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Editorial: Live feed for early ontogenetic development in marine fish larvae

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Editorial on the Research Topic:
Live feed for early ontogenetic development in marine fish larvae

1 Preface

Live feeds hold the key to a stable and expanding marine aquaculture. In this editorial, we briefly review the history of live feed production for marine larviculture and summarize the latest contribution issued in the research topic – Live Feed for Early Ontogenetic Development in Marine Fish Larvae. With the current research that were submitted to this research topic, we see trends into many different aspects of live feed production. We are ensured that some of the remaining bottlenecks will be solved in a near future, providing a diverse and ecological sound marine aquaculture sector to flourish.

2 Larviculture of marine fish species

The marine aquacultures are expanding with an increasing diversity of fish species across different regions. Most commercial marine fish larvae require live feed as first feeding diet, such as European seabass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*), turbot (*Scophthalmus maximus*), Atlantic halibut (*Hippoglossus hippoglossus*) and cod (*Gadus* spp.) in Mediterranean and East Atlantic regions (Sweetman, 1992; Reitan et al., 1993; Moksness and Støle, 1997; Shields, 2001; Evjemo et al., 2003; Oie et al., 2017) milkfish (*Chanos chanos*), groupers (*Epinephelus* spp.), cobia (*Rachycentron canadum*), snappers (*Lutjanus* spp.), sea bass (*Lates calcarifer* and *Lateolabrax japonicus*), sea breams (*Acanthopagrus* spp. and *Pagrus* spp.) and pompano (*Trachinotus* spp.) in Asian Pacific and Oceanian regions (Chen and Long, 1991;

Fushimi, 2001; Liao et al., 2001; Marte, 2003; Le Moullac et al., 2003; Palmer et al., 2007); grey mullet (*Mugil cephalus*), Pacific threadfin (*Polydactylus sexfilis*), snook (*Centropomus* sp.) and red drum (*Sciaenops ocellatus*) in American regions (Lee and Ostrowski, 2001; Cerqueira and Tsuzuki, 2009). Besides the edible fish species, the larviculture of many ornamental reef fish are already or under commercialization stage, such as clownfish (*Amphiprion* spp.), tang fish (*Paracanthurus* spp. and *Zebrasoma* spp.) and angelfish (*Centropyge* spp. and *Pomacanthus* spp.). Furthermore, several emerging species with difficult early larval stages (i.e., very tiny mouth or perception toward mobile pattern of specific live feed) are targeted by specialised research institutions and conservation programs developed by aquariums, such as Rising Tide Conservation, Tropical Aquaculture Laboratory, University of Florida; Oceanic Institute of Pacific University in Hawaii (Degidio et al., 2017; Callan et al., 2018), James Cook University in Australia (Chen et al., 2020), National Museum of Marine Biology & Aquarium and National Dong Hwa University in Taiwan (Chiu and Leu, 2021; Leu et al., 2022). Private sectors, such as Bali Aquarich based in Indonesia, De Jong Marinelife in the Netherlands and Biota in the USA have also contributed extensively to the captive breeding of marine ornamental fish for the demand of the aquarium market.

Techniques of live feed production is a subject that is commonly interesting for larviculture industry worldwide, but the selection of live feed is related to what fish species and developmental stages they are targeted for. For warm water fish species, such as groupers (*Epinephelus* spp.), duration of larviculture (hatch to metamorphosis) is around 20-30 days. Live feeds selected for the three-stage larviculture are categorized as follows: (1) first feeding stage (3-10 day post hatch, dph): fertilized oyster egg (<70 µm), nauplii of small copepod species (<120 µm) and SS-type *Brachionus* rotifer (<150 µm); (2) secondary feeding stage (8-20 dph): S-type *Brachionus* rotifer (<200 µm), newly-hatched *Artemia* nauplii (<450 µm) and copepodites (200-600 µm); (3) pre-weaning stage (18-30 dph): *Artemia* metanauplii (<600 µm), adult copepods (800-1200 µm) and micro pellet diets (Marte, 2003; Zhang et al., 2015). This example indicates that the size and type of live feeds are primarily critical, and highlights that the establishments of live feed production should be diversified for various fish species. Most of the marine fish larvae are born with a small mouth gape and incomplete digestive system (Yúfera and Darias, 2007). Those larvae need tiny live feed with great bioavailable nutrients to sustain survival, growth and other metabolic functions. Indeed, live feeds are essential for larval ontogeny due to their superior nutritional value, palatability and mobility-triggered predatory attraction. This research topic aims to establish a collection of articles that tackles different issues related to the recent progress of live feed production, and their implications in marine larviculture.

3 Live feed production for marine larviculture

In general, marine fish have poor capability to synthesize essential fatty acids, which indicates that a dietary supplement of these nutrients is crucial (Coutteau et al., 1997; Rainuzzo et al., 1997; Mejri et al.). The specific requirement on highly unsaturated fatty acids (HUFAs) could be a consequence of evolution in marine trophic relationships. In nature, phytoplankton capture solar energy *via* photosynthesis, and biosynthesize HUFAs and other organic nutrients. These components are ingested, sometimes bio-transformed and accumulated by zooplankton, and then by larvae of most marine fish. Indeed, phytoplankton and zooplankton are natural food items for marine larvae, thus they are also produced as live feed for larval nourishment in marine hatchery (Reitan et al., 1997). Based on the species availability and climate condition, applications of live feeds (species selection and production techniques) are diverse and region-specific (Lee et al., 2008; Conceição et al., 2010; Nielsen et al., 2017; Pan et al.).

Phytoplankton has been long used as live feed for zooplankton and larvae of several marine organisms, and as water conditioning element (i.e., green water technique) (De Pauw et al., 1984; Borowitzka, 1997; Pan et al.). One of the focuses in research and development (R&D) for microalgae is the photobioreactor, which could facilitate an efficient and high-density microalgae production. Many companies and institutions have well demonstrated the industrial scale photobioreactor system for marine hatcheries (Naumann et al., 2013; Tibbetts et al., 2020). Several studies have showed that manipulations in culture conditions and composition of fertilizer could affect microalgal productivity and nutritional quality (Harrison et al., 1990; Reitan et al., 1994; Reitan et al., 1997; Cañavate, 2019; Han et al., 2019; Cañavate and Fernández-Díaz, 2022). In this research topic, Latsos et al. revealed a significant increase of fatty acid levels in the microalga *Rhodomonas* sp. inducing by nitrogen starvation. The authors proposed a two-phase culture system, which firstly accelerates the cell growth and subsequently induces PUFAs accumulation by nitrogen starvation in commercial scale cultivation.

Ciliates are a group of micrometre-sized protists that show great potential for feeding small-mouthed reef fish larvae, such as cleaner goby (*Gobiosoma evelynae*), purple fire-fish (*Nemateleotris decora*) and blue-striped angelfish (*Chaetodontoplus septentrionalis*) (Olivotto et al., 2005; Madhu and Madhu, 2014; Leu et al., 2015). Ciliate production relies on the suspending organic matters and bacteria as their dietary resources, which makes their cultivation relatively easy. In addition, the fact of ciliate's bacterivorous feature has facilitated their implication as bio-effector. In this research topic, Lin et al. revealed an innovative method of using the ciliate *Strombidium* sp. to reduce pathogenic *Vibrio* sp., which leads to a significant increase of the survival rate in juvenile grouper *Epinephelus coioides*.

Rotifers in aquaculture largely belongs to the genus *Brachionus*, and it has been extensively produced as live feed for marine larviculture since the 1960-70s in Japan and Norway (Watanabe et al., 1983; Pejler, 1998; Lubzens et al., 2001). Rotifer cultures are well established, and intensive culture systems are available in the industry worldwide (Lubzens et al., 1989; Fu et al., 1997; Odo et al., 2015; Pan et al.). One of the advantages of rotifer production is the feasibility of reaching ultra-high densities. For instance, Yoshimura et al. (2003) reported a rotifer density over 10^5 individuals ml^{-1} in a culture equipped with a membrane filtration system. Research in rotifer enrichment and its effect on marine fish larviculture are still an area of interest. In this research topic Ghaderpour and Estevez shows a close relationship between the composition of the dominant phospholipids between Meager larvae (*Argyrosomus regius*) and its live feed, rotifers respectively. Another example from the research topic is the ongoing research in effects of novel enrichment protocols for rotifers. Safiin et al. showed promising results when incorporating palm oil into enrichment diets of rotifers that were fed to *L. calcarifer* larvae. Furthermore, Fu et al. also investigated the effects of different commercially available enrichment products on rotifers to reveal how the enrichment impact survival, growth, fatty acid composition and jaw deformities of Golden Pompano larvae (*Trachinotus ovatus*).

The harvest of brine shrimp *Artemia* dormant cysts or biomass was first commercialized in San Francisco Bay, California and the Great Salt Lake, Utah, USA during 1950s for the aquarium market, and further exploited for the increased demands from the marine larviculture industries in 1970s (Lavens and Sorgeloos, 2000). Until now, *Artemia* cyst production relies mainly on natural resources, thus new harvest sites (e.g., southern Siberia and central Asia) have been investigated and established for cyst production industry in the recent decades (Litvinenko et al., 2015; Le et al., 2019; Camara, 2020; Pan et al.). Another manner of brine shrimp cyst production is the cultivation in inland saltworks or hypersaline ponds (e.g., China, Kenya and Vietnam), which facilitates a better biological and environmental controls to achieve sustainable cyst production (Baert et al., 1997; Van Stappen et al., 2020). Owning the significant role of *Artemia* in the industry, two UN-recognized Artemia Reference Centres has been established at Ghent University, Belgium and Tianjin University of Science and Technology, China, where scientists are working on the pioneer aspects of genomic sequencing, genetic and microbial regulations, nutrition and exploitation of new cyst resources (Sorgeloos, 1980; FAO, 2017; Sorgeloos and Roubach, 2021; Duan et al., 2022). Based on the great scientific contributions, the supply chain of *Artemia* cysts and their relevant products are successfully marketed by several companies, such as INVE Aquaculture Co. in Belgium and Ocean Star International Co. in the USA. Research in *Artemia* is still very relevant when used as starter diets for fish larvae of some species. In this research topic, Planas et al. and Planas et al.) studies different breeder and pre-breeding diets, with *Artemia*, and their effect on new-born seahorses.

Copepods are natural food items for marine organisms, and are either harvested from field or intentionally cultivated for marine larviculture (Støttrup, 2000; Drillet et al., 2011; Hansen, 2017). The capacity of PUFA bioconversion or accumulation in copepod determines their nutritional benefit for larval feeding (Nielsen et al., 2019). Research protocols addressing nutritional manipulations of copepods are issued in this research topic (Camus et al.; Dayras et al.; Matsui et al.; Wang et al.). Focuses on other cultivation parameters are also included in the research topic, such as salinity, temperature, and photoperiod (Choi et al.; Wang et al.; Yoshino et al.). On the other hand, the risk of epibionts on copepods in the indoor intensive culture system was reported by Pan et. al. This study revealed a significant decline of egg production in diatom-infested *Acartia tonsa*, suggesting the prevention of epibiosis should be carried out in copepod cultures.

The intensive mono-species production of copepods is still in its' pre-industrial stage, but the commercial production is estimated economically feasible (Abate et al., 2015; Abate et al., 2016). Some temperate species, in particular the calanoid *Acartia tonsa* (strain DFH.AT1) has been intensively studied for aquaculture purposes (Støttrup et al., 1986; Drillet et al., 2006; Jepsen et al., 2007; Hagemann et al., 2016; Pan et al., 2020). Its capacity to easily produce resting eggs that can be stored at low temperature encouraged the R&D projects (e.g., IMPAQ in Denmark, COPREST and STARTRENS in Norway) and the industrialisation initiatives. Since few years, the C-FEED company based in Norway, produces resting eggs of *A. tonsa* that are easily shipped for larviculture. On the other hand, many local suppliers in Asia that provide live copepods (e.g., *Apocyclops royi* and *Pseudodiaptomus annandalei*) cultivated in outdoor ponds for commercial fish hatcheries (Su et al., 1997; Blanda et al., 2015; Grønning et al., 2019). The optimal mass culture protocols of the listed copepod species have been long developed and reviewed in several projects and publications (Drillet et al., 2011; Rasdi and Qin, 2016; Hansen, 2022). Although these millimetre-sized copepod species (adult size at around 1-2 mm) are commercially used, the research and development of novel and micrometre-sized copepod species (adult size < 800 μm) are needed. Based on the articles collected in our research topic, there are trends in that the search for the desirable copepod candidates are micrometre-sized calanoid (Camus et al.; Choi et al.; Yoshino et al.; Wang et al.) and cyclopoid copepods (Dayras et al.). These successful accomplishments, coupled with the previous contributions from Australia, Hawaii and South Korea (McKinnon et al., 2003; Kline and Laidley, 2015; Lee and Choi, 2016), have implicated the potential breakthrough of using micrometre-sized copepods e.g. *Bestiolina* sp., *Parvocalanus* sp., and *Paracyclopina* sp. as first feeding diets for the emerging marine aquaculture fish species with particularly tiny mouth gapes.

4 Enrichment techniques in rotifer and *Artemia* for marine fish larviculture

One of the focuses addressed in this research topic is nutritional enrichment protocols for fish larval feeding (Fu et al.; Matsui et al.; Planas et al.; Planas et al.; Safiin et al.; Vo et al.). This is especially relevant for live feed organisms with poor capacity in bioconversion and accumulation of nutrients e.g., *Artemia* and rotifers. High availability and easy maintenance make these animals commercial live feeds, even though none of them appear naturally in the marine food web. As unnatural food items, *Artemia* and rotifer are often poor in essential fatty acids. Fortunately, these organisms demonstrate non-selective feeding behaviour which makes them excellent vectors of nutritious elements for farmed larvae (Léger et al., 1987; Fernández-Reiriz et al., 1993; Ghaderpour and Estevez). In this research topic, processing of alternative ingredients and enrichment protocols are reported for the larval feeding of many emerging fish species, such as Asian Seabass (*L. calcarifer*), Atlantic cod (*Gadus morhua*), Golden Pompano (*T. ovatus*), Long-snouted seahorse (*H. guttulatus*), Slender seahorse (*Hippocampus reidi*), Meager (*A. regius*), and Red Sea Bream (*Pagrus major*) (Fu et al.; Matsui et al.; Planas et al.; Planas et al.; Safiin et al.; Vo et al.). The choice of live feeds, enrichment products and protocols differ among these studies, and the suitability of various designs are highly correlated to the nutritional requirement of the farmed species. Another research focus is to replace fish oil in the enrichment emulsion for sustainability and cost-down management. Indeed, the alternative ingredients (e.g., palm oil) could reduce over-reliance in fish oil and open a new avenue in regional-specific industry of live feed enrichment products (Safiin et al.).

5 Perspectivation

Industrial production for micrometre-sized copepod is particularly worth to be invested for the supplement or replacement of the rotifer and the price-increasing *Artemia*. Selective breeding on preferable aquaculture traits (e.g., high productivity, nutritional value) could further improve the economic feasibility of the available live feed strains (Souissi et al., 2016; Pan et al., 2017). Genomic or genetic studies of live feed organisms should be addressed to clarify the effects of culture managements on their physiology (e.g., nutrient synthesis, stress tolerance) (Nielsen et al., 2019; Lee et al., 2022). The production of other live feed candidates, such as the recent successful larviculture project of ballan wrasse (*Labrus bergylta*) using cryo-preserved barnacle nauplii called "CryoPlankton" from Planktonic Co., Norway (Malzahn et al., 2022), and gelatinous zooplankton (e.g., flame jellyfish *Rhopilema esculentum* for feeding larval silver pomfret *Pampus argenteus* and larvacean for eel larvae) should be developed to support the successful larviculture of emerging aquaculture species (MochiokaIwamizu, 1996; Liu et al., 2015). Overall, the optimization of live feed production, conservation for shipment (e.g., cryopreservation or artificially-induced resting stages) and the improvement of enrichment techniques are still hot topics. Commercialization of the relevant research contributions are encouraged to be accomplished under collaborations between academia and industry (Hansen et al., 2017). Another important step is continuous meetings between academia and industry at international levels in relevant forums e.g., LARVI-conference and the European Aquaculture Society to further support the development of the marine aquaculture (Hansen and Møller, 2021).

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All authors were responsible for the idea of this special volume, wrote, and reviewed this editorial topic. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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.

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