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δ^{13}C and δ^{15}N in Humboldt squid beaks: understanding potential geographic population connectivity and movement

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Abstract

We quantified the δ^{13}C and δ^{15}N values in the lower beaks of Humboldt squid, *Dosidicus gigas*, collected from international waters off Costa Rica, Ecuador, Peru and Chile by Chinese squid jigging vessels during 2009, 2010 and 2013. There was a significant difference in the isotopic values among regions with the lowest value off Ecuador and the highest off Chile, which were interpreted as a function of trophic effects as well as baseline values. However, constant trophic level of *D. gigas* across its geographic range showed that spatial variation in the baseline of primary production is the main driver responsible for the observed geographic isotope variability. Inter-regional difference and intra-regional convergence of isotope values indicated squid off Costa Rica, Ecuador and Chile belong to different geographically segregated populations, which were previously proved by integrated population identifying method. In contrast, the higher variations in δ^{13}C and δ^{15}N values in a given size group suggest the squid off Peru move and forage in different places. Moreover, potential population exchange could be responsible for the overlap of the isotope values between the squid off Peru and off Chile. On the whole, the spatial difference in isotopic values of Humboldt squid beaks improves our understanding of potential geographic population connectivity and movement.

Key words: stable isotope, *Dosidicus gigas*, beaks, geographic variability, trophic level, the eastern Pacific Ocean

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1 Introduction

The Humboldt squid (*Dosidicus gigas*) widely inhabits coastal and pelagic waters in the eastern Pacific Ocean (Nigmatullin et al., 2001). The squid is considered to be ecologically and economically important on a global scale, owing to its large geographic range, extremely high fecundity, flexible feeding strategies, tolerance of environmental extremes and great fishery potential (Gilly and Markaida, 2007). Distribution of the Humboldt squid is related to not only its principal prey (Nevárez-Martínez et al., 2000) but also top predators (Jaquet et al., 2003), which means it plays an important trophic role in the ecosystem (Rosas-Luis et al., 2008). *Dosidicus gigas* supports the largest cephalopod fisheries in the eastern Pacific Ocean including the Gulf of California (Nevárez-Martínez et al., 2000), the coastal and oceanic waters of Peru (Taipe et al., 2001; Chen and Zhao, 2006) and Chile (Zúñiga et al., 2008; Liu et al., 2010) and the Costa Rica Dome (Ichii et al., 2002; Chen et al., 2014) as well as in equatorial regions (Chen et al., 2012).

Stable isotope analysis (SIA) of carbon and nitrogen (δ^{13}C and δ^{15}N) has been widely used in the study of habitat use, movement patterns and trophic position of marine organisms including marine mammals (Mendes et al., 2007), sea birds (Jaquemet et al., 2008), fishes (McMahon et al., 2011) and cephalopods (Ruiz-Cooley et al., 2004, 2013). Stable isotopes in cephalopod soft tissues (e.g., mantle and buccal mass) may reveal environmental information prior to capture because of their relatively quick turnover (Stowasser et al., 2006). In contrast, hard tissues (e.g., beaks, gladii and eye lenses) are metabolically inactive structures with new molecules being continuously laid down and with no turnover after synthesis (Xavier et al., 2015). Consequently, δ^{13}C and δ^{15}N within these structures combine the feeding ecology of cephalopods over their lifespan.

Spatial patterns of stable isotopes in organism tissues reflect variations in baseline values as well as trophic effects. Consequently, geographic variation in δ^{13}C and δ^{15}N in tissues of marine species to some extent represents differences in both habitats and trophic positions. In general, δ^{13}C values reflect the source of primary producers, since they typically only increase by

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0.5‰ to 1.5‰ per trophic level (DeNiro and Epstein, 1978). Therefore, the δ^{13}C values in the marine ecosystem are commonly used to discriminate between inshore vs. offshore or pelagic vs. benthic feeding, as well as the lower- vs. higher-latitude plankton (Sherwood and Rose, 2005; Cherel and Hobson, 2007). In contrast, consumers are enriched in ^{15}N by 2‰ to 3.5‰ relative to their food (DeNiro and Epstein, 1981), which provides a possible mechanism to estimate trophic position (Hobson and Welch, 1992).

SIA in cephalopods hard structures is becoming increasingly helpful for countering the paucity of life history data because its inactive metabolism leads to little elemental turnover after formation. Cephalopod bodies contain few hard structures, and the beak is an important one that has been widely used in the application of age determination (Hernández-López et al., 2001), population identification (Martínez et al., 2002) as well as dietary analysis (Xavier et al., 2011). Recently, δ^{13}C and δ^{15}N values in cephalopod beaks have been widely used in the investigation of trophic positions, energy pathways and migrations (Cherel and Hobson, 2005; Hobson and Cherel, 2006; Cherel et al., 2009, 2011).

In this study, we analysed the δ^{13}C and δ^{15}N values in *D. gigas* beak samples from four geographic regions (Costa Rica, Ecuador, Peru and Chile) in the eastern Pacific Ocean. Our primary aims were to evaluate variability of the stable isotope values across different regions and to identify the potential geographic populations connectivity and movement of such an intra-specific complex species based on the detected spatial difference in isotopes. The yield results will benefit an understanding of the life history of this squid.

2 Materials and methods

2.1 *Squid sampling*

A total of 82 squids (17 off Costa Rica, 28 off Ecuador, 20 off Peru and 17 off Chile) with mantle lengths (ML) ranging between 183 mm and 534 mm were collected from 39 stations in the eastern Pacific Ocean, during 2009 to 2013 (Fig. 1). All samples were immediately frozen at sea and dissected in the laboratory after being defrosted. Beaks were extracted, washed and then kept in 75% ethanol until isotopic analysis.

2.2 *Stable isotope analysis*

Lower beaks were selected for stable isotope analysis. To reduce the interference of possible contaminants, lower beaks were first rinsed in MilliQ water for 5 min and subsequently dried in an oven at 60°C for 48 h prior to isotope analysis. After drying, beaks were homogenized to fine powder with an agate mortar and pestle. Approximately 1–2 mg of samples were weighed into 0.3 mg tin capsules and analyzed 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 the Key Laboratory of Sustainable Exploitation of Oceanic Fisheries Resources, Ministry of Education, at Shanghai Ocean University. The δ^{13}C and δ^{15}N of the samples are expressed in standard notation as the following functions (Fry, 2006):

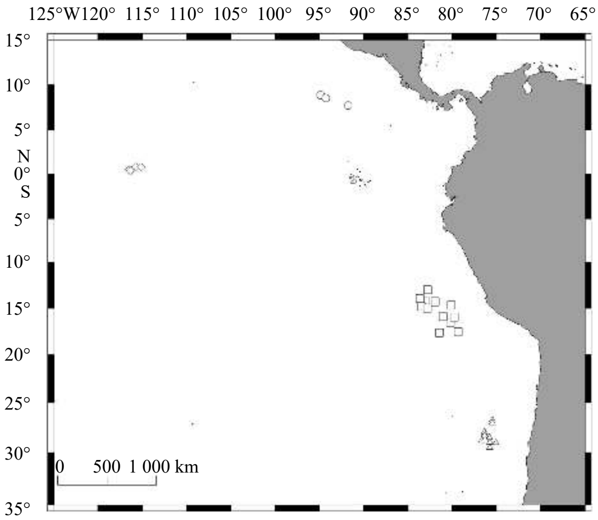

Fig. 1. Sampling stations in this study.

$$\delta^{13}\mathrm{C} = \left(\frac{(^{13}\mathrm{C}/^{12}\mathrm{C})_{\mathrm{sample}}}{(^{13}\mathrm{C}/^{12}\mathrm{C})_{\mathrm{standard}}} - 1\right) \times 1\,000,$$

$$\delta^{15}\mathrm{N} = \left(\frac{(^{15}\mathrm{N}/^{14}\mathrm{N})_{\mathrm{sample}}}{(^{15}\mathrm{N}/^{14}\mathrm{N})_{\mathrm{standard}}} - 1\right) \times 1\,000,$$

where ^{13}C/^{12}C and ^{15}N/^{14}N are the atomic ratios of ^{13}C and ^{15}N in the sample and standards, respectively, and δ is the measure of the ratio of heavy to light isotopes in the sample. The standard reference material for carbon is Vienna Pee Dee Belemnite (V-PDB) and for nitrogen is atmospheric nitrogen (N_2). USGS 24 (–16.049‰ V-PDB) was used as the primary standard for ^{13}C, and USGS 26 (53.7‰ N_2) was used to quantify ^{15}N. To assess the associated errors within and between runs, repeated analyses of internal laboratory reference standards (Protein (–26.98‰ V-PDB and 5.96‰ N_2)) were performed every ten samples. The analytical precision was less than 0.1‰ for both carbon and nitrogen.

2.3 *Adjustment for δ^{15}N baseline values*

To correctly compare the δ^{15}N values among different regions, we adjusted all specimen δ^{15}N values (referred to as baseline-adjusted δ^{15}N values, BA-δ^{15}N) by subtracting phytoplankton δ^{15}N values obtained for each sampling station (Fig. 1) from a global coupled ocean circulation-biogeochemistry-isotope model with a horizontal resolution of 1.8° (latitude)×3.6° (longitude) and 19 vertical levels (Somes et al., 2010; Navarro et al., 2013).

2.4 *Statistical analysis*

An analysis of variance (ANOVA) and subsequent Tukey's pairwise comparison tests were conducted to determine the difference in δ^{13}C and δ^{15}N when multiple comparisons between regions were possible. Bayesian ellipses stable isotope analysis was conduct to compare isotopic niche widths among communities (Jackson et al., 2011). All statistical tests were performed using SPSS 15.0, and significance was determined at α=0.05 level.

3 Results

3.1 *Stable isotope values*

The δ^{13}C in the lower beaks of squid from Costa Rica, Ecuador, Peru and Chile had a relatively small range from –18.2‰ to –17.7‰ (–17.9‰±0.1‰), –19.2‰ to –18.5‰ (–19.0‰±0.1‰), –17.8‰ to –15.9‰ (–17.0‰±0.6‰) and –17.1‰ to –16.1‰ (–16.6‰±0.3‰), respectively. The δ^{15}N ranges in the lower beaks of squid from waters off these countries were much larger from 5.9‰ to 7.9‰ (6.9‰±0.5‰), 2.9‰ to 4.7‰ (3.6‰±0.5‰), 6.1‰ to 15.4‰ (9.7‰±2.6‰) and 14.2‰ to 17.0‰ (15.5‰±0.8‰), respectively (Table 1).

Table 1. δ^{13}C, δ^{15}N and BA-δ^{15}N values within lower beaks of *D. gigas* off Costa Rican, Ecuadorian, Peruvian and Chilean waters

Study area	Coordinate	Sample size	ML/mm		δ^{13}C/‰			δ^{15}N/‰			BA-δ^{15}N/‰		
			Range	Mean(SD)	Range	Mean(SD)	*T*	Range	Mean(SD)	*T*	Range	Mean(SD)	*T*
Costa Rica	7°46′–8°55′N, 91°48′–94°55′W	17	285–347	311(14)	–18.2 to –17.7	–17.9(0.1)	a	5.9–7.9	6.9(0.5)	a	1.0–2.8	1.9(0.5)	a
Ecuador	1°18′N–0°32′S, 114°59′–118°52′W	28	256–344	295(21)	–19.2 to –18.5	–19.0(0.1)	b	2.9–4.7	3.6(0. 5)	b	1.1–2.9	1.8(0.4)	a
Peru	12°54′–17°33′S, 79°49′–83°41′W	20	183–534	350(102)	–17.8 to –15.9	–17.0(0.6)	c	6.1–15.4	9.7 (2.6)	c	2.4–11.5	5.8(2.6)	b
Chile	26°41′–29°25′S, 75°05′–76°39′W	17	318–511	430(59)	–17.1 to –16.1	–16.6(0.3)	d	14.2–17.0	15.5 (0.8)	d	12.8–15.6	14.0(0.7)	c
Total	8°55′N –29°25′S, 75°05′–118°52′W	82	183–534	342(78)	–19.2 to –15.9	–17.8(1.0)		2.9–17.0	8.3(4.6)		1.0–15.6	5.3(5.0)	

Note: SD represents standard deviation, *T* result from Tukey's HSD with significant differences (P<0.05) indicated by letters (a, b, c, d).

3.2 *Spatial difference in stable isotope values*

ANOVA showed a significant spatial difference both in δ^{13}C and δ^{15}N among regions (Table 2; ANOVA, P<0.05 for both δ^{13}C and δ^{15}N), and the squid samples off Chile had the highest, while the samples from Ecuador had the lowest values (Fig. 2). Pairwise analysis showed that there was a significant inter-regional difference in isotope values between regions (Table 1; Tukey HSD, P<0.05). Scatter plots of δ^{13}C and δ^{15}N showed that squid from different regions were separated clearly except for those from Peru which, to some extent, shared considerable overlaps with those in Costa Rica and Chile (Fig. 3).

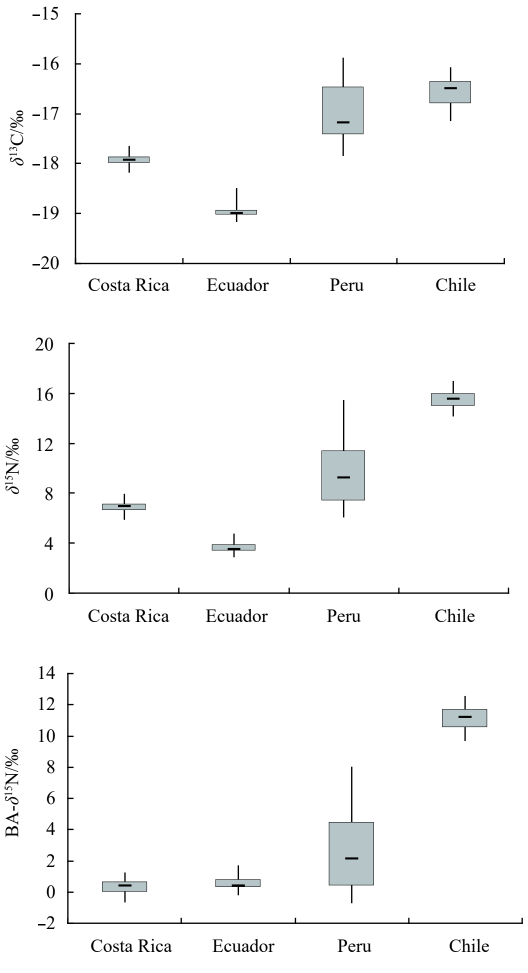

Fig. 2. Box-whisker plot of the δ^{13}C, δ^{15}N and BA-δ^{15}N values in beaks of *D. gigas* off Costa Rica, Ecuador, Peru and Chile. Box shows first and third quartile, whisker lower and upper values, and bold bar median value.

Table 2. Analysis of variance comparing beak isotopes of *D. gigas* from the four regions in the eastern Pacific Ocean

Isotope	Mean squares		*F*	*P*
	Four regions	Error		
δ^{13}C	25.194	0.124	203.884	0.000
δ^{15}N	522.357	1.874	278.775	0.000

3.3 *Stable isotope values correlating with squid size*

There was no clear increase in δ^{13}C and δ^{15}N values was apparent with ascending squid size for a given study area, except for the sample squid for Peru ($F_{1,6}$=55.2, P=0.000<0.05 for δ^{13}C and $F_{1,6}$=11.7, P=0.014<0.05 for δ^{15}N). The δ^{13}C and δ^{15}N values of squid off Peru varied greatly in a given size group, while the range of isotopes off Costa Rica, Ecuador and Chile for a given size group were small (Fig. 4).

4 Discussion

The δ^{13}C and δ^{15}N values in different tissues (muscle, gladii, beaks and eye lenses) of *D. gigas* were reported over its distribution ranges (Table 3). However, the isotope values in beaks were only reported in the Gulf of California (Ruiz-Cooley et al., 2006, 2011) and coastal Peru (Ruiz-Cooley et al., 2011), which are not covered in this study. A comparative experiment shows that muscle isotope values were higher than isotope values for beak by 1‰ for δ^{13}C and 4‰ for δ^{15}N (Ruiz-Cooley et al., 2006). By adding these enrichment factors to beak values, the adjusted isotope values off Chile and Peru in this study are similar to conclusions previously reported for muscle (Hückstädt et al., 2007; Argüelles et al., 2012). Off Costa Rica and Ecuador, the δ^{15}N values were reported in another chitin structure, the gladius, and were slightly higher than those found in the beaks in this study (Ruiz-Cooley et al., 2010).

4.1 *Geographic difference in isotopic values*

The potential influence of trophic as well as baseline effects might be responsible for the significant spatial difference in the δ^{15}N values of the Humboldt squid among the four sampling regions in this study. A similar significant geographic variation was also detected in the muscle (Ruiz-Cooley and Gerrodette, 2012) of the same species and also in the squid *Sthenoteuthis oualaniensis* (Takai et al., 2000). Indeed, dietary studies showed *D. gigas* has a relatively constant trophic level about 4 across its geographic range, although it can feed on a large range of food (Markaida and Sosa-Nishizaki, 2003; Field et al., 2007; Tam et al., 2008). Therefore, spatial variation in the baseline of primary production (4.96‰–5.10‰ in Costa Rica, 1.83‰ in Ecuador,

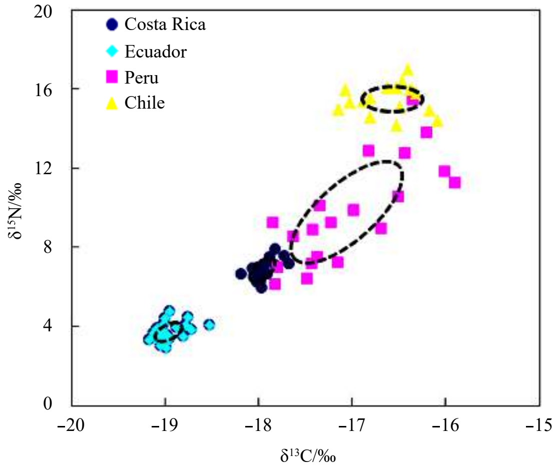

Fig. 3. Scatter plots of the δ^{13}C and δ^{15}N values in beaks of *D. gigas* off Costa Rica, Ecuador, Peru and Chile.

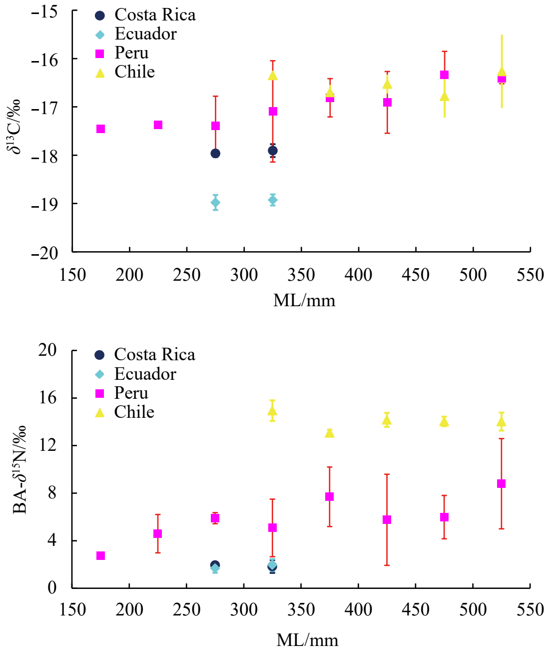

Fig. 4. The relationships between δ^{13}C and δ^{15}N values versus mantle length (ML) of *D. gigas* off Costa Rica, Ecuador, Peru and Chile.

3.65‰–4.51‰ in Peru and 0.04‰–2.27‰ in Chile) is likely responsible for the observed geographic variability of δ^{15}N values rather than trophic level.

Baseline adjustment is a good approach only able to account for variability in baseline values in the areas of capture. Thus, regional δ^{15}N difference should disappear after baseline adjustment, if *D. gigas* are resident in each area. To test the hypothesis, we adjusted all specimen δ^{15}N values (referred to as baseline-adjusted δ^{15}N values, BA-δ^{15}N) by subtracting phytoplankton δ^{15}N values obtained for each sampling station from a global coupled ocean circulation-biogeochemistry-isotope model (Somes et al., 2010). However, the BA-δ^{15}N values still show significant inter-regional variation except for between Costa Rica and Ecuador (Fig. 2). Therefore, if any of these squids are recent migrants to the areas where they were captured, a baseline correction would not properly adjust their isotope values. Given that whole beaks integrate feeding over the entire lifespan, any feeding and movements across different isoscapes during their lifespans would create spatial variability in beak isotope values and some degree of spatial baseline bias from areas outside of the capture location is certainly possible (Young et al., 2015).

Unlike δ^{15}N, there was a more restricted range (3.3‰) in the δ^{13}C values with a gradual increase from −19.2‰ (Ecuador) to −15.9‰ (Peru). In the marine ecosystem, the δ^{13}C values commonly reflect sources of primary productivity and vary greatly with latitude and distance to shore (Cherel and Hobson, 2007). Therefore, in the current study, a significant difference in δ^{13}C values may reflect a geographic shift. For example, in this study, Ecuador samples with lower δ^{13}C values (−19.0‰) were taken more offshore than the samples from other regions, and Chile squid from higher latitudes had higher δ^{13}C values (−16.6‰) than those from Peru (−17.0‰) and Costa Rica (−17.9‰), which is consistent with the conclusion revealed in *D. gigas* muscle by Ruiz-Cooley and Gerrodette (2012). Such geographic differences were also reported in both muscle and beaks of the same species in the Gulf of California (Ruiz-Cooley et al., 2006), and are consistent with the findings reported for the common Japanese squid (*Todarodes pacificus*) corresponding to two different sampling regions which were also associated with different geographic populations (Ikeda et al., 1998).

4.2 *Population connectivity and movement*

Although the isotopic values of squid sampled off Peru shared some overlaps with Costa Rica and Chile (Fig. 3), the sampled squid could be clearly grouped by region according to the significantly different δ^{13}C and BA-δ^{15}N values found in the beaks. The intra-regional similarity of BA-δ^{15}N values in the beaks of the squid from Chile, Costa Rica and Ecuador (Fig. 3), indicated that the squid from a given area tend to have a small range of food. While large range of BA-δ^{15}N values indicates squid from Peru occupy a large range of trophic level.

Similar δ^{13}C values for individuals within a given region except for Peru (with range of 0.5‰ for Costa Rica, 0.6‰ for Ecuador and 1.1‰ for Chile) would be consistent with local feeding linked to a common source of primary production. This indicate that individuals from a given region might share similar primary productivity values over their life, or they experience regionally-distinct isotope values at early life but would have spent a sufficient period of time at the location-of-capture to influence the whole beak isotope values. Previous studies successfully identified these squid as spatially segregated populations based on the analysis of geographic heterogeneous isotopic values in gladius (Ruiz-Cooley et al., 2010), and elemental signature in early ontogenetic statolith (Liu et al., 2015a) as well as spatial variation in beak size (Liu et al., 2015b). Consequently, inter-regional differences and intra-regional convergence of δ^{13}C and δ^{15}N values in the beak indicate that the squid from Costa Rica, Ecuador and Chile belong to different geographic populations.

Off Ecuador, there were no overlaps of the isotope values with other regions (Fig. 3) which led us to believe that the squid off Ecuador are an independent population and do not have any exchange with other populations. Yan et al. (2011) reported that the squid off Ecuador have significant genetic differentiation from both the squid off Peru and those from Costa Rica, and this was further proved by Liu (2014) who recommended that the high geographic genetic diversities and significant genetic differentiation were caused by ocean currents and historical factors.

Elemental signature analyses have shown that *D. gigas* off

Table 3. The δ^{13}C and δ^{15}N values of *D. gigas* reported in previous studies

Study area	Tissue	Mean(SD)		Reference
		δ^{13}C/‰	δ^{15}N/‰	
Gulf of California	muscle	−16.2 to −14.2	14.5–17.9	Ruiz-Cooley et al. (2004)
Gulf of California	muscle (large)	−14.9(0.6)	16.9(0.7)	Ruiz-Cooley et al. (2006)
	muscle (medium)	−16.2(0.3)	14.7(0.4)	
	beak (large)	−15.6(0.3)	13.0(1.0)	
	beak (medium)	−17.3(0.5)	10.6(0.7)	
	beak (from stomach of sperm whale)	−16.5(1.2)	12.4(0.8)	
Central Chile inshore waters	muscle		18.5	Hückstädt et al. (2007)
Gulf of California	muscle	~−18.2	~14	Drazen et al. (2008)
USA inshore waters	gladius	−18.4(0.5)	10.5(0.6)	Ruiz-Cooley et al. (2010)
Gulf of California		−17.6(0.2)	14.1(0.4)	
Costa Rica inshore waters		−17.6(0.2)	8.9(0.7)	
Columbia inshore waters		−17.5 (0.2)	9.2(0.2)	
Ecuador inshore waters		−17.3(0.3)	7.0(0.1)	
Ecuador oceanic waters		−18.1 (0.2)	4.9(0.4)	
Gulf of California and Peru	beak	−18.1 to −17.4	7.0–12.0	Ruiz-Cooley et al. (2011)
Offshore of Northern Peru	muscle	−16.0(0.3)	13.8(2.5)	Lorrain et al. (2011)
	gladius	−16.2(0.5)	9.0(2.3)	
Northern Humboldt Current System	muscle	−19.1 to −15.1	7.4–20.5	Argüelles et al. (2012)
South Chile inshore waters	muscle	~−15.9	~17.5	Ruiz-Cooley and Gerrodette (2012)
Peru inshore and oceanic waters		~−16.3	~11.5	
Eastern Pacific Warm Pool		~−16.8	~13.5	
Gulf of California		~−16.5	~18.5	
Mexico inshore waters		~−17. 1	~15.3	
USA inshore waters		~−17.7	~14.5	
Canada inshore waters		~−17.5	~15.4	
Northern California Current system	gladius		9–13.3	Ruiz-Cooley et al. (2013)
Northern California Current system	muscle	−19.1(0.2)	13.9(0.5)	Miller et al. (2013)
Gulf of California	eye lens	−18.6(0.7)	13.5(1.0)	Onthank (2013)
USA inshore waters		−18.4(0.5)	12.8(0.8)	
Canada inshore waters		−18.2(0.3)	14.1(1.6)	

Costa Rica is a specific population having a narrow movement (Liu et al., 2015a) which is in agreement with our view in this study. This conclusion was also presented by Ruiz-Cooley et al. (2010), because the squid spawn off the Costa Rica Dome and nursery and feed in the vicinity (Chen et al., 2013; Liu et al., 2015a).

Off Peru, a higher variation in δ^{13}C and BA-δ^{15}N values within a given size group (Fig. 4) indicated that intra-regional differences in isotope values from the same size group was more likely caused by baseline shifts than squid size. Thus, larger δ^{13}C and BA-δ^{15}N ranges indicate that these squid consequently move and feed in different places and subsequently display distinct isotopic values as they grow. Therefore, we hypothesize that the squid off Peru might move and forage in different places with distinct isotopic values as they grow, where squid movement is strongly influenced by the Humboldt Current (Anderson and Rodhouse, 2001). This multivariate inter-individual migration pattern can also been seen in the offshore waters of Northern Peru (Lorrain et al., 2011).

Scatter plots of δ^{13}C and BA-δ^{15}N values (Fig. 3) seem to indicate some of these origins were from Chile and Costa Rica. However, natural tags and molecular methods proved that *D. gigas* from the southern and northern hemispheres should have different natal origins (Sandoval-Castellanos et al., 2007, 2010; Staaf et al., 2010). Molecular methods analysis showed significant genetic differentiation between the squid off Costa Rica and off Peru (Yan et al., 2011). Therefore, the overlap of the isotope values between the squid off Peru and off Costa Rica was not a result of population exchange but experience of the same baseline isotopic history. Although variation in beak size and statolith elemental signature implies that *D. gigas* off Peru and Chile were interpreted as different geographic populations (Liu et al., 2015b), considerable overlaps indicated that they should, to a certain extent, have experienced population exchange. This was genetically verified by Sandoval-Castellanos et al. (2007) and Ibáñez et al. (2011), who suggested that Peru currents and its countercurrents were responsible for the population interchange. Thus, the exchange is likely to be responsible for the overlap of the isotope values between the squid off Peru and off Chile in this study.

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