).

Féral compared his results with Lange's observations and proposed that the same stages occur in all three species examined, i.e., *S. officinalis*, *Sepiola atlantica* and *O. vulgaris*. Specifically wound healing corresponds to **stage 1**; blastema formation and early growth **to stage 2**; later growth to **stage 3**; differentiation and morphogenesis **to stage 4 and 5**; and functional recovery **to stage 6** (Lange, 1920; Féral, 1977, 1979).

At the beginning of this century, interest in the ability of cephalopods to regenerate appendages has been rekindled (e.g., Rohrbach and Schmidtberg, 2006; Fossati et al., 2011, 2013, 2015; Tressler et al., 2014; Imperadore et al., 2017; Zullo et al., 2017). Recent studies largely confirm with the results obtained by Lange and Féral, albeit with some differences, particularly with regard to the timing of each stage.

Tentacle regeneration in *S. officinalis* has been shown to proceed via the same six stages as arm regeneration in the same species, although with a delay in sucker regrowth. In this instance, sucker regeneration in adults appears to proceed through the same steps of sucker formation as cuttlefish embryos, with the process again delayed by comparison (Rohrbach and Schmidtberg, 2006).

A similar process was also proposed for *O. vulgaris* during the study of arm development in embryos (Nödl et al., 2015). Apparently, both development and regeneration of the arm involve similar steps, including "a shift from an early isotropic, mesenchymal cell proliferation to a distally regionalized cell division pattern, as well as the formation of suckers as a single row of rounded papillae" (Nödl et al., 2015, p. 14).

Impairment of function after arm amputation in cuttlefish (*S. officinalis* and *Sepia pharaonis*) has only been reported by Tressler et al. (2014). Indeed, soon after an arm is injured, the motions associated with swimming, prey manipulation and posture are altered. Recovery of function occurs a few days later, long before complete regeneration of the arm, which is reported to require about 40 days. The stages of regeneration appear to be similar to those reported by Lange and Féral, with some differences in the length of each stage. This, as well as other variations in the timing of regeneration stages observed in these studies could be attributed to several factors, including differences in animal age, diet, rearing conditions, water temperature, surgical procedures or anesthesia employed.

Fossati et al. (2013, 2015) describe the morphological changes involved in arm regeneration in *O. vulgaris*, with a focus on the involvement of the enzyme acetylcholinesterase (AchE). The authors found that AchE expression decreases during wound healing, when proliferation activity is intense and rises again above basal level at 3–4 weeks post-lesion. Another decrease is observed 42 days after damage, with a return to basal level 130 days later, when all structures have been regenerated. AchE appears to have a similar expression pattern during regeneration and arm development, suggesting the involvement of this enzyme in functions other than classical synaptic transmission, such as tissue morphogenesis (Fossati et al., 2013, 2015).

Regeneration of the Male Cephalopod's "sexual" Arm

The hectocotylus is the differentiated-specialized extremity of the "sexual" arm of a male cephalopod. This structure was studied by Sereni (1929a, 1932) who investigated the possibility that a sex hormone controls regeneration of this specialized arm. To answer this question, specimens of *O. vulgaris* were castrated and then had either the hectocotylus tip (males) or the corresponding arm tip (females) removed (Callan, 1940). Complete regeneration of the original structures was observed in both sexes suggesting that the regeneration of both sexual and non-sexual arms do not rely on hormone secretions of the reproductive system. These findings were later confirmed by Taki (1944).

Regeneration of the "sexual arm" was also investigated in later studies.

For example, O'Dor and Wells (1978), induced gonadotropin release by the optic gland, thus forcing sexual maturation of *O. vulgaris* individuals, after which arm-cropping was performed. It was found that in general, faster-maturing octopus of both sexes regenerate their arms more slowly than control animals and, more importantly, that hectocotylized arms regenerate faster than the other arms on the same animal.

In addition, the hectocotylus seems to be less susceptible to injury in the first place in comparison to other arms (Steenstrup, 1857; Bello, 1995). Indeed, some cephalopod species are known to hold this arm close to the body while foraging, presumably to reduce the chances of injury. More rapid regeneration and protection of this specialized arm appear to be due to its importance in mating and reproduction (Huffard et al., 2008). There is even a striking case of a specimen of *Abdopus* sp. which had lost all its arms except the hectocotylus (Wada, 2017).

Regeneration Events in the Cephalopod Central Nervous System

Information regarding the ability of cephalopods to regenerate central nervous tissue is provided by the definitive work of John Z. Young and his co-workers (summarized in Young, 1971). Many experiments involving the removal or lesioning of specific areas of the brain of *O. vulgaris* were carried out with the aim of evaluating subsequent impairment in learning capabilities. In reporting these experiments, Young described the formation of scar tissue above the surface of the brain after removal of a specific brain lobe. He also identified regenerating nerve fibers 34 days after surgery. According to Young, some of these fibers originate from the optic tract, while others from other areas such as the cerebral tract and the palliovisceral system. Regenerating nerve fibers were also identified four days after removal of the subvertical lobe and 16–29 days after bilateral section of the optic tracts.

The distances traveled by the regenerating fibers in the central nervous system of the octopus seem quite remarkable, and further investigation is required to confirm and better describe the phenomenon of neural rewiring. To the best of our knowledge, Young's are, unfortunately, the only available accounts of nerve fiber regeneration in the central nervous system of cephalopods.

Pallial and Stellar Nerves

Fredericq (1878) first discovered and described the "phenotypic" effect of transecting one of the two pallial nerves while studying *O. vulgaris* physiology. This pair of nerves connects the brain to the periphery (i.e., the mantle) through the stellate ganglia. Each ganglion gives rise to 25–40 stellar nerves which innervate chromatophores and respiratory muscles in the mantle. Fredericq observed complete paralysis of these muscles and paling of the skin due to the effect of denervation of chromatophores on the mantle, ipsilateral to the lesion. Lesioning of both nerves led to the death of the animal, due to blockage of respiratory movements (Fredericq, 1878).

Many years later, Sereni (1929b) and Young (1929) conducted a series of systematic observations of the consequences of the transecting the pallial and stellar nerves in *O. vulgaris, Octopus macropus, and E. moschata*, as well as the removal of the entire stellate ganglion. After transection of both pallial and stellar nerves, degeneration of nervous fibers and accumulation of lipid material in the nerve stumps was observed. In addition, clot formation occurred between the two stumps of the lesioned nerve (Young, 1929).

After lesioning of the pallial nerve, structural changes were observed in the cells of the subesophageal mass of the brain, where the majority of the fibers originate, but never in the stellate ganglion. Transection of the stellar nerves demonstrated, instead structural changes of the cells inside the ganglion. No signs of regeneration or restoration of function were detected (Young, 1929). Aside from providing a basis for subsequent and more detailed investigations of regeneration, these studies allowed an initial interpretation of the neural pathways and connections between central and peripheral nervous systems via the pallial nerve in cephalopods.

The proof that these nerves are actually able to regenerate was obtained only in 1932, when more than 200 animals representing seven different cephalopod species (both decapods and octopods) were surveyed in an in-depth investigation of the degenerative and regenerative phenomena occurring after pallial and stellar nerve lesioning (Sereni and Young, 1932; Young, 1932). One of the main findings was that scar tissue, mainly produced by amoebocytes, forms between the transected ends of a nerve, and these cells also infiltrate the two stumps and proliferate amitotically. They have also been observed to actively phagocytose and become filled with granules of fat.

Degeneration of axons is mainly observed in the peripheral stump, which breaks into lumps, whereas closer to the lesion, tip ends swell and later branch. Breaking axons produce large spheres which are probably invaded by amoebocytes and which persist even after functional regeneration occurs. Degenerating spheres are also observed after double sectioning of the pallial nerve on both ends of the isolated nerve portion. Regeneration is visible in the few intact fibers of the peripheral stump, though it is much more evident in the central stump, with a calculated growth rate of between 7 and 18 μm per hour. Fibers are able to grow either through the scar, toward the peripheral stump, or laterally and backwards, without a well-defined direction. From 11 to 18 days post lesion, vigorous regeneration of the peripheral stump is also observed. While this is occurring, connective tissue becomes highly disorganized (mainly in the peripheral stump) with nuclei undergoing changes in shape close to the lesion (Sereni and Young, 1932).

Regarding the effect of lesions on the stellate ganglion, it was observed that retrograde degeneration occurs in ganglion cells if the lesion is performed on stellar nerves, while no effect is visible in these cells if the lesion is performed at the level of the pallial nerve; degeneration of the nerve fibers never seems to extend beyond a synapse (Young, 1932). However, transection of the pallial nerve does result in the filling of the ventral neuropil of the ganglion with fine granules which disappear in about 4–5 days. Degeneration is also observed inside the neuropil and in the dorsal roots of the stellar nerves (probably comprising chromatophore fibers, which do not form synapses in the ganglion). At 7 days post-lesion, the neuropil shrinks, resulting in a reduction in the size of the stellate ganglion. After a stellar nerve lesion, no degeneration of the ventral neuropil occurs, though some takes place in the fibers of the dorsal neuropil of certain axons in the pallial nerve (Young, 1972).

Regenerative and degenerative processes appear to correlate strongly with seawater temperature; the speed of both processes has been observed to increase at higher temperatures (Sereni and Young, 1932; Young, 1972).

During these studies, observations of the behavioral effects of lesions to the skin were also carried out. At first, chromatophore muscles are relaxed and thus appear pale, but then they gradually re-expand, showing the ability to re-establish coloration of the skin again 3–5 days after denervation, in a manner independent of the central nervous system (Sereni, 1929b). A "wave effect" is also sometimes observed; this is due to the hyperexcitability of chromatophores (Sereni, 1929b; Sereni and Young, 1932). This phenomenon was described in greater detail by Packard (1992) who named these waves "wandering clouds," as they propagate randomly over the denervated skin and can last for weeks or even months.

Sereni and Young (1932) observed the first signs of true functional regeneration 65 days post-lesioning, though the majority of the animals required 3–4 months for complete recovery.

Young and his co-workers later focused on the ability of *O. vulgaris* to regain lost function after crushing or cutting one of the pallial nerves (Sanders and Young, 1974). The return of control of color patterning, papillae and mantle muscle contraction was observed over 126 days after surgery by tracking a specific chromatic pattern, the "conflict mottle" (see definition of "Broad Conflict Mottle" as reviewed in Borrelli et al., 2006). This was elicited by placing an animal in a conflict situation, using for example a 10 V shock each time the animal tried to attack a crab prompting uncertainty as to whether to of attack or desist. Eight to ten weeks were required for the complete recovery of pattern production after crushing of the nerve. No animal showed any signs of color pattern recovery until at least 50 days after surgery, in both summer and autumn. Six out of 10 animals recovered the full color pattern (most between 60 and 69 days), while only two out of 10 recovered papillae function (between 30 and 50 days).

When the pallial nerve was cut, only four in 10 animals recovered color patterning, and for these, although some signs of recovery where visible at 30 days, a complete recovery of function required 109 days. By contrast, seven out of 10 animals recovered the ability to raise their papillae. In two of these animals, a 2 cm portion of the nerve was removed during surgery. The skin did not undergo any color changes during the 109 days post-surgery, with chromatophores remaining hyper-excitable and dark spots appearing at random.

Electrical stimulation demonstrated that in these two cases no functional regeneration occurred, while stimulation of the cut pallial nerve after 126 days yielded mantle muscle contractions in three out of three instances, and chromatophore contraction in two out of three instances (in the third instance only a partial response was elicited). Histological analysis of the samples showed pronounced differences in the response of fibers to crushing versus cutting. In the former instance, degenerating axoplasm is confined to the connective tubes and remains visible for months. Fibers were seen to grow in a much more regular fashion compared to crisscrossing of fibers in the cut nerve, despite the fact that in some cases the peripheral stump had been reached. Strikingly, stump-reconnection after cutting often did not lead to functional recovery whereas after crushing it often did. An explanation that has been posited for functional recovery after resection is that the nerve fibers reconnect with their target end-organs. However, the possibility that each individual fiber could both recognize its own specific tube and innervate its original target organ seems quite remote. An alternative possibility is that one axon innervates all the chromatophores of a particular component of the body pattern, rather than just one or a few chromatophores.

Recently, cell proliferation after pallial nerve transection was investigated by Imperadore et al. (2017), who described the mitotic division of circulating hemocytes which migrate to the injury site and continue to proliferate even after infiltrating the stumps. Hemocyte infiltration and proliferation among nerve fibers appears to follow a specific pattern that is correlated with fiber regeneration, suggesting a role for these cells in fostering axonal regrowth. Connective tissue cells also undergo intense proliferation in the nerve, and at 2 weeks post-lesion, these proliferating cells are also positively marked with the neuronal marker NF200, potentially indicating the differentiation of unlabeled stem/progenitor cells (or glial cells). A role for the connective tissue in driving regenerating fibers toward target tissue has also been suggested, resulting in the formation of a spike-like structure in the stump still connected to the brain (Imperadore et al., 2017).

The effect of chromatophore modulation on the skin after denervation was also examined. About 7 days after lesioning, animals at rest are able to produce a homogeneous chromatic pattern on both side of the mantle. Local control exerted by skin receptors was suggested to be involved in the process, as the possibility of target re-innervation can be excluded at such an early stage of regeneration (Imperadore et al., 2017).

Other Tissues and Body Parts: Cornea, Lens, Brachial Gland and Brachial Hearts

There are only two known accounts of a cephalopod surviving and recovering from lesions to the eyes. A brief appendix is presented in Lange (1920), in which there is mention of the effect of lens extirpation. Survival of animals is greatly affected by surgery, though Lange reports that some animals lived for up to 10 weeks post-surgery (Lange, 1920). Soon after injury, these animals lost the ability to perceive light; the faculty was regained 8 weeks later.

Interestingly, there are two reports of rapid corneal regeneration in two species of octopus, *O. vulgaris* and *Enteroctopus dofleini* (Dingerkus and Santoro, 1981). In the case of *E. dofleini,* the damage had occurred in the wild, with one cornea completely missing. Ten days were required to completely regenerate it, and ultimately, the new cornea was indistinguishable from that of contralateral uninjured eye. To further confirm this finding, the same researchers ablated a single cornea in two *O. vulgaris* females and found that they completely regenerated in 9 and 10 days, respectively. Interestingly, regeneration time was similar for the two species even though they were maintained at very different water temperatures (4–7°C for *E. dofleini* and 22°C for *O. vulgaris*).

At the beginning of the twentieth century, many invertebrate researchers focused on the identification of organs with endocrine functions. Sereni (1932); Mitolo (1938) and Taki (1944) initiated such investigations in cephalopods. They focused on the anatomy and function of the branchial gland in particular, uncovering clues that hinted at an endocrine function (Taki, 1964). These studies reported evidence that the gland often presented signs of necrosis in the animals examined, which apparently was the result of a physiological phenomenon, but that the affected area is continuously replaced by regenerating tissue.

The branchial gland and the branchial heart are also subject to anemic infarct, from which they are able to recover via scar-healing orchestrated by amoebocytes. In the words of the Iwao Taki: "The healing of the infarct is due to the amoebocytes which enter the morbid tissue; they first clean the lesion by devouring the residue tissue, and aggregate together to develop a new tissue. The outer part of the healed tissue is crowded by many fibroblasts containing elongate nuclei, while the inner part is formed by a loose parenchymatous tissue consisting of spherical, undifferentiated cells. In a vigorous animal, the healing proceeds in due course and the secretory activity is resumed" (Taki, 1964, p. 390). In addition, if the function of the branchial gland is suppressed, arm regeneration appears greatly delayed, though never completely inhibited (Taki, 1964).

CLOSING REMARKS

Studies conducted over the last 160 years and summarized here demonstrate the incredible regenerative abilities of cephalopods. Species of cuttlefish, squid and octopus all appear capable of recovering the structure and function of a variety of damaged or lost tissues, including appendages, peripheral nerves, the cornea, and even aspects of the central nervous system. Ultimately, the regenerated tissues are indistinguishable from the original structures.

But, despite the fact that great effort has been expended in the exploration of cephalopod regenerative abilities, the underlying molecular and cellular pathways remain largely uncharacterized. The majority of relevant findings are based on histological analysis, with more recent publications reporting mainly macroscopic and microscopic observations.

Though technical limitations continue to impede attempts to understand regenerative abilities in cephalopods, a number of important findings have been obtained nonetheless.

Among these, one of the most important has been establishment of the role of hemocytes, the circulating cellular components that form the basis of the cephalopod immune system (for review see Gerdol et al., 2018), in various phases of the regeneration process (Lange, 1920; Sereni and Young, 1932; Féral, 1978, 1979, 1988; Polglase et al., 1983; Imperadore et al., 2017). Almost all studies of regeneration in cephalopods report the involvement of hemocytes which rush to the site of the lesion to form a scar, and although this tissue forms a protective plug against pathogens, it does not present a physical barrier to regenerative phenomena (Lange, 1920; Polglase et al., 1983; Féral, 1988). Indeed, in the case of an arm wound, this plug contributes to the formation of the so-called primary blastema, thought to supply material for the regenerating stump (Lange, 1920). A scar also forms between the two stumps of a transected pallial or stellar nerve, but as is the case in non-nervous tissue, a regenerating nerve fiber eventually passes through the scar to re-connect with target tissue.

During healing and regeneration, hemocytes are also involved in removing necrotic tissues by actively phagocytizing debris. They also appear to transdifferentiate into other cell types (Lange, 1920; Féral, 1979, 1988; Polglase et al., 1983). It has been suggested that during arm regeneration, new muscles and nervous cells can only originate from dedifferentiated cells of the same type; by contrast, hemocytes are capable of transforming from round to spindle-shaped (Lange, 1920; Féral, 1979, 1988; Polglase et al., 1983) and apparently to differentiate from fibrocytes (Féral, 1979).

It has also been assumed that chromatophores and iridophores in the skin of a regenerating arm are derived through the dedifferentiation of another cell type, most likely hemocytes or fibrocytes. Both of these cell types have the potential to serve either as chromatophores or iridophores due to their position inside the blastema, close to the epidermis. The possibility that cephalopod hemocytes can transdifferentiate into another cell-type has already been proposed by Jullien et al. (1956), whose findings were later confirmed by Féral's work. However, it must be pointed out that these hypotheses are based only on circumstantial evidence and lack any direct confirmation.

The proliferation of hemocytes during regeneration is another common finding of the studies reviewed here. Early investigations attributed this to amitotic division (Lange, 1920; Sereni and Young, 1932), while more recent accounts noted mitotic cell division (Féral, 1979; Imperadore et al., 2017). This ambiguity might be explained by the different approaches employed: early studies were based only on histology and macroscopic observations with some additional microscopic examination, while more recent work, including that of Imperadore et al. (2017), have benefitted from the use of cellular markers.

Amitosis is a process in which cell division results from nuclear restriction, giving rise to two daughter cells that differ from each other and from the parent cell (e.g., Child, 1907a,b,c,d), because no homogenous segregation of chromosomes occurs (see first description in Remak, 1841 cited in: Lucchetta and Ohlstein, 2017). This process appears to be widespread among invertebrates and vertebrates alike, though its actual function remains unexplained. Recently amitosis has been reported to be involved in stem cell replacement during gut regeneration in *Drosophila melanogaster* (Lucchetta and Ohlstein, 2017).

It is probable that both mitosis and amitosis take place during tissue regeneration in cephalopods as two alternative modes of replenishing degrading tissues and as a source of stem or progenitor cells. However, further investigation is required to elucidate the mechanisms involved.

Lens regeneration and cornea repair have been observed in vertebrates such as newts, frogs and salamanders (e.g., Carinato et al., 2000; Henry and Tsonis, 2010; Henry et al., 2012), but the occurrence of cornea regeneration after complete extirpation has so far only been reported in two species of octopus (*O. vulgaris* and *E. dofleini*, Dingerkus and Santoro, 1981). If documented, widespread occurrence of this ability in octopuses would support their use as models of this phenomenon, leading to further insights that might be applicable even to "higher" vertebrates and human medicine.

Peripheral nerve lesions, which cause severe impairment to affected animals, have also been made in cephalopods in order to observe putative regenerative phenomena. After unilateral pallial nerve transection, animals lose control of breathing muscles and chromatophores on the ipsilateral side of the mantle. Wallerian degeneration is observed in the distal stump of the nerve and chromatolysis is detected in brain cells, as happens also in mammals after nerve injury. However, in the case of cephalopods, nerve regeneration begins a few hours after lesioning, and continues until nerves are reconnected to end target tissues and function is completely recovered. A process of differentiation in stem/progenitor cells thought to enable this regeneration, but this remains speculation (Imperadore et al., 2017).

The potential of this molluscan class to enlighten the study of regeneration is clear, and new tools and techniques that have recently been developed should facilitate its study in the near future.

Despite limited availability of tools allowing more advanced genomic/proteomic analyses, gene function inactivation, and cell labeling, to cite some, researchers are committed in establishing new strategies for the study of regeneration in this taxon.

Label-free multiphoton microscopy (Imperadore et al., 2018) and micro-PET imaging (Zullo et al., 2018) have been recently applied to *O. vulgaris* to follow regeneration after pallial nerve transection (Imperadore et al., 2018) and arm regrowth after amputation (Zullo et al., 2018). The two methods appear very promising: multiphoton microscopy does not rely on any specific marker or dye, allowing the detection of structures and cells usually not revealed with classical staining; micro-PET imaging possibly enable detection of proliferating cells in regenerating tissues and might allow, in the next future, *in vivo* and minimally invasive imaging investigations.

The effort in developing alternative methodologies and/or adapting tools to cephalopod research is very promising and require integration of different scientific communities and fields.

AUTHOR CONTRIBUTIONS

PI drafted an earlier version of the manuscript. PI and GF contributed to the final writing of the manuscript.

FUNDING

This work has been supported by the Stazione Zoologica Anton Dohrn (RITMARE Flagship Project - MIUR & SZN to GF), and by the Association for Cephalopod Research CephRes to PI.

ACKNOWLEDGMENTS

The authors are greatly indebted to Drs. Caitlin E. O'Brien and Giovanna Ponte for critical discussions and reading of this manuscript. We thank Dr Christiane Groeben (http://independent.academia.edu/ChristianeGroeben) for her assistance and for transmitting her passion for the history of science. The Library of the Stazione Zoologica Anton Dohrn (Napoli, Italy) and its staff are also acknowledged for their support and for providing access to Zoological Record and all library holdings.

This work was initiated following an oral presentation given by the junior Author at the Regeneration Workshop 2013 which was organized by Association for Cephalopod Research - CephRes (Naples, Italy; September 25th and 26th, 2013).

Finally, the authors recognize the opportunity provided by the COST Action FA1301 – CephsInAction in facilitating cephalopod science networking capabilities and their growth.

REFERENCES

Adam, W. (1937). Notes sur les Céphalopodes. VII. Sur un cas de regeneration chez Sepioteuthis lessoniana Lesson, 1830. *Bulletin de l'Institut Royal des Sciences Naturelles de Belgique* 13:4.

Aldrich, F. A., and Aldrich, M. M. (1968). On regeneration of the tentacular arm of the giant squid Architeuthis dux Steenstrup (Decapoda, Architeuthidae). *Can. J. Zool.* 46, 845–847. doi: 10.1139/z68-120

Alejo-Plata, M. D. C., and Méndez, O. V. (2014). Arm Abnormality in Octopus hubbsorum (Mollusca: Cephalopoda: Octopodidae). *Am. Malacol. Bull.* 32, 217–219. doi: 10.4003/006.032.0212

Appellof, A. (1893). Teuthologische Beitrage. IV. Ueber einen fall von doppelseitiger Hektokotylisation bei Eledone cirrhosa. *Bergen Mus. Aarbog. I*, 15.

Aristotle, I. (1910). *Historia Animalium, english translation by D'Arcy Wenthworth Thompson*. Oxford: Clarendon Press.

Arnold, J. M. (1985). Shell growth, trauma, and repair as an indicator of life history for Nautilus. *Veliger* 27, 386–396.

Barrande, J. (1877). *Céphalopodes: Etudes Générales. Extraits du Système Silurien du Centre de la Bohême.* Prague; Paris: Selbstverl. des Verf.

Bello, G. (1995). Hectocotylus regeneration in wild-caught sepiolid squids. *J. Marine Biol. Assoc. U.K.* 75, 491–494. doi: 10.1017/S0025315400018336

Bello, G., and Paparella, P. (2003). "Scar-bearing cuttlebones in wild-collected Sepia orbignyana (Cephalopoda: Sepiidae) and the effects of scar occurrence on morphometric relationships," in *Coleoid Cephalopods Through Time*, ed K. H. B. S. Warnke K (Berlin: Berliner Paläobiol Abh), 13–18.

Bond, P. N., and Saunders, W. B. (1989). Sublethal injury and shell repair in Upper Mississippian ammonoids. *Paleobiology* 15, 414–428. doi: 10.1017/S0094837300009593

Borrelli, L., Gherardi, F., and Fiorito, G. (2006). *A Catalogue of Body Patterning in Cephalopoda*. Napoli: Stazione Zoologica A. Dohrn; Firenze University Press.

Brewer, R. S., and Seitz, A. C. (2013). A Unique Case of Bilateral Hectocotylization in the North Pacific Giant Octopus (Enteroctopus dofleini). *Malacologia* 56, 297–300. doi: 10.4002/040.056.0216

Brock, J. (1887). Indische Cephalopoden. *Zool. Jahrb* 2, 591–614.

Buckman, S. S. (1891). Notes on Nautili and Ammonites. *Quart. J. Geol. Soc. Lond. XLVII*, 1–165.

Budelmann, B. U. (1998). Autophagy in Octopus. *South Afr. J. Marine Sci.* 20, 101–108. doi: 10.2989/025776198784126502

Bullock, A. M., Polglase, J. L., and Phillips, S. E. (1987). The wound healing and haemocyte response in the skin of the lesser octopus Eledone cirrhosa (Mollusca: Cephalopoda) in the presence of Vibrio tubiashii. *J. Zool.* 211, 373–385. doi: 10.1111/j.1469-7998.1987.tb01540.x

Bush, S. L. (2006). Autotomy as a deep-sea squid defense. *Integr. Comp. Biol.* 46, E19–E19.

Bush, S. L. (2012). Economy of arm autotomy in the mesopelagic squid Octopoteuthis deletron. *Mar. Ecol. Prog. Ser.* 458, 133–140. doi: 10.3354/meps09714

Callan, H. G. (1940). The absence of a sex-hormone controlling regeneration of the hectocotylus in *Octopus vulgaris* Lam. *Pubbl. Staz. Zool. Napoli* 18, 15–19.

Carinato, M. E., Walter, B. E., and Henry, J. J. (2000). Xenopus laevis gelatinase B (Xmmp-9): Development, regeneration, and wound healing. *Develop. Dyn.* 217, 377–387. doi: 10.1002/(SICI)1097-0177(200004)217:4<377::AID-DVDY5>3.0.CO;2-U

Child, C. M. (1907a). Studies on the relation between Amitosis and Mitosis: I. Development of the ovaries and oögenesis in Moniezia. *Biol. Bull.* 12, 89–114. doi: 10.2307/1535772

Child, C. M. (1907b). Studies on the relation between Amitosis and Mitosis: II. Development of the Testes and Spermatogenesis in Moniezia (Continued). *Biol. Bull.* 12, 191–212+214–224.

Child, C. M. (1907c). Studies on the relation between Amitosis and Mitosis: III. Maturation, Fertilization, and Cleavage in Moniezia. *Biol. Bull.* 13, 138–148.

Child, C. M. (1907d). Studies on the relation between Amitosis and Mitosis: IV. Nuclear division in the Somatic structures of the Proglottids of Moniezia. V. General Discussion and Conclusions concerning Amitosis and Mitosis in Moniezia. *Biol. Bull.* 13, 165–184.

Clarke, M. R. (1966). A review of the systematics and ecology of oceanic squids. *Adv. Mar. Biol.* 4, 91–300. doi: 10.1016/S0065-2881(08)60314-4

Dingerkus, G., and Santoro, E. D. (1981). Cornea regeneration in the Pacific giant octopus, Octopus dofleini, and the common octopus *O. vulgaris. Experientia* 37, 368–369. doi: 10.1007/BF01959867

Dinsmore, C. E. (1991). *A History of Regeneration Research: Milestones in the Evolution of a Science.* Cambridge: Cambridge University Press.

Farley, J. (2001). "Steenstrup, J Japetus S," in *eLS* (John Wiley & Sons, Ltd). doi: 10.1038/npg.els.0002383

Féral, J. P. (1977). *La régénération des bras de Sepia officinalis L. (Mollusque, Céphalopode, Coléoïdé).* Thèse Doctorat de Spécialité, Université Pierre et Marie Curie.

Féral, J.-P. (1978). La regeneration des bras de la seiche Sepia officinalis L.(Cephalopoda: Sepioidea) I. Etude morphologique. *Cahiers de Biologie Marine* 19, 355–361.

Féral, J.-P. (1979). Regeneration of the arms of *Sepia officinalis* L. (Cephalopoda, Sepioidea). 2. Histologic and cytologic study. *Cah. Biol. Mar.* 20, 29–42.

Féral, J.-P. (1988). Wound healing after arm amputation in *Sepia officinalis* (Cephalopoda: Sepioidea). *J. Invertebr. Pathol.* 52, 380–388. doi: 10.1016/0022-2011(88)90049-3

Fiorito, G., Affuso, A., Anderson, D. B., Basil, J., Bonnaud, L., Botta, G., et al. (2014). Cephalopods in neuroscience: regulations, research and the 3Rs. *Invert. Neurosci.* 14, 13–36. doi: 10.1007/s10158-013-0165-x

Florini, M., Fiorito, G., Hague, T., and Andrews, P. L. R. (2011). "Monco": a natural model for studying arm usage and regeneration in *Octopus vulgaris*. *J. Shellfish Res.* 30, 1002–1002.

Fossati, S. M., Candiani, S., Nodl, M. T., Maragliano, L., Pennuto, M., Domingues, P., et al. (2015). Identification and expression of Acetylcholinesterase in *Octopus vulgaris* arm development and regeneration: a conserved role for ACHE? *Mol. Neurobiol.* 52, 45–56. doi: 10.1007/s12035-014-8842-2

Fossati, S. M., Carella, F., De Vico, G., Benfenati, F., and Zullo, L. (2013). Octopus arm regeneration: role of acetylcholinesterase during morphological modification. *J. Exp. Mar. Biol. Ecol.* 447, 93–99. doi: 10.1016/j.jembe.2013.02.015

Fossati, S., Carella, F., Benfenati, F., and Zullo, L. (2011). "Octopus arm regeneration and its potential implication in reparative pathways," in *EuroCeph 2011 "Cephalopod BiologyResearch in the 21st Century—A European Perspective"*. eds J.o.S. Research (Vico Equense: National Shellfisheries Association), 1003.

Fredericq, L. (1878). Recherches sur la Physiologie du poulpe commun. *Arch. Zool. Exp.Gén.* 7, 535–583.

Gerdol, M., Gomez-Chiarri, M., Castillo, M., Figueras, A., Fiorito, G., Moreira, R., et al. (2018). "Immunity in molluscs: recognition and effector mechanisms, with a Focus on Bivalvia," in *Advances in Comparative Immunology*, ed E. L. Cooper (Springer), 100–180.

Gleadall, I. G. (1989). An octopus with only seven arms: anatomical details. *J. Molluscan Stud.* 55, 479–487. doi: 10.1093/mollus/55.4.479

González, Á. F., and Guerra, Á. (2008). First observation of a double tentacle bifurcation in cephalopods. *Mar. Biodiv. Records* 1, E44. doi: 10.1017/S175526720600529X

Gordon, M. (1964). *Carboniferous cephalopods of Arkansas*. Washington, DC: United States Geological Survey Professional Paper, 460.

Grimpe, G. (1928). "Pflege, Behandlung und Zucht der Cephalopoden fur zoologische und physiologische Zwecke," in *Handbuch der Biologischen Arbeitsmethoden,* ed E. Äberhalden (Berlin; Wien: Verlag Urban & Schwarzenberg), 331–402.

Guex, J. (1967). Contribution â l'etude des blessures chez les ammonites. *Bull. Lab. Geol. Mineral, Geophys. Mus. Geol. Univ. Lausanne* 165, 1–16.

Hanko, B. (1913). Über den gespaltenen Arm eines *Octopus vulgaris*. *Archiv. Für. Entwicklungsmechanik der Organ.* 37, 217–221. doi: 10.1007/BF02289930

Hanlon, R. T., Forsythe, J. W., Cooper, K. M., Dinuzzo, A. R., Folse, D. S., and Kelly, M. T. (1984). Fatal penetrating skin ulcers in laboratory-reared octopuses. *J. Invertebr. Pathol.* 44, 67–83. doi: 10.1016/0022-2011(84)90047-8

Harms, C. A., Lewbart, G. A., McAlarney, R., Christian, L. S., Geissler, K., and Lemons, C. (2006). Surgical excision of mycotic (*Cladosporium* sp.) granulomas from the mantle of a cuttlefish (*Sepia officinalis*). *J. Zoo Wildl. Med.* 37, 524–530. doi: 10.1638/06-033.1

Haven, N. (1972). The ecology and behavior of *Nautilus pompilius* in the Philippines. *Veliger* 15, 75–80.

Henry, J. J., and Tsonis, P. A. (2010). Molecular and cellular aspects of amphibian lens regeneration. *Prog. Retin. Eye Res.* 29, 543–555. doi: 10.1016/j.preteyeres.2010.07.002

Henry, J. J., Thomas, A. G., Hamilton, P. W., Moore, L., and Perry, K. J. (2012). "Cell signaling pathways in vertebrate lens regeneration," in *New Perspectives in Regeneration,* eds E. Heber-Katz and D. Stocum (Berlin; Heidelberg: Springer), 75–98.

Hielscher, B., Ruth, P., and Boletzky von, S. (1996). Cytobiologische Untersuchungen zu Struktur und Regeneration der Tentakel von *Sepia officinalis* L. *Verhandlungen der Deutschen Zoologischen Gesellschaft* 89:213.

Higashide, R., Sakai, Y., and Hashimoto, H. (2007). On freak minor octopus, Octopus minor, found out in Imabari fish market, Ehime Prefecture. *J. Grad. Sch. Biosphere Sci. Hiroshima Univ.* 46, 15–19.

Hochberg, F. G., Norman, M. D., and Finn, J. (2006). Wunderpus photogenicus n. gen. and sp., a new octopus from the shallow waters of the Indo-Malayan Archipelago (Cephalopoda: Octopodidae). *Molluscan Res.* 26, 128–140.

Hoffmann, R., and Keupp, H. (2015). "Ammonoid Paleopathology," in *Ammonoid Paleobiology: From Anatomy to Ecology*, eds C. Klug, D. Korn, K. De Baets, I. Kruta, and R. H. Mapes (Dordrecht: Springer), 877–926.

Hölder, H. (1973). Miscellanea cephalopodica. Ein belemnitisches Proostrakum aus dem Unteren Lias. *Münstersche Forschungen zur Geologie und Paläontologie* 29, 39–76.

Hölder, H. (1977). Zwei ungewöhnliche Erscheinungsformen anomaler Jura-Ammoniten der forma aegra verticata. *Paläontologische Zeitschrift* 51, 254–257. doi: 10.1007/BF02986573

Huffard, C. L., Caldwell, R. L., and Boneka, F. (2008). Mating behavior of Abdopus aculeatus (d'Orbigny 1834) (Cephalopoda: Octopodidae) in the wild. *Mar. Biol.* 154, 353–362. doi: 10.1007/s00227-008-0930-2

Imperadore, P., Shah, S. B., Makarenkova, H. P., and Fiorito, G. (2017). Nerve degeneration and regeneration in the cephalopod mollusc *Octopus vulgaris*: the case of the pallial nerve. *Sci. Rep.* 7:46564. doi: 10.1038/srep46564

Imperadore, P., Uckermann, O., Galli, R., Steiner, G., Kirsch, M., and Fiorito, G. (2018). Nerve regeneration in the cephalopod mollusc *Octopus vulgaris*: label-free multiphoton microscopy as a tool for investigation. *J. R. Soc. Inter.* 15:20170889. doi: 10.1098/rsif.2017.0889

Jullien, A., Cardot, J., Ripplinger, J., and Claudey, D. (1956). Reactions inflammatoires provoquees experimentalement dans le foie des cephalopodes decapodes. *Comptes Rendus Hebdomadaires des Seances de L'Academie des Sciences* 243, 456–458.

Kakabadzé, M. V., and Sharikadzé, M. Z. (1993). On the mode of life of heteromorph ammonites (heterocone, ancylocone, ptychocone) *Geobios* 26, 209–215 doi: 10.1016/S0016-6995(06)80375-X

Keupp, H. (1976). Neue Beispiele für den Regenerationsmechanismus bei verletzten und kranken Ammoniten. *Paläontologische Zeitschrift* 50, 70–77. doi: 10.1007/BF03001973

Keupp, H. (1977). Paläopathologische Normen bei Amaltheiden (Ammonoidea) des Fränkischen Lias. *Jahrbuch der Coburger Landesstiftung* 263–280.

Keupp, H. (1998). Mundsaumverletzungen bei Pleuroceras (Ammonoidea). *Fossilien* 1, 37–42.

Keupp, H. (2000). Anomale muskelleisten bei ammoniten. *Berliner Geowiss Abh E.* 34, 279–289.

Keupp, H. (2006). Sublethal punctures in body chambers of Mesozoic ammonites (forma aegrafenestra nf), a tool to interpret synecological relationships, particularly predator-prey interactions. *Paläontologische Zeitschrift* 80, 112–123. doi: 10.1007/BF02988971

Kier, W. M. (2016). The musculature of coleoid cephalopod arms and tentacles. *Front. Cell Develop. Biol.* 4:10. doi: 10.3389/fcell.2016.00010

Kier, W. M., and Smith, K. K. (1985). Tongues, tentacles and trunks: the biomechanics of movement in muscular - hydrostats. *Zool. J. Linn. Soc.* 83, 307–324. doi: 10.1111/j.1096-3642.1985.tb01178.x

Klug, C. (2007). Sublethal injuries in Early Devonian cephalopod shells from Morocco. *Acta Palaeontol. Pol.* 52, 749–759.

Kröger, B. (2002a). Antipredatory traits of the ammonoid shell. Indications from Jurassic ammonoids with sublethal injuries. *Paläontologische Zeitschrift* 76, 223–234. doi: 10.1007/BF02989859

Kröger, B. (2002b). On the efficiency of the buoyancy apparatus in ammonoids: evidences from sublethal shell injuries. *Lethaia* 35, 61–70. doi: 10.1111/j.1502-3931.2002.tb00068.x

Kröger, B. (2004). Large shell injuries in Middle Ordovician Orthocerida (Nautiloidea, Cephalopoda). *GFF* 126, 311–316. doi: 10.1080/11035890401263311

Kröger, B. (2011). Size matters–Analysis of shell repair scars in endocerid cephalopods. *Fossil Record* 14, 109–118. doi: 10.5194/fr-14-109-2011

Kröger, B., and Keupp, H. (2004). A paradox survival–report of a repaired syn vivo perforation in a nautiloid phragmocone. *Lethaia* 37, 439–444. doi: 10.1080/00241160410002171

Kruta, I., and Landman, N. H. (2008). Injuries on Nautilus jaws: implications for the function of ammonite aptychi. *Veliger* 50:241.

Kumpf, H. E. (1960). Arm abnormality in Octopus. *Nature* 4709, 334–335. doi: 10.1038/185334a0

Kurosaka, K., Yamashita, H., Ogawa, M., Inada, H., and Arimoto, T. (2012). Tentacle-breakage mechanism for the neon flying squid Ommastrephes bartramii during the jigging capture process. *Fish. Res.* 121, 9–16. doi: 10.1016/j.fishres.2011.12.016

Landman, N. H., and Lane, J. A. (1997). Foldlike irregularities on the shell surface of Late Cretaceous ammonoids. *Am. Mus. Novit.* 3197.

Landman, N. H., and Waage, K. M. (1986). Shell abnormalities in scaphitid ammonites. *Lethaia* 19, 211–224. doi: 10.1111/j.1502-3931.1986.tb00734.x

Lange, M. M. (1920). On the regeneration and finer structure of the arms of the cephalopods. *J. Exper. Zool.* 31, 1–57. doi: 10.1002/jez.1400310102

Lehmann, U. (1975). Über Biologie und Gehäusebau bei Dactylioceras (Ammonoidea) aufgrund einer Fraktur-Analyse. *Mitt. Inst. Geol. Palaeontol. Hamb.* 44, 195–206.

Lo Bianco, S. (1909). Notizie biologiche riguardanti specialmente il periodo di maturità sessuale degli animali del Golfo di Napoli. *Mittheilungen Aus Der Zoologischen Station zu Neapel* 19, 513–763.

Lönnberg, E. (1898). On the cephalopods collected by the Swedish expedition to Tierra del Fuego 1895–96. *Svenska Expeditionen till Magellansländerna* 2, 49–64.

Lucchetta, E. M., and Ohlstein, B. (2017). Amitosis of polyploid cells regenerates functional stem cells in the drosophila intestine. *Cell Stem Cell* 20, 609–620. doi: 10.1016/j.stem.2017.02.012

Mapes, R. H. (1979). *Carboniferous and Permian Bactritoidea (Cephalopoda) in North America*. Lawrence: The University of Kansas Paleontological Institute.

Mapes, R. H., and Chaffin, D. T. (2003). "Predation on cephalopods," in *Predator - Prey Interactions in the Fossil Record*, eds Kelley, H. Patricia, Kowalewski, Michal, Hansen, and A. Thor (Boston, MA: Springer), 177–213.

Margheri, L., Ponte, G., Mazzolai, B., Laschi, C., and Fiorito, G. (2011). Non-invasive study of Octopus vulgaris arm morphology using ultrasound. *J. Exp. Biol.* 214, 3727–3731. doi: 10.1242/jeb.057323

Massy, A. L. (1916). The Cephalopoda of the Indian Museum. *Records Indian Museum* 12, 185–247.

May, R. M. (1933). La formation des terminaisons nerveuses dans les ventouses du bras régénéré du céphalopode *Octopus vulgaris* Lam. *Ann. St. océanogr. Salammbò* 7, 1–15.

Meenakshi, V. R., Martin, A. W., and Wilbur, K. M. (1974). Shell repair in *Nautilus macromphalus*. *Mar. Biol.* 27, 27–35. doi: 10.1007/BF00394757

Mietchen, D., Keupp, H., Manz, B., and Volke, F. (2005). Non-invasive diagnostics in fossils-Magnetic Resonance Imaging of pathological belemnites. *Biogeosciences* 2, 133–140. doi: 10.5194/bg-2-133-2005

Mitolo, M. (1938). Sulla funzione dei corpi branchiali nei cefalopodi. *Arch. Sci. Biol. Napoli* 24, 35–58.

Morard, A. (2002). Post-pathological keel-loss compensation in ammonoid growth. *Lethaia* 35, 21–31. doi: 10.1080/002411602317345830

Müller, D. (1976). Japetus Smith Steenstrup (1813–1897), zoology. *Dict. Sci. Biogr.* 13, 9–10.

Murata, M., Ishii, M., and Osako, M. (1981). On the regeneration of tentacle of the oceanic squid, *Ommastrephes bartrami* (Lesueur) [in northwest Pacific]. *Bull. Hokkaido Reg. Fish. Res. Lab. (Jpn)* 46, 1–14.

Nair, K. P., and Rao, B. N. (1985). "Instances of regeneration in the cuttlefish Sepia pharaonis Ehrenberg and in the squid Loligo duvaucelii Orbigny from Indian waters," in *Cephalopod Bionomics, Fisheries and Resources of the Exclusive Ecomomic zone of India*. (Cochin: Indian Council of Agricultural Research), 160–164.

Nödl, M. T., Fossati, S. M., Domingues, P., Sanchez, F. J., and Zullo, L. (2015). The making of an octopus arm. *Evodevo* 6:19. doi: 10.1186/s13227-015-0012-8

Norman, M. D. (1992). Ameloctopus litoralis, gen. et sp. nov.(Cephalopoda: Octopodidae), a new shallow-water octopus from tropical Australian waters. *Inverteb. Syst.* 6, 567–582. doi: 10.1071/IT9920567

Norman, M. D., and Finn, J. (2001). Revision of the Octopus horridus species-group, including erection of a new subgenus and description of two member species from the Great Barrier Reef, Australia. *Invertebr. Syst.* 15, 13–35. doi: 10.1071/IT99018

O'Dor, R. K., and Wells, M. J. (1978). Reproduction versus somatic growth: hormonal control in *Octopus vulgaris*. *J. Exper. Biol.* 77, 15–31.

Odunze, S., and Mapes, R. H. (2013). Nearly circular, oval and irregular holes in Cretaceous ammonoids from Nigeria. *Lethaia* 46, 409–415. doi: 10.1111/let.12019

Okada, Y. K. (1937). An occurrence of branched arms in the decapod cephalopod, Sepia esculenta Hoyle. *Annot. Zool. Jpn.* 17, 93–94

Okada, Y. K. (1965a). On Japanese octopuses with branched arms, with special reference to their captures from 1884 to 1964. *Proc. Jpn. Acad.* 41, 618–623.

Okada, Y. K. (1965b). Rule of arm-branching in Japanese octopuses with branched arms. *Proc. Jpn. Acad.* 41, 624–629.

Packard, A. (1988). "The skin of cephalopods (coleoids): general and special adaptations," in *The Mollusca*, ed E. R. C. M. R. Trueman (San Diego, CA: Academic Press), 37–67.

Packard, A. (1992). A note on dark waves (wandering clouds) in the skin of *Octopus vulgaris*. *J. Physiol.* 446:40.

Palacio, F. J. (1973). On the double hectocotylization of octopods. *Nautilus* 87, 99–102.

Parona, C. (1900). Sulla Dicotomia delle braccia nei Cefalopodi. *Bollettino dei Musei di Zoologia e Anatomia Comparata Della Reale Università di Genova* 96, 1–7.

Polglase, J. L. (1980). A preliminary report on the thraustochytrid(s) and labyrinthulid(s) associated with a pathological condition in the lesser octopus Eledone cirrhosa. *Bot. Mar.* 23, 699–706. doi: 10.1515/botm-1980-1106

Polglase, J. L., Bullock, A., and Roberts, R. (1983). Wound healing and the haemocyte response in the skin of the Lesser octopus Eledone cirrhosa (Mollusca: Cephalopoda). *J. Zool.* 201, 185–204. doi: 10.1111/j.1469-7998.1983.tb04269.x

Portmann, A. (1952). Les bras dorsaux de Tremoctopus violaceus Delle Chiaje. *Revue Suisse De Zoologie* 59, 288–293. doi: 10.5962/bhl.part.75371

Reimschuessel, R., and Stoskopf, M. K. (1990). Octopus automutilation syndrome. *J. Invertebr. Pathol.* 55, 394–400. doi: 10.1016/0022-2011(90)90083-I

Richiardi, S. (1881). Sulla riproduzione delle braccia dell'Octopus vulgaris Lamk. e sulla mostruosità di una conchiglia della Sepia officinalis Linn. *Zool. Ant.* 406–407.

Riggenbach, E. (1901). Beobachtungen uber Selbsteverstummelung. *Zool. Anzeiger.* 24, 587–593.

Robison, B. H., Reisenbichler, K. R., Hunt, J. C., and Haddock, S. H. (2003). Light production by the arm tips of the deep-sea cephalopod *Vampyroteuthis infernalis*. *Biol. Bull.* 205, 102–109. doi: 10.2307/1543231

Robson, G. C. (1929). A *Monograph* of the *Recent* Cephalopoda *Based* on the *Collections* in the British Museum (Natural History). *Part I Octopodinae*. London: Order of the Trustees of the British Museum (Natural History).

Rohrbach, B., and Schmidtberg, H. (2006). Sepia arms and tentacles: model systems for studying the regeneration of brachial appendages. *Vie et Milieu* 56, 175–190.

Sanders, G. D., and Young, J. Z. (1974). Reappearance of specific colour patterns after nerve regeneration in octopus. *Proc. R. Soc. Lond. B. Biol. Sci.* 186, 1–11. doi: 10.1098/rspb.1974.0031

Saunders, W. B. (1973). *Upper Mississippian Ammonoids from Arkansas and Oklahoma*. Boulder, CO: Geological Society of America.

Saunders, W. B., Knight, R. L., and Bond, P. N. (1991). Octopus predation on nautilus: evidence from Papua New Guinea. *Bull. Mar. Sci.* 49, 280–287.

Saunders, W. B., Spinosa, C., and Davis, L. E. (2010). "Predation on Nautilus," in *Nautilus. Topics in Geobiology,* eds W. B. Saunders and N. H. Landman (Dordrecht: Springer Verlag), 201–212.

Sereni, E. (1929a). Correlazioni umorali nei Cefalopodi. *Am. J. Physiol.* 90:512.

Sereni, E. (1929b). Fenomeni fisiologici consecutivi alla sezione dei nervi nei cefalopodi. *Boll. Soc. It. Biol. Sperim* 4, 736–740.

Sereni, E. (1932). Sulla funzione dei corpi branchiali dei Cefalopodi. *Arch. Zool. Ital*. 16, 941–947.

Sereni, E., and Young, J. Z. (1932). Nervous degeneration and regeneration in Cephalopods. *Pubbl. Staz. Zool. Napoli* 12, 173–208.

Shaw, T. J., Osborne, M., Ponte, G., Fiorito, G., and Andrews, P. L. (2016). Mechanisms of wound closure following acute arm injury in Octopus vulgaris. *Zool. Lett.* 2:8. doi: 10.1186/s40851-016-0044-5

Slotta, F., Korn, D., Klug, C., Kröger, B., and Keupp, H. (2011). Sublethal shell injuries in Late Devonian ammonoids (Cephalopoda) from Kattensiepen (Rhenish Mountains). *Neues Jahrbuch für Geologie und Paläontologie-Abhandlungen* 261, 321–336. doi: 10.1127/0077-7749/2011/0173

Smith, E. A. (1907). LVI.—Notes on an "Octopus" with branching arms. *J. Nat. Hist.* 20, 407–410. doi: 10.1080/00222930709487360

Steenstrup, J. (1856). Hectocotyldannelsen hos Octopodslaegterne Argonauta og Tremoctopus, oplyst ved Iagttagelse af lignende Dannelser hos Blaekspruntterne Almindelighed. *Kongelige danske Videnskabernes Selskabs Skrifter* 4, 186–215.

Steenstrup, J. (1857). Hectocotylus-formation in Argonauta and Tremoctopus explained by observations on similar formation in the Cephalopoda in general. *Ann. Mag. Natl. Hist.* 116, 81–114. doi: 10.1080/00222935709487882

Taki, I. (1941). On keeping octopods in an aquarium for physiological experiments, with remarks on some operative techniques. *Venus* 10, 140–156.

Taki, I. (1944). Studies on Octopus. 2. Sex and genital organs of females and males. *Jpn. J. Malacol.* 13, 267–316.

Taki, I. (1964). On the morphology and physiology of branchial gland in Cephalopoda. *J. Fac. Fish Anim. Husb. Hiroshima Univ.* 5, 345–417.

Tanabe, K., Shinomiya, A., and Fukuda, Y. (1988). "Notes on shell breakage in Nautilus pompilius from Fiji," in *Occasional Papers* (Kagoshima).

Toll, R. B., and Binger, L. C. (1991). Arm anomalies: cases of supernumerary development and bilateral agenesis of arm pairs in Octopoda (Mollusca, Cephalopoda). *Zoomorphology* 110, 313–316. doi: 10.1007/BF01668021

Trego, K. D. (1993). Examples of damage repair in the shell of the cephalopod genus Argonauta. *Veliger* 36, 200–202.

Tressler, J., Maddox, F., Goodwin, E., Zhang, Z., and Tublitz, N. (2014). Arm regeneration in two species of cuttlefish *Sepia officinalis* and *Sepia pharaonis*. *Inv. Neurosci* 14, 37–49. doi: 10.1007/s10158-013-0159-8

Tsujino, Y., and Shigeta, Y. (2012). Biological response to experimental damage of the phragmocone and siphuncle in *Nautilus pompilius* Linnaeus. *Lethaia* 45, 443–449. doi: 10.1111/j.1502-3931.2012.00306.x

Tucker, J. K., and Mapes, R. H. (1978). Possible predation on *Nautilus pompilius*. *Veliger* 21, 95.

Verrill, A. E. (1881). Regeneration of lost parts in the squid (Loligo Pealei). *J. Nat. Hist.* 7, 489–490. doi: 10.1080/00222938109459561

Verrill, A. E. (1882). *Report on the Cephalopods of the Northeastern Coast of America*. Washington, DC: U.S. Government Printing Office.

Voight, J. R. (1992). Movement, injuries and growth of members of a natural population of the Pacific pygmy octopus, *Octopus digueti*. *J. Zool.* 228, 247–264. doi: 10.1111/j.1469-7998.1992.tb04606.x

von Boletzky, S., and Overath, H. (1991). "Shell fracture and repair in the cuttlefish Sepia officinalis," in *La Seiche, The Cuttlefish,* ed E. Boucaud-Camou (Caen: Centre de Publications, Universitè de Caen), 69–78.

Wada, T. (2017). Size-assortative mating and arm loss in the wild shallow-water octopus Abdopus sp. (Cephalopoda: Octopodidae). *J. Natl. Hist.* 51, 2635–2644. doi: 10.1080/00222933.2016.1252069

Yacob, J., Lewis, A. C., Gosling, A., St Hilaire, D. H., Tesar, L., McRae, M., et al. (2011). Principles underlying chromatophore addition during maturation in the European cuttlefish, *Sepia officinalis*. *J. Exp. Biol.* 214, 3423–3432. doi: 10.1242/jeb.055251

Yomogida, S., and Wani, R. (2013). Higher risk of fatality by predatory attacks in earlier ontogenetic stages of modern *Nautilus pompilius* in the Philippines: evidence from the ontogenetic analyses of shell repairs. *Lethaia* 46, 317–330. doi: 10.1111/let.12010

Young, E. R., and Vecchione, M. (2016). *Octopoteuthis Ruppell 1844 [Online]. The Tree of Life Web Project.* Available online at: http://tolweb.org/Octopoteuthis/19839/2016.11.16

Young, J. Z. (1929). Fenomeni istologici consecutivi alla sezione dei nervi nei cefalopodi. *Boll. Soc. It. Biol. Sperim*. 4, 741–744.

Young, J. Z. (1932). Memoirs: on the cytology of the neurons of cephalopods. *J. Cell Sci.* 75, 1–47.

Young, J. Z. (1971). *The Anatomy of the Nervous System of Octopus vulgaris*. London: Oxford University Press.

Young, J. Z. (1972). The Organization of a Cephalopod Ganglion. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 263, 409–429. doi: 10.1098/rstb.1972.0005

Zato,n, M. (2010). Sublethal injuries in Middle Jurassic ammonite shells from Poland. *Geobios* 43, 365–375. doi: 10.1016/j.geobios.2009.11.004

Zullo, L., Buschiazzo, A., Massollo, M., Riondato, M., Democrito, A., Marini, C., et al. (2018). 18F-FDG micro-PET imaging for research investigations in the *Octopus vulgaris*: applications and future directions in invertebrate neuroscience and tissue regeneration. *J. Nucl. Med. Jnumed*. 117:205393. doi: 10.2967/jnumed.117.205393

Zullo, L., Fossati, S. M., Imperadore, P., and Nödl, M. T. (2017). Molecular determinants of Cephalopod muscles and their implication in muscle regeneration. *Front. Cell Develop. Biol.* 5:53. doi: 10.3389/fcell.2017.00053

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Physiology

ORIGINAL RESEARCH
published: 23 July 2018
doi: 10.3389/fphys.2018.00954

Development of Swimming Abilities in Squid Paralarvae: Behavioral and Ecological Implications for Dispersal

Erica A. G. Vidal[1]*, *Louis D. Zeidberg*[2] *and Edward J. Buskey*[3]

[1] *Center for Marine Studies, University of Parana, Pontal do Paraná, Brazil,* [2] *School of Natural Sciences, Chapman Science Academic Center, California State University, Monterey Bay, Seaside, CA, United States,* [3] *Marine Science Institute, The University of Texas at Austin, Port Aransas, TX, United States*

This study investigates the development of swimming abilities and its relationship with morphology, growth, and nourishment of reared *Doryteuthis opalescens* paralarvae from hatching to 60 days of age. Paralarvae (2.5–11 mm mantle length – ML) were videotaped, and their behavior quantified throughout development using computerized motion analysis. Hatchlings swim dispersed maintaining large nearest neighbor distances (NND, 8.7 ML), with swimming speeds (SS) of 3–8 mm s^{-1} and paths with long horizontal displacements, resulting in high net to gross displacement ratios (NGDR). For 15-day-old paralarvae, swimming paths are more consistent between jets, growth of fins, length, and mass increases. The swimming pattern of 18-day-old paralarvae starved for 72 h exhibited a significant reduction in mean SS and inability to perform escape jets. A key morphological, behavioral, and ecological transition occurs at about 6 mm ML (>35-day old), when there is a clear change in body shape, swimming performance, and behavior, paths are more regularly repeated and directional swimming is evident, suggesting that morphological changes incur in swimming performance. These squid are able to perform sustained swimming and hover against a current at significantly closer NND (2.0 ML), as path displacement is reduced and maneuverability increases. As paralarvae reach 6–7 mm ML, they are able to attain speeds up to 562 mm s^{-1} and to form schools. Social feeding interactions (kleptoparasitism) are often observed prior to the formation of schools. Schools are always formed within areas of high flow gradient in the tanks and are dependent on squid size and current speed. Fin development is a requisite for synchronized and maneuverable swimming of schooling early juveniles. Although average speeds of paralarvae are within intermediate Reynolds numbers ($Re < 100$), they make the transition to the inertia-dominated realm during escape jets of high propulsion ($Re > 3200$), transitioning from plankton to nekton after their first month of life. The progressive development of swimming capabilities and social interactions enable juvenile squid to school, while also accelerates learning, orientation and cognition. These observations indicate that modeling of the lifecycle should include competency to exert influence over small currents and dispersal patterns after the first month of life.

Keywords: cephalopod, *Doryteuthis opalescens*, Reynolds number, swimming behavior, schooling, social interaction, starvation

Edited by:
Richard Emlet,
University of Oregon, United States

Reviewed by:
William McKee Kier,
University of North Carolina at Chapel Hill, United States
Akira Sakurai,
Georgia State University, United States
Joseph T. Thompson,
Franklin & Marshall College, United States

****Correspondence:***
Erica A. G. Vidal
ericavidal2000@yahoo.com.br

Specialty section:
This article was submitted to Invertebrate Physiology, a section of the journal Frontiers in Physiology

Received: *02 March 2018*
Accepted: *28 June 2018*
Published: *23 July 2018*

Citation:
Vidal EAG, Zeidberg LD and Buskey EJ (2018) Development of Swimming Abilities in Squid Paralarvae: Behavioral and Ecological Implications for Dispersal. Front. Physiol. 9:954. doi: 10.3389/fphys.2018.00954

INTRODUCTION

The California market squid, *Doryteuthis opalescens* (Berry, 1911), is an important fishery resource and a key forage species that lives in the nearshore pelagic environment. The species has complex spawning behavior laying benthic eggs that incubate for 3–9 weeks depending on temperature. Upon hatching, planktonic paralarvae are negatively geotactic (Sidie and Holloway, 1999), passive drifters and become entrained within 3 km of shore by interacting with tidally reversing currents and cyclonic gyres. Paralarvae perform vertical diel migration (Zeidberg and Hamner, 2002), and their greatest abundance is associated with cooler sea surface temperatures (SST) during La Niña events (Van Noord and Dorval, 2017). Juveniles are nektonic, live on the shelf (Zeidberg, 2014) and move to the slope or over deep water with development. Adults return to the shelf to aggregate into spawning groups of millions of individuals, where they are the focus of the largest cephalopod fishery in the United States (Ryley, 2015).

Squids are highly active social animals and form schools at an early-life stage (Sugimoto and Ikeda, 2012; Vidal and Boletzky, 2014). Social behavior and the ability to gather in schools where individuals synchronize to each other's SS in parallel orientation (polarization) is a major ecological and behavioral adaptation, as it requires complex swimming and cognitive skills (Hurley, 1978; Sugimoto and Ikeda, 2012; Sugimoto et al., 2013; Yasumuro et al., 2015). The underlying mechanisms allowing squid to make the transition from passive planktonic drifters to active schoolers early in life have received little attention. Schooling is believed to provide many benefits to individuals by improving foraging strategies, reducing predation risk, accelerating learning, and improving orientation skills (Masuda et al., 2003; Oshima et al., 2016).

Throughout their life cycle, squid use a dual mode system for swimming, combining pulsed jetting and fin-flapping (Bartol et al., 2008, 2016; Stewart et al., 2010). However, the hydrodynamic efficiency of this dual mode locomotive system changes considerably during ontogeny due to changes in relative size and shape of the body and fins, mantle muscle structure, and flow regimen (Hoar et al., 1994; Thompson and Kier, 2001; Bartol et al., 2008, 2016; Thompson et al., 2010). Paralarvae experience intermediate Re, a fluid regime in which both viscous and inertial flow forces have important effects, compared with the high Re regime of adult animals. Hatchlings swim predominantly using high-volume, low-velocity pulsed jets as they have rudimentary fins and larger relative funnel apertures, moving proportionately a greater distance with each jet (Bartol et al., 2008, 2009; Staaf et al., 2014). It was shown that the propulsive efficiency (thrust) of the exhalant phase of the jet was significantly greater in newly hatched *Doryteuthis pealeii* than in adults (Bartol et al., 2008, 2009). Little has been reported, however, on the progressive improvement of these swimming abilities during early ontogeny. In adults, the jet propulsive efficiency improves at higher SSs (Anderson and Grosenbaugh, 2005) and fins have a fundamental role in generating thrust (Anderson and Demont, 2005), stabilizing the body, and also providing net lift (Stewart et al., 2010; Olcay and Malazi, 2016).

Understanding how swimming abilities develop during early ontogeny in squid is of paramount importance to evaluate the extent to which active swimming influences dispersal, distribution, and sizes of a population as the environment changes from year to year. Dispersal is recognized as a consequence of planktonic development (Scheltema, 1986). It was recently demonstrated that the developmental mode (planktonic or benthic) of cephalopods influences their dispersal ability to such an extent that it can determine the broader distributional range of species with planktonic hatchlings (Villanueva et al., 2016). As paralarvae develop, the interplay between their swimming abilities, behavior, and the local currents dictates distribution, dispersal or retention, growth, and survival.

The end of the planktonic dispersive phase will be determined by the ability of paralarvae to hold a position against a current (sustained swimming) and to form schools. This ability should be regulated by morphology and size and ultimately nourishment. Starvation is considered one of the major regulators of larval growth and survival in the sea (Boidron-Métairon, 1995), and while previous studies have confirmed that *D. opalescens* paralarvae are extremely sensitive to starvation (Vidal et al., 2002b, 2006), little work has been directed toward understanding the effects of food availability on the swimming performance of paralarvae. More precisely, how does food availability impact paralarvae swimming ability and pattern? What are the main developmental processes and morphological attributes that allow squid to transition from plankton to nekton and to swim in schools?

Upon hatching, *D. opalescens* is dispersed by currents and relies on jet-and-sink swimming, but within the first 2 months of life they develop the ability to form schools. We used motion analysis combined with morphological and growth data from reared *D. opalescens* paralarvae to provide an assessment of swimming performance and uncover interconnected ecological and behavioral milestones. Specifically, this study sought to(1) examine the development of swimming abilities of paralarvae and their interplay with growth, morphological attributes, and behavior, (2) evaluate the effects of starvation on the swimming performance of paralarvae, and (3) investigate how and when squid achieve the transition from plankton to nekton.

MATERIALS AND METHODS

Field Collection and Experiments

Eggs of *D. opalescens* were collected by SCUBA divers on the spawning grounds (15–30 m) in Monterey Bay (36°60′N, 121°80′W) and Southern California (34°7′N, 119°05′W), United States. After collection, the eggs were placed in sealed plastic bags with seawater, filled with pure O_2, and air-shipped

---

Abbreviations: FW, fin width; GR, growth rate; ML, mantle length; MW, mantle width; NGDR, net to gross displacement ratio; NND, nearest neighbor distance; RCD, rate of change of direction; Re, Reynolds number; SS, displacement swimming speed.

in a cooler box with frozen ice packs to the National Resource Center for Cephalopods, University of Texas Medical Branch, Galveston, TX, United States, where three experiments took place. In all experiments, eggs and paralarvae were reared at 16 ± 0.5°C in a recirculating system consisting of seven 220-l cylindrical tanks (0.95 m diameter × 0.4 m height). The water inflow of the rearing tanks was maintained at a rate of 5.7 l min^{-1} generating a counter-clockwise current of ∼1 cm s^{-1} that promoted an even distribution of the paralarvae and their prey (Vidal et al., 2002b).

The number of paralarvae per tank ranged from 800 to 3000 and the food offered was *Artemia* spp. nauplii enriched with SUPER SELCO (INVE®), mysid shrimp (*Americamysis almyra*), and wild zooplankton (mainly copepods) at densities of 50–200 prey l^{-1} (Vidal et al., 2002b). In the starvation experiment, paralarvae were kept in the hatching tank up to day 14 after hatching when a total of 2100 were randomly transferred to three other tanks (700 paralarvae in each tank) and exposed to different periods of starvation. The first day of the experiment was day 15 after hatching and each experimental tank constituted an experimental group that was exposed to 24, 48, and 72 h of starvation, for the purpose of comparing the swimming performance of fed paralarvae with those exposed to one, two, and three days of starvation.

Video Recording of Behavior

During the experiments, paralarvae ranging in size from 2.5 to 11 mm ML were filmed between 14:00 to 20:00 h in the rearing tanks at 0, 5, 15, 16, 17, 18, 40, 50, and 60 days after hatching. In total, 17 h of filming was performed, from 1 to 2 h for each age. Videos were recorded with a Sony CCD-TR930 Hi8 camcorder operating at 30 frames s^{-1} fitted with a #1.5 close-up lens. The camera was mounted on a tripod at a 90° angle to the glass window on the side of the tanks, the frame of view for filming was 3.6 cm × 3.6 cm. Distance calibration was performed prior to each filming session. A small thin ruler was positioned inside the tanks facing the window. The camera was set to operate in manual mode and the autofocus and zoom functions were turned off, then the focus of the camera was adjusted to the ruler with a focal distance 1–3 cm in toward the center of the tank from the window. The lens aperture was also locked to maintain a constant depth of field (3 mm). Paralarvae were videotaped when they came into the frame of view and were in focus for the small depth of field. Errors resulting from the positioning of the paralarvae along the optical axis were estimated to be below 15% for hatchlings and decreased as paralarvae increased in size. During filming, the camera was connected to a TV set to allow monitoring of behavior without disturbing the paralarvae.

The water inflow of the tanks was turned off until the current had reduced to 1 mm s^{-1}, and then video-taping occurred for 20 min. The same procedure was repeated in at least three other tanks, holding same aged squid. Current speed was measured with a flowmeter (Flo-Mate, Marsh-McBirney, Frederick, Md, model 2000; Vidal et al., 2002b). Thus, SSs could be underestimated by up to 1 mm s^{-1}, but this effect would be similar for all ages.

Paralarvae Swimming Speed and Behavior by Motion Analysis

To describe the swimming pattern and behavior of *D. opalescens*, paralarvae video recordings of 0-, 5-, 15-, 40-, 50-, and 60-day-old-fed paralarvae and of 16-, 17-, and 18-day-old starved paralarvae were played back through a Motion Analysis VP-110 video-to-digital processor, and digitized outlines of the paralarvae were sent to a computer at a rate of 15 or 30 frames s^{-1}, depending on the mean displacement SS of the paralarvae. Slower swimming paralarvae (younger) were sampled at 15 frame s^{-1}, but faster swimming paralarvae (older) were sampled at the full 30 frames s^{-1}, for higher temporal resolution. Approximately 10 min of combined swimming behavior from at least 15 paralarvae of each age filmed were examined (∼9000 measurements at 15 frames s^{-1}; Buskey et al., 1993). The swimming patterns were quantified in a two-dimensional representation of a three-dimensional swimming pattern; thus, if a paralarva was swimming toward or away from the camera, it would appear stationary; however, these errors were reduced by focusing the camera to a shallow depth of field, and only using images that were in focus. Thus, only trajectories perpendicular to the camera were precisely recorded.

Paths of movement and motion parameters were calculated using Expertvision Cell-Track computer software. Mean SS (mm s^{-1}) and mean RCDs (° s^{-1}) were calculated from paths of movements during each interval. RCD is an index of turning rate irrespective of direction, and is measured as the absolute value of the angular velocity. The change in both X and Y positions were used to measure the displacement between two central locations for the paralarvae over a known time interval and this change in location over time was used to calculate speed. The tendency of paralarvae to remain within an area by changing their turning behavior was indicated by the mean NGDRs of successive cumulative segments of their paths of travel. NGDR is an index of path tortuousness or convolution; a ratio of net displacement (the linear distance between starting and ending points of a path) to gross displacement (the total distance traveled by the path over the same time interval). Basically, a straight path gives an NGDR value of 1 and a closed circular path a value of 0. This ratio was measured repeatedly, as the swimming path lengthened over time. Mean parameters are based on multiple paths from several paralarvae of each age. Mann–Whitney–Wilcoxon tests were used to compare SS, NGDR, and RCD, according to Zar (1996).

Mantle Length, Fin Width, and Dry Weight Measurements

Random samples of 5–10 paralarvae were collected from the rearing tanks for each age after recording swimming behavior. Sampled paralarvae were anesthetized with magnesium chloride and both ML and FW were measured according to Roper and Voss (1983) to the nearest 0.01 mm under a dissecting microscope equipped with an ocular micrometer. Then, dry body mass (dry weight – DW) were obtained individually from 5 to 10 paralarvae after placement in an oven at 60 °C for 24 h, using a microbalance to the nearest 0.01 mg (as in Vidal et al., 2002a).

Also, samples of 5–10 other age paralarvae (0-, 1-, 2-, 3-, 4-, 6-, 7-, 10-, 11-, 13-, 17-, 19-, 23-, 24-, 25-, 27-, 31-, 33-, 35-, 37-, 42-, 45-, 47-, and 55-day-old) were sampled to obtain a more precise relationship between DW and ML and DW and age.

Length–Weight Relationship and Length–Fin Width Relationship

The regression of the length–weight relationship was calculated from dry weight of paralarvae versus ML as in the formula:

$$\mathrm{DW} = a\mathrm{ML}^{b}$$

where DW is dry weight, ML is mantle length, a is a constant, and b the allometric factor. DW and ML were log transformed to produce a linear relationship, and then a and b were estimated by least square regression. The relative growth between FW and ML was analyzed by the allometric equation:

$$\mathrm{FW} = a\mathrm{ML}^{b}$$

where b is the allometric constant and a is the initial index. After logarithmic transformation of *FW* and *ML*, a and b were estimated by least square regression. Growth relationship between the two linear variables indicate negative allometry when $b < 1$, showing that FW grows less rapidly than the ML, positive allometry when $b > 1$, and isometry when $b = 1$. A significance test for comparison of the slope against 1 was applied (Sokal and Rohlf, 1995).

Survival Rates and Growth Relationships

Mortality was determined daily by counting the number of dead paralarvae in each replicate tank during the experiments. Survival was calculated as the percentage of live paralarvae left in each replicate tank versus the initial number, excluding the paralarvae sampled for data collection. Final survival rates were expressed as the minimum and maximum values obtained from all the replicate tanks in each experiment.

Growth rates were expressed as instantaneous GRs and were calculated using the standard exponential function:

$$Y = Y_1 e^{bd}$$

where Y is the mean body DW or ML, Y_1 is the mean DW or ML obtained on hatching day, e is the natural logarithm, b is the slope, and d is age in days post-hatching. The instantaneous relative GRs expressed in % body DW day^{-1} and in % ML day^{-1} were calculated using the formula: $\mathrm{GR} = 100 \times (eb^{-1})$. Both age–weight and age–size relationships were fitted to the exponential equation. The time required for a squid to double its weight or size (doubling time) was calculated by dividing the natural log of 2 by the GR value obtained based on the hatching weight and that at 60 days (Forsythe and Hanlon, 1988).

Fin Beats, Mantle Width, Swimming Angle Measurements, and Reynolds Number Calculations

Fin beat frequency and MW were measured from image analysis of 15–25 paralarvae of 0, 15, 40, and 60 days of age. These measurements were captured from a video camera positioned above a small round aquarium (7 cm H, 33 cm diameter) that was a miniature model of the rearing tanks. The MW measurements were obtained according to Roper and Voss (1983) from still frame images to evaluate morphological changes in the mantle with development. During filming, a scale was positioned at the bottom of the aquarium to set the scale for each image and distance calibration was performed prior to filming as described above. Measurements were performed by digitizing a line on the image, and the width values were stored.

To evaluate schooling behavior, the angular orientation and the NND were measured from filming performed through the window of the rearing tanks. A protractor was used to obtain the angular orientation by comparing the horizon to a line drawn from the eye lenses and the posterior tip of the mantle of paralarvae as reference points (**Figure 1**). The NND was measured as the distance between the eyes of randomly selected paralarvae and was standardized to the mean ML of measured squid. Both the angles and the NND were obtained from 5 to 22 paralarvae of each age. To ensure precision and accuracy of measurements, squid were only measured when within a predefined distance and orientation to the camera, when their eyes were exactly parallel to the video camera and in focus. T-tests were used to compare the orientation angles and NND (Sokal and Rohlf, 1995).

The MLs were used to calculate Re, using the following equation:

$$\mathrm{Re} = UL/\nu$$

where L is ML in meters, U is swimming velocity obtained from motion analysis (see above) in m s^{-1}, and ν is the kinematic viscosity of water. The kinematic viscosity, ν, is 1.155614E−06 $\mathrm{m}^2\ \mathrm{s}^{-1}$ in 16°C seawater of 33 g kg^{-1} (Sharqawy et al., 2010). Small paralarvae yield lower intermediate Re values

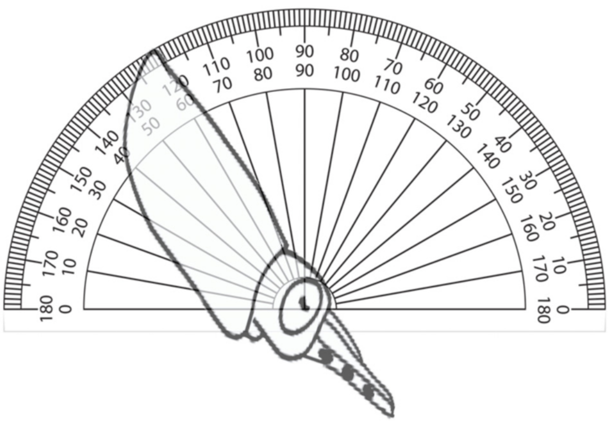


FIGURE 1 | Diagram of angular orientation of paralarvae. Angles of orientation were measured by comparing the horizon to a line drawn from the eye lenses and the posterior tip of the mantle of paralarvae as reference points. The angle shown is 60°.

indicating that the effects of viscosity on swimming performance are significant.

Ethics Statement

This study has been conducted in compliance with recommendations of the ARRIVE Guideline (Kilkenny et al., 2010) for reporting *in vivo* experiments with research animals. The Institutional Animal Care and Use Committee (IACUC) of the University of Texas did not require researchers to submit protocols for the ethical treatment of invertebrate larvae at the time this research was performed.

RESULTS

Swimming Performance, Paths, and Speeds of Fed Paralarvae

The swimming of a newly hatched squid with a mean ML of 2.65 mm was characterized by the predominance of a short pulsed jet-and-sink motion (vertical bobbing). This resulted from mantle contractions causing jetting up and mantle expansion and negative buoyancy causing sinking. The typical swimming paths showed large speed variation, with frequent change from low speed to several peaks of higher speed in time intervals of 0.1–0.2 s (**Figure 2A**). These changes took place within a narrow speed range (4–8 mm s^{-1}) with mean SS of 5.70 mm s^{-1} (**Figures 2A**, **3A**). Hatchlings drift with the current and showed paths with relatively long horizontal displacements and short excursion in the vertical plane, resulting in high NGDR values and mean RCD around 300° s^{-1}. The relative SS was 2.2 ML s^{-1} and the Re was 13 (**Table 1**). The swimming paths observed for 5-day-old paralarvae were very similar to those of newly hatched paralarvae, but there was more variance in speed (**Table 1** and **Figure 3B**).

In 15-day-old paralarvae (3.78 mm ML), the swimming paths exhibited longer and more consistent time intervals between jets (**Figure 2B**). These paralarvae showed enhanced activity, with more variation in speed and a significantly higher mean speed (7.50 mm s^{-1}), than newly hatched and 5-day-old paralarvae ($p < 0.05$, **Table 1**) and maximum speeds of 208 mm s^{-1} (**Figures 2B**, **3C**). The NGDR and RCD were not significantly different from early paralarvae, but Re numbers were fourfold higher for maximum speed when compared to hatchlings (**Table 1**).

As paralarvae reached 40 days of age and 6–7 mm ML, they were able to perform very fast changes of speed, accelerating from 5 to 50 mm s^{-1} in approximately 0.2 s (**Figure 2C**). The path displacement was more circuitous when compared with 15-day-old paralarvae. Due to more powerful jets, the distance traveled by paralarvae during vertical displacement was larger than the linear distance between the start and ending points of the paths (**Figure 2C**). This resulted in significantly lower NGDR values ($p < 0.05$, **Table 1**), half of those measured for early paralarvae (**Table 1**). Mean RCD was higher ($p < 0.05$) and doubled in paralarvae from 15 to 40 days. This was a reflection of considerable changes in the swimming pattern, as older squid spent more time hovering. By doing so, they remain in the same area for longer periods of time by maneuvering and adjusting their orientation to other squid. Mean speed was 8.84 mm s^{-1} with greater variance (**Figure 3D**), and maximum speeds reached 562 mm s^{-1}. The relative SS decreased to 1.3 ML s^{-1}, due to hovering.

Paralarvae of 6–7 mm ML were able to jet in all directions at much higher speeds than 15-day-old paralarvae, performing faster horizontal displacements, both during predatory (jetting forward) and escape behavior (jetting backward). A more regularly repeated (cyclic) SS pattern became evident during jetting due to hovering, with short periods (∼5 s) jetting at mean speed of 15 mm s^{-1}, interspersed with other periods of jetting at 8 mm s^{-1} (**Figure 2C**). The Re for maximum speed quadrupled between days 15 and 40, demonstrating that 40-day-old squid are able to attain a wide range of speeds and Re in the inertial realm (**Table 1**).

The swimming paths observed for 60-day-old squid (9.81 mm ML) were similar to those of 40-day-old squid (**Figure 2C**), but reflected enhanced maneuverability, stability and jetting control (**Table 1**). Squid spent most of the time hovering; therefore, the relative path displacement was greatly reduced. The mean SS was 11.16 mm s^{-1} and the distribution of speeds was strongly skewed to the right, showing the capacity for attaining and maintaining higher speeds (**Table 1** and **Figure 3E**). Due to the relatively small field of view when compared to the total size of 60-day-old squid, it was not possible to document maximum speed.

Swimming Behavior and Social Interactions of Fed Paralarvae

Newly hatched paralarvae had internal yolk sacs that occupy a relatively large portion of the mantle cavity. Hatchlings swam in random directions, dispersed and maintaining a mean distance of 8.7 ML from each other by jetting and sinking continuously, oriented at 45° angles (**Figures 4**, **5**). They drift with the current and the short horizontal displacements against the current were primarily due to prey inspection and attack, when fins were mainly used, beats average 2.8 s^{-1} (**Table 1**). Hatchlings often displayed aggressive behavior toward one another, chasing and attacking other paralarvae that swam nearby with knocks of arms and tentacles tips. Often after a paralarva had captured a large prey (mysid shrimp) relative to its size, the prey was attacked by several other paralarvae (up to 7) and all feed on the same prey (**Figure 6**). This kleptoparasitism is intensified 6–7 days after hatching, when yolk reserves are fully absorbed.

Fifteen-day-old paralarvae still swam randomly, although they were observed more often in the same angular orientation (**Figure 4**), maintaining a mean distance from one another of 5.5 ML (**Figure 5**). They were able to swim up and down in the water column easily, and more often horizontally, both with and against the current. Horizontal displacements were often related to prey inspection, pursuit, and attacks. During the phase of positioning prior to a prey attack, paralarvae remained quasi-stationary by means of rapid fin beats, averaging 3.62 beats s^{-1} (**Table 1**). Kleptoparasitism remained frequent.

Between 30 and 40 days of age, paralarvae showed a considerable change in swimming behavior, holding position

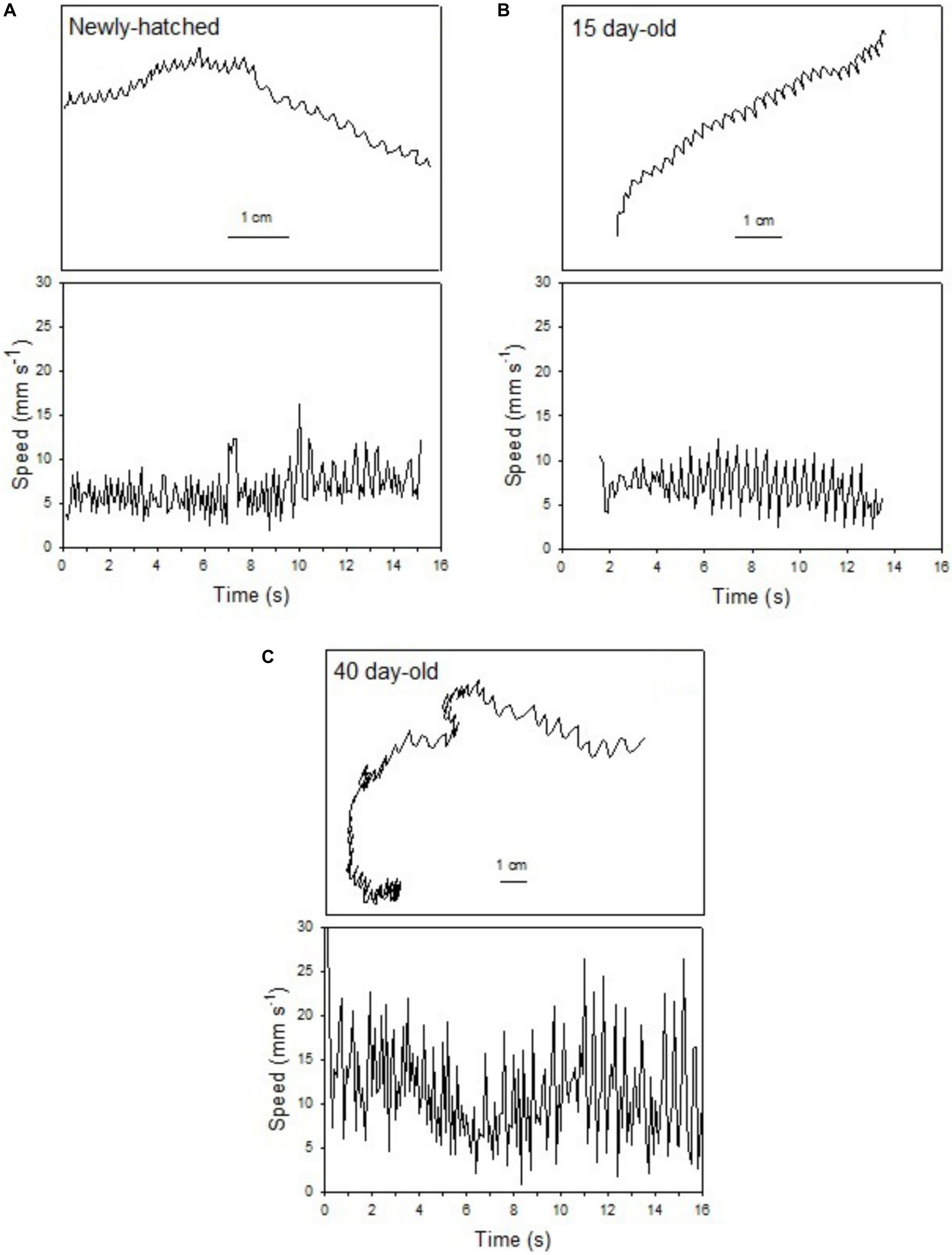


FIGURE 2 | *Doryteuthis opalescens.* Representative swimming path records in the vertical plane and swimming speed patterns (14–16 s duration) of fed paralarvae. **(A)** Newly hatched, **(B)** 15-day-old, and **(C)** 40-day-old. Paralarva is traveling from left to right as recording time increases.

against the current, and swimming close to each other. Concomitantly, aggressive behavior, toward other squid swimming nearby was reduced. Kleptoparasitism also was reduced likely because prey size relative to squid size diminished. Schooling behavior is defined here as a group of at least three squid swimming in the same direction in parallel orientation and

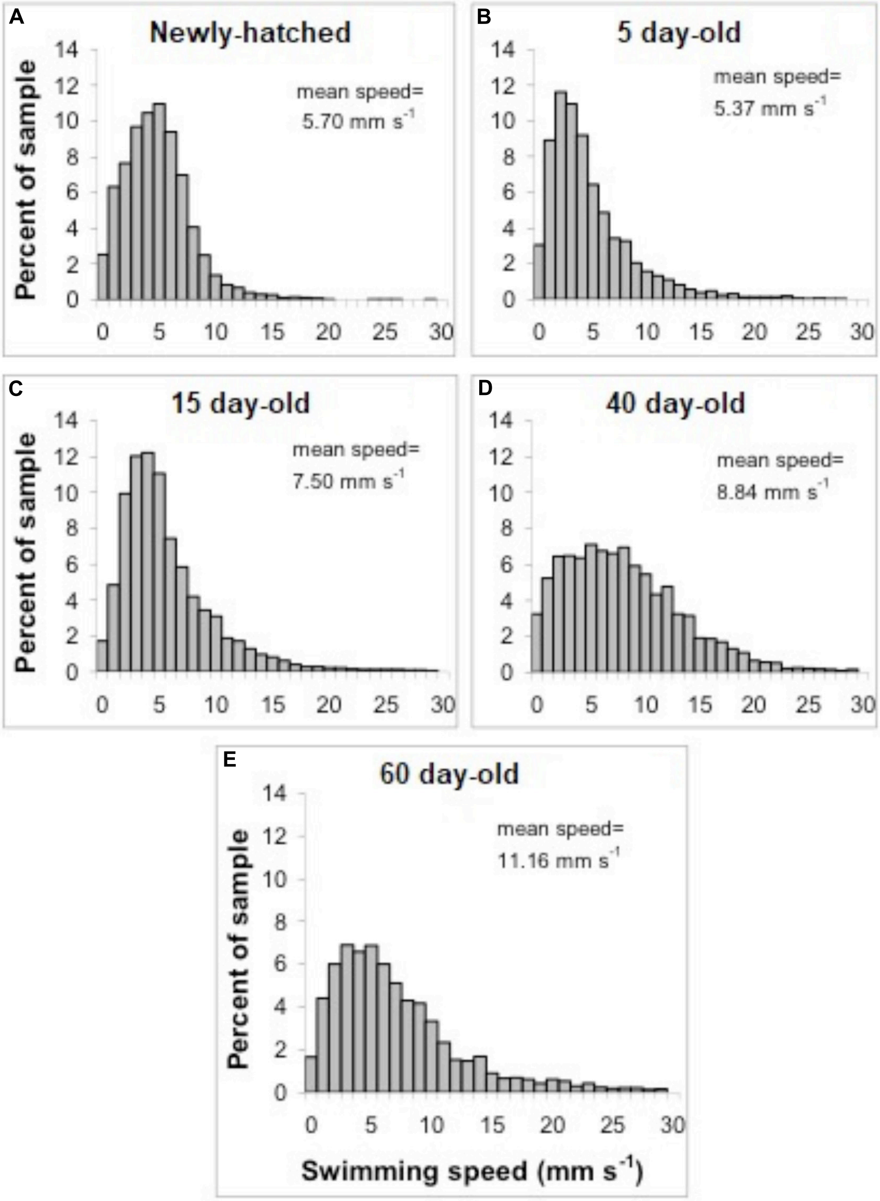


FIGURE 3 | *Doryteuthis opalescens.* Distribution of swimming speeds of paralarvae. **(A)** Newly hatched, **(B)** 5-day-old, **(C)** 15-day-old, **(D)** 40-day-old, and **(E)** 60-day-old.

positioned within one to three ML of each other (Pitcher and Parrish, 1993; Sugimoto and Ikeda, 2012). Schooling was first observed in 35- to 45-day-old paralarvae (6.0–8.0 mm ML). The largest paralarvae in the tanks were the first to swim against the current and to form schools. The size of the school progressively increased from 5 to 15–40 squid and they were always formed in the same place, close to the surface, against the current and underneath the water inlet spray bar, where the current was at its maximum velocity. Sometimes, more than one school could be seen in a tank, but each school was sorted by size. The smallest paralarvae in the tanks did not take part in the schools, as they were not able to hold a position against the current. Although the same age as the squid forming schools, these paralarvae swam randomly close to the surface. The larger schooling squid often vigorously attacked small squid that approached the school.

Forty-day-old schooling squid spent most of the time hovering significantly closer to one another (2.0 ML; **Table 1** and **Figures 4**, **5**). Nevertheless, they were able to swim horizontally both forward (arms first) and backward (tails first) crossing the tank (0.8 m) in fractions of a second (0.2 s). Forward horizontal displacements were mainly caused by interactions with prey or with other squid, during which fins utilization increased, especially during the positioning phase prior to prey capture; fin beats averaged 5.12 beats s^{-1} (**Table 1**).

TABLE 1 | *Doryteuthis opalescens.*

Age (d)	ML (mm)	Swimming speed (mm s^{-1})	Maximum speed (mm s^{-1})	ML s^{-1}	Fin beats s^{-1}	NGDR	RCD (° s^{-1})	Reynolds number swimming speed	Reynolds number maximum speed
0	2.65 ± 0.07	5.70 ± 0.4^{a}	67	2.2	2.8 ± 0.85	0.63 ± 0.10^{a}	298 ± 47^{a}	13	154
5	2.70 ± 0.13	5.37 ± 0.7^{a}	84	2.0	–	0.64 ± 0.07^{a}	297 ± 70^{a}	13	196
15	3.78 ± 0.24	7.50 ± 1.5^{b}	208	2.0	3.6 ± 0.47	0.71 ± 0.04^{a}	275 ± 21^{a}	26	680
40	6.70 ± 0.92	8.84 ± 1.6^{b}	562	1.3	5.1 ± 1.76	0.36 ± 0.07^{b}	564 ± 107^{b}	51	3258
50	7.23 ± 1.15	$6.16 \pm 1.6^{a,b}$	–	0.9	–	0.37 ± 0.08^{b}	571 ± 89^{b}	39	–
60	9.81 ± 1.41	11.16 ± 3.0^{b}	–	1.2	6.5 ± 3.06	0.36 ± 0.05^{b}	586 ± 68^{b}	95	–

Mantle lengths (ML), swimming speed, net to gross displacement ratio (NGDR), and rate of change of direction (RCD) of paralarvae reared from 0 to 60 days after hatching. Values are means of 5–15 individuals ± SD. Means with same superscript letters denote no statistical difference (Mann–Whitney–Wilcoxon test, $p > 0.05$). Reynolds numbers are calculated from the mean speed (swimming or maximum) U, ML, and v, the kinematic viscosity of seawater at 16°C and 33 g kg^{-1} (Sharqawy et al., 2010), Re = UL/v.

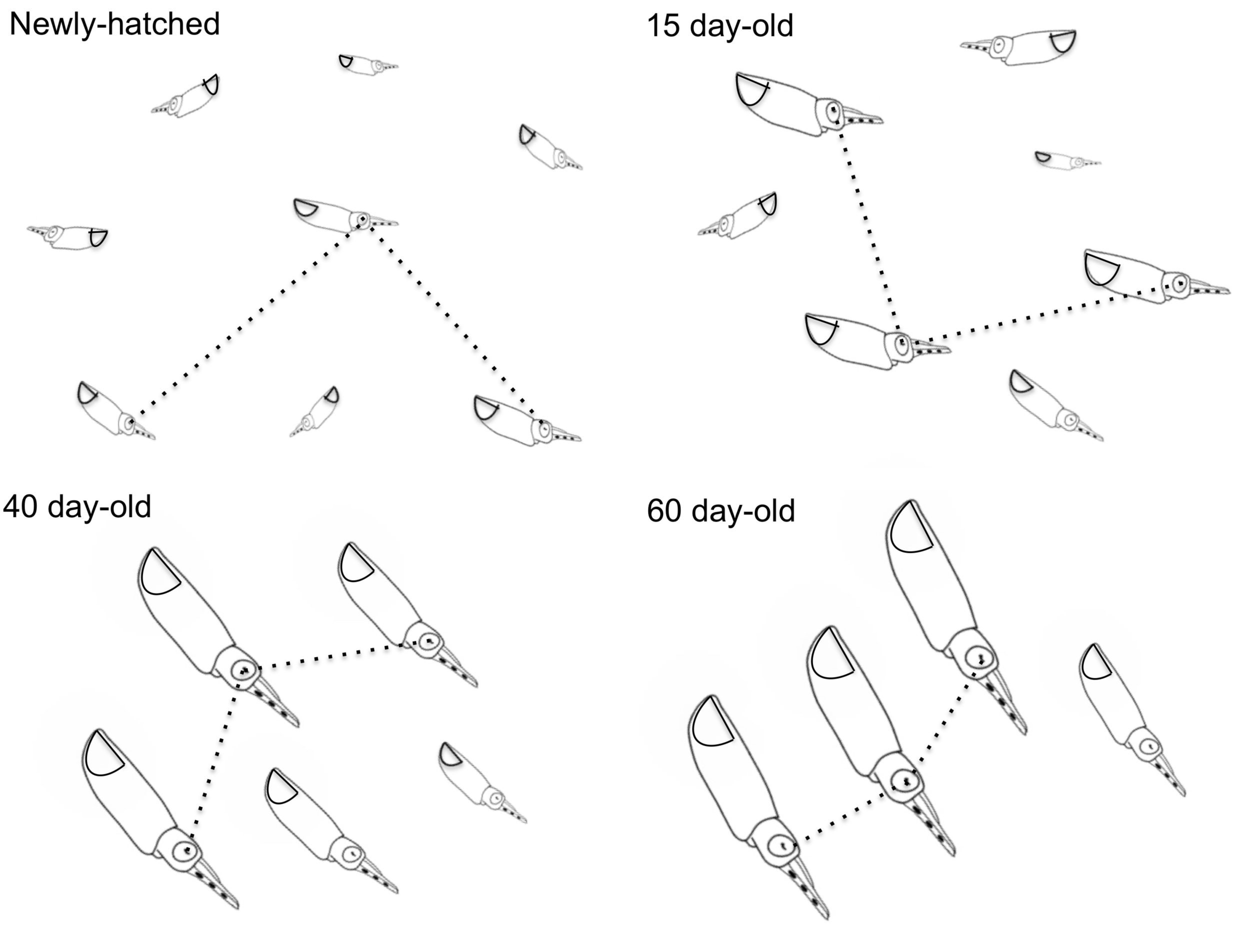


FIGURE 4 | *Doryteuthis opalescens.* Stereotyped swimming behavior patterns of paralarvae reared for 60 days after hatching. The dotted lines indicate the nearest-neighbor distance (NND) measured as the distance between the eyes of randomly selected paralarvae.

Sixty-day-old schooling squid showed enhanced swimming control and parallel orientation (polarization) when compared with 40-day-old squid. They swam in a more vertical orientation (62°) with mean fin beats of 6.51 beats s^{-1} (**Table 1** and **Figure 5**). These skilled movements permitted swimming closer to other squid, resulting in mean NND of 1.5 ML (**Figures 4**, **5**), and the highest level of synchrony and fine-scale movements powered by fins and jet propulsion. This synchrony was particularly evident when any disturbance occurred, as for example, a shadow from an observer or when any squid in the school changed orientation or speed in an escape reaction. Immediately after such disturbances, the squid responded similar to a flash explosion, scattering from a polarized orientation by jetting backward without colliding and disrupting the school, dispersing briefly and then reassembling.

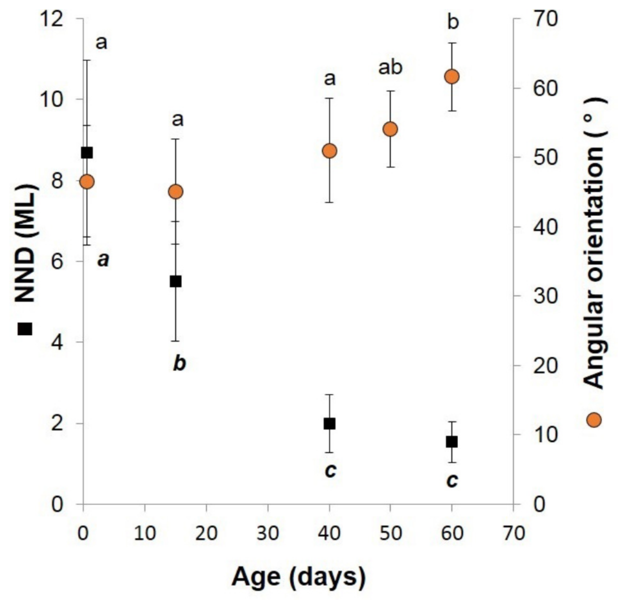


FIGURE 5 | *Doryteuthis opalescens*. Relationships between the nearest-neighbor distance (NND) and swimming angles of orientation and age in squid reared for 60 days after hatching. The NND was measured as the distance between the eyes of randomly selected paralarvae and was standardized to the mean ML of measured squid. Symbols and bars indicate mean and SD, respectively; means with same superscript letters denote no statistical difference (t-test, $p > 0.05$).

Swimming Paths and Speeds, and Behavior of Starved Paralarvae

The swimming paths of 16-, 17-, and 18-day-old paralarvae starved for 24, 48, and 72 h, respectively, showed an erratic pattern with speeds ranging from nearly 0 to 10 mm s^{-1} (**Table 2**). The distribution of SS of starved paralarvae showed marked changes when compared to that of 15-day-old fed paralarvae. The speed was distributed mainly between 5 and 8 mm s^{-1} slightly skewed to the right (**Figures 7, 8**). Mean SS of 15-day-old fed paralarvae and 16-day-old paralarvae starved for 24 h were not statistically different (Mann–Whitney–Wilcoxon test, $p > 0.05$; **Figure 8** and **Tables 1**, **2**). A reduction in mean RCD values was also observed, showing that starved paralarvae changed direction less often when compared to 15-day-old fed paralarvae and even to newly hatched squid (**Table 1**, **2**), suggesting decrease in swimming activity. Increasing the starvation period led to a significant reduction in the mean SS of 18-day-old paralarvae and a significant reduction in mean RCD for 16- and 17-day-old starved paralarvae (Mann–Whitney–Wilcoxon test, $p < 0.05$; **Table 2** and **Figures 7**, **8**). Similarly, the NGDR was significantly different between 15-day-old-fed and 16- and 17-day-old starved paralarvae, but not to 18-day-old starved paralarvae.

Sixteen-day-old paralarvae starved for 24 h spent most of the time searching for food. They frequently swam horizontally and were observed to inspect, attack, and often capture small particles that were released immediately after determination as a non-prey item. The arms and tentacles spread out and extended in repeated searching. This behavior had the effect of increasing horizontal displacement. The swimming behavior observed for 17- and 18-day-old paralarvae starved for 48 and 72 h, respectively, changed considerably when compared to 16-day-old paralarvae. The main swimming behavior was the jet and sink of hatchlings, but with even lower speeds (**Figure 8**). These paralarvae showed a lack of interest in particles, and had fewer horizontal movements. Indeed, they moved little, jetting passively and swimming close to the bottom most of the time, rarely close to the surface.

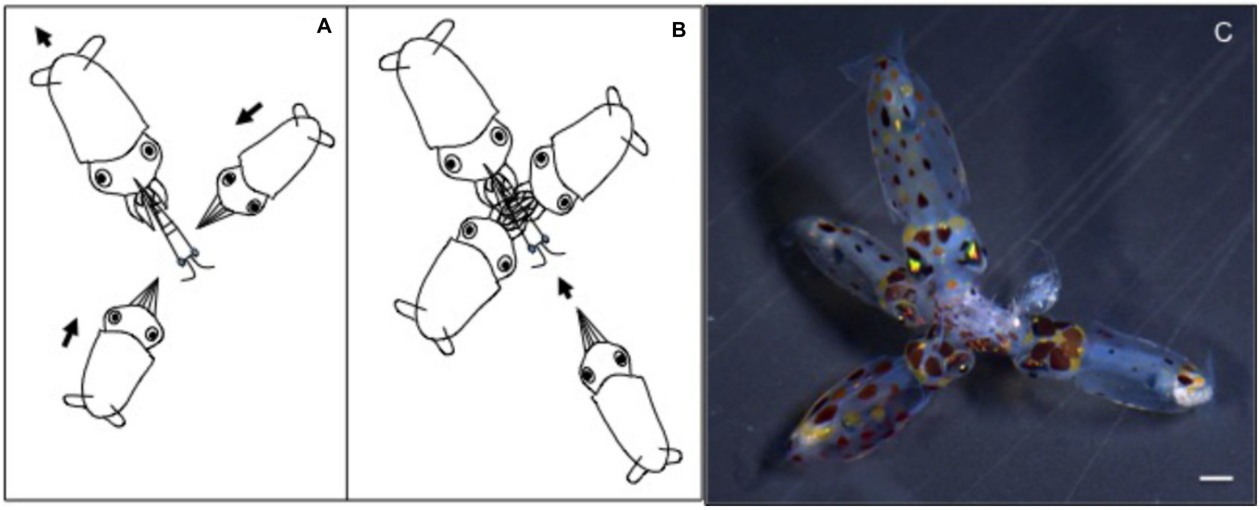


FIGURE 6 | *Doryteuthis opalescens.* Stereotyped group feeding behavior of paralarvae as a result of kleptoparasitism, a competition involving stealing of already-captured prey in possession of another paralarvae (host). **(A)** A large paralarva captures a large prey (mysid shrimp), which cannot be enclosed within the arms. **(B)** Other smaller paralarvae attack the captured prey. **(C)** Several paralarvae feed on the same prey.

TABLE 2 | *Doryteuthis opalescens*.

Age (d)	Starvation period (h)	ML (mm)	Swimming speed (mm s^{-1})	Maximum speed (mm s^{-1})	ML s^{-1}	NGDR	RCD (° s^{-1})
15	–	3.78 ± 0.24	7.50 ± 1.5^a	208	2.00	0.71 ± 0.04^a	275 ± 21^a
16	24	3.91 ± 0.22	8.43 ± 1.8^a	23	2.16	0.74 ± 0.09^b	166 ± 30^b
17	48	3.72 ± 0.35	7.06 ± 1.5^a	18	1.90	0.81 ± 0.05^b	192 ± 50^b
18	72	3.55 ± 0.41	6.07 ± 1.3^b	18	1.82	0.69 ± 0.09^a	303 ± 80^a

Age, mean mantle lengths (ML), swimming speeds, net to gross displacement ratio (NGDR), and rate of change of direction (RCD) of 15 day-old paralarvae starved for 24, 48, and 72 h. Values are means ± SD and means with same superscript letters denote no statistical difference (Mann–Whitney–Wilcoxon test, $p > 0.05$).

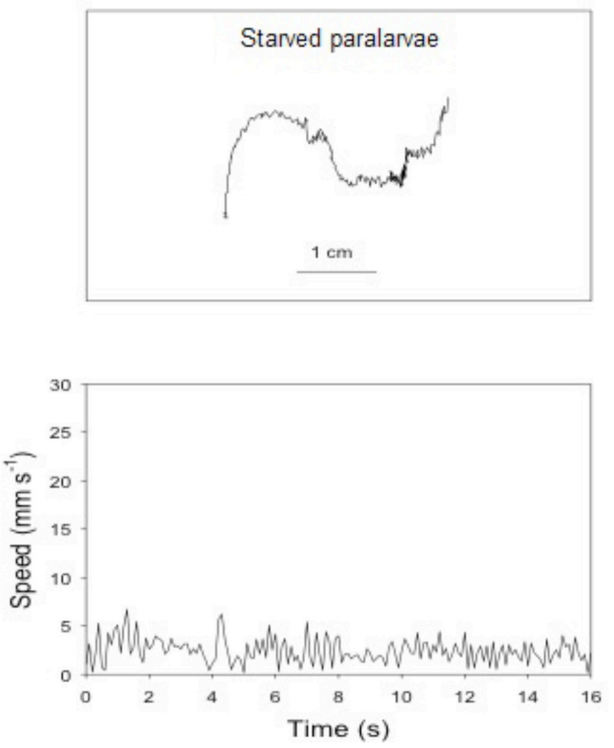


FIGURE 7 | *Doryteuthis opalescens.* Representative swimming path records in the vertical plane and swimming speed patterns (14–16 s duration) of 18-day-old paralarvae starved for 72 h.

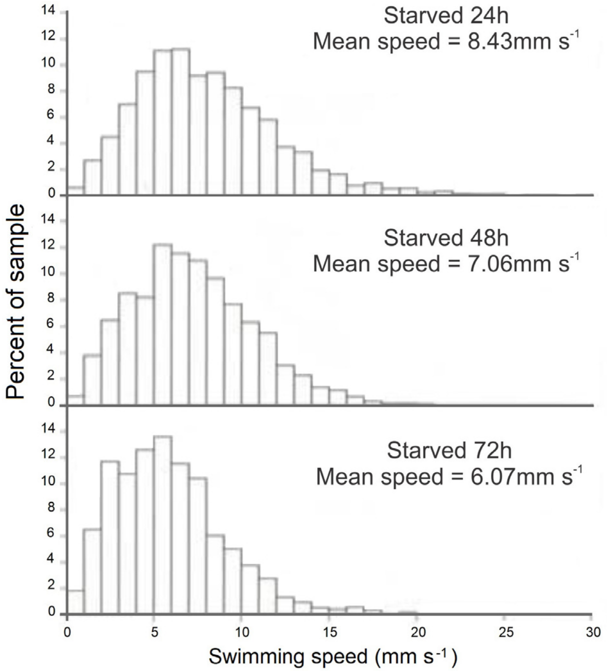


FIGURE 8 | *Doryteuthis opalescens.* Distribution of swimming speeds for 15-day-old paralarvae starved for 24, 48, and 72 h.

Survival Rates

Survival rates at day 10 after hatching were between 70 and 78.4%, and decreased to 54.3–67% on day 20. From then on, survival decreased relatively slowly, reaching 48.2–60.7 at day 40. Final survival rates were between 42 and 59% at day 60. The highest mortality was observed during the first 10 days after hatching and coincided with the no net growth phase during first feeding. A second peak of mortality occurred at day 40.

Relative Growth and Development of Fins

The relative growth of fins was examined in 104 paralarvae from 2.5 to 8.2 mm ML. In hatchlings, the FW represented from 55 to 72% of ML, but in 40-day-old paralarvae (>6.0 mm ML), the FW/ML decreases to 43% and this ratio increases again to 50% by day 50 (**Figure 9** and **Table 3**). No discontinuity point was detected ($F = 3.166$, $p = 0.779$), and the relationship between FW and ML showed an allometric constant of 1.43 (**Figure 9**). Relative GRs for FW were low soon after hatching (0.52% day^{-1}), but increased to 1.21% day^{-1} between 5 and 15 days after hatching and reached even higher rates in 40- and 50-day-old paralarvae, 1.38 and 1.95% day^{-1}, respectively, demonstrating that fins grows faster than the ML in paralarvae older than 15 days of age (>4.0 mm ML; **Table 3**).

Growth in Length and Weight

A decrease in body mass took place soon after hatching due to the exponential rate of yolk utilization. As a result, 5-day-old paralarvae lost 13% of their hatching DW and showed the highest GR in MW, 1.81% day^{-1} (**Figure 10** and **Table 3**). The weight loss was regained over the next days. Thus, no significant

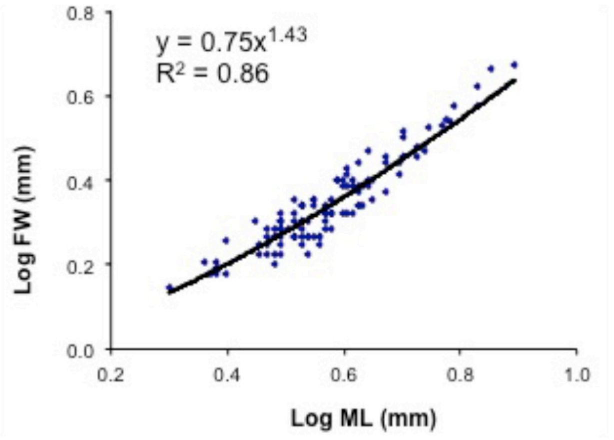


FIGURE 9 | *Doryteuthis opalescens*. Relationship between mantle length (ML) and fin width (FW) in paralarvae reared for 60 days after hatching (n = 104).

increase in weight was observed until day 10 (**Figure 10B**). This represents the no net-growth phase that lasted approximately 10 days at 16°C (Vidal et al., 2002a). By day 15, paralarvae were almost threefold heavier than the newly hatched squid with the highest relative GRs for ML and DW for the study (**Table 3**). At day 40, MW had the lowest relative growth (**Table 3**), indicating that the proportion of MW to ML was decreasing, as the mantle was growing more slender. The second highest period of relative GRs occurred at day 60. At this time, the squid were growing more rapidly in length and weight (**Table 3**). After the no net-growth phase, growth was exponential at a rate of 6.2% body DW day^{-1} and 2.1% ML day^{-1} (**Figures 10A,B**). Mean body DW reached 16.5 mg at day 60 (**Table 3**). Thus, during the first 60 days of life, *D. opalescens* hatchlings double their mean DW five times and mean ML twice, at 12 and 31 days, respectively (**Figure 10**). The parameters from the length–weight relationships were a = 0.03 and b = 2.80, indicating slightly negative allometric growth ($b < 3$; **Figure 10C**).

TABLE 3 | *Doryteuthis opalescens*.

Age (d)	ML (mm)	ML growth rates (mm day^{-1})	DW (mg)	DW$^{(1/3)}$/ML	DW growth rates (mg day^{-1})	FW (mm)	FW/ML	FW growth rates (mm day^{-1})	MW (mm)	MW/ML	MW growth rates (mm day^{-1})
0	2.65 ± 0.07	–	0.46 ± 0.07	0.29	–	1.90 ± 0.13	0.72	–	1.92 ± 0.26	0.78 ± 0.07	–
5	2.70 ± 0.13	0.37	0.40 ± 0.06	0.27	–2.69	1.95 ± 0.14	0.72	0.52	2.10 ± 0.55	0.72 ± 0.07	1.81
15	3.78 ± 0.24	3.42	1.21 ± 0.32	0.28	11.70	2.20 ± 0.20	0.58	1.21	2.32 ± 0.29	0.63 ± 0.05	0.98
40	6.70 ± 0.92	2.06	5.40 ± 1.94	0.26	6.17	2.90 ± 0.53	0.43	1.38	2.66 ± 0.73	0.45 ± 0.05	0.55
50	7.23 ± 1.15	1.35	8.20 ± 2.01	0.28	4.27	3.60 ± 0.81	0.50	1.95	–	–	–
60	9.81 ± 1.41	3.17	16.50 ± 2.39	0.26	7.24	–	–	–	–	0.37 ± 0.02	1.11

Growth rates of paralarvae reared from 0 to 60 days after hatching. Means were obtained from samples of 15 to 40 paralarvae per age. Mantle length (ML), dry weight (DW), fin width (FW), mantle width (MW).

DISCUSSION

Swimming Ability of Paralarvae and Implications for Dispersal

This study documents the progressive development of swimming abilities in *D. opalescens* paralarvae as they undergo complex morphological, behavioral, and ecological changes that enable them to swim in schools. We detailed the transition from the pulsed jet-and-sink motion of paralarvae to the burst-and-coast swimming of juveniles. This significant improvement of swimming ability occurs within the first 2 months of life. Swimming faster and with more control co-occurs with growth (rapid increase of ML and fin size, changes in mantle muscle morphology; Preuss et al., 1997; Thompson and Kier, 2001, 2006), social interactions, and cognition capabilities. These three factors lead to the shift in paralarvae physiological ecology from plankton to nekton. Most importantly, the ecological implications of the swimming performance of paralarvae convey that they are competent to exert influence over fine-scale distribution and population dispersal by active swimming.

During ontogeny, an important factor changing as paralarvae increase in size is their hydrodynamic environment and the varying effects of viscosity and inertia expressed in the Re number. Hatchlings have bell shape (the highest MW/ML ratio), rudimentary fins when compared to juveniles and large yolk reserves (40–60% of their body dry weight; Vidal et al., 2002a) that restrict the volume of water held in their mantle cavities. Therefore, they are almost completely dependent upon constant short pulsed jet for locomotion (Bartol et al., 2009). The shape and size of hatchlings and their limited swimming ability are ideal traits for exploiting passive transport and dispersal by currents.

After yolk is fully absorbed, jetting is improved as paralarvae can hold proportionally more water in their mantle cavities; simultaneously predatory behavior (Vidal et al., 2002a), social interactions, and escape responses are intensified. The expression of these crucial activities results in more horizontal displacements and often demand burst SS. Average cruising speeds of paralarvae are within intermediate Re numbers ($10 < Re < 200$), in which both viscous and inertial flow forces play important roles. However, we have shown that >6 mm ML squid move in the inertia-dominated realm during escape jets (i.e., the zone of Re >200, reaching Re >3200).

A major change in shape and swimming performance of paralarvae takes place between 15 and 40 days post-hatching. Squid >40 days (>6 mm ML) develop the adult rocket-shaped body and are able to attain higher speeds. Indeed, average and maximum speeds increased considerably with age and size, allowing late paralarvae to cover short horizontal distances at higher speed (562 mm s^{-1}). This substantial improvement of swimming performance allows paralarvae to occasionally escape from the intermediate Re realm during burst speed and operate under different flow regimes, suggesting that morphological changes in body shape incur in swimming performance. Late paralarvae high-speed escape ability should provide squid significant ecological advantage in terms of evading their predators.

We have demonstrated that swimming abilities of squid develop early in life, emphasizing that the passive drifting dispersal period of paralarvae is brief, as they form schools at 35–40 days of age (>6 mm ML). Studies have shown that *D. opalescens* paralarvae migrate to the surface in the first 6 h after hatching (Sidie and Holloway, 1999) and perform vertical diel migrations from 30 m (day) to the surface (night) by 14 days (Zeidberg and Hamner, 2002). The highest abundances of paralarvae are found in the neuston layer at night associated with cooler SST (13–16.5°C; Koslow and Allen, 2011; Van Noord and Dorval, 2017). Our experimental results are consistent with field studies and attest that paralarvae can actively influence their fine-scale distribution by migrating vertically, becoming aggregated in areas of high food availability and adjust their dispersal patterns in the field.

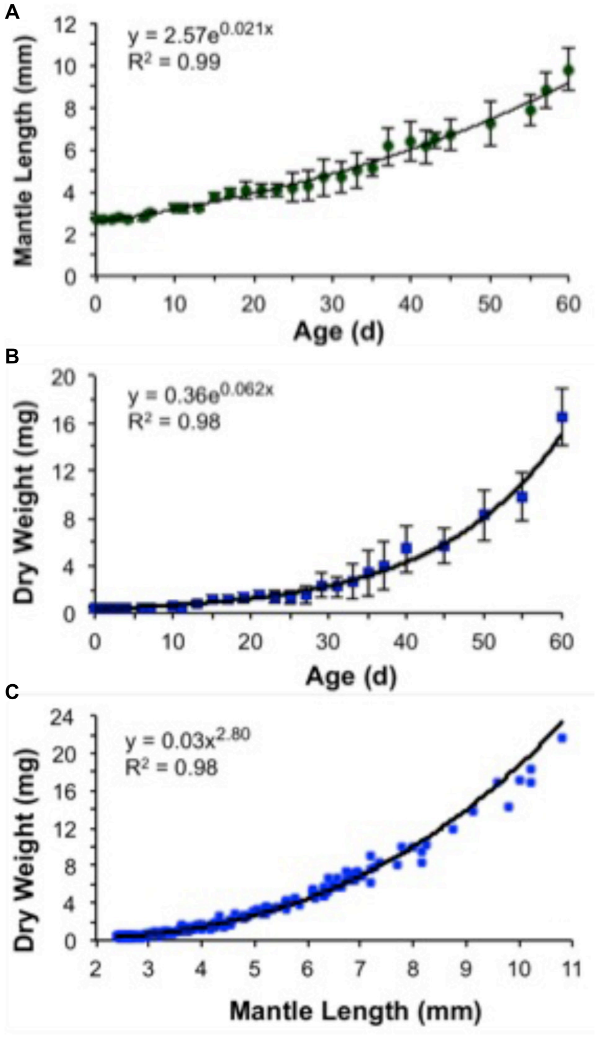


FIGURE 10 | *Doryteuthis opalescens*. Growth rates of paralarvae reared for 60 days after hatching at 16 ± 1°C. **(A)** Mantle length versus age (values are means of 5–7 paralarvae ± SD), **(B)** dry weight versus age (values are means of 5–7 paralarvae ± SD), and **(C)** dry weight versus mantle length ($n = 185$).

Survival, Growth, and Swimming Performance

During the first 60 days of this study, paralarvae grew from 3 to 10 mm ML and doubled their DW five times (a near 40-fold increase). GRs were obtained at a rearing temperature of 16°C and would change depending on the mean temperature experienced by paralarvae in nature. Survival was the highest ever registered during any rearing experiments with loliginid squid (Yang et al., 1986; Vidal et al., 2002b). However, two main points of mortality were observed at 10 and 40 days after hatching; the first was related to the transition to exogenous feeding and the second possibly caused by the need for larger prey types (Vidal et al., 2002b).

The swimming performance and the motion paths of paralarvae changed considerably with development. Hatchlings drift with the current, exhibiting a more random motion and no coordinated swimming with nearby squid. During development, the interplay between fins, mantle contractions, and funnel action of paralarvae shifts from a physiology evolved for aerobic depth maintenance to inertia based gliding with the occasional need for anaerobic bursts of speed (Bartol, 2001; Thompson and Kier, 2001). There is a clear transition in swimming behavior with the ability to perform sustainable swimming and to school.

The large size of the fins relative to the ML in >40-day-old paralarvae promote enhanced swimming control necessary for the synchronized and fine-motor control swimming of schooling squid. Fins act as stabilizers and allow rapid braking by producing drag, generate lift at lower speeds to lessen negative buoyancy (Thompson et al., 2010; Bartol et al., 2016). The rate of fin beating nearly doubled between days 0 and 40, and then increased again by 27% by day 60, which occurs concurrently with significant changes in the motion pattern parameters and NND. Indeed, by 60 days, the swimming path has become finely controlled.

Fins are versatile – playing different roles depending on the swimming orientation and velocity. This was demonstrated in a study with adult *Lolliguncula brevis*, when squid are swimming backward in the tail-first position, fins function as stabilizers at low speeds, and propulsors at high speeds, while also providing net lift to hold the squid in a vertical position; but when squid are swimming forward arms-first, fins provide mainly lift and thrust to assist jetting (Anderson and Demont, 2005; Stewart et al., 2010). Squid are highly maneuverable and the coordination between jet and fins are the primary drivers of turning performance, which are involved both in prey capture and escape behavior (Jastrebsky et al., 2016). Accordingly, our results have shown a progressive development of fins during ontogeny, demonstrating that fin development is a requisite for sustained swimming and dynamic stability (Stewart et al., 2010) and the synchronized and maneuverable swimming of schooling early juveniles.

The SS obtained in the present study were similar to some other studies of cephalopod paralarvae, with average speed of 5–11 mm s^{-1} and maximum speed reaching 560 mm s^{-1}. Maximum speed in *Octopus vulgaris* increased from 79.7 mm s^{-1} in hatchlings (2.0 mm ML) to 456.7 mm s^{-1} in 30-day-old paralarvae (4.5 mm ML) and then decreased when they were close to settlement (Villanueva et al., 1996). *Loligo forbesi* hatchlings (3.7 mm ML) can swim at average speeds of 6–20 mm s^{-1} and are able to attain a maximum speed of 250 mm s^{-1} (Zuev, 1964), while those of *L. vulgaris* can reach 160 mm s^{-1} in backward jets (Packard, 1969), and *D pealeii* (1.8 mm ML) have an average speed of 8.3 mm s^{-1} (Zakroff et al., 2018), but can reach up to 30.5 mm s^{-1} (Bartol et al., 2009). Ommastrephid hatchlings are among the smallest of the cephalopods; however, *Illex illecebrosus* (∼1.2 mm ML) and *Dosidicus gigas* can attain average and maximum speeds of 10 and 50 mm s^{-1}, respectively (O'Dor et al., 1986; Staaf et al., 2008). By contrast, large hatchlings such as *Sepioteuthis lessoniana* (6.0 mm ML) are able to attain higher average speeds (60–150 mm s^{-1}) during the first 2 months of life (Sugimoto and Ikeda, 2012).

Swimming Performance of Starved Paralarvae

Starved paralarvae progressively lost the ability to swim (**Table 2**). Paralarvae starved for 48–72 h did not perform escape jets and behavior regressed to that similar to newly hatched paralarvae with short swimming paths and an inability to overcome a current (**Table 2**). Paralarvae were able to recover from 48 and 72 h of starvation with a survival rate of 60 and 37%, respectively after 8 days (Vidal et al., 2006). These results emphasize the importance of nutritional condition, as well as yolk reserves at hatching on studies of swimming behavior. Most importantly, they suggest that depending on the duration of the starvation period, unnourished squid would not be able to keep pace with schooling squid and thus would be at higher threat of predation without the protection of the school.

Formation of Schools and Ecological Implications

Schooling was first noticed in 35- to 45-day-old paralarvae (6–7 mm ML). It is noteworthy that schools were always formed against the current, near the site of the inflow of water, where the currents were at their maximum velocity. This provides evidence that paralarvae might be capable of sensing flow (current shear) and are attracted to it, or their natural swimming pattern caused them to become aggregated in the highest gradients of flow. Loliginid squid have analogs of lateral lines on their heads and arms that contain epidermal hair cells that directly respond to sinusoidal water movements, which correspond to the sensitivity of fish lateral lines (Budelmann and Bleckmann, 1988) and ablation of these receptors increase the chance of predation in dark environments (York and Bartol, 2014).

Squid paralarvae are negatively buoyant (Sidie and Holloway, 1999; Martins et al., 2014), as a result, paralarvae sink when swimming stops and must constantly jet to stay suspended and this is energetically costly. Forming schools early in life in areas of gradients of flow (vertical turbulent mixing) could assist paralarvae in holding position, minimizing the energy allocated to jetting and aggregate them with their food in upwelling areas (Zuev, 1964; Koslow and Allen, 2011; Van Noord and Dorval, 2017). Indeed, *D. opalescens* paralarvae have been found aggregated within cyclonic gyres and eddy-induced upwelling in the Southern California Bight, where vertical mixing occurs at the frontal zone of warm and cold waters (Zeidberg and Hamner, 2002).

Schools were formed positioned against the current, which seems advantageous, as planktonic prey drifting with the current are brought toward the arms of squid positioned side-by-side. This suggests that there may be a close interplay between swimming performance, flow gradients, and foraging tactics of juvenile squid, representing a highly interesting topic for future investigations.

Yang et al. (1986) reared market squid through their lifecycle in larger tanks with higher current speeds. These researchers first noticed the ability to hold position at 40–45 days (10 mm ML) and to form schools by days 60–80 (15 mm ML). Our tanks had lower flow rates (1.0 cm s^{-1}), thus the earliest possible school formation seems to be determined by size and the local currents, both related to sustained swimming ability (position holding). Nevertheless, there is a similarity between the sizes at which both *D. opalescens* and *Illex argentinus* form schools to forage. An exceptionally high density of *I. argentinus* juveniles of ∼10 mm ML was found associated with elevated plankton production in an upwelling area off southern Brazil (Vidal et al., 2010). This highlights the importance of size, rather than age, in the ability of squid to swim against a current and to form schools. Interestingly, by evaluating the effects of mantle and funnel aperture in a theoretical model of squid jet propulsion, Staaf et al. (2014) have proposed that the maximum efficiency of squid jet propulsion is found at 10 mm ML, which is a ML slightly larger than the ML size at which both *I. argentinus* and *D. opalescens* start to swim in schools. As the capability to rear squid improves the next step would be to study the key transition that occurs at 20–30 mm ML (Vidal, 1994; Moltchaniwskyj, 1995; Zeidberg, 2004). This is probably the point where squid attain a size that affords them inertial dominated movement for cruising speeds and a shift to a fish diet (Karpov and Cailliet, 1979).

Swimming Behavior and Social Interactions

Changes in paralarvae swimming pattern and ability during ontogeny were accompanied by changes in social interactions associated with the formation of schools. These interactions provided insights into how squid acquire complex cognitive and fine-motor skills during ontogeny. Early paralarvae (0- to 15-day-old) swim with a relatively large NND and display aggressive behavior toward other paralarvae swimming nearby. Aggressive behavior and NND decrease progressively with growth. When paralarvae grow to a size that allows sustained swimming, they hover in place longer and often assume a similar body orientation as close neighbors. The close proximity and the parallel orientation soon promote enhanced polarization and synchronization, culminating with the formation of schools. These findings are consistent with the observations of Sugimoto and Ikeda (2012) for reared *S. lessoniana*. In the present study, however, the first squid to form schools swim in a more vertical orientation, perhaps a reflection of the slow current speed and tank configuration. Nevertheless, when the schools dispersed due to any disturbance, juveniles swam backward horizontally (lower angles) at higher speeds.

Another important feature of *D. opalescens* schools is that although more than one school was observed in a tank, each school was size assorted and the largest squid were frequently at the front, similar to the hierarchical structure observed in adult *S. lessoniana* (Boal and González, 1998). This hierarchical social organization underscores enhanced learning and cognitive capabilities (Ikeda, 2009; Sugimoto and Ikeda, 2012; Oshima et al., 2016). Indeed, social feeding interactions are often observed before the formation of schools in same-aged but different sized paralarvae. Large paralarvae frequently capture larger prey, which cannot be enclosed within the arms, facilitating kleptoparasitism (**Figure 6**). This social interaction provides smaller paralarvae the possibility of feeding on large prey items that they could not capture alone and that large paralarvae would not ingest entirely, maximizing resources (Vidal and Boletzky, 2014). Furthermore, this behavior denotes enhanced foraging repertoire based on learning through social interactions and could, ultimately, determine the hierarchical social organization of schools; large faster growing squid are the first to perform sustainable swimming and others follow. Kleptoparasitism is also common during rearing of *O. vulgaris* paralarvae (Vidal, EAG pers. obs.) and was recently reported for captive *Todarodes pacificus* schooling adults (Vijai et al., 2017). Nevertheless, field studies are required to provide a better understanding of this behavior.

Hunting behaviors develop in paralarvae in concert with changes in growth, muscle structure, swimming refinement, and social interactions. Paralarvae learn to catch copepods by honing predatory behaviors with a large increase in mortality at each developmental transition (Chen et al., 1996). It is perhaps significant that the adult-like tentacular strike behavior is only employed in prey capture in squid older than 40 days (Chen et al., 1996; Kier, 1996). The expression of this important behavior correlates well with our results on fin GR relative to the ML (**Table 3**), the ability to form schools and attain SSs that corresponds to Re >3200.

The progressive development of swimming abilities and social interactions described here may enable squid to accelerate learning, orientation, and cognition by amplifying fundamental social information from an early life stage. Morphological, neurophysiological, and sensory capabilities are required to perform parallel synchronized swimming with nearest neighbors while schooling (Pitcher and Parrish, 1993; Oshima et al., 2016). Earlier studies with fish have shown that vision along with the lateral line are of major importance for maintaining a particular position and orientation with respect to neighbors, while the lateral line has a key role in monitoring SSs and direction of other fish in the school (Partridge and Pitcher, 1980; Pitcher and Parrish, 1993).

The highly coordinated motion of juvenile squid observed in the present study during school formation, when the school scatters due to a disturbance and then reassembles, represents a rapid assimilation of group information. Schooling squid are constantly synchronizing and fine-tuning their behavior to what their neighbors are doing. Development of group dynamics from individual social interactions is crucial to the understanding of the mechanisms of social behavior. A recent study has shown that schooling decisions and group behavior in *D. pealeii* are influenced by the presence or absence of injured individuals (Oshima et al., 2016). Social interactions early in life may serve important adaptive functions of squid schools (Adamo and Weichelt, 1999; Sugimoto and Ikeda, 2012; Sugimoto et al., 2013).

CONCLUSION

This study documented key events of survival and growth during early ontogeny of *D. opalescens*. Paralarvae undergo major and complex morphological, behavioral, and ecological changes during their first month of life, which may be requisites for schooling behavior. Our results revealed a progressive development of swimming abilities in squid from the random jet-and-sink swimming pattern of hatchlings with large NND to the finely controlled, parallel, and synchronized movements of schooling early juveniles at shorter NND. We have also shown that the feeding condition influences the swimming performance and behavior of paralarvae. In addition, the high relative GRs of mantle and fins in >15-day-old paralarvae lead to transformation in body shape (from a bell to a rocket) and enhanced SS and control, suggesting that morphological changes incur in swimming performance, enabling squid to perform sustained swimming. This event represents a key ecological and behavioral transition that occurs at about 6 mm ML and correlates well with the ability of paralarvae to reach high Re (>3200) during escape jets, transitioning to the inertia-dominated realm and from plankton to nekton. The main features of *D. opalescens* schools formed at an early age (35- to 45-day-old) were their hierarchical configuration (with the largest squid swimming at front), site of formation (at the highest gradients of flow inside the tanks), and positioned against the current. This provides evidence that paralarvae might be capable of sensing flow and deserves future investigations to improve our understanding of their sensory capabilities and fine-scale distribution. The results also suggest that the passive drifting period of squid is brief as paralarvae are competent to control their distribution and dispersal just after their first month of life. Social interactions prior to and during schooling provided insights into how squid acquire sophisticated cognitive and fine motor skills during ontogeny. Formation of schools at an early-life stage seems to be an adaptation for optimizing energy employed in foraging tactics and amplifying social information, thus serving important ecophysiological functions.

AUTHOR CONTRIBUTIONS

EAGV conceived and designed the study, conducted the experiments, collected and analyzed the data, and drafted the article. LZ helped with data analysis to draft the article and made improvements to the article. EB conceived and designed the study and helped with data analysis. All authors worked together to interpret the findings and approved the final version.

FUNDING

EAGV was supported by the Brazilian National Research Council (CNPq; Grant # 311183/2014-0). This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

ACKNOWLEDGMENTS

We would like to thank the substantial contributions made by the reviewers and the handling Editor, Dr. Richard Emlet. EAGV also thanks the Brazilian National Research Council – CNPq.

REFERENCES

Adamo S. A., and Weichelt K. J. (1999). Field observations of schooling in the oval squid, *Sepioteuthis lessoniana* (Lesson, 1830). *J. Molluscan. Stud.* 65, 377–380. doi: 10.1093/mollus/65.3.377

Anderson, E. J., and Demont, M. E. (2005). The locomotory function of the fins in the squid *Loligo pealei*. *Mar. Freshw. Behav. Phys.* 38, 169–189. doi: 10.1080/10236240500230765

Anderson, E. J., and Grosenbaugh, M. A. (2005). Jet flow in steadily swimming adult squid. *J. Exp. Biol.* 208, 1125–1146. doi: 10.1242/jeb.01507

Bartol, I. K. (2001). Role of aerobic and anaerobic circular muscle fibers in swimming squid: electromyography. *Biol. Bull.* 200, 59–66. doi: 10.2307/1543085

Bartol, I. K., Krueger, P. S., Jastrebsky, R. A., Williams, S., and Thompson, J. T. (2016). Volumetric flow imaging reveals the importance of vortex ring formation in squid swimming tail-first and arms-first. *J. Exp. Biol.* 219, 392–403. doi: 10.1242/jeb.129254

Bartol, I. K., Krueger, P. S., Stewart, W. J., and Thompson, J. T. (2009). Pulsed jet dynamics of squid hatchlings at intermediate reynolds numbers. *J. Exp. Biol.* 212, 1506–1518. doi: 10.1242/jeb.026948

Bartol, I. K., Krueger, P. S., Thompson, J. T., and Stewart, W. J. (2008). Swimming dynamics and propulsive efficiency of squids throughout ontogeny. *Integr. Comp. Biol.* 48, 720–733. doi: 10.1093/icb/icn043

Berry, S. S (1911). Notes on some cephalopods in the collection of the University of California. *Univ. Calif. Publ. Zool.* 8, 301–310.

Boal, J. G., and González, S. A. (1998). Social behavior of individual oval squids (Cephalopoda,Teuthoidea, Loliginidae, *Sepioteuthis lessoniana*) within a captive school. *Ethology* 104, 161–178. doi: 10.1111/j.1439-0310.1998.tb00059.x

Boidron-Métairon, I. F. (1995). "Larval nutrition," in *Ecology of Marine Invertebrate Larvae*, ed. L. R. McEdward (Boca Raton, FL: CRC Press), 223–248.

Budelmann, B. U., and Bleckmann, H. (1988). A lateral line analogue in cephalopods: water waves generate microphonic potentials in the epidermal head lines of sepia and lolliguncula. *J. Comp. Physiol. A* 164, 1–5. doi: 10.1007/BF00612711

Buskey, E. J., Coulter, C., and Strom, S. (1993). Locomotory patterns of microzooplankton: potential effects on food selectivity of larval fish. *Bull. Mar. Sci.* 53, 29–43.

Chen, D. S., Van Dykhuizen, G., Hodge, J., and Gilly, W. F. (1996). Ontogeny of copepod predation in juvenile squid (*Loligo opalescens*). *Biol. Bull.* 190, 69–81. doi: 10.2307/1542676

Forsythe, J. W., and Hanlon, R. T. (1988). Behavior, body patterning and reproductive biology of *Octopus bimaculoides* from California. *Malacologia* 29, 1–55.

Hoar, J. A., Sim, E., Webber, D. M., and O'Dor, R. K. (1994). "The role of fins in the competition between squid and fish," in *Mechanics and Physiology of Animal Swimming*, eds L. Maddock, Q. Bone, and J. M. C. Rayner (Cambridge: University Press), 27–33. doi: 10.1017/CBO9780511983641.004

Hurley, A. C. (1978). School structure of the squid *Loligo opalescens*. *Fish. Bull.* 76, 433–442.

Ikeda, Y. (2009). A perspective on the study of cognition and sociality of cephalopod mollusks, a group of intelligent marine invertebrates. *Jpn. Psychol. Res.* 51, 146–153. doi: 10.1111/j.1468-5884.2009.00401.x

Jastrebsky, R. A., Bartol, I. K., and Krueger, P. S. (2016). Turning performance in squid and cuttlefish: unique dual- mode, muscular hydrostatic systems. *J. Exp. Biol.* 219, 1317–1326. doi: 10.1242/jeb.126839

Karpov, K. A., and Cailliet, G. M. (1979). Prey composition of the market squid, *Loligo opalescens* Berry, in relation to depth and location of capture, size of squid, and sex of spawning squid. *CaCOFI Rep.* 20, 51–57.

Kier, W. M. (1996). Muscle development in squid: ultrastructural differentiation of a specialized muscle fiber type. *J. Morphol.* 229, 271–288. doi: 10.1002/(SICI)1097-4687(199609)229:3

Kilkenny, C., Browne, W. J., Cuthill, I. C., Emerson, M., and Altman, D. G. (2010). The ARRIVE guidelines animal research: reporting in vivo experiments. *PLoS Biol.* 8:e1000412. doi: 10.1371/journal.pbio.1000412

Koslow, J. A., and Allen, C. (2011). The influence of the ocean environment on the abundance of market squid, *Doryteuthis (Loligo) opalescens*, paralarvae in the southern California bight. *CalCoFi Rep.* 52, 205–213. doi: 10.1371/journal.pone.0118992

Martins, R. S., Roberts, M. J., Chang, N., Verley, P., Moloney, C. L., and Vidal, E. A. G. (2014). Effect of yolk utilization on the specific gravity of chokka squid (*Loligo reynaudii*) paralarvae: implications for dispersal on the Agulhas Bank, south Africa. *Fish. Ocean* 23, 116–131. doi: 10.1093/icesjms/fsq098

Masuda, R., Shoji, J., Nakayama, S., and Tanaka, M. (2003). Development of schooling behavior in Spanish mackerel *Scomberomorus niphonius* during early ontogeny. *Fish. Sci.* 69, 772–776. doi: 10.1046/j.1444-2906.2003.00685.x

Moltchaniwskyj, N. A. (1995). Multiple spawning in the tropical squid *Photololigo* sp.: what is the cost in somatic growth? *Mar. Biol.* 124, 127–135. doi: 10.1007/BF00349154

O'Dor, R. K., Foy, E. A., Helm, P. L., and Balch, N. (1986). The locomotion and energetics of hatchling squid, *Illex illecebrosus*. *Am. Mal. Bull.* 4, 55–60.

Olcay, A. B., and Malazi, M. T. (2016). The effects of a longfin inshore squid's fins on propulsive efficiency during underwater swimming. *Ocean Eng.* 128, 173–182. doi: 10.1016/j.oceaneng.2016.10.037

Oshima, M., von Treuheim, T. P., Carroll, J., Hanlon, H. T. T., Walters, T. E., and Crook, R. J. (2016). Peripheral injury alters schooling behavior in squid, *Doryteuthis pealeii*. *Behav. Process.* 128, 89–95. doi: 10.1016/j.beproc.2016.04.008

Packard, A. (1969). Jet propulsion and the giant fibre response of *Loligo*. *Nature* 221, 875–877. doi: 10.1038/221875a0

Partridge, B. L., and Pitcher, T. J. (1980). The sensory basis of fish schools: relative roles of lateral line and vision. *J. Comp. Physiol.* 135, 315–325. doi: 10.1007/BF00657647

Pitcher, T. J., and Parrish, J. K. (1993). "Functions of shoaling behavior in teleosts," in *Behavior of Teleost Fishes*, 2nd Edn, ed. T. J. Pitcher (London: Chapman and Hall), 363–439. doi: 10.1007/978-94-011-1578-0_12

Preuss, T., Lebaric, Z. N., and Gilly, W. F. (1997). Post-hatching development of circular mantle muscles in the squid *Loligo opalescens*. *Biol. Bull.* 192, 375–387. doi: 10.2307/1542747

Roper, C. F. E., and Voss, G. L. (1983). Guidelines for taxonomic descriptions of cephalopod species. *Mem. Natl. Mus. Vic.* 44, 48–63. doi: 10.24199/j.mmv.1983.44.03

Ryley, L. (2015). Review of selected California fisheries for 2014: coastal pelagic finfish, market squid, groundfish, pacific herring, dungeness crab, ocean salmon, true smelts, hagfish, and deepwater ROV surveys of MPAs and surrounding nearshore habitat. *CalCOFI Rep.* 56, 1–30.

Scheltema, R. S. (1986). On dispersal and planktonic larvae of benthic invertebrates: an eclectic overview and summary of problems. *Bull. Mar. Sci.* 39, 290–322.

Sharqawy, M. H., Lienhard, J. H., and Zubair, S. M. (2010). Thermophysical properties of seawater: a review of existing correlations and data. *Desalin. Water Treat.* 16, 354–380. doi: 10.5004/dwt.2010.1079

Sidie, J., and Holloway, B. (1999). Geotaxis in the squid hatchling *Loligo pealei*. *Am. Zool.* 39:74A.

Sokal, R. R., and Rohlf, F. J. (1995). *Biometry: The Principles and Practice of Statistics in Biological Research*. New York, NY: W.H. Freeman.

Staaf, D. J., Camarillo-Coop, S., Haddock, S. H. D., Nyack, A. C., Payne, J., Salinas-Zavala, C. A., et al. (2008). Natural egg mass deposition by the humboldt squid (*Dosidicus gigas*) in the gulf of California and characteristics of hatchlings and paralarvae. *J. Mar. Biol. Assoc. U.K.* 88, 759–770. doi: 10.1017/S0025315408001422

Staaf, D. J., Gilly, W. F., and Denny, M. W. (2014). Aperture effects in squid jet propulsion. *J. Exp. Biol.* 217, 1588–1600. doi: 10.1242/jeb.082271

Stewart, W. J., Bartol, I. K., and Krueger, P. S. (2010). Hydrodynamic fin function of brief squid, *Lolliguncula brevis*. *J. Exp. Biol.* 213, 2009–2024. doi: 10.1242/jeb.039057

Sugimoto, C., and Ikeda, Y. (2012). Ontogeny of schooling behavior in the oval squid *Sepioteuthis lessoniana*. *Fish. Sci.* 78, 287–294. doi: 10.1007/s12562-011-0464-2

Sugimoto, C., Yanagisawa, R., Nakajima, R., and Ikeda, Y. (2013). Observations of schooling behavior in the oval squid *Sepioteuthis lessoniana* in coastal waters of Okinawa Island. *Mar. Biodivers. Rec.* 6:e34. doi: 10.1017/S1755267213000067

Thompson, J. T., Bartol, I. K., Baksi, A. E., Li, K. Y., and Krueger, P. S. (2010). The ontogeny of muscle structure and locomotory function in the long-finned squid *Doryteuthis pealeii*. *J. Exp. Biol.* 213, 1079–1091. doi: 10.1242/jeb.034553

Thompson, J. T., and Kier, W. M. (2001). Ontogenetic changes in mantle kinematics during escape-jet locomotion in the oval squid, *Sepioteuthis lessoniana* Lesson, 1830. *Biol. Bull.* 201, 154–166. doi: 10.2307/1543330

Thompson, J. T., and Kier, W. M. (2006). Ontogeny of mantle musculature and implications for jet locomotion in oval squid, *Sepioteuthis lessoniana*. *J. Exp. Biol.* 209, 433–443. doi: 10.1242/jeb.02017

Van Noord, J. E., and Dorval, E. (2017). Oceanographic influences on the distribution and relative abundance of market squid paralarvae (*Doryteuthis opalescens*) off the southern and central California coast. *Mar. Ecol.* 38:e12433. doi: 10.1111/maec.12433

Vidal, E. A. G. (1994). Relative growth of paralarvae and juveniles of *Illex argentinus* (Castellanos, 1960) in southern Brazil. *Antarct. Sci.* 6, 275–282. doi: 10.1017/S0954102094000416

Vidal, E. A. G., and Boletzky, S. V. (2014). "*Loligo vulgaris* and *Doryteuthis opalescens*," in *Cephalopod Culture*, eds J. Iglesias, L. Fuentes, and R. Villanueva (London: Springer-Verlag), 271–314.

Vidal, E. A. G., DiMarco, F. P., and Lee, P. G. (2006). Effects of starvation and recovery on the survival, growth and RNA: DNA ratio in loliginid squid paralarvae. *Aquaculture* 260, 94–105. doi: 10.1016/j.aquaculture.2006.05.056 doi: 10.1016/j.aquaculture.2006.05.056

Vidal, E. A. G., DiMarco, F. P., Wormuth, J. H., and Lee, P. G. (2002a). Influence of temperature and food availability on the survival, growth and yolk utilization in hatchling squid. *Bull. Mar. Sci.* 71, 915–931.

Vidal, E. A. G., DiMarco, F. P., Wormuth, J. H., and Lee, P. G. (2002b). Optimizing rearing conditions of hatchling loliginid squid. *Mar. Biol.* 140, 117–127. doi: 10.1007/s002270100683

Vidal, E. A. G., Haimovici, M., and Hackbart, V. (2010). Distribution of paralarval and small juvenile cephalopods in relation to primary production in an upwelling area off southern Brazil. *ICES J. Mar. Sci.* 67, 1346–1352. doi: 10.1093/icesjms/fsq080

Vijai, D., Puneeta, P., Matsui, H., Morooka, M., and Sakurai, I. (2017). Intraspecific kleptoparasitism in squid school and schooling behavior of its prey. *Mar. Biodiv.* 47, 637–638. doi: 10.1007/s12526-016-0549-3

Villanueva, R., Nozais, C., and Boletzky, S. V. (1996). Swimming behavior and food searching in planktonic *Octopus vulgaris* cuvier from hatching to settlement. *J. Exp. Mar. Biol. Ecol.* 208, 169–184. doi: 10.1016/S0022-0981(96)02670-6

Villanueva, R., Vidal, E. A. G., Fernández-Álvarez, F. Á, and Nabhitabhata, J. (2016). Early mode of life and hatchling size in cephalopod molluscs: influence on the species distributional ranges. *PLoS One* 11:e0165334. doi: 10.1371/journal.pone.0165334

Yang, W. T., Hanlon, R. H., Krejci, M. E., Hixon, R. F., and Hulet, W. H. (1986). Growth, behavior, and sexual maturation of the market squid, *Loligo opalescens*, cultured through the life cycle. *Fish. Bull.* 84, 771–798.

Yasumuro, H., Nakatsuru, S., and Ikeda, Y. (2015). Cuttlefish can school in the field. *Mar. Biol.* 162, 763–771. doi: 10.1007/s00227-015-2622-z

York, C. A., and Bartol, I. K. (2014). Lateral line analogue aids vision in successful predator evasion for the brief squid, *Lolliguncula brevis*. *J. Exp. Biol.* 217, 2437–2439. doi: 10.1242/jeb.102871

Zakroff, C., Mooney, T. A., and Wirth, C. (2018). Ocean acidification responses in paralarval squid swimming behavior using a novel 3D tracking system. *Hydrobiologia* 808, 83–106. doi: 10.1007/s10750-017-3342-9

Zar, J. H. (1996). *Biostatistical Analysis*. Upper Saddle River, NJ: Prentice-Hall.

Zeidberg, L. D. (2004). Allometry measurements can determine the size and swimming speeds of juvenile and adult *Loligo opalescens* (Cephalopoda: Myopsida) squid from in situ video. *J. Exp. Biol.* 207, 4195–4203. doi: 10.1242/jeb.01276

Zeidberg, L. D. (2014). "*Doryteuthis opalescens*, opalescent inshore squid," in *Advances in Squid Biology, Ecology and Fisheries, Part I Myopsida Squids*, eds R. Rosa, G. Pierce, and R. O'Dor (New York, NY: Nova Science Publishers), 159–204.

Zeidberg, L. D., and Hamner, W. M. (2002). Distribution of squid paralarvae, *Loligo opalescens* (Cephalopoda: Myopsida), in the southern California bight in the three years following the 1997 El Niño. *Mar. Biol.* 141, 111–122. doi: 10.1007/s00227-002-0813-x

Zuev, G. V. (1964). On the ability of cephalopod larvae to active movement. *Zool. Z.* 43, 1440–1445.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

ORIGINAL RESEARCH
published: 01 December 2017
doi: 10.3389/fphys.2017.00981

Maternal and Embryonic Stress Influence Offspring Behavior in the Cuttlefish *Sepia officinalis*

Caitlin E. O'Brien, Christelle Jozet-Alves, Nawel Mezrai, Cécile Bellanger, Anne-Sophie Darmaillacq and Ludovic Dickel*

Normandie Univ., UNICAEN, Rennes 1 Univ., UR1, CNRS, UMR 6552 ETHOS, Caen, France

Stress experienced during prenatal development—either applied to reproducing females (maternal stress), directly to developing offspring (embryonic stress) or in combination—is associated with a range of post-natal behavioral effects in numerous organisms. We conducted an experiment to discern if maternal and embryonic stressors affect the behavior of hatchlings of the cuttlefish *Sepia officinalis*, a species with features that allow for the examination of these stress types in isolation. Separating the impact of stress transmitted through the mother vs. stress experienced by the embryo itself will help clarify the behavioral findings in viviparous species for which it is impossible to disentangle these effects. We also compared the effect of a naturally-occurring (predator cue) and an "artificial" (bright, randomly-occurring LED light) embryonic stressor. This allowed us to test the hypothesis that a threat commonly faced by a species (natural threat) would be met with a genetically-programmed and adaptive response while a novel one would confound innate defense mechanisms and lead to maladaptive effects. We found that the maternal stressor was associated with significant differences in body patterning and activity patterns. By contrast, embryonic exposure to stressors increased the proportion of individuals that pursued prey. From these results, it appears that in cuttlefish, maternal and embryonic stressors affect different post-natal behavior in offspring. In addition, the effect of the artificial stressor suggests that organisms can sometimes react adaptively to a stressor even if it is not one that has been encountered during the evolutionary history of the species.

Keywords: body patterning, predation, visual lateralization, activity, threat response

Edited by:
Graziano Fiorito,
Stazione Zoologica Anton Dohrn, Italy

Reviewed by:
Cinzia Chiandetti,
University of Trieste, Italy
Enrico Alleva,
Istituto Superiore di Sanità, Italy

***Correspondence:**
Ludovic Dickel
ludovic.dickel@unicaen.fr

Specialty section:
This article was submitted to Invertebrate Physiology, a section of the journal Frontiers in Physiology

***Received:** 08 September 2017*
***Accepted:** 16 November 2017*
***Published:** 01 December 2017*

Citation:
O'Brien CE, Jozet-Alves C, Mezrai N, Bellanger C, Darmaillacq A-S and Dickel L (2017) Maternal and Embryonic Stress Influence Offspring Behavior in the Cuttlefish Sepia officinalis. Front. Physiol. 8:981. doi: 10.3389/fphys.2017.00981

INTRODUCTION

Stress responses occur in reaction to any external or anticipated threat. In response to a predator, for instance, an animal may increase its metabolism and divert resources to its muscles and away from less critical functions like digestion and foraging behavior—the "fight or flight" stress response (Cannon, 1939). Other kinds of stressors will induce different reactions. In response to food scarcity, for instance, an animal may have the opposite reaction, prioritizing digestive processes to extract the maximum amount of energy from food items and even undertaking risky foraging behavior (Wang et al., 2006). While stress responses have presumably evolved to increase survival in the face of an immediate stressor, there is an increasing awareness that stress responses come with a host of negative fitness consequences. This often depends on whether the stressor causing the response is acute or chronic: A short, single experience of a stressor (e.g., a single encounter with a predator) often produces a short-term, adaptive response while long-term or repeated exposure to stressors (e.g., prolonged food shortage) can have lasting negative impacts on fitness (Jones, 1996; Miller et al., 2007). These costs come from the energetic tradeoffs involved in maintaining the response or in the form of missed opportunities (e.g., lost foraging time, mating opportunities). Chronic and repeated stressors are often associated with reductions in immune function, the advent of various diseases, negative impacts on psychological health and disruptions to normal biological functions (e.g., Katz et al., 1981; Miller et al., 2007; Favreau-Peigné et al., 2014). Thus, understanding the underlying causes and effects of stress responses has implications for medicine, psychology and developmental biology, and is studied in a number of animal models.

The long-term effects of stress that occurs during the embryonic development of an organism are known to be especially significant. Research in a number of vertebrate taxa demonstrates that stress responses in reproducing females can have a strong impact on the behavior of her offspring. In some cases, such stress may serve as an indicator of prospective environment, prompting adaptive changes to the offspring phenotype that help it cope with future challenges. Stress responses can also be associated with reduced offspring fitness; normal developmental processes can be disrupted and the animal may be more susceptible to disease (Gluckman and Hanson, 2004). While the effects of prenatal stress have been relatively well-documented in a number of taxa, it is often unclear if effects observed are the direct result of a stress response in the offspring or a maternally–transmitted effect. One potential mechanism for prenatal stress effects in offspring is the transfer of "stress hormones" (e.g., glucocorticoids, catecholamines) from mother to developing embryo. Such hormones are secreted by animals in response to stressors and affect physiology, behavior and metabolism. Their transfer to offspring via the placenta or egg yolk could explain many of the alterations to offspring phenotype that are sometimes observed (Hayward and Wingfield, 2004; Groothuis et al., 2005; Weinstock, 2008).

Alternatively or in parallel, embryos could be experiencing stressors directly and generating their own stress responses. Where most authors use the term "prenatal stress" to refer to an offspring's response to any stressor experienced during embryonic development, we distinguish between effects of stressors applied to the mother ("maternal stress") and those applied to the offspring themselves ("embryonic stress"). Investigations of stressors applied directly to developing embryos are much less numerous than studies of maternally-applied stress, largely for logistical reasons. By necessity, prenatal stressors must be applied to pregnant or brooding females in many behavioral models, since their embryos develop viviparously or ovoviviparously. Moreover, it has only recently become widely recognized that the embryos of many species are able to perceive and react to stimuli in the surrounding environment, and that this sensory input could provide essential information to prepare for challenges in the postnatal environment (e.g., Mathis et al., 2008). One way to gauge the relative contributions of maternal and embryonic stress responses is to compare their effects in experimental isolation using animal models that are oviparous and autonomous at birth (e.g., many fish, amphibians, precocial birds, and invertebrates). For example, experiments have demonstrated that rainbow trout eggs exposed to stress hormones (comparable to what a stressed mother might produce) result in offspring that are more fearful 5 months after hatching than control animals, although no differences were seen at 2 months (Colson et al., 2015). Likewise, when eggs of the same species were isolated from their mothers and subjected to conspecific alarms cues they demonstrated greater behavioral plasticity than non-stressed controls (Poisson et al., 2017). Therefore, it seems that both maternal and embryonic stressors affect behavior in this species. However, experiments with another species of trout failed to show any differences induced by prenatal stress, suggesting that susceptibility to prenatal stress is not universal across this subfamily (Ghio et al., 2016). By comparing these three studies, we can see that stress effects differ depending on stress type, species, context and age, a finding that likely holds true for other groups as well.

Despite their potential as good study models, there is an unfortunate lack of work with invertebrates, perhaps because invertebrates are sometimes considered unsophisticated and thus unworthy of behavioral study, and because experiments are complicated by the existence of larval phases in many species. The cuttlefish *Sepia officinalis* (Linnaeus, 1758) has neither of these issues. Like other coleoid cephalopods, it is neurologically and behaviorally sophisticated but unlike other coleoids and invertebrates, it has no pelagic larval stage, settling directly on the bottom after hatching (Hanlon and Messenger, 1998). Even more importantly for a potential model for the study of prenatal stress, this species is known to perceive and learn from within the egg (Romagny et al., 2012). A number of embryonic influences have already been identified in cuttlefish. For instance, embryos can develop post-hatching prey preferences and behavioral asymmetries from visual or odor cues (Darmaillacq et al., 2008; Jozet-Alves and Hebert, 2012) and habituate to repeated sensory stimuli, such as light, odor and tactile cues (Romagny et al., 2012). Documenting the effects of maternal and embryonic stress in this species may elucidate general principals about how animal offspring are affected by different types of stress, or indicate that the impact differs according to phylum. In addition, a better understanding of the effects of maternal and embryonic stress in *S. officinalis* would have direct implications for the welfare of cephalopods in aquaculture, laboratories and aquaria. This is important as cephalopods are increasingly recognized as advanced organisms capable of pain and suffering and were recently added to the list of protected animal groups covered by European welfare legislation (Directive 2010/63/EU).

In order to determine whether prenatal stress affects cuttlefish behavior, we subjected reproducing female cuttlefish and their eggs to stressful stimuli. Our primary goal was to determine if female cuttlefish transmit stress effects to their offspring.

Abbreviations: UM-C, Unstressed Mother Control eggs; SM, Stressed Mother eggs; WM, Wild Mother eggs; UM-PE, Unstressed Mother Predator-Exposed eggs; UM-LE, Unstressed Mother Light-Exposed eggs; HI, Heterogeneity Index.

To this end, we compared the offspring of "unstressed" and "stressed" captive females. We also included a group of "wild" eggs in order to assess whether captivity during egg-laying exerts any effects. Our secondary goal was to assess the relevance of stressor type to offspring. We tested the hypothesis that stress responses depend on stress type, particularly how "familiar" it is to the species. We predicted that a naturally-occurring stressor like odor cues from a co-occurring predator species would elicit an adaptive anti-predator response genetically programmed by natural selection. In contrast, we predicted that an artificial stressor would confound innate defense mechanisms and provoke behavioral responses with largely negative effects on fitness. We tested this hypothesis by comparing the effects of an artificial stressor (randomized bouts of bright LED light) to a naturally-occurring one (predator odor) applied to developing embryos. Experiments had already demonstrated that prenatal exposure to predator odor affect the post-natal behavioral lateralization of cuttlefish (Jozet-Alves and Hebert, 2012). LED light was selected as the artificial stressor since it can be detected by late-stage embryos (Romagny et al., 2012) and is likely to be present in aquacultural facilities and laboratories. Immediately after hatching, the offspring from each of these stress groups were tested in a battery of behavioral tests. These tests were chosen to assess a wide range of behaviors thought to be crucial to survival in the wild: body patterning, predation ability, brain lateralization, baseline activity and activity in response to an imminent threat. Behavior was tested during the first 10 days after hatching as this is thought to be the time of highest mortality in the lifecycle of cuttlefish (Bloor et al., 2013).

METHODS

Two different experiments were conducted, one testing for the potential transfer of the effects of captivity or stress from reproducing females to their offspring, and a second exploring the impact of stressors applied directly to developing embryos. In the first experiment, we exposed spawning female cuttlefish to daily removal from the water. We then compared the behavior of their offspring (SM) to that of offspring of a group of captive but unstressed mother controls (UM-C). We also compared both of these groups to offspring from naturally-spawned eggs collected from the wild (WM). While the maternal experience of these eggs was unknown and uncontrolled, their inclusion gives a sense of the effects of maternal capture and captivity (**Figure 1**).

In the second experiment testing embryonic stress, we subdivided eggs from the unstressed control mothers into three groups in order to investigate the effects of stimuli applied directly to embryos. We applied two kinds of stressors: a naturally-occurring stressor consisting of odor cues from common predatory fish (UM-PE) and an artificial stressor consisting of high intensity LED light timed randomly and unpredictably throughout the day and night (UM-LE). These two groups were compared to the unstressed mother control (UM-C) group used in the maternal stress comparisons (**Figure 1**).

After hatching, the effects of prenatal stress treatments on offspring were assessed with a battery of tests covering various aspects of the cuttlefish behavioral repertoire, including body patterning, visual lateralization, predation, activity patterns and fear response. These tests allowed us to make a broad assessment as to whether stressors affect offspring behavior and to make general comparisons between embryonic and maternal stress and between a natural and an artificial stressor. We predicted that the direct experience of an embryonic stressor would have a stronger effect on offspring behavior than maternal stress, which consists of information that must be transmitted indirectly to offspring through the mother. We also expected that cuttlefish would have evolved adaptive responses to the natural embryonic stressor (predator odor), but would demonstrate inappropriate and likely maladaptive responses to the artificial stressor since its response to this stimulus could not have been shaped by natural selection.

Animal Collection and Housing

Adult Females

Cuttlefish traps were set off the coast of France in the English Channel. Thirty seven adult female cuttlefish (*S. officinalis*) were captured between May and June, 2015 and 28 were captured in May, 2016 and transported to the Centre de Recherches en Environnement Côtier (CREC, marine station of the University of Caen, Luc-sur-Mer, France). These females were mated with males and then placed in treatment tanks in a semi-open flow-through seawater system (15 ± 1°C) under a 16:8 h light/dark cycle.

Captured females were split randomly into two groups, and eggs collected from them were designated as "unstressed mother—control" and "stressed mother." The females designated as unstressed mothers (six in 2015 and 12 in 2016) were maintained in large (1,000 L), round tanks enriched with stones, plastic algae, floating objects and plenty of shaded area. In 2015, these females were housed in these tanks singly, but in 2016, the capture of two dozen cuttlefish on a single day necessitated housing in groups of three. Those females assigned to be in the stressed mothers group were isolated in bare tanks (65 L) with a water depth of 19 cm and subjected to randomized 10-s removals from the water three times a day using a specially-made mesh platform. Eggs spawned by these stressed captive females after at least 1 week in these conditions were collected. Four unstressed mothers and four stressed mothers spawned between May 15 and June 9, 2015 and 11 unstressed mothers and eight stressed mothers spawned between May 14 and 29, 2016.

Eggs and Hatchlings

Wild mother eggs (WM) were collected by SCUBA divers from pre-placed tethers in the English Channel (49°19.667N-0°18.767W) in June, 2015 from a depth of 13.7 m. These, along with eggs collected from unstressed and stressed mothers in captivity, were moved to floating trays in 65L tanks (80 × 60 × 40 cm) after 8 h of steady temperature habituation (from 15° to 20°C). These were housed in a darkened room with exposure to the natural light cycle and supplied with seawater from a gently flowing open system and aerated by an airstone. A randomly-selected third of the control mother eggs, designated as controls (UM-C), along with WM and stressed mother SM eggs, were not treated any further. The other two thirds of the control mother

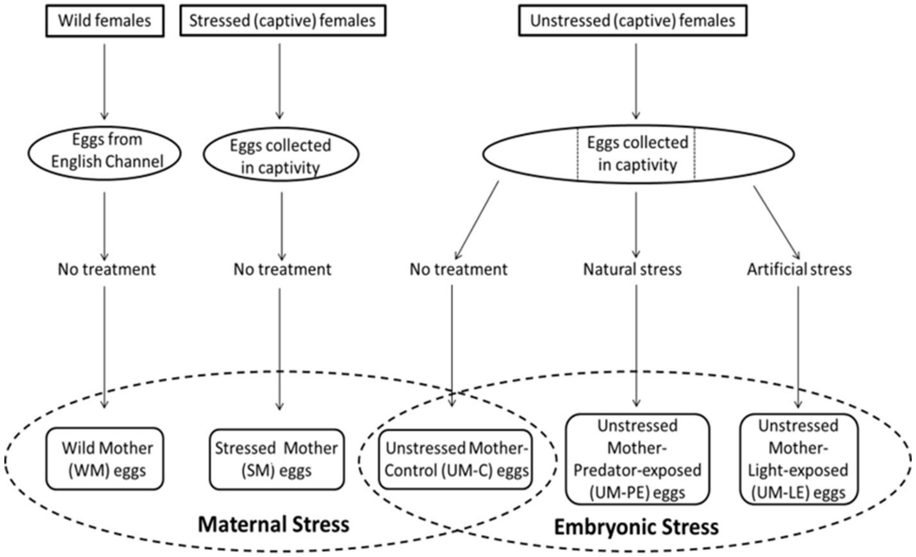


FIGURE 1 | Schematic representation of the experimental design.

eggs were divided randomly into predator-exposed (UM-PE) and light-exposed (UM-LE) groups. Three sea bass (Linnaeus, 1758; Dicentrarchus labrax; total length = 25–30 cm) were housed with UM-PE eggs, separated by a mesh barrier that allowed the eggs chemosensory and visual exposure to the predatory fish. Light-exposed eggs experienced strong LED illumination (20.7klux, approximately 10 cm from surface of water) for 90 min a day (six randomly-timed periods of 15 min). All eggs were gently agitated once a day to remove detritus and discourage parasite growth.

Hatchlings were recorded and collected at 08:00 each morning between June 29 and August 5, 2015, and July 2–24, 2016, and then transferred to a new tank to remove them from any further exposure to the stress treatments. Between experiments, hatchlings and juveniles were maintained in individually-labeled compartments to preserve identity. These compartments were situated in an aerated open seawater system (19–23°C) with a water depth of 7 cm. Sex determination was not possible at this age. All hatchlings born on a single day comprised a daily cohort. A total of 22 cohorts (numbering up to 12 individuals each) were hatched and tested daily between July and August. In 2015, after the predation experiment on Day 4, individuals were fed a single shrimp (Crangon crangon; Linnaeus, 1758) per day. In 2016, hatchlings were fed *ad libitum* starting on Day 4.

Behavioral Experiments

Following the 2 months of prenatal stress treatments described in the previous section, the resulting offspring were subjected to a battery of tests conducted during the first 10 days after hatching (**Figure 2**). These behavioral tests were selected to determine whether the stress treatments had affected certain key aspects of the behavioral phenotype—body patterning, predation ability, brain lateralization, activity level and response to a threat. The data resulting from these tests were analyzed in R, GraphPad (Prism®) and StatXact®7 (Cytel Inc.). All *p*-values are two-tailed and alpha was set at 0.05.

Body Patterning

In 2015, on the day of hatching (Day 1), between 9:00 and 10:30, up to 12 cuttlefish at a time were placed in randomized order in small uniform gray ("uniform background") circular compartments with slanted sides to minimize shadows (radius = 2.9 cm bottom, 3.35 cm top, length of sides = 2.5 cm; mean gray value = 101 ± 3.9) under white LED light (0.63 to 0.88 klux) and photographed at 0, 5, 15, and 30 min after placement on the background with a Panasonic HDC-SD60 camera. On Day 2, between 10:30-12:00, cuttlefish order was re-randomized and each was photographed four times (0, 5, 15, and 30 min after placement) against a checkered pattern ("disruptive background"). The check size of the disruptive background was selected to be approximately the size of a hatchling's main body-patterning component, the dorsal mantle square (3 × 3 mm), since previous studies have shown that this usually elicits a disruptive pattern in cuttlefish (Chiao et al., 2015).

ImageJ was used to assess the heterogeneity index (HI), a measure of body pattern disruptiveness, of individuals from the photographs. By selecting the outline of the mantle by hand and measuring the "standard deviation," HI was calculated from the standard deviation between the mean gray values of every individual pixel (x) comprising the dorsal mantle ($\bar{x}$), and the total number of pixels (N) selected, with higher values indicating higher overall disruptiveness of body patterning (see methodological description in Di Poi et al., 2014).

$$\mathrm{HI} = \sqrt{\frac{1}{N}\sum(x-\bar{x})^2} \quad (1)$$

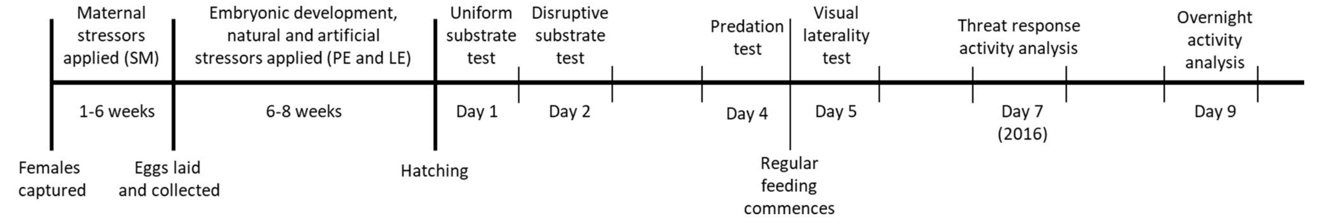


FIGURE 2 | Timeline of stress treatments and behavioral tests. All tests except for the threat response activity analysis occurred in 2015.

Only photographs in which cuttlefish had settled and remained motionless were used for these measurements. Because there was little variation over time in individuals' HI, the values from the four time points were averaged and used to calculate group means for each background type. In total, 55 WM, 41 UM-C, 43 SM, 44 UM-PE, and 39 UM-LE offspring were measured. HI values conformed to parametric assumptions as determined by visual inspection of histograms and normality plot, and were compared with the "anova" function in the "nlme" R package. *Post hoc* comparisons were made using the "glht" function in the "multcomp" R package.

Initial Prey Encounter

Food was withheld until Day 4, when individuals were gently moved from their compartments and placed in circular open-field arenas (radius = 5.9 cm, 250 mL) between 21:00 and 23:00, corresponding to peak feeding time (twilight) for this species (Quintela and Andrade, 2002). Each cuttlefish was allowed 15 min to habituate to the new environment, after which time filming commenced for 15 min (Panasonic HDC-SD60) and a single shrimp (C. crangon, total length 0.7—1.4 cm) was introduced. Videos were analyzed using VLC Media Player and ImageJ to collect data. The moment that cuttlefish orientated toward shrimp with their body was defined as the "time of detection" while the moment that tentacles touched the shrimp and subdued it successfully was defined as the "time of capture." Most caught shrimp on the first attempt, although any tentacle extensions without successful capture of the shrimp were recorded as a "failed capture attempt." Seven variables were calculated from this information: latency to detection (time between prey introduction and detection), latency to attack (time between detection and first strike at prey), latency to capture (time between detection and capture), distance of detection (distance between nearest cuttlefish eye and shrimp at time of detection), attempted capture rate (percentage of cuttlefish that attempted capture), capture rate (percentage of cuttlefish attempting capture that succeeded in capturing the shrimp) and success rate (percentage of attempted captures that were successful). In total, 56 WM, 37 UM-C, 40 SM, 38 UM-PE, and 42 UM-LE offspring were tested. Latencies and distance of detection did not meet parametric assumptions, so groups were compared with exact Kruskal-Wallis tests by Monte Carlo sampling followed by *post hoc* exact permutation tests (with sequential Bonferroni correction). The variables "attempted capture rate," "capture rate," and "success rate" were compared with chi square exact tests.

Visual Laterality Test

These tests were conducted between 10:00 and 22:00 5 days after hatching. The testing apparatus consisted of a start box (3.5 × 5 cm), a movable transparent barrier and two darkened shelters (3.5 × 4 cm) located 15 cm apart (see Jozet-Alves et al., 2012). Each shelter contained blue aquarium gravel and was shaded with a plastic cover. The apparatus was filled with seawater (renewed between trials) and placed under a bright fluorescent lamp (5.5 lux at the surface of the arena). In order to determine if stress induced a population-level eye-use preference, individuals were tested for shelter choice (in randomized order) by gently positioning them in the start box in such a way that it could view both shelters. Once the cuttlefish was in position, the transparent barrier was removed and the cuttlefish was allowed free access to the entire arena. Bright light is unpleasant to cuttlefish, and thus they were highly motivated to exit the start box and seek one of the darkened shelters. In total, 43 WM, 40 UM-C, 43 SM, 42 UM-PE, and 41 UM-LE offspring were tested. Within-group comparisons (the proportion turning right vs. left) were made with binomial tests and between-group comparisons (whether the proportion of those turning left differed between maternal or embryonic stress groups) were analyzed with chi square exact tests.

Overnight Activity Analysis

At midnight of Day 9, four cuttlefish from each daily cohort were randomly selected and placed in a circular open-field arena (radius = 5.9 cm, depth = 2.3 cm, 250 mL) made of opaque white plastic (sides) and a glass base. Illuminated from below by infrared light (which is not visible to the cuttlefish but is recorded by the camera), each individual was filmed from overhead for 6 h with a software-specific camera in a darkened room. This period corresponds with the times at which cuttlefish have been found to be most active (Denton and Gilpin-Brown, 1961; Jäckel et al., 2007; Frank et al., 2012; Oliveira et al., 2017). Videos were analyzed with Ethovision (Noldus®), a software package for behavioral tracking. The total distance traveled, time spent moving, and mean meander were recorded for each individual. Some individuals were unusable due to poor lighting and were excluded. In total, 20 WM, 10 UM-C, 15 SM, 8 UM-PE and eight UM-LE offspring were analyzed. These data did not conform to parametric assumptions, so were analyzed with exact Kruskal-Wallis tests followed by *post hoc* exact permutation tests (sequential Bonferroni correction).

Threat Response Activity Analysis

At noon on Day 7, two pairs of treatment- and age-matched cuttlefish were randomly selected from the daily cohort. They were placed in the open-field arena described in the previous paragraph and recorded and tracked in the same manner. After 1 h of filming, 50 ml of "blank" water from the UM-C egg tank was added to the arena of one member of each pair and 50 ml of "predator odor" water from the UM-PE egg tank containing the three seabass (*D. labrax*) was added to their counterparts' arenas. This was accomplished using tubes already present beneath the waterline of each arena in order to minimize the disturbance of the addition of water. The total distance traveled and time spent moving were recorded for each individual in the same manner as described above. To control for individual differences, post treatment values are expressed as a percentage of the initial hour for each individual (baseline). In total, groups of 10 UM-C, SM, UM-PE, and UM-LE offspring were divided into "blank" ($n = 5$ per stress group) and "predator odor" treatments ($n = 5$ per stress group). These data did not conform to parametric assumptions, so were analyzed with a non-parametric analysis of longitudinal data (R package "nparLD") followed by *post hoc* exact permutation tests (sequential Bonferroni correction).

Ethical Note

This research followed the guidance given by Directive 2010/63/EU, and French regulations regarding the use of animals for experimental procedures, and was approved by the Regional Ethical Committee Cenomexa (Committee agreement number: 54; project agreement number: A14384001). The experiment was designed to decrease animal distress by minimizing the number of animals. Enrichment was provided to unstressed captive adult cuttlefish. After spawning, adult females died naturally following senescence (June/July). After the completion of behavioral experiments, juvenile cuttlefish were anesthetized in 17.5g/L MgCl2 and euthanized with an overdose of ethanol (2%) for neurobiological testing (results not detailed here).

RESULTS

Body Patterning

In the maternal stress groups, a repeated measures ANOVA revealed a significant effect of the background type (i.e., uniform vs. disruptive: $p = 0.001$; $F = 11.299$), and of the treatment groups ($p < 0.001$; $F = 15.66$). As no interaction was found ($p = 0.915$; $F = 0.089$), this analysis showed that mean HI are higher on the disruptive background whatever the group considered (**Figure 3**). Pairwise *post hoc* comparisons showed that mean HI values are lower in UM-C eggs than in WM eggs ($p < 0.001$) and SM eggs ($p = 0.034$). There was no significant difference between WM and SM HI scores ($p = 0.021$).

In the embryonic stress groups, a repeated measures ANOVA revealed a significant effect of the background type (i.e., uniform vs. disruptive: $p = 0.007$; $F = 7.493$), but not of stress treatment groups ($p = 0.066$; $F = 2.733$). As no interaction was found ($p = 0.893$), this analysis indicates that mean HIs are higher on the disruptive background in all groups (data not shown).

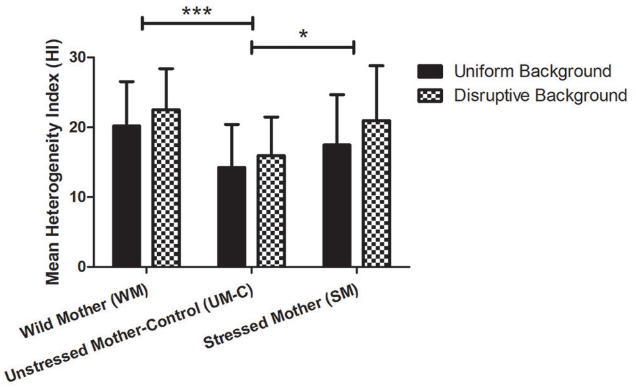


FIGURE 3 | Heterogeneity Index (HI) ± s.d. of maternal stress groups on uniform and disruptive backgrounds. Between groups, WM offspring ($n = 55$) and SM ($n = 43$) had significantly higher HI than UM-C ($n = 41$; $p < 0.001$ and $= 0.034$). Significant differences between groups are indicated by connecting brackets. *$p < 0.05$; ***$p < 0.001$.

Initial Prey Encounter

In the maternal stress groups, there were no significant differences between groups for any of the variables measured (data not included).

Among the embryonic stress groups, there were no significant differences between groups in latency of detection, latency to attack, latency to capture or success rate (data not included). However, distance of detection was significantly different among the treatment groups (exact Kruskal-Wallis test: $p = 0.0178$; $H = 7.636$). Pairwise *post hoc* tests showed that this distance was significantly lower in UM-PE than in UM-LE (exact permutation test, sequential Bonferroni correction: $p = 0.008$; see **Table 1**). Attempted capture rate was also significantly different among the treatment groups (chi-square test: $p < 0.001$; $X^2 = 18.795$). Pairwise *post hoc T*-tests showed that this rate was higher in UM-LE than in UM-C and UM-PE groups (**Table 1**).

Visual Laterality Test

In the maternal stress groups, 72.1% of WM ($n = 43$), 47.5% of UM-C ($n = 40$) and 60.5% of SM ($n = 43$) offspring chose the shelter viewed in their left visual field (**Figure 4**). This group-level bias was only significant in WM group (exact binomial tests: $p = 0.005$). The proportion of individuals choosing the shelter located in their left or their right visual field was not significantly different between groups (chi square exact test: $p = 0.083$; $X^2 = 5.237$).

In the embryonic stress groups, 47.5% of UM-C ($n = 40$), 59.5% of UM-PE ($n = 42$) and 61.0% of UM-LE ($n = 41$) offspring chose the shelter perceived in their left visual field (data not included). No group-level bias was found, whatever the group considered (binomial tests). The proportion of individuals choosing the shelter located in their left or their right visual field was not significantly different between groups (chi square exact test: $p = 0.434$; $X^2 = 1.797$).

Overnight Activity Analysis

In the maternal stress groups, the distance traveled and time spent moving (**Figures 5A,B**) were significantly different

TABLE 1 | Attempted capture rate (percentage of cuttlefish that attempted captured), capture rate (percentage of cuttlefish that captured shrimp), success rate (the percentage of successful captures) of embryonic stress groups during the initial prey encounter.

	UM-Control $n = 35$	**UM-Predator Exposed (natural stressor)** $n = 37$	**UM-Light Exposed (artificial stressor)** $n = 34$	**Group comparisons**	***Post hoc* tests**
Attempted capture rate (%)	40.0	48.65	88.24	$p < 0.001$, $X^2 = 18.795$	UM-C vs. UM-LE: $p < 0.001$ UM-LE vs. UM-PE: $p = 0.008$
Capture rate (%)	85.71	88.89	96.67	$p = 0.492$, $X^2 = 1.862$	
Success rate (%)	85.71	84.21	96.67	$p = 0.333$, $X^2 = 2.568$	

Both group comparisons and post hocs are chi squared exact tests (sequential Bonferroni correction).

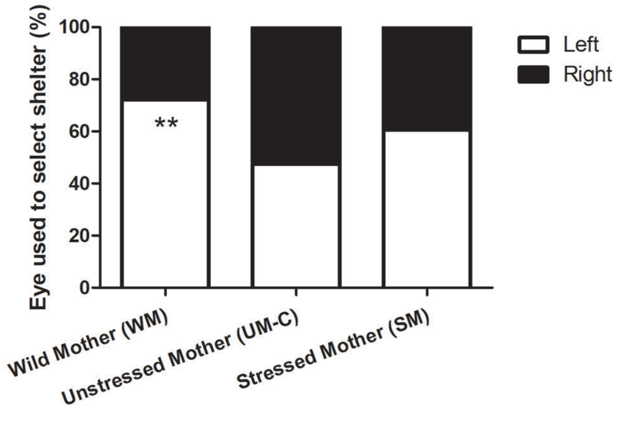


FIGURE 4 | Eye used to select shelter in maternal stress groups. More WM ($n = 43$) chose the shelter in their left visual field (binomial test; $p = 0.005$, signified by asterisks) while no preference was found in UM-C ($n = 40$) or SM ($n = 43$). The proportions were not significantly different between groups ($p = 0.08$).

between groups (Kruskal-Wallis tests: distance: $p = 0.009$; $H = 8.982$; time moving: $p = 0.028$; $H = 7.036$). Pairwise *post hoc* comparisons showed that both variables were significantly greater in SM ($n = 15$) than in UM-C offspring ($n = 10$) (exact permutation tests: distance: $p = 0.002$; time: $p = 0.005$). Finally, no significant differences existed between groups in mean meander (Kruskal-Wallis test: $p = 0.374$; $H = 1.965$; **Figure 5C**). In addition, WM showed a statistical trend for higher distance traveled than UM-C (exact permutation tests: $p = 0.058$).

In the embryonic stress groups, there were no significant differences between groups for any of the variables measured (Kruskal-Wallis tests; data not included).

Threat Response Activity Analysis

In the maternal stress groups, the non-parametric analysis for longitudinal data revealed a significant difference within groups according to time (i.e., before vs. after water addition), but not according to treatment groups (i.e., WM, SM, and UM-C) or cue type (i.e., blank water vs. predator odor), for both distance traveled ($p < 0.001$; $F = 32.666$; **Figure 6A**) and time moving ($p < 0.001$; $F = 25.284$; **Figure 6B**). As no interaction was found, this analysis showed that mean distance traveled and time spent moving are decreasing after adding water whatever the treatment group and the cue type considered.

In the embryonic stress groups, the non-parametric analysis for longitudinal data revealed a significant difference within groups according to time (i.e., before vs. after water addition), but not according to treatment groups (i.e., UM-C, UM-PE, and UM-LE) or cue type (i.e., blank water vs. predator odor), for both distance traveled ($p < 0.001$; $F = 37.982$; **Figure 7A**) and time moving ($p < 0.001$; $F = 32.437$; **Figure 7B**). As no interaction was found, this analysis showed that mean distance traveled and time spent moving decrease after adding water whatever the treatment group and the cue type considered.

DISCUSSION

We conducted this experiment with the aim of determining if prenatal stress affects cuttlefish behavior, and to compare various stressor types. We found that maternal stress was associated with differences in offspring body patterning and activity patterns. By contrast, offspring exposed to a natural stressor, predator odor, showed no differences from controls, while embryos exposed to an artificial stressor, bright light, differed in their predation behavior. In addition, we found that maternal captivity during spawning may affect visual laterality (summarized in **Table 2**).

Body Patterning

In all groups, the mean HI (disruptiveness) on the disruptive background was consistently higher than that of the uniform one, suggesting that all cuttlefish adjusted their body patterns to the background. Significant differences were also seen between groups: In our experiment, maternal stress increased the mean disruptiveness of the body pattern displayed. Our results also suggest that female captivity during egg-laying can induce a group bias for higher disruptiveness in her offspring, since the offspring of wild mothers had the highest HI overall. Previous experiments with cuttlefish hatchlings have detected similar differences in body patterning between groups incubated in different environments (O'Brien et al., 2016a) and exposed to certain pharmaceuticals during development (Di Poi et al., 2014;

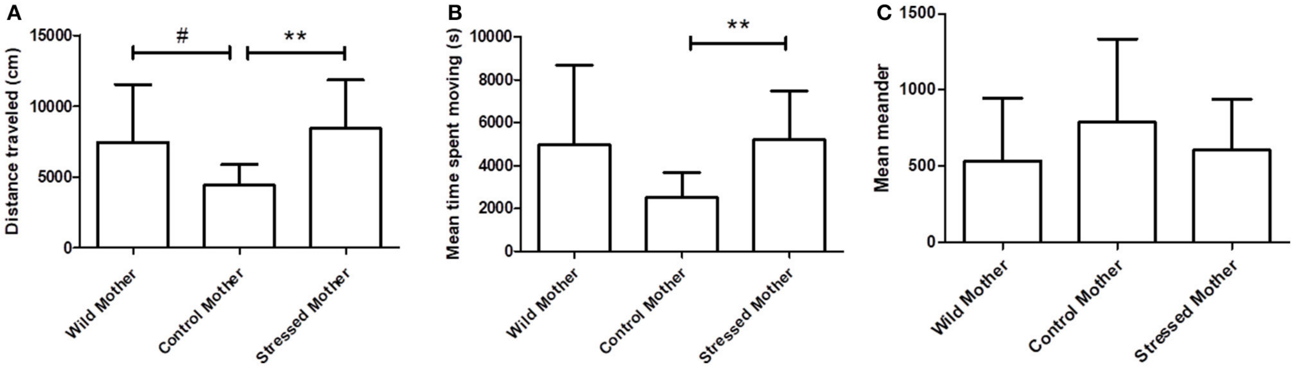


FIGURE 5 | The total distance traveled **(A)**, time spent moving **(B)** and mean meander (turn angle/distance traveled; **(C)** ± s.d. of maternal stress groups in the overnight activity analysis test. Significant differences (indicated by connecting brackets with asterisks) exist between UM-C ($n = 10$) and SM ($n = 15$) in both distance traveled and time spent moving ($p = 0.009$ and 0.005; *post hoc* asymptotic permutation tests with sequential Bonferroni correction. WM $n = 20$. **$p < 0.01$; #indicates a statistical tendency ($p < 0.08$).

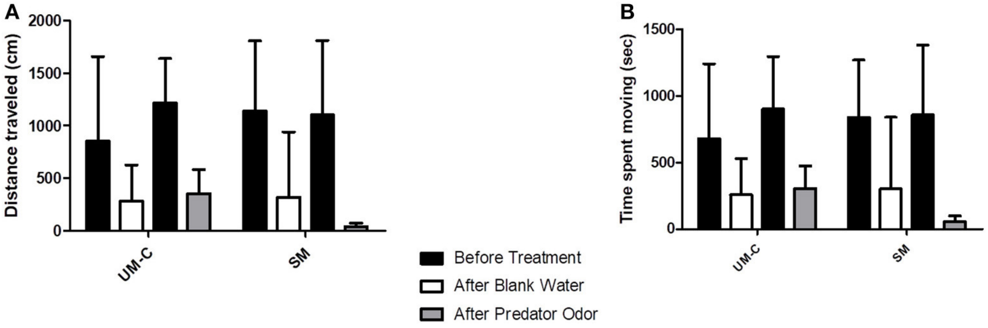


FIGURE 6 | The total distance traveled **(A)** and time moving **(B)** ± s.d. for maternal stress groups in the threat response activity analysis. Differences within groups are indicated by connecting bars; $n = 5$ for all bars.

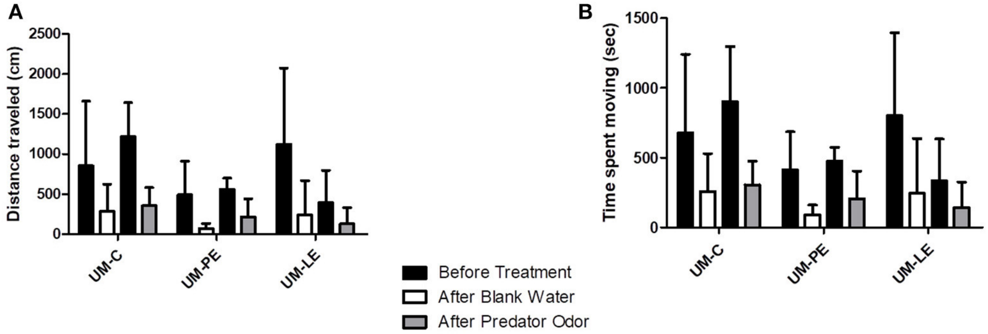


FIGURE 7 | The total distance traveled **(A)** and time moving **(B)** ± s.d. for embryonic stress groups in the threat response activity analysis. Differences within groups are indicated by connecting bars; $n = 5$ for all bars.

Bidel et al., 2016). The existence of similar differences between maternal stress groups in this experiment indicates that maternal experience can also affect this behavior, and may be adaptive for their offspring—higher disruptiveness could potentially improve camouflage on the variegated backgrounds often present in the natural environment.

Where the tactic of adult cuttlefish is often to match the background by expressing more uniform patterns in response to uniform backgrounds and more disruptive patterns in response to disruptive ones (Mathger et al., 2007; Barbosa et al., 2008), young cuttlefish usually display a fairly chronic body pattern that often clashes with the background (Hanlon and Messenger,

TABLE 2 | Summary of behavioral test results in comparison to the unstressed control mothers.

	Body Patterning	Predatory Behavior	Visual Laterality	Activity Patterns	Threat Response
	2015 data	**2015 data**	**2015 data**	**2015 data**	**2016 data**
Wild Mother offspring (WM)	Higher disruptiveness	No effect	Group-level left bias not observed in control group	Statistical tendency for higher distance traveled	Not tested
Stressed Mother offspring (SM)	Higher disruptiveness	No effect	No effect	Greater distance traveled and time spent moving	No effect
Natural stressor: Predator-exposed as eggs (UM-PE)	No effect	No effect	No effect	No effect	No effect
Artificial stressor: Light exposed as eggs (UM-LE)	No effect	Higher attempted capture rate	No effect	No effect	No effect

1988; Poirier et al., 2005). The ability to produce a uniform body pattern emerges during the first few months of life (see O'Brien et al., 2016b), and the results of the present experiments suggest that maternal stress and environment may delay the emergence of this ability.

Predation

Almost twice as many UM-LE offspring attempted capture than UM-C or UM-PE. Light is known to influence the timing of hatching (Paulij et al., 1991), and it is possible that these offspring had higher feeding motivation at the same age than other hatchlings because of increased energetic needs due to accelerated embryonic development. Faster development could also have accelerated visual maturation, leading UM-LE hatchlings to be better than their siblings at detecting prey. Indeed, UM-LE were able to detect prey at a significantly greater distance than UM-PE. It is worth noting however, that although a greater proportion of UM-LE captured shrimp, they were not better predators than the other groups, since the capture and success did not differ significantly between groups (close to 100%). This is in accordance with early experiments suggesting that prey capture operates using a highly-stereotyped program that improves little with age or experience (Wells, 1958). Despite not being better at hunting, young cuttlefish with higher feeding motivation would likely grow faster from consuming more prey.

Visual Laterality

In our experiment, no group-level bias was found in the control group. This is in accordance with previous experiments showing that a left eye-use preference for shelter seeking is not fully developed until a month after hatching (Jozet-Alves et al., 2012). Among all other groups, only WM group displayed a group-level preference toward the left side on Day 5. These results do conflict somewhat with the findings of Jozet-Alves and Hebert (2012); in that study, the authors showed that prenatal exposure to predator odor induced a left preference 3 days after hatching. However, this preference was slight, and it was necessary to test each cuttlefish more than once to detect it. Our experiment used a single trial per individual, a method formerly utilized in birds (Pittet et al., 2009), and it is possible that running only one trial did not allow us to detect the presence of the fledgling eye-use preference seen in the other groups.

The fact that eye-use preference did exist in the WM group suggests that when egg-laying and early development occur in the wild, the maturation of the left eye use preference is faster. Being lateralized from hatching may have an adaptive advantage by rendering WM offspring able to dual task (Vallortigara and Rogers, 2005). For example, while using their right eye for hunting (Schnell et al., 2016) they can simultaneously "keep an eye out" for shelter with their left should the need for a rapid escape arise.

Overnight Activity

In our assessment of baseline activity level, we found no differences between embryonic stress groups, while stressed mother offspring were associated with greater activity than control mother offspring, and similar to that of WM. We also observed a statistical tendency for WM hatchlings to travel a greater distance than UM-C. Activity levels and open field behavior have been used in behavioral research as a means of quantifying the impacts of various prenatal stressors in a variety of animals. No previously-published studies have measured this behavior in cuttlefish hatchlings, but we can draw insight from other species.

Some species, including rhesus monkeys and salmon, demonstrate decreases in overall activity after maternal or embryonic stress (Schneider, 1992; Clarke et al., 1996; Espmark et al., 2008), while others, including blue foxes and Japanese quails, show increases in activity and steps taken in open field tests (Braastad, 1998; Guibert et al., 2011). The effects of prenatal stress on activity have been studied most extensively in rodents, especially rats, and results are mixed. Some authors (Masterpasqua et al., 1976; Peters, 1986; Hilakivi et al., 1989; Sandi et al., 1996; Wilson et al., 2013) report increases in exploration and open field activity. Others report no or little effect of stress (Chapman and Stern, 1979; Van den Hove et al., 2005), or even opposite effects according to sex (Alonso et al., 1991). The majority of studies however, find decreases in movement and "exploration" in the offspring of females subjected to a variety of stressors during pregnancy (Hockman, 1961; Fride et al., 1986; Suchecki and Neto, 1991; Poltyrev et al., 1996; Vallee et al., 1997; Fujioka et al., 2001; Patin et al., 2004). Thus it seems that cuttlefish may differ in this respect from most vertebrate models and could therefore serve as a means to explore the factors driving the evolution of this response in different animal groups.

Based on insight from the studies in other animals that do show activity increases (cited above), the greater activity level observed in SM may reflect a search for shelter or food or an urge to escape. This could be advantageous by allowing young cuttlefish to avoid predation and to grow more quickly. It is also worth noting that an open field test conducted under laboratory conditions may not reflect "natural" behavior that would be seen in the wild. Indeed, a study in lab mice that compared open field behavior in the lab to the same test conducted in an outdoor grassy field found marked limitation in the number of behaviors expressed in the artificial setting (Fiore et al., 1995).

The group differences observed suggests that the stress experienced by the females during egg-laying was transmitted to their offspring and altered behavioral patterns. Physiologically, such an increase might be the result of slower vertical lobe maturation. This is the area of the brain potentially responsible for behavioral inhibition (Dickel et al., 2001, 2006), and a less mature VL would permit a higher level of basal activity. This experiment provides a starting point for future activity analyses with hatchling cuttlefish.

Threat Response

Many animals strongly alter their activity patterns in response to predator odor, especially in aquatic ecosystems. In particular, there is an extensive amount of literature documenting the behavioral responses of numerous aquatic gastropod and bivalve species (the extant molluscan groups most closely related to cephalopods) to waterborne predator odors, including escape responses such as crawling out of the water or burying (e.g., Snyder and Snyder, 1971; Jacobsen and Stabell, 2004; Dalesman et al., 2006), as well as reductions in movement such as cessation of filter feeding or decreases in foraging and migration (e.g., Reimer and Tedengren, 1997; Smee and Weissburg, 2006). Adult cuttlefish are known to react to predators with increases in escape behavior (Staudinger et al., 2013) and numerous body pattering displays (Adamo et al., 2006). Cuttlefish embryos are able to detect odors starting during the final third of embryonic development and respond to it in various ways, including embryonic increases in breathing rate (Romagny et al., 2012; Mezrai et al., in preparation), as well as post-natal behavioral lateralization (Jozet-Alves and Hebert, 2012) and changes in prey preference (Guibé et al., 2010). Thus, the ability to detect waterborne predator cues is present before hatching. The existence of odor-induced anti-predator responses in other molluscs, coupled with chemosensory abilities of embryonic cuttlefish, led us to predict that a change in activity pattern would be observable in response to predator odor in young hatchlings. The predator cue we utilized came from sea bass, which are known to prey on hatchling cuttlefish in the wild (Blanc and Daguzan, 1999), and thus represent an imminent threat to survival which should elicit a change in movement.

A reduction in activity was observed in all groups after the addition of either predator odor or blank water. This was a continuation of a pattern of progressively decreasing activity over time, and no group's reaction to predator odor differed from that of their response to blank water. Thus, it seems that unlike many other molluscs and adult cuttlefish, hatchling cuttlefish do not possess a marked locomotory threat response. Perhaps they rely exclusively on burying and/or body patterning to avoid predation. Unfortunately, the video quality and lack of sand necessary for the behavioral tracking software to function optimally prevented us from observing any burying or body patterning response. Researchers should take advantage of evolving video analysis technology to incorporate these possibilities into future tests of activity and threat response.

Maternal vs. Embryonic Stress

Body patterning and activity levels were both affected by maternal stress, while embryonic stress only affected one aspect of predatory behavior. Additionally, the differences between WM and UM-C in activity and turning bias suggest that the environment in which eggs are laid can also affect offspring behavior. In sum, maternal stress and spawning environment resulted in more post-natal behavioral changes than the direct experience of stressors in the egg. The greater post-natal reaction to the maternal stimuli suggests that mothers' experience might be a more reliable indicator of future prospects than stressors experienced by the embryos directly.

Maternal experience is known to "program" offspring in many other species; most commonly, the offspring of mothers exposed to a particular predator showed adaptive responses when encountering that predator itself (reviewed in Agrawal et al., 1999; Storm and Lima, 2010). In birds and mammals, such maternal stress effects are likely mediated by the transfer of stress hormones in the egg or placenta (Hayward and Wingfield, 2004; Groothuis et al., 2005; Weinstock, 2008). Since cuttlefish lack a planktonic larval phase and their dispersal abilities are likely limited by their size, any dangers present at or near the spawning site are likely to be a threat to cuttlefish at hatching. Anticipating and preparing for these threats makes adaptive sense. The higher disruptiveness and greater activity levels of stressed mother offspring and the higher disruptiveness and left turning bias of wild mothers could be advantageous to hatchlings by improving camouflage and facilitating escape from predators.

The effects of maternal environment and stress should be taken into account when planning, conducting and interpreting future laboratory experiments with cuttlefish—the behaviors observed may differ depending on how subjects were obtained (i.e., bred in captivity or collected from the wild) and handled, and experimenters should carefully consider their experimental priorities (i.e., whether they are trying to assess natural behavior) before they source cuttlefish eggs for experiments. More broadly, further experimentation in other oviparous species is important in understanding the results obtained in viviparous and ovoviviparous species for which maternal and embryonic effects cannot be disassociated.

Artificial vs. Natural Embryonic Stressors

Sea bass (*D. labrax*) are a particularly relevant stressor to cuttlefish since they have long co-existed in the English Channel and readily predate on hatchling and juvenile cuttlefish (Blanc and Daguzan, 1999). Sensing sea bass odor in the natal environment is a direct signal of post-natal threat for hatchling cuttlefish. Because of this, selective pressure for embryos to detect and prepare for this threat is presumably strong. Indeed, embryonic exposure to seabass odor is associated with increased lateralization in cuttlefish hatchlings, a behavioral adaptation which can facilitate rapid escape (Jozet-Alves and Hebert, 2012). In these five experiments however, the predator cues had no discernable behavioral effect.

It is possible that embryos habituated to the predator odor. In our experiment, UM-PE embryos were housed in tanks with seabass for most of development, and had the ability to sense odor cues for the last seven (of 30) stages of embryonic development (Romagny et al., 2012). Thus, they had at least several weeks of chemosensory exposure to these predators. Post-natal studies in other animals, including fish, rats and lizards, have shown that while acute stress exposure can result in adaptive changes (e.g., increased predator avoidance behavior or HPA-axis sensitivity), long-term or repeated exposure can actually reduce or eliminate the adaptive response (Dielenberg and McGregor, 1999; Weinberg et al., 2009). On the other hand, some studies show a lack of habituation to predator odor applied long-term (e.g., Epple et al., 1993). If habituation to predator odor is indeed occurring in cuttlefish, the evolutionary reason for this merits further scrutiny. One possibility is that because the predator odor was not paired with alarm cues from injured conspecifics in our experiment, the cuttlefish embryos learned to regard it as benign. Such a phenomenon occurs in harbor seals, which learn to distinguish between the calls of fish-eating and seal-eating orca populations and behave accordingly (Deecke et al., 2002).

In parallel, we tested an "artificial" stressor that could be compared to the effect of predator odor. We selected an artificial light source (LED panels) at a high intensity to penetrate the opaque egg membrane. The timing of the light regime was randomized and mimicked what might occur in some artificial settings. Though this stressor was a completely artificial stimulus and not indicative of a threat, it was associated with a strong, seemingly adaptive effect on predation behavior. Thus, our prediction of positive effects in response to predator odor and of disruptive effects in response to LED light was not supported by these results. This suggests that the evolutionary "familiarity" of a stressor (i.e., whether the species has encountered it before) is not the only explanation for fitness differences in the stressor response. The fact that we found an effect of light (increased predation) and no effect of predator odor may instead be explained by the relevance of the sensory modalities engaged by each stressor. While both odor and light can be perceived and responded to by embryos, cuttlefish are highly visual animals (Darmaillacq et al., 2017), and thus visual cues are likely to be more relevant to them than odor cues. Alternatively, this behavior may simply reflect a physiological improvement in visual acuity due to the wider ranges of light intensity experienced during embryonic development. Further testing exploring the role of different cues and sensory modalities are ongoing (Mezrai, in preparation).

CONCLUSION

The results reported here can serve as a basis for future behavioral tests examining prenatal stress and other embryonic influences. The tests utilized were non-invasive methods and, when employed as a battery, cover a broad range of behaviors critical to survival that give a rough measure of offspring fitness and treatment group differences. In particular, the activity analyses and threat response test were the first to be conducted with hatchling cuttlefish, and should offer valuable baseline data for researchers hoping to utilize such tests in the future. Further experimentation with other sources of prenatal stress will elaborate on the results reported here and could reveal previously-unknown prenatal pressures driving offspring behavior.

At the same time, greater effort should be made to account for the effects of spawning environment and early stimulation when planning and interpreting laboratory experiments and in the welfare of this regulated species. It is well-established that environmental enrichment is crucial to early cognitive development in cuttlefish (Dickel et al., 2000) and is recommended for the welfare of adults (Fiorito et al., 2015). The results presented here underscore the importance of maintaining a stimulating environment for reproducing females and even potentially their eggs. Researchers should strive to maintain at least a basic level of sensory enrichment for captive adults, and carefully consider the environmental cues experienced by developing eggs. Future guidelines will hopefully standardize a basic level of enrichment for all European cuttlefish research. It may even be beneficial to include predator cues and other mild stressors to encourage the development of certain aspects of the behavioral phenotype (e.g., hunting ability). Carefully adapting captive enclosures to cuttlefish needs will ensure the psychological well-being of individuals and the reliability of experimental results, promote growth in aquaculture and yield more savvy offspring for future hatch and release programs.

SUMMARY STATEMENT

The effects of several chronic prenatal stressors (maternal stress, embryonic exposure to predator odor or bright light) on hatchling cuttlefish are compared in five tests.

AUTHOR CONTRIBUTIONS

CO: Primary data collector and author of article; CJ-A: Assisted with data analysis, experimental design and editing of manuscript; NM: Data collector, editor of manuscript; CB: Data collector, editor of manuscript; A-SD: Supervisor, editor of manuscript; LD; Primary supervisor, editor of manuscript.

ACKNOWLEDGMENTS

This work was supported by the Agence Nationale de la Recherche (ANR) and the Région Basse-Normandie as part of the research project PReSTO'Cog (ANR-13-BSV7-0002). We are grateful for the assistance of Olivier Basuyaux and Sébastien Pien of the Synergie Mer et Littoral (SMEL), Nadège Villain-Naud and Céline Thomasse of NECC Ethos (Université de Caen), Jean Paul Lehodey, David Liegard, Frédéric Guyon, and David Lemeille of the Centre de Recherches en Environnement Côtier (CREC, Université de Caen), as well as the numerous students who worked with us, including Kevin Bairos-Novak, Héloise Duretz, Camille Auger, Chloe Jane Way, Pauline Billiard, and Justine Lequesne. We also appreciate the excellent suggestions made by two reviewers. The manuscript was proofread by Barbara O'Brien.

REFERENCES

Adamo, S. A., Ehgoetz, K., Sangster, C., and Whitehorne, I. (2006). Signaling to the enemy? Body pattern expression and its response to external cues during hunting in the cuttlefish *Sepia officinalis (Cephalopoda). Biol. Bull.* 210, 192–200. doi: 10.2307/4134557

Agrawal, A. A., Laforsch, C., and Tollrian, R. (1999). Transgenerational induction of defences in animals and plants. *Nature* 401, 60. doi: 10.1038/43425

Alonso, S. J., Arevalo, R., Afonso, D., and Rodriguez, M. (1991). Effects of maternal stress during pregnancy on forced swimming test behavior of the offspring. *Physiol. Behav.* 50, 511–517. doi: 10.1016/0031-9384(91)90538-Y

Barbosa, A., Mäthger, L. M., Buresch, K. C., Kelly, J., Chubb, C., Chiao, C.-C., et al. (2008). Cuttlefish camouflage: the effects of substrate contrast and size in evoking uniform, mottle or disruptive body patterns. *Vis. Res.* 48, 1242–1253. doi: 10.1016/j.visres.2008.02.011

Bidel, F., Di Poi, C., Imarazene, B., Koueta, N., Budzinski, H., Van Delft, P., et al. (2016). Pre-hatching fluoxetine-induced neurochemical, neurodevelopmental, and immunological changes in newly hatched cuttlefish. *Environ. Sci. Pollut. Res.* 23, 5030–5045. doi: 10.1007/s11356-015-4591-7

Blanc, A., and Daguzan, J. (1999). Young cuttlefish *Sepia officinalis* (Mollusca: Sepiidae) in the Morbihan Bay (south Brittany, France): accessory prey of predators. *J. Mar. Biol. Assoc. U.K.* 79, 1133–1134. doi: 10.1017/S0025315499001435

Bloor, I. S. M., Wearmouth, V. J., Cotterell, S. P., McHugh, M. J., Humphries, N. E., Jackson, E. L., et al. (2013). Movements and behaviour of European common cuttlefish *Sepia officinalis* in english channel inshore waters: first results from acoustic telemetry. *J. Exp. Mar. Biol. Ecol.* 448, 19–27. doi: 10.1016/j.jembe.2013.06.013

Braastad, B. O. (1998). Effects of prenatal stress on behaviour of offspring of laboratory and farmed mammals. *Appl. Anim. Behav. Sci.* 61, 159–180. doi: 10.1016/S0168-1591(98)00188-9

Cannon, W. B. (1939). T*he Wisdom of the Body. 1932*. New York, NY: Norton.

Chapman, R. H., and Stern, J. M. (1979). Failure of severe maternal stress or ACTH during pregnancy to affect emotionality of male rat offspring: implications of litter effects for prenatal studies. *Dev. Psychobiol.* 12, 255–267. doi: 10.1002/dev.420120309

Chiao, C.-C., Chubb, C., and Hanlon, R. T. (2015). A review of visual perception mechanisms that regulate rapid adaptive camouflage in cuttlefish. *J. Comp. Physiol. A* 201, 933–945. doi: 10.1007/s00359-015-0988-5

Clarke, A. S., Soto, A., Bergholz, T., and Schneider, M. L. (1996). Maternal gestational stress alters adaptive and social behavior in adolescent rhesus monkey offspring. *Infant Beh. Dev.* 19, 451–461. doi: 10.1016/S0163-6383(96)90006-5

Colson, V., Valotaire, C., Geffroy, B., and Kiilerich, P. (2015). Egg cortisol exposure enhances fearfulness in larvae and juvenile rainbow trout. *Ethology* 121, 1191–1201. doi: 10.1111/eth.12437

Dalesman, S., Rundle, S. D., Coleman, R. A., and Cotton, P. A. (2006). Cue association and antipredator behaviour in a pulmonate snail, *Lymnaea stagnalis*. *Anim. Behav.* 71, 789–797. doi: 10.1016/j.anbehav.2005.05.028

Darmaillacq, A.-S., Lesimple, C., and Dickel, L. (2008). Embryonic visual learning in the cuttlefish, *Sepia officinalis*. *Anim. Behav.* 76, 131–134. doi: 10.1016/j.anbehav.2008.02.006

Darmaillacq, A.-S., Mezrai, N., O'Brien, C. E., and Dickel, L. (2017). Visual ecology and the development of visually guided behavior in the cuttlefish. *Front. Physiol.* 8:402. doi: 10.3389/fphys.2017.00402

Deecke, V. B., Slater, P. J., and Ford, J. K. (2002). Selective habituation shapes acoustic predator recognition in harbour seals. *Nature* 420:171. doi: 10.1038/nature01030

Denton, E. J., and Gilpin-Brown, J. B. (1961). The effect of light on the buoyancy of the cuttlefish. *J. Mar. Biol. Assoc. U.K.* 41, 343–350. doi: 10.1017/S002531540002395X

Di Poi, C., Bidel, F., Dickel, L., and Bellanger, C. (2014). Cryptic and biochemical responses of young cuttlefish *Sepia officinalis* exposed to environmentally relevant concentrations of fluoxetine. *Aqua. Toxicol.* 151, 36–45. doi: 10.1016/j.aquatox.2013.12.026

Dickel, L., Boal, J. G., and Budelmann, B. U. (2000). The effect of early experience on learning and memory in cuttlefish. *Dev. Psychobiol.* 36, 101–110. doi: 10.1002/(SICI)1098-2302(200003)36:2<101::AID-DEV2>3.0.CO;2-L

Dickel, L., Chichery, M.-P., and Chichery, R. (2001). Increase of learning abilities and maturation of the vertical lobe complex during postembryonic development in the cuttlefish, Sepia. *Dev. Psychobiol.* 39, 92–98. doi: 10.1002/dev.1033

Dickel, L., Darmaillacq, A.-S., Poirier, R., Agin, V., Bellanger, C., and Chichery, R. (2006). Behavioural and neural maturation in the cuttlefish *Sepia officinalis*. *Vie et Milieu* 56, 89–95.

Dielenberg, R. A., and McGregor, I. S. (1999). Habituation of the hiding response to cat odor in rats (*Rattus norvegicus*). *J. Comp. Psychol*. 113, 376–387. doi: 10.1037/0735-7036.113.4.376

Epple, G., Mason, J. R., Nolte, D. L., and Campbell, D. L. (1993). Effects of predator odors on feeding in the mountain beaver (*Aplodontia rufa*). *J. Mammal*. 74, 715–722. doi: 10.2307/1382293

Espmark, A. M., Eriksen, M. S., Salte, R., Braastad, B. O., and Bakken, M. (2008). A note on pre-spawning maternal cortisol exposure in farmed Atlantic salmon and its impact on the behaviour of offspring in response to a novel environment. *Appl. Anim. Behav. Sci.* 110, 404–409. doi: 10.1016/j.applanim.2007.04.003

Favreau-Peigné, A., Calandreau, L., Constantin, P., Gaultier, B., Bertin, A., Arnould, C., et al. (2014). Emotionality modulates the effect of chronic stress on feeding behaviour in birds. *PLoS ONE* 9:e87249. doi: 10.1371/journal.pone.0087249

Fiore, M., Dell'Omo, G., Alleva, E., and Lipp, H.-P. (1995). A comparison of behavioural effects of prenatally administered oxazepam in mice exposed to open-fields in the laboratory and the real world. *Psychopharmacology* 122, 72–77.

Fiorito, G., Affuso, A., Basil, J., Cole, A., de Girolamo, P., D'Angelo, L., et al. (2015). Guidelines for the Care and Welfare of Cephalopods in Research -A consensus based on an initiative by CephRes, FELASA and the Boyd Group. *Lab. Anim.* 49, 1–9. doi: 10.1177/0023677215580006

Frank, M. G., Waldrop, R. H., Dumoulin, M., Aton, S., and Boal, J. G. (2012). A preliminary analysis of sleep-like states in the Cuttlefish *Sepia officinalis*. *PLoS ONE* 7:e38125. doi: 10.1371/journal.pone.0038125

Fride, E., Dan, Y., Feldon, J., Halevy, G., and Weinstock, M. (1986). Effects of prenatal stress on vulnerability to stress in prepubertal and adult rats. *Physiol. Behav.* 37, 681–687. doi: 10.1016/0031-9384(86)90172-1

Fujioka, T., Fujioka, A., Tan, N., Chowdhury, G. M. I., Mouri, H., Sakata, Y., et al. (2001). Mild prenatal stress enhances learning performance in the non-adopted rat offspring. *Neuroscience* 103, 301–307. doi: 10.1016/S0306-4522(00)00582-0

Ghio, S. C., Leblanc, A. B., Audet, C., and Aubin-Horth, N. (2016). Effects of maternal stress and cortisol exposure at the egg stage on learning, boldness and neophobia in brook trout. *Behaviour* 153, 1639–1663. doi: 10.1163/1568539X-00003377

Gluckman, P. D., and Hanson, M. A. (2004). Developmental origins of disease paradigm: a mechanistic and evolutionary perspective. *Pediatr. Res.* 56, 311–317. doi: 10.1203/01.PDR.0000135998.08025.FB

Groothuis, T. G., Müller, W., von Engelhardt, N., Carere, C., and Eising, C. (2005). Maternal hormones as a tool to adjust offspring phenotype in avian species. *Neuroscience Biobehav. Rev.* 29, 329–352. doi: 10.1016/j.neubiorev.2004.12.002

Guibé, M., Boal, J. G., and Dickel, L. (2010). Early exposure to odors changes later visual prey preferences in cuttlefish. *Dev. Psychobiol.* 52, 833–837. doi: 10.1002/dev.20470

Guibert, F., Richard-Yris, M.-A., Lumineau, S., Kotrschal, K., Bertin, A., Petton, C., et al. (2011). Unpredictable mild stressors on laying females influence the composition of Japanese quail eggs and offspring's phenotype. *Appl. Anim. Behav. Sci.* 132, 51–60. doi: 10.1016/j.applanim.2011.03.012

Hanlon, and Messenger, J. B. (1988). Adaptive coloration in young cuttlefish (*Sepia officinalis* L.): the morphology and development of body patterns and their relation to behaviour. *Philos. Trans. R. Soc. B* 320, 437–487.

Hanlon, and Messenger, J. B. (1998). *Cephalopod Behaviour*. Cambridge, UK: Cambridge University Press.

Hayward, L. S., and Wingfield, J. C. (2004). Maternal corticosterone is transferred to avian yolk and may alter offspring growth and adult phenotype. *Gen. Comp. Endocrinol.* 135, 365–371. doi: 10.1016/j.ygcen.2003.11.002

Hilakivi, L. A., Ota, M., and Lister, R. (1989). Effect of isolation on brain monoamines and the behavior of mice in tests of exploration, locomotion, anxiety and behavioral 'despair.' *Pharmacol. Biochem. Behav.* 33, 371–374. doi: 10.1016/0091-3057(89)90516-9

Hockman, C. H. (1961). Prenatal maternal stress in the rat: its effects on emotional behavior in the offspring. *J. Comp. Physiol. Psychol.* 54:679. doi: 10.1037/h0041945

Jäckel, G., Mark, F. C., Gutowska, M., Claireaux, G., Ellington, C. P., and Pörtner, H. O. (2007). Analysis of diurnal activity patterns and related changes in metabolism in the cephalopod Sepia officinalis. *Ann. Soc. Exp. Biol.* 146A(4Suppl):S83. doi: 10.1016/j.cbpa.2007.01.109

Jacobsen, H. P., and Stabell, O. B. (2004). Antipredator behaviour mediated by chemical cues: the role of conspecific alarm signalling and predator labelling in the avoidance response of a marine gastropod. *Oikos* 104, 43–50. doi: 10.1111/j.0030-1299.2004.12369.x

Jones, R. B. (1996). Fear and adaptability in poultry: insights, implications and imperatives. *World's Poultry Sci. J.* 52, 131–174. doi: 10.1079/WPS19960013

Jozet-Alves, C., and Hebert, M. (2012). Embryonic exposure to predator odour modulates visual lateralization in cuttlefish. *Proc. R. Soc. B* 280:20122575. doi: 10.1098/rspb.2012.2575

Jozet-Alves, C., Viblanc, V. A., Romagny, S., Dacher, M., Healy, S. D., and Dickel, L. (2012). Visual lateralization is task and age dependent in cuttlefish, *Sepia officinalis*. *Anim. Behav.* 83, 1313–1318. doi: 10.1016/j.anbehav.2012.02.023

Katz, R. J., Roth, K. A., and Carroll, B. J. (1981). Acute and chronic stress effects on open field activity in the rat: implications for a model of depression. *Neurosci. Biobehav. Rev.* 5, 247–251. doi: 10.1016/0149-7634(81)90005-1

Linnaeus, C., von. (1758). *Systema Naturae, 10th Edn,* Vol. 1. Stockholm: L. Salvii.

Masterpasqua, F., Chapman, R. H., and Lore, R. K. (1976). The effects of prenatal psychological stress on the sexual behavior and reactivity of male rats. *Dev. Psychobiol.* 9, 403–411. doi: 10.1002/dev.420090503

Mathger, L. M., Chiao, C.-C., Barbosa, A., Buresch, K. C., Kaye, S., and Hanlon, R. T. (2007). Disruptive coloration elicited on controlled natural substrates in cuttlefish, *Sepia officinalis*. *J. Exp. Biol.* 210, 2657–2666. doi: 10.1242/jeb.004382

Mathis, A., Ferrari, M. C., Windel, N., Messier, F., and Chivers, D. P. (2008). Learning by embryos and the ghost of predation future. *Proc. R. Soc. Lond. B* 275, 2603–2607. doi: 10.1098/rspb.2008.0754

Miller, G. E., Chen, E., and Zhou, E. S. (2007). If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. *Am. Psychol. Assoc.* 133, 25–45. doi: 10.1037/0033-2909.133.1.25

O'Brien, C. E., Bowie, M., Billard, P., Darmaillacq, A.-S., Jozet-Alves, C., Behaim, D., et al. (2016a). The effect of an artificial incubation environment on hatchling size and behavior in the cuttlefish, *Sepia officinalis*. *Vie et Milieu Life Environ.* 66, 1–9.

O'Brien, C. E., Mezrai, N., Darmaillacq, A.-S., and Dickel, L. (2016b). Behavioral development in embryonic and early juvenile cuttlefish (*Sepia officinalis*). *Dev. Psychobiol.* 59, 145–160. doi: 10.1002/dev.21476

Oliveira, C. C., Grano-Maldonado, M. I., Gonçalves, R. A., Frias, P. A., and Sykes, A. V. (2017). Preliminary results on the daily and seasonal rhythms of cuttlefish *Sepia officinalis* (Linnaeus, 1758) locomotor activity in captivity. *Fishes* 2:9. doi: 10.3390/fishes2030009

Patin, V., Vincent, A., Lordi, B., and Caston, J. (2004). Does prenatal stress affect the motoric development of rat pups? *Dev. Brain Res.* 149, 85–92. doi: 10.1016/j.devbrainres.2003.12.008

Paulij, W. P., Herman, P. M. J., Roozen, M. E. F., and Denuce, J. M. (1991). The Influence of Photoperiodicity on Hatching of *Sepia Officinalis*. *J. Mar. Biol. Assoc. U.K.* 71, 665. doi: 10.1017/S0025315400053224

Peters, D. A. (1986). Prenatal stress: effect on development of rat brain serotonergic neurons. *Pharmacol. Biochem. Behav.* 24, 1377–1382. doi: 10.1016/0091-3057(86)90198-X

Pittet, F., Blois-Heulin, C., Houdelier, C., Richard-Yris, M.-A., and Lumineau, S. (2009). "Sexual display laterality in male of Japanese quail," in *Behavior 2009-31st International Ethological Conference* (Rennes).

Poirier, R., Chichery, R., and Dickel, L. (2005). Early experience and postembryonic maturation of body patterns in cuttlefish (*Sepia officinalis*). *J. Comp. Psychol.* 119, 230–237. doi: 10.1037/0735-7036.119.2.230

Poisson, A., Valotaire, C., Borel, F., Bertin, A., Darmaillacq, A.-S., Dickel, L., et al. (2017). Embryonic exposure to a conspecific alarm cue triggers behavioural plasticity in juvenile rainbow trout. *Anim. Behav.* 133, 35–45. doi: 10.1016/j.anbehav.2017.09.013

Poltyrev, T., Keshet, G. I., Kay, G., and Weinstock, M. (1996). Role of experimental conditions in determining differences in exploratory behavior of prenatally stressed rats. *Develop. Psychobiol.* 29, 453–462.

Quintela, J., and Andrade, J. P. (2002). Diel feeding rhythms, daily ration and gastric evacuation rates of *Sepia officinalis* in the Ria Formosa lagoon (South Portugal). *Bull. Mar. Sci.* 71, 665–680.

Reimer, O., and Tedengren, M. (1997). Predator-induced changes in byssal attachment, aggregation and migration in the blue mussel, *Mytilus edulis*. *Mar. Freshw. Behav. Phys* 30, 251–266. doi: 10.1080/10236249709379029

Romagny, S., Darmaillacq, A.-S., Guibe, M., Bellanger, C., and Dickel, L. (2012). Feel, smell and see in an egg: emergence of perception and learning in an immature invertebrate, the cuttlefish embryo. *J. Exp. Biol.* 215, 4125–4130. doi: 10.1242/jeb.078295

Sandi, C., Venero, C., and Guaza, C. (1996). Novelty-related rapid locomotor effects of corticosterone in rats. *Eur. J. Neurosci.* 8, 794–800. doi: 10.1111/j.1460-9568.1996.tb01264.x

Schneider, M. L. (1992). Prenatal stress exposure alters postnatal behavioral expression under conditions of novelty challenge in rhesus monkey infants. *Dev. Psychobiol.* 25, 529–540. doi: 10.1002/dev.420250706

Schnell, A. K., Hanlon, R. T., Benkada, A., and Jozet-Alves, C. (2016). Lateralization of eye use in cuttlefish: opposite direction for anti-predatory and predatory behaviors. *Front. Physiol.* 7:620. doi: 10.3389/fphys.2016.00620

Smee, D. L., and Weissburg, M. J. (2006). Hard clams (Mercenaria mercenaria) evaluate predation risk using chemical signals from predators and injured conspecifics. *J. Chem. Ecol.* 32, 605–619. doi: 10.1007/s10886-005-9021-8

Snyder, N. F., and Snyder, H. A. (1971). Defenses of the Florida apple snail *Pomacea paludosa*. *Behaviour* 40, 175–214. doi: 10.1163/156853971X00384

Staudinger, M. D., Buresch, K. C., Mäthger, L. M., Fry, C., McAnulty, S., Ulmer, K. M., et al. (2013). Defensive responses of cuttlefish to different teleost predators. *Biol. Bull.* 225, 161–174. doi: 10.1086/BBLv225n3p161

Storm, J. J., and Lima, S. L. (2010). Mothers forewarn offspring about predators: a transgenerational maternal effect on behavior. *Am. Nat.* 175, 382–390. doi: 10.1086/650443

Suchecki, D., and Neto, J. P. (1991). Prenatal stress and emotional response of adult offspring. *Physiol. Behav.* 49, 423–426. doi: 10.1016/0031-9384(91)90259-Q

Vallee, M., Mayo, W., Dellu, F., Le Moal, M., Simon, H., and Maccari, S. (1997). Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: correlation with stress-induced corticosterone secretion. *J. Neurosci.* 17, 2626–2636.

Vallortigara, G., and Rogers, L. J. (2005). Survival with an asymmetrical brain: advantages and disadvantages of cerebral lateralization. *Behav. Brain Sci.* 28, 575–588. doi: 10.1017/S0140525X05000105

Van den Hove, D. L. A., Blanco, C. E., Aendekerk, B., Desbonnet, L., Bruschettini, M., Steinbusch, H. P., et al. (2005). Prenatal restraint stress and long-term affective consequences. *Dev. Neurosci.* 27, 313–320. doi: 10.1159/000086711

Wang, T., Hung, C. C., and Randall, D. J. (2006). The comparative physiology of food deprivation: from feast to famine. *Annu. Rev. Physiol.* 68, 223–251. doi: 10.1146/annurev.physiol.68.040104.105739

Weinberg, M. S., Bhatt, A. P., Girotti, M., Masini, C. V., Day, H. E., Campeau, S., et al. (2009). Repeated ferret odor exposure induces different temporal patterns of same-stressor habituation and novel-stressor sensitization in both hypothalamic-pituitary-adrenal axis activity and forebrain c-fos expression in the rat. *Endocrinology* 150, 749–761. doi: 10.1210/en.2008-0958

Weinstock, M. (2008). The long-term behavioural consequences of prenatal stress. *Neurosci. Biobehav. Rev.* 32, 1073–1086. doi: 10.1016/j.neubiorev.2008.03.002

Wells, M. J. (1958). Factors affecting reactions to Mysis by newly hatched Sepia. *Behaviour* 13, 96–111. doi: 10.1163/156853958X00055

Wilson, C. A., Vazdarjanova, A., and Terry, A. V. (2013). Exposure to variable prenatal stress in rats: effects on anxiety-related behaviors, innate and contextual fear, and fear extinction. *Behav. Brain Res.* 238, 279–288. doi: 10.1016/j.bbr.2012.10.003

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

ORIGINAL RESEARCH
published: 29 March 2018
doi: 10.3389/fphys.2018.00299

Ontogenetic and Experience-Dependent Changes in Defensive Behavior in Captive-Bred Hawaiian Bobtail Squid, *Euprymna scolopes*

Kia Seehafer[1], Samantha Brophy[2], Sara R. Tom[2] and Robyn J. Crook[2]*

[1] Department of Biology, Sacramento State University, Sacramento, CA, United States, [2] Department of Biology, San Francisco State University, San Francisco, CA, United States

Cephalopod molluscs are known for their extensive behavioral repertoire and their impressive learning abilities. Their primary defensive behaviors, such as camouflage, have received detailed study, but knowledge is limited to intensive study of relatively few species. A considerable challenge facing cephalopod research is the need to establish new models that can be captive bred, are tractable for range of different experimental procedures, and that will address broad questions in biological research. The Hawaiian Bobtail Squid (*Euprymna scolopes*) is a small, tropical cephalopod that has a long history of research in the field of microbial symbiosis, but offers great promise as a novel behavioral and neurobiological model. It can be bred in the laboratory through multiple generations, one of the few species of cephalopod that can meet this requirement (which is incorporated in regulations such as EU directive 2010/63/EU). Additionally, laboratory culture makes *E. scolopes* an ideal model for studying ontogeny- and experience-dependent behaviors. In this study, we show that captive bred juvenile and adult *E. scolopes* produce robust, repeatable defensive behaviors when placed in an exposed environment and presented with a visual threat. Further, adult and juvenile squid employ different innate defensive behaviors when presented with a size-matched model predator. When a 10-min training procedure was repeated over three consecutive days, defensive behaviors habituated in juvenile squid for at least 5 days after training, but memory did not appear to persist for 14 days. In contrast, adult squid did not show any evidence of long-term habituation memory. Thus we conclude that this species produces a range of quantifiable, modifiable behaviors even in a laboratory environment where ecologically-relevant, complex behavioral sequences may not reliably occur. We suggest that the lack of long-term memory in adult squid may be related to their less escalated initial response to the mimic, and thus indicates less motivation to retain memory and not necessary inability to form memory. This is the first demonstration of age-related differences in defensive behaviors in *Euprymna*, and the first record of habituation in this experimentally tractable genus of squid.

Keywords: defensive behavior, *Euprymna*, habituation, learning, memory, squid

OPEN ACCESS

Edited by:
Graziano Fiorito,
Stazione Zoologica Anton Dohrn, Italy

Reviewed by:
Jean-Pierre Bellier,
Shiga University of Medical Science,
Japan
Christine Huffard,
Monterey Bay Aquarium Research
Institute, United States

***Correspondence:**
Robyn J. Crook
rcrook@sfsu.edu

Specialty section:
This article was submitted to
Invertebrate Physiology,
a section of the journal
Frontiers in Physiology

Received: 06 September 2017
Accepted: 13 March 2018
Published: 29 March 2018

Citation:
Seehafer K, Brophy S, Tom SR and
Crook RJ (2018) Ontogenetic and
Experience-Dependent Changes in
Defensive Behavior in Captive-Bred
Hawaiian Bobtail Squid, Euprymna
scolopes. Front. Physiol. 9:299.
doi: 10.3389/fphys.2018.00299

INTRODUCTION

Cephalopod molluscs have received intensive study of their behaviors and nervous systems, due to their vertebrate-like cognitive abilities, neurally-controlled skin pigmentation that enables rapid camouflage and signaling, and dynamic behavioral repertoires that are produced reliably in captive settings. Molluscan models, including cephalopods, have also been instrumental in advancing our understanding of the cellular mechanisms that underlie behavioral plasticity, from the simplest forms of learning (Glanzman, 2010) to more complex cognitive processes, including problem solving and observational learning (Fiorito and Scotto, 1992; Richter et al., 2016). Cephalopods' behaviors are readily modified by experience, including exposure to threats (Crook and Basil, 2008; Crook et al., 2009, 2011; Alupay et al., 2014; Oshima et al., 2016), but due to the difficulty of culturing most cephalopod species from eggs in laboratory settings, less is known about ontogenetic changes to behavior, particularly in squid.

The Hawaiian bobtail squid, *Euprymna scolopes* is a small, tropical cephalopod that has been well studied for its symbiotic relationship with the bioluminescent bacteria, *Vibrio fischeri* (Nyholm and McFall-Ngai, 2004; Lee et al., 2009). *E. scolopes* is one of the few cephalopod species that can be reared successfully through multiple generations in laboratory settings, and is experimentally tractable and relatively easy to keep. It is therefore a promising model for behavioral and neurobiological studies (Zepeda et al., 2017), but currently there is limited literature on its behavior.

The genus *Euprymna* contains at least six species, with cosmopolitan distribution throughout southeast Asia and Australasia and robust local population structures (Kimbell et al., 2002). Previous studies examining the defensive behaviors and personality traits of *Euprymna tasmanica* suggest that innate behavioral responses to threatening stimuli remain fairly constant across the squid's lifespan (Sinn, 2005; Sinn and Moltschaniwskyj, 2005; Sinn et al., 2008, 2010), but experience-dependent effects have not been tested. Wild observations of *E. scolopes* by divers suggest these squid employ a range of defensive behaviors, which may be combined or performed in dynamic sequences when animals were presented with persistent threats (Moynihan, 1983; Anderson and Mather, 1996). Here, for the first time, we examine ontogenetic and experience-dependent effects on defensive behaviors in captive bred *Euprymna scolopes*. By reducing environmental complexity and presenting a stereotyped threat stimulus over multiple trials, we show that juveniles are more reactive to threats than adults, and that juvenile responses can be habituated both within and across days of training. In contrast, adult squid displayed low-level responses to the presented threat, and did not show evidence of within- or across-day habituation under identical training conditions to juveniles.

METHODS

Animals

First and second generation, captive bred squid originated from six wild-caught *E. scolopes,* collected in the waters surrounding O'ahu, Hawaii. Subjects were reared from birth in the laboratory, in continuously circulating artificial seawater maintained at a temperature of 24.5°C. Hatchling and juvenile squid were fed *ad libitum* on a combination of live mysid shrimp (*Amerimysis bahia*) and grass shrimp (*Palaemonetes*)spp. and adult squid were fed live grass shrimp twice per day. *E scolopes* is short lived, growing to sexual maturity at around 55–70 days post hatching, and entering senescence between 90 and 120 days (Lee et al., 2009). Juvenile squid used in this study were 30–37 days post hatching at the outset of experiments, and adults ranged in age from 62 to 90 days post hatching.

Squid were housed in groups of 4–6 in round enclosures 26 cm in diameter. About 2.5 cm of sand covered the mesh bottom of the enclosure and water level was maintained at ∼24 cm, flowing in from an overhead pipe and out through the mesh bottom. At the end of experiments animals remained in the lab in the breeding colony, until they died of natural causes between 120 and 241 days post hatching.

In the United States, cephalopods are not included in federal laws governing the use and welfare of research animals, thus no protocol or approval number was required for this study. As such, all applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Experimental Apparatus

For behavioral tests, one squid was removed from its home enclosure and transferred to a round crystallization dish ("100 mm" size for juveniles and "125 mm" size for adults), filled with water taken from the home tank system but containing no internal structure or substrate. The test arena was enclosed in a white blind that prevented the squid from seeing the predator mimic before it was immersed in the trial arena. A camcorder (Sony, HDR-XR200) was positioned 70 cm directly above the arena, and all trials were filmed for later behavioral analysis. The entire experimental setup was enclosed inside a black curtained sub-room that visually isolated the squid from the experimenters and other stimuli.

We used two predator mimics, size matched to the two age classes of squid, with each ∼4x the body length of the squid being tested (**Figure 1A**). We based the shape and size of the mimic on initial observations of squid not used in the subsequent experiments, that showed strong, innate defensive reactions to a looming visual stimulus that was longer than it was tall, and little to no response to a stimulus that was tall and narrow. We considered that these two orientations may have resembled benthic and pelagic fish predators, respectively. Mimics were constructed from white duct tape attached to a laboratory measuring spoon, cut to a size and shape that produced strong defensive reactions in initial testing. Mimics were colored black with permanent marker to contrast strongly with the white arena surround. A trained experimenter controlled the movement of the mimic. We chose to move mimics by hand instead of by an automated movement. Animals were unrestrained and moved freely about the arena, and it was crucial that the mimic was presented directly in front of the squid at each presentation, to accurately represent a looming predator. Although this undoubtedly

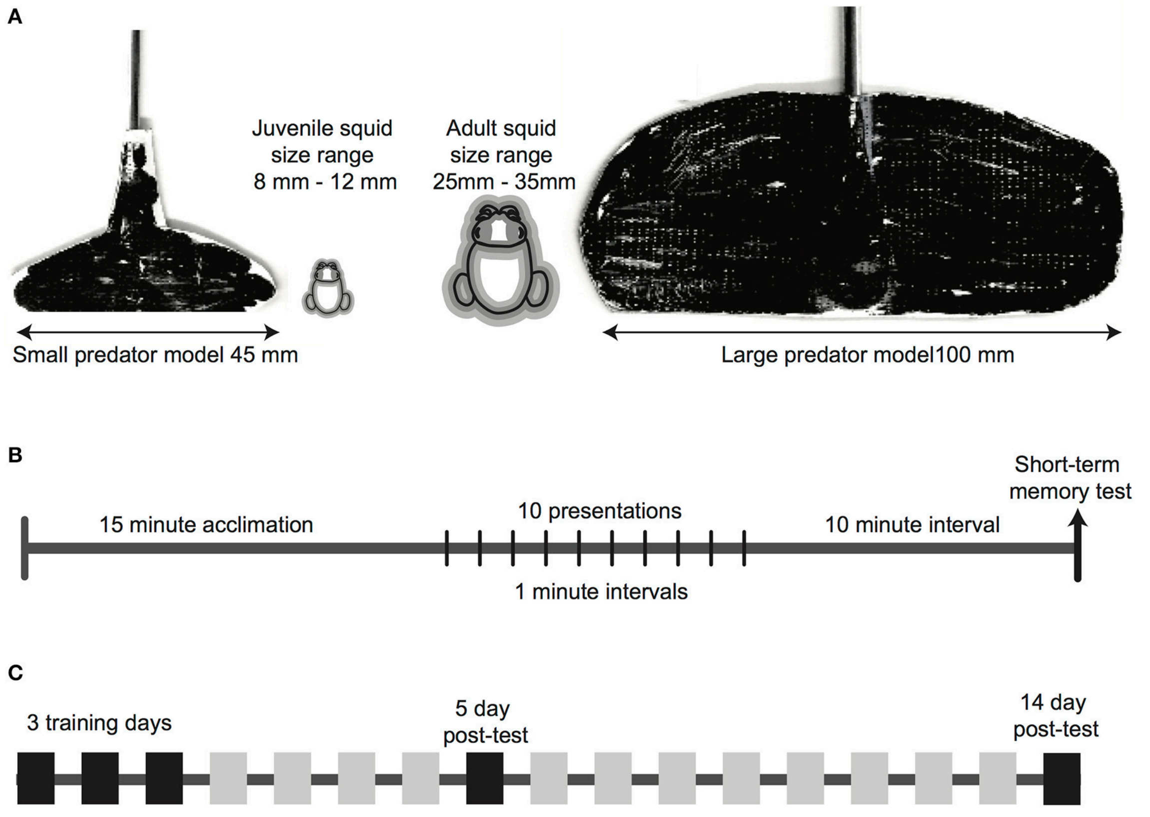


FIGURE 1 | Methodological details of the experiment. **(A)** Predator mimics shown alongside a squid outline, showing the relative size of the subject and the model. Squid outlines show the average squid size (black line), with the gray zone showing the range of sizes in each age class. **(B)** Timeline of each experimental trial, which was repeated on each experiment day. A 15-min acclimation period was followed by 10 presentations of the mimic, with a 1-min inter-presentation interval. A single presentation, which functioned as a short-term memory test, was given 10 min after the conclusion of the training sequence. **(C)** Timeline of the full experiment. Trials occurred on three consecutive training days, and long-term memory was tested 5 days, and again at 14 days after the third training day.

introduced some variation into the speed and positioning of the mimic, we considered that this was preferable to an automated movement that may have collided with the moving squid, or traveled in a fixed line that did not necessarily approach the squid's position. Typical approach speeds were measured at ∼2 cm/s.

Procedure

Each experimental trial followed the same sequence (**Figure 1B**). Squid were given a 15-min acclimation period after being moved into the test arena, in which there was no visual or physical disturbance. Video recording commenced in the final minute of acclimation and continued throughout the trial. After the acclimation period ended, 10 presentations of the predator mimic were given, with a 1-min inter-presentation interval. Mimics were always introduced to the arena at the edge directly opposite the squid, moved across the arena until directly in front of the squid (within 10 mm of the arms), then removed, without making contact with the squid's body.

A single short-term memory test was given 10 min after the final presentation of the mimic. This test presentation was identical to all other presentations.

We repeated this procedure for three consecutive "training days" (**Figure 1C**), then tested for long-term memory retention 5 days after the final training day, and again at 14 days after the final training day, so the longest unreinforced interval tested was 9 days (between the 5 and 14th day). Experimental procedures on the two long-term test days were identical to the training days.

Data Analysis and Statistical Procedures

Behavioral Coding

We categorized defensive behaviors into six classes, and ranked them from lowest to highest level, based on previously published reports (Moynihan, 1983; Sinn and Moltschaniwskyj, 2005; Sinn et al., 2008, 2010). Ranks (**Figure 2**) were: no response (rank = 0; not shown on **Figure 2**), color change (rank = 1), avoidance swimming using the fins (rank = 2), escape jetting using the mantle (rank = 3), a distinctive arm posture we term "arm fan" (rank = 4), and inking (rank = 5). For each presentation, we recorded whether or not a squid performed any of these behaviors, and allocated a score to each response that corresponded to the most escalated behavior observed (for example, a squid that changed color,

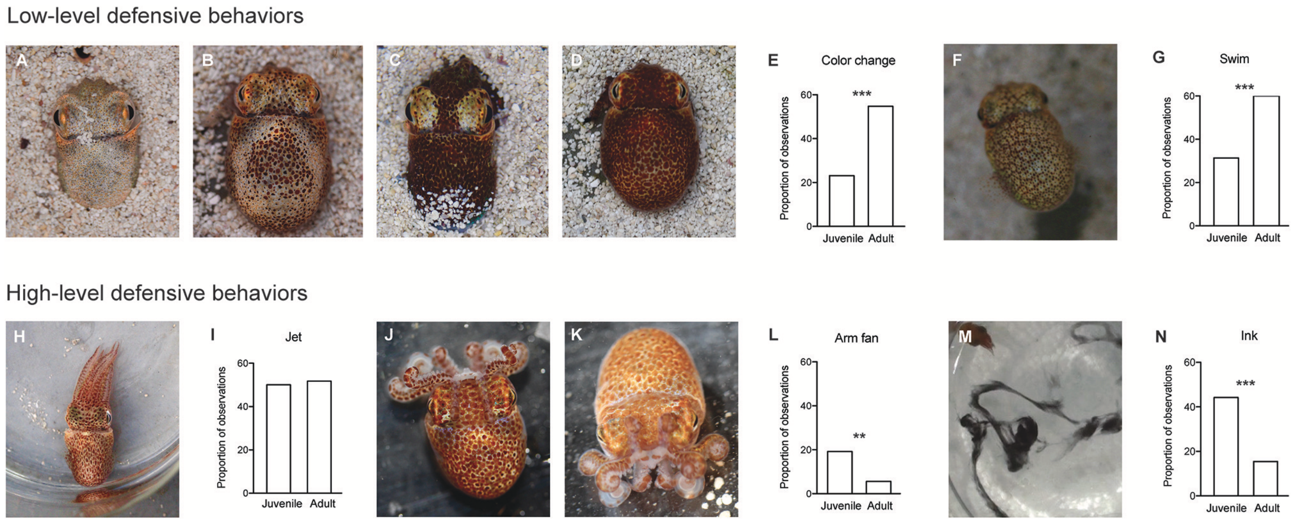


FIGURE 2 | Behaviors produced in response to the predator mimic were classed as high- or low-level responses. On the first training day, adults were more likely to employ low-level responses, while juveniles were more likely to produce high-level responses. Top panel: **A–D** a color change was recorded when the animal changed from any one of four defined patterns to another. We grouped the continuous color change variable into four discrete patterns: **(A)** Pale, **(B)** Dice (see Moynihan, 1983), **(C)** Mottled or **(D)** Dark. Photographs show a squid on sandy substrate for illustrative purposes only. Trials were conducted with no substrate. **(E)** Color change was more likely to be employed by adult squid ($n = 15$) compared with juveniles ($n = 12$) (Chi-square test, $p < 0.001$). **(F)** Fin swimming, another low leve defensive behavior, was characterized by slow movement (<1 bodylength/s) extended, undulating fins, and rounded mantle. **(G)** Adults employed fin swimming more frequently than juveniles ($p < 0.001$). Bottom Panel: we recorded three high-level defensive behaviors. **(H)** Jetting was characterized by extended arms, rapid movement (>1 bodylength/second), folded fins and contracted mantle. **(I)** There was no difference in the frequency of jetting in response to the predator mimic between juvenile and adult squid. **(J,K)** A characteristic arm posture "Arm Fan" employed by *E.scolopes* in response to the predator mimic. **(J,K)** show two different angles. **(L)** Arm fan was produced more frequently by juveniles ($p = 0.02$). **(M)** Inking was the highest level defensive response we recorded. **(N)** Inking was more frequent in juveniles than in adults ($p < 0.001$).

swam away and then produced an arm fan would receive an escalation score of 4). While squid often employed several behaviors in response to one presentation of the stimulus (as has been described previously by Anderson and Mather, 1996), we chose a simplified method of recording only the single most escalated response, as we hypothesized that this would be most likely to capture declines in responses due to habituation learning. We also measured the time the squid took after each presentation to return to a quiescent state, usually characterized by sitting on the base of the arena or hovering in place using fin swimming. Because squid occasionally stopped and then restarted responses to the stimulus, we considered quiescence to have occurred when the squid remained still for at least 5 s.

Behavioral data from video records was coded independently by at least two experimenters, and inter-rater reliability exceeded 90%. In addition, 4 "untrained" observers re-scored ∼10% of all video files, and were given only written and still-image descriptions of the behaviors recorded by trained observers. Untrained scorers had observed *E. scolopes* behavior informally in the lab, but did not take part in experiments and were blind to expectations and previous data collection for the study. "Untrained" observers replicated the trained observers scores in 82% of the observations, a level we deemed acceptable. Where mismatched scores for either trained or untrained observers were recorded, the senior author (RJC) re-scored those observations, and acted as the "tie-breaker" in determining the final score for that presentation.

Statistical Analyses

Behavioral responses were recorded for every presentation in the first training trial to compute response proportions (**Figure 2**). Statistical analyses were conducted in GraphPad Prism 6.0. We used a Chi-Square test with Yates' correction to compare frequencies between adults and juveniles for each behavior.

For ease of interpretation, on figures we show only behavioral responses for the first, tenth and test presentation for each day. Ranked escalation scores (**Figure 3**) were compared with Wilcoxon signed-ranks tests for comparisons made between the first and tenth minute of each test, and the first minute and test presentations. We also compared scores between the first minute on the first training day, and each of the first minutes on each subsequent day. Sequential Bonferroni corrections were applied to planned comparisons.

To compare the proportions of "response vs. no response" (**Figure 4**), we used Fisher's Exact Tests. Latency to quiescence measures (**Figure 5**) conformed to the normal distribution (Bartlett's Test: adults: $p = 0.23$, juveniles; $p = 0.13$) and were analyzed with ANOVA followed by paired t-tests for within and across-trial comparisons. A $p < 0.05$ was considered significant.

RESULTS

Frequencies of High- and Low-Level Defensive Behaviors Vary With Age

Innate responses to the predator model varied between juvenile and adult squid. On the first day of training, when squid

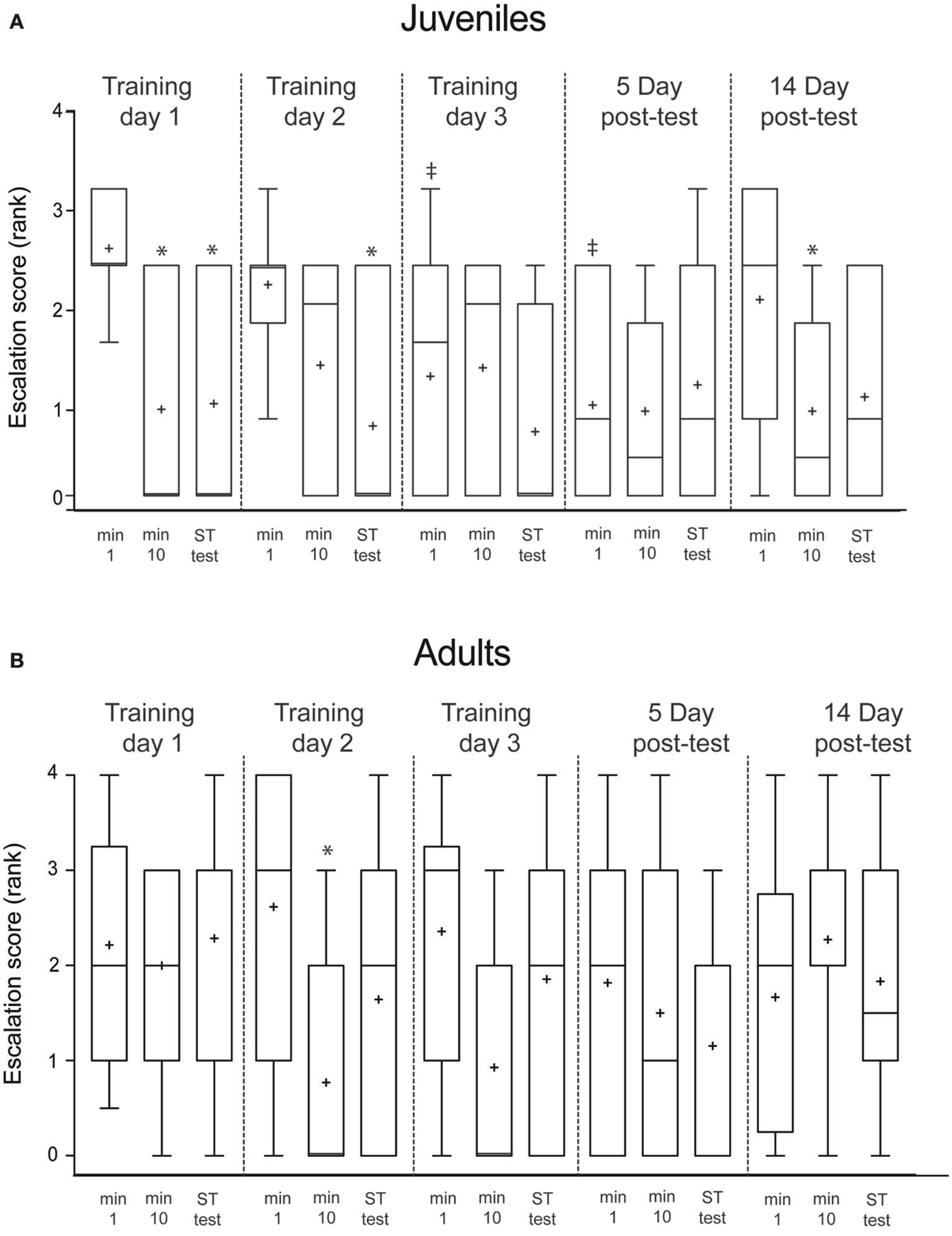


FIGURE 3 | Escalation score (the highest ranked behavior produced in response to presentation of the predator mimic) declined in juveniles both within and across days, but did not decline in adults. Ranks were: no response = 0, color change = 1, avoidance swim = 2, jet = 3, arm fan = 4, ink = 5. **(A)** Mean escalation scores of juveniles ($n = 12$) plotted for the first minute, final (tenth) minute and short-term test (10 min after training) for each day of the experiment. **(B)** The same intervals plotted for adult squid ($n = 15$). Boxes show 25/75 percentiles, whiskers are 10–90th percentiles. Median is indicated with a line and mean with +. Comparisons are made with Wilcoxon signed-ranks tests. Comparisons within each day (min 1 vs. min 10 or vs. test, indicating short-term memory aquired within a trial) are denoted with *. Across trial comparisons for the first minute of Day 1 vs. first minutes on subsequent days (indicating long-term memory) are denoted with ‡.

were experiencing the predator mimic for the first time, adult squid were more likely to display low level defensive behaviors of color changes ($\chi^2 = 34.9$, $p < 0.001$) and fin swimming, ($\chi^2 = 27.3$, $p < 0.001$; See **Figures 2A–G**) compared with juveniles, while the high level defensive behaviors of arm postures and inking were more frequently employed by juveniles than by adults (arm fan: $\chi^2 = 13.5$, $p = 0.002$; ink $\chi^2 = 34.2$, $p < 0.001$; **Figures 2J–N**). Instances of Jetting did not differ between the two age classes (**Figures 2H,I**).

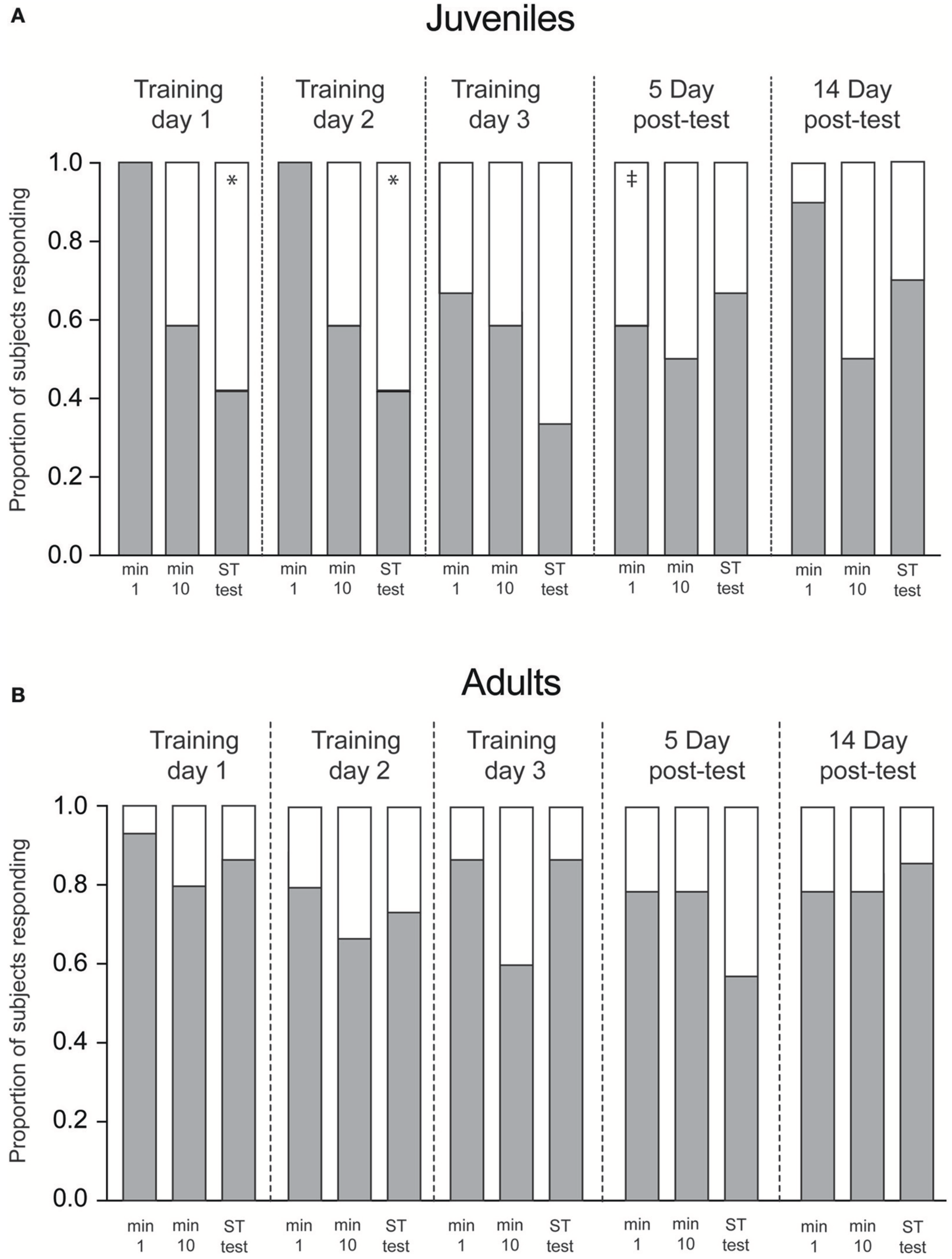


FIGURE 4 | Proportion of response (gray) and non-response (white) to Presentation 1, 10 and short-term tests on each training day. **(A)** Juveniles ($n = 12$) showed significant declines in responses from the first presentation to the short-term memory test on Day 1 and Day 2. Long-term memory was present at the 5-day interval, but not the 14-day interval. **(B)** Adult squid showed no change in response/nonresponse rate over the training sequence. Bars show proportion of squid responding on each trial. Statistical comparisons made with Fisher's Exact Tests Comparisons within each day (min 1 vs. test, indicating short-term memory acquired within a trial) are denoted with *. Significant across trial comparisons for the first minute of Day 1 vs. first minutes on subsequent days (indicating long-term memory) are denoted with ‡.

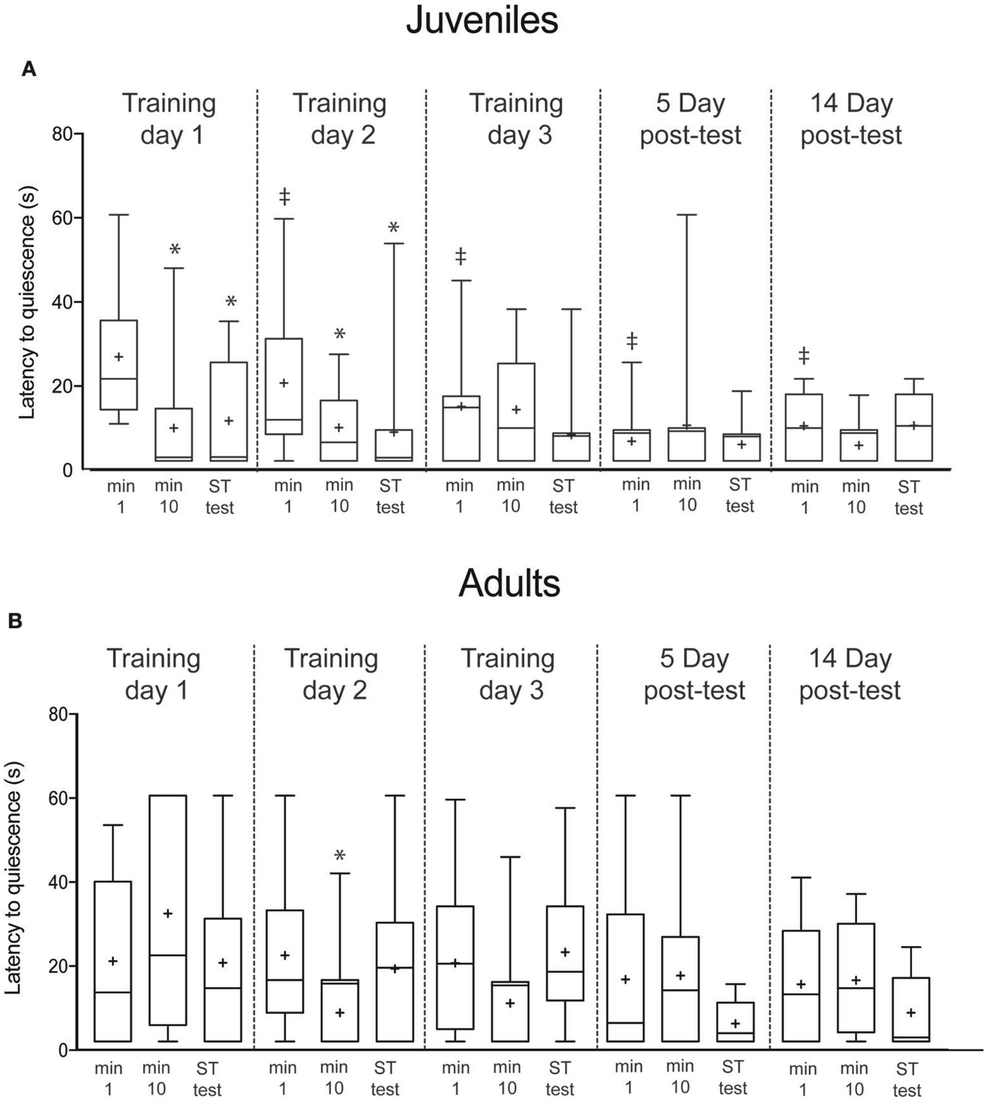


FIGURE 5 | Latency to quiescence after the first, tenth and short-term test presentation of the predator mimic. **(A)** showed significant declines in responses from the first to last training trials on Day 1 and Day 2, and showed short-term retention on each day. Long-term memory appeared on Day 3 and was present at the 5-day interval, but not the 14-day interval. **(B)** Adult squid showed no sustained changes in response/nonresponse rate over the training sequence. Boxes show 25/75 percentiles, whiskers are 10–90th percentiles. Median is indicated with a line and mean with +. Statistical comparisons made with paired *t*-tests. Comparisons within each day (min 1 vs. min 10 or vs. test, indicating short-term memory acquired within a trial) are denoted with *. Across trial comparisons for the first minute of Day 1 vs. first minutes on subsequent days (indicating long-term memory) are denoted with ‡.

Juvenile Squid Show Consistent Evidence of Short- and Long-Term Defensive Habituation, but Adults Do Not

In juvenile squid, the average escalation score (the rank value of the most highly escalated behavior produced at each presentation) declined significantly from the first to the tenth minute of training on the first training day ($p = 0.03$; **Figure 3A**), indicating rapid habituation to the predator mimic. Reduced responses persisted at the short-term memory test ($p = 0.03$). There was no long-term memory apparent after a single day of training, as the escalation score for the first presentation on the second day was not lower than that on the first day. Scores declined again over the course of the second day of training, with short-term memory apparent ($p = 0.02$). By the third training day, long term habituation memory was present, as the initial score was significantly lower than that of the first training day ($p = 0.03$) Escalation scores remained low at the first presentation of the 5-day post-test (0.02), indicating stable long-term memory. Escalation scores on day 14 were not significantly different than for Day 1, indicating no memory of the procedure persisted at this interval.

The results for adult squid differed in that there was no apparent habituation on Day 1 of training, and short-term memory first appeared on the second training day ($p = 0.03$; **Figure 3B**). Unlike in juvenile squid, long-term (>24 h) reduction in escalation scores was not apparent in adult squid.

We also measured learning by tracking the proportion of presentations to which squid made any measureable response, vs. no response at all (**Figure 4**). Similar to results described above, juvenile squid showed both within- and across-trial habituation to the predator mimic (**Figure 4A**), with positive responses declining over the first day of training ($p = 0.02$) and the second day of training (p=0.016). Proportions of responses were intermediate for the third training day, but long-term memory was apparent at the 5-day post-test, where response rate was significantly lower than on the first trial of the first training day ($p = 0.04$). By the 14th day after training proportions were not different from Day 1, matching the results observed for escalation scores. Among adult squid, response rates were generally high across all trials, with no significant declines observed at any point in the experimental sequence.

Lastly, we recorded the latency for squid to return to quiescence after each presentation of the predator mimic. Among juvenile squid, latency to quiescence declined in the first trial ($t = 4.9$, $p = 0.004$; **Figure 5A**), and remained low for the short-term memory test ($t = 3.1$, $p = 0.01$). The same pattern was observed on Day 2 (min 1 vs. min 10, $t = 2.2$, $p = 0.047$; min 1 vs. ST memory test, $t = 3.8$, $p = 0.003$). By Day 3 long-term memory was apparent (min 1 Day 1 vs. min 1 Day 3, $t = 2.1$, $p = 0.04$). Latencies remained significantly reduced on both Day 5 (min 1 Day 1 vs. min 1 Day 5, $t = 3.6$, $p = 0.0006$) and Day 14 after training (min 1 Day 1 vs. min 1 Day 14, $y = 2.8$, $p = 0.006$).

Latency to quiescence for adults showed a somewhat different pattern to escalation and overall response rate. Although there was no learning apparent on Day 1 (**Figure 5B**), on the second training day a significant reduction in latency occurred by the tenth training trial ($t = 2.3$, $p = 0.02$), however, this short-term effect did not persist at the 10-min memory test, nor was there a similar significance pattern on Day 3. There was no evidence of across-trial reductions that would indicate long-term memory acquisition.

DISCUSSION

Here we show that *Euprymna scolopes* displays age-related changes to innate defensive behaviors, and also exhibits age-related differences in short-and long-term habituation. More broadly, we show that changes to the behaviors of these captive bred squid can be captured by relatively simple metrics, suggesting that this species is a promising behavioral and neurobiological model that warrants additional development and investigation.

Juvenile squid displayed escalated innate defensive behavior more frequently than adult squid, (where "innate" defensive behavior was defined as any behavior exhibited on the first day of training, given that squid were all captive bred and had no experience with real or simulated predators prior to experiments). Previous findings from a closely related species, *Euprymna tasmanica,* suggest similar ontogenetic shifts in defensive behavior, where juveniles exhibit strong defensive responses, sub adults a wide variety of responses, and adults solidify their responses as either "shy" or "bold" squid (Sinn and Moltschaniwskyj, 2005; Sinn et al., 2008). It is possible that since we tested different animals in the juvenile and adult age groups, the adults' physical (developmental) environment, which included frequent handling and constant presence of laboratory personnel, may have contributed to their less reactive defensive behavior, or that adult squid of this species are bolder in general. Although the juvenile squid in our study were sexually immature, in other respects their behavior and physiology appears largely indistinguishable from that of adults; their hunting behaviors and general diurnal patterns are identical to those of adults, and we observed no evidence of immaturity in their sensory or motor systems that might explain the differences in behaviors between adults and juveniles.

Escalation score and response/non-response data (**Figures 3**, **4**) indicate that juveniles habituate in the short-term over each trial, and exhibit signs of long-term memory at the 5-day retention interval. Although it is possible that the within-trial declines we observed might be due to fatigue or some other non-mnemonic factor, such effects cannot account for the across-trial effect of reduced responses on the first presentation on subsequent days. Both within- and across-trial habitation have been demonstrated in other cephalopods (Angermeier and Dassler, 1992; Kuba et al., 2006a,b), and in a large number of other invertebrate species (see Byrne and Hawkins, 2015). We have recently demonstrated stable long-term associative memory in the species (Zepeda et al., 2017) but to our knowledge this is the first demonstration of long-term, non-associative learning in sepiolid squid.

While adult squid also showed some inconsistent signs of short-term habituation to the predator model, they never exhibited long-term retention. We propose several explanations for why adults failed to commit the predator mimic to long-term memory. First, it is possible that *E. scolopes* becomes less cognitively capable of consolidating long-term memory as it ages. Studies of cuttlefish (*S. officinalis*), show that two year old (geriatric) animals were slower to learn an avoidance task than one year old cuttlefish (Mather, 2008), and that in senescent cuttlefish, initial neural decline was motor and not sensory (Mather, 2008). This suggests that older or senescent cephalopods may have a failure to react to stimuli, even though they register it.

Second, it is possible that the predator mimic we used mimicked a predator specifically of juvenile squid. It is likely that wild *E. scolopes* face different predators at different size classes, but there is little available literature on predators of adult *E. scolopes.* Other studies on cephalopods have shown that familiarization with specific prey throughout early time periods leads to specific prey preferences (Darmaillacq et al., 2006a,b), which could be true also for predator recognition. It is possible that adult squid are not predated by benthic predators in the wild, and thus our mimic was less ecologically relevant to adults than to juveniles. Predation risk is generally related to body size, and as individuals grow they face different predators (Werner and Gilliam, 1984) Additionally, the adult *E. scolopes* in this study had no prior experience of predators; perhaps the lack of exposure during an early "sensitive period" in which predator recognition is solidified, contributed to the minimal responses we observed here.

Third, it is possible that our study was unable to clearly identify adult defensive behaviors. For example, adults may engage in burying as a defensive behavior more often than highly escalated and highly visible behavior such as inking; since our experimental set-up did not include substrate, we may have been unable to clearly distinguish burying behavior in adults from avoidant swimming or jetting.

CONCLUSION

Captive bred *Euprymna* express ecologically relevant, easily quantifiable defensive behaviors in controlled experimental settings. *E. scolopes* exhibits age-dependent, innate defensive behaviors in response to a simulated predator threat test, and age-dependent differences in patterns of learning and memory. We propose that our study provides several important advances for the field of cephalopod research, which is currently challenged to meet higher ethical standards than in the past (see EU directive 2010/63/EU), to broaden its scope to improve the diversity of species typically used in studies, and to produce novel findings that enlighten broad questions in biological research. Here, we demonstrate the feasibility of using first and second generation captive bred specimens of *E. scolopes* in behavioral research, a more ethical alternative than using wild-caught specimens. Further, we show that squid, which are rarely used in behavioral experiments, express robust non-associative learning and memory, a finding that broadens knowledge of the diversity of behaviors in cephalopods. Finally, we suggest that studies such as those described here have the possibility of enlightening broad debates about origins of intelligence, the neuroanatomical and physiological underpinnings of neural plasticity, and the evolution of complex behaviors.

AUTHOR CONTRIBUTIONS

KS and RC designed experiments; KS, SB, and ST conducted experiments; all authors analyzed data and conducted statistical analysis, all authors wrote the paper.

ACKNOWLEDGMENTS

We thank members of the Crook Laboratory at SF State for assistance with animal husbandry and for additional data analysis. The Nyholm Lab at the University of Connecticut provided us with *Euprymna* eggs, and Eric Koch at the University of Hawaii at Manoa supplied us with adult *Euprymna*, to initiate our breeding colony. Bret Grasse from the Monterrey Bay Aquarium provided invaluable advice on *Euprymna* husbandry and breeding.

This study was funded by start-up funding to RC from SFSU, and KS was supported by NSF-REU grant DBI-1659175.

REFERENCES

Alupay, J. S., Hadjisolomou, S. P., and Crook, R. J. (2014). Arm injury produces long-term behavioral and neural hypersensitivity in octopus. *Neurosci. Lett.* 558, 137–142. doi: 10.1016/j.neulet.2013.11.002

Anderson, R. C., and Mather, J. A. (1996). Escape responses of *Euprymna scolopes* Berry, 1911 (*Cephalopoda*: *Sepiolidae*). *J. Molluscan Stud.* 62, 543–545. doi: 10.1093/mollus/62.4.543

Angermeier, W. F., and Dassler, K. (1992). Inhibitory learning and memory in the lesser octopus (*Eledone cirrhosa*). *Bull. Psychon. Soc.* 30, 309–310. doi: 10.3758/BF03330474

Byrne, J. H., and Hawkins, R. D. (2015). Nonassociative learning in invertebrates. *Cold Spring Harb. Perspect. Biol.* 7:a021675. doi: 10.1101/cshperspect.a021675

Crook, R., and Basil, J. (2008). A biphasic memory curve in the chambered nautilus, *Nautilus pompilius* L. (Cephalopoda: Nautiloidea). *J. Exp. Biol.* 211, 1992–1998. doi: 10.1242/jeb.018531

Crook, R. J., Hanlon, R. T., and Basil, J. A. (2009). Memory of visual and topographical features suggests spatial learning in nautilus (*Nautilus pompilius* L.). *J. Comp. Psychol.* 123, 264–274. doi: 10.1037/a0015921

Crook, R. J., Lewis, T., Hanlon, R. T., and Walters, E. T. (2011). Peripheral injury induces long-term sensitization of defensive responses to visual and tactile stimuli in the squid *Loligo pealeii*, Lesueur 1821. *J. Exp. Biol.* 214, 3173–3185. doi: 10.1242/jeb.058131

Darmaillacq, A. S., Chichery, R., and Dickel, L. (2006a). Food imprinting, new evidence from the cuttlefish Sepia officinalis. *Biol. Lett.* 2, 345–347. doi: 10.1098/rsbl.2006.0477

Darmaillacq, A. S., Chichery, R., Shashar, N., and Dickel, L. (2006b). Early familiarization overrides innate prey preference in newly hatched *Sepia officinalis* cuttlefish. *Anim. Behav.* 71, 511–514. doi: 10.1016/j.anbehav.2005.04.019

Fiorito, G., and Scotto, P. (1992). Observational learning in *Octopus vulgaris*. *Science* 256, 545–547. doi: 10.1126/science.256.5056.545

Glanzman, D. L. (2010). Common mechanisms of synaptic plasticity in vertebrates and invertebrates. *Curr. Biol.* 20, R31–R36. doi: 10.1016/j.cub.2009.10.023

Kimbell, J. R., McFall-Ngai, M. J., Roderick, G. K., Crespi, B., Liu, H., and Flook, P. (2002). Two genetically distinct populations of bobtail squid, *Euprymna scolopes*, exist on the Island of O'ahu. *Pacific Sci.* 56, 347–355. doi: 10.1353/psc.2002.0024

Kuba, M. J., Byrne, R. A., Meisel, D. V., and Mather, J. A. (2006a). Exploration and habituation in intact free moving *Octopus vulgaris*. *Int. J. Comp. Psychol.* 19, 426–438.

Kuba, M. J., Byrne, R. A., Meisel, D. V., and Mather, J. A. (2006b). When do octopuses play? Effects of repeated testing, object type, age, and food deprivation on object play in *Octopus vulgaris*. *J. Comp. Psychol.* 120, 184–190. doi: 10.1037/0735-7036.120.3.184

Lee, P. N., McFall-Ngai, M. J., Callaerts, P., and de Couet, H. G. (2009). The Hawaiian bobtail squid (*Euprymna scolopes*): a model to study the molecular basis of eukaryote-prokaryote mutualism and the development and evolution of morphological novelties in cephalopods. *Cold Spring Harb. Protoc.* 2009:pdb.emo135. doi: 10.1101/pdb.emo135

Mather, J. A. (2008). Cephalopod consciousness: behavioural evidence. *Conscious. Cogn.* 17, 37–48. doi: 10.1016/j.concog.2006.11.006

Moynihan, M. (1983). Notes on the behavior of *Euprymna scolopes* (*Cephalopoda*: *Sepiolidae*). *Behaviour* 85, 25–41. doi: 10.1163/156853983X00020

Nyholm, S. V., and McFall-Ngai, M. (2004). The winnowing: establishing the squid–vibrio symbiosis. *Nat. Rev. Microbiol.* 2, 632–642. doi: 10.1038/nrmicro957

Oshima, M., di Pauli von Treuheim, T., Carroll, J., Hanlon, R. T., Walters, E. T., and Crook, R. J. (2016). Peripheral injury alters schooling behavior in squid, Doryteuthis pealeii. *Behav. Processes* 128, 89–95. doi: 10.1016/j.beproc.2016.04.008

Richter, J. N., Hochner, B., Kuba, M. J., Hochner, B., Segev, I., and Hochner, B. (2016). Pull or push? octopuses solve a puzzle problem. *PLoS ONE* 11:e0152048. doi: 10.1371/journal.pone.0152048

Sinn, D. L. (2005). *From Individuals to Populations: Personality Traits in Southern Dumpling Squid (Euprymna tasmanica Pfeffer, 1884) and Their Life History Correlates*. University Tasmania. Available online at: http://eprints.utas.edu.au/255 (Accessed July 17, 2017).

Sinn, D. L., Gosling, S. D., and Moltschaniwskyj, N. A. (2008). Development of shy/bold behaviour in squid: context-specific phenotypes associated with developmental plasticity. *Anim. Behav.* 75, 433–442. doi: 10.1016/j.anbehav.2007.05.008

Sinn, D. L., and Moltschaniwskyj, N. A. (2005). Personality traits in dumpling squid (*Euprymna tasmanica*): context-specific traits and their correlation with biological characteristics. *J. Comp. Psychol.* 119, 99–110. doi: 10.1037/0735-7036.119.1.99

Sinn, D. L., Moltschaniwskyj, N. A., Wapstra, E., and Dall, S. R. X. (2010). Are behavioral syndromes invariant? Spatiotemporal variation in shy/bold behavior in squid. Behav. *Ecol. Sociobiol.* 64, 693–702. doi: 10.1007/s00265-009-0887-2

Werner, E. E., and Gilliam, J. F. (1984). The ontogenetic niche and species interactions in size-structured populations. *Annu. Rev. Ecol. Syst.* 15, 393–425. doi: 10.1146/annurev.es.15.110184.002141

Zepeda, E. A., Veline, R. J., and Crook, R. J. (2017). Rapid associative learning and stable long term memory in the squid, *Euprymna scolopes*. *Biol. Bull.* 232, 212–218. doi: 10.1086/693461

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Physiology

PERSPECTIVE
published: 21 August 2018
doi: 10.3389/fphys.2018.01160

The Cephalopod Large Brain Enigma: Are Conserved Mechanisms of Stem Cell Expansion the Key?

*Astrid Deryckere and Eve Seuntjens**

Laboratory of Developmental Neurobiology, Department of Biology, KU Leuven, Leuven, Belgium

Within the clade of mollusks, cephalopods have developed an unusually large and complex nervous system. The increased complexity of the cephalopod centralized "brain" parallels an amazing amount of complex behaviors that culminate in one order, the octopods. The mechanisms that enable evolution of expanded brains in invertebrates remain enigmatic. While expression mapping of known molecular pathways demonstrated the conservation of major neurogenesis pathways and revealed neurogenic territories, it did not explain why cephalopods could massively increase their brain size compared to other mollusks. Such an increase is reminiscent of the expansion of the cerebral cortex in mammalians, which have enlarged their number and variety of neurogenic stem cells. We hypothesize that similar mechanisms might be at play in cephalopods and that focusing on the stem cell biology of cephalopod neurogenesis and genetic innovations might be smarter strategies to uncover the mechanism that has driven cephalopod brain expansion.

Keywords: stem cell, neurogenesis, cephalopod, brain development, invertebrate neuron

OPEN ACCESS

Edited by:
Graziano Fiorito,
Stazione Zoologica Anton Dohrn, Italy

Reviewed by:
Lindy Holden-Dye,
University of Southampton,
United Kingdom
Pamela Imperadore,
Association for Cephalopod
Research – CephRes, Italy
Thomas Hummel,
Universität Wien, Austria

***Correspondence:**
Eve Seuntjens
eve.seuntjens@kuleuven.be

Specialty section:
This article was submitted to
Invertebrate Physiology,
a section of the journal
Frontiers in Physiology

Received: 13 February 2018
Accepted: 02 August 2018
Published: 21 August 2018

Citation:
Deryckere A and Seuntjens E (2018)
The Cephalopod Large Brain Enigma:
Are Conserved Mechanisms of Stem
Cell Expansion the Key?
Front. Physiol. 9:1160.
doi: 10.3389/fphys.2018.01160

THE COMPLEX BRAIN OF CEPHALOPODS

Among mollusks and even among all invertebrates, cephalopods have a large and complex brain that is highly centralized (Nixon and Young, 2003). The brain encircles the esophagus and is divided into 25 major lobes further subdivided in 37 or 38 lobes in octopods and decapods, respectively. These lobes control different functionalities, including motor function, feeding and color change, but also sensory information processing and higher cognitive functioning (Young, 1963, 1971; Budelmann, 1995; Nixon and Young, 2003). The adult cephalopod brain has a typically invertebrate ganglia-like structure with densely packed neural cell bodies lying in the outer, perikaryal layer and branched processes and synapses centered in the neuropil (Matheson, 2002; Richter et al., 2010). It however seems that the cephalopod brain has a cordal origin, meaning that the initially formed cluster of neurons is longitudinally stretched rather than densely packed. This cordal organization is similar to the more primitive aculiferans like the chiton instead of a ganglionic origin shared by conchifera such as gastropods and bivalves (Richter et al., 2010; Shigeno et al., 2015). In this simplistic system of cords, neurons are allocated in rope-like territories in the neurectoderm, spanning the midline of the early embryo. The brain then develops further by increasing the size of these cordal territories by proliferation and migration of neuroblasts and by global transitions to centralize the cords (Yamamoto et al., 2003; Richter et al., 2010; Shigeno et al., 2015). Along with ensheathing neuron fibers with myelin to increase conduction velocity, which is widespread in vertebrates but also invertebrates (Boullerne, 2016), this centralization allows faster information processing leading to more complex behavior (Budelmann, 1995).

Similar to a proposed scenario of nervous system evolution (Arendt et al., 2016), cephalopods might have adopted a simple neural organization (nerve net) and expanded its size (to cord and brain lobe) by enhancing neurogenesis. It is tempting to speculate that an increase in neuronal number would allow complex behaviors and enhanced cognitive capacity. The common octopus for example has the largest and most complex brain of all cephalopods, allowing amazing problem solving capacity (Young, 1971; Fiorito et al., 1990; Nixon and Young, 2003). Its nervous system accounts for about 500 million nerve cells (Young, 1963; Budelmann, 1995) which is seven times more compared to the mouse brain (Herculano-Houzel et al., 2006) and comparable to the marmoset, a small primate (Herculano-Houzel et al., 2007).

Also in vertebrate evolution, increased learning and memory is paralleled by a massive expansion of the cerebral cortex (Abdel-Mannan et al., 2008). What remains puzzling is how certain cephalopods such as cuttlefish, squid and in particular octopus were able to immensely increase their neuronal numbers to 100s of millions, whereas other mollusks (e.g., *Aplysia*: 10,000 neurons) or invertebrates (e.g., *Drosophila*: 135,000 neurons) did not. In this Perspective, we discuss the potential mechanisms that could lead to increased neuronal production in cephalopods from an evolutionary viewpoint and suggest routes for future investigation.

CONSERVED GENETIC PATHWAYS FOR NEUROGENESIS: WHAT DO WE LEARN?

Several studies on the fruit fly *Drosophila melanogaster*, the annelid *Platynereis dumerilii* and the mouse indicate that divergent species have chosen a similar molecular blueprint to establish their central nervous system (CNS) (Martín-durán et al., 2018). Transcription factors and secreted morphogens that determine the anterior–posterior (Otx-Pax-Hox) as well as dorso-ventral (BMP-Msx-Nkx) patterning of the CNS have been evolutionary conserved and ensure the organized development and position of the CNS in invertebrate and vertebrate species (Hirth, 2010). For example, signaling molecules and transcription factors such as *Nkx2.1*/*Nkx2.2*, *Pax6*, and *Otx2* are expressed in a comparable pattern along the anterior–posterior and dorsoventral axes in the neurectoderm of *D. melanogaster* and *P. dumerilii* and the dorsal neural plate of vertebrates (Holland et al., 2013; Martín-durán et al., 2018).

In cephalopods, the expression of these and other general neuroectodermal patterning transcription factors has also been conserved (Navet et al., 2014; Wollesen et al., 2014). *Pax2/5/8* expression in the CNS of the pygmy squid *Idiosepies notoides* demarcates roughly comparable anterior–posterior patterning as *Drosophila* and mouse, positioning the structures that are responsible for higher cognitive functioning and signal integration, such as the superior frontal and the vertical lobe, at the most anterior end (Wollesen et al., 2015). A similar study in *Sepia officinalis* shows that the mediolateral patterning of the CNS marked by *Nkx2.1*, *Pax2-8*, *Gsx*, and *Msx* seems grossly conserved, although the orientation has been reversed (Buresi et al., 2016). Furthermore, the collinear anterior to posterior expression pattern of Hox genes is preserved in the CNS of the squid *Euprymna scolopes* (Lee et al., 2003). Shh, a morphogen and the transcription factor Pax6 have been extensively studied in vertebrates and *Drosophila* where they steer eye formation and are involved in nervous system development by specifying dorsoventral identity (Echelard et al., 1993; Halder et al., 1995; Ericson et al., 1997). In the cuttlefish *S. officinalis* and squids *Loligo opalescens* and *E. scolopes*, *Pax6* expression is found in the developing eyes, suckers of the arms and in the optic lobes (Tomarev et al., 1997; Hartmann et al., 2003; Navet et al., 2009). In *S. officinalis*, expression was also observed in visceral and cerebral ganglia, but unlike in vertebrates, *Pax6* expression is not restricted to the dorsal area of the CNS and *Shh* is constrained to tissues surrounding the optic area (Navet et al., 2009, 2014).

Taken together, several conserved transcription factors and morphogens are expressed in developing cephalopod brains, in patterns that remain grossly similar to other invertebrates. However, signaling factors such as Wnt, TGF-β, Hedgehog, FGF, and Notch as well as transcription factors such as SoxB and proneural basic helix-loop-helix proteins have been found to be implicated in neurogenesis and the formation of neural networks in *Nematostella vectensis*, a cnidarian without a centralized and expanded brain (Rentzsch et al., 2017). It therefore, remains questionable whether the presence of conserved neurogenic factors in itself will be key to reveal the mechanism behind the remarkable expansion of neural tissue in coleoid cephalopods. Indeed, not only the presence of such factors is important, their function needs to be preserved as well. The latter is not always the case: bivalves and gastropods adopted the expression of posterior markers of brain development such as *Gbx* to develop a shell: a different, typical mollusk feature that is absent in coleoid cephalopods (Wollesen et al., 2017). This finding indicates we might not discover the (molecular) mechanism driving neurogenic expansion by examining merely the presence of conserved molecular pathways. In addition, it will be required to investigate functional conservation. Furthermore, as will be explained below, the neurogenic process itself could be studied more from a cell biological viewpoint, especially in species that evolved out of the ordinary.

MECHANISMS TO INCREASE NEURONAL CELL NUMBER

Examples of neural expansion in terms of cell number can be found most prominently in vertebrates, in the most anterior part of their CNS, the telencephalon. Shortly after neurulation, the neural tube extends in a lateral fashion by symmetric divisions of the neuroepithelial precursors. This leads to an expansion of the neurogenic domain and happens before the generation of neurons (Fish et al., 2008). Such a lateral expansion goes beyond typical neuroectodermal invaginations observed in other deuterostomes and ecdysozoans (Hartenstein and Stollewerk, 2015). A broad neurogenic domain is also apparent during cephalopod development. The cephalopod brain emerges from four pairs of ectodermal placodes in the equatorial zone of the embryo, that develop into rope-like territories (Yamamoto et al., 2003). At the onset of organogenesis, these neurogenic precursor regions occupy a major part of the cephalopod embryonic ectoderm as was shown by expression of *SoxB1* (Buresi et al., 2016) and represented by color-marked areas in **Figure 1A**. At the same time, there is evidence of early post-mitotic neurons expressing synaptotagmin or *NeuroD* (**Figure 1B**; Shigeno et al., 2015; Buresi et al., 2016). Interestingly, the cephalopod neurogenic territory is layered, and post-mitotic neurons (pm) form a distinctive band toward the inside (**Figure 1C**), whereas progenitors form a distinct sheet on top (**Figure 1D**; Shigeno et al., 2015). A similar division occurs in mammalian cortical neurogenesis (**Figure 1E**), where post-mitotic neurons (marked by *NeuroD*, **Figures 1F,G**) migrate radially outwards to form the cortical plate, leaving the progenitors (marked by Neurogenin2, **Figure 1H**) as an apical layer surrounding the ventricle (**Supplementary Material**). Also in the teleost fish telencephalon, post-mitotic neurons migrate radially inwards and progenitors remain as a distinctive layer at the outside apical border (Abdel-Mannan et al., 2008; Furlan et al., 2017). Neurogenesis in the vertebrate cerebral cortex is marked by a switch from symmetric to asymmetric divisions, in which the neurogenic stem cell (also known as radial glia, blue cell in **Figure 2**) self-renews and generates a daughter cell that either becomes post-mitotic (direct neurogenesis), or an intermediate precursor (indirect neurogenesis) (**Figure 2**; Paridaen and Huttner, 2014). These intermediate progenitors (multipolar pink cells in **Figure 2**) divide a few times to generate the bulk of the post-mitotic neurons (labeled green in **Figure 2**), that actively migrate out of the progenitor domain. In vertebrates, the increasing ratio between indirect and direct neurogenesis determines the radial expansion of the cortex seen over evolution (Florio and Huttner, 2014; Taverna et al., 2014). Besides controlling the decision between proliferative symmetric over neurogenic asymmetric divisions, and between direct versus indirect neurogenesis, diversifying the nature of the intermediate progenitors is a third way particularly managed by mammals (including primates and human) to vastly increase neuronal number. Evidence exists that duplication of the radial glial neurogenic stem cell layer resulting in the formation of basal (or outer) radial glia (orange cells in **Figure 2**) lies at the basis of gyrification of the cerebral cortex (Florio and Huttner, 2014; Fernández et al., 2016). The columnar organization of the amacrine cells around their bundled trunks in octopods we observe in drawings from both of Gray and Young, and the folded structure of the vertical lobe (Gray, 1968; Young, 1971), are reminiscent of the primate cerebral cortex structure (Hubel and Wiesel, 1969). Compared to other invertebrates such as *Drosophila*, that also has different types of neurogenic precursors (Homem and Knoblich, 2012; Hartenstein and Stollewerk, 2015), cephalopods might have increased neuronal output applying vertebrate-like mechanisms (symmetric divisions to laterally expand the neurogenic stem cell field, larger diversity of progenitors to increase indirect neurogenesis and active neuronal migration).

Regulation of the cell cycle is obviously important in the context of neurogenesis. A prolonged period of active cell cycling in neural stem cells would be an additional mechanism to increase neuronal output. In humans, primary microcephaly, which is due to lower cortical cell number and manifests as a reduction in cortical size, is caused by mutations in genes important in mitotic cell division, such as spindle formation and centrosome function (Gilmore and Walsh, 2013). The fact that these mutations primarily affect brain development suggests that factors that control cell cycle will predominantly impact the number of neurons produced in an animal. Assuming that a basic process such as the cell cycle is regulated by similar factors in all bilaterian animals, has very recently been put into question. The planarian *Schmidtea mediterranea* seems to have lost *MAD1*, *MAD2* and several other genes implicated in the spindle assembly checkpoint (Grohme et al., 2018). These factors have conserved functions from yeast to mammalians, yet seem not that essential to planaria, that have retained mitotic checkpoint function. Amazingly, planarians display whole-body regeneration potential while overproliferation conditions such as cancer have not been reported. Similar gene losses have been described in *Drosophila* that should affect DNA repair, yet this process is not really impacted either (Sekelsky, 2017). Clearly, our knowledge on basic cellular processes such as the cell cycle is far from complete, in particular in cephalopods.

ADULT NEUROGENESIS

The cephalopod brain continues to grow over the entire lifetime of the animal (Wirz, 1959; Young, 1963; Dickel et al., 1997) whereas particular regions such as the vertical lobe and superior frontal lobe increase in size in response to learning (Dickel et al., 2001). This growth is paralleled -at least in *O. vulgaris*- by a linear increase in DNA content and number of nuclei (Packard and Albergoni, 1970; Giuditta et al., 1971), suggesting that also beyond embryogenesis, neurons are generated. In mammals, adult neurogenesis is steered by neural stem cells in the ventricular-subventricular zone lining the lateral ventricle and in the subgranular zone of the hippocampal dentate gyrus (Zhao et al., 2008; Altman, 2011). Non-mammalian vertebrates like teleosts bear neural progenitors in multiple neurogenic regions. These continuously produce new neurons that migrate and integrate in the mature brain (Kizil et al., 2012). Adult neural stem cells in invertebrates have also extensively been studied (reviewed by Simões and Rhiner, 2017). *D. melanogaster* only shows a low level of adult neurogenesis by a dispersed population of neural progenitors in the optic lobes. These progenitors are mainly quiescent (Fernández-Hernández et al., 2013), but can start local proliferation upon acute tissue damage (Heisenberg et al., 1995; Moreno et al., 2015).

To date, little information exists on adult neurogenesis in cephalopods. Buresi et al. (2013) suggest a prolongation of proliferative capacities of the ganglia in cephalopod hatchlings which implies preservation of quiescent stem cells to allow delayed adult neurogenesis (Baratte and Bonnaud, 2009). Excitotoxic lesion by kainic acid in the vertical lobe of *S. officinalis* induced proliferation as measured by BrdU incorporation (Graindorge et al., 2008) and recently, Di Cosmo et al. (2018) observed active proliferation in the *O. vulgaris* nervous system

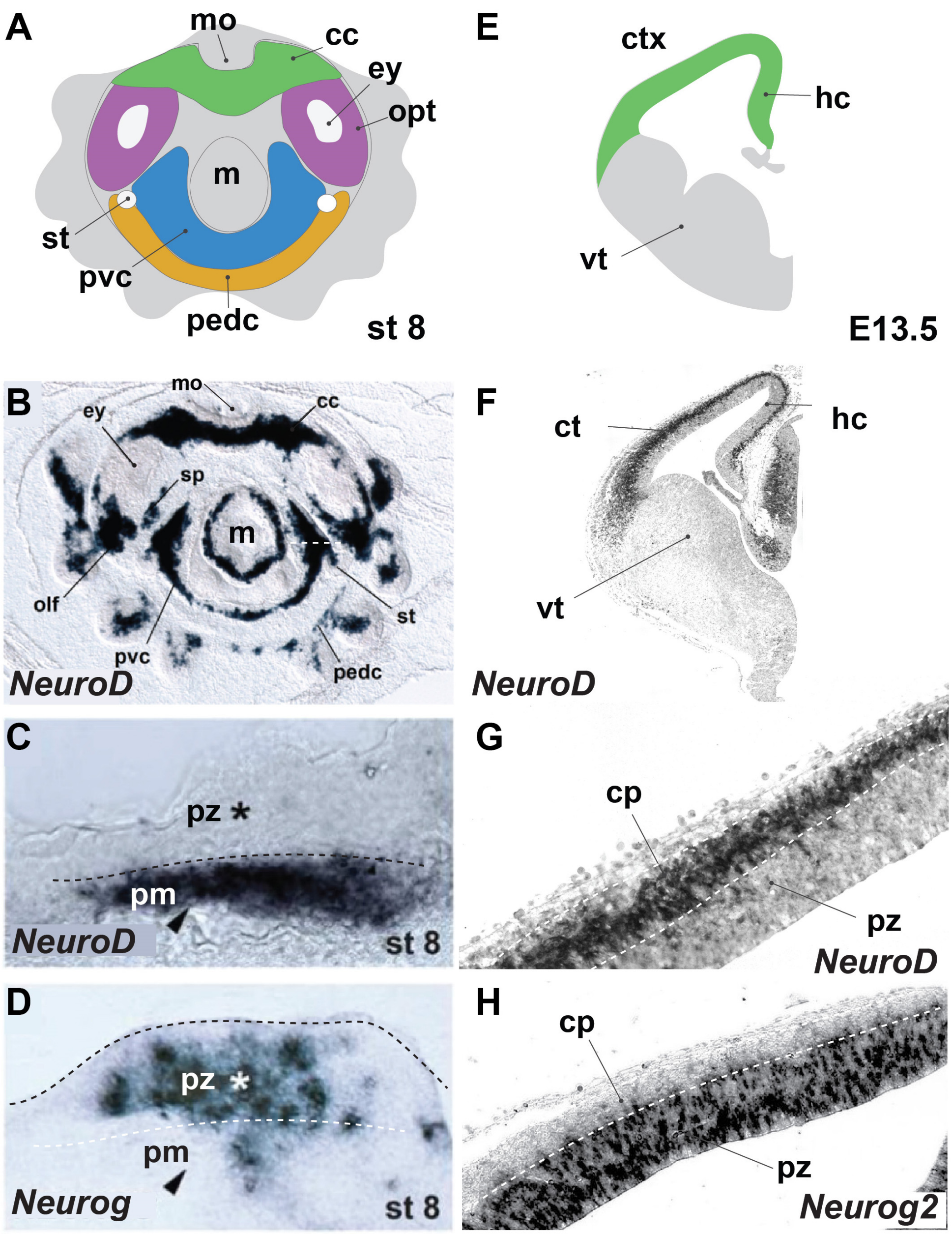


FIGURE 1 | *Octopus bimaculoides* and mouse neurogenesis occurs in similarly laminated neuroectoderm. **(A)** Schematic top–down overview of the neurogenic territories in the stage 8 *Octopus bimaculoides* embryo. All color-marked areas are neurogenic, cord-like regions. **(B)** Whole-mount *in situ* hybridization for NEUROD, a marker of young post-mitotic neurons. **(C,D)** Higher magnification of the neurogenic area (white dashed line in **B**) demarcating a laminated structure with post-mitotic neurons (pm, arrowhead, marked by *NEUROD*, **C**) separated from progenitors (pz, star, marked by *NEUROG*, **D**) (dashed line). **(E)** Schematic view of a coronal section through the mouse telencephalon at E13.5, demarcating the ventral telencephalon (vt, gray) and dorsally placed cortex (ctx) and hippocampal (hc) areas (green). **(F)** *In situ* hybridization of *Neurod*, a post-mitotic neuron proneural transcription factor. **(G,H)** Higher magnification of the cortical laminated structure (dashed lines), with a progenitor zone (pz, marked by *Neurog2*, **H**) lining the ventricle and a post-mitotic cortical plate (cp, marked by *NeuroD*, **G**). **(B–D)** Adapted from Shigeno et al. (2015). cc, cerebral cord; cp, cortical plate; ctx, cerebral cortex; ey, eye; hc, hippocampus; m, mantle; mo, mouth; olf, olfactory organ; opt, optic lobe; pedc, pedal cord; pvc, palliovisceral cord; pz, progenitor zone; sp, subpedunculate tissue; st, statocyst; vt, ventral telencephalon; ve, ventricle.

after *in vitro* administration of BrdU. PCNA levels seem to increase in the vertical and frontal lobes of *O. vulgaris* housed in an enriched environment suggesting active cell division takes place (Bertapelle et al., 2017), however, leaving the reader in the dark on the precise cellular location of the presumptive raise in mitotic activity. Whether these findings reveal a true self-renewing population of stem cells and whether neurons are generated is therefore not yet proven. Measurements of DNA content per nucleus in different adult *O. vulgaris* brain lobes indicated an amount that exceeded the estimated DNA

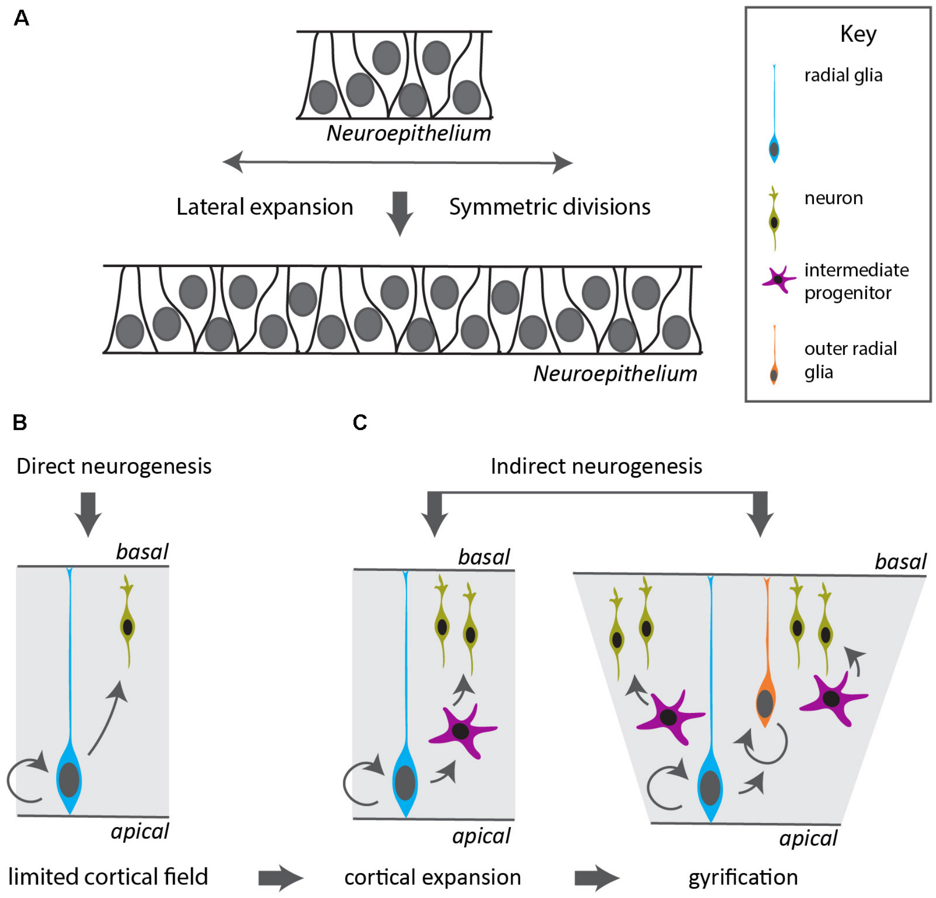


FIGURE 2 | Modes of neurogenesis in the vertebrate cerebral cortex. **(A)** Before the onset and during early stages of neurogenesis, the neuroepithelium divides symmetrically to expand in a lateral fashion, increasing the neurogenic domain. **(B)** In vertebrates with a small cortical field, the radial glia divide asymmetrically to generate neurons in a direct manner. **(C)** Indirect neurogenesis generates intermediate progenitors that divide symmetrically resulting in increased neuronal output and expansion of the cerebral cortex. The appearance of a duplication of the radial glia layer in outer radial glia allows further radial and lateral expansion and gyrification of the cortex.

content of diploid cells, suggesting polyploidy in a number of cells (Giuditta et al., 1971). Polyploidy might indicate active cell cycling (tetraploidy during G2 phase). Intriguingly, a recent report showed that during starvation stress, stem cells can be generated from polyploid cells by amitosis in *Drosophila* (Lucchetta and Ohlstein, 2017). This alternative mechanism of cell division that is characterized by nuclear division without spindle formation, has been shown to occur in many species ranging from plants and ciliates to mammals (Miller, 1980; Kuhn et al., 1991; Magelhães et al., 1991; Prescott, 1994) and might be induced by physiological and pathological stressors (Chen and Wan, 1986). Lange (1920) already suggested a role for direct division or amitosis in octopus arm regeneration after amputation. She did not observe infiltrating cells nor mitotic spindles in the blastema-like structure, but instead found several nuclei in different stages of amitotic division (Lange, 1920). Given that such alternative mechanisms to mitosis might exist, and quiescence of adult progenitors might "hide" neurogenesis, more extensive exploration of adult neurogenesis that goes beyond demonstration of mitosis is necessary. An interesting alternative route of neurogenesis was described recently. In crustaceans, the adult pool of neurons is supplied from the hematopoietic system that act as true stem cells to sustain neurogenesis in the adult animal (Benton et al., 2014).

GENETIC INNOVATIONS MIGHT DRIVE COMPLEX NEURAL SYSTEM DEVELOPMENT

Sequencing of *Octopus bimaculoides* revealed an extremely large genome size [∼2.7 versus ∼1.6 Gb for *Mytilus* (mussel)] (Albertin et al., 2015; Murgarella et al., 2016). Unexpectedly, this increase is not due to simple duplication, but by expansion of a few specific gene families including protocadherins and C_2H_2 zinc finger proteins, as well as interleukin-17-like genes, G-protein coupled receptors, sialins and chitinases (Albertin et al., 2015). A similar protocadherin gene expansion has been found in coleoid cephalopods (Liscovitch-Brauer et al., 2017), whereas cadherin expression is enriched in suckers, such as for instance the unique *CDHX* (Wang and Ragsdale, 2017). Protocadherins have predominant functions in the development and maintenance of the nervous system of vertebrates and are highly enriched in neural tissue of *O. bimaculoides*, but are absent in *Drosophila* (Zipursky and Sanes, 2010; Liu et al., 2014; Wang et al., 2014; Albertin et al., 2015). Furthermore, Albertin et al. (2015) identified three copies from the disc large family members in the *O. bimaculoides* genome. Members of this family function in post-synaptic scaffolding and have four copies in the mammalian genome whereas *Drosophila* only has one (Nithianantharajah et al., 2013; Albertin et al., 2015). The independent expansion of these and more genes in both vertebrates and *O. bimaculoides* and their enrichment in neural tissues, suggest a convergent evolution on the molecular basis and might be related to an increasingly complex brain. Having a reference genome at hand, we can now hunt for the innovations in the octopus genetic information that might explain their unique neural expansion.

FUTURE PERSPECTIVES

Cephalopods have developed an expanded and centralized CNS that allows amazing behavior and complex cognition. Studying the onset and precise timing of neurogenesis in relation to the diversity of progenitors and neurons will be fundamental to further map out the molecular mechanisms driving cephalopod neural expansion. Hereto, an extra effort to sequence the genomes of cephalopods is essential.

Developing tools for cell biological analysis such as stem cell or explant cultures would allow analysis of cell cycle parameters and neurogenesis. The general lack of information on stem cells for the whole mollusk phylum including around 85,000 extant species hinders setting up *in vitro* cell cultures (Rosenberg, 2014; Hartenstein and Stollewerk, 2015). Indeed, cell culture has not been very successful in mollusks, and only one cell line (Bge cells) has been established so far, derived from embryonic tissue of the snail *Biomphalaria glabrata*, whereas over 500 cell lines of insects exist (Lynn, 2007; Yoshino et al., 2013). Recently, Maselli et al. cultured adult *O. vulgaris* neurons and showed that successful adhesion and neurite extension is limited to 4 days *in vitro* (Maselli et al., 2018). Besides cell culture, brain slice culture has been successfully used to measure long term potentiation in the adult brain (Hochner et al., 2003). These methods deserve further exploration in the context of neurogenesis as well.

Finally, recent genome data of regenerating animals reveal that our knowledge on the regulation of the cell cycle, and by extension the regulation of neurogenesis, is far from complete (Grohme et al., 2018; Nowoshilow et al., 2018). The careful analysis of cell cycle regulation and the prevalence of potentially alternative mechanisms in cephalopods merits further attention.

Taken together, generation and exploitation of additional genome and transcriptome data will yield more insight into the molecular mechanisms of neural expansion, whereas the establishment of (stem) cell culture methods will boost deeper understanding of cell cycle regulation and neurogenesis. Such studies should go hand-in-hand with *in vivo* analysis of the cell biology of neurogenesis during development and in adult life, to understand how this process contributes to brain expansion and plasticity.

ETHICS STATEMENT

All animal experiments were carried out on euthanized animals in compliance with the most recent European regulations and Belgian law and according to the guidelines of the Animal Care Committee of KU Leuven (ECD No. P153/2012).

AUTHOR CONTRIBUTIONS

AD and ES wrote the manuscript and approved the final document. ES performed the experiments and drew the figures.

FUNDING

AD was supported by a SB Ph.D. fellowship of Fonds Wetenschappelijk Onderzoek (FWO) Belgium (Project No. 1S19517N). ES was supported by funding of the FWO (G0B5916N and G0C2618N) and the KUL (C14/16/049). Publication cost was covered by the KU Leuven Fund for Fair Open Access.

ACKNOWLEDGMENTS

We thank G. Ponte for critical discussions and Q. Ma and J. Lee for providing plasmids for Ngn2 and NeuroD probes, respectively, for *in situ* hybridization.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2018.01160/full#supplementary-material

REFERENCES

Abdel-Mannan, O., Cheung, A. F. P., and Molnár, Z. (2008). Evolution of cortical neurogenesis. *Brain Res. Bull.* 75, 398–404. doi: 10.1016/j.brainresbull.2007.10.047

Albertin, C. B., Simakov, O., Mitros, T., Wang, Z. Y., Pungor, J. R., Edsinger-Gonzales, E., et al. (2015). The octopus genome and the evolution of cephalopod neural and morphological novelties. *Nature* 524, 220–224. doi: 10.1038/nature14668

Altman, J. (2011). "The discovery of adult mammalian neurogenesis," in *Neurogenesis in the Adult Brain I*, eds T. Seki, K. Sawamoto, J. M. Parent, and A. Alvarez-Buylla (Dordrecht: Springer), doi: 10.1007/978-4-431-53933-9_1

Arendt, D., Tosches, M. A., and Marlow, H. (2016). From nerve net to nerve ring, nerve cord and brain-evolution of the nervous system. *Nat. Rev. Neurosci.* 17, 61–72. doi: 10.1038/nrn.2015.15

Baratte, S., and Bonnaud, L. (2009). Evidence of early nervous differentiation and early catecholaminergic sensory system during Sepia officinalis embryogenesis. *J. Compar. Neurol.* 517, 539–549. doi: 10.1002/cne.22174

Benton, J. L., Kery, R., Li, J., Noonin, C., Söderhäll, I., and Beltz, B. S. (2014). Cells from the immune system generate adult-born neurons in crayfish. *Dev. Cell* 30, 322–333. doi: 10.1016/j.devcel.2014.06.016

Bertapelle, C., Polese, G., and Di Cosmo, A. (2017). Enriched environment increases PCNA and PARP1 levels in octopus vulgaris central nervous system: first evidence of adult neurogenesis in lophotrochozoa. *J. Exp. Zool. Part B Mol. Dev. Evol.* 328, 347–359. doi: 10.1002/jez.b.22735

Boullerne, A. I. (2016). The history of myelin. *Exp. Neurol.* 283(Pt B), 431–445. doi: 10.1016/j.expneurol.2016.06.005

Budelmann, B. U. (1995). The cephalopod nervous system: what evolution has made of the molluscan design. *Nervous Syst. Invertebrates Evolut. Compar. Approach* 72, 115–138. doi: 10.1007/978-3-0348-9219-3

Buresi, A., Andouche, A., Navet, S., Bassaglia, Y., Bonnaud-Ponticelli, L., and Baratte, S. (2016). Nervous system development in cephalopods: how egg yolk-richness modifies the topology of the mediolateral patterning system. *Dev. Biol.* 415, 143–156. doi: 10.1016/j.ydbio.2016.04.027

Buresi, A., Canali, E., Bonnaud, L., and Baratte, S. (2013). Delayed and asynchronous ganglionic maturation during cephalopod neurogenesis as evidenced by Sof-elav1 expression in embryos of Sepia officinalis (Mollusca, Cephalopoda). *J. Compar. Neurol.* 521, 1482–1496. doi: 10.1002/cne.23231

Chen, Y., and Wan, B. (1986). A study on amitosis of the nucleus of the mammalian cell. I. A study under the light and transmission electron microscope. *Acta Anat. (Basel.)* 127, 69–76. doi: 10.1159/000146240

Di Cosmo, A., Bertapelle, C., Porcellini, A., and Polese, G. (2018). Magnitude assessment of adult neurogenesis in the Octopus vulgaris brain using a flow cytometry-based technique. *Front. Physiol.* 9:1050. doi: 10.3389/fphys.2018.01050

Dickel, L., Chichery, M.-P., and Chichery, R. (1997). Postembryonic maturation of the vertical lobe complex and early development of predatory behavior in the cuttlefish (Sepia officinalis). *Neurobiol. Learn. Mem.* 67, 150–160.

Dickel, L., Chichery, M.-P., and Chichery, R. (2001). Increase of learning abilities and maturation of the vertical lobe complex during postembryonic development in the cuttlefish, Sepia. *Dev. Psychobiol.* 39, 92–98. doi: 10.1002/dev.1033

Echelard, Y., Epstein, D. J., St-Jaques, B., Shen, L., Mohler, J., Mcmahon, J. A., et al. (1993). Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. *Cell* 75, 1417–1430. doi: 10.1016/0092-8674(93)90627-3

Ericson, J., Rashbass, P., Schedl, A., Brenner-Morton, S., Kawakami, A., Van Heyningen, V., et al. (1997). Pax6 controls progenitor cell identity and neuronal fate in response to graded Shh signaling. *Cell* 90, 169–180. doi: 10.1016/S0092-8674(00)80323-2

Fernández, V., Llinares-Benadero, C., and Borrell, V. (2016). Cerebral cortex expansion and folding: what have we learned? *EMBO J.* 35, 1021–1044. doi: 10.15252/embj.201593701

Fernández-Hernández, I., Rhiner, C., and Moreno, E. (2013). Adult neurogenesis in *Drosophila*. *Cell Rep.* 3, 1857–1865. doi: 10.1016/j.celrep.2013.05.034

Fiorito, G., Von Planta, C., and Scotto, P. (1990). Problem solving ability of octopus-vulgaris lamarck (Mollusca, Cephalopoda). *Behav. Neural Biol.* 53, 217–230. doi: 10.1016/0163-1047(90)90441-8

Fish, J. L., Dehay, C., Kennedy, H., and Huttner, W. B. (2008). Making bigger brains – The evolution of neural-progenitor-cell division. *J. Cell Sci.* 121, 2783–2793. doi: 10.1242/jcs.023465

Florio, M., and Huttner, W. B. (2014). Neural progenitors, neurogenesis and the evolution of the neocortex. *Development* 141, 2182–2194. doi: 10.1242/dev.090571

Furlan, G., Cuccioli, V., Vuillemin, N., Dirian, L., Muntasell, A. J., Coolen, M., et al. (2017). Life-long neurogenic activity of individual neural stem cells and continuous growth establish an outside-in architecture in the teleost pallium. *Curr. Biol.* 27, 3288.e3–3301.e3. doi: 10.1016/j.cub.2017.09.052

Gilmore, E. C., and Walsh, C. A. (2013). Genetic causes of microcephaly and lessons for neuronal development. *Wiley Interdiscipl. Rev. Dev. Biol.* 2, 461–478. doi: 10.1002/wdev.89

Giuditta, A., Libonati, M., Packard, A., and Prozzo, N. (1971). Nuclear counts in the brain lobes of Octopus vulgaris as a function of body size. *Brain Res.* 25, 55–62. doi: 10.1016/0006-8993(71)90566-X

Graindorge, N., Jozet-Alves, C., Chichery, R., Dickel, L., and Bellanger, C. (2008). Does kainic acid induce partial brain lesion in an invertebrate model: sepia officinalis? Comparison with electrolytic lesion. *Brain Res.* 1238, 44–52. doi: 10.1016/j.brainres.2008.08.002

Gray, E. G. (1968). Electron microscopy of the glio-vascular organization of the brain of *octopus*. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 255, 13–32. doi: 10.1098/rstb.1969.0002

Grohme, M. A., Schloissnig, S., Rozanski, A., Pippel, M., Young, G. R., Winkler, S., et al. (2018). The genome of *Schmidtea mediterranea* and the evolution of core cellular mechanisms. *Nature* 554, 56–61. doi: 10.1038/nature25473

Halder, G., Callaerts, P., and Gehring, W. J. (1995). New perspectives on eye evolution. *Curr. Opin. Genet. Dev.* 5, 602–609. doi: 10.1016/0959-437X(95)80029-8

Hartenstein, V., and Stollewerk, A. (2015). The evolution of early neurogenesis. *Dev. Cell* 32, 390–407. doi: 10.1016/j.devcel.2015.02.004

Hartmann, B., Lee, P. N., Kang, Y. Y., Tomarev, S., de Couet, H., and Callaerts, P. (2003). Pax6 in the sepiolid squid *Euprymna scolopes*: evidence for a role in eye, sensory organ and brain development. *Mech. Dev.* 120, 177–183. doi: 10.1016/S0925

Heisenberg, M., Heusipp, M., and Wanke, C. (1995). Structural plasticity in the *Drosophila* brain. *J. Neurosci.* 15, 1951–1960. doi: 10.1523/JNEUROSCI.15-03-01951.1995

Herculano-Houzel, S., Collins, C. E., Wong, P., and Kaas, J. H. (2007). Cellular scaling rules for primate brains. *Proc. Natl. Acad. Sci. U.S.A.* 104, 3562–3567. doi: 10.1073/pnas.0611396104

Herculano-Houzel, S., Mota, B., and Lent, R. (2006). Cellular scaling rules for rodent brains. *Proc. Natl. Acad. Sci. U.S.A.* 103, 12138–12143. doi: 10.1073/pnas.0604911103

Hirth, F. (2010). On the origin and evolution of the tripartite brain. *Brain Behav. Evol.* 76, 3–10. doi: 10.1159/000320218

Hochner, B., Brown, E. R., Langella, M., Shomrat, T., and Fiorito, G. (2003). A learning and memory area in the octopus brain manifests a vertebrate-like long-term potentiation. *J. Neurophysiol.* 90, 3547–3554. doi: 10.1152/jn.00645.2003

Holland, L. Z., Carvalho, J. E., Escriva, H., Laudet, V., Schubert, M., Shimeld, S. M., et al. (2013). Evolution of bilaterian central nervous systems: a single origin? *EvoDevo* 4, 1–20. doi: 10.1186/2041-9139-4-27

Homem, C. C. F., and Knoblich, J. A. (2012). *Drosophila* neuroblasts: a model for stem cell biology. *Development* 139, 4297–4310. doi: 10.1242/dev.080515

Hubel, D., and Wiesel, T. (1969). Anatomical demonstration of columns in the monkey striate cortex. *Nature* 221, 747–750. doi: 10.1038/221747a0

Kizil, C., Kaslin, J., Kroehne, V., and Brand, M. (2012). Adult neurogenesis and brain regeneration in zebrafish. *Dev. Neurobiol.* 72, 429–461. doi: 10.1002/dneu.20918

Kuhn, E. M., Therman, E., and Susman, B. (1991). Amitosis and endocycles in early cultured mouse trophoblast. *Placenta* 12, 251–261. doi: 10.1016/0143-4004(91)90006-2

Lange, M. M. (1920). On the regeneration and finer structure of the arms of the cephalopods. *J. Exp. Zool.* 31, 1–57. doi: 10.1002/jez.1400310102

Lee, P. N., Callaerts, P., De Couet, H. G., and Martindale, M. Q. (2003). Cephalopod Hox genes and the origin of morphological novelties. *Nature* 424, 1061–1065. doi: 10.1038/nature01872

Liscovitch-Brauer, N., Alon, S., Porath, H. T., Elstein, B., Unger, R., Ziv, T., et al. (2017). Trade-off between transcriptome plasticity and genome evolution in cephalopods. *Cell* 169, 191.e11–202.e11. doi: 10.1016/j.cell.2017.03.025

Liu, H., Chang, L. H., Sun, Y., Lu, X., and Stubbs, L. (2014). Deep vertebrate roots for mammalian zinc finger transcription factor subfamilies. *Genome Biol. Evol.* 6, 510–525. doi: 10.1093/gbe/evu030

Lucchetta, E. M., and Ohlstein, B. (2017). Amitosis of polyploid cells regenerates functional stem cells in the *Drosophila* intestine. *Cell Stem Cell* 20, 609.e6–620.e6. doi: 10.1016/j.stem.2017.02.012

Lynn, D. E. (2007). "Available lepidopteran insect cell lines," in *Baculovirus and Insect Cell Expression Protocols*, ed. D. W. Murhammer (Totowa, NJ: Humana Press), 117–137. doi: 10.1007/978-1-59745-457-5_6

Magelhães, M., Pignatelli, D., and Magelhães, M. (1991). Amitosis in human adrenal cells. *Histol. Histopathol.* 6, 251–256.

Martín-durán, J. M., Pang, K., Børve, A., Lê, H. S., and Furu, A. (2018). Convergent evolution of bilaterian nerve cords. *Nature* 553, 45–50. doi: 10.1038/nature25030

Maselli, V., Xu, F., Syed, N., Polese, G., and Di Cosmo, A. (2018). A novel approach to primary cell culture for Octopus vulgaris neurons. *Front. Physiol.* 9:220. doi: 10.3389/FPHYS.2018.00220

Matheson, T. (2002). Invertebrate nervous systems. *Encycl. Life Sci.* 10, 1–6. doi: 10.1002/cphy.cp130211

Miller, R. H. (1980). Amitosis and endocytogenesis in the fruit of *Malus sylvestris*. *Ann. Bot.* 46, 567–575. doi: 10.1093/oxfordjournals.aob.a085955

Moreno, E., Fernandez-Marrero, Y., Meyer, P., and Rhiner, C. (2015). Brain regeneration in *Drosophila* involves comparison of neuronal fitness. *Curr. Biol.* 25, 955–963. doi: 10.1016/j.cub.2015.02.014

Murgarella, M., Puiu, D., Novoa, B., Figueras, A., Posada, D., and Canchaya, C. (2016). A first insight into the genome of the filter-feeder mussel *Mytilus galloprovincialis*. *PLoS One* 11:e0151561. doi: 10.1371/journal.pone.0160081

Navet, S., Andouche, A., Baratte, S., and Bonnaud, L. (2009). Shh and Pax6 have unconventional expression patterns in embryonic morphogenesis in Sepia officinalis (Cephalopoda). *Gene Expr. Patterns* 9, 461–467. doi: 10.1016/j.gep.2009.08.001

Navet, S., Baratte, S., Bassaglia, Y., Andouche, A., Buresi, A., and Bonnaud, L. (2014). Neurogenesis in cephalopods: "Eco-evo-devo" approach in the cuttlefish Sepia officinalis (mollusca-cephalopoda). *J. Mar. Sci. Technol. (Taiwan)* 22, 15–24. doi: 10.6119/JMST-013-0820-1

Nithianantharajah, J., Komiyama, N. H., McKechanie, A., Johnstone, M., Blackwood, D. H., Clair, D. S., et al. (2013). Synaptic scaffold evolution generated components of vertebrate cognitive complexity. *Nat. Neurosci.* 16, 16–24. doi: 10.1038/nn.3276

Nixon, M., and Young, J. Z. (2003). *The Brains and Lives of Cephalopods*. Oxford: OUP Oxford.

Nowoshilow, S., Schloissnig, S., Fei, J.-F., Dahl, A., Pang, A. W. C., Pippel, M., et al. (2018). The axolotl genome and the evolution of key tissue formation regulators. *Nature* 554, 50–55. doi: 10.1038/nature25458

Packard, A., and Albergoni, V. (1970). Relative growth, nucleic acid content and cell numbers of the brain in Octopus vulgaris (Lamarck). *J. Exp. Biol.* 52, 539–552.

Paridaen, J. T., and Huttner, W. B. (2014). Neurogenesis during development of the vertebrate central nervous system. *EMBO Rep.* 15, 351–364. doi: 10.1002/embr.201438447

Prescott, D. M. (1994). The DNA of ciliated protozoa. *Microbiol. Rev.* 58, 233–267. doi: 10.1016/0022-2836(72)90095-2

Rentzsch, F., Layden, M., and Manuel, M. (2017). The cellular and molecular basis of cnidarian neurogenesis. *Wiley Interdiscipl. Rev. Dev. Biol.* 6, 1–19. doi: 10.1002/wdev.257

Richter, S., Loesel, R., Purschke, G., Schmidt-Rhaesa, A., Scholtz, G., Stach, T., et al. (2010). Invertebrate neurophylogeny: suggested terms and definitions for a neuroanatomical glossary. *Front. Zool.* 7:29. doi: 10.1186/1742-9994-7-29

Rosenberg, G. (2014). A new critical estimate of named species-level diversity of the recent mollusca. *Am. Malacol. Bull.* 32, 308–322. doi: 10.4003/006.032.0204

Sekelsky, J. (2017). DNA repair in *Drosophila*: mutagens, models, and missing genes. *Genetics* 205, 471–490. doi: 10.1534/genetics.116.186759

Shigeno, S., Parnaik, R., Albertin, C. B., and Ragsdale, C. W. (2015). Evidence for a cordal, not ganglionic, pattern of cephalopod brain neurogenesis. *Zool. Lett.* 1, 1–13. doi: 10.1186/s40851-015-0026-z

Simões, A. R., and Rhiner, C. (2017). A cold-blooded view on adult neurogenesis. *Front. Neurosci.* 11:1–7. doi: 10.3389/fnins.2017.00327

Taverna, E., Götz, M., and Huttner, W. B. (2014). The cell biology of neurogenesis: toward an understanding of the development and evolution of the neocortex. *Annu. Rev. Cell Dev. Biol.* 30, 465–502. doi: 10.1146/annurev-cellbio-101011-155801

Tomarev, S. I., Callaerts, P., Kos, L., Zinovieva, R., Halder, G., Gehring, W., et al. (1997). Squid Pax-6 and eye development. *Proc. Natl. Acad. Sci. U.S.A.* 94, 2421–2426. doi: 10.1073/pnas.94.6.2421

Wang, C., You, Y., Qi, D., Zhou, X., Wang, L., Wei, S., et al. (2014). Human and monkey striatal interneurons are derived from the medial ganglionic eminence but not from the adult subventricular zone. *J. Neurosci.* 34, 10906–10923. doi: 10.1523/JNEUROSCI.1758-14.2014

Wang, Z. Y., and Ragsdale, C. W. (2017). Cadherin genes and evolutionary novelties in the octopus. *Semin. Cell Dev. Biol.* 69, 151–157. doi: 10.1016/j.semcdb.2017.06.007

Wirz, K. (1959). Etude biometrique du systeme nerveux des Cephalopodes. *Bull. Biol. France Belg.* 93, 78–117.

Wollesen, T., McDougall, C., Degnan, B. M., and Wanninger, A. (2014). POU genes are expressed during the formation of individual ganglia of the cephalopod central nervous system. *EvoDevo* 5:41. doi: 10.1186/2041-9139-5-41

Wollesen, T., Rodríguez Monje, S. V., Todt, C., Degnan, B. M., and Wanninger, A. (2015). Ancestral role of Pax2/5/8 in molluscan brain and multimodal sensory system development. *BMC Evol. Biol.* 15:231. doi: 10.1186/s12862-015-0505-z

Wollesen, T., Scherholz, M., Rodríguez Monje, S. V., Redl, E., Todt, C., and Wanninger, A. (2017). Brain regionalization genes are co-opted into shell field patterning in Mollusca. *Sci. Rep.* 7, 1–11. doi: 10.1038/s41598-017-05605-5

Yamamoto, M., Shimazaki, Y., and Shigeno, S. (2003). Atlas of the embryonic brain in the pygmy squid, *Idiosepius paradoxus*. *Zool. Sci.* 20, 163–179. doi: 10.2108/zsj.20.163

Yoshino, T. P., Bickham, U., and Bayne, C. J. (2013). Molluscan cells in culture: primary cell cultures and cell lines. *Can. J. Zool.* 91, 391–404. doi: 10.1139/cjz-2012-0258

Young, J. Z. (1963). The number and sizes of nerve cells in Octopus. *Proc. Zool. Soc.*140, 229–254. doi: 10.1111/j.1469-7998.1963.tb01862.x

Young, J. Z. (1971). *The Anatomy of the Nervous System of Octopus vulgaris*. Oxford: Oxford university press.

Zhao, C., Deng, W., and Gage, F. H. (2008). Mechanisms and functional implications of adult neurogenesis. *Cell* 132, 645–660. doi: 10.1016/j.cell.2008.01.033

Zipursky, S. L., and Sanes, J. R. (2010). Chemoaffinity revisited: dscams, protocadherins, and neural circuit assembly. *Cell* 143, 343–353. doi: 10.1016/j.cell.2010.10.009

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

PERSPECTIVE
published: 14 January 2019
doi: 10.3389/fphys.2018.01905

In silico Identification and Expression of Protocadherin Gene Family in *Octopus vulgaris*

Ruth Styfhals[1,2]*, Eve Seuntjens[2], Oleg Simakov[3], Remo Sanges[1,4] and Graziano Fiorito[1]

[1] Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Naples, Italy, [2] Laboratory of Developmental Neurobiology, Department of Biology, KU Leuven, Leuven, Belgium, [3] Department of Molecular Evolution and Development, University of Vienna, Vienna, Austria, [4] Computational Genomics Laboratory, Neuroscience Area, International School for Advanced Studies (SISSA), Trieste, Italy

Connecting millions of neurons to create a functional neural circuit is a daunting challenge. Vertebrates developed a molecular system at the cell membrane to allow neurons to recognize each other by distinguishing self from non-self through homophilic protocadherin interactions. In mammals, the protocadherin gene family counts about 50 different genes. By hetero-multimerization, protocadherins are capable of generating an impressive number of molecular interfaces. Surprisingly, in the California two-spot octopus, *Octopus bimaculoides*, an invertebrate belonging to the Phylum Mollusca, over 160 protocadherins (PCDHs) have been identified. Here we briefly discuss the role of PCDHs in neural wiring and conduct a comparative study of the protocadherin gene family in two closely related octopus species, *Octopus vulgaris* and *O. bimaculoides*. A first glance at the expression patterns of protocadherins in *O. vulgaris* is also provided. Finally, we comment on PCDH evolution in the light of invertebrate nervous system plasticity.

Keywords: protocadherins, DSCAM, plasticity, neural wiring, octopus, cephalopod

Edited by:
Fernando Ariel Genta,
Fundação Oswaldo Cruz (Fiocruz),
Brazil

Reviewed by:
Angelique Christine Paulk,
Massachusetts General Hospital,
Harvard Medical School,
United States
David B. Edelman,
Dartmouth College, United States

****Correspondence:***
Ruth Styfhals
ruth.styfhals@szn.it

Specialty section:
This article was submitted to
Invertebrate Physiology,
a section of the journal
Frontiers in Physiology

Received: *24 June 2018*
Accepted: *18 December 2018*
Published: *14 January 2019*

Citation:
Styfhals R, Seuntjens E, Simakov O, Sanges R and Fiorito G (2019) In silico Identification and Expression of Protocadherin Gene Family in Octopus vulgaris. Front. Physiol. 9:1905. doi: 10.3389/fphys.2018.01905

NEURAL WIRING AND NEURONAL RECOGNITION: PROTOCADHERINS AND DOWN SYNDROME CELL ADHESION MOLECULE

Neurons are capable of recognizing each other through a neuronal barcode-like mechanism (i.e., chemoaffinity hypothesis, Sperry, 1963). The establishment of a molecular identity allows neurons to form connections with appropriate "partners" and to discriminate self from non-self, an essential feature to build-up neural networks during development and/or structural remodeling in the adult (Christensen et al., 2013; Schreiner et al., 2017). Various molecules such as the immunoglobulins and cadherins have been implicated in this synaptic specificity (de Wit and Ghosh, 2016).

The Protocadherin Gene Family

Protocadherins (PCDHs) are cell-adhesion molecules and represent the largest subgroup of the cadherin superfamily. PCDHs contain six or seven extracellular cadherin (EC) repeats, and are considered a chordate innovation (Hulpiau and van Roy, 2011). They are expressed mainly in the nervous system and seem to be involved in both nervous system development and functioning (reviewed by Peek et al., 2017). The majority of mammalian PCDHs are located together on the genome in three gene clusters (i.e., PCDHa, PCDHb, PCDHg; for review see Hirayama and Yagi, 2017). It has been suggested that vertebrates utilize clustered PCDHs to generate neuronal identities essential for synaptic specificity. For instance, the differential expression of PCDHs, through alternative promoter choice and tetramerization at the cell surface, allows the 22 PCDHγ genes to generate over 234,256 different extracellular regions (Schreiner and Weiner, 2010). The non-clustered protocadherins are scattered throughout the genome. They are expressed in specific neural regions in the mammalian brain, while the clustered PCDHs are broadly expressed throughout various brain regions, although they exhibit a certain cell-type specificity (e.g., Zou et al., 2007). The observed PCDH expression patterns are related to their function; the non-clustered PCDHs are known to be involved during early stages such as axon outgrowth and path-finding, while clustered PCDHs are essential for axon terminal formation and dendritic self-avoidance, thus helping the establishment of neural-specific connections (Goodman et al., 2017; Peek et al., 2017).

PCDHs are also known to be continuously expressed in adult mammalian brains, with elevated expression levels in the hippocampus, cerebellum and cortex (e.g., Hertel et al., 2008, 2012; Junghans et al., 2008; Nuernberger et al., 2008; Kim et al., 2010; Krishna-K et al., 2011), suggesting a role in adult brain functioning, beyond the establishment of neural connectivity.

DSCAM, an Alternative to PCDHs in Invertebrates

In the insect *Drosophila melanogaster* protocadherins found their counterpart in the repertoire of DSCAM (Down syndrome cell adhesion molecule) isoforms. While *D. melanogaster* lacks PCDHs completely, over 19,008 unique DSCAM isoforms are formed through extensive alternative splicing (Schmucker et al., 2000; Schmucker and Chen, 2009; Zipursky and Sanes, 2010). *D. melanogaster* DSCAMs act in the recognition of neural self vs. non-self (Hattori et al., 2008). DSCAM is known to be required for axon guidance and for the formation of axon pathways in the nervous system, and their molecular diversity is suggested to contribute to the specificity of neuronal connectivity (Schmucker et al., 2000; Hummel et al., 2003; Zhan et al., 2004; Zhu et al., 2006; Matthews et al., 2007). In analogy to what is known for clustered PCDHs, axons expressing the same set of DSCAM isoforms repel each other, thus ensuring neural branch segregation (Zhan et al., 2004). Intriguingly, the arthropods developed two different molecular mechanisms to generate neuronal diversity. Hexapods and crustaceans possess the same hypervariable DSCAM gene, and isoforms are generated as in *D. melanogaster* (Brites et al., 2008; Armitage et al., 2012). In contrast, in Chelicerata DSCAM developed a genomic organization similar to vertebrate PCDHs, which arose through duplication events (Yue et al., 2016). Instead of generating Chelicerata-DSCAM isoforms through splicing, different DSCAMs are expressed through alternative promoter choice (Cao et al., 2018).

Which mechanism a species uses to generate their repertoire of cell-recognition molecules, therefore, appears less important. What seems to be more essential is the available number of cell-recognition molecules and how these molecules convey the signal that is generated upon cell-cell interaction. The similarities on a functional, genomic and molecular level between the clustered protocadherins and the Chelicerata-DSCAM are highly intriguing considering the fact that these proteins share no sequence homology (for review see Jin and Li, 2018).

PROTOCADHERINS: A SHORT OVERVIEW THROUGHOUT THE ANIMAL KINGDOM

The protocadherin gene clusters are considered to be a vertebrate innovation and their diversity among species (i.e., lineage-specific duplication, gene conversion, adaptive variation in diversified ectodomains) has been suggested to drive the substantial increase in central nervous system complexity in vertebrates relative to other species (Noonan et al., 2004b).

The human genome contains 12 non-clustered and 53 clustered PCDHs. Although mammalian protocadherins are known to be orthologous, differences can even be found between humans and chimpanzees. Open reading frame-changing nucleotide insertions in no less than three PCDH genes have been found (Wu, 2005). Sequence differences among orthologous PCDHs in several vertebrate lineages appear to reflect adaptive differences in protocadherin function that contribute to clade-specific structural and functional specializations of the nervous system.

Protocadherins in humans, mice, rats, lizards, elephant sharks, and coelacanths are similarly organized in 3–4 clusters on a single locus (Wu and Maniatis, 1999; Noonan et al., 2004a; Yu et al., 2008; Jiang et al., 2009; but see for *Xenopus* Etlioglu et al., 2016). Teleosts exhibit an intriguing increase in clustered PCDHs due to fish-specific whole genome duplications (Wu, 2005; Yu et al., 2008). Their genomes contain two PCDHα and two PCDHγ clusters located on two different loci, but lack the PCDHβ cluster completely. Until recently, it was thought that clustered genomic organization was maintained throughout vertebrate evolution. However, cyclostomes are known to possess only non-clustered protocadherins (Ravi et al., 2015).

In an attempt to summarize the relative distribution of PCDHs in the animal kingdom, we surveyed data in the literature to illustrate the relative abundance of protocadherins in different chordate and non-chordate species (**Figure 1A**). In invertebrates, only low abundances (or absence) of protocadherins have been detected in the genomes of several species such as *Lottia gigantea*, *Caenorhabditis elegans*, *D. melanogaster*, *Ciona intestinalis*, and *Strongylocentrotus purpuratus* to mention some (**Figure 1A**). The PCDHs identified in the genomes of invertebrates are generally non-clustered protocadherins, the exceptions being *L. gigantea* and cephalopods (see below).

CEPHALOPOD PROTOCADHERINS

The recent genome sequencing of the cephalopod mollusc *Octopus bimaculoides* (Albertin et al., 2015) and the data provided

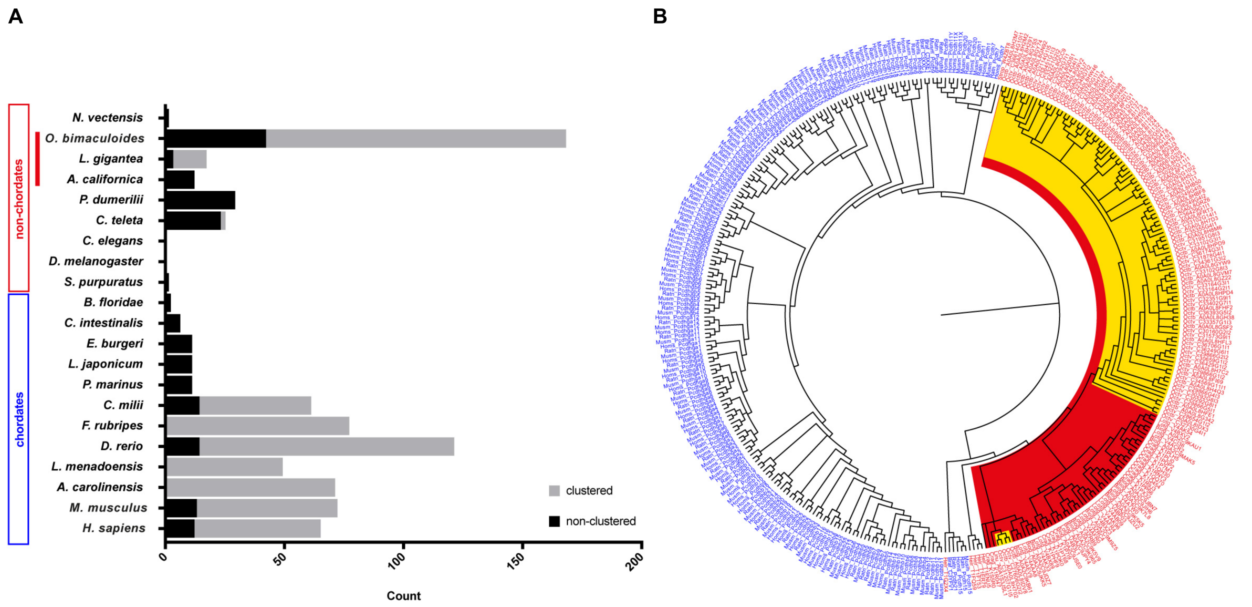


FIGURE 1 | Distribution and evolution of the protocadherin gene family in metazoans. **(A)** Abundance of protocadherins in the genomes of different chordate and non-chordate species. Data are derived from: Wu and Maniatis (1999), Noonan et al. (2004a,b), Wu (2005), Whittaker et al. (2006), Yu et al. (2007), Noda and Satoh (2008), Yu et al. (2008), Jiang et al. (2009), Hulpiau and van Roy (2011), Albertin et al. (2015), and Ravi et al. (2015). The attribution to clustered vs. non-clustered PCDHs in the graph (*Lottia gigantea* and *Octopus bimaculoides*) is derived from Authors' estimation (Albertin et al., 2015). **(B)** Bayesian phylogenetic reconstruction of the evolutionary relationships between protocadherins in different species. Chordate PCDHs are visualized in blue and non-chordate PCDHs are shown in red. Molluscan PCDHs are highlighted in red. The included cephalopod species are *Octopus vulgaris* and *O. bimaculoides,* which are highlighted in yellow. Octopus protocadherins interdigitate on the tree (see **Supplementary Figure S2**).

for the Longfin inshore squid *Doryteuthis pealeii* identified a large amount of clustered PCDH in cephalopods (Albertin et al., 2015; see also Wang and Ragsdale, 2017). In particular, the *O. bimaculoides* genome was found to encode over 120 clustered protocadherins and about 50 non-clustered PCDHs (168 multi-exonic PCDH genes, Albertin et al., 2015). Furthermore, 155 PCDHs have been identified in transcriptomes of the squid *D. pealeii* (Albertin et al., 2015). Interestingly, they showed that the expansion of protocadherins occurred independently in squid and octopus (Albertin et al., 2015). Octopus PCDHs are characterized as clustering together on the genome, an organization that includes a head-to-tail arrangement, analogous to what has been documented in the case of mammalian clustered PCDHs (Chen and Maniatis, 2013; Wang and Ragsdale, 2017). According to the summary provided by Wang and Ragsdale (2017), the three largest octopus clusters comprise 31, 17, and 10 PCDHs, while more than twenty scaffolds include at least two protocadherins. In their analysis of the known intracellular domain-motifs in octopus PCDHs, they were unable to find any analogy with vertebrates. Expression analysis showed that Ob-PCDHs are particularly enriched within the nervous system, mainly within the optic lobes and the axial nerve cord (Albertin et al., 2015; Wang and Ragsdale, 2017). It is also intriguing that cadherins have been identified in *O. bimaculoides* including one with 77 EC domains, that appears highly expressed in octopus suckers (Wang and Ragsdale, 2017).

Recent *de novo* transcriptomes of other cephalopod species (i.e., *S. officinalis, Octopus vulgaris* and *O. bimaculoides*) have provided evidence of a variable but large number (spanning from 127 to 251) of protocadherin open reading frames (Liscovitch-Brauer et al., 2017). This work confirms also previous recent evidence of the existence of RNA-editing in cephalopods, and suggests that RNA-editing is more extensive in protocadherins with respect to other genes in cephalopods. Interestingly, in the transcriptome of *Nautilus*, which had significantly less RNA editing sites, only 28 PCDH open reading frames have been recognized (Liscovitch-Brauer et al., 2017). We speculate that the very low abundance of PCDHs within *Nautilus* may simply reflect a less complex nervous system within the cephalopod clade (Nixon and Young, 2003). In *Callistoctopus minor* over 300 genes are reported as protocadherins (Kim et al., 2018).

The above-mentioned account of the PCDH gene family expansion in one representative taxon of the Lophotrochozoa, i.e., cephalopods (Albertin et al., 2015), *de facto* challenges the view that protocadherins are a vertebrate innovation (Yu et al., 2008). It seems that protocadherins expanded independently in two very distant clades, namely Lophotrochozoa and Vertebrata. This is confirmed by the enrichment of protocadherins in the nervous system of both coleoid cephalopods and vertebrates, representing a striking case of convergent evolution.

PCDHs in the Common Octopus, *Octopus vulgaris*

To further contribute to the knowledge of PCDHs in cephalopods, we explored the available transcriptome of another cephalopod species, the common octopus *O. vulgaris,* obtained from the research groups of Drs. R. Sanges and G. Fiorito at the Stazione Zoologica Anton Dohrn, Naples, Italy. The *O. vulgaris* transcriptome was based on RNA-Seq studies carried out on the central nervous system (i.e., optic lobes, supra-esophageal and sub-esophageal masses), proximal and distal extremities of arm (including muscular and/or nervous tissues), and other nervous system ganglia (Petrosino, 2015). The resulting transcriptome identified more than a hundred thousand expressed transcripts from different neural structures, significantly extending previously available transcriptome data for this species (Zhang et al., 2012; but see also Liscovitch-Brauer et al., 2017). By mining the *O. vulgaris* transcriptome for sequences containing four, five, six, or seven cadherin repeats, we identified 53 unique putative protocadherin gene sequences which can be used for future gene expression analysis (see **Supplementary Information**). This number is likely an underestimation, given the stringency of the analysis and the fact that we relied on a transcriptome assembly.

A phylogenetic tree of PCDHs comparing different vertebrate and invertebrate species, illustrates that the PCDH repertoire in two different octopus species (*O. vulgaris* and *O. bimaculoides*; characterized by different life cycles) did not evolve independently. The PCDH expansion occurred before speciation in octopus, thus suggesting that they are orthologous (**Figure 1B** and **Supplementary Figure S2**). The clustered Ob-PCDHs have extremely similar sequences, which is possibly due to recent gene duplications or gene conversions (Albertin et al., 2015). In addition, the specific phylogenetic tree of *O. vulgaris* (**Supplementary Figure S3**) shows that protocadherins possessing seven EC repeats are significantly different from Ov-PCDH possessing less repeats, which is reminiscent of the non-clustered δ1-PCDH subfamily in vertebrates. It would be interesting to see whether this convergence also exists at a genomic and functional level.

Moreover, two of these δ1-PCDH-like genes seem to cluster together with other molluscs (**Figure 1B** and **Supplementary Figure S2**), which would suggest that they are ancestral to other Ov-PCDHs. This observation supports the previous hypothesis that ancient PCDHs possessed more EC domains, which got lost or rearranged during evolution (Hulpiau and van Roy, 2011). Various Ov-PCDH and Ob-PCDH seem to possess very

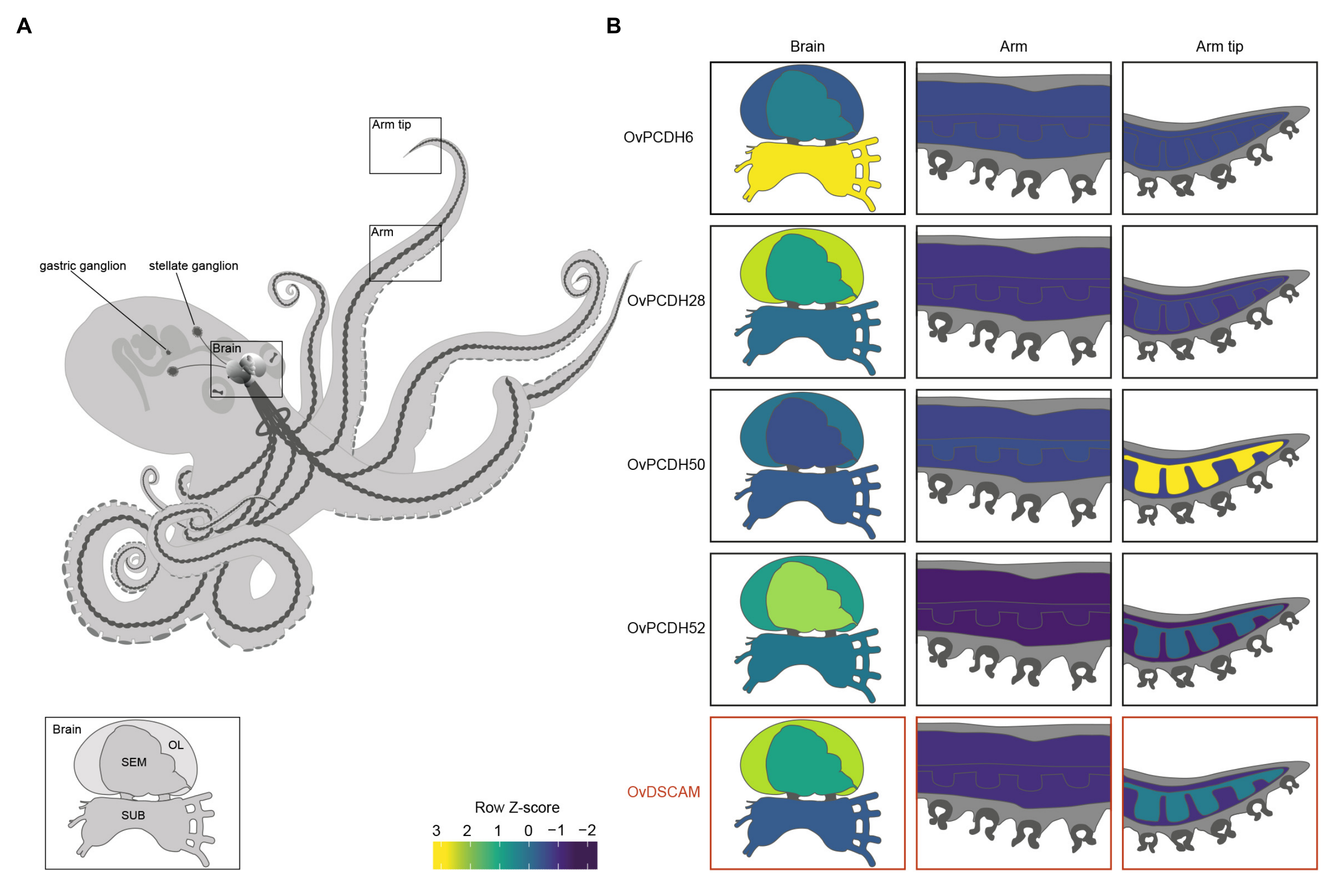


FIGURE 2 | Protocadherin and Dscam expression in *O. vulgaris*. **(A)** A schematic overview of the octopus and the main components of its nervous system. The octopus brain (SEM: supra-esophageal mass; SUB: sub-esophageal mass; OL: optic lobe), arm nerve cord, stellate and gastric ganglia are visualized. **(B)** Relative expression levels (coded according to Row Z-score) of selected Ov-PCDHs (see **Supplementary Information** and **Supplementary Figure S1**) and Ov-DSCAM are shown in the brain (supra-esophageal mass; sub-esophageal mass; optic lobe), arm (muscle tissue and axial nerve cord) and arm tip.

short extracellular regions (5 EC, data not shown) compared to vertebrate PCDHs (6 EC or 7 EC). According to Hulpiau and van Roy (2011), this would suggest that short octopus PCDHs are more evolved than those of the vertebrates.

Not much is known about the intracellular partners of PCDHs in vertebrates (Weiner and Jontes, 2013). Whether these intracellular interactions are conserved in *O. vulgaris* remains unexplored. Based on our current data, we have no evidence for the presence of the cytoplasmic domains that characterize vertebrate δ1-PCDH (CM1, CM2, CM3) in *O. vulgaris*, thus suggesting that Ov-PCDHs may have developed different intracellular pathways (see **Supplementary Information**). Octopus-specific motifs identified by Albertin et al. (2015) were found in the Ov-PCDHs (**Supplementary Table S1**).

Based upon the presence of cadherin repeats, we propose conserved extracellular interactions of Ov-PCDH. It is probable that they will act as cell-adhesion molecules, although nothing is known regarding their adhesion specificity. After alignment of the first EC repeat, we found around 30% identity with vertebrate protocadherins at the protein level, an expected value for non-orthologous proteins (see **Supplementary Table S1** and **Supplementary Figure S5**). Based upon alignment of Ov-PCDH transcripts we show around 98% identity at a protein level between protocadherins in *O. vulgaris* and *O. bimaculoides* (See **Supplementary Information**: Sequence Alignments).

Previous PCDH expression analysis in Albertin et al. (2015) showed increased expression within the nervous system, suggesting that cephalopod protocadherins play an important role in the nervous system of these organisms. The same disparity can be observed between neural and non-neural tissue in *O. vulgaris* (**Figure 2** and **Supplementary Figure S1**). As in vertebrates, we found few PCDHs expressed in non-neural octopus tissues. Our findings, based on *in silico* data, highlight the lower expression in the sub-esophageal mass, possibly explained by the presence of fewer neurons in comparison to the supra-esophageal mass and the optic lobes. However, it is also possible that less active reorganization of the neural circuitry is required in adults within brain areas controlling basic motor patterns. We also found an elevated PCDH expression of three different genes (6 or 7 EC) in the arm tip (**Figure 2** and **Supplementary Figure S1**), a region that may require continuous growth and rewiring of newly developing sensory systems. Moreover, protocadherins appear differentially expressed in the supra-esophageal mass, sub-esophageal mass, optic lobe and the stellate ganglion of *O. vulgaris*.

Octopus DSCAM

We applied the same strategy (see **Supplementary Information**) for the identification of DSCAM in *O. vulgaris*. Our assembled *O. vulgaris* transcriptome possesses only one DSCAM transcript, while the genome of *O. bimaculoides* presents two different isoforms of the same gene.

Based on the phylogenetic reconstruction, octopus DSCAM shows close identity with DSCAMs in other molluscs (**Supplementary Figure S4**). Ov-DSCAM and Ov-PCDHs have similar expression patterns throughout the nervous system of *O. vulgaris* (**Figure 2** and **Supplementary Figure S1**). It is speculated here that Ov-DSCAM has a similar role to vertebrate DSCAM, and exerts its function in a complementary manner to the PCDH gene clusters. DSCAM and DSCAM-L1 in vertebrates have been shown to be essential for neurite self-avoidance, but not for synaptic specificity (Fuerst et al., 2009).

CLOSING REMARKS

Here we show for the first time the presence of a large number of protocadherins in the transcriptome of the cephalopod mollusc *O. vulgaris*. Our data reveal the existence of differential expression of PCDHs in different brain lobes of the nervous system of an adult octopus. The increased expression of some PCDHs in the supra-esophageal mass and the optic lobes is intriguing since these are the areas where most of neural computation is achieved, including processes such as learning and memory (Young, 1991; Hochner et al., 2006; Marini et al., 2017; Turchetti-Maia et al., 2017).

Based on the expression of protocadherins in brain of adult mammals, such as the hippocampus and cerebellum (e.g., Hertel et al., 2008, 2012; Junghans et al., 2008; Nuernberger et al., 2008; Kim et al., 2010; Krishna-K et al., 2011), we propose a role for protocadherins in adult octopus brain functioning. Several examples are available in support of the hypothesis that PCDHs are involved in neural plasticity. First, electroconvulsive shocks induce neural activity evoking structural rearrangements through neurogenesis and synaptogenesis (Scott et al., 2000), as well as altered non-clustered PCDH-gene expression (Kim et al., 2010). Second, several non-clustered PCDHs, belonging to the δ1-subgroup, are known to affect synaptic plasticity through a conserved motif "RRVTF" in their cytoplasmic domain (Vanhalst et al., 2005). Protein phosphatase1-α specifically binds to this motif, thereby regulating synaptic plasticity at three different levels (for review see: Winder and Sweatt, 2001; Vanhalst et al., 2005). Third, an antibody against Arcadlin, the rat homolog of PCDH8, interfered with long-term potentiation in slice preparations of the rat hippocampus (Yamagata et al., 1999). Fourth, PCDH10 has been implicated in complex molecular cascades regulating synapse elimination in the mouse hippocampus (Tsai et al., 2012). Additionally, the intracellular domain of PCDHα genes can interact with a tyrosine kinase, *fyn* (Kohmura et al., 1998). In the mouse hippocampus, *fyn* is involved in inducing NMDA receptor-dependent long-term potentiation (Grant et al., 1992). Last but not least, the human-specific gene pair PCDH11X/Y has been recognized to play a role in the development of human language (Speevak and Farrell, 2011; Priddle and Crow, 2012, 2013). To the best of our knowledge, the examples provided above represent known cases of vertebrate PCDH involvement in neural plasticity. Furthermore, synaptic activity has been shown to modulate protein turnover, which allows change and thus plasticity at the level of the synapse (Bingol and Sheng, 2011; Alvarez-Castelao and Schuman, 2015; Cohen and Ziv, 2017).

We suggest that synaptic plasticity can be achieved through PCDH synthesis and degradation. By replacing the protocadherin repertoire at its cell surface, each neuron would be theoretically capable of forming new synaptic connections, thereby mediating structural plasticity in the adult (de Wit and Ghosh, 2016).

We speculate that the expansion of the protocadherin gene family in vertebrates and in cephalopods can be linked to the development of brain complexity and the increased plasticity in the adult brains. Uncovering expression patterns of both DSCAM and PCDHs in octopus will yield insights into their potential function. We expect that protocadherins that are involved in synaptic specificity will be expressed in a mosaic pattern distributed across the entire brain, whereas localized expression suggests a role in target recognition and axonal outgrowth. This seems to be the case in *O. vulgaris* since differential expression can be observed in different brain regions (**Figure 2** and **Supplementary Figure S1**). In addition to elucidation and analysis of these patterns in various parts of the octopus brain (e.g., the supra-esophageal mass and the optic lobes), the investigation of PCDH expression patterns during development and regeneration in *O. vulgaris* (e.g., Imperadore et al., 2017; Zullo et al., 2017) will be central in future studies.

The increased expression of a number of protocadherins in the stellate ganglion of *O. vulgaris* suggests that PCDHs are involved in plasticity related to the neural control of the chromatophores; key neuro-muscular organs involved in body patterning.

The biological role of DSCAM in the octopus is also an interesting problem whose future elucidation may facilitate comparative evolutionary analysis.

Finally, the putative differential expression of different PCDHs in octopus (and cephalopods generally) opens up a new avenue of studies aimed at deciphering the contribution of these adhesion molecules to neural wiring and neural plasticity in the adult, as in the case of the higher vertebrates.

ETHICS STATEMENT

The study is *in silico* only, based on samples obtained from *Octopus vulgaris* RNA-seq experiments collected in 2011 and 2012, thus well before the entry into force of Directive 2010/63/EU.

AUTHOR CONTRIBUTIONS

RSt, ES, and GF conceived this manuscript. RSt carried out the analysis and drafted the manuscript. OS and RSa provided guidance on the bioinformatic analysis. All authors discussed the results, contributed to writing and commented on the manuscript at all stages, and read and approved the submitted manuscript.

FUNDING

RSt is supported by the Stazione Zoologica Anton Dohrn (SZN) and the Katholieke Universiteit Leuven (KU Leuven; C14/16/049) for her Ph.D. project. This work was also supported through the COST Action FA$_{1301}$ Cephs*In*Action to RSt. RITMARE Flagship Project and Progetto Premiale 'MolEcOC' (Italian Ministry of Research-MIUR and SZN) also provided funding to RSa and GF.

ACKNOWLEDGMENTS

We would like to thank Massimiliano Volpe for his assistance on the phylogenetic reconstructions. We are also in debt to Dr. Giovanna Ponte for continuous advice.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2018.01905/full#supplementary-material

REFERENCES

Albertin, C. B., Simakov, O., Mitros, T., Wang, Z. Y., Pungor, J. R., Edsinger-Gonzales, E., et al. (2015). The octopus genome and the evolution of cephalopod neural and morphological novelties. *Nature* 524, 220–224. doi: 10.1038/nature14668

Alvarez-Castelao, B., and Schuman, E. M. (2015). The regulation of synaptic protein turnover. *J. Biol. Chem.* 290, 28623–28630. doi: 10.1074/jbc.R115.657130

Armitage, S. A., Freiburg, R. Y., Kurtz, J., and Bravo, I. G. (2012). The evolution of Dscam genes across the arthropods. *BMC Evol. Biol.* 12:53. doi: 10.1186/1471-2148-12-53

Bingol, B., and Sheng, M. (2011). Deconstruction for reconstruction: the role of proteolysis in neural plasticity and disease. *Neuron* 69, 22–32. doi: 10.1016/j.neuron.2010.11.006

Brites, D., McTaggart, S., Morris, K., Anderson, J., Thomas, K., Colson, I., et al. (2008). The Dscam homologue of the crustacean *Daphnia* is diversified by alternative splicing like in insects. *Mol. Biol. Evol.* 25, 1429–1439. doi: 10.1093/molbev/msn087

Cao, G., Shi, Y., Zhang, J., Ma, H., Hou, S., Dong, H., et al. (2018). A chelicerate-specific burst of nonclassical Dscam diversity. *BMC Genomics* 19:66. doi: 10.1186/s12864-017-4420-0

Chen, W. V., and Maniatis, T. (2013). Clustered protocadherins. *Development* 140, 3297–3302. doi: 10.1242/dev.090621

Christensen, R., Shao, Z., and Colón-Ramos, D. A. (2013). The cell biology of synaptic specificity during development. *Curr. Opin. Neurobiol.* 23, 1018–1026. doi: 10.1016/j.conb.2013.07.004

Cohen, L. D., and Ziv, N. E. (2017). Recent insights on principles of synaptic protein degradation. *F1000Research* 6:675. doi: 10.12688/f1000research.10599.1

de Wit, J., and Ghosh, A. (2016). Specification of synaptic connectivity by cell surface interactions. *Nat. Rev. Neurosci.* 17, 22–35. doi: 10.1038/nrn.2015.3

Etlioglu, H. E., Sun, W., Huang, Z., Chen, W., and Schmucker, D. (2016). Characterization of a single genomic locus encoding the clustered protocadherin receptor diversity in *Xenopus tropicalis*. *G3* 6, 2309–2318. doi: 10.1534/g3.116.027995

Fuerst, P. G., Bruce, F., Tian, M., Wei, W., Elstrott, J., Feller, M. B., et al. (2009). DSCAM and DSCAML1 function in self-avoidance in multiple cell types in the developing mouse retina. *Neuron* 64, 484–497. doi: 10.1016/j.neuron.2009.09.027

Goodman, K. M., Rubinstein, R., Dan, H., Bahna, F., Mannepalli, S., Ahlsén, G., et al. (2017). Protocadherin cis-dimer architecture and recognition unit diversity. *Proc. Natl. Acad. Sci. U.S.A.* 114, E9829–E9837. doi: 10.1073/pnas.1713449114

Grant, S. G., O'dell, T. J., Karl, K. A., Stein, P. L., Soriano, P., and Kandel, E. R. (1992). Impaired long-term potentiation, spatial learning, and hippocampal development in fyn mutant mice. *Science* 258, 1903–1910. doi: 10.1126/science.1361685

Hattori, D., Millard, S. S., Wojtowicz, W. M., and Zipursky, S. L. (2008). Dscam-mediated cell recognition regulates neural circuit formation. *Annu. Rev. Cell Dev. Biol.* 24, 597–620. doi: 10.1146/annurev.cellbio.24.110707.175250

Hertel, N., Nuernberger, M., and Redies, C. (2008). A cadherin-based code for the divisions of the mouse basal ganglia. *J. Comp. Neurol.* 508, 511–528. doi: 10.1002/cne.21696

Hertel, N., Redies, C., and Medina, L. (2012). Cadherin expression delineates the divisions of the postnatal and adult mouse amygdala. *J. Comp. Neurol.* 520, 3982–4012. doi: 10.1002/cne.23140

Hirayama, T., and Yagi, T. (2017). Regulation of clustered protocadherin genes in individual neurons. *Semin. Cell Dev. Biol.* 69, 122–130. doi: 10.1016/j.semcdb.2017.05.026

Hochner, B., Shomrat, T., and Fiorito, G. (2006). The octopus: a model for a comparative analysis of the evolution of learning and memory mechanisms. *Biol. Bull.* 210, 308–317. doi: 10.2307/4134567

Hulpiau, P., and van Roy, F. (2011). New insights into the evolution of metazoan cadherins. *Mol. Biol. Evol.* 28, 647–657. doi: 10.1093/molbev/msq233

Hummel, T., Vasconcelos, M. L., Clemens, J. C., Fishilevich, Y., Vosshall, L. B., and Zipursky, S. L. (2003). Axonal targeting of olfactory receptor neurons in *Drosophila* is controlled by Dscam. *Neuron* 37, 221–231. doi: 10.1016/S0896-6273(02)01183-2

Imperadore, P., Shah, S. B., Makarenkova, H. P., and Fiorito, G. (2017). Nerve degeneration and regeneration in the cephalopod mollusc *Octopus vulgaris*: the case of the pallial nerve. *Sci. Rep.* 7:46564. doi: 10.1038/srep46564

Jiang, X.-J., Li, S., Ravi, V., Venkatesh, B., and Yu, W.-P. (2009). Identification and comparative analysis of the protocadherin cluster in a reptile, the green anole lizard. *PLoS One* 4:e7614. doi: 10.1371/journal.pone.0007614

Jin, Y., and Li, H. (2018). Revisiting Dscam diversity: lessons from clustered protocadherins. *Cell. Mol. Life Sci*. doi: 10.1007/s00018-018-2951-4 [Epub ahead of print].

Junghans, D., Heidenreich, M., Hack, I., Taylor, V., Frotscher, M., and Kemler, R. (2008). Postsynaptic and differential localization to neuronal subtypes of protocadherin β16 in the mammalian central nervous system. *Eur. J. Neurosci.* 27, 559–571. doi: 10.1111/j.1460-9568.2008.06052.x

Kim, B.-M., Kang, S., Ahn, D.-H., Jung, S.-H., Rhee, H., Yoo, J. S., et al. (2018). The genome of common long-arm octopus *Octopus minor*. *Gigascience* 7:giy119. doi: 10.1093/gigascience/giy119

Kim, S., Mo, J., Han, S., Choi, S., Han, S., Moon, B., et al. (2010). The expression of non-clustered protocadherins in adult rat hippocampal formation and the connecting brain regions. *Neuroscience* 170, 189–199. doi: 10.1016/j.neuroscience.2010.05.027

Kohmura, N., Senzaki, K., Hamada, S., Kai, N., Yasuda, R., Watanabe, M., et al. (1998). Diversity revealed by a novel family of cadherins expressed in neurons at a synaptic complex. *Neuron* 20, 1137–1151. doi: 10.1016/S0896-6273(00)80495-X

Krishna-K, K., Hertel, N., and Redies, C. (2011). Cadherin expression in the somatosensory cortex: evidence for a combinatorial molecular code at the single-cell level. *Neuroscience* 175, 37–48. doi: 10.1016/j.neuroscience.2010.11.056

Liscovitch-Brauer, N., Alon, S., Porath, H. T., Elstein, B., Unger, R., Ziv, T., et al. (2017). Trade-off between transcriptome plasticity and genome evolution in cephalopods. *Cell* 169, 191–202.e11. doi: 10.1016/j.cell.2017.03.025

Marini, G., De Sio, F., Ponte, G., and Fiorito, G. (2017). "Behavioral analysis of learning and memory in cephalopods," in *Learning and Memory: A Comprehensive Reference*, 2nd Edn, ed. J. H. Byrne (Amsterdam: Academic Press, Elsevier), 441–462. doi: 10.1016/B978-0-12-809324-5.21024-9

Matthews, B. J., Kim, M. E., Flanagan, J. J., Hattori, D., Clemens, J. C., Zipursky, S. L., et al. (2007). Dendrite self-avoidance is controlled by Dscam. *Cell* 129, 593–604. doi: 10.1016/j.cell.2007.04.013

Nixon, M., and Young, J. Z. (2003). *The Brains and Lives of Cephalopods*. New York, NY: Oxford University.

Noda, T., and Satoh, N. (2008). A comprehensive survey of cadherin superfamily gene expression patterns in *Ciona intestinalis*. *Gene Expr. Patterns* 8, 349–356. doi: 10.1016/j.gep.2008.01.004

Noonan, J. P., Grimwood, J., Danke, J., Schmutz, J., Dickson, M., Amemiya, C. T., et al. (2004a). Coelacanth genome sequence reveals the evolutionary history of vertebrate genes. *Genome Res.* 14, 2397–2405. doi: 10.1101/gr.2972804

Noonan, J. P., Grimwood, J., Schmutz, J., Dickson, M., and Myers, R. M. (2004b). Gene conversion and the evolution of protocadherin gene cluster diversity. *Genome Res.* 14, 354–366. doi: 10.1101/gr.2133704

Nuernberger, M., Weth, F., and Redies, C. (2008). Layer-specific expression of multiple cadherins in the developing visual cortex (V1) of the ferret. *Cereb. Cortex* 19, 388–401. doi: 10.1093/cercor/bhn090

Peek, S. L., Mah, K. M., and Weiner, J. A. (2017). Regulation of neural circuit formation by protocadherins. *Cell. Mol. Life Sci.* 74, 4133–4157. doi: 10.1007/s00018-017-2572-3

Petrosino, G. (2015). *The Transcriptional Landscape of the Nervous System of Octopus vulgaris*. Ph.D. thesis, Università degli Studi di Napoli Federico II, Naples.

Priddle, T. H., and Crow, T. J. (2012). Protocadherin 11X/Y a human-specific gene pair: an immunohistochemical survey of fetal and adult brains. *Cereb. Cortex* 23, 1933–1941. doi: 10.1093/cercor/bhs181

Priddle, T. H., and Crow, T. J. (2013). The protocadherin 11X/Y (PCDH11X/Y) gene pair as determinant of cerebral asymmetry in modern *Homo sapiens*. *Ann. N. Y. Acad. Sci.* 1288, 36–47. doi: 10.1111/nyas.12042

Ravi, V., Yu, W.-P., Pillai, N. E., Lian, M. M., Tay, B.-H., Tohari, S., et al. (2015). Cyclostomes lack clustered protocadherins. *Mol. Biol. Evol.* 33, 311–315. doi: 10.1093/molbev/msv252

Schmucker, D., and Chen, B. (2009). Dscam and DSCAM: complex genes in simple animals, complex animals yet simple genes. *Genes Dev.* 23, 147–156. doi: 10.1101/gad.1752909

Schmucker, D., Clemens, J. C., Shu, H., Worby, C. A., Xiao, J., Muda, M., et al. (2000). *Drosophila* Dscam is an axon guidance receptor exhibiting extraordinary molecular diversity. *Cell* 101, 671–684. doi: 10.1016/S0092-8674(00)80878-8

Schreiner, D., Savas, J. N., Herzog, E., Brose, N., and de Wit, J. (2017). Synapse biology in the 'circuit-age'—paths toward molecular connectomics. *Curr. Opin. Neurobiol.* 42, 102–110. doi: 10.1016/j.conb.2016.12.004

Schreiner, D., and Weiner, J. A. (2010). Combinatorial homophilic interaction between γ-protocadherin multimers greatly expands the molecular diversity of cell adhesion. *Proc. Natl. Acad. Sci. U.S.A.* 107, 14893–14898. doi: 10.1073/pnas.1004526107

Scott, B. W., Wojtowicz, J. M., and Burnham, W. M. (2000). Neurogenesis in the dentate gyrus of the rat following electroconvulsive shock seizures. *Exp. Neurol.* 165, 231–236. doi: 10.1006/exnr.2000.7458

Speevak, M. D., and Farrell, S. A. (2011). Non-syndromic language delay in a child with disruption in the Protocadherin11X/Y gene pair. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 156, 484–489. doi: 10.1002/ajmg.b.31186

Sperry, R. W. (1963). Chemoaffinity in the orderly growth of nerve fiber patterns and connections. *Proc. Natl. Acad. Sci. U.S.A.* 50, 703–710. doi: 10.1073/pnas.50.4.703

Tsai, N.-P., Wilkerson, J. R., Guo, W., Maksimova, M. A., DeMartino, G. N., Cowan, C. W., et al. (2012). Multiple autism-linked genes mediate synapse elimination via proteasomal degradation of a synaptic scaffold PSD-95. *Cell* 151, 1581–1594. doi: 10.1016/j.cell.2012.11.040

Turchetti-Maia, A., Shomrat, T., and Hochner, B. (2017). "The vertical lobe of cephalopods. A brain structure ideal for exploring the mechanisms of complex forms of learning and memory," in *The Oxford Handbook of Invertebrate Neurobiology*, ed. J. J. Byrne (Oxford: Oxford University Press), 1–27.

Vanhalst, K., Kools, P., Staes, K., Van Roy, F., and Redies, C. (2005). δ-Protocadherins: a gene family expressed differentially in the mouse brain. *Cell. Mol. Life Sci.* 62, 1247–1259. doi: 10.1007/s00018-005-5021-7

Wang, Z. Y., and Ragsdale, C. W. (2017). Cadherin genes and evolutionary novelties in the octopus. *Semin. Cell Dev. Biol.* 69, 151–157. doi: 10.1016/j.semcdb.2017.06.007

Weiner, J. A., and Jontes, J. D. (2013). Protocadherins, not prototypical: a complex tale of their interactions, expression, and functions. *Front. Mol. Neurosci.* 6:4. doi: 10.3389/fnmol.2013.00004

Whittaker, C. A., Bergeron, K.-F., Whittle, J., Brandhorst, B. P., Burke, R. D., and Hynes, R. O. (2006). The echinoderm adhesome. *Dev. Biol.* 300, 252–266. doi: 10.1016/j.ydbio.2006.07.044

Winder, D. G., and Sweatt, J. D. (2001). Roles of serine/threonine phosphatases in hippocampel synaptic plasticity. *Nat. Rev. Neurosci.* 2, 461–474. doi: 10.1038/35081514

Wu, Q. (2005). Comparative genomics and diversifying selection of the clustered vertebrate protocadherin genes. *Genetics* 169, 2179–2188. doi: 10.1534/genetics.104.037606

Wu, Q., and Maniatis, T. (1999). A striking organization of a large family of human neural cadherin-like cell adhesion genes. *Cell* 97, 779–790. doi: 10.1016/S0092-8674(00)80789-8

Yamagata, K., Andreasson, K. I., Sugiura, H., Maru, E., Dominique, M., Irie, Y., et al. (1999). Arcadlin is a neural activity-regulated cadherin involved in long term potentiation. *J. Biol. Chem.* 274, 19473–19479. doi: 10.1074/jbc.274.27.19473

Young, J. Z. (1991). Computation in the learning system of cephalopods. *Biol. Bull.* 180, 200–208. doi: 10.2307/1542389

Yu, W.-P., Rajasegaran, V., Yew, K., Loh, W.-L., Tay, B.-H., Amemiya, C. T., et al. (2008). Elephant shark sequence reveals unique insights into the evolutionary history of vertebrate genes: a comparative analysis of the protocadherin cluster. *Proc. Natl. Acad. Sci. U.S.A.* 105, 3819–3824. doi: 10.1073/pnas.0800398105

Yu, W.-P., Yew, K., Rajasegaran, V., and Venkatesh, B. (2007). Sequencing and comparative analysis of fugu protocadherin clusters reveal diversity of protocadherin genes among teleosts. *BMC Evol. Biol.* 7:49. doi: 10.1186/1471-2148-7-49

Yue, Y., Meng, Y., Ma, H., Hou, S., Cao, G., Hong, W., et al. (2016). A large family of Dscam genes with tandemly arrayed 5′ cassettes in Chelicerata. *Nat. Commun.* 7:11252. doi: 10.1038/ncomms11252

Zhan, X.-L., Clemens, J. C., Neves, G., Hattori, D., Flanagan, J. J., Hummel, T., et al. (2004). Analysis of Dscam diversity in regulating axon guidance in *Drosophila* mushroom bodies. *Neuron* 43, 673–686. doi: 10.1016/j.neuron.2004.07.020

Zhang, X., Mao, Y., Huang, Z., Qu, M., Chen, J., Ding, S., et al. (2012). Transcriptome analysis of the *Octopus vulgaris* central nervous system. *PLoS One* 7:e40320. doi: 10.1371/journal.pone.0040320

Zhu, H., Hummel, T., Clemens, J. C., Berdnik, D., Zipursky, S. L., and Luo, L. (2006). Dendritic patterning by Dscam and synaptic partner matching in the *Drosophila* antennal lobe. *Nat. Neurosci.* 9, 349–355. doi: 10.1038/nn1652

Zipursky, S. L., and Sanes, J. R. (2010). Chemoaffinity revisited: dscams, protocadherins, and neural circuit assembly. *Cell* 143, 343–353. doi: 10.1016/j.cell.2010.10.009

Zou, C., Huang, W., Ying, G., and Wu, Q. (2007). Sequence analysis and expression mapping of the rat clustered protocadherin gene repertoires. *Neuroscience* 144, 579–603. doi: 10.1016/j.neuroscience.2006.10.011

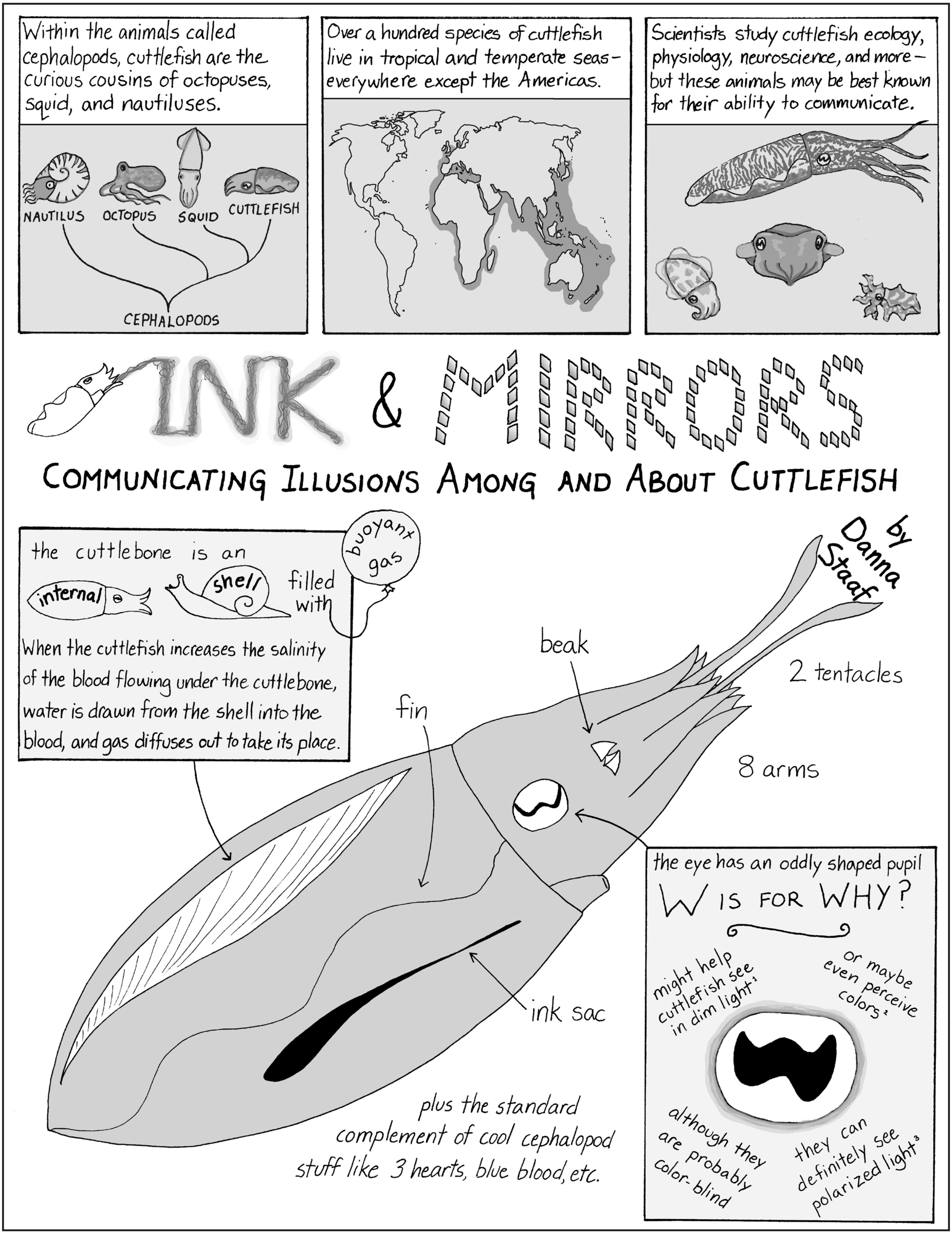
Zullo, L., Fossati, S. M., Imperadore, P., and Nödl, M. T. (2017). Molecular determinants of Cephalopod muscles and their implication in muscle regeneration. *Front. Cell Dev. Biol.* 5:53. doi: 10.3389/fcell.2017.00053

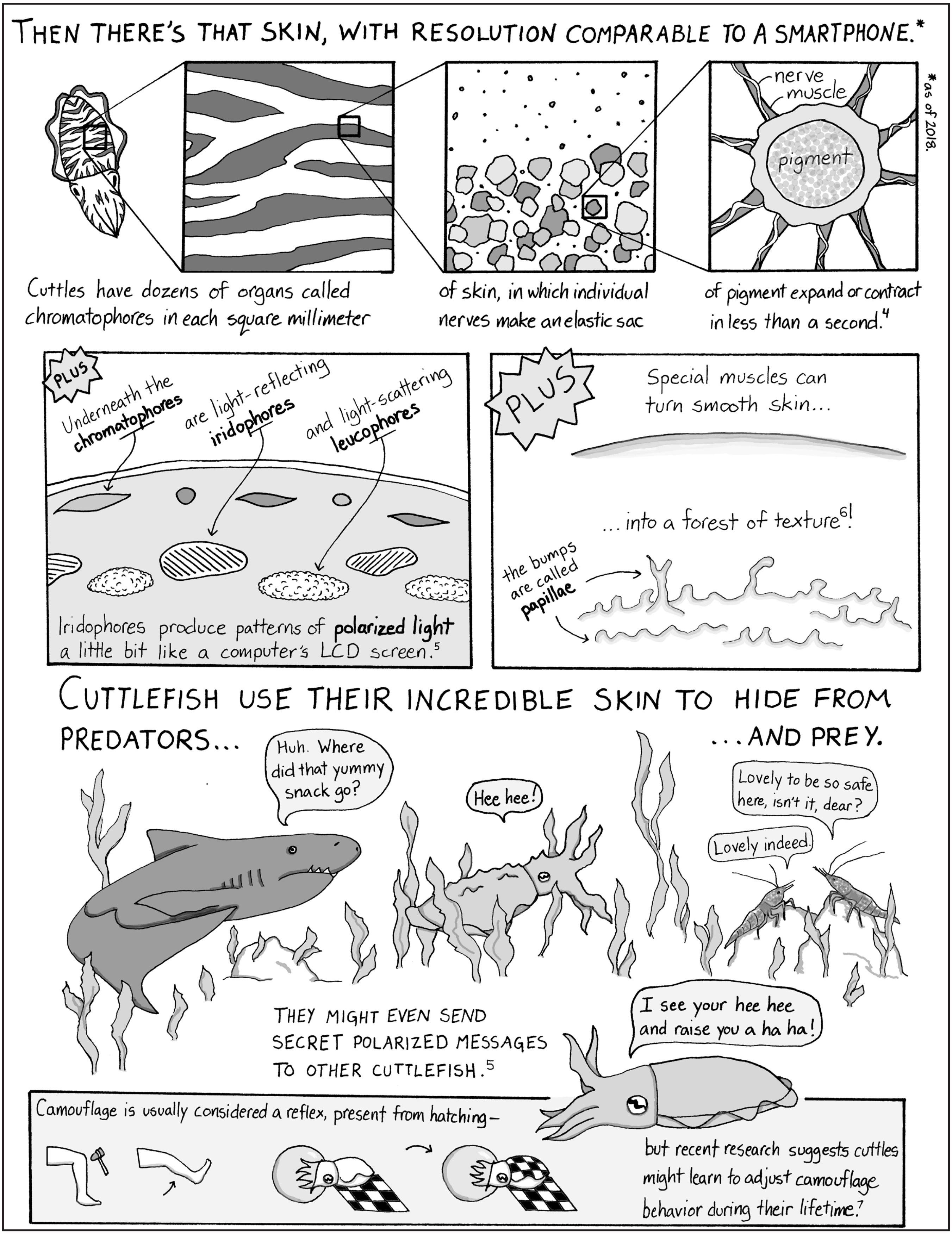
Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

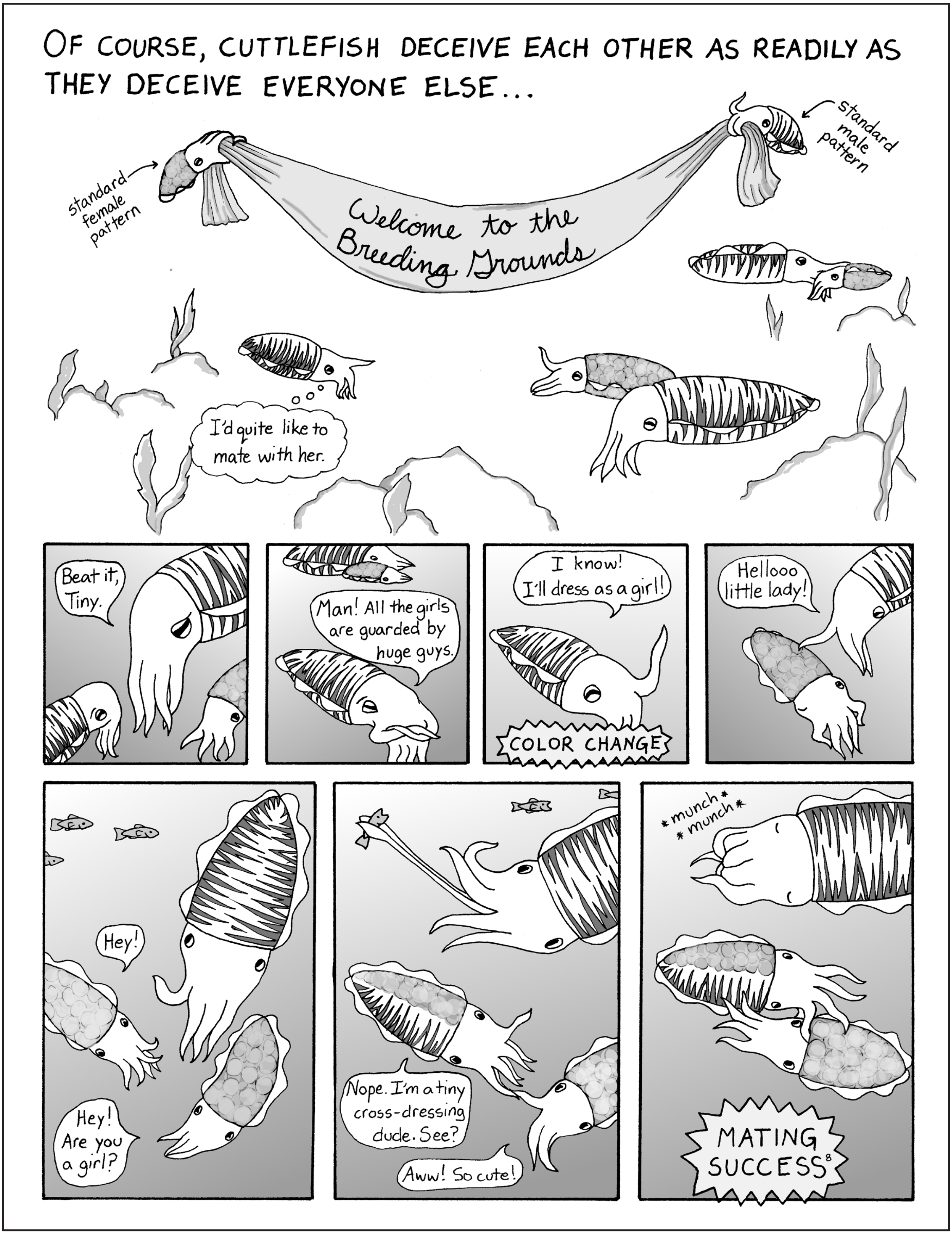
Ink and Mirrors: Communication Between and About Cuttlefish

Danna Staaf

National Association of Science Writers, United States
dannajoy@gmail.com


Within the animals called cephalopods, cuttlefish are the curious cousins of octopuses, squid, and nautiluses.
NAUTILUS
OCTOPUS
SQUID
CUTTLEFISH
CEPHALOPODS
Over a hundred species of cuttlefish live in tropical and temperate seas – everywhere except the Americas.
Scientists study cuttlefish ecology, physiology, neuroscience, and more – but these animals may be best known for their ability to communicate.
INK & MIRRORS
COMMUNICATING ILLUSIONS AMONG AND ABOUT CUTTLEFISH
by Danna Staaf
the cuttlebone is an internal shell filled with buoyant gas
When the cuttlefish increases the salinity of the blood flowing under the cuttlebone, water is drawn from the shell into the blood, and gas diffuses out to take its place.
beak
fin
2 tentacles
8 arms
ink sac
plus the standard complement of cool cephalopod stuff like 3 hearts, blue blood, etc.
the eye has an oddly shaped pupil
W IS FOR WHY?
might help cuttlefish see in dim light¹
or maybe even perceive colors²
although they are probably color-blind
they can definitely see polarized light³


THEN THERE'S THAT SKIN, WITH RESOLUTION COMPARABLE TO A SMARTPHONE.*
*as of 2018.
nerve
muscle
pigment
Cuttles have dozens of organs called chromatophores in each square millimeter
of skin, in which individual nerves make an elastic sac
of pigment expand or contract in less than a second.4
PLUS
Underneath the chromatophores
are light-reflecting iridophores
and light-scattering leucophores
Iridophores produce patterns of polarized light a little bit like a computer's LCD screen.5
PLUS
Special muscles can turn smooth skin...
... into a forest of texture6!
the bumps are called papillae
CUTTLEFISH USE THEIR INCREDIBLE SKIN TO HIDE FROM PREDATORS...
...AND PREY.
Huh. Where did that yummy snack go?
Hee hee!
Lovely to be so safe here, isn't it, dear?
Lovely indeed.
THEY MIGHT EVEN SEND SECRET POLARIZED MESSAGES TO OTHER CUTTLEFISH.5
I see your hee hee and raise you a ha ha!
Camouflage is usually considered a reflex, present from hatching–
but recent research suggests cuttles might learn to adjust camouflage behavior during their lifetime.7


OF COURSE, CUTTLEFISH DECEIVE EACH OTHER AS READILY AS THEY DECEIVE EVERYONE ELSE...
standard female pattern
standard male pattern
Welcome to the Breeding Grounds
I'd quite like to mate with her.
Beat it, Tiny.
Man! All the girls are guarded by huge guys.
I know! I'll dress as a girl!
COLOR CHANGE
Hellooo little lady!
Hey!
Hey! Are you a girl?
Nope. I'm a tiny cross-dressing dude. See?
Aww! So cute!
munch *munch*
MATING SUCCESS[8]

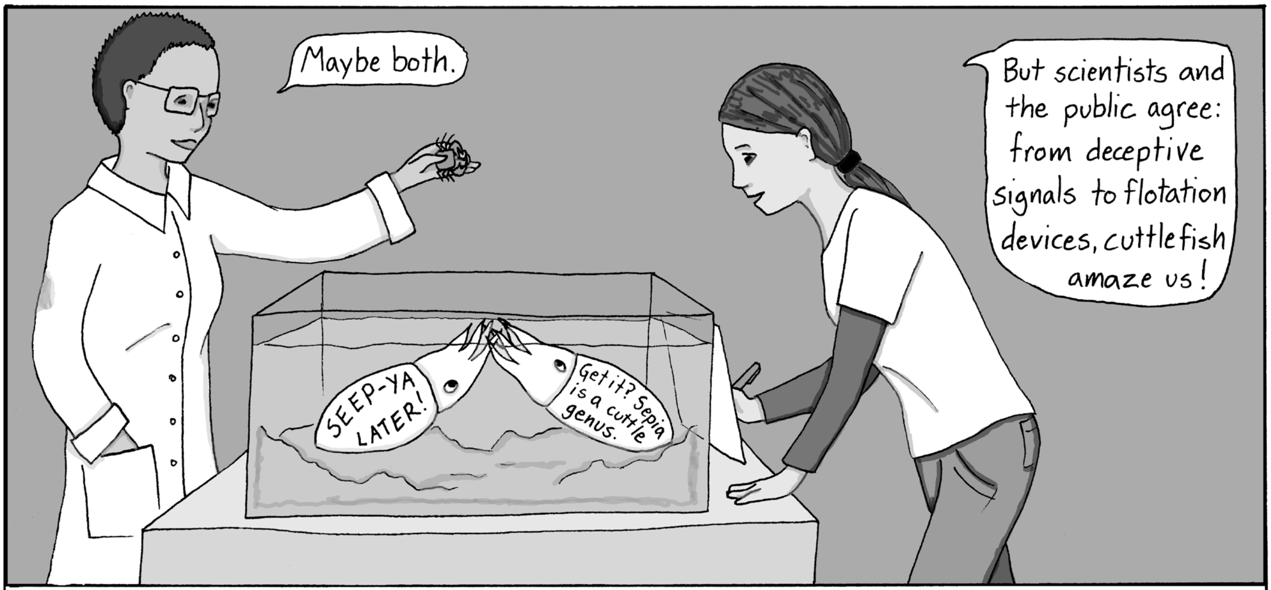
Self-identified cephalopod fans were asked what they liked about cuttlefish.
Their answers, used with permission, illustrate how easily humans can relate to these animals,

from behavior...

to appearance...

to their very name.

Does anthropomorphism prevent us from seeing things as they are?[9]
Or do analogies aid our understanding?[10]



Widespread fascination with cuttlefish offers abundant opportunities-and pitfalls-for science communication.

1. Mäthger, Hanlon, Häkansson, + Nilsson. 2013. "The W-shaped pupil in cuttlefish: functions for improving horizontal vision." *Vision Research*
2. Stubbs + Stubbs. 2016. "Spectral discrimination in color blind animals via chromatic aberration and pupil shape." *PNAS*
3. Shashar. 2014. "Polarization vision in cephalopods." In *Polarized Light and Polarization Vision in Animal Sciences*.
4. Messenger. 2001. "Cephalopod chromatophores: neurobiology and natural history." *Biological Reviews*.
5. Mäthger, Shashar, + Hanlon. 2009. "Do cephalopods communicate using polarized light reflections from their skin?" *Journal of Experimental Biology*.
6. Panetta, Buresch, + Hanlon. 2017. "Dynamic masquerade with morphing three-dimensional skin in cuttlefish." *Biology Letters*.
7. Hough, Case, + Boal. 2016. "Learned control of body patterning in cuttlefish *Sepia officinalis* (Cephalopoda)." *Journal of Molluscan Studies*.
8. Brown, Garwood, + Williamson. 2012. "It pays to cheat: tactical deception in a cephalopod social signalling system." *Biology Letters*.
9. Davies. "Anthropomorphism in science." 2010. *EMBO Reports*.
10. Zohar + Ginossar. 1998. "Lifting the taboo regarding teleology and anthropomorphism in biology education-heretical suggestions." *Science Education*.

THANKS to OctoNation (Warren, James, Alexis, Meghan) and Cephalopods-Present and Past (Denise, Andrea, Christian, Rich)

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read
for greatest visibility
and readership

FAST PUBLICATION
Around 90 days
from submission
to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative,
and constructive
peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers
acknowledged by name
on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data
and methods to enhance
research reproducibility

DIGITAL PUBLISHING
Articles designed
for optimal readership
across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics
track visibility across
digital media

EXTENSIVE PROMOTION
Marketing
and promotion
of impactful research

LOOP RESEARCH NETWORK
Our network
increases your
article's readership