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Sexual dimorphism in feeding apparatus and niche partitioning in juvenile jumbo squid *Dosidicus gigas*

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ABSTRACT: In a wide range of sexually reproducing marine organisms, males and females coexist through niche separation to reduce intraspecific competition and maximize survival; however, sexual segregation in mesopelagic cephalopods is poorly understood. To evaluate ontogenetic differences in diet and habitat partitioning between juvenile female and male jumbo squid *Dosidicus gigas*, we determined age and maturity, quantified stable isotope ratios along gladii bulk tissue and individual amino acids (AAs), and analyzed morphometrics of feeding apparatuses. *D. gigas* of 24 to 33 cm gladius length (GL) were collected in the northern Humboldt Current. Results showed that females had larger feeding apparatuses than males and higher $\delta^{15}N$ values in bulk tissue and trophic AAs, but relatively similar source-AA $\delta^{15}N$ values at same GLs. These results indicate that females catch prey of higher trophic position at earlier maturing stages than males, but tend to share similar habitat. This behavior likely reduces intraspecific competition, promotes ontogeny of sexual niche partitioning, and maximizes energy intake. Our study reveals that sexual segregation in feeding habits occurs in juvenile *D. gigas* during ontogenesis in the northern Humboldt Current, a strategy that can help elucidate its complex population dynamics and adaptation to fluctuating environmental conditions.

KEY WORDS: *Dosidicus gigas* · Stable isotope analysis · Compound-specific isotope analysis · Amino acid · Niche partitioning · Energy allocation

INTRODUCTION

Species that reproduce sexually have developed a wide range of strategies to maximize survival, feeding and reproductive success (Conradt 2005, Ruckstuhl & Clutton-Brock 2005). In many group-living species, females and males coexist by using habitat differently or performing different social behaviors, thereby reducing intraspecific competition and favoring niche separation (Dayan & Simberloff 2005, Bearhop et al. 2006). This strategy is often associated with sexual variability in size, social behavior, and nutrient requirements (Ruckstuhl & Neuhaus 2002). Investigating the mechanisms driving sexual niche partitioning is vital for understanding population dynamics, specifically, how male and female foraging strategies change in response to environmental variability (Quillfeldt et al. 2008, Wearmouth & Sims 2008). There is a burgeoning literature documenting sexual niche partitioning in marine organisms; however, as most studies have focused on vertebrates, the potential sexual foraging segregation has not

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been studied extensively in marine invertebrates. This is especially true for pelagic cephalopods, which are an important component in marine ecosystems (Clarke 1996, Ménard et al. 2013), and have economic importance for neritic and oceanic fisheries around the world (Hunsicker et al. 2010).

Evidence suggests that sexual foraging segregation exists in some squid species, for example, female and male Patagonian squid *Loligo Gahi* forage at different depths (Arkhipkin & Middleton 2002), and sex-specific predation behaviors were observed in Japanese pygmy squid *Idiosepius paradoxus* (Takeshita & Sato 2016). However, little being known about sex-specific foraging strategies for pelagic cephalopods at any stage of ontogenesis, data are limited to a few squid species of commercial importance (Arkhipkin & Middleton 2002, Kato et al. 2016). In addition, different nutrient requirements, one of the mechanisms underlying sexual segregation (Wearmouth & Sims 2008), have been recently reported for cephalopods such as arrow squid *Nototodarus gouldi* (McGrath Steer & Jackson 2004), southern calamari *Sepioteuthis australis* (Pecl & Moltschaniwskyj 2006) and Argentine shortfin squid *Illex argentines* (Lin et al. 2015). In these 3 species, females and males showed divergent nutrient requirements for growth or reproduction.

Jumbo squid *Dosidicus gigas* supports important commercial fisheries off the coasts of western South and Central America and the Gulf of California, with the commercial annual catch exceeding 1 million tons in 2014 (FAO 2016). This squid species is a voracious predator, highly abundant, and endemic to the eastern Pacific Ocean (Nigmatullin et al. 2001). Coincident with the strong 1997–1998 El Niño event, *D. gigas* expanded its distribution range limits from 30° to 50° N, and from 40° to 46° S (Zeidberg & Robison 2007, Keyl et al. 2008). During this range expansion, *D. gigas* consumed a wide range of prey items, including species of ecological and commercial importance, such as Pacific hake *Merluccius productus* and market squid *Doryteuthis opalescens* (Field et al. 2007, Ibáñez & Cubillos 2007). In the eastern Pacific, a disproportionate number of female *D. gigas* compared to males (~2.5:1) was consistently found in catch data from 1990 to 2008, even in the same body size range (Ibáñez & Cubillos 2007, Tafur et al. 2010). A better understanding of sex-specific strategies, e.g. spatial sexual segregation of feeding areas or trophic niche partitioning between coexisting individuals, in this squid species can be useful for implementing regulations and determining whether fisheries require sex-based harvest strategies to reduce skewed mortality, especially given the rapid rise in squid landings.

Differentiating potential foraging strategies between female and male *D. gigas* is often difficult in empirical studies. Stomach content analysis has a long history as a tool for investigating squid feeding habits. It is effective for identifying prey items and quantifying diet composition (Markaida & Sosa-Nishizaki 2003, Field et al. 2007, 2013). However, it can also be biased due to squid feeding behavior, in which fish heads and vertebrae of larger prey are eliminated because these items cannot pass through the esophagus. This behavior affects the reliable estimation of the relative frequency and size of larger prey (Hanlon & Messenger 1996, Dawe et al. 1997). Also, stomach contents usually reflect recent diet over a relatively short time period and underestimate prey items that are more easily digested (Wallace et al. 1981). In addition, *D. gigas* exhibits both horizontal and vertical movements between depths of hypoxic oxygen minimum layer and surface waters and forages in both shallow shelf and deeper waters (Bazzino et al. 2010); thus sexual niche partitioning may occur in a 3-dimensional space, which is difficult to observe and to sample.

Carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$, respectively) are efficient tracers for elucidating resource partitioning at intra- and inter-specific levels (Bearhop et al. 2006), and can be a key approach for understanding foraging strategies between sexes of mesopelagic cephalopods. $\delta^{13}C$ is often used to reveal carbon sources at the base of the food web, allowing identification of foraging locations because $\delta^{13}C$ changes little (0–1‰) during trophic transfers (Post 2002). $\delta^{15}N$ shows a stepwise enrichment in consumer tissues (2.5–3.4‰) in a given food web, providing a method to estimate trophic position (TP). However, differences in oceanographic and biogeochemical processes affect $\delta^{15}N$ baseline values, which are propagated up into a squid's tissues through the diet (Ruiz-Cooley et al. 2010, 2013, Lorrain et al. 2011). Amino acid (AA) compound-specific nitrogen isotopic analysis (CSIA-AA) has been incorporated in ecological studies to estimate TP using tissue samples from the consumer of interest and without the need to collect samples near the base of the food web (McClelland & Montoya 2002, Chikaraishi et al. 2009, 2014). This is possible because the $\delta^{15}N$ values of source-AAs exhibit little isotopic fractionation and are proxies of primary producer isotope values, whereas trophic-AAs exhibit large isotopic fractionation depending upon the animal diet and nutritional requirements (Chikaraishi et al. 2009, O'Connell 2017). Integrating isotope data from both bulk tissue and individual AAs can unravel complex dynamics of species inhabiting remote habitats or that are highly migratory, identify ontogenetic changes in diet and habitat, and estimate TP (Choy et al. 2012, Seminoff et al. 2012, Ruiz-Cooley et al. 2013).

The squid gladius is a continuously growing, metabolically inert tissue that records dietary information in the feeding areas used by individuals. Compared to stomach content analysis mentioned above (Markaida & Sosa-Nishizaki 2003, Field et al. 2007, 2013), stable isotope analysis (SIA) along squid gladii can reflect ontogenetic patterns associated with changes in diet and habitat during tissue synthesis (Ruiz-Cooley et al. 2010, 2013). In the northern Pacific and waters off Peru, medium to large sized (~20–80 cm) *D. gigas* exhibited different ontogenetic δ^{13}C and δ^{15}N patterns along gladii associated with available prey items and squid dynamics in a given region (Ruiz-Cooley et al. 2010, 2013, Lorrain et al. 2011), but differences between males and females have not been investigated. Using growth information obtained from the squid statolith, Li et al. (2017) reported that time-based consecutive sampling of the gladius can back-calculate events over the lifetime of *D. gigas* off Peru. Hence, sequential δ^{13}C and δ^{15}N values along a gladius can be used to identify the time at which sexual segregation began in a squid's life, and together with other biological data, identify the factors driving niche partitioning.

Morphometric characteristics of squid feeding apparatuses (such as arms, tentacles, and beaks) can be valuable for investigating foraging behavior because they are directly associated with the size and type of ingested prey. Squid use arms and tentacles to seize and restrain their prey, and longer arms and tentacles may increase the potential to capture large prey (including larger prey sizes) from greater distances (Hanlon & Messenger 1996), perhaps in relation to increasing swimming speed with prey size (Ware 1978). The squid beak is one of the main feeding apparatuses inside the mouth that relates to its biting force (Kear 1994) and represents a morphologic indicator for explaining resource use (Franco-Santos & Vidal 2014). Moreover, squid energy allocation has evolved to meet the concurrent energy demands associated with somatic growth and reproduction, due to their rapid growth, short life, and monocyclic reproductive pattern (Nigmatullin et al. 2001, Moltschaniwskyj & Carter 2013). Both somatic growth and gonad development can have major demands for nutrient requirements (Peig & Green 2009), and consequently, ontogenetic shifts in energy allocation occur over different squid life stages (McGrath Steer & Jackson 2004, Lin et al. 2015).

The overarching goal of this study was to determine whether there is evidence of sexual foraging segregation as a potential means to partition resources and reduce intraspecific competition in a given large marine ecosystem. We use both SIA along gladii and morphometric analyses to evaluate the potential for sexual foraging segregation in *D. gigas* as a function of gladius length and age. Specifically, we hypothesize that *D. gigas* from the northern Humboldt Current ecosystem exhibits sex-specific foraging strategies, potentially driven by sexual size dimorphism in feeding apparatuses and/or different energetic demands that would maximize feeding success.

MATERIALS AND METHODS

Squid sampling and processing

Fieldwork was conducted on commercial jigging vessels operating during 2013, 2014 and 2015 off the Peruvian exclusive economic zone (EEZ), which is part of the northern Humboldt Current ecosystem (10°–16° S, Fig. 1). A total of 1277 squid were collected fresh and stored frozen (−20°C) on board, then defrosted in the laboratory. Dorsal mantle length (ML) and body weight (BW) were measured to the nearest 1 mm and 1 g, respectively. All individuals were dissected and the sex and maturity stage (stages I and II, immature; stages III, maturing) were determined according to Lipiński & Underhill (1995). Wet weight of gonads and soma were recorded to the nearest 1 g. The gladius and beak were removed and washed using distilled water for 5 min in an ultrasonic cleaner. Gladii or beaks which appeared to be damaged were eliminated. The statolith was extracted for age determination following protocols from Arkhipkin et al. (2014). All values presented are mean ± SD.

Morphometric analysis

The gladius length (GL) was measured for each specimen. The frequency distributions of GL for individual maturity stages were analyzed to identify possible periods of reproductive development. The frequency was calculated by each interval size of 3 cm GL, from 18 to 39 cm (n = 351, Table S1 in the Supplement at http://www.int-res.com/articles/suppl/m607p099_supp.pdf). Individuals with intact feeding ap-

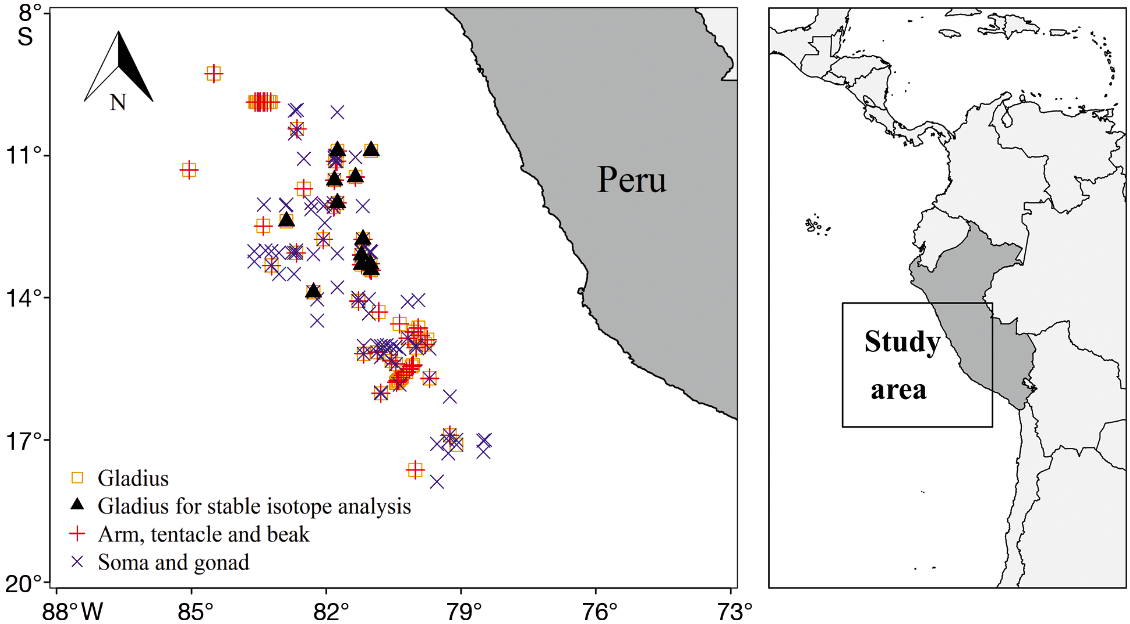

Fig. 1. Study area and sampling locations off the Peruvian exclusive economic zone. Symbols mark different sets of data obtained from *Dosidicus gigas* samples

paratuses (arms, tentacles, upper and lower beaks) were selected prior to analysis (n = 258). Six morphometric characteristics of arms and tentacles (Table S1, Fig. S1 in the Supplement) were recorded in accordance with Moltschaniwskyj (1995): the length of each of the 4 arms (A1–A4), tentacular club length (CL) and tentacle length (TL). Where possible, measurements were taken on the right-hand side of each individual. In addition, 8 length measurements of the beak (Fig. S1) were taken with a vernier micrometer following Clarke (1986): upper hood length (UHL), upper crest length (UCL), upper rostrum length (URL), upper wing length (UWL), lower hood length (LHL), lower crest length (LCL), lower rostrum length (LRL), and lower wing length (LWL). Principal component analysis (PCA) was used to determine the major features of shape variation in the arms, tentacles, and beaks.

To evaluate the impacts of size divergence on sexual segregation more objectively, analysis of covariance (ANCOVA) was used to remove the allometric effects of body size with major morphometric characteristics of morphometric variables as the dependent variables, GL as the covariate, and sex as the categorical variable. This analytical approach tests for differences between sexes in morphometry between sexes at a given GL, and thus is useful for comparing sexual dimorphism while controlling for the effect of body size. Statistical analyses were carried out using SPSS version 19.0 (IBM 2010).

Bulk stable isotope analysis

A total of 25 individuals (29.4 ± 4.8 cm mantle length) were selected from a larger sample size collected in the same overall region for the summer/autumn hatching cohort in 2013 and 2014 (see Table S2 in the Supplement for detailed sampling information). Squid age information was determined using statoliths, i.e. counting the number of growth increments and assuming one increment is deposited daily (Arkhipkin et al. 2015). The approximate hatching date was identified by calculating the difference between sampling date and age.

Age-based sampling was used to evaluate the potential for sexual niche partitioning. The proostraca were cut consecutively starting from the position of 130 d following the growth equation developed for *D. gigas* proostracum off Peru and each section may represent a roughly 10 d period of growth (Li et al. 2017) because both sexes have similar growth rates during the studied lifespan (Liu et al. 2013, Gong et al. 2018). To identify if sexual niche partitioning occurred in early stages, possibly paralarval stages, 5 mm sections were cut beginning from the posterior tip of each proostracum in 43 individuals (23 females and 20 males).

The $\delta^{13}C$ and $\delta^{15}N$ values of each proostracum section were quantified using an IsoPrime 100 isotope ratio mass spectrometer (IsoPrime) and vario ISOTOPE cube elemental analyzer (Elementar Analysensysteme) in the stable isotope laboratory of Shanghai Ocean University. International reference materials (USGS 24 [$\delta^{13}C = -16.049‰$], USGS 26 [$\delta^{15}N = 53.7‰$]) and laboratory running standard (protein [$\delta^{13}C = -26.98‰$ and $\delta^{15}N = 5.96‰$]) were used to calibrate the system and compensate for drift. Measurement errors of $\delta^{13}C$ and $\delta^{15}N$ were approximately 0.05 and 0.06‰, respectively.

As a metabolically inert tissue, each proostracum section recorded a 10 d period of squid growth, and therefore the sequential isotopic values provide a continuous time series of diet (Li et al. 2017). The temporal variation of isotopic values was estimated at the cohort level, meaning that all male or female data were grouped by the same period. To estimate the isotopic niche of *D. gigas* in the $\delta^{13}C$–$\delta^{15}N$ space, we used the stable isotope Bayesian ellipses (SIBER) package in R (Jackson et al. 2011, R Core Team 2018). Specifically, the standard ellipse corrected area (SEA_C) is a metric that allows for statistical comparisons between different age-classes, which was calculated for each 20 d interval using our 10 d isotopic sampling, to minimize the uncertainty (Syväranta et al. 2013), from an age of 130 to 230 d. Sexual niche partitioning was estimated as the percentage of SEA_C overlap and sequential isotopic values among different age-classes and sexes.

Amino acid isotope analysis

From the 25 squid initially selected for bulk SIA, we randomly selected 8 individuals (4 males and 4 females) for CSIA-AA (Table S2). For each squid, we analyzed $\delta^{15}N$ values of individual amino acids in each proostracum section. In total, we analyzed 37 proostracum sections (Table S3 in the Supplement). Gladius samples were hydrolyzed and AAs were derivatized in accordance with the established methods (Yarnes & Herszage 2017). Briefly, 3 mg of dried, ground proostracum tissues were subjected to acid hydrolysis (6 M HCl) at 150°C for 70 min. The hydrolysates and amino acid mixtures were dried under a stream of N_2, followed by the addition of an internal standard (L-Norleucine). The derivatization followed the N-acetyl isopropyl esters procedure outlined by Yarnes & Herszage (2017). CSIA-AA was conducted at the University of California, Davis Stable Isotope Facility. The $\delta^{15}N$ values of AAs were determined by gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) using a Thermo Trace GC Ultra interfaced with a Delta V Advantage IRMS (both from Thermo Scientific) and equipped with a combustion reactor with Ni/NiO/CuO catalyst. The capillary column was a DB-1301 (60 m × 0.25 mm × 1 mm, Agilent Technologies). The injection was performed at 240°C. The separation of AA derivatives was achieved under the following temperature program: 70°C held for 2 min, then increased to 140°C at 15°C min^{-1}, and finally raised at 8°C min^{-1} to 255°C and held for 35 min. L-Norleucine was used as an internal standard to calculate provisional values for each sample. $\delta^{15}N$ values of the following 12 AAs could be quantified using the methods described: alanine (Ala), aspartic acid (Asp), glutamic acid (Glu), glycine (Gly), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), and valine (Val). All sample were run twice to evaluate the variability. The measurement error was approximately 0.37‰.

Based on previous findings (McClelland & Montoya 2002, Chikaraishi et al. 2009), the previously listed 12 AAs were originally classified as either trophic-AAs (Tro-AAs: Ala, Asp, Glu, Leu, Pro and Val) or source-AAs (Src-AAs: Gly, Met, Lys, Phe, Ser, and Thr). However, recent studies reported that $\delta^{15}N$ values of Gly, Ser, and Thr exhibited significant variability during trophic transfer (Nielsen et al. 2015, McMahon & McCarthy 2016) and they are not included in the Src-AAs anymore. Therefore, these 3 AAs were not used in the further analysis. We evaluated variability in squid relative TPs by using $\delta^{15}N$ values of the obtained Tro-AAs and Src-AAs by calculating the difference between the mean values of Tro-AA and Src-AA ($\Delta\delta^{15}N_{\text{Tro-Src}}$), which serve as TP indicators (Choy et al. 2015). We used correlation analysis between $\delta^{15}N$ values of bulk tissues and average Src-AA, and bulk tissue and TP indicator ($\Delta\delta^{15}N_{\text{Tro-Src}}$) to evaluate the degree of association between bulk $\delta^{15}N$ values and baseline $\delta^{15}N$ values or TP. Lastly, regression analyses were conducted to evaluate linear relationships between ages. The differences between females and males were tested using 1-way ANOVA and paired *t*-tests.

Condition index

A total of 1166 individuals were used to determine differences in gonad and somatic condition during different life stages. A condition index, called the Scaled mass index, was calculated using the following formula based on weight data and morphometric measures (Peig & Green 2009):

$$W_i^* = W_i \left[\frac{ML_0}{ML_i} \right]^{b_{\text{SMA}}} \tag{1}$$

where W is the gonad or somatic weight, W_i^* is the predicted mass value for individual i, W_i and ML_i are the observed values of W and ML for individual i, respectively, and ML_0 is the arithmetic mean for the study population. b_{SMA} is the scaling exponent estimated through the standardized major axis (SMA) regression of ln(W) on ln(ML), which is predicted from the following formulas:

$$\ln(W) = \ln(a) + b_{OLS}\ln(ML) \quad (2)$$

$$b_{SMA} = \frac{b_{OLS}}{r} \quad (3)$$

where a is the parameter to be estimated, and b_{OLS} and r are the slope and Pearson's correlation coefficient of the ordinary least squares regression of ln(W) on ln(ML), respectively. This approach allowed us to quantify the energy capital accumulated in the squid as a result of feeding and compare the gonad and somatic conditions (Peig & Green 2009). Variations in gonad or somatic condition with maturity stage were investigated using ANOVA and pairwise post hoc comparisons.

RESULTS

Gladius growth with gonad development

Maturity stages II and III were not observed in female *D. gigas* with a GL less than 24 cm, but were common for females larger than 30 cm. In contrast, males showed an early period in gonad development as indicated by the presence of maturity stages II and III, from 21 to 24 cm GL. Across all size intervals (18–39 cm), consistent increases in maturity trends were detected between levels of gonad investment and GL for both sexes (Fig. 2).

Sexual dimorphism in feeding organs

The PCA of squid arm, tentacle, and beak morphometric characteristics retained 2 components with eigenvalues greater than 1 and explaining 80.68% of the variance in the original dataset. The 12 main morphometric variables (A1–A4, CL, TL, UHL, UCL, URL, UWL, LHL, and LRL) showed significant positive relationships with GL ($r > 0.67$ for males and females; Table 1). In addition, the female individuals showed higher values in the main morphometric variables than males, specifically in the GL range of individuals for SIA (i.e. <33.8 cm, see Table S2).

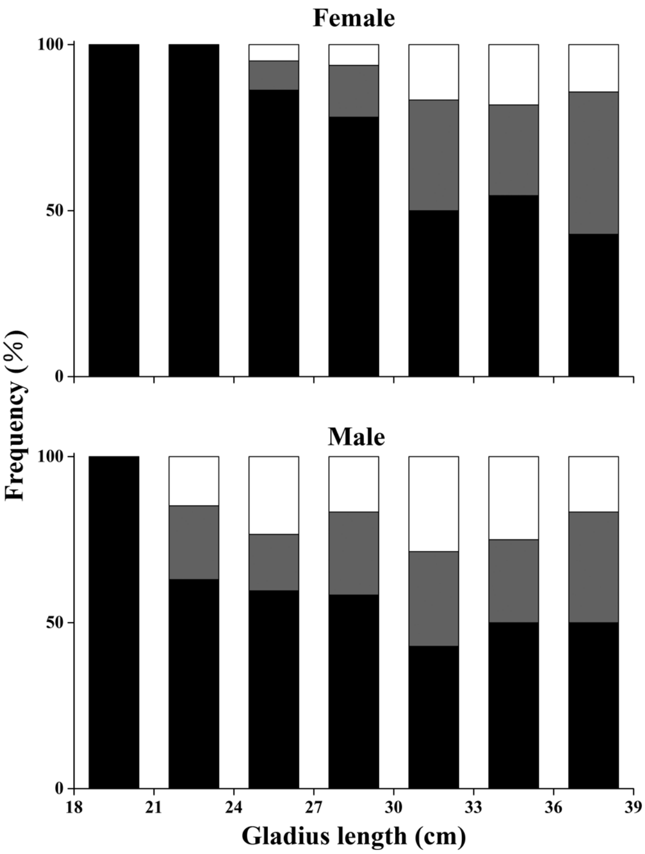

Fig. 2. Frequency distribution of females and males of *Dosidicus gigas* by gladius length grouped into 3 cm intervals. Maturity stage: black, stage I (immature); grey, stage II (immature); white, stage III (maturing)

Stable isotope analysis in bulk tissue

Squid used for SIA had similar mean MLs and ages between females (30.3 ± 2.9 cm and 220 ± 22 d, respectively) and males (28.4 ± 2.3 cm and 214 ± 17 d) (ANOVA, ML: $F_{1,23} = 0.55$, p = 0.46; age: $F_{1,23} = 3.34$, p = 0.08, Table S2). A total of 232 gladius sections were obtained from the 25 analyzed proostraca. For both sexes, most individuals showed decreasing but variable shifts in δ^{13}C and δ^{15}N values as a function of gladius length and age (Fig. 3a,b). For all individuals, no significant difference in isotope values was observed between 2013 and 2014 squid (ANOVA, δ^{13}C: $F_{1,230} = 0.367$, p = 0.55 and δ^{15}N: $F_{1,230} = 0.002$, p = 0.96, respectively). Analysis per sex, however, showed that the δ^{15}N values of females collected in 2014 were significantly higher than females sampled in 2013 (ANOVA, $F_{1,103} = 75.26$, p < 0.01; paired t-test, $t = -14.10$, df = 9, p < 0.01), while males did not differ between years (ANOVA, $F_{1,74} =$ 0.04, p = 0.84; paired t-test, $t = 0.29$, df = 5, p = 0.79).

Table 1. Correlations for identifying sexual dimorphism in arm, tentacle and beak shape with gladius length. The number of samples is given in parentheses. The 12 morphometric measurements included: tentacle length (TL), club length (CL), length of each of the 4 arms (A1–A4) on the right-hand side of each individual and 6 length measures of beak: upper hood length (UHL), upper crest length (UCL), upper rostrum length (URL), upper wing length (UWL), lower hood length (LHL), and lower rostrum length (LRL). For the ANCOVA, morphometric characteristics were used as dependent variables, and gender was the categorical variable. Gladius length was used as a covariate to control for body size differences. Statistical significance at *p < 0.05 and **p < 0.01 is shown

	Female (148)			Male (110)			ANCOVA	
	r	Slope	Intercept	*r*	Slope	Intercept	*F*	p
TL	0.81	1.00	3.08	0.73	1.20	−4.26	12.93	**
CL	0.84	0.59	−4.40	0.67	0.55	−4.83	17.82	**
A1	0.85	0.63	−3.23	0.79	0.72	−6.32	7.64	*
A2	0.91	0.71	−3.97	0.90	0.76	−6.53	23.12	**
A3	0.90	0.69	−3.08	0.90	0.75	−5.84	22.03	**
A4	0.91	0.63	−3.12	0.90	0.65	−4.67	20.75	**
UHL	0.92	0.74	−1.74	0.92	0.77	−3.65	37.46	**
UCL	0.90	0.92	−2.05	0.92	0.91	−3.88	29.85	**
URL	0.88	0.26	−0.45	0.86	0.28	−1.30	14.96	**
UWL	0.78	0.22	−0.37	0.86	0.21	−0.35	11.99	**
LHL	0.63	0.21	0.07	0.87	0.18	0.50	7.50	*
LRL	0.81	0.25	−0.42	0.86	0.24	−0.48	19.40	**

At cohort level, females (−17.34 ± 0.76‰; range −19.61 to −15.72‰) and males (−17.48 ± 0.64‰; range −19.00 to −15.56‰) had equivalent mean proostracum δ^{13}C values (ANOVA, $F_{1,230}$= 2.07, p = 0.15; Fig. 3c, Table S2), but differed in mean proostracum δ^{15}N values. Males had a much wider range of δ^{15}N values than females during the study period, and most males had lower and narrower δ^{15}N values than females at older age groups (>190 d) ($\delta^{15}N_{female}$: 11.82 ± 1.95‰, range 7.53 to 16.48‰; $\delta^{15}N_{male}$: 9.95 ± 2.18‰, range 6.19 to 15.37‰; ANOVA, $F_{1,230}$ = 47.06, p < 0.01; Fig. 3b,d). Males and females varied in their isotopic niches through the 6 age classes analyzed in this study (Table 2, Fig. 4). Essentially, the overlap of isotopic niches (Fig. 4) between females and males exhibited a significant decreasing trend with age (i.e.

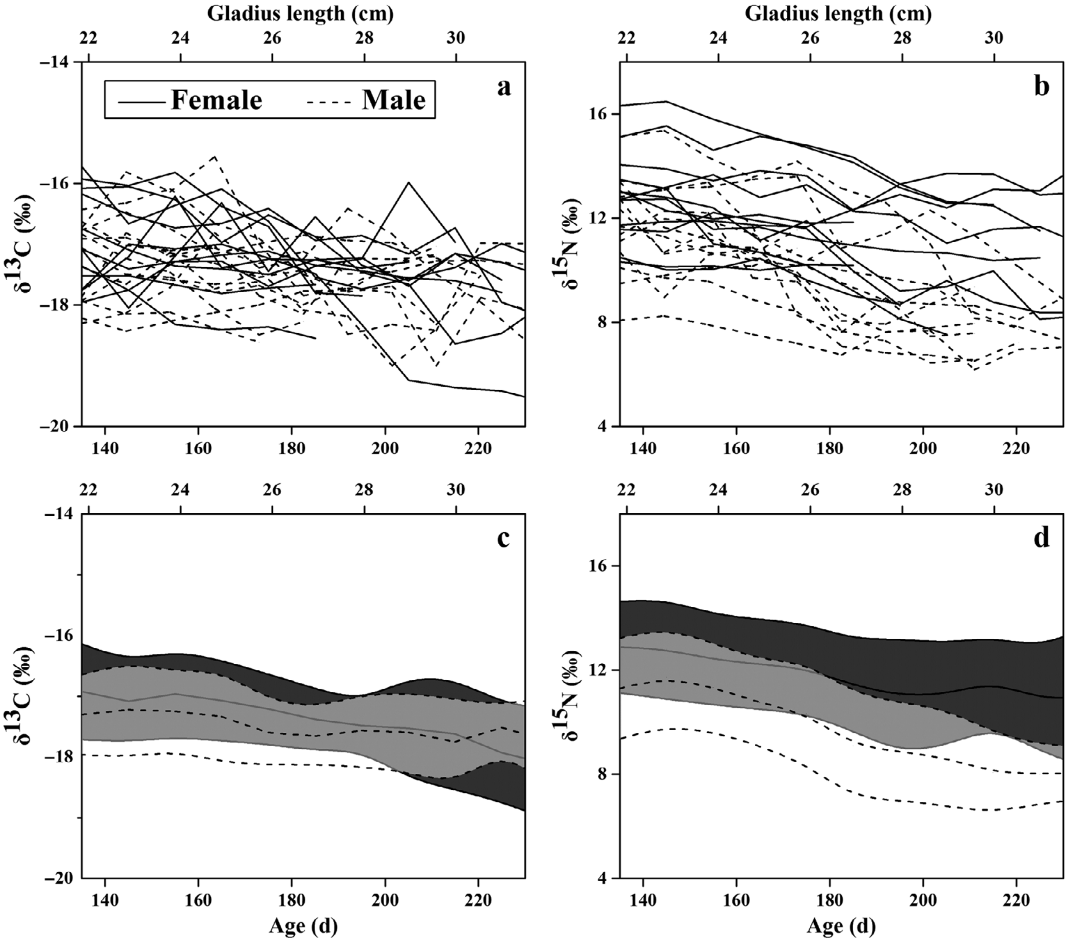

Fig. 3. Sequential (a) δ^{13}C and (b) δ^{15}N; and (c,d) mean (±1 SD) values of female and male *Dosidicus gigas*

Table 2. Sex-specific isotopic niches. Sexual segregation was estimated as the percentage of the standard ellipse corrected area (SEA_C) overlap. Paralarval = paralarval stages

Age class (d)	Gladius length (cm)	SEA_C (‰^{2}) Female	SEA_C (‰^{2}) Male	Overlap size (‰^{2})	SEA_C overlap (%) Female	SEA_C overlap (%) Male
Paralarval	0.0–0.5	3.22	3.54	2.60	80.55	73.36
130–150	21.8–23.3	3.55	4.17	1.49	41.91	35.70
151–170	23.3–25.1	3.74	3.78	1.82	48.69	48.26
171–190	25.1–27.0	3.06	3.26	1.07	35.08	32.97
191–210	27.0–29.0	4.12	3.46	1.16	28.06	33.47
211–230	29.0–31.1	4.43	2.54	0.01	0.28	0.49

from similar niches in early, presumably paralarval, stages to low overlap in core isotopic niches at older age classes; $r = -0.84$, $p < 0.01$), which was mainly driven by males shifting isotopic niches (i.e. decreasing $\delta^{15}N$ isotope values) through time, while females remained at relatively similar $\delta^{15}N$ values.

Amino acid isotope analysis

$\delta^{15}N$ values for females from all AAs ranged from 3.25 to 32.01‰ and from 1.89 to 30.23‰ for males (Table S3). The $\delta^{15}N_{Tro\text{-}AA}$ values of females (range 14.63 to 32.01‰) were significantly higher than that of male $\delta^{15}N_{Tro\text{-}AA}$ values (range 13.99 to 30.23‰) (ANOVA, $F_{1,428} = 7.31$, $p < 0.01$). There was no significant difference between $\delta^{15}N_{Src\text{-}AA}$ values of females and males (range from 3.25 to 18.60‰ and 1.89 to 20.17‰, respectively, ANOVA, $F_{1,204} = 1.67$, $p = 0.19$). Furthermore, mean $\delta^{15}N_{Tro\text{-}AA}$ values for females of each age-class were significantly higher than those of males (paired *t*-test, $t = 10.74$, df = 4, $p < 0.01$), while no differences in $\delta^{15}N_{Src\text{-}AAs}$ values was observed between sex (paired *t*-test, $t = 1.58$, df = 4, $p = 0.19$) (Fig. 5a). Females had higher TP proxies than males; however, the tendency was inconsistent throughout age classes (Fig. 5b). For example, the mean $\Delta\delta^{15}N_{Tro\text{-}Src}$ value of females 180–190 d old was lower than males at the same ages. In addition, there was a strong positive relationship between $\delta^{15}N_{Src}$ with bulk $\delta^{15}N$ values ($r = 0.80$, $p < 0.01$), while a weak but significant correlation was found between $\Delta\delta^{15}N_{Tro\text{-}Src}$ with bulk $\delta^{15}N$ values ($r = 0.36$, $p = 0.03$).

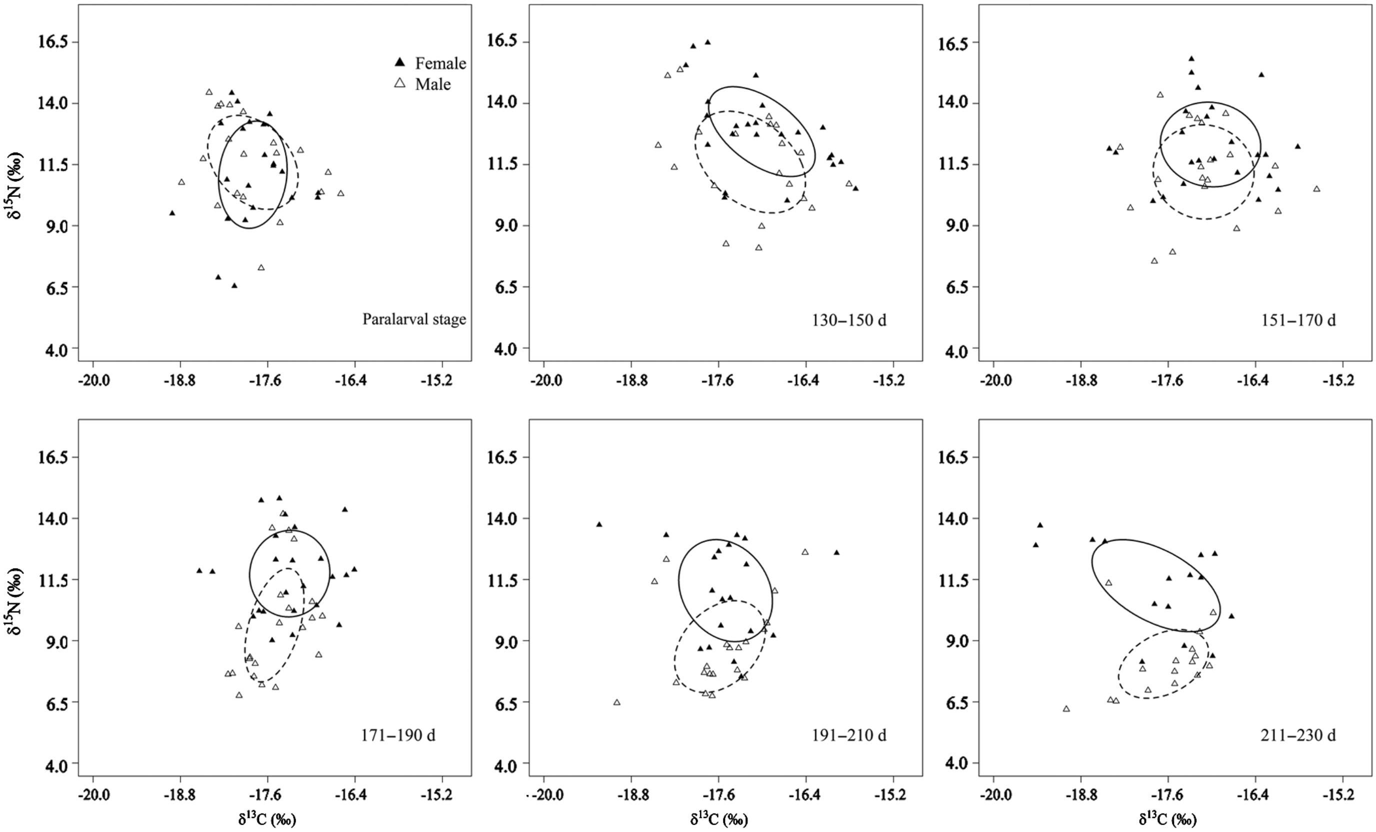

Fig. 4. Isotopic niche areas (calculated with SEA_C) for female (black circle) and male (dashed circle) *Dosidicus gigas* during 6 different age classes. See Table 2 for explanation of the ellipse areas and overlap area values

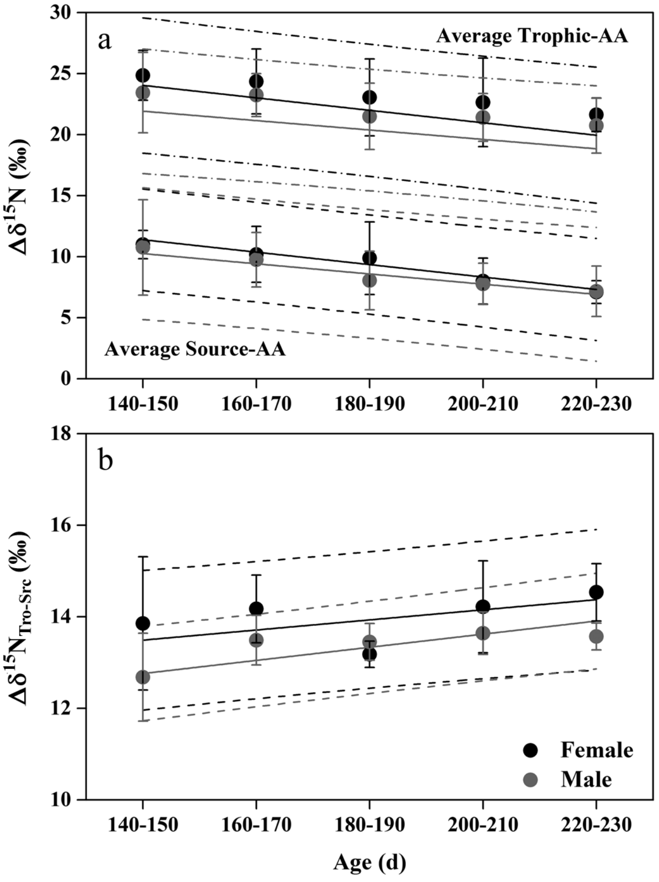

Fig. 5. (a) Paired plots show $\delta^{15}N$ values (±1 SD) of average trophic amino acids (i.e. Ala, Asp, Glu, Leu, Pro, and Val; trophic-AAs) and source amino acids (i.e. Lys, Met, and Phe; source-AAs) of female and male *Dosidicus gigas*. (b) Sequential relative trophic position ($\Delta\delta^{15}N_{Tro\text{-}Src}$) estimates as the weighted mean approach (the difference between the mean of trophic-AAs and source-AAs. See description in 'Materials and methods: Amino acid isotope analysis' for abbreviations

Energy allocation in early maturity stages

Females and males of similar maturity stages exhibited different body or somatic conditions throughout their development. Female somatic condition did not vary greatly between maturity stages (ANOVA, $F_{2,823} = 0.01$, $p = 0.99$; Fig. 6a). In contrast, significant differences ($F_{2,322} = 9.74$, $p < 0.01$; Fig. 6b) were found in male somatic condition using post hoc Tukey's HSD comparisons, indicating that values for maturity stage I (326.78 ± 38.95) were significantly higher than those of males in stage II (307.09 ± 39.72) and stage III (302.74 ± 45.30), with no significant difference between the last 2 stages.

Significant differences in gonad condition values were detected between maturity stages for both sexes (Fig. 6b,d). For females, no significant difference was observed between stages I and II (ANOVA, $F_{2,823} = 202.61$, $p < 0.01$; Tukey's HSD, $p = 0.24$). However, the gonad conditions of stages I and II were significantly lower than those of individuals in stage III ($p < 0.01$). Unlike females, males exhibited a progressive change in gonad condition through all maturity stages (ANOVA, $F_{2,322} = 99.67$, $p < 0.01$)

DISCUSSION

We used morphometric analyses of squid feeding structures (arm, tentacle, and beak), age determination using statoliths, and stable isotope ratios along gladii to evaluate trophic niche partitioning between female and male *D. gigas* during ontogenesis. We also determined maturity and gonad and somatic condition to evaluate variability between sexes at different maturity stages. Together, our results indicate that *D. gigas* exhibited ontogenetic variability in sexual niche partitioning, and this pattern seems to increase as squid age. Our study reveals different isotopic patterns between females and males indicating trophic niche differentiation, possibly driven by sex-specific diets and differences in energy allocation between the sexes across early maturity stages. Below, we discuss our results in detail, and propose the underlying mechanisms driving sexual segregation for the highly dynamic mesopelagic squid species, *D. gigas.*

Size dimorphism and sexual niche partitioning

Morphometric analyses indicate that sexual size dimorphism occurred in the arm, tentacle, and beak of *D. gigas* with the same gladius length (GL), which scales with body size. The observed size divergence in feeding apparatuses was not equal between females and males as they grew, but changed progressively over the range of GL. In particular, our results indicate that female feeding apparatuses were larger than those of males even for individuals with the same body size. As functional feeding apparatuses, the observed sexual size variations in arm, tentacle, and beak indicate differences in food resource use between female and male squid (Hanlon & Messenger 1996, Franco-Santos & Vidal 2014). Although detailed studies on the relationships between these body parts and prey size are lacking, the scale of these feeding apparatuses can be related to the amount of pulling and bite force (Kear 1994, Hanlon & Messenger 1996). Using quantile regression analysis, Field et al. (2013)

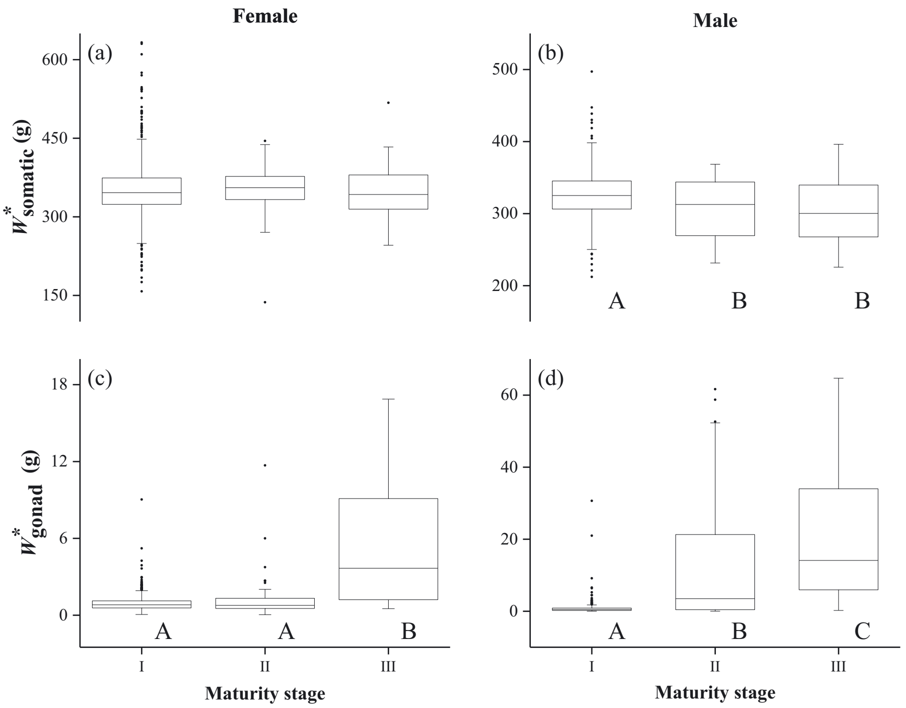

Fig. 6. Shifts in somatic or gonad condition of *Dosidicus gigas* (n = 1166) estimated by the condition index method called the scaled mass index (W^*; see Peig & Green 2009). There were no significant differences in female somatic conditions between maturity stages. Where appropriate, alphabetic characters (A, B, C) below each plot summarize pairwise post hoc comparisons among maturity stages (maturity stages not sharing letters were statistically different). Values exceeding 1.5 times the interquartile range of boxplots are marked with black dots

indicated an increase in the size of prey items with increasing *D. gigas* body size in the California Current ecosystem; this relation was not found in the Gulf of California, where small myctophids and cephalopods were the main prey items across different squid sizes, ranging from 14.5 to 87.5 cm MLs (Markaida et al. 2003). However, other studies from the same area and using stable isotope ratios of jumbo squid muscle tissue, beak and gladius have shown a trend toward higher prey trophic position with squid size (Ruiz-Cooley et al. 2004, 2006, 2010). According to the optimal foraging theory, animals should maximize their energy acquisition within a given period of time spent foraging and simultaneously reduce costs (MacArthur & Pianka 1966). Perhaps bigger feeding apparatuses favor female *D. gigas* capturing larger prey items or prey at a higher trophic position than males as they become larger and older, reducing intraspecific competition by exploiting different food resources. These findings contradict to some extent the results derived from stomach content analysis in *D. gigas* from the northern Humboldt Current (Alegre et al. 2014) and Ecuador (Rosas-Luis & Chompoy-Salazar 2016). Both of these studies showed that females and males have similar diet composition; however, differences in prey size were not explicitly evaluated. Remnant hard tissues in *D. gigas* stomachs are commonly used to estimate prey size (Markaida et al. 2003, Field et al. 2007, 2013). However, squid have a unique feeding behavior: any food must pass through the very narrow esophagus, which is located in the middle of the brain. Therefore, prey of any size is rapidly sliced into small pieces by the combined actions of the beak and radula (Hanlon & Messenger 1996). Researchers analyze these food remains to identify prey at the species level, but there is a bias in prey identification due to different digestion and passage rates for hard and soft tissues, and diet analyses can underestimate the occurrence and size of larger prey (Rodhouse & Nigmatullin 1996, Field et al. 2013). Estimating prey size using stomach content analysis is very difficult, especially for the wide variety of prey types consumed by *D. gigas* off Peru (Lorrain et al. 2011, Alegre et al. 2014) and elsewhere (Field et al. 2013). Nonetheless, differences in the relative frequency of prey items were found between female and male *D. gigas* in the Gulf of California, e.g. more *Leachia* sp. were found in male stomachs (Markaida & Sosa-Nishizaki 2003).

Using SIA, we found variable shifts in bulk δ^{13}C and δ^{15}N values along the proostracum for both sexes from 21 to 33 cm GLs (Fig. 3). The lack of differences in bulk δ^{13}C values between females and males and between years along proostraca suggests that both sexes shared the same overall habitat and perhaps moved horizontally and vertically within the same range during growth, since they were caught in the same months of both 2013 and 2014 in an area of approximately 3° × 3° (Fig. 1), and were hatched in the same seasons (Table S2). However, the overall range of bulk δ^{13}C and δ^{15}N values exhibited by females and males throughout their lives suggest complex foraging and migratory behavior. For instance, δ^{13}C and δ^{15}N values varied latitudinally along the northern Humboldt Current ecosystem with a difference of ~4.0 and 9.0‰, respectively, in *D. gigas* muscle bulk tissue (which reflects integrated information from a few weeks) and copepods collected from 3.5° to 18.3°S (Argüelles et al. 2012). Therefore, the observed variability in $\delta^{13}C$ and $\delta^{15}N$ values among individuals and age-classes suggests that *D. gigas* possibly moved and fed in different regions of the same overall ecosystem.

Except for a few females, most individuals had decreasing but variable bulk $\delta^{15}N$ values along the proostracum (Fig. 3b), suggesting that they moved from ^{15}N-enriched to ^{15}N-depleted areas, possibly from southern nearshore areas to more northerly offshore waters (Alegre et al. 2014, Li et al. 2017). These results are consistent with the decreasing $\delta^{15}N$ values from source-AAs as males and females grew, and the strong correlations between $\delta^{15}N_{Src}$ and bulk $\delta^{15}N$ values supporting baseline-driven $\delta^{15}N$ differences. Nonetheless, females consistently exhibited higher bulk $\delta^{15}N$ values than males along gladii. Assuming that both sexes shared the same habitat and had similar movement patterns, differences in prey items can probably be the major determinant of sex-specific $\delta^{15}N$ values. Higher bulk $\delta^{15}N$ values (mean ± SD: 11.82 ± 1.95‰) indicate that females possibly fed at a higher trophic level than males (9.95 ± 2.18‰, Fig. 3d). This hypothesis is supported by CSIA-AA results because the TP indicator ($\Delta\delta^{15}N_{Tro\text{-}Src}$) was higher for females than males for most age-classes (Fig. 5b), except for 180–190 d. This anomaly may be due to changes in prey availability, which remains to be elucidated. The mean difference in bulk $\delta^{15}N$ values between males and females along the proostracum is 1.87‰, less than 1 trophic level, assuming a trophic enrichment factor of 2.75‰ between squid and their prey (Caut et al. 2009). This variability in bulk $\delta^{15}N$ values suggests that females captured prey of higher trophic position (possibly larger prey or prey at higher trophic positions; see Espinoza et al. 2017) than males, which may maximize feeding success and fulfill energetic demands, especially for growing maturing females. Our morphometric analysis and isotope data from bulk tissue and amino acids support this hypothesis for the region and period of study, but it is unknown if this feeding strategy occurs in other years, environmental conditions and ecosystems.

Ontogeny of trophic niche segregation

Comparisons among isotopic niches between females and males demonstrated niche partitioning during ontogeny, since isotopic niches were similar at early age classes but differed for older classes (Table 2, Fig. 4). As a voracious predator, resource competition between female and male *D. gigas* is likely to increase during their rapid growth. In general, predators can alleviate intraspecific competition by food partitioning (Dayan & Simberloff 2005). Therefore, sexual niche segregation displayed by the changing isotopic patterns among age classes (Table 2, Fig. 4) indicate higher partitioning of food resources at larger body sizes and increasing age. These patterns suggest that females and males can coexist by foraging in the same area and catching different prey types or similar prey types but at different sizes. Given the positive relationships between the depths of occurrence and sizes of mesopelagic fishes (Willis & Pearcy 1980), the sexual niche partitioning might also result from potential sex-specific vertical habitat use (Arkhipkin & Middleton 2002). Sexual segregation would likely maximize feeding success, reduce competition and ultimately increase reproductive success. Ontogeny of isotopic niche differentiation corroborates the presence of food partitioning possibly resulting from sexual size dimorphism in this study. However, the considerable overlaps shown in Fig. 4 (e.g. 130–210 d) suggest that females and males, to some extent, had similar diet composition or similar prey type but different prey sizes.

Besides size dimorphism, nutrient requirement is another factor that can drive sexual separation. Differences in nutrient needs have been recently found in female and male cephalopods (McGrath Steer & Jackson 2004, Pecl & Moltschaniwskyj 2006, Lin et al. 2015), possibly due to divergent energetic needs for growth and reproduction in different life-stages. In our study, this previous pattern could result in a step-change isotopic niche partitioning, since the isotopic variability along the proostracum would reflect the shifts in *D. gigas* foraging patterns during different maturity stages. While maturity stages in studied squid were differentiated only by qualitative attributes, the isotopic variation between males and females through ontogenesis was evident (Figs. 3 & 4), and both sexes showed an increase in gonad investment with maturity stages. Specifically, the variability in male gonads and somatic condition (Fig. 6) suggests that males require relatively less energy for reproduction compared with females (McGrath Steer & Jackson 2004). This pattern may increase energetic allocation towards gonad development, while they subsequently decrease somatic condition, resulting in lower body weight of males than females at a given length (Liu et al. 2013). In females, higher nutrient requirements combined with the greater potential of fecundity were observed as *D. gigas* aged (Nigmatullin & Markaida 2009). Our isotopic results and morphometric analysis indicate that they can meet energetic requirements for somatic growth while simultaneously allocating more energy for gonad development. This is possibly due to their food partitioning, e.g. males may adjust their energy allocation to suit different feeding conditions or food availability (i.e. smaller size of prey). This pattern could also result in a divergence in metabolic rates as squid mature, which would consequently influence isotopic fractionation (Gaye-Siessegger et al. 2004, Grigoriou & Richardson 2009). Meanwhile, the differences in energy demands between sexes may influence squid feeding behavior, and female *D. gigas* may undertake relatively larger energy costs for the growth of functional feeding apparatuses than males. This phenomenon is also found in another ommastrephidae species (*Illex illecebrosus*) and a nearshore loliginid squid (*Loligo chinensis*). In these 2 species, the sex with higher energetic requirements has larger feeding apparatuses (Fang et al. 2017, Y. Gong unpubl. data). In addition, sexual size dimorphism also apparently increases with increasing gonad investment and maturity stages. The sexual dimorphism in feeding apparatuses in female and male squid could be considered as an adaptation to different nutrient requirements. However, given the high plasticity in *D. gigas* growth in relation to environmental conditions and its opportunistic feeding behavior, it is unknown if the observed sexual trophic niche partitioning for small to medium squid is true for larger animals, other Peruvian regions and ecosystems, and other years. A recent study using *D. gigas* beaks from small to large sized squid indicates differences in isotopic values between females and males, suggesting differences in diet and distribution in some years in the central Gulf of California (Trasviña-Carrillo et al. 2018). Perhaps sexual segregation is stronger in larger mature squid and in specific environmental conditions and productivity regimes.

CONCLUSION

Pelagic squid are an important linkage in food webs and respond quickly to environmental variation (Clarke 1996, Ménard et al. 2013). Integrating morphometrics of feeding apparatuses and stable isotope analysis of bulk and AAs of sequential sections along squid gladii can determine sexual foraging partitioning in a given period of time and area. In our study waters off Peru, we identified variability in ontogenetic isotopic patterns between males and females, indicative of food partitioning, especially for larger body size and older age classes. This behavior could be driven by size dimorphism in feeding apparatuses, sexual responses in energy allocation, and different nutrient requirements. However, further research is needed to understand differences in physiological response and predation behavior between males and females for large individuals and from other ecosystems. It is unknown whether sexual segregation in *D. gigas* is prevalent across its geographical range or what environmental conditions trigger sexual segregation in habitat and/or diet composition. Trophic niche partitioning between females and males of a commercially valuable squid like *D. gigas* may have important implications for their population dynamics and resilience to human exploitation.

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