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Sexual niche partitioning of jumbo squids in the Southeast Pacific Ocean

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## 1. Introduction

Sexes are thought to coexist by differences in habitat use or social behaviors, thereby reducing the level of intraspecific competition and results in niche separation (Bearhop et al., 2006; Nifong et al., 2015; Kernaléguen et al., 2016). This sexual segregation occurs in a wide range of animal species, usually assumed to be related to size dimorphism and (or) nutrient requirements (Bearhop et al., 2006; Kernaléguen et al., 2016). Understanding the mechanisms driving sexual behavior variation is important in attempts to successfully manage and conserve animal populations, since sexual segregation may result in spatial-temporal dynamics (see review by Wearmouth and Sims, 2008). There is a burgeoning literature documenting sexual segregation in marine organisms, but have focused mainly on vertebrates, such as seabird (Bearhop et al., 2006), teleost (Kock et al., 2013), and mammals (Kernaléguen et al., 2016). Information on sex-specific diet, foraging area or habitat use of cephalopods are less frequently reported (but see Arkhipkin and Middleton, 2002; Kato et al., 2016) and in general would add to the debate. Especially the pelagic cephalopods that are a dominant component in marine ecosystems both as predators and as valuable sources for top predators (Clarke, 1996; Ménard et al., 2013). They also have economic importance for neritic and oceanic fisheries around the world (Hunsicker et al., 2010).

There is evidence associated with potential sexual segregation in oceanic squids though their sex-specific foraging strategies were unknown. For example, differences in nutrient requirements between sexes of three squid species were recently reported: *Nototodarus gouldi* (Steer and Jackson, 2004), *Sepioteuthis australis* (Pecl and Moltschaniwskyj, 2006) and *Illex argentines* (Lin et al., 2015). In these cases, females and males within the population show divergent energetic needs for growth or reproduction. Moreover, social status is another hypothesis that has been assumed to explain the sexual segregation (Bearhop et al., 2006; Kernaléguen et al., 2016). Indeed, a disproportionate number of female squid was often found

in *Dosidicus gigas* based on catch data (Ibáñez and Cubillos, 2007; Tafur et al, 2010) despite unbiased fishing method, which possibly indicated females dominate the sex ratio by prioritizing foraging behavior in the same habitat (Griffiths et al., 2014). Understanding sexual segregation of these species is of fundamental importance for their management, for instance, whether they require sex-biased harvest strategies to reduce skewed fishing mortality with the rapid rise in their landings.

As a voracious and highly migratory predator, differentiating the foraging strategies between female and male cephalopod is often difficult in empirical studies. Sexual segregation may occur in a three-dimensional space, since they perform horizontal movements and vertical migration at the same time (Arkhipkin and Middleton, 2002; Bazzino et al., 2010). Moreover, cephalopod esophagus passes through the brain, is usually narrow. This means that prey is not swallowed whole but shred by beak and radula (Hanlon and Messenger, 1996 P48). This mode of feeding, therefore, causes the potential bias in estimation of the stomach contents (Rodhouse and Nigmatullin, 1996), notably the coexisting females and males with similar prey at the same space and time. Moreover, stomach contents usually reflect diet over relatively short time period and potentially underestimate the preys that are more easily digested.

The jumbo squid (*D. gigas*) is the most abundant ommastrephid squid in the eastern Pacific Ocean (Anderson and Rodhouse, 2001). It supports the important commercial fisheries off the coasts of western South and Central America and the Gulf of California (Nevárez-MartíNez et al., 2000; Taipe et al., 2001; Rocha and Vega, 2003). *D. gigas* has been known to expanse its range in both hemispheres (Field et al., 2007; Ibáñez and Cubillos, 2007), having influence on the other commercial species in these ecosystems. Recent developments in stable isotope analysis and Bayesian isotope mixing models (Jackson et al. 2011) provide an excellent opportunity to investigate the underlying mechanisms explaining ontogeny of sexual segregation in *D. gigas*. Carbon and nitrogen stable isotopes are efficient trophic tracers and used to elucidate the trophic patterns between sexes (Bearhop et al., 2006; Nifong et al., 2015; Kernaléguen et al., 2016). The carbon stable isotope ratio ($\delta^{13}C$) is often used to reveal the ultimate carbon source, allowing identification of foraging locations, since $\delta^{13}C$ changes little (0.75‰) during trophic transfers. While, the nitrogen stable isotope ratio ($\delta^{15}N$) shows a

stepwise enrichment in consumer tissues (2.75‰), providing a method to quantify trophic position (Caut et al., 2009). *D. gigas* gladius is a continuously growing, metabolically inert tissue enables us to reveal the diet at the time of tissue synthesis, providing the ontogenetic shift and migration patterns (Lorrain et al., 2011; Ruiz-Cooley et al., 2013; Li et al., 2017). Indeed, marine organisms always exhibit variability in their habitat use during different life stages (Carlisle et al., 2015; Kernaléguen et al., 2016). Hence, sequential isotopic signatures along the gladius can back-calculate when sexual segregation occurs in squid's life and compare with the factors causing the niche partitioning (e.g. size dimorphism and nutrient requirements).

It seems logical to use morphometric characteristics of feeding organs (e.g. arm, tentacle and beak) to investigate foraging ability of cephalopod since these organs are correlate with the size of food items ingested (Saporiti et al., 2016). Unlike most marine organisms, cephalopod can use arms and tentacles to seize and restrain their prey, longer arms or tentacles may exert stronger pulling force (Hanlon and Messenger, 1996 P47). Beak is one of the main feeding structures inside the squid mouth, which related to its bite force (Kear, 1994) and can be used as a morphologic indicator to explain resource use (Franco-Santos et al., 2014). Moreover, coleoid cephalopods have evolved an adaptive response to the energy demands of somatic growth while simultaneously reproduction investment, due to their biological characteristics such as rapid growth, short life, and monocyclic reproductive pattern (Moltschaniwskyj and Carter, 2012). Both somatic growth and gonad development can be expected to have major consequences for nutrient requirements (Peig and Green, 2009). Hence, ontogenetic shifts in allocation of energy have often been found between different life stages in squid (Steer and Jackson, 2004; Lin et al., 2015).

Here, combining the trophic morphology and stable isotope analysis, we evaluate the sexual size dimorphism in feeding organs and ontogeny of isotopic niche partitioning of *D. gigas*. Specifically, we asked the following questions: (i) Does sexual segregation occurs in *D. gigas* from Northern Humboldt Current ecosystem? (ii) Whether sexual dimorphism and (or) sex-specific nutrient requirements are the main factors resulting in the ontogeny of sexual segregation? Such questions answered could provide new information relating to how and why these coexisting female and male pelagic cephalopod compete or partition resources.

## 2. Materials and methods

### 2.1. Squid sampling and processing

Fieldwork was conducted on commercial jigging vessels operating during 2013, 2014 and 2015 off the Peruvian Exclusive Economic Zone, which is the part of Northern Humboldt Current Ecosystem, from 10°S to 16°S (Fig. 1). Squid were collected fresh and frozen 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 were determined according to Lipinski and Underhill. (1995). Wet weight of gonad and somatic were recorded to the nearest 1 g. The statolith was extracted for age determination by counting the number of growth increments (Arkhipkin et al., 2014). The gladius and beak were removed and washed using distilled water for 5 minutes in an ultrasonic cleaner. Gladii or beaks which appeared to be damaged were eliminated.
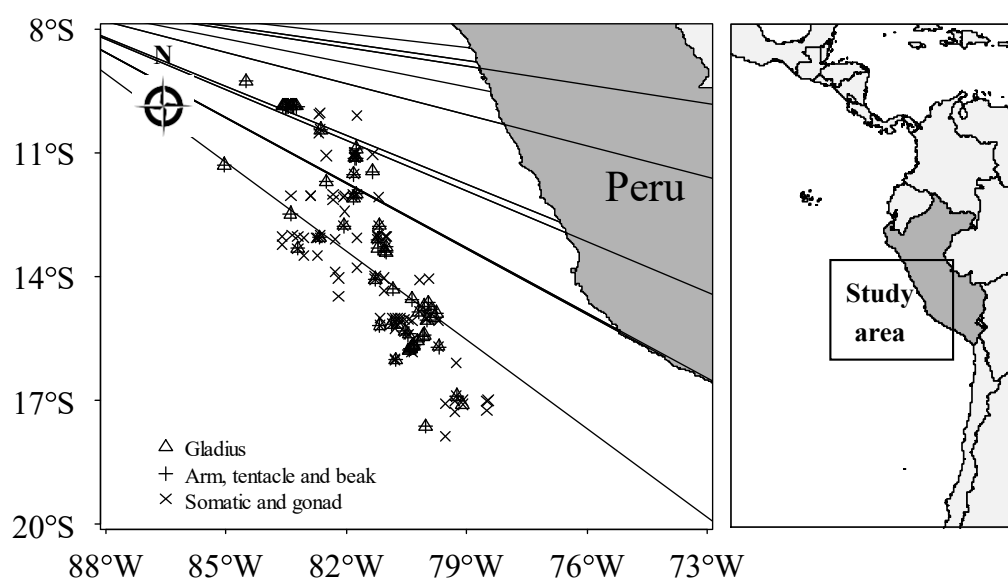


Fig. 1. Study area and sampling locations.

### 2.2. Morphometric analysis

The length of proostracum (PL), one of the morphological part of gladius, was measured for each specimen. PL frequency distribution of maturity stages was analyzed to identify possible period of reproductive development. The frequency was calculated by each interval size of 2.5 cm PL, from 15.4 to 37.5 cm (N = 351, Table 1).

Table 1. Summary information of sampled *Dosidicus gigas* (N = sample size for each tissue).

Values are mean ± SD with ranges (minimum, maximum). ML: dorsal mantle length; BW:

Body weight; F: females; M: males.

| Tissue | N (F, M) | Sampling date | Coordinates | ML (cm) | BW (g) |
|---|---|---|---|---|---|
| gladius | 171 (125, 46) | Jul. to Oct. 2013 | 79°57' - 83°24'W 10°54′ - 15°09'S | 27.2 ± 3.6 (20.9, 39.6) | 575.5 ± 361.6 (225.0, 1647.0) |
| | 81 (51, 30) | Feb. to Sep. 2014 | 74°57′ - 83°13′W 10°26′ - 17°38′S | 27.9 ± 7.1 (19.1, 48.5) | 749.4 ± 705.6 (177.0, 3361.0) |
| | 99 (56, 43) | Jun. to Sep. 2015 | 79°45' - 85°03'W 9°16′ - 15°48'S | 28.1 ± 6.0 (20.7, 49.0) | 712.8 ± 626.7 (225.0, 3095.0) |
| arm, tentacle and beak | 123 (76, 47) | Jul. to Oct. 2013 | 79°57' - 83°24'W 10°54′ - 15°09'S | 27.2 ± 3.6 (20.9, 39.1) | 576.9 ± 268.1 (268.0, 1647.0) |
| | 54 (35, 19) | Apr. to May. 2014 | 74°57′ - 83°13′W 10°26′ - 17°38′S | 27.9 ± 7.5 (19.2, 48.5) | 851.1 ± 773.0 (234.0, 3361.0) |
| | 81 (45, 36) | Jun. to Sep. 2015 | 79°45' - 85°03'W 9°16′ - 15°48'S | 28.6 ± 6.4 (20.7, 49.0) | 759.0 ± 680.6 (225.0, 3095.0) |
| somatic and gonad | 336 (284, 52) | Jul. to Oct. 2013 | 79°57' - 83°24'W 10°54′ - 15°09'S | 27.1 ± 3.3 (20.9, 38.8) | 576.0 ± 251.0 (207.0, 2647.0) |
| | 830 (539, 291) | Feb. to Sep. 2014 | 74°57′ - 83°36′W 10°26′ - 17°38′S | 27.7 ± 5.9 (18.0, 57.5) | 719.9 ± 723.5 (206.0, 4851.0) |

Six morphometric characteristics of arms and tentacles of 258 individuals (Table 1) were

recorded in accordance with Moltschaniwskyj. (1995). These measurements included length

of each of the four 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,

eight length measures of beak were measured with 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 main features of shape variation in these organs.

In order to evaluate the impacts of size divergence on sexual segregation more objectively, the differences in body-size effects are removed in this study. Since the latter has a strong influence on factors such as predation risk and activity budgets (Wearmouth and Sims, 2008), which may bias the interpretation of our results. We used an analysis of covariance (ANCOVA) with efficient morphometric characteristics as the dependent variables, PL as the covariate and gender as the categorical variable. This analytical approach tests for differences between sexes in shape data at a given PL, and thus is useful for comparing sexual dimorphism while controlling for the effect of body size. Statistical analyses were carried out by SPSS vers. 19.0 (IBM Corp, 2010).

## 2.3. Stable isotope analysis

To explore ontogenetic sexual differences in foraging, a total of 25 maturing squid of each sex were used in the subsequent SIA. Table S1 shows the detailed information of the samples. The proostraca were cut consecutively every ten days starting from the position of 130 d, according to Li et al. (2017). Meanwhile, to evaluate the degree of potential sexual segregation in embryonic and paralarval stages, 5 mm section was cut beginning from the posterior tip of each proostracum from 43 individuals (23 females and 20 males).

The $\delta^{13}C$ and $\delta^{15}N$ values of each proostracum section were determined by IsoPrime 100 isotope ratio mass-spectrometer (IsoPrime Corporation, Cheadle, UK) and vario ISOTOPE cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). International reference materials (USGS 24 (-16.049‰v PDB), USGS 26(53.7‰v N2)) 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.

Since each proostracum section recorded a ten-day period of squid growth, the ontogeny of isotopic values may provide a relative measure of the dietary during the early-life stages (Li et al., 2017). The temporal variations of isotopic values were estimated at the population level, meaning that all male or female data were grouped by the same time period.

We also used Stable Isotope Bayesian Ellipses (SIBER) package in R statistical software, vers. 3.4.0 (R Core Team, 2017), to estimated isotopic niche in the $\delta^{13}C$-$\delta^{15}N$ space. The standard ellipse corrected area (SEA$_C$) is a metric that is robust for comparisons between

small and unbalanced sample sizes (Jackson et al., 2011). This approach providing a measure of uncertainty and allowing for statistical comparisons between different age-classes and sexes. Total area was not used as it is the area of the convex hull that encompasses all individuals and therefore always increases with sample size. Sexual segregation was estimated as the percentage of $SEA_C$ overlap and sequential isotopic values among different age-classes and sexes. All results were presented as mean ± SD.

## 2.3. Condition index

A total of 1166 individuals were used to determine differences in levels of 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 (see Peig and Green 2009 for details):

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

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

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

$$b_{SMA} = \frac{b_{OLS}}{r}$$

$a$ is a parameter to be estimated. $b_{OLS}$ and r are the slope and Pearson's correlation coefficient of the ordinary least squares regression of $\ln(W)$ on $\ln(ML)$, respectively.

Variations in gonad or somatic condition with maturity stage were investigated using one-way analysis of variance (ANOVA) and pairwise post-hoc comparisons.

## 3. Results

### 3.1. Proostracum growth with gonad development

No maturity stage II or III female was found before 20 cm PL in this study population. In contrast, males showed an early period in the development of the gonad from 17.5 to 20.0 cm PL. Across all interval sizes, consistently increasing trends were detected between levels of gonad investment and PL for both sexes (Fig. 2).
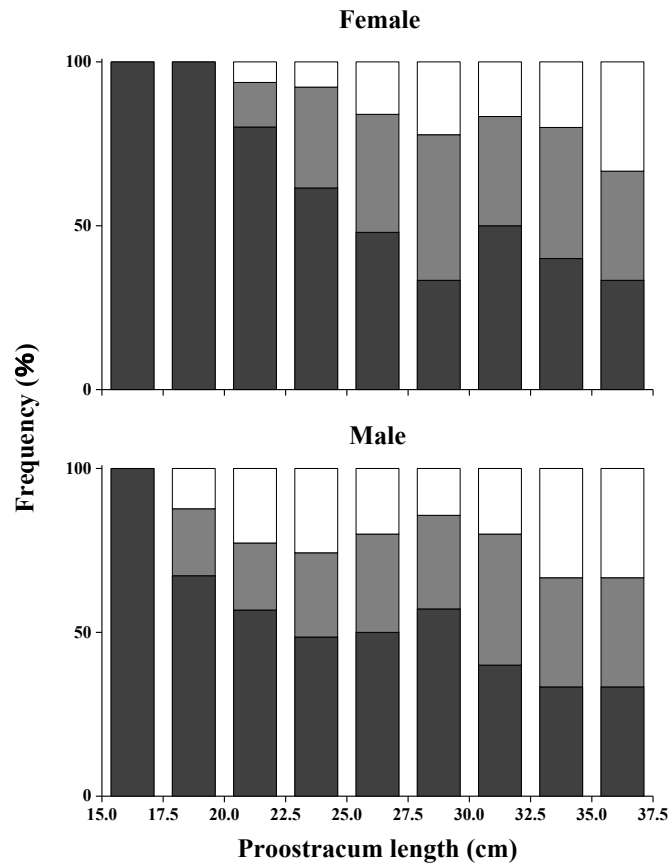


Fig. 2. Frequency distribution of females and males by proostracum length grouped into 2.5 cm intervals of *Dosidicus gigas*. Maturity stage: dark grey, stage I; grey, stage II; white, stage III.

## 3.2. Sexual dimorphism in feeding organs

The PCA retained two components, having eigenvalues greater than 1 and explaining 80.68% of the variance or information contained in the original data set. Meanwhile, twelve main variables (A1-A4, CL, TL, UHL, UCL, URL, UWL, LHL and LRL) of shape variation in arm, tentacle and beak were showed in the PCA results. All these variables showed significant positive relationships with PL (Table 2). In addition, the difference in main morphometric variables between sexes was consistent over their range of PL. The results might be indicating that the size of female feeding organs was significantly greater than males.

Table 2. Correlations to identify the sexual dimorphism in beak shape with proostracum length. Twelve morphometric measurements included: length of each of the four arms (A1-A4) on the right-hand side of each individual, club length (CL), tentacle length (TL) and six 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 analysis of covariance (ANCOVA), these morphometric characteristics were used as dependent variables, and gender as the categorical variable. Proostracum length was used as the covariate to control for body size differences. * and ** reflect statistical significance $p < 0.05$ and $p < 0.01$, respectively.

| | Female | | | Male | | | ANCOVA | |
|---|---|---|---|---|---|---|---|---|
| N | 156 | | | 102 | | | | |
| | $r$ | slope | intercept | $r$ | slope | intercept | $F$ | $p$ |
| TL | 0.76 | 1.35 | 0.78 | 0.80 | 1.45 | -3.46 | 14.31 | ** |
| CL | 0.83 | 0.72 | -4.22 | 0.81 | 0.64 | -4.05 | 32.80 | ** |
| A1 | 0.79 | 0.82 | -3.87 | 0.87 | 0.92 | -6.93 | 8.18 | ** |
| A2 | 0.89 | 0.91 | -4.59 | 0.90 | 0.96 | -6.86 | 18.36 | ** |
| A3 | 0.89 | 0.89 | -3.80 | 0.89 | 0.94 | -5.94 | 18.29 | ** |
| A4 | 0.90 | 0.80 | -3.65 | 0.89 | 0.76 | -3.96 | 29.24 | ** |
| UHL | 0.92 | 0.97 | -2.94 | 0.93 | 1.00 | -4.68 | 18.91 | ** |
| UCL | 0.91 | 1.18 | -3.27 | 0.91 | 1.17 | -4.53 | 29.88 | ** |
| URL | 0.85 | 0.34 | -0.78 | 0.86 | 0.33 | -0.99 | 25.81 | ** |
| UWL | 0.84 | 0.28 | -0.48 | 0.81 | 0.26 | -0.50 | 15.84 | ** |
| LHL | 0.86 | 0.27 | -0.13 | 0.71 | 0.26 | -0.09 | 11.31 | * |
| LRL | 0.87 | 0.33 | -0.85 | 0.85 | 0.32 | -0.98 | 5.37 | ** |

### 3.2. Ontogeny of isotopic values and niche segregation

Isotopic values for all samples are presented in Fig. 3. Females and males exhibited similar $\delta^{13}C$ values (-17.34 ± 0.76 ‰ and -17.47 ± 0.64 ‰, respectively, ANOVA, $F_{1, 231} = 2.07$, $p = 0.15$). However, they differed in their $\delta^{15}N$ values. Males had a much wider range in $\delta^{15}N$ values (Fig.3B), such that most males showing lower values than females during the last age groups ($\delta^{15}N_{female}$: 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, $F_{1, 231} = 47.06$, $p < 0.01$).

Both sexes exhibited alterations in their isotopic niches (Fig.4). Furthermore, the overlap of isotopic niches between females and males exhibited a significantly decreasing trend through time/age ($r$ = -0.84, $p$ < 0.01). From similar niches in embryonic and paralarval stages to a rarely overlap in core isotopic niche for females and males (Fig.4, Table4).
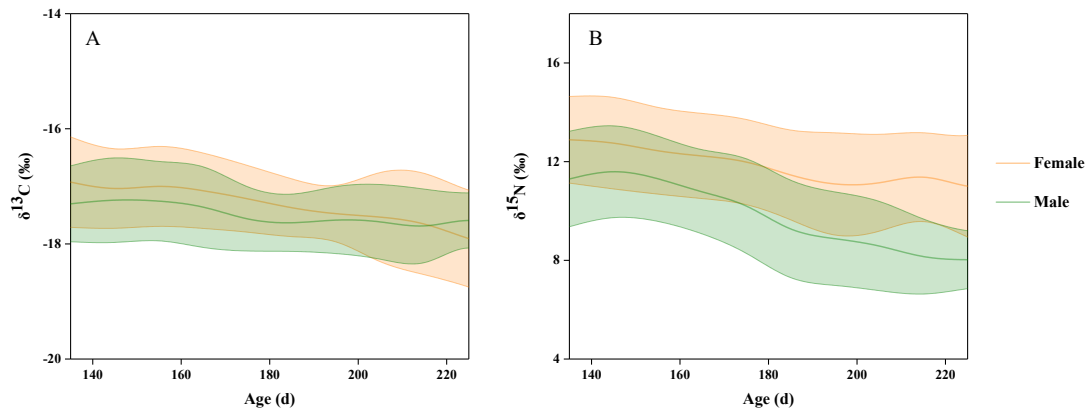


Fig. 3. Ontogenetic time series of $\delta^{13}C$ and $\delta^{15}N$ and mean (1SD) values.
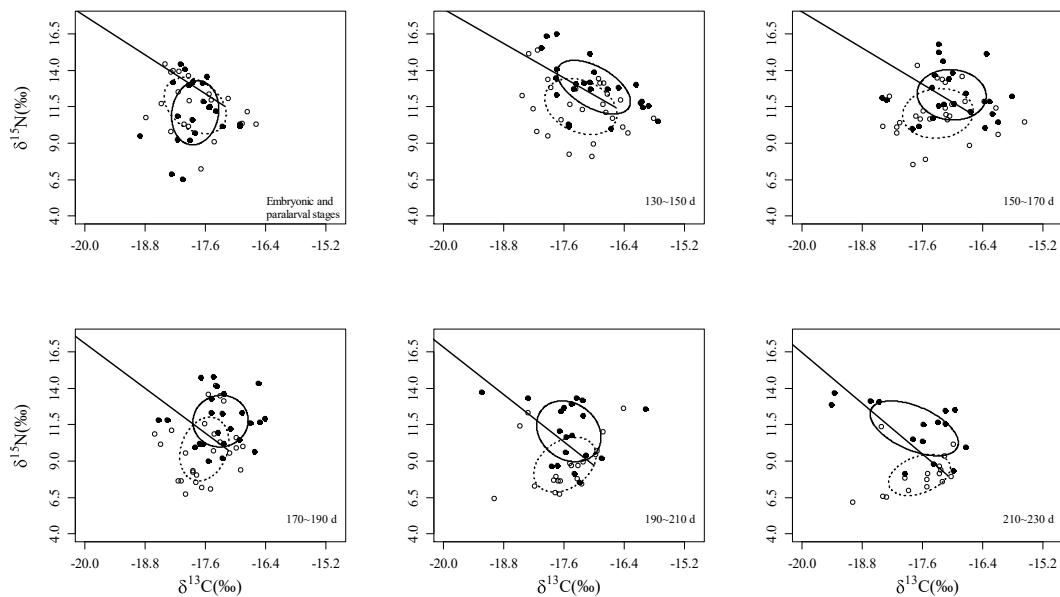


Fig. 4. Isotopic niche areas (calculated with $SEA_C$) for female (black circle) and male (white circle) *Dosidicus gigas* during different age-classes (see Table 3 for the ellipse areas and overlap area values).

## 3.4. Energy allocation in early maturity stages

Females and males of similar maturity stages exhibited a different body or somatic condition throughout their development. Female somatic condition did not vary greatly

between maturity stages (ANOVA, $F_{2, 823}$ = 0.01, $p$ = 0.99, Fig5A). In contrast, highly significant ($F_{2, 322}$ = 9.74, $p < 0.01$, Fig5B) was found in male somatic condition with post hic Tukey HSD comparisons, indicating that values for maturity stage I (326.78 ± 38.95) were significantly higher than those of females in stage II (307.09 ± 39.72) and stage III (302.74 ± 45.30), with no significant difference between the last two stages.

Significant differences in gonad condition values were detected between maturity stages for both sexes (Fig5B and 5D). For females (ANOVA, $F_{2, 823}$ = 202.61, $p < 0.01$), no significant difference was observed between stage I and II (Tukey HSD, $p$ = 0.24). However, the gonad conditions of both stages were significantly lower than those of individuals in stage III ($p < 0.01$). Unlike females, males exhibited a progressive change in gonad condition through maturity stages (ANOVA, $F_{2, 322}$ = 99.67, $p < 0.01$)

## 4. Discussion

Combining arm, tentacle and beak size data and gladius stable isotope signatures, this study provides new information relating to the understanding of ontogenetic patterns and food partitioning between female and male *D. gigas* from Northern Humboldt Current Ecosystem. Due to their size dimorphism in feeding organs and different nutrient requirements, ontogeny of sexual segregation was detected: greater foraging abilities and higher $\delta^{15}$N values of females than males; ontogeny of isotopic niche differentiation along with sexual responses in energy allocation.

### 4.1. Size dimorphism on sexual segregation

For *D. gigas*, sexual size dimorphism was found in arm, tentacle and beak with the same PL, which is a part of gladius generally scales with body size. Moreover, this size divergence between sexes was progressive change over their range of PL. The results indicated that the size of female feeding organs was significantly greater than the males even when they have the same body size. As functional feeding organs, arm, tentacle and beak can be used as morphologic indicators to explain the differences in resource use (Hanlon and Messenger, 1996 P47; Franco-Santos et al., 2014). Although detailed studies on relationship between these organs and prey size are lacking, the scale of these organs are related to the degree of its pulling and bite forces (Kear, 1994; Hanlon and Messenger, 1996 P47). According to the optimal foraging theory, predators should maximize their energy gain while simultaneously

minimizing costs (Stephens and Krebs, 1986). It appears that the stronger foraging ability of female *D. gigas* allows them to capture larger prey items than the males during early life-stages, and potentially they have different resource use. This conclusion contrasts to some extent with stomach contents of *D. gigas* collected from Northern Humboldt Current (Alegre et al., 2014, Rosas-Luis and Chompoy-Salazar, 2016), which suggested that female and male *D. gigas* have similar diets instead of having the size of preys considered. Cephalopod has its special feeding behavior, any food must pass through the esophagus, which located in the middle of the brain. Different sizes of prey were sliced into small pieces by beak while the radula further shred them, which are used for identification in stomach content analysis (Hanlon and Messenger, 1996). This mechanism, therefore, causes the potential bias in estimation of the stomach contents (Rodhouse and Nigmatullin, 1996), and hardly reflect the size of prey, especially the coexisting females and males forage for a wide variety of prey types (Lorrain et al., 2011; Alegre et al., 2014).

SIA has proved to be an alternative approach to explore differences in foraging between sexes (Bearhop et al., 2006; Nifong et al., 2015; Kernaléguen et al., 2016). Moreover, sequential isotopic signatures along *D. gigas* gladius provide higher resolution in dietary and migration than muscle (Ruiz-Cooley et al., 2013; Li et al., 2017), the latter may take several weeks for integration (Lorrain et al., 2011). In this study, the $\delta^{13}C$ and $\delta^{15}N$ values showed variable isotopic shifts along the proostracum for both sexes (Fig. 3). Even if there was no difference in $\delta^{13}C$ values between female and male *D. gigas*, the large range of $\delta^{13}C$ values exhibited by females (3.89 ‰) and males (3.44 ‰), still revealed its complex foraging and migratory behavior. The gradients in $\delta^{13}C$ values in the Northern Humboldt Current Ecosystem have been well recognized (Argüelles et al., 2012; Ruiz-Cooley et al., 2012). Therefore, the $\delta^{13}C$ values can be considered as an indicator of *D. gigas* foraging area, namely the females and males had similar migration pattern in the period studied, may be due to its collective behavior. In contrast to $\delta^{13}C$ values, a significant higher $\delta^{15}N$ values was revealed in females than those of males. Since both sexes had similar migration pattern, the differences in habitat use are probably the major determinant of sex-specific $\delta^{15}N$ values. These results indicating that the females feeding at a higher trophic level than males. Besides, the mean difference of $\delta^{15}N$ values is 1.82 ‰, less than 1 trophic level (2.75‰, Caut et al., 2009),

suggesting that the variation comes from differences in the relative amounts of different prey items ingested, and both sexes have the similar diet types. This conclusion confirmed our hypothesis that female *D. gigas* can capture larger prey items than males.

### 4.2. Ontogeny of trophic niche segregation

Comparison of the isotopic niches of females and males exhibited ontogeny of niche partitioning, from similar isotopic niche to a rarely overlap (Fig. 4, Table 3). As a voracious predator, resource competition between female and male *D. gigas* may progressively intense during its rapid growth. In general, predators can alleviate intraspecific competition by food partitioning (Dayan and Simberloff, 2005). Therefore, sexual niche segregation found in the changing patterns in our study presumably reflecting the greater partitioning of resources when *D. gigas* have larger body size, suggesting that females and males can coexist through foraging more extensive area or prey types. This pattern would be likely to reduce the sexual resource competition and maintain their functional roles in community, which has been widely observed in marine organisms (Bearhop et al., 2006; Kernaléguen et al., 2016). Ontogeny of isotopic niche differentiation confirmed the presence of food partitioning results from sexual size dimorphism in this study. However, this factor does not fully explain the considerable overlaps as shown in Fig. 4, even if there is size divergence in feeding organs between sexes.

Table 3. Sex-specific isotopic niches. Sexual segregation was estimated as the percentage of SEA$_C$ overlap.

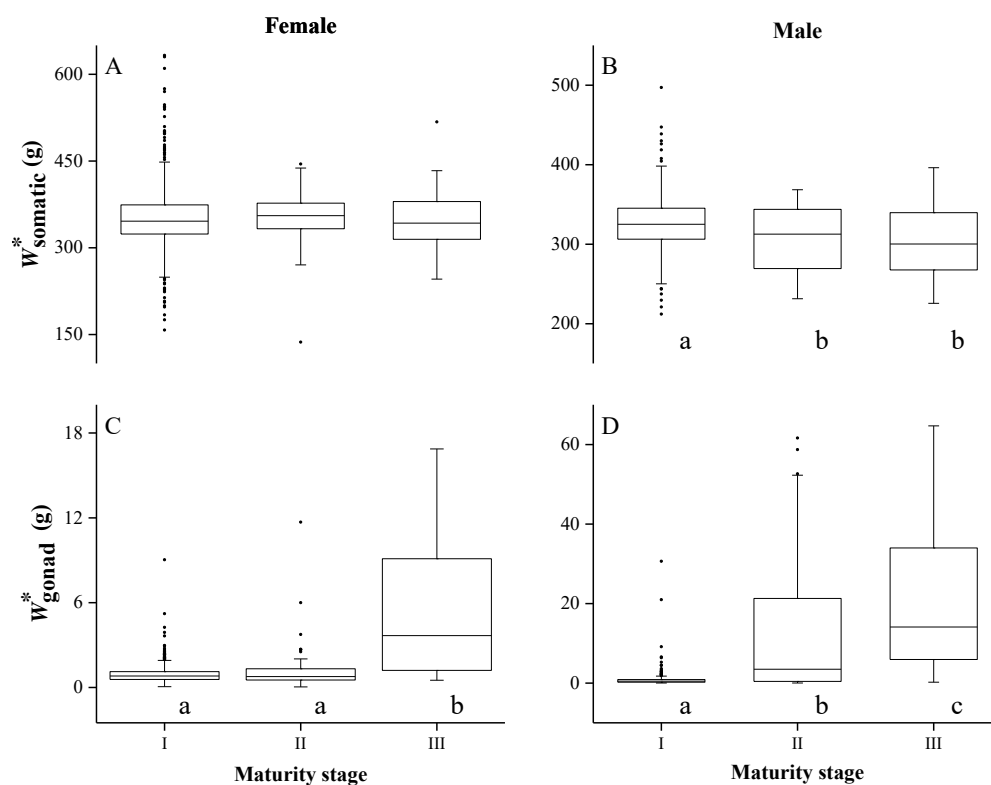| Age class (d) | SEA$_C$ (‰$^2$) | | Overlap size (‰$^2$) | SEA$_C$ Overlap (%) | |
|---|---|---|---|---|---|
| | Female | Male | | Female | Male |
| Embryonic and paralarval stages | 3.22 | 3.54 | 2.60 | 80.55 | 73.36 |
| 130-150 | 3.55 | 4.17 | 1.49 | 41.91 | 35.70 |
| 150-170 | 3.74 | 3.78 | 1.82 | 48.69 | 48.26 |
| 170-190 | 3.06 | 3.26 | 1.07 | 35.08 | 32.97 |
| 190-210 | 4.12 | 3.46 | 1.16 | 28.06 | 33.47 |
| 210-230 | 4.43 | 2.54 | 0.01 | 0.28 | 0.49 |

Fig. 5. Ontogenetic trends in somatic or gonad condition estimated by the condition index method called the Scaled mass index (Peig and Green 2009). There was 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.

Except size dimorphism, nutrient requirement is another main factor results in sexual separation (Kernaléguen et al., 2016). Indeed, differences in nutrient requirements always been found in female and male cephalopod (Steer and Jackson, 2004; Pecl and Moltschaniwskyj, 2006; Lin et al., 2015), they proposed that these alterations reflect the divergent energetic needs for growth and reproduction in different life-stages. Meanwhile, this pattern may 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. In this study, both sexes showed an ascent in gonad investment with maturity stages. Though males require relatively fewer energy for reproduction compared with females

(Stearns, 1992), a negative correlation between gonad and somatic conditions was observed (Fig. 5). This suggests males, which increase energetic allocation towards gonad development, subsequently decrease somatic condition. In contrast, females can supply energetic requirements for somatic growth while simultaneously allocate more energy for gonad development. This possibly due to their food partitioning and energy allocation, males may forage more times to suit their lower food availability (i.e. smaller size of prey), which may also increase the energy consumption.

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## Appendix

Table S1. Characteristics and isotopic values of *Dosidicus gigas* sampled for stable isotope analysis. F: females; M: males.

| Serial Number | Sampling location | Sampling date | Body mass (g) | Mantle length (cm) | Sex | $\delta^{13}C$ | | | | $\delta^{15}N$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Mean | SD | Maximum | Minimum | Mean | SD | Maximum | Minimum |
| 1 | 81°21′W11°27′S | 2013/08/25 | 987 | 33.4 | F | -16.96 | 0.67 | -15.72 | -17.73 | 9.30 | 0.84 | 10.50 | 8.16 |
| 2 | 81°45′W10°54′S | 2013/08/18 | 496 | 26.6 | F | -17.65 | 0.11 | -17.51 | -17.81 | 10.17 | 0.10 | 10.32 | 10.00 |
| 3 | 81°45′W12°00′S | 2013/08/09 | 843 | 30.8 | F | -17.37 | 0.36 | -16.74 | -18.20 | 12.16 | 1.01 | 13.67 | 10.60 |
| 4 | 81°11′W12°46′S | 2013/09/19 | 467 | 26.6 | F | -18.12 | 0.46 | -17.36 | -18.54 | 12.19 | 0.46 | 13.06 | 11.81 |
| 5 | 81°49′W11°31′S | 2013/09/15 | 1090 | 33.8 | F | -17.38 | 0.23 | -17.08 | -17.79 | 11.32 | 0.85 | 12.74 | 10.38 |
| 6 | 81°49′W11°31′S | 2013/09/15 | 1229 | 35.4 | F | -17.03 | 0.68 | -16.17 | -18.23 | 10.44 | 1.78 | 13.00 | 8.13 |
| 7 | 81°45′W10°54′S | 2013/08/18 | 587 | 28.0 | F | -16.64 | 0.86 | -15.82 | -17.85 | 11.06 | 1.27 | 12.22 | 8.65 |
| 8 | 82°53′W12°23′S | 2014/08/21 | 661 | 28.1 | F | -17.22 | 0.44 | -16.21 | -17.76 | 10.43 | 2.15 | 13.49 | 7.53 |
| 9 | 79°42′W15°42′S | 2014/06/12 | 910 | 31.3 | F | -18.23 | 1.08 | -17.00 | -19.61 | 13.36 | 0.56 | 14.06 | 12.31 |
| 10 | 80°00′W15°03′S | 2014/06/08 | 743 | 29.0 | F | -17.23 | 0.51 | -16.32 | -18.05 | 13.96 | 1.37 | 15.55 | 11.53 |
| 11 | 80°43′W15°10′S | 2014/06/16 | 855 | 31.5 | F | -17.17 | 0.92 | -16.05 | -18.64 | 12.45 | 0.80 | 14.23 | 11.67 |
| 12 | 80°27′W15°24′S | 2014/06/24 | 678 | 29.6 | F | -16.96 | 0.57 | -15.98 | -17.95 | 14.65 | 1.54 | 16.84 | 12.54 |
| 13 | 81°00′W13°25′S | 2013/09/03 | 483 | 26.0 | M | -17.72 | 0.65 | -16.83 | -18.49 | 10.89 | 0.58 | 11.69 | 10.18 |
| 14 | 81°00′W13°25′S | 2013/09/03 | 410 | 24.9 | M | -18.24 | 0.26 | -17.93 | -18.61 | 10.09 | 0.51 | 10.86 | 9.51 |
| 15 | 81°01′W13°17′S | 2013/08/30 | 441 | 25.4 | M | -17.66 | 0.16 | -17.49 | -17.93 | 11.69 | 1.03 | 13.39 | 10.65 |
| 16 | 82°17′W13°53′S | 2014/08/18 | 768 | 26.7 | M | -17.90 | 0.43 | -17.17 | -18.43 | 9.91 | 1.67 | 12.28 | 7.94 |
| 17 | 82°17′W13°53′S | 2014/08/18 | 864 | 30.2 | M | -16.96 | 0.29 | -16.47 | -17.51 | 10.23 | 1.44 | 11.97 | 7.74 |
| 18 | 82°17′W13°53′S | 2014/08/18 | 643 | 27.9 | M | -17.40 | 0.45 | -16.81 | -18.15 | 10.09 | 2.59 | 13.37 | 7.46 |
| 19 | 82°53′W12°23′S | 2014/08/21 | 925 | 28.6 | M | -17.89 | 0.58 | -17.13 | -19.00 | 9.21 | 2.27 | 12.74 | 6.46 |

| 20 | 82°53′W12°23′S | 2014/08/21 | 819 | 31.4 | M | -17.23 | 1.07 | -15.56 | -19.00 | 8.98 | 2.07 | 12.35 | 6.19 |
| 21 | 82°53′W12°23′S | 2014/08/21 | 985 | 32.8 | M | -17.57 | 0.66 | -16.41 | -18.57 | 11.67 | 3.17 | 15.37 | 7.32 |
| 22 | 82°53′W12°23′S | 2014/08/21 | 703 | 29.6 | M | -17.12 | 0.74 | -16.09 | -18.32 | 8.11 | 1.38 | 10.10 | 6.53 |
| 23 | 82°53′W12°23′S | 2014/08/21 | 641 | 29.0 | M | -17.51 | 0.32 | -17.03 | -17.99 | 7.68 | 0.47 | 8.25 | 6.75 |
| 24 | 82°53′W12°23′S | 2014/08/21 | 811 | 29.1 | M | -17.49 | 0.62 | -16.89 | -18.48 | 11.98 | 1.58 | 13.60 | 8.89 |
| 25 | 82°17′W13°53′S | 2014/08/18 | 725 | 28.2 | M | -17.19 | 0.24 | -16.63 | -17.49 | 9.47 | 0.97 | 10.86 | 8.13 |