



Ontogenetic and Spatiotemporal Changes in Isotopic Niche of Jumbo Squid (*Dosidicus gigas*) in the Southeastern Pacific

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Jumbo squid (*Dosidicus gigas*) plays an important role in food webs throughout the eastern Pacific; however, its trophic ecology is complex, because it is a highly migratory and opportunistic predator. In this study, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were measured along the lateral wall of lower beak to investigate the ontogenetic and spatiotemporal feeding strategy shifts of jumbo squid, respectively. Both year and area had significant influences on $\delta^{13}\text{C}$ ($p < 0.01$), while sex and stage had no significant influence on $\delta^{13}\text{C}$ ($p > 0.05$). All explanatory variables (sex, stage, year, and area) had significant influences on $\delta^{15}\text{N}$ ($p < 0.05$). The differences of niche among life stages indicated that the habitat and feeding habit were likely changed with the growth of individuals. Compared with normal years, the habitat of squids might change during the El Niño year. However, during the strong El Niño year, habitat location changed and niche size decreased compared to normal years. The trophic niche of *D. gigas* differed between different areas. Stable isotopes in early life stages were most effective for distinguishing different geographic populations. Females and males were likely to inhabit similar geographic areas throughout ontogeny. Unexpectedly, we found evidence to suggest females may have larger habitats and wider trophic niche than males. This study revealed the ontogenetic and spatiotemporal plasticity of habitat and feeding strategy of *D. gigas*.

Keywords: *Dosidicus gigas*, isotopic niche, stable isotope, ontogeny, spatiotemporal changes, sex, El Niño

INTRODUCTION

Jumbo squid is widely distributed from California to Chile in the eastern Pacific (Nigmatullin et al., 2001; Taïpe et al., 2001; Field et al., 2007). It is a main prey item of various fishes, marine birds, and mammals (Nigmatullin et al., 2001; Ruiz-Coolley et al., 2006; Espinoza et al., 2017), and is also an active predator of numerous prey species such as copepods, shrimps, crabs, and fishes (Rodhouse and Nigmatullin, 1996;

Markaida, 2006; Field et al., 2007). It grows fast and is the largest and one of the most abundant species of the family Ommastrephidae (Nigmatullin et al., 2001; Arkhipkin et al., 2014). It is an important commercial target species off the coasts of western South and Central America and the Gulf of California (Gong et al., 2018a). It is an opportunistic voracious predator with a high capability to adapt to environmental variability (Keyl et al., 2008; Ruiz-Cooley et al., 2013). Furthermore, distribution and habitat for jumbo squid have been shown to change with changes in marine environmental factors (Yu et al., 2016; Trasviña-Carrillo et al., 2018; Yu and Chen, 2018; Hu et al., 2019). Range expansion of *D. gigas* occurred during a strong 1997–1998 El Niño event, which was suggested to be correlated with wider distribution for spawning eggs and higher success in egg development during anomalously high-temperature events, coupled with migration of smaller squid (Ruiz-Cooley et al., 2013). Yu and Chen (2018) found that ocean warming might shrink the habitat of *D. gigas* and correspond with a southeastward range-shift in the Southeast Pacific Ocean. Coincident with the variability of the prey items in new habitats, the feeding strategies and diets of *D. gigas* may shift (Ruiz-Cooley et al., 2006; Ruiz-Cooley et al., 2010; Lorrain et al., 2011). Ruiz-Cooley et al. (2010) analyzed stable isotopic signatures along the proostracum of the gladius, and revealed the ontogenetic and geographic variability of foraging for *D. gigas*. In this study, we attempted to investigate the shift of feeding strategies of *D. gigas* by analyzing the ontogenetic and spatiotemporal changes in isotopic niche using the beak lateral wall.

Stable isotope analysis (mainly of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) is a useful tool for investigating the trophic ecology of marine animals, including fishes (Trujillo-Olvera et al., 2018; Varela et al., 2018), marine birds (Castillo et al., 2019), cephalopods (Cherel and Hobson, 2005; Ruiz-Cooley et al., 2006; Ruiz-Cooley et al., 2010; Ruiz-Cooley et al., 2013; Merten et al., 2017; Gong et al., 2018b; Hu et al., 2019; Gong et al., 2020), and mammals (Mendes et al., 2007; Ruiz-Cooley et al., 2012). $\delta^{13}\text{C}$ is used as an indicator of the primary sources of organic matter in the food chain (Post, 2002; McCutchan et al., 2003; Cherel and Hobson, 2007). High $\delta^{13}\text{C}$ values correspond to zones of high productivity in low latitude and coastal waters (Rau et al., 1989; Miller et al., 2008). It can be used to trace the animals' habitats and movements, because $\delta^{13}\text{C}$ changes little (0–1‰) with trophic level (Post, 2002). $\delta^{15}\text{N}$ tends to enrich stepwise from prey to predators (2–5‰), providing a method to estimate a species' trophic level (Post, 2002; Bode et al., 2004). However, the $\delta^{15}\text{N}$ baseline value may be influenced by the oceanographic and biogeochemical processes, which can propagate differently into individual tissues (Ruiz-Cooley and Gerrodette, 2012). The direction and variability of trophic niche among different life stages can reveal shifts of ecological niche. For some species, tissues that grow continuously can record the information of trophic ecology over an individual's whole life span (Mendes et al., 2007; Hunsicker et al., 2010; Kernaléguen et al., 2016).

Cephalopod beaks are hard structures composed of chitin-protein complexes (Miserez et al., 2007; Miserez et al., 2010). This chemical composition makes them resistant to digestion. Beaks encountered in predator stomachs can be used for identification down to species level (Lu and Ickeringill, 2002; Hu et al., 2018). Though the gladius was demonstrated to be a

good tissue to investigate the trophic ecology of squid (Ruiz-Cooley et al., 2010; Lorrain et al., 2011; Gong et al., 2018b), the beak has greater potential because it could be collected from both squids and predator stomachs (Ruiz-Cooley et al., 2006; Hu et al., 2019). Moreover, the beak grows continuously throughout the cephalopod's lifetime by an incremental accretion of non-reactive biological material (Guerra et al., 2010; Canali et al., 2011; Queirós et al., 2018). Due to these characteristics, beak can be a useful material to investigate cephalopod ontogenetic niche shifts. However, in most previous studies, only whole beak isotopic values have been analyzed, making it difficult to deduce an individual's trophic shift and/or movement (Ruiz-Cooley et al., 2006; Cherel et al., 2009). The stable isotope signatures along the upper beak hood were used to reveal the ontogenetic shift in diet and migration (Guerra et al., 2010). However, there is no evidence that the increments deposited along the beak hood. Daily deposition of increments on the lateral wall of beaks from the tip of rostrum to the edge of lateral wall has been validated (Perales-Raya et al., 2010). The isotopic values along the lateral wall of beaks could therefore reveal the information spanning the whole lifetime of squid. Queirós et al. (2018) analyzed the isotopic values of different sections on the lateral wall of beaks of *Kondakovia longimana*, and found that habitat of squid spanned a wide latitudinal range, and trophic level increases throughout its lifetime.

Several literatures suggested that equatorial undercurrent became more intense and pushed warm equatorial surface waters closer to the coast during El Niño events in the southeastern Pacific, and narrowing the suitable habitat of *D. gigas* (Keyl et al., 2008; Yu and Chen, 2018). Therefore, we hypothesized that the trophic niche of the squid would become smaller and the competition between females and males would become greater during El Niño events. In this study, the samples were collected from both normal years (2013 and 2014) and El Niño years (2015 and 2016). We attempted to analyze the variability of trophic niche between normal years and El Niño years by both life stage and sex.

In this study, we quantified stable isotope values along the lower beak lateral wall of *D. gigas*, collected from the southeastern Pacific, to (1) explore the feasibility to investigate the trophic ecology of *D. gigas* using lower beaks lateral wall, and to (2) evaluate ontogenetic and spatiotemporal variability of habitat and feeding strategies for *D. gigas*. This study improves the knowledge of ecological niche shifts of *D. gigas* in four perspectives (sex, ontogeny, annual, and geography).

MATERIALS AND METHODS

Sampling

All jumbo squid were randomly sampled from the catch of Chinese commercial jigging vessels in the Southeastern Pacific. A total of 137 (95 females and 42 males) samples were collected off Peruvian EEZ (exclusive economic zone) from June to September from 2013 to 2016 (**Table 1**, **Figure 1**). Forty females were sampled off Chilean EEZ from October to

TABLE 1 | Sample information of *D. gigas* in southeastern Pacific (ML corresponds to the mantle length; LLWL corresponds to the lower beak lateral wall length; “Peru 2013” represents samples caught off Peruvian EEZ in 2013, “Chile 2015” represents samples caught off Chilean EEZ in 2015, and so on).

| Group | Date | Sex | N | ML/mm | | | | LLWL/mm | | | |
|------------|---------------|-----|----|-------|-----|--------|-------|---------|-------|-------|------|
| | | | | Min | Max | Mean | SD | Min | Max | Mean | SD |
| Peru 2013 | 19 Jul–27 Sep | F | 25 | 257 | 362 | 315.84 | 41.34 | 15.66 | 24.34 | 20.34 | 2.54 |
| | 15 Jul–27 Sep | M | 11 | 264 | 396 | 323.45 | 44.20 | 16.11 | 23.73 | 19.52 | 2.69 |
| Peru 2014 | 12 Jun–13 Sep | F | 21 | 240 | 524 | 369.10 | 83.80 | 14.32 | 35.66 | 21.99 | 5.96 |
| | 12 Jun–18 Jul | M | 8 | 408 | 575 | 477.38 | 64.99 | 21.00 | 37.91 | 27.06 | 5.14 |
| Peru 2015 | 06 Jul–17 Sep | F | 25 | 250 | 456 | 367.40 | 66.75 | 13.26 | 28.68 | 21.91 | 4.07 |
| | 10 Aug–17 Sep | M | 11 | 286 | 490 | 414.91 | 65.03 | 15.87 | 27.66 | 23.60 | 3.61 |
| Peru 2016 | 08 Aug–28 Sep | F | 24 | 288 | 358 | 308.54 | 13.73 | 16.40 | 23.51 | 18.71 | 1.60 |
| | 08 Aug–28 Sep | M | 12 | 231 | 319 | 294.58 | 22.73 | 12.10 | 19.20 | 16.93 | 1.78 |
| Chile 2015 | 20 Oct–23 Dec | F | 40 | 308 | 479 | 367.00 | 40.19 | 21.03 | 28.94 | 24.17 | 1.96 |

November 2015. In this study, 2013 and 2014 were normal years, followed by El Niño events during 2015 and 2016. The El Niño event in 2016 was much stronger than that in 2015 (Jacox et al., 2016).

All specimens were immediately frozen on board. After defrosting the samples in the laboratory, the mantle length and body weight were measured. Meanwhile, sex was identified. The beaks of squids were extracted and stored in 70% alcohol, which does not significantly affect the isotopic composition of beaks (Ruiz-Coolley et al., 2011). Morphometric measurements were conducted for beaks using digital calipers.

Beak Processing and Stable Isotope Analysis

Five sections (the tip of the rostrum, 5.0 mm, 10.0 mm, and 15.0 mm along the lateral wall, and the edge of the lateral wall) were cut along the lower lateral wall from the tip to edge following the “V” shape of the growth lines, which represent five different stages of the squid (Figure 2). Each section’s width is 1.0 mm. All sections were cleaned in ultrasonic cleaner for 10 min and rinsed with distilled water. They were dried and homogenized into fine powder for stable isotope analysis.

A total of 876 subsamples were prepared for SIA. One-milligram powder of each sample was pooled in tin capsule. The SIA was conducted using an ISOPRIME 100 isotope ratio mass spectrometer (Isoprime Corporation, Cheadle, UK) and a vario ISOTOPE cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) at Shanghai Ocean University. To calibrate the system and compensate for drift, the international reference materials [USGS 24 (−16.04‰ v PDB), USGS 26 (53.7‰ v N₂) and a laboratory reference (protein, −26.98‰ v PDB and 5.96‰ v N₂)] were used for calibration every ten samples. The measurement errors of δ¹³C and δ¹⁵N were less than 0.1‰ for both δ¹³C and δ¹⁵N.

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where X is ¹³C or ¹⁵N, and R_{sample} and R_{standard} are the ratios of ¹³C/¹²C or ¹⁵N/¹⁴N of the sample and the standard, respectively.

Generalized Linear Model

Generalized linear model (GLM) is a mathematical extension of linear models that allow for non-linearity and non-constant variance structures. These traits enable GLMs to be commonly used in ecological studies (Guisan et al., 2002; Kim et al., 2012; Kato et al., 2016). To investigate the effects of sex, ontogeny, year, and area on the stable isotopes in beaks, GLMs were built as follows:

$$SI = \text{Sex} + \text{Stage} + \text{Year} + \text{Area} + \epsilon$$

where SI is the measured isotopic ratio (δ¹³C or δ¹⁵N) in the beak; Stage is the life stage of squid; Year is the year of sampling; Area is the sampling area of squid; Sex is the sex of squid; and ε is

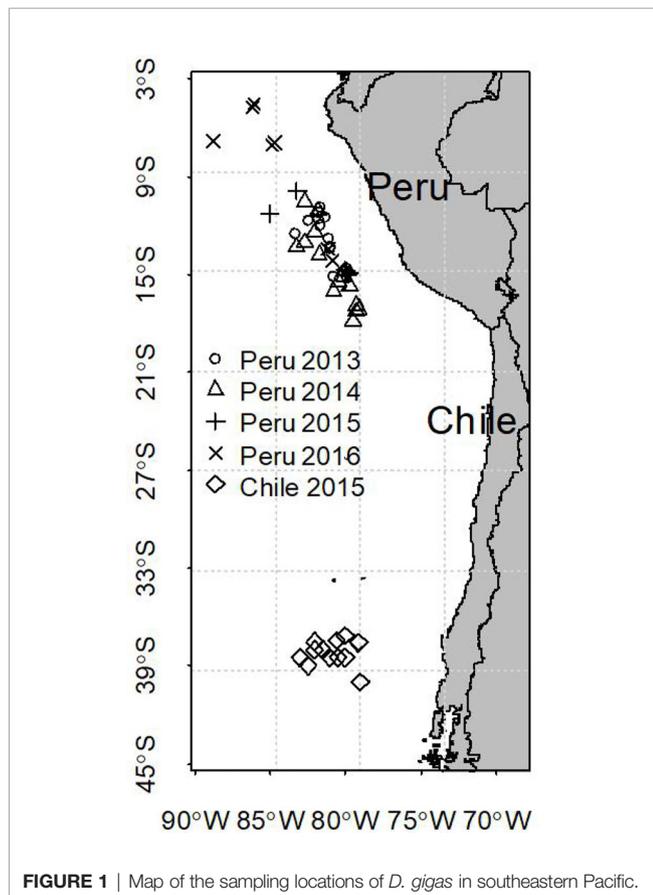


FIGURE 1 | Map of the sampling locations of *D. gigas* in southeastern Pacific.

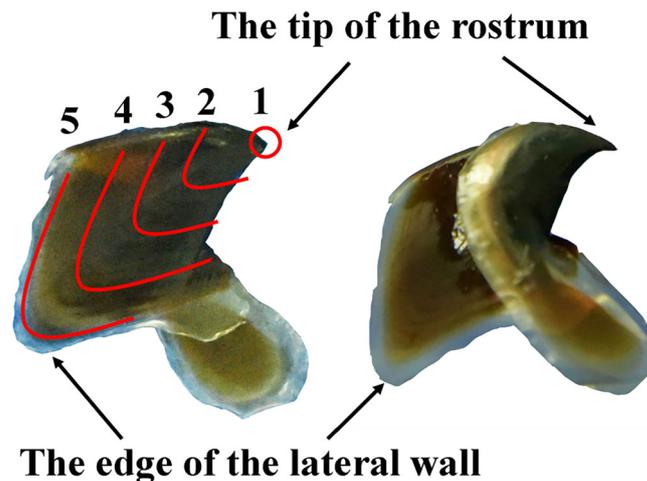


FIGURE 2 | Lateral view of the lateral wall for lower beak (1–5 represent the tip of the rostrum, 5.0 mm, 10.0 mm, and 15.0 mm along the lateral wall, and the edge of the lateral wall, respectively).

the error of the model. Statistical analyses were carried out using the `glm2` package in R 3.5.1.

Variation in Isotopic Values

The gender variability in isotopic values was analyzed using a two-tailed *t*-test for a given area, year, and life stage. The ontogenetic variability in isotopic values was determined using ANOVA for a given area, year, and sex, and the pairwise comparisons among five life stages were evaluated using Tukey HSD (honest significant difference) test. For interannual variability, the same procedure was followed, except a Tukey HSD test considered pairwise comparisons between years.

We analyzed the geographic variability between Peru and Chile in isotopic values using a two-tailed *t*-test for a given sex and life stage. Statistical analyses were performed using SPSS 19.0.

Comparison of Isotopic Niches

The standard ellipse corrected area (SEAc) was used to determine the isotopic niche width and overlap between genders, life stages, years, and areas. This approach is unbiased with sample size and allows robust comparison among data sets (Jackson et al., 2011). Variation in SEAc size was statistically evaluated by comparing the posterior distributions of standard ellipse area using the SIBER package in R. Statistical analyses were performed using R 3.5.1.

RESULTS

Variability in Isotopic Values

The results of GLMs suggested that both year and area had significant influences on $\delta^{13}\text{C}$ ($p < 0.001$), while sex and stage had no significant influence on $\delta^{13}\text{C}$ ($p > 0.05$) (Table 2). All explanatory variables (sex, stage, year, and area) had significant influences on $\delta^{15}\text{N}$ ($p < 0.05$) (Table 2).

For the sexual variability off Peru, $\delta^{13}\text{C}$ significantly differed between genders for stage 3 and 5 in 2013 (*t*-test, $p < 0.05$), while no significant difference was found for other groups (*t*-test, $p > 0.05$) (Table 3 and Figure 3). $\delta^{15}\text{N}$ significantly differed between genders for stage 2, 3, and 4 in 2014 (*t*-test, $p < 0.05$), and no significant difference was found for other groups (*t*-test, $p > 0.05$) (Table 3 and Figure 3).

For the ontogenetic variability, We found significant differences in $\delta^{13}\text{C}$ among five stages for Peru 2016 and Chile 2015 (ANOVA, $p < 0.001$). No significant difference was found for Peru 2013, 2014, and 2015 (ANOVA, $p > 0.05$) (Table 3 and Figure 3). $\delta^{15}\text{N}$ differed among five stages for Peru 2014 and 2016 and Chile 2015 (ANOVA, $p < 0.001$), and no significant difference was found for Peru 2013 and 2015 (ANOVA, $p > 0.05$) (Table 3 and Figure 3).

For the annual variability off Peru, $\delta^{13}\text{C}$ significantly differed among 4 years for stage 1 and 2 of females and for stage 1, 2, and

TABLE 2 | Statistical outputs of generalized linear model in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for *D. gigas*.

| Explanatory variable | $\delta^{13}\text{C}$ SE | <i>t</i> value | <i>p</i> | $\delta^{15}\text{N}$ SE | <i>t</i> value | <i>p</i> |
|----------------------|-----------------------------|----------------|----------|-----------------------------|----------------|----------|
| Sex | 0.057 | -0.336 | 0.737 | 0.183 | 2.316 | 0.021 |
| Stage | 0.016 | -0.098 | 0.922 | 0.052 | -4.969 | 8.11e-07 |
| Year | 0.023 | 5.589 | 3.10e-08 | 0.074 | -14.700 | < 2e-16 |
| Area | 0.059 | 8.169 | 1.10e-15 | 0.189 | 9.720 | < 2e-16 |

TABLE 3 | The summary of isotopic values in lower beaks for *D. gigas*.

| Group | Sex | Stage | $\delta^{13}\text{C}$ (‰) | | $\delta^{15}\text{N}$ (‰) | |
|------------|-----|-------|---------------------------|------|---------------------------|------|
| | | | Mean | SD | Mean | SD |
| Peru 2013 | F | 1 | -18.02 | 0.52 | 9.36 | 1.76 |
| | | 2 | -17.50 | 0.34 | 10.16 | 1.67 |
| | | 3 | -17.22 | 0.36 | 10.97 | 1.48 |
| | | 4 | -17.30 | 0.40 | 10.38 | 1.89 |
| | | 5 | -18.27 | 0.46 | 8.72 | 1.92 |
| | M | 1 | -18.21 | 0.47 | 10.44 | 2.14 |
| | | 2 | -17.63 | 0.37 | 10.83 | 1.69 |
| | | 3 | -17.56 | 0.43 | 11.78 | 1.73 |
| | | 4 | -17.45 | 0.32 | 11.22 | 1.98 |
| | | 5 | -18.59 | 0.40 | 8.99 | 2.27 |
| Peru 2014 | F | 1 | -18.45 | 0.54 | 8.32 | 3.12 |
| | | 2 | -17.99 | 0.51 | 10.42 | 2.45 |
| | | 3 | -17.93 | 0.55 | 11.42 | 2.20 |
| | | 4 | -17.84 | 0.62 | 11.81 | 2.50 |
| | | 5 | -18.24 | 0.59 | 10.08 | 3.20 |
| | M | 1 | -18.19 | 0.26 | 10.15 | 2.56 |
| | | 2 | -17.94 | 0.28 | 12.27 | 1.49 |
| | | 3 | -17.97 | 0.27 | 12.99 | 1.31 |
| | | 4 | -17.85 | 0.38 | 13.73 | 0.98 |
| | | 5 | -17.98 | 0.66 | 11.84 | 1.36 |
| Peru 2015 | F | 1 | -17.40 | 0.56 | 8.43 | 1.85 |
| | | 2 | -16.82 | 0.44 | 9.00 | 1.41 |
| | | 3 | -16.73 | 0.49 | 9.84 | 1.33 |
| | | 4 | -16.70 | 0.44 | 9.18 | 1.16 |
| | | 5 | -17.57 | 0.50 | 7.71 | 1.14 |
| | M | 1 | -17.21 | 0.73 | 7.74 | 1.35 |
| | | 2 | -16.73 | 0.66 | 8.74 | 2.20 |
| | | 3 | -16.77 | 0.81 | 9.75 | 1.90 |
| | | 4 | -16.61 | 0.67 | 9.60 | 2.19 |
| | | 5 | -17.70 | 0.38 | 8.12 | 1.67 |
| Peru 2016 | F | 1 | -17.62 | 0.43 | 7.66 | 0.68 |
| | | 2 | -17.44 | 0.39 | 7.44 | 0.65 |
| | | 3 | -17.24 | 0.41 | 8.02 | 0.70 |
| | | 4 | -17.41 | 0.47 | 7.26 | 0.76 |
| | | 5 | -18.40 | 0.75 | 5.74 | 0.76 |
| | M | 1 | -17.46 | 0.40 | 7.25 | 0.92 |
| | | 2 | -17.39 | 0.41 | 7.19 | 0.41 |
| | | 3 | -17.27 | 0.44 | 7.78 | 0.43 |
| | | 4 | -17.46 | 0.39 | 7.05 | 0.51 |
| | | 5 | -18.44 | 0.55 | 5.61 | 1.01 |
| Chile 2015 | F | 1 | -17.87 | 0.69 | 12.17 | 2.02 |
| | | 2 | -16.78 | 0.32 | 10.90 | 2.10 |
| | | 3 | -16.82 | 0.38 | 11.03 | 2.65 |
| | | 4 | -16.63 | 0.36 | 9.99 | 2.28 |
| | | 5 | -17.07 | 0.38 | 7.73 | 2.35 |

"Peru 2013" represents the samples collected off Peru during 2013, and so on.

3 of males (ANOVA, $p < 0.05$) (Table 3 and Figure 3). $\delta^{15}\text{N}$ significantly differed among 4 years for all stages of both females and males (ANOVA, $p < 0.01$) (Table 3 and Figure 3).

For the geographic variability, $\delta^{13}\text{C}$ had significant differences between Peru and Chile in 2015 for stage 1 and 5 of females (t -test, $p < 0.01$) (Table 3 and Figure 3). $\delta^{15}\text{N}$ had significant differences between Peru and Chile in 2015 for stage 1, 2, and 3 of females (t -test, $p < 0.05$) (Table 3 and Figure 3).

Ontogenetic Variation in Isotopic Niche

Off Peru, the niche overlaps between stage 1 and stages 3 and 4 in 2013, 2014, and 2015 ranged from 0 to 19% for females and

from 0 to 23% for males, while overlaps for the same life stages in 2016 ranged from 33% to 59% for females and from 55% to 92% for males (Tables 4, 5 and Figure 4). The niche overlaps between stages 1 and 5 for Peru 2013, 2014, and 2015 ranged from 62% to 66% for females and from 29% to 57% for males, while there was no overlap for those in 2016 (Tables 4, 5 and Figure 4). Except for 2014, there was no overlap between stage 5 and stages 2, 3, and 4 for squids off Peru (Tables 4, 5 and Figure 4).

Off Chile, no overlap was found between stage 1 and stages 2, 3, 4, and 5 for females in 2015, while overlaps ranged from 17% to 99% between other stages (Tables 4, 5 and Figure 4).

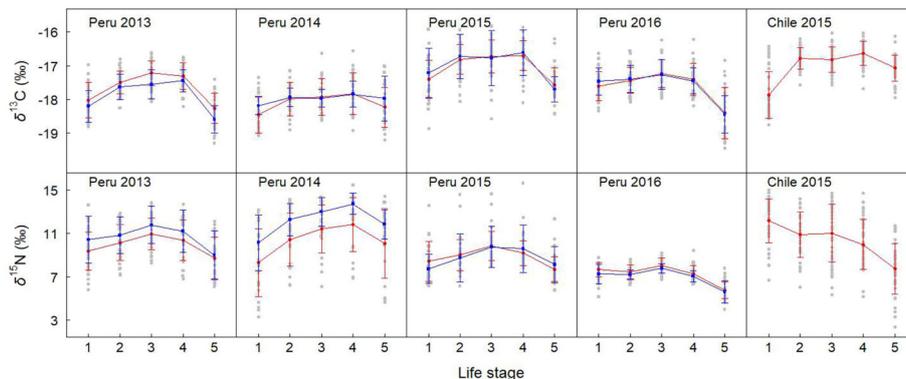


FIGURE 3 | Mean ± standard deviation isotopic values in lower beaks of females (red solid circle) and males (blue solid square) in each life stage. “Peru 2013” represents the samples collected off Peru during 2013, and so on.

TABLE 4 | Ontogenetic variation in isotopic niche of females for different groups.

| Group | | Stage 1 | Stage 2 | Stage 3 | Stage 4 | Stage 5 |
|------------|---------|---------|---------|---------|---------|---------|
| Peru 2013 | Stage 1 | | 0.027 | 0.003 | 0.247 | 0.419 |
| | Stage 2 | 15/25 | | 0.200 | 0.119 | 0.043 |
| | Stage 3 | 0/0 | 21/27 | | 0.023 | 0.008 |
| | Stage 4 | 8/9 | 77/55 | 89/49 | | 0.312 |
| | Stage 5 | 60/63 | 0/0 | 0/0 | 0/0 | |
| Peru 2014 | Stage 1 | | 0.163 | 0.135 | 0.386 | 0.363 |
| | Stage 2 | 29/40 | | 0.467 | 0.243 | 0.099 |
| | Stage 3 | 13/19 | 69/72 | | 0.215 | 0.082 |
| | Stage 4 | 10/11 | 64/52 | 94/74 | | 0.269 |
| | Stage 5 | 66/59 | 86/57 | 72/46 | 52/42 | |
| Peru 2015 | Stage 1 | | 0.223 | 0.020 | 0.333 | 0.142 |
| | Stage 2 | 7/11 | | 0.097 | 0.367 | 0.034 |
| | Stage 3 | 0/0 | 48/47 | | 0.047 | 0.001 |
| | Stage 4 | 4/6 | 72/74 | 64/68 | | 0.065 |
| | Stage 5 | 37/62 | 0/0 | 0/0 | 0/0 | |
| Peru 2016 | Stage 1 | | 0.421 | 0.156 | 0.119 | 0.003 |
| | Stage 2 | 65/73 | | 0.111 | 0.082 | 0.001 |
| | Stage 3 | 33/32 | 40/34 | | 0.439 | 0.059 |
| | Stage 4 | 59/46 | 90/64 | 40/33 | | 0.080 |
| | Stage 5 | 0/0 | 0/0 | 0/0 | 0/0 | |
| Chile 2015 | Stage 1 | | 0.002 | 0.088 | 0.499 | 0.357 |
| | Stage 2 | 0/0 | | 0.079 | 0.002 | 0.010 |
| | Stage 3 | 0/0 | 99/68 | | 0.096 | 0.162 |
| | Stage 4 | 0/0 | 68/55 | 50/59 | | 0.362 |
| | Stage 5 | 0/0 | 17/14 | 20/25 | 19/20 | |

For each group, bottom left values are percentages of SEAc overlaps (the first value is the overlap of the younger stage over the older stage, and the second value is the overlap of the older stage over the younger stage), and top right values are p-values of SEAc comparisons. “Peru 2013” represents the samples collected off Peru during 2013, and so on.

Annual and Geographic Variation in Isotopic Niche

For the annual variation of females off Peru, there were relatively large overlaps between 2013 and 2014 throughout the life stages, and the maximum overlap (98%) was observed during stage 5 (Table 6, Figure 4). The overlap was 62% between 2015 and 2016 during stage 1, then no overlap was found from stage 2 to 5. The overlap between 2015 and 2013 and 2014 ranged from 6% to 37% and 0 to 5% throughout the life stage. There was no overlap between 2016 and 2013, 2014, and 2015 after stage 1. Except for

stages 3 and 5, SEAcS in 2016 were significantly smaller than those in 2013 ($p < 0.01$). SEAcS in 2016 were significantly smaller than those in 2014 throughout the life stage ($p < 0.001$).

For the annual variation of males off Peru, largest overlaps occurred during stage 1 between 2013 and 2014 (86%), and 2015 and 2016 (95%) (Table 7, Figure 4). The isotopic niches in 2015 and 2016 were separated after stage 1. There were overlaps between 2013 and 2015 during stages 2, 3, and 4, while no overlap was observed between 2016 and 2013 and 2014 throughout the life stage. All SEAcS in 2016 were smaller than

TABLE 5 | Ontogenetic variation in isotopic niche of males for different groups.

| Group | | Stage 1 | Stage 2 | Stage 3 | Stage 4 | Stage 5 |
|-----------|---------|---------|---------|---------|---------|---------|
| Peru 2013 | Stage 1 | | 0.094 | 0.175 | 0.117 | 0.395 |
| | Stage 2 | 14/26 | | 0.356 | 0.459 | 0.144 |
| | Stage 3 | 4/7 | 58/50 | | 0.411 | 0.253 |
| | Stage 4 | 0/0 | 60/56 | 74/80 | | 0.185 |
| | Stage 5 | 31/35 | 0/0 | 0/0 | 0/0 | |
| Peru 2014 | Stage 1 | | 0.222 | 0.140 | 0.111 | 0.394 |
| | Stage 2 | 26/38 | | 0.375 | 0.331 | 0.157 |
| | Stage 3 | 14/23 | 64/76 | | 0.452 | 0.092 |
| | Stage 4 | 0/0 | 23/29 | 46/50 | | 0.078 |
| | Stage 5 | 29/27 | 68/44 | 44/24 | 0/0 | |
| Peru 2015 | Stage 1 | | 0.111 | 0.079 | 0.488 | 0.031 |
| | Stage 2 | 23/26 | | 0.403 | 0.108 | 0.001 |
| | Stage 3 | 0/0 | 42/60 | | 0.068 | 0.001 |
| | Stage 4 | 0/0 | 42/49 | 79/64 | | 0.031 |
| | Stage 5 | 35/57 | 0/0 | 0/0 | 0/0 | |
| Peru 2016 | Stage 1 | | 0.028 | 0.005 | 0.136 | 0.324 |
| | Stage 2 | 45/94 | | 0.244 | 0.195 | 0.063 |
| | Stage 3 | 29/55 | 22/20 | | 0.065 | 0.015 |
| | Stage 4 | 48/92 | 82/75 | 15/15 | | 0.261 |
| | Stage 5 | 0/0 | 0/0 | 0/0 | 0/0 | |

For each group, bottom left values are percentages of SEAc overlaps (the first value is the overlap of the younger stage over the older stage, and the second value is the overlap of the older stage over the younger stage), and top right values are p-values of SEAc comparisons. "Peru 2013" represents the samples collected off Peru during 2013, and so on.

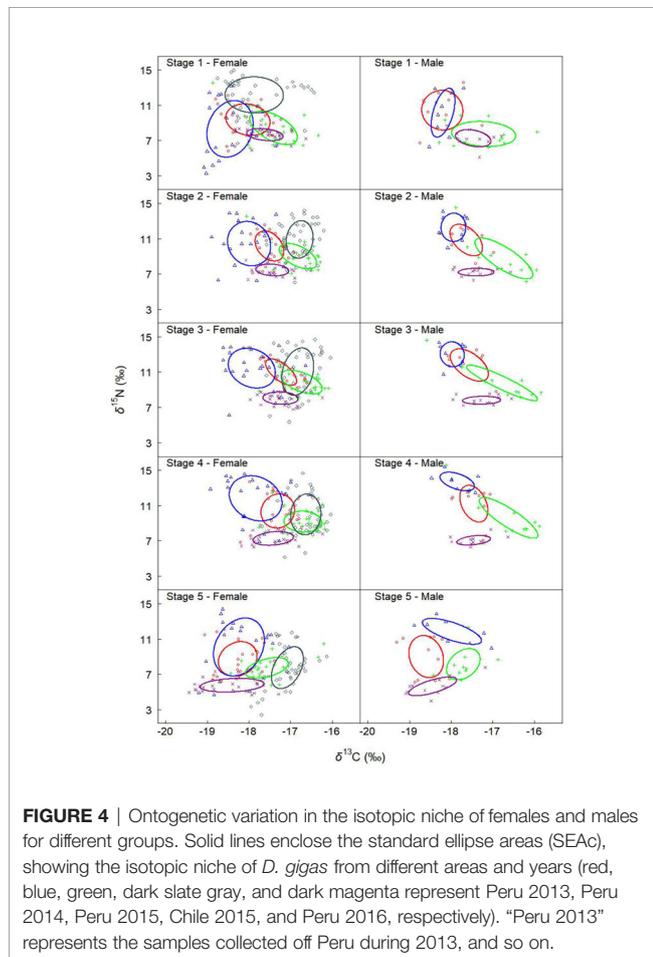


FIGURE 4 | Ontogenetic variation in the isotopic niche of females and males for different groups. Solid lines enclose the standard ellipse areas (SEAc), showing the isotopic niche of *D. gigas* from different areas and years (red, blue, green, dark slate gray, and dark magenta represent Peru 2013, Peru 2014, Peru 2015, Chile 2015, and Peru 2016, respectively). "Peru 2013" represents the samples collected off Peru during 2013, and so on.

those in 2014 and significantly less than those in 2013 throughout the life stage ($p < 0.05$).

For the geographic variation of females in 2015, only 1% of niche overlapped between Peru and Chile during stage 1, while overlaps ranged from 29% to 81% after stage 1 (**Table 6**, **Figure 4**). Except for stage 2, SEAc off Chile were significantly greater than those off Peru ($p < 0.05$).

Sexual Variation in Isotopic Niche

Off Peru, niche overlaps between females and males ranged from 55% to 76%, 68% to 79%, 67% to 100%, and 78% to 99% in 2013, 2014, 2015, and 2016, respectively (**Table 8** and **Figures 5, 6**). In 2013 and 2016, no significant difference was found for SEAc between females and males. In 2014, SEAc of females were significantly greater than those of males ($p < 0.05$). In 2015, SEAc of females were significantly less than those of males during stages 3 and 4 ($p < 0.01$), while there was no significant difference between females and males during stages 1, 2, and 5.

DISCUSSION

Ontogenetic Variation in Isotopic Niche

We found that life stage had no significant influence on $\delta^{13}\text{C}$, which might be because *D. gigas* did not migrate over large geographic areas (Ruiz-Cooley et al., 2010). However, life stage had strong significant influences on $\delta^{15}\text{N}$. $\delta^{15}\text{N}$ fluctuated with stage off Peru from 2014 to 2016 and decreased off Chile in 2015, which contrasted to some previous studies that $\delta^{15}\text{N}$ was positively related with the growth of squids (Ruiz-Cooley et al., 2006; Ruiz-Cooley et al., 2010; Espinoza et al., 2017; Trastviña-Carrillo et al., 2018). *D. gigas* is an opportunistic predator and a

TABLE 6 | Annual and geographic variation in isotopic niche of females during different life stages.

| Stage | | Peru 2013 | Peru 2014 | Peru 2015 | Peru 2016 | Chile 2015 |
|-------|------------|-----------|-----------|-----------|-----------|------------|
| 1 | Peru 2013 | | 0.023 | 0.444 | 0.000 | |
| | Peru 2014 | 60/32 | | 0.013 | 0.000 | |
| | Peru 2015 | 31/33 | 1/3 | | 0.000 | 0.033 |
| | Peru 2016 | 9/31 | 1/9 | 20/62 | | |
| | Chile 2015 | | | 1/1 | | |
| 2 | Peru 2013 | | 0.004 | 0.506 | 0.005 | |
| | Peru 2014 | 48/21 | | 0.005 | 0.000 | |
| | Peru 2015 | 6/6 | 0/0 | | 0.004 | 0.214 |
| | Peru 2016 | 0/0 | 0/0 | 0/0 | | |
| | Chile 2015 | | | 34/27 | | |
| 3 | Peru 2013 | | 0.001 | 0.172 | 0.094 | |
| | Peru 2014 | 22/7 | | 0.004 | 0.000 | |
| | Peru 2015 | 37/28 | 0/0 | | 0.011 | 0.014 |
| | Peru 2016 | 0/0 | 0/0 | 0/0 | | |
| | Chile 2015 | | | 69/39 | | |
| 4 | Peru 2013 | | 0.010 | 0.090 | 0.006 | |
| | Peru 2014 | 45/22 | | 0.000 | 0.000 | |
| | Peru 2015 | 8/12 | 0/0 | | 0.092 | 0.033 |
| | Peru 2016 | 0/0 | 0/0 | 0/0 | | |
| | Chile 2015 | | | 81/51 | | |
| 5 | Peru 2013 | | 0.005 | 0.037 | 0.066 | |
| | Peru 2014 | 98/45 | | 0.000 | 0.000 | |
| | Peru 2015 | 3/6 | 1/5 | | 0.386 | 0.044 |
| | Peru 2016 | 0/0 | 0/0 | 0/0 | | |
| | Chile 2015 | | | 29/19 | | |

For each group, bottom left values are percentages of SEAc overlaps (the first value is the overlap of the earlier year over the later year, and the second value is the overlap of the earlier year over the later year), and top right values are p-values of SEAc comparisons. "Peru 2013" represents the samples collected off Peru during 2013, and so on.

TABLE 7 | Annual variation in isotopic niche of males during different life stages.

| Stage | | Peru 2013 | Peru 2014 | Peru 2015 | Peru 2016 |
|-------|------------|-----------|-----------|-----------|-----------|
| 1 | Peru 2013 | | 0.160 | 0.471 | 0.009 |
| | Peru 2014 | 53/86 | | 0.172 | 0.113 |
| | Peru 2015 | 0/0 | 0/0 | | 0.011 |
| | Peru 2016 | 0/0 | 0/0 | 34/95 | |
| | Chile 2015 | | | 0.093 | 0.002 |
| 2 | Peru 2013 | | 0.262 | 0.033 | 0.025 |
| | Peru 2014 | 30/38 | | 0.033 | 0.000 |
| | Peru 2015 | 10/6 | 0/0 | | |
| | Peru 2016 | 0/0 | 0/0 | 0/0 | |
| | Chile 2015 | | | 0.105 | 0.285 |
| 3 | Peru 2013 | | 0.105 | 0.038 | 0.002 |
| | Peru 2014 | 24/41 | | 0.038 | 0.075 |
| | Peru 2015 | 24/24 | 0/0 | | 0.000 |
| | Peru 2016 | 0/0 | 0/0 | 0/0 | |
| | Chile 2015 | | | 0.114 | 0.162 |
| 4 | Peru 2013 | | 0.114 | 0.024 | 0.004 |
| | Peru 2014 | 7/12 | | 0.024 | 0.089 |
| | Peru 2015 | 10/8 | 0/0 | | 0.000 |
| | Peru 2016 | 0/0 | 0/0 | 0/0 | |
| | Chile 2015 | | | 0.320 | 0.195 |
| 5 | Peru 2013 | | 0.320 | 0.359 | 0.048 |
| | Peru 2014 | 0/0 | | 0.359 | 0.136 |
| | Peru 2015 | 0/0 | 0/0 | | 0.225 |
| | Peru 2016 | 0/0 | 0/0 | 1/2 | |
| | Chile 2015 | | | | |

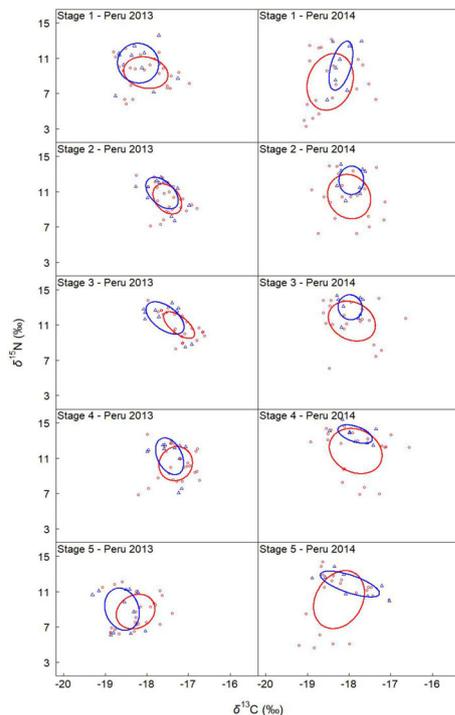
For each group, down left values are percentages of SEAc overlaps (the first value is the overlap of the earlier year over the later year, and the second value is the overlap of the earlier year over the later year), and top right values are p-values of SEAc comparisons. "Peru 2013" represents the samples collected off Peru during 2013, and so on.

highly migratory species (Nigmatullin et al., 2001; Ruiz-Cooley and Gerrodette, 2012). $\delta^{15}\text{N}$ in beaks could be influenced not only by the available food but also by the baseline $\delta^{15}\text{N}$ value (Lorrain et al., 2011; Li et al., 2017; Liu et al., 2018). Moreover, $\delta^{15}\text{N}$ was observed to fluctuate and decreased with growth of individual either in previous studies (Lorrain et al., 2011; Li et al.,

2017), indicating high diversity of prey items and different migratory routes *D. gigas* (Ruiz-Cooley et al., 2013). The stomach content analysis showed that the food of *D. gigas* off Peru was dominated by fish (in particular *Vinciguerria* sp., Nomeidae) and cephalopods (*D. gigas* and other species), but crustaceans such as euphausiids can also make a large

TABLE 8 | Sexual variation in isotopic niche off Peru in different years ("Peru 2013" represents the samples collected off Peru during 2013, and so on).

| Group | Stage | SEAc Overlap (%) | | SEAc ($\% \text{ } ^2$) | | p-value |
|-----------|-------|------------------|------|---------------------------|------|---------|
| | | Female | Male | Female | Male | |
| Peru 2013 | 1 | 67 | 56 | 3.0 | 3.5 | 0.378 |
| | 2 | 76 | 69 | 1.7 | 1.9 | 0.413 |
| | 3 | 63 | 38 | 1.3 | 2.2 | 0.106 |
| | 4 | 57 | 67 | 2.4 | 2.1 | 0.288 |
| | 5 | 55 | 50 | 2.8 | 3.1 | 0.449 |
| Peru 2014 | 1 | 28 | 71 | 5.5 | 2.2 | 0.015 |
| | 2 | 25 | 68 | 4.1 | 1.5 | 0.011 |
| | 3 | 25 | 75 | 3.9 | 1.3 | 0.003 |
| | 4 | 19 | 79 | 5.0 | 1.2 | 0.001 |
| | 5 | 26 | 69 | 6.1 | 2.3 | 0.023 |
| Peru 2015 | 1 | 69 | 57 | 2.8 | 3.4 | 0.455 |
| | 2 | 100 | 55 | 1.7 | 3.1 | 0.055 |
| | 3 | 76 | 62 | 1.8 | 2.2 | 0.002 |
| | 4 | 67 | 42 | 1.7 | 2.7 | 0.000 |
| | 5 | 73 | 58 | 1.7 | 2.1 | 0.217 |
| Peru 2016 | 1 | 78 | 58 | 0.9 | 1.2 | 0.231 |
| | 2 | 61 | 85 | 0.8 | 0.6 | 0.160 |
| | 3 | 60 | 88 | 0.9 | 0.6 | 0.129 |
| | 4 | 55 | 99 | 1.2 | 0.6 | 0.052 |
| | 5 | 60 | 80 | 1.8 | 1.4 | 0.236 |

**FIGURE 5** | Sexual variation in the isotopic niche off Peru in 2013 and 2014. Solid lines enclose the standard ellipse areas (SEAc), showing the isotopic niche of females and males (red and blue represent female and male, respectively). "Peru 2013" represents the samples collected off Peru during 2013, and so on.

contribution to the diet (up to 45% by mass) (Lorrain et al., 2011). Pardo-Gandarillas et al. (2014) found that the main prey species in the stomachs of *D. gigas* from coastal and oceanic waters of Chile were *Macruronus novaezelandiae* and *Trachurus murphyi*, respectively, which contributed 36.9% and 84.5% by mass to the diet, respectively.

Off Peru, niche overlaps between stage 1 and stages 3 and 4 in 2013, 2014, and 2015 were very small, while overlaps between stage 1 and stages 2 and 5 were large (Tables 4, 5 and Figure 4). We suggested that squids likely lived in a similar habitat during stages 1 and 2, then moved to a different habitat during stages 3 and 4. Previous studies suggested that majority of paralarvae are carried northward in the Humboldt Current, and then juveniles grow in the Humboldt Current and will eventually be carried to the west in the South Equatorial Current (Anderson and Rodhouse, 2001; Keyl et al., 2008). Then, adult squid migrate southward and come back to the spawning grounds. In 2016, overlaps between stage 1 and stages 2, 3, and 4 were large, while no overlap was observed between stages 1 and 5. The overlaps between life stages in 2016 indicated that squids inhabited and foraged in a similar area from paralarval to adult phase and experienced a short horizontal migration, which was probably caused by the strong El Niño event. The temperature of sea water has a strong influence on the abundance and distribution of squids (Waluda and Rodhouse, 2006; Waluda et al., 2006; Yu et al., 2016; Yu and Chen, 2018). During the strong El Niño event in 2016, the equatorial undercurrent became more intense and pushed warm equatorial surface waters closer to the coast, which weakened the coastal upwelling current and narrowed the suitable habitat for squid (Keyl et al., 2008; Yu and Chen, 2018). Yu and Chen (2018) evaluated the effects of ocean

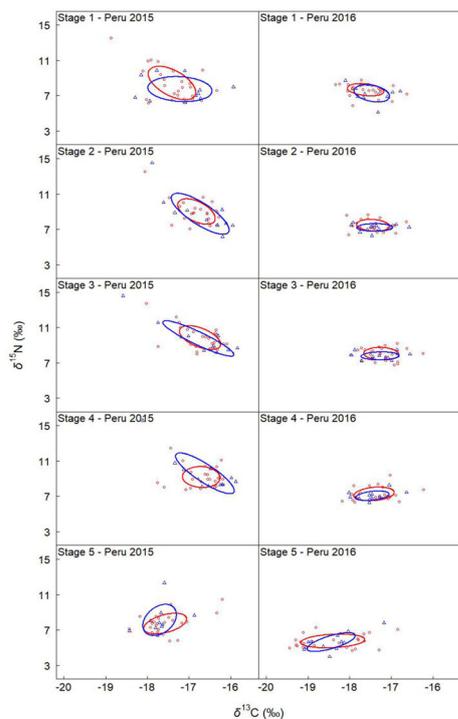


FIGURE 6 | Sexual variation in the isotopic niche off Peru in 2015 and 2016. Solid lines enclose the standard ellipse areas (SEAC), showing the isotopic niche of females and males (red and blue represents female and male, respectively). “Peru 2013” represents the samples collected off Peru during 2013, and so on.

warming to the habitat quality and spatial distribution of *D. gigas* off Peru, and found that the potential habitat of *D. gigas* tended to shrink and move southeastward under ocean warming.

Off Chile, the $\delta^{13}\text{C}$ value in this study was similar to the results of Gong et al. (2018a) using muscle tissue but the $\delta^{15}\text{N}$ value was much lower. In this study, both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ significantly differed among ontogenetic stages. $\delta^{13}\text{C}$ obviously increased from stage 1 to 2, and became stable from stage 2 to 4, then apparently decreased from stage 4 to 5, indicating that the squids lived in a similar area during stages 2–4. $\delta^{15}\text{N}$ decreased from stage 3 to 4, which was interpreted as a decrease in the trophic position of squids. Besides, there was no overlap for trophic niche between stage 1 and stages 2, 3, 4, and 5 of the *D. gigas*, indicating that the habitat of the squids changed after paralarval phase. Squids off Chile were suggested to carry out both a south-northward movement within coastal waters and an east-westward movement between oceanic and coastal waters (Nigmatullin et al., 2001; Liu et al., 2016).

Annual Variation in Isotopic Niche

GLMs showed that year had significant influences on both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, which contrasted a previous study (Argüelles et al., 2012). We suggested that it is probably because the El Niño event occurred during 2015 and 2016 (Li et al., 2017; Hu et al., 2019). For the annual variability off Peru, $\delta^{13}\text{C}$ significantly differed

among years during stages 1, 2, and 3, while $\delta^{15}\text{N}$ significantly differed among years during all stages. Therefore, compared with $\delta^{13}\text{C}$, year effects on $\delta^{15}\text{N}$ tended to be greater. Li et al. (2017) also found that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ had significant differences among years. The variance in $\delta^{13}\text{C}$ indicated differences of habitat squids experienced among years (Ruiz-Cooley et al., 2010; Lorrain et al., 2011), while the annual variability in $\delta^{15}\text{N}$ was likely caused by both the variance of habitat and trophic position in different years (Ruiz-Cooley and Gerrodette, 2012; Liu et al., 2018). Except for stage 1 females in 2014, the average $\delta^{15}\text{N}$ in 2013 and 2014 was greater than those in 2015 and 2016 during every stage (Tables 4, 5 and Figure 4). We suspect that the El Niño event during 2015 and 2016 changed the migration route and reduced the trophic position of squids.

For the annual variation off Peru, relatively large overlaps were observed between 2013 and 2014 (normal years) throughout the life stage. Trophic niches were separated between normal years (2013 and 2014) and El Niño years (2015 and 2016) with the growth of squids. Moreover, we found that there was no overlap between 2015 and 2016 after the paralarvae phase. Therefore, we suggested that the habitat of squids changed during El Niño years and differed between 2015 and 2016. Other studies have noted that regional differences in environmental variables were different between 2015 and 2016 (Jacox et al., 2016). Oceanographic changes during El Niño events may have shortened and changed the horizontal migration routes and reduced the habitat of squids (Keyl et al., 2008; Yu and Chen, 2018). We found that isotopic niche width in 2016 was generally less than this in 2013 and 2014 throughout the life stage. Therefore, for squids, not only habitat changed but also niche size decreased in 2016. The impacts of El Niño event on squids in 2016 were greater than 2015 (only habitat of squids changed in 2015, while both habitat and niche size changed in 2016). The El Niño event was much stronger during the periods squids experienced in 2016 than that in 2015 (Jacox et al., 2016).

Geographic Variation in Isotopic Niche

In this study, GLMs showed that area had significant influences on both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, which was in agreement with previous studies (Argüelles et al., 2012; Ruiz-Cooley and Gerrodette, 2012). Gong et al. (2018a) analyzed the stable isotopes of muscle tissue of *D. gigas* in the offshore waters of the central eastern Pacific and off Peru and Chile, and found that the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were different among origins.

These differences were probably due to the variability in food composition and baseline values across different geographic areas (Ruiz-Cooley and Gerrodette, 2012; Gong et al., 2018a). Latitudinal gradients in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values have been recognized in the eastern Pacific and can be propagated up into the squid tissues through the diet (Argüelles et al., 2012; Ruiz-Cooley and Gerrodette, 2012). Differences of nearly 4.0‰ in $\delta^{13}\text{C}$ values and 8.0‰ in $\delta^{15}\text{N}$ values were investigated in *D. gigas* muscle tissues along a 15° latitudinal gradient in the Humboldt Current Ecosystem (Argüelles et al., 2012). $\delta^{15}\text{N}$ not only provides information of the trophic position but also can be used to discriminate squids among areas (Gong et al., 2018a). The variations of $\delta^{15}\text{N}$ among life stages off Chile were greater

than those off Peru, which was probably caused by differences of assimilated diet and biochemical cycle between different areas (Ruiz-Cooley et al., 2010; Gong et al., 2020). Only 1% overlap was observed during stage 1 between Peru and Chile in 2015. Compared with other stages, stable isotopes during early life history stage best distinguishes different areas. Several studies also noted that stable isotope can be used for distinguishing geographic populations of *D. gigas* (Ruiz-Cooley et al., 2010; Gong et al., 2018a; Gong et al., 2020).

Sexual Variation in Isotopic Niche

Sex had no significant influence on $\delta^{13}\text{C}$. Females and males probably live and forage together in the similar habitat (Post, 2002), which was consistent with a previous study of gladius stable isotopes (Gong et al., 2018b). Moreover, niche overlaps between females and males were large off Peru from 2013 to 2016, further indicating that females and males shared a similar habitat. Gong et al., (2018b) investigated the sexual variability of niche using gladius stable isotope, and also found that the niche overlaps between females and males were large from paralarval stage to 210 days. The isotopic niches between genders only differed during 211–230 days. Results of GLMs showed sex-specific differences in $\delta^{15}\text{N}$, and *t*-test showed that differences in $\delta^{15}\text{N}$ between females and males only occurred in 2014, which indicated that trophic level of prey items was probably different between females and males (Cherel and Hobson, 2005; Lorrain et al., 2011; Kernaléguen et al., 2016; Trasviña-Carrillo et al., 2018). In 2014, niche width of females was significantly greater than that of males during all life stages. Compared with males, we supposed that females likely had larger habitats and more available food in 2014. Compared with normal years (2013 and 2014), the niche overlaps were generally greater between females and males during El Niño years (2015 and 2016). This might be due to the smaller suitable habitats during El Niño events (Keyl et al., 2008; Yu and Chen, 2018), resulting in greater competition between females and males.

In conclusion, we investigated the ontogenetic and spatiotemporal effects on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in lower beaks of *D. gigas*, and compared the trophic niches by sex, life stage, year, and area. $\delta^{15}\text{N}$ was significantly influenced by all explanatory variables, while $\delta^{13}\text{C}$ was only influenced by year and area. The habitat and feeding habit of squids likely changed due to the niche differentiation. In a weak El Niño year (2015), the habitat of squids changed. However, during a strong El Niño year (2016), habitat changed and niche size decreased. Stable isotope in early life stages was most effective for distinguishing different geographic populations. Females and males probably

inhabited a similar area. However, compared with males, females might have larger habitats and more available food. This study improves the understanding of habitat and feeding strategies of *D. gigas*.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by Institutional animal care and use Committee of Shanghai Ocean University.

AUTHOR CONTRIBUTIONS

GH, RB, and XC conceived and designed the experiments. XC and JL provided the tissue samples. GH performed the experiments and analyzed the data with the help of ZZ and JL. GH wrote the manuscript with the advice of RB and XC. All authors contributed to the manuscript revision and approved the submitted version.

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