



Discrete Spawning Aggregations of the Loliginid Squid *Doryteuthis gahi* Reveal Life-History Interactions of a Dwarf Morphotype at the Center of Its Distribution Range

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Heterogeneous environmental conditions along the Humboldt Current System (HCS) influence the life-history strategy of a variety of species in different ways. There is limited information on latitudinal traits of coastal cephalopods as part of the interacting species in pelagic and benthic environments. The present study used the loliginid squid *Doryteuthis gahi* as a model organism to: (1) evaluate latitudinal traits on egg laying patterns, (2) characterize a particular spawning ground, (3) quantify the isotopic variation from different tissues, (4) evaluate potential trophic ontogenetic changes, (5) determinate trophic position, and (6) isotopically estimate the relative importance of putative preys in the squid's assimilated diet. Results evidenced that egg-masses collected between 2014 and 2020 presented similar patterns along northern-central Chile (27–36°S), with females attaching small egg-capsules (10–50 mm length) through the year and over a variety of anthropogenic and natural substrates. At a small scale (Coquimbo; 29°S), early life history traits showed distinct patterns depending on SST, with warmer collection periods (~18°C) evidencing larger capsules and smaller embryos, although small paralarvae were obtained over the 4-year sampling period. In this site, sampling of older ontogenetic stages supported the constant presence of small-sized squids (19–77 mm ML). Males had larger mean sizes compared to females and undetermined specimens, with a high proportion of mature stages. Observations in captivity were extended for up to 110 d, validating that small females (45–64 mm ML) spawn the small egg-capsules typically observed in the field. Differences in stable isotope composition between beaks and soft tissues of adult squids were lower for $\delta^{13}\text{C}$ values (1.1 vs. 1.4‰, respectively) and higher for $\delta^{15}\text{N}$ values (5.3 vs. 4.7‰, respectively). Isotopic composition through ontogeny found similar $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, suggesting that carbon sources (pelagic origin) and trophic position did not change significantly, with copepods, euphausiids and nereid polychaetes being the most important preys. This study unveils the permanent occurrence of a small reproductive morphotype of *D. gahi*

in shallow coastal habitats of northern-central Chile (i.e., center of the distribution range), providing the first insights for understanding the species' potential adaptations to heterogeneous conditions in the HCS and the unexplored distribution gap between the two centers of abundance (Peru and the Falkland/Malvinas islands).

Keywords: egg capsules, egg size, paralarvae, stable isotopes, trophic ecology, Loliginidae

INTRODUCTION

The effects of environmental changes, either natural or anthropogenic, usually result in direct consequences for reproductive traits, including age at first reproduction, fecundity, parental investment, and spawning habitats (Byrne, 2011). In species with complex life-history strategies, like most marine species, development in a multi-stressor world can change at any stage; therefore, understanding the onset of environmental fluctuations on planktonic (e.g., embryos, larvae) and benthic/pelagic (e.g., juvenile, adults) life-history stages is crucial to identify their ontogenetic-associated vulnerabilities (Byrne, 2011). Consequently, evaluating species' latitudinal and/or longitudinal distributions, and their associated reproductive traits, may provide important information on how different species (and so populations) will respond to environmental gradients (Fernández et al., 2007).

Among the diversity of marine species dealing with environmental variability, cephalopods are thought to be especially sensitive as they have a unique set of life-history characteristics such as rapid and labile growth, short lifespans, and strong life-history plasticity; that allow them to quickly adapt to changing environmental conditions (Pierce et al., 2008). The extreme flexibility exhibited through their life-cycles, the increased population abundances (Doubleday et al., 2016), and the rapid ways of turnover at the population level (e.g., invasive range expansion of the Humboldt squid *Dosidicus gigas*; Zeidberg and Robison, 2007), suggest that cephalopods' responses to changing environmental conditions are likely to be much more complex than in other co-occurring taxa, as fish species, especially during larval and juvenile phases (Pecl and Jackson, 2008; Pierce et al., 2008; Pimentel et al., 2012).

In this context, the Southeast Pacific provide an interesting scenario to evaluate differential effects of environmental variability at latitudinal and longitudinal gradients. Along the Chilean coast (~3000 km of coastline), nutrient-rich, cold waters characterize the environmental conditions in the Humboldt Current System (HCS; Thiel et al., 2007), providing an appropriate environment for ~86 species of cephalopods between 18° and 56°S (Ibáñez et al., 2009). However, most information on this group has been focused only on commercial species, including three octopuses (*Octopus mimus*, *Enteroctopus megalocyathus*, and *Robsonella fontaniana*), and two squids (*Dosidicus gigas*, *Doryteuthis gahi*) (see Rocha and Vega, 2003; Ibáñez et al., 2010). Among coastal species, the loliginid squid *D. gahi*, is the only one inhabiting through the entire southeastern Pacific, from Northern Peru to Southern Chile. The distribution of this small species [130–170 mm dorsal mantle length (ML)] has been proposed from southern Peru and Chile in the

Pacific Ocean, to southern Argentina and the Falklands/Malvinas Islands in the Atlantic Ocean (Jereb and Roper, 2010), with two population units being detected, the Peruvian group and the Chilean – Falklands/Malvinas group (Ibáñez and Poulin, 2014; McKeown et al., 2019). In fact, it has been assumed that populations off Peru and Chile are connected by squids that occur in northern Chile (Jereb and Roper, 2010; Arkhipkin et al., 2015b); however, there are only few records of *D. gahi* occurrence in the region between 20°S and 36°S (Ibáñez et al., 2012; Carrasco and Pérez-Matus, 2016).

Members of the family Loliginidae, such as the Patagonian long-finned squid *D. gahi*, have a sessile benthic egg stage and tend to be neritic in habit, with seasonal distributions that shift between shallow coastal waters and deeper offshore waters along continental shelves (Boyle and Rodhouse, 2005; Arkhipkin et al., 2013). Consequently, loliginid populations are physically structured by shallow water topography and seasonal variations in water temperature, affecting embryonic development, growth rates, and so the timing at which early life stages would be subject to intense predation (Arkhipkin et al., 2013). In the case of *D. gahi*, the biology and ecology of the species is mostly known from the Falkland/Malvinas population (Arkhipkin et al., 2013), with few studies describing life-history traits along its Pacific distribution (Guerra et al., 2001; Villegas, 2001; Cardoso et al., 2005; Ibáñez et al., 2005; Carrasco et al., 2016).

Through the Southeast Pacific distribution range, *D. gahi* has been recognized as a prey item for a variety of coastal fishes (González and Chong, 1997; Cubillos et al., 2003; González and Oyarzún, 2003; Chong et al., 2006) and one mammal (Sielfeld et al., 2018); nonetheless, its role as an opportunistic and efficient predator has been scarcely assessed through pelagic food webs in the HCS (Guerra et al., 1991). Information regarding the species diet has been mostly obtained from the South Atlantic region, evidencing that planktonic crustaceans (e.g., euphausiids, amphipods, and copepods) are the most important components, whereas fish may have a minor contribution as a prey (Guerra et al., 1991; Arkhipkin et al., 2013). However, in the Eastern Pacific (Peru), the fish *Odontesthes regia* and nereid polychaetes (*Pseudonereis gallapagensis* and *Nereis callaona*) were identified as important components of stomach contents (Cardoso et al., 1998). In Chilean waters, the trophic role of *D. gahi* in coastal food webs is still unknown, being practically absent from ecological studies regarding pelagic and/or benthic communities from the HCS (see Thiel et al., 2007; Espinoza et al., 2017; Pérez-Matus et al., 2017; Pizarro et al., 2019).

Considering the overlooked ecological interactions involving the squid *D. gahi* in coastal environments of the HCS, and especially in the distribution gap between 12 and 34°S, this study aimed to: (1) provide latitudinal observations on spawning

habitats and egg laying patterns along the Chilean coast, (2) characterize a particular spawning ground based on different ontogenetic stages collected from the field, (3) quantify and determine variability of isotopic composition among beaks and soft tissues (arms and mantle), (4) evaluate potential trophic ontogenetic changes, (5) determine the trophic position of this coastal squid in relation to other pelagic consumers, and (6) isotopically estimate the relative importance of putative preys on the squid's assimilated diet.

According to the latest information available for *D. gahi* regarding spawning habitats in central Chile (Carrasco and Pérez-Matus, 2016) and population genetic studies through most of the distribution range (McKeown et al., 2019), we hypothesized that: (1) The existing distribution gap from southern Peru to central Chile will be characterized by the occurrence of small (= low fecundity) squids, which is evidenced in the small size of the egg capsules (with few encapsulated embryos) permanently observed through the northern-central Chilean coast (i.e., "abundant edge distribution"), and (2) other than evident differences in size (and so reproductive traits), the ecological and/or functional role of the small Humboldt specimens (e.g., diet, trophic position) will be comparable to that in larger specimens occurring in the main abundance centers.

MATERIALS AND METHODS

Latitudinal Traits on Spawning Habitats

In addition to previous published records, updated information on sporadic collections of egg masses of *D. gahi* along the Chilean coast from 2014 to 2020 [i.e., ~3,500 km of coast; from Caldera (27°S) to Punta Arenas (53°S); **Figure 1a**] was summarized and typified using actual photographic records from the field and morphometric approaches. Whenever possible, the collected egg masses were evaluated in laboratory including the following standard measurements: capsule length (mm), number of encapsulated embryos, egg size (mm), and paralarvae size (mantle length; ML, mm) (for details on methods see Carrasco and Pérez-Matus, 2016).

Characterization of a Spawning Ground

Early Life Stages

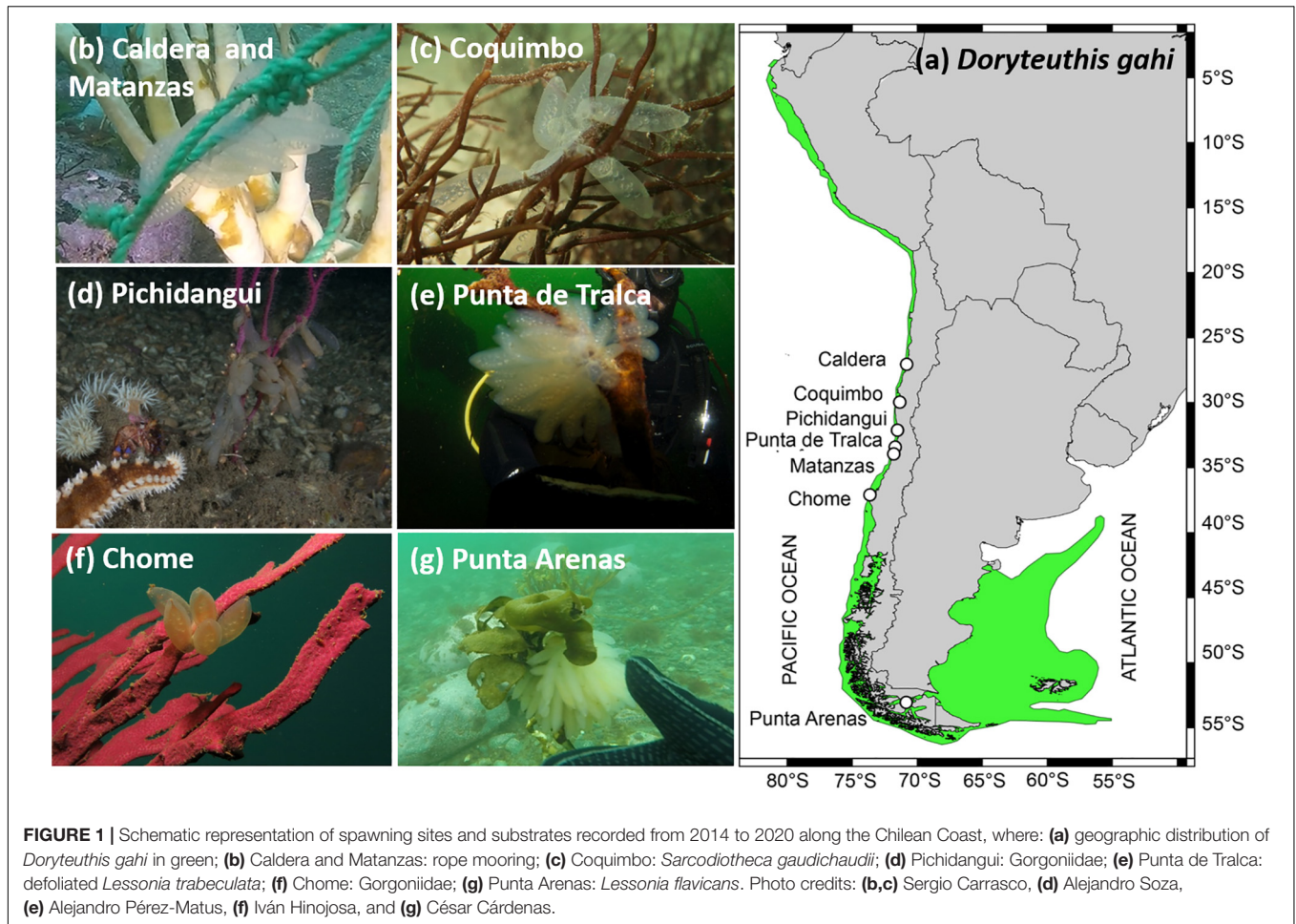
From all locations presented above, and due to its proximity to the laboratory, a permanent sampling site was selected at La Herradura Bay, Coquimbo, northern-central Chile (29°S; **Figure 1a**). In this site, fifteen subtidal surveys were carried out from May 2016 to March 2020, and whenever possible, egg masses were hand-collected by SCUBA diving at depths between 6 and 30 m. Samples were transported in seawater to the laboratory and each egg mass placed in a 20 L plastic container with constantly flowing fresh seawater at ambient temperature (range: 12–15°C in winter, and 16–20°C in summer). For each sampling date, sea surface temperature (SST, °C) was also obtained from the Hydrographic and Oceanographic Service of the Chilean Navy¹ databases, allowing further contrasts

with the early life stages analyzed. Morphometric traits were evaluated in fresh samples, including number of capsules per egg mass, capsule size (mm), number of encapsulated embryos, egg size (mm), and paralarvae size (mm). Only eggs in early developmental stages (i.e., stage 16–17; see Guerra et al., 2001) and recently hatched paralarvae [i.e., 48 h post-hatch; mantle length (ML, mm)] were considered for morphometry (see Carrasco and Pérez-Matus, 2016). All measurements were performed by using a manual caliper and a dissecting scope at 20X magnification (Zeiss Stemi 2000-C) at Sala de Colecciones Biológicas, Universidad Católica del Norte (SCBUCN). At this time, egg capsules and paralarvae were frozen at –20°C for further stable isotope analyses (see below sections). In order to assess differences in early life-stages (i.e., capsules, eggs, and paralarvae size) among sampling temperatures (SST), independent Generalized Least Square Models (GLS) were performed for the evaluated traits. In each case, normality and homoscedasticity were previously checked by using Shapiro–Wilk and Levene's tests, respectively.

Juvenile and Adult Stages

Collections of juvenile and adult stages were conducted during four nocturnal surveys (20:30 PM to 01:30 AM) carried out between October 2018 and March 2020 in different points of the same bay (La Herradura). Squids were attracted to the boat by light fishing, carefully collected with a hand net, and individualized into 10 or 20 L plastic containers depending on the specimens' size. In the case of collecting a considerable fraction of the school (i.e., up to 60 individuals), specimens were maintained at a maximum density of 3–4 individuals per 20 L container, and carefully transported to the laboratory located nearby within the same bay. In general, squids from low density collections remained individualized into the same 20 L containers with running seawater at ambient temperature (16.05 ± 1.4°C; mean ± SD). However, at high density collections, individuals were separated into two size classes (20–40 and 50–70 mm ML) and placed in two 3,000 L flow-through tanks, allowing them to maintain schools of around 30 individuals each. In all cases, squids were fed at a daily basis with amphipods, decapod megalopae, shrimps, and fish (depending on availability). Survival was also evaluated at that time, with any dead individual being removed from the tank to be sexed, measured with a manual caliper to the nearest millimeter (ML, mm), and weighed (g) using a digital balance (Radwag WTC 2000). All specimens were frozen at –20°C. Stomach content was also visually inspected by ventrally dissecting six adult specimens (i.e., 1–2 survival days in laboratory); however, considering that all samples evidenced highly digested food with no recognizable prey remains, no further analyses were carried out. Cannibalized squids from the laboratory ($n = 20$) were excluded from the analysis as only the mantles (i.e., no head or arms/tentacles) were recovered. With this information, a length-weight relationship was calculated using most specimens collected (46 out of 76) regardless the survival time in captivity (from 1 to 110 days). A power function model was used to evaluate the relationship between ML and weight. Additionally, ML of males, females, and undetermined

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juveniles, was contrasted with an ANOVA test after normality and homoscedasticity were evaluated using Shapiro–Wilk and Levene’s tests, respectively. Frequency distributions of macroscopic maturity stages (immature, maturing, and mature) between male and females were analyzed using a 2-sample test for equality of proportions with Yate’s correction (Proportion test). Accordingly, the maturing stage was characterized by the absence of spermatophores in males and the development of the nidamental glands in females.

Stable Isotope Analyses

Sample Collection and Preparation

As described above, benthic egg capsules of *D. gahi* were collected during subtidal surveys, whereas juvenile/adult squids, small juvenile fishes, zooplankton (e.g., copepods, euphausiids, brachyuran zoeae), and emergent benthic fauna (e.g., amphipods, nereid polychaetes) were collected during nocturnal surveys. For squids’ egg capsules (post-spawning capsules without embryos) and newly hatched paralarvae, complete individuals were considered in the analyses. In the case of juvenile and adult squids, mantle, arms, and lower beaks were dissected to compare potential differences in stable isotopes values. Samplings from lower beaks were collected as suggested for several species of

squids (Cherel and Hobson, 2005; Cherel et al., 2009a,b, 2019; Xavier et al., 2015) and benthic octopuses (Guerreiro et al., 2015; Matias et al., 2019). For juvenile fish, muscle pieces were sampled, whereas for euphausiids, amphipods and polychaetes, complete individuals were used. Due to their small sizes and weights, samples of copepods and brachyuran zoeae were obtained by pooling ~ 10 individuals to complete the necessary mass. In all cases, around 10 mg of wet tissue (in order to get ~1 mg dry mass) was obtained, washed with Milli-Q water, placed in pre-combusted vials, and dried in an oven (60°C) for 48 h to be grounded into a fine powder with an agate mortar. Small amounts (~0.5 mg) were placed in pre-weighed tin capsules and stored in a desiccator until the shipment for stable isotope analyses. When possible, voucher specimens were preserved in 95% ethanol and deposited at Sala de Colecciones Biológicas Universidad Católica del Norte (SCBUCN) for further taxonomic corroboration and genetic analyses. These ethanol-preserved specimens are also suitable for future isotopic analyses (see Pauli et al., 2017).

Carbon ($\delta^{13}\text{C}$) and Nitrogen ($\delta^{15}\text{N}$) Stable Isotope Ratios

Analyses of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope ratios were conducted at UC Davis Stable Isotope Facility, using a

PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (IRMS, Sercon Ltd., Cheshire, United Kingdom). Samples were combusted at 1000 °C in a reactor packed with chromium oxide and silvered copper oxide. Following combustion, oxides were removed in a reduction reactor (reduced copper at 650°C). The helium carrier then flowed through a water trap (magnesium perchlorate and phosphorous pentoxide). N₂ and CO₂ were separated on a Carbosieve GC column (65°C, 65 mL/min) before entering the IRMS. Stable isotope ratios were reported in the δ notation as the deviation relative to international standards (Vienna Pee Dee Belemnite for $\delta^{13}\text{C}$ and atmospheric N₂ for $\delta^{15}\text{N}$), so $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1]$, where R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$, respectively. Typical precision of the analyses was $\pm 0.2\%$ for $\delta^{13}\text{C}$ and $\pm 0.3\%$ for $\delta^{15}\text{N}$.

Lipid Normalization

Since lipids are ^{13}C -depleted relative to protein (DeNiro and Epstein, 1977), lipid normalization was applied for invertebrate and fish using C:N ratios (molar) [Eq. (3) in Post et al., 2007]. This correction was applied to samples with C:N values > 3.5 ($> 5\%$ lipid content) according to the following equation:

$$\delta^{13}\text{C}' = \delta^{13}\text{C} + (0.99) \times (\text{C} : \text{N}) - 3.32$$

Stable Isotope Composition on Squids' Tissues and Ontogenetic Variability

In order to determine differences in mean values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among different squid's body parts (beaks, mantle, and arms), stable isotope values were independently compared through analyses of variance (ANOVA). Previous to the analyses, normality and homoscedasticity were tested with Shapiro-Wilk and Levene's tests, respectively. Parametric *post hoc* Tukey's tests were used to determine statistical differences in isotopic values among tissues.

To detect potential changes in prey selection and trophic position of *D. gahi* through ontogeny, Pearson's correlation coefficient was used to determine the relationship between mantle length (i.e., 2 to 70 mm ML) with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from mantle tissues, respectively. Mantle tissue was selected because it is more stable through ontogeny than arm tissue, which could be damaged and regenerated due agonistic and/or predatory-prey interactions. Data normality was tested through the Shapiro-Wilk test.

Estimating Trophic Position

To assess the status of *D. gahi* within the pelagic food web, we estimated the trophic position for different consumers (e.g., squids, invertebrates, and fish) using the "oneBaseline" model, incorporating uncertainty in the trophic discrimination factor (TDF) for muscle nitrogen (mean \pm SD $\Delta^{15}\text{N} = 3.4 \pm 0.1\%$) (Post, 2002), as well as baseline and consumer $\delta^{15}\text{N}$ values through Bayesian inference with *tTrophicPosition* package (Quezada-Romegialli et al., 2018). Assuming that pelagic species of squids, invertebrates, and fish, could be supported mainly by pelagic carbon and nitrogen (Thiel et al., 2007), copepods were used as baseline with a mean trophic position of 2.2 (see Chen

et al., 2018). Models were run with 2 chains, 20,000 adapting samplings, and 20,000 iterations.

Contribution of Putative Preys to Squid's Assimilated Diet

To evaluate the relative contribution of six potential preys ($n \geq 3$ individuals), including four invertebrates (i.e., *Euphausia mucronata*, *Heterophoxus oculatus*, *Pseudonereis gallapagensis*, and cyclopoida copepods) and two fishes (*Odontesthes regia* and *Hypsoblennius sordidus*) to the assimilated diet of *D. gahi* population (estimated for both mantle and arm tissue), Bayesian stable isotope mixing models were used (MixSiar; Stock et al., 2018) employing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values as tracers and elemental concentration from preys (concentration-dependence model). The TDF values for muscle (see trophic position methods above) were taken from a global meta-analyses (Post, 2002). The error term "Process* Residual" was selected for the mixture (i.e., consumers). The residual error incorporates the potential variation associated with consumers (e.g., different metabolic rate, assimilation efficiency or digestibility), whereas the process error incorporates the variation associated to sampling process and consumer specialization (Stock and Semmens, 2016). Uninformative priors (all values between 0 and 1 are likely equally) were used in the models. MixSiar were run using a "normal" chain length through Markov Chain Monte Carlo (MCMC) and assessed through Gelman-Rubin and Geweke diagnostic test (Stock and Semmens, 2016).

All statistical analyses were performed in RStudio (R Studio Team, 2020).

RESULTS

Latitudinal Traits on Spawning Habitats

Field samplings and observations over the last 6 years (2014–2020) evidenced that egg masses of *D. gahi* presented similar laying patterns along the Chilean coast (27°S to 53°S), with females attaching the translucent and gelatinous egg capsules through the year and over a variety of structures, including anthropogenic substrates such as rope mooring in Caldera and Matanzas (**Figure 1b**), and natural substrates such as the red algae *Sarcodiotheca gaudichaudii* in Coquimbo (**Figure 1c**), cold-water corals Family Gorgoniidae in Pichidangui and Chome (**Figures 1d,f**), heavily grazed stipes of kelp *Lessonia trabeculata* in Punta de Tralca (**Figure 1e**), and solitary stipes of kelp *Lessonia flavicans* in Punta Arenas (**Figure 1g**) (see details in **Table 1**). In general, morphological traits of egg capsules and early stages of development from northern-central Chile evidenced similar patterns, with typical egg capsules ranging from 10 to 40 mm length (**Table 1**). The presence of larger capsules seems to be consistent in the coldest and southernmost site (Punta Arenas; 53°S), although one egg mass recorded in northern Chile (Coquimbo; 29°S) also contained large capsules ($n = 10$ capsules; range: 72–96 mm; **Table 2**). Accordingly, the number of encapsulated embryos and paralarvae size (ML, mm) from Punta Arenas were 3-fold and 1.1-fold larger than the bigger

TABLE 1 | Morphometric traits on egg capsules and early developmental stages (i.e., eggs and paralarvae) of *Doryteuthis gahi* recorded in different spawning sites and substrates along the Chilean Coast from 2014 to 2020 (27° to 53°S; **Figure 1**).

Spawning sites	Collection date	Substrate	No of capsules sampled	Capsule size (mean \pm SD, mm)	Capsule size (range, mm)	No of embryos per capsule (range)	Egg size (mm)	Paralarvae size (ML range, mm)	References
Caldera	20/09/16	Rope mooring	4	25.18 \pm 3.82	20–30	–	–	1.6–2.3*	Present study
Coquimbo	20/05/16–09/03/20	<i>Sarcodiotheca gaudichaudii</i>	59	25.28 \pm 4.75	15–38.5	1–19	1.0–2.0	1.8–2.8	Present study
Valparaíso	18/05/14	Rope mooring	27	32.26 \pm 4.13	23.6–38	7–25	1.9–2.9	2.3–2.8	Carrasco and Pérez-Matus, 2016
Punta de Talca	19/08/14–06/12/14	<i>Lessonia trabeculata</i>	123	22.82 \pm 4.50	10.1–33.6	4–26	2.2–2.6	2.3–2.9	Carrasco and Pérez-Matus, 2016
Matanzas	08/02/16	Rope mooring	28	19.64 \pm 4.46	10–28.5	–	–	2–2.7	Present study
Magellan Strait	10/06/08–20/05/11	<i>Macrocystis pyrifera</i>	7	61 \pm 1.9	61	56	2.5	2.5	Rosenfeld et al., 2014
Punta Arenas	15/10/14	<i>Lessonia flavicans</i>	27	64 \pm 5.97	54–83.8*	34–76*	–	2.2–3.2*	Present study

The symbol (*) indicates that measurements were performed in ethanol-preserved samples.

counterparts from central Chile (Valparaíso; 32°S) (i.e., 76 vs. 25; and 3.2 vs. 2.8 mm, respectively) (**Table 1**).

Characterization of a Spawning Ground Early Life Stages

As in latitudinal observations above, when considering a particular spawning site (i.e., La Herradura bay, Coquimbo), egg capsules were obtained from field and laboratory conditions during different months of the 4-year observation period. Overall, successful collections were recorded in January, March, April, May, June, July, August, October, and December (**Table 2**), with early life history traits (i.e., capsule, eggs, and paralarvae) evidencing distinct patterns depending on SST at the specific collection date. Egg capsules showed a significant positive association with sampling temperatures ($\beta = 6.51$; $t = 11.41$; $p < 0.001$), with larger capsules being recorded during warmer periods of around 18°C experienced during 2020 (range: 19–96 mm; $n = 62$; **Table 2**; and **Figure 2a**), compared with smaller capsules recorded during colder periods of around ~15.5 during years 2016, 2018, and 2019 (range: 15–38 mm; $n = 87$; **Table 2**; and **Figure 2a**). Conversely, egg size evidenced a significant negative association with sampling temperatures ($\beta = -0.10$; $t = -34.20$; $p < 0.001$), with smaller eggs being observed during warmer periods of 2020 (range: 1.33–1.52; $n = 87$), in comparison with egg sizes observed during the colder years 2018 (range: 1.00–2.14 mm; $n = 509$) and 2019 (range: 1.00–2.00 mm; $n = 559$; **Table 2**; and **Figure 2b**). In the other hand, paralarvae size showed a weak but significant negative association with sampling temperatures ($\beta = -0.032$; $t = -3.60$; $p < 0.001$), although small individuals (<2 mm ML) were spread over the 4-year sampling period ($n = 172$; **Table 2**; and **Figure 2c**).

In general, egg capsules were attached to a variety of available substrata, including the typical rope moorings (from 6 to 20 m depth), the red alga *Sarcodiotheca gaudichaudii* in shallow waters up to 10 m, and a mix of unidentified porifera and erect bryozoans in a deep collection of around 30 m (**Table 2**). Egg capsules (range: 15–96 mm) and paralarvae (range: 1.8–2.9 mm ML) presented similar morphological traits compared to previous collections from central Chile (**Table 1**), suggesting that similar adult sizes would be responsible for reproductive interactions and egg laying in these coastal environments.

Juvenile and Adult Stages

Samplings of older ontogenetic stages evidenced the constant presence of juvenile/adult squids through the year. In sum, 76 squids were collected with sizes ranging from around 19 to 77 mm ML and total wet weights from 0.3 to 15 g (**Table 3**). When combining all specimens collected (regardless survival time in laboratory), the power function model evidenced a strong positive relationship between squids' mantle length (ML, mm) and weight (gr) ($TW = 0.0014 \text{ ML}^{2.642}$; $t_{1,44} = 19.256$, $p < 0.001$; **Figure 3a**).

From the forty-six specimens evaluated, 43% corresponded to females ($n = 20$), 33% to males ($n = 15$), and 24% to small juveniles of undetermined sex ($n = 11$). Mantle lengths (mean \pm SD) evidenced significant differences (ANOVA, $F_{2,41} = 8.912$; $p < 0.001$), with males showing slightly larger

TABLE 2 | Morphometric traits of egg capsules and early developmental stages (i.e., eggs and paralarvae) of *Doryteuthis gahi* collected from field and laboratory spawnings between 2016 and 2020 (Coquimbo; 29° 58' S; **Figure 1**).

Collection Dates	Substrate	Sea surface temperature SST (°C)	Egg mass	N° of capsules per clump	Capsule size (mm) (mean ± SD)	Capsule size (mm) (range)	Number of encapsulated embryos (mean ± SD)	Number of encapsulated embryos (range)	Egg size (mm) (mean ± SD)	Egg size (mm) (range)	Paralarvae size (ML, mm) (mean ± SD)	Paralarvae size (ML, mm) (range)
20/05/2016	<i>Sarcodiotheca</i>	15.2	1	12	22.62 ± 5.00	15.00–31.10	12 ± 4.11	1–17	–	–	2.28 ± 0.17	1.92–2.56
25/07/2018	Rope mooring	13.3	2	17	23.74 ± 2.11	19.10–27.10	16 ± 1.81	11–18	1.72 ± 0.22	1.29–2.14	–	–
30/10/2018	Laboratory	14.9	3	6	19.82 ± 2.48	16.50–22.60	17 ± 3.69	11–20	1.83 ± 0.14	1.50–2.00	2.32 ± 0.16	2.10–2.57
30/10/2018	Laboratory	14.9	4	5	39.25 ± 6.84	33.00–47.50	27 ± 10.50	12–36	1.73 ± 0.12	1.50–2.00	2.46 ± 0.12	2.24–2.67
12/12/2018	<i>Sarcodiotheca</i>	16.9	5	8	24.04 ± 4.29	17.20–29.70	17 ± 5.19	6–22	1.28 ± 0.09	1.00–1.43	–	–
29/01/2019	<i>Sarcodiotheca</i>	17.9	6	7	31.46 ± 3.63	27.60–38.50	17 ± 2.06	13–19	1.25 ± 0.09	1.00–1.57	2.29 ± 0.14	1.81–2.52
21/03/2019	<i>Sarcodiotheca</i>	17.3	7	11	27.85 ± 3.98	21.00–34.80	16 ± 1.51	13–17	1.43 ± 0.09	1.29–1.71	2.53 ± 0.14	2.10–2.71
29/08/2019	<i>Sarcodiotheca</i>	13.5	8	9	26.01 ± 1.62	23.10–27.70	14 ± 3.18	6–17	1.68 ± 0.17	1.00–2.00	2.54 ± 0.14	2.14–2.81
29/08/2019	Briozoo/Porifera	13.5	9	12	22.28 ± 2.94	16.50–28.60	12 ± 2.02	7–14	1.66 ± 0.12	1.29–2.00	2.64 ± 0.12	2.38–2.86
03/03/2020	Rope mooring	18.1	10	45	51.94 ± 8.69	33.50–69.10	28 ± 5.29	16–40	–	–	2.46 ± 0.11	2.19–2.57
09/03/2020	Rope mooring	18.1	11	43	83.02 ± 6.87	72.20–96.60	–	–	–	–	2.30 ± 0.36	2.38–2.90
23/04/2020	Laboratory	16.1	12	52	35.96 ± 3.49	32.90–41.60	17 ± 7.92	10–26	1.45 ± 0.04	1.33–1.52	2.33 ± 0.18	2.10–2.52
03/06/2020	Laboratory	14.2	13	5	22.20 ± 4.10	19.30–25.10	11 ± 2.12	9–12	–	–	–	–

Type of substrate and sea surface temperature (SST; °C) for each particular date is also provided.

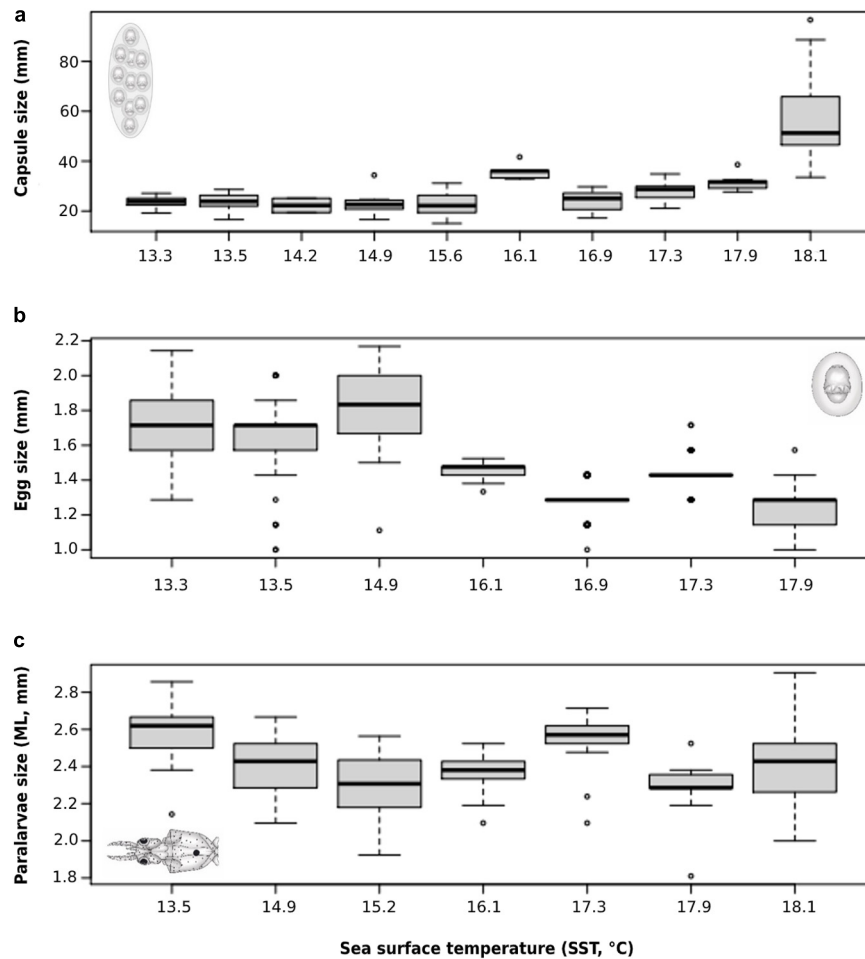


FIGURE 2 | Early life-history traits of *D. gahi* collected at Herradura Bay, Coquimbo, between 2016 and 2020 under different sea surface temperature (SST) conditions, where: **(a)** capsule size (mm), **(b)** egg size (mm), and **(c)** paralarvae size (ML, mm). Central horizontal lines indicate median.

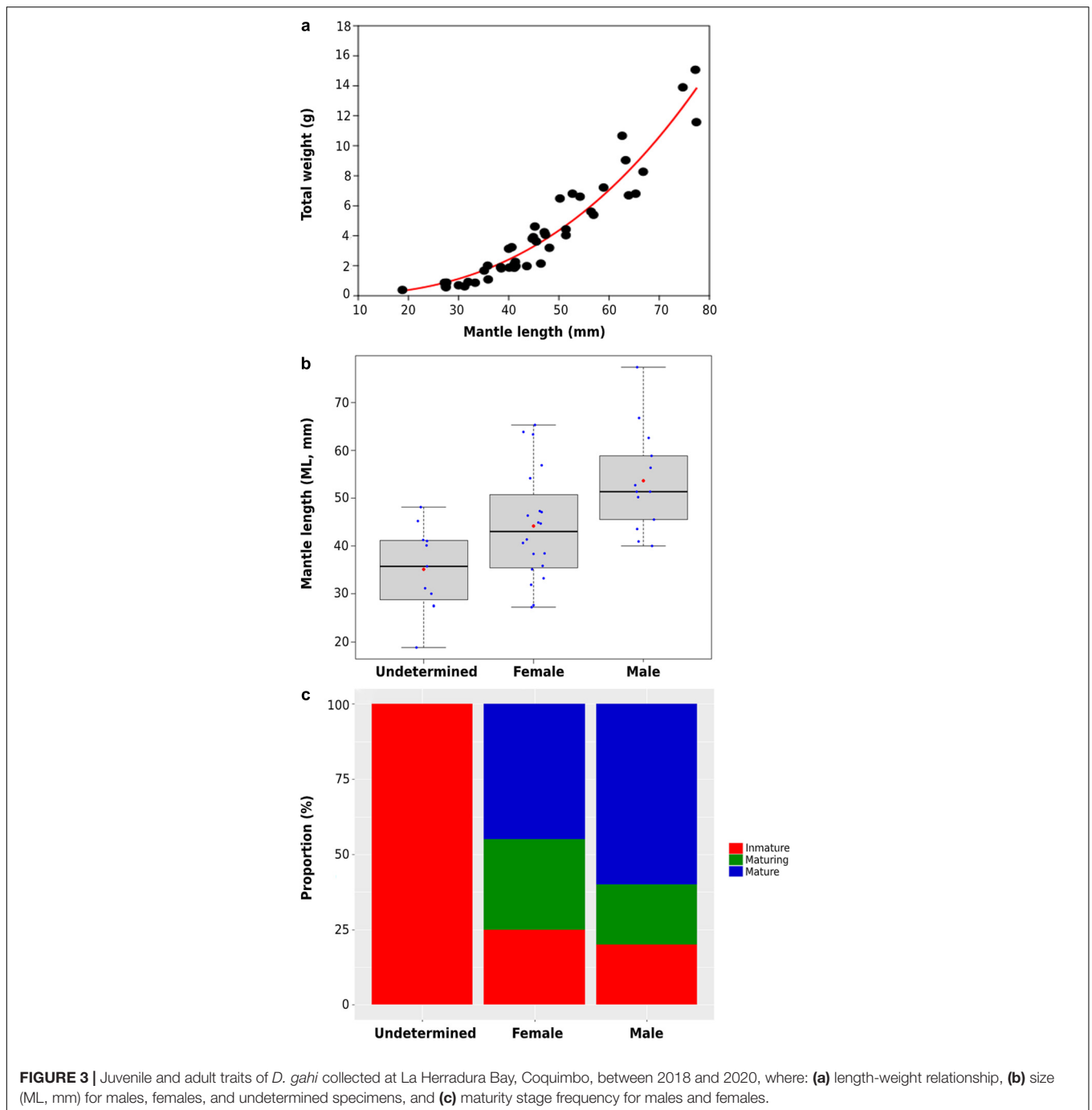
TABLE 3 | Morphometric and gravimetric traits of juvenile and adult *Doryteuthis gahi* collected between 2018 and 2020 at a single spawning site, La Herradura bay, Coquimbo (29° 58' S).

Collection Dates	N	Survival (Range)	Mantle length (DML, mm) (Mean ± SD)	Mantle length (DML, mm) (Range)	Weight (gr) (Mean ± SD)	Weight (gr) (Range)
26-10-2018	9	1–10	47.5 ± 9.2	31.2–63.9	4.8 ± 2.1	0.6–6.8
30-08-2019	3	1–3	57.9 ± 20	35.8–74.7	8.3 ± 5.9	2.0–13.9
21-02-2020	4	2–25	56.7 ± 16.5	41.4–77.2	7.8 ± 6.1	1.9–15.1
01-03-2020	60	6–111	43.1 ± 13.4	18.8–77.4	3.1 ± 2.7	0.4–11.6

sizes (54 ± 11 mm ML) compared to females (44 ± 11 mm ML; Tukey's test: $p = 0.045$) and undetermined specimens (35 ± 9 mm) (Tukey's test: $p < 0.001$) (Figure 3b). Frequency distribution of maturity stages evidenced that a high proportion of squids (i.e., 60% males and 45% females) corresponded to “mature” individuals, with sex evidencing no significant differences (Proportion test, $\chi^2 = 0.288$, $df = 1$, $p = 0.59$; Figure 3c). Similar results were observed between “maturing” (20% males and 30% females; Proportion test,

$\chi^2 = 0.077$, $df = 1$, $p = 0.78$), and “immature” stages (20% males and 25% females; Proportion test, $\chi^2 < 0.001$, $df = 1$, $p = 1$) (Figure 3c).

In captivity, individualized squids from first collections (2018–2019) did not survive longer than 10 days (Table 3); however, two females of different size (45 and 64 mm ML) laid eggs in the tank's walls and in the artificial structures provided. Morphometrics on those egg masses and paralarvae have been provided in Table 2 (see egg masses 3 and 4, respectively).



In the other hand, squids maintained in schools (20–40 and 50–70 mm ML) survived for up to 110 days (Table 3), which to our knowledge correspond to the longest captivity time recorded for *D. gahi* to date. Females of both size classes laid eggs between 2 and 3 months under these controlled conditions, although larger specimens produced more and larger egg capsules (and so more eggs per capsule) compared with smaller squids (see egg masses 12 and 13, respectively; Table 2). Within each of the two size classes, nearly 20% of the mortality was due to cannibalism over smallest sizes, suggesting that

similar behaviors (reproductive and trophic) could be structuring schools in the wild.

Stable Isotopes

Isotopic Composition of Beaks and Soft Tissues

The mean (\pm SD) $\delta^{13}\text{C}$ values evidenced significant differences among the analyzed structures (ANOVA, $F_{2,33} = 4.708$, $p = 0.0159$), with beaks being significantly more ^{13}C -enriched than arms ($-16.1 \pm 1.2\text{‰}$ vs. $-17.6 \pm 1.2\text{‰}$; Tukey's test, $p = 0.019$). Mantle tissue had intermediate values

($-17.4 \pm 1.5\text{‰}$), but did not show significant differences with arms or beaks (Tukey's test, $p > 0.05$ in both cases; **Figure 4a**). The $\delta^{15}\text{N}$ evidenced significant differences in the mean values (\pm SD) of the structures analyzed (ANOVA, $F_{2,33} = 186.9$, $p < 0.001$), with beaks being significantly lower ($12.2 \pm 0.7\text{‰}$) in contrast to arms ($16.9 \pm 0.8\text{‰}$) and mantle ($17.5 \pm 0.6\text{‰}$) (Tukey's test, $p < 0.001$ in both cases; **Figure 4b**). Intra-individual differences of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between lower beak and soft tissues (i.e., mantle, arms) (correction factor) evidenced low differences for mean $\delta^{13}\text{C}$ values ($1.1 \pm 0.6\text{‰}$ vs. $1.4 \pm 0.7\text{‰}$, respectively; **Table 4**) and higher for $\delta^{15}\text{N}$ values ($5.3 \pm 0.5\text{‰}$ vs. $4.7 \pm 0.4\text{‰}$, respectively; **Table 4**).

Ontogenetic Changes on Squids' Isotopic Composition

Pearson's regressions evidenced a positive but weak relationship between squids' mantle length (ML, mm) and both $\delta^{13}\text{C}$ ($y = 0.013x - 18.27$; $r^2 = 0.029$, $df = 12$, $p = 0.55$; **Figure 5a**) and

$\delta^{15}\text{N}$ values ($y = 0.0197x + 16.382$; $r^2 = 0.22$, $df = 12$, $p = 0.09$; **Figure 5b**). These findings suggest that through ontogeny (e.g., 2, 40, and 60 mm ML; see **Figures 5c–e**, respectively), *D. gahi* may present similar carbon sources (i.e., pelagic origin) and only slight increments in their trophic position.

Trophic Position of *D. gahi* Within the Pelagic Community

Nocturnal pelagic species evidenced a wide variation of $\delta^{13}\text{C}$ (7.3‰) and $\delta^{15}\text{N}$ values (6.4‰), with the most ^{13}C -depleted values (mean \pm SD) reported in the blenniid *Hypsoblennius sordidus* ($-21.7 \pm 0.7\text{‰}$) and the common krill *Euphausia mucronata* ($-20.3 \pm 0.7\text{‰}$), whereas the most ^{13}C -enriched values were detected in the polychaete *Pseudonereis gallapagensis* ($-15.5 \pm 3.9\text{‰}$) and the amphipod *Heterophoxus oculatus* ($-14.5 \pm 0.3\text{‰}$) (**Figure 6** and **Table 5**). The $\delta^{15}\text{N}$ values were ^{15}N -depleted in *E. mucronata* ($12.2 \pm 1.3\text{‰}$) and brachyuran zoeae (12.5‰), and ^{15}N -enriched in *P. gallapagensis*

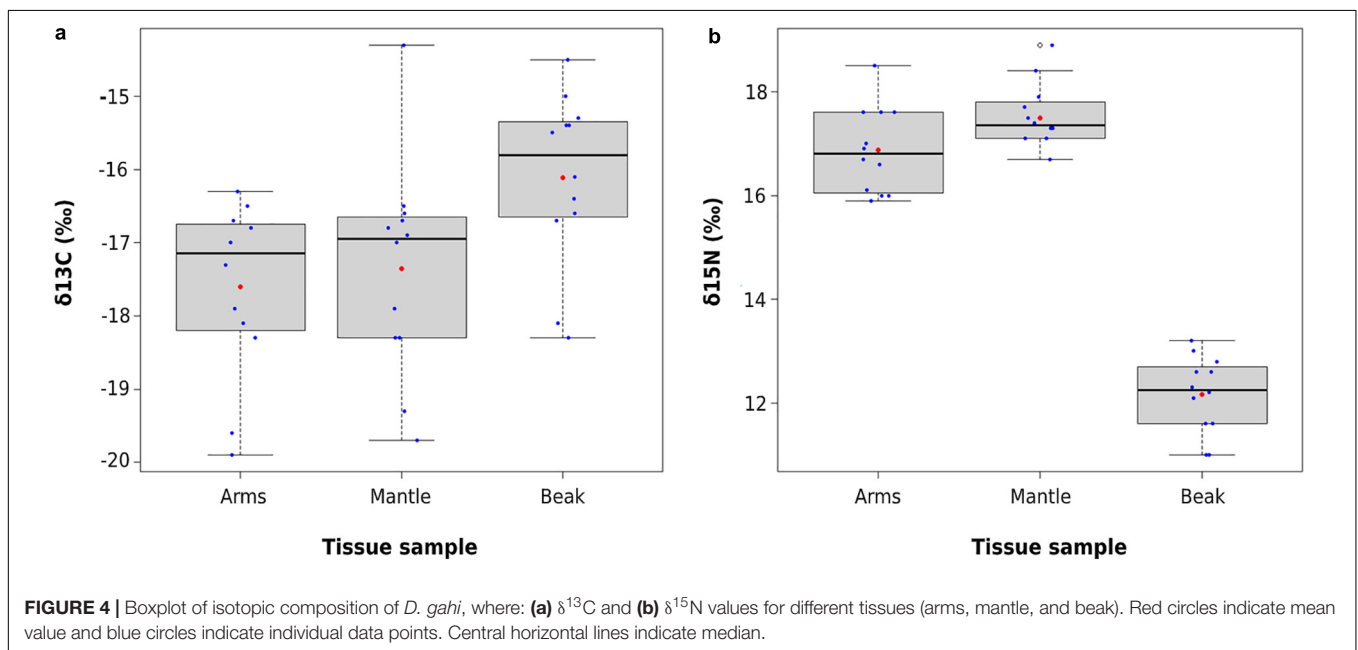


TABLE 4 | Summary of estimated differences of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (correction factor) between hard (beak) and soft tissues (e.g., mantle, arms and buccal mass) from *Doryteuthis gahi* and other cephalopods obtained from the literature.

Species	Structures compared	$\delta^{13}\text{C}$ (‰)		$\delta^{15}\text{N}$ (‰)		References
		Mean	SD	Mean	SD	
<i>Doryteuthis gahi</i>	Arms – lower beak	1.4	0.7	4.7	0.4	This study
<i>Doryteuthis gahi</i>	Mantle – lower beak	1.1	0.6	5.3	0.5	This study
<i>Psychoteuthis gracilis</i>	Arms – lower beak	-0.6	0.3	3.8	0.3	Cherel and Hobson, 2005
<i>Todaropsis eblanae</i>	Mantle – lower beak	0.8	-	4.4	-	Hobson and Cherel, 2006
<i>Illex coindetii</i>	Mantle – lower beak	0.8	-	5.2	-	Hobson and Cherel, 2006
<i>Loligo vulgaris</i>	Mantle – lower beak	0.7	-	6.2	-	Hobson and Cherel, 2006
<i>Sepia officinalis</i>	Mantle – lower beak	0.9	-	4.8	-	Hobson and Cherel, 2006; Golikov et al., 2019
<i>Todarodes filippovae</i>	Mantle – lower beak	-	-	3.5	-	Cherel et al., 2009a
<i>Dosidicus gigas</i>	Buccal mass – lower beak	1.2	0.9	4.0	0.7	Ruiz-Cooley et al., 2006
1 architeuthid + 4 omastrephid (pooled)	Mantle – lower beak	-0.6	0.4	4.2	0.3	Logan and Lutcavage, 2013

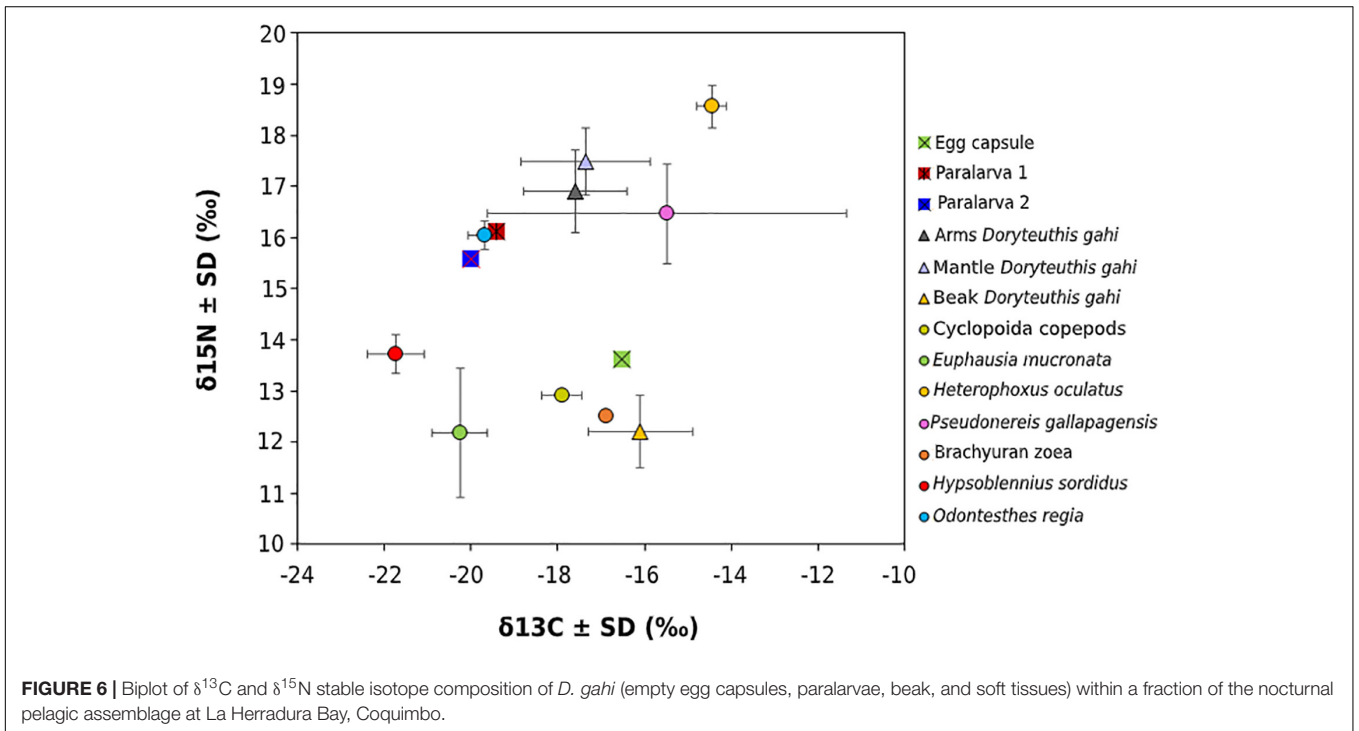
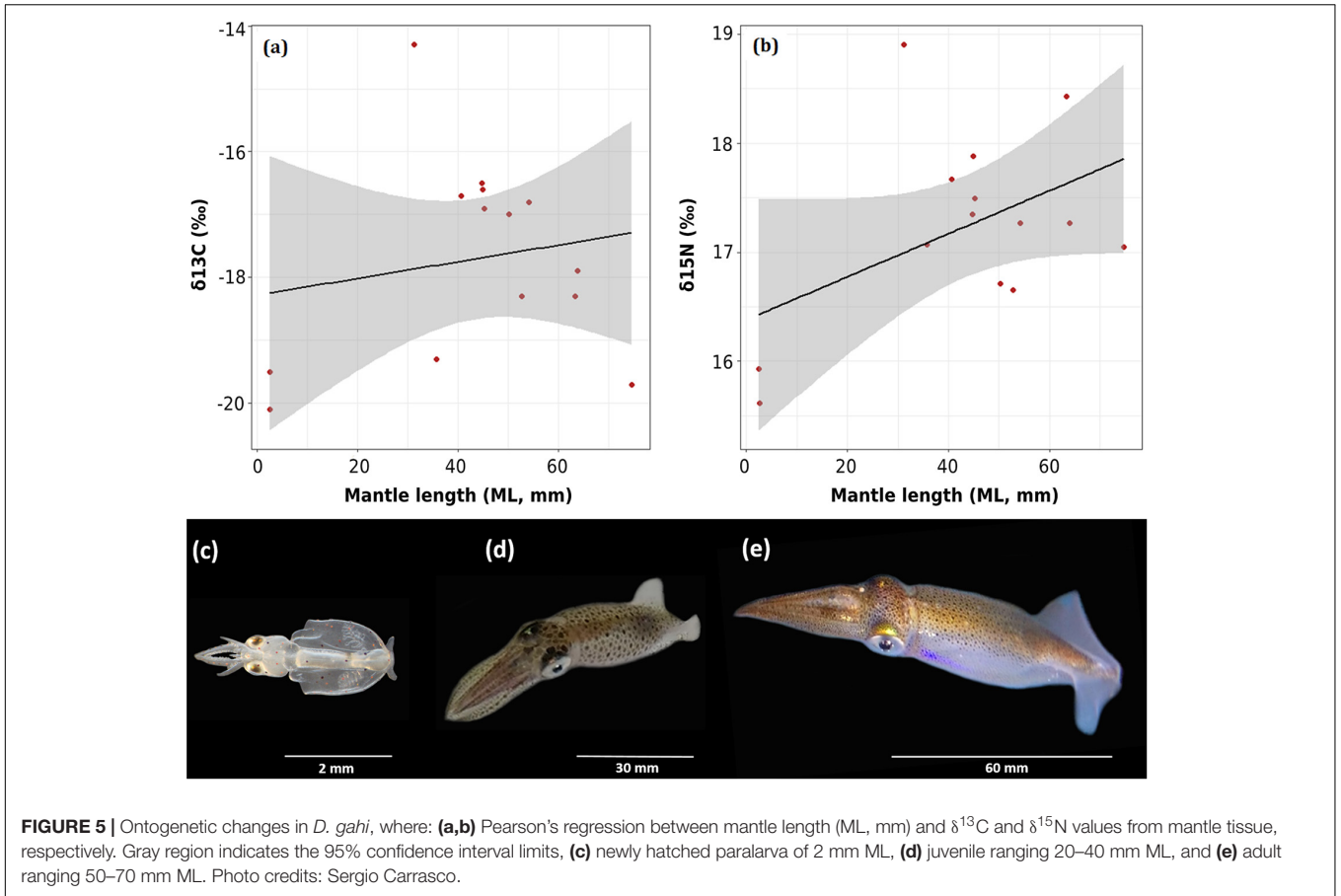


TABLE 5 | Summary of $\delta^{13}\text{C}$, $\delta^{13}\text{C}'$ (lipid-corrected values), $\delta^{15}\text{N}$ isotopes composition, and C:N ratio for the squid *Doryteuthis gahi* [i.e., egg capsules, paralarvae, soft tissues (mantle and arms), and lower beaks] and nocturnal pelagic species.

Species	$\delta^{13}\text{C}$ (‰)	SD	$\delta^{13}\text{C}'$ (‰)	SD	$\delta^{15}\text{N}$ (‰)	SD	n	C:N	SD	TP _{mode}	95% CI
<i>Doryteuthis gahi</i>											
Egg capsule	-18.9	-	-16.5	-	13.6	-	1	5.7	-	-	-
Paralarva 1	-20.6	0.1	-19.4	0.0	16.1	0.1	7	4.6	0.1	-	-
Paralarva 2	-21.3	0.1	-20.0	0.1	15.6	0.0	6	4.6	0.1	-	-
Lower beak	-17.1	1.2	-16.1	1.2	12.2	0.7	12	4.4	0.1	-	-
Mantle	-18.2	1.5	-17.4	1.5	17.5	0.6	12	4.2	0.1	3.6	2.8–4.5
Arms	-18.5	1.7	-17.6	1.2	16.9	0.8	12	4.5	0.3	3.7	2.7–4.7
Putative preys											
Invertebrates											
<i>Euphausia mucronata</i>	-21.3	0.6	-20.3	0.7	12.2	1.3	4	4.4	0.1	2.2	1.1–3.8
Brachyuran zoeae	-19.8	-	-16.9	-	12.5	-	1	6.3	-	-	-
Cyclopoida copepods	-19.0	0.5	-17.9	0.1	12.9	0.1	4	4.5	0.4	2.2*	-
<i>Pseudonereis gallapagensis</i>	-16.6	4.1	-15.5	3.9	16.5	1.0	5	4.5	0.2	3.4	2.6–4.6
<i>Heterophoxus oculatus</i>	-16.4	0.2	-14.5	0.3	18.6	0.4	3	5.3	0.2	4.2	2.6–5.5
Fishes											
<i>Hypsoblennius sordidus</i>	-22.4	0.7	-21.7	0.7	13.7	0.4	5	4.1	0.1	2.7	1.8–3.6
<i>Odontesthes regia</i>	-20.4	0.4	-19.7	0.5	16.0	0.3	5	4.0	0.1	3.3	2.4–4.4

Trophic position (TP) was estimated from *D. gahi* (mantle tissue) and pelagic consumers. The symbol (*) means that baseline consumer and putative trophic position was according to Chen et al. (2018).

($16.5 \pm 1.0\text{‰}$) and *H. oculatus* ($18.6 \pm 0.4\text{‰}$) (Figure 6 and Table 5).

Estimations of trophic position indicated that primary consumers included *E. mucronata* (TP_{mode} = 2.2; 95% credible interval [CI] = 1.1–3.8), and *H. sordidus* (TP_{mode} = 2.7; 95% CI = 1.8–3.6; Table 5), followed by secondary consumers that included *O. regia* juveniles (TP_{mode} = 3.3; 95% CI = 2.4–4.4), *P. gallapagensis* (TP_{mode} = 3.4; 95% CI = 2.6–4.6) and *D. gahi* (mantle: TP_{mode} = 3.6; 95% CI = 2.8–4.5, arms: TP_{mode} = 3.7; 95% CI = 2.7–4.7; Table 5), and tertiary consumers such as *H. oculatus* (TP_{mode} = 4.2; 95% CI = 2.6–5.5; Table 5).

Bayesian Mixing Models Outputs

The Bayesian mixing models indicated that among the putative preys included in this study, Cyclopoida copepods could be the most important prey item assimilated in the diet of *D. gahi*, with a median estimate of 37.9% in the mantle (95% CI = 5.5–62.4; Figure 7a; Table 6), and 42.5% in the arm tissue (95% CI = 5.8–73.3%; Figure 7b; Table 6). The euphausiid *E. mucronata* was also relatively important in the mantle (median = 16.8%; 95% CI = 1.0–46.3) and arm tissue (median = 22.2; 95% CI = 1.7–53). For both tissues (mantle and arms), the contribution of pelagic fishes was in general low, with *O. regia* showing a 7.9% and 5.4%, respectively (Table 6), followed by *H. sordidus* with 6.7 and 5.0%, respectively (Table 6).

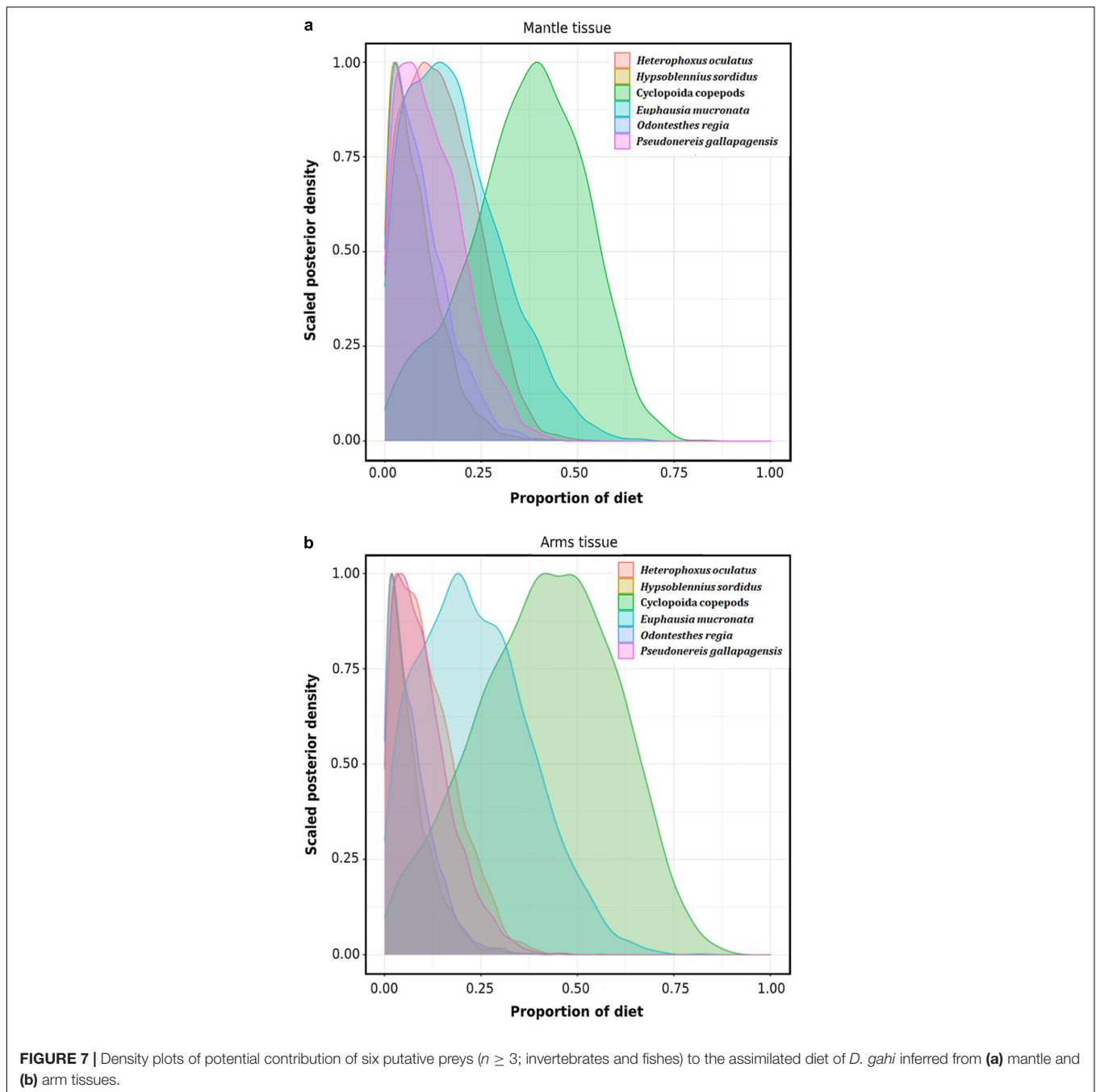
DISCUSSION

Latitudinal Traits on Spawning Habitats

Observations carried out during the preset surveys allowed identifying the use of different areas along the Chilean coast by females *D. gahi* to spawn their egg-capsules, suggesting that

plasticity in the substrate selected for egg-capsule deposition may play an important role allowing the occurrence of these discrete spawning areas in sites with contrasting structural properties (e.g., erected kelp forests or cold-water corals, branched bottom algae, anthropogenically disturbed sites). Specific traits of egg-capsules associated to different spawning habitats will still require larger datasets at different scales (and specially in northern and southern Chile) to fully understand latitudinal and/or selection patterns influencing females' reproductive outputs.

Nonetheless, latitudinal patterns of egg-laying were similar to those previously described in central Chile (Carrasco and Pérez-Matus, 2016), corresponding to small egg-capsules (10–50 mm length) deposited at depths between 6 and 30 m. Larger capsules (up to 90 mm length) were evidenced only in one occasion during the 4-year observation period (March 2020; see Table 2), being considered uncommon. Unlike this pattern, records from the northernmost geographic distribution of the species (Peru) have evidenced the presence of at least 2-fold larger egg capsules attached on coarse sand (up to 169 and 220 mm length; Cardoso et al., 2005 and Argüelles et al., 2008a, respectively), suggesting that larger females (i.e., 100–400 mm ML; Villegas, 2001) could be responsible for spawning these type of egg-capsules. Similarly, at the southernmost distribution sites, larger egg-capsules have also been consistently recorded in the Magellanic channels and Fjords, Chile (up to 60 and 90 mm length; Rosenfeld et al., 2014 and present study, respectively), the Argentinean Patagonia (based on the number of embryos per capsule; average 69, and up to 154; Barón, 2001), and the Falkland/Malvinas Islands (up to 86 mm length; Arkhipkin et al., 2000). As in northern sites, larger egg capsules could therefore be attributable to larger (and so high fecundity) mature females (80–300 mm ML) (Laptikhovskiy et al., 2002).



Although information on changes in life history traits owing latitudinal (and so temperature) differences have been described for several species through the HCS (e.g., Fernández et al., 2007; Rivadeneira et al., 2010; Haye et al., 2019), large-scale variation in life-history traits of coastal cephalopods along the SE Pacific coast has not been previously evaluated. In this context, *D. gahi* has been described as the only loliginid squid adapted to reproduce in cold-water environments (ca. 6–9°C; see Arkhipkin et al., 2000; Arkhipkin and Middleton, 2003); however, current observations suggest that higher temperatures experienced in the northernmost distribution limit (i.e., Northern Peru;

17–24°C)² might not be generating marked differences in some life-history traits (e.g., adult size, egg-capsule size, number of encapsulated embryos) when compared with specimens from the Falklands/Malvinas islands, which correspond to a different genetic population unit (McKeown et al., 2019).

Evaluating inshore spawning aggregations from a latitudinal perspective has filled important gaps in the life-history of *D. gahi* along SE Pacific waters, agreeing with our assumptions and suggesting that groups inhabiting the northern and southern

²<https://www.bio-oracle.org/>

TABLE 6 | Statistical summary of concentration dependence Bayesian mixing models (median and 95% credibility intervals) for contribution of five putative preys to the assimilated diet of *Doryteuthis gahi* inferred from soft tissues (mantle and arms).

Putative preys	Mixing models outputs			
	Mantle		Arms	
	Median	95% CI	Median	95% CI
Invertebrates				
Cyclopoida copepods	37.9	5.5–62.4	42.5	5.8–73.3
<i>Euphausia mucronata</i>	16.8	1.0–46.3	22.2	1.7–53
<i>Pseudonereis gallapagensis</i>	11.1	0.6–32	8.7	0.4–28.2
<i>Heterophoxus oculatus</i>	13.9	0.7–33.8	9.5	0.5–2.9
Fishes				
<i>Hypsoblennius sordidus</i>	6.7	0.3–25.2	5	0.2–22
<i>Odontesthes regia</i>	7.9	0.4–27	5.4	0.2–21.6

distribution ends are characterized by larger adult sizes that are able to lay larger egg capsules with more encapsulated embryos, fitting the “abundant edge distribution” pattern described for several marine invertebrate species (Sagarin and Gaines, 2002). These findings are also consistent with the higher abundances and larger sizes necessary to maintain the important fishery developed in both geographic regions since early 80’s (Arkhipkin et al., 2015b). Potential mechanisms driving the observed differences in life-history traits of *D. gahi* between both extremes and the center of the distribution (e.g., variable plastic responses, inadequate levels of successful reproduction, offspring survival; see Sagarin et al., 2006; Gaston, 2009), as well as the persistence of these traits along the HCS, is a work in progress.

Characterization of a Spawning Ground

The permanent evaluation of a single spawning site evidenced similar patterns as in latitudinal observations described above, with the typical small capsules (developmental stages 16–17; Guerra et al., 2001) being selectively attached to a variety of natural and anthropogenic substrates (i.e., rope mooring, red algae, bryozoans) devoid of structures that could produce mechanical disturbance to the gelatinous egg masses (see Carrasco and Pérez-Matus, 2016). Similar to what has been described for *D. gahi* in some areas around the Falkland/Malvinas islands (Arkhipkin et al., 2000), the occurrence of egg masses in the present study site was patchy and variable through the sampling period, with several negative dives (not reported in this manuscript) evidencing the sporadic nature of these findings. An increase of underwater sampling hours would probably help improving the reduced data set.

The presence of two egg masses containing more capsules than usual (43 and 45) is not rare in field collections, as up to 57 capsules per clump have been previously recorded (Carrasco and Pérez-Matus, 2016). However, the large capsule sizes observed in those egg masses (up to 69 and 96 mm length, respectively) is considered uncommon for these latitudes. Although there are no other field observations to compare these findings, two possible scenarios could be expected: first, one or multiple large-sized females (>70 mm ML) would be responsible for egg capsule deposition, and second, the small reproductive females commonly observed in the wild

(~50 mm ML) would be able to invest, at least under some circumstances, a higher reproductive effort in generating these larger capsules deposited communally. Despite evident differences in capsule size, eggs and paralarvae morphometrics seems not to differ from previous collections. In fact, after embryonic development occurred in the laboratory (from 10 to 28 days), newly hatched paralarvae evidenced similar sizes (1.8–2.9 mm ML) compared with those recorded in Peru (1.9–2.8 mm ML; Cardoso et al., 2005), central Chile (2.3–2.9 mm ML; Carrasco and Pérez-Matus, 2016), and Magellanic channels (2.5 mm ML; Rosenfeld et al., 2014), but smaller than those from the Falkland/Malvinas islands (3.1–3.4 mm ML; Arkhipkin et al., 2000).

Although the variable nature of field collections may limit the ecological interpretation of these local spawning events (29°S), it is interesting to point out that larger capsules were recorded in warmer conditions experienced during 2020 (SST: ~18°C), contrasting with the generally lower temperatures during previous sampling years (2016, 2018, and 2019; SST: ~15.5°C). Considering that larger egg capsules tend to have more encapsulated embryos (Arkhipkin et al., 2000; Carrasco and Pérez-Matus, 2016), and eggs seems to be larger in colder conditions (Arkhipkin et al., 2000; Laptikhovskiy, 2006; present study), it would be plausible to expect that the relationship capsule size/embryo size is operating similarly in different sections of the distribution range of the species regardless obvious differences in temperature between both locations (i.e., Falkland/Malvinas islands and northern-central Chile, respectively). In this context, and as described for several cephalopod species (Boyle and Rodhouse, 2005; Pierce et al., 2008; Pimentel et al., 2012; Rosa et al., 2012) including *D. gahi* (Cinti et al., 2004; Cardoso et al., 2005), the temperature experienced during these early developmental stages would directly influence different paralarval traits, including hatchling size and performance. In fact, previous laboratory manipulation of thermal conditions during the early development of *D. gahi* (i.e., 9, 12, and 16°C) agree with those observations, evidencing that embryos incubated at lower temperatures had longer developmental times, larger hatching sizes, and longer survival times than siblings incubated at higher temperatures (Carrasco et al., 2016).

Therefore, it would be possible to hypothesize that paralarvae *D. gahi* should be present in the coastal habitats of northern-central Chile through the year, experiencing and surviving different temperature conditions ranging from 9 to 17°C, corresponding to common mean daily SST recorded at these latitudes (see Wieters, 2005; Aravena et al., 2014). However, other than the present results, there is no field information to validate this assumption, evidencing that the entire life-cycle of the species (from their encapsulated embryos to adult reproductive stages) has been completely overlooked as a component of coastal pelagic communities along the SE Pacific.

The possibility to collect and maintain juvenile and adult stages of *D. gahi* in controlled laboratory conditions for up to 110 days, allowed demonstrating that mature stages can be found at small sizes of around 40 mm ML in both sexes, being corroborated by the presence of well-developed spermatophores and eggs in males and females, respectively. Accordingly, laboratory spawns allowed confirming that females as small as 45 mm ML are fully mature and fertilized, depositing egg capsules that developed normally until hatching and that resemble the small-sized capsules typically described in northern-central Chile (Carrasco and Pérez-Matus, 2016). Considering that the minimum size recorded for mature females is about 90 mm ML in Concepción, southern-central Chile (Ibáñez et al., 2005) and 130 mm ML in Falkland/Malvinas islands (Arkhipkin et al., 2013), the present study describes the smallest egg-laying female *D. gahi* (45 mm ML) recorded through the complete distributional range of the species. Since a length-weight power function on adult specimens revealed a coefficient <3, these findings also support the dwarf morphotype hypothesis, suggesting that a small reproductive morphotype is consistently occurring along the HCS between the two genetically different centers of abundance, the Chilean – Falklands/Malvinas and the Peruvian groups (Ibáñez and Poulin, 2014; McKeown et al., 2019). Similar morphological changes owing environmental variability have also been described in males *D. gahi* from the Patagonian shelf population (Jones et al., 2019) and also in the jumbo squid *D. gigas* from Peruvian waters (Argüelles et al., 2008b; Keyl et al., 2011; Arkhipkin et al., 2015a), enhancing the role of cephalopod plasticity when facing variable environmental conditions. Ongoing work on this Humboldt morphotype of *D. gahi* will complement population genetic studies as well as behavioral components associated to reproductive and trophic interactions in captive conditions.

Trophic Ecology of *D. gahi* in Northern-Central Chile

There was no evidence of a marked relationship between ML and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, suggesting that carbon sources and trophic position, respectively, may not change significantly through the size range analyzed (2–70 mm ML). In fact, $\delta^{15}\text{N}$ values from hatched paralarvae were slightly ^{15}N -depleted than arms and mantle tissue from larger squids, reflecting a relatively constant diet through the ontogenetic stages evaluated. Similar to these findings, recent research

exploring trophic allometries in cephalopods have also suggested that there are some species or Families (i.e., Cranchiidae) that do not exhibit ontogenetic trophic position shifts across different size classes, differing from the general description of cephalopod as voracious predators. These low activity level cephalopods were characterized by having low trophic positions, feeding largely upon herbivorous zooplankton and do not shifting to higher levels of piscivory until larger sizes (see Murphy et al., 2020).

Accordingly, previous studies have suggested similar changes in the diet of *D. gahi*, shifting from crustaceans in small-sized individuals (5.1–15 cm ML) to fish in larger sizes (15.1–25 cm ML) (Rosas-Luis et al., 2014). Changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values through the gladius sections have also supported dietary observations, suggesting important trophic changes through ontogeny (Rosas-Luis et al., 2017). Therefore, our findings are consistent with both observations, demonstrating a common source of food and trophic position in small and large-sized squids (via stable isotope analyses), and directly corroborating in laboratory that larger specimens *D. gahi* are able to prey upon different fish species, as well as cannibalize other squids, if predator and prey sizes are appropriate (i.e., ~50% of squid's ML; SA. Carrasco, unpublished data).

The trophic position (TP) estimated here for *D. gahi* (TP = 3.6 to 3.7) corroborate the trophic role of the species as a zooplanktivore (secondary consumer) in pelagic food webs. When contrasting TP values with other pelagic species occurring in the Humboldt Current system (all through C and N stable isotopes), these values were comparable to other pelagic species such as the mackerel *Trachurus murphyi* (TP = 3.8; Pizarro et al., 2019), the anchovy *Engraulis ringens* (TP = 3.7), and the jumbo squid *Dosidicus gigas* (TP = 3.9) (Espinoza et al., 2017). These estimations were comparable among studies given the use of the same trophic discrimination factor (TDF: $\pm 3.4\%$; Post, 2002), except the trophic position of copepods (2.2) that was slightly lower than the value 2.5 used by Espinoza et al. (2017). In the other hand, TP estimated for *D. gahi* through different sections of the gladius evidenced higher values for juveniles (TP = 4.2) and adult stages (TP = 4.3) (Rosas-Luis et al., 2017); however, these values matched the error measurements from the TP models of mantle and arms.

Despite stomach content in the six specimens analyzed here only evidenced a liquefied content where no further taxonomic identifications of the dietary items were possible, the importance of planktonic crustaceans in the diet of *D. gahi* has been confirmed through stomach content in other areas of the distribution range, including Peru (Cardoso et al., 1998), Falkland/Malvinas Islands (Guerra et al., 1991), and South West Atlantic (Arkhipkin et al., 2013; Rosas-Luis et al., 2014). Bayesian mixing models were consistent with those observations and agreed with preliminary expectations, suggesting that copepods and euphausiids would be the most important putative preys, followed in a minor proportion by amphipods and polychaetes. In this context, the Coquimbo Bay system is located within an important upwelling center

(Moraga et al., 2001; Thiel et al., 2007), with copepods dominating the zooplankton community structure, while euphausiids are frequently observed in lower abundances (Mattos and Mujica, 2012; Torreblanca et al., 2016) as response to extensive diel vertical migration into the oxygen minimum zone (Thiel et al., 2007). The presence of amphipods and swimming nereid polychaetes in nocturnal pelagic assemblages is not rare, and has been associated to the incidence of artificial lights in shallow depths (<30 m) in the former (Carrasco et al., 2017), or to the presence of reproductive stages (i.e., epitokes) attracted by lunar phases in the latter (Fong, 1993). Consistently with the present records, the presence of the polychaete *P. gallapagensis* has been reported in stomach content of individuals *D. gahi* from Peru (Cardoso et al., 1998) as well as in other members of the Family Loliginidae (Rocha et al., 1994), whereas amphipods have been recorded in the Atlantic Ocean (Arkhipkin et al., 2013). Therefore, the potential contribution of polychaetes and amphipods in the diet of *D. gahi* may provide temporal trophic subsidize to juvenile and adult squids in these coastal waters via emergent macrobenthic fauna (see Carrasco et al., 2017).

In the other hand, the trophic role of cephalopods as preys has been better understood given the presence of chitinous beaks within the stomach contents of upper consumers, including fishes, birds, mammals and other squids (reviewed in Arkhipkin et al., 2013). Differences in stable isotope composition between beaks and soft tissues (mantle and arms) observed in the present study show the same pattern reported in other cephalopods, with beaks tending to be slightly ^{13}C -enriched and significantly ^{15}N -depleted compared with soft tissues (Cherel and Hobson, 2005; Hobson and Cherel, 2006). The lower $\delta^{15}\text{N}$ values in beaks compared to soft tissues could be explained by the presence of ^{15}N -depleted chitin molecules (Cherel et al., 2009a). Therefore, differences in the present values for *D. gahi* could be useful for obtaining corrections factors for beaks found in different predators' stomach contents, improving our understanding on the trophic role of the species along the HCS.

Overall, the present study unveils the permanent occurrence of a dwarf reproductive morphotype of *D. gahi* in shallow coastal habitats along the Humboldt Current System, providing the first insights for understanding potential adaptations to heterogeneous conditions in different sections of the distribution range (e.g., temperature, food availability), but also through the life cycle of the species (e.g., egg-capsules, paralarvae, adults). The combination of direct underwater surveys, isotopic composition, and behavioral components of alive organisms in laboratory, provided a strong approach for teasing out some of the ecological mechanisms underlying spatial connectivity patterns and the species' role in a crucial (but unexplored) section of its geographic range, which connect the two genetically distinct centers of abundance, the Chilean – Falklands/Malvinas and the

Peruvian. The incorporation of pelagic/benthic nutrients from small invertebrates (e.g., copepods, amphipods, euphausiids, and nereid polychaetes) and its transmission to upper trophic levels (fish, mammals, seabirds, and humans), enhance the role of *D. gahi* as an important nutritional link in these coastal environments and potentially through the complete distributional range.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article are available from the corresponding author (SC), upon reasonable request.

ETHICS STATEMENT

The use of animals during this study was reviewed and approved by the Comité Ético Científico de la Facultad de Medicina de la Universidad Católica del Norte, Coquimbo, Chile (CECFAMED – UCN N° 18/2020).

AUTHOR CONTRIBUTIONS

SC conceived the idea, designed the study, and led the writing of the manuscript. SC and GZ-H performed samplings. GZ-H, SC, and CI analyzed the data. MB reviewed samples, generated databases, and assisted in specimens' maintenance in laboratory. All authors have read and commented on the manuscript.

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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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