Ontogenetic variation of δ^{13}C and δ^{15}N recorded in the gladius of the jumbo squid *Dosidicus gigas*: geographic differences

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ABSTRACT: Cephalopods play an important role in marine food webs, but their feeding ecology has been mostly examined by traditional methods. In this study, δ^{13}C and δ^{15}N values were measured along the proostracum (a morphological part of the gladius) to reconstruct squid feeding variations. Gladii or the internal shell of jumbo squid *Dosidicus gigas* was collected at 6 sites from the eastern Pacific Ocean to evaluate trophic shifts as a function of size. The results showed that C- and N-isotopic values varied throughout proostracum length for all squid; changes that could be determined by temporal variation in prey consumption. Both δ^{13}C and δ^{15}N significantly increased with length and supported ontogenic shifts in diet. Detectable differences in δ^{15}N values were found at all sites. This δ^{15}N-oceanic variation may be associated with variations in squid assimilated diet, but may also be due to differences in the biochemical cycle among areas. Isotopic values only overlapped among the nearest sites. These results suggest that *D. gigas* did not migrate over large geographic areas, but rather moved within narrower latitudinal ranges. We recommend the use of stable isotopic analyses along the gladius as an effective approach to tracing foraging variations and also in geographically differentiating subpopulations.

KEY WORDS: Gladius · Squid · Stable isotopes · Trophic variation · Geographic variation

INTRODUCTION

Knowledge about the ecology of mesopelagic and bathypelagic cephalopods is scarce due to the difficulty of sampling and observing them in their deep-water habitat (Rodhouse & Nigmatullin 1996). Some pelagic species, such as the jumbo Humboldt squid *Dosidicus gigas*, migrate vertically from deep to near the sea surface at night to forage (Yatsu et al. 1999), allowing scientists to recover information about their biology. The varying sizes of jumbo squid may determine their relationship to diverse taxa in nearshore and pelagic communities: while *D. gigas* are voracious predators (Nigmatullin et al. 2001), larger squid may become the prey of apex predators such as sperm whales (Clarke et al. 1988, Clarke & Paliza 2001, Ruiz-

Cooley et al. 2004, 2006), and smaller squid may be consumed by other predators. Hence, investigating the trophic variation of *D. gigas* of different sizes is essential to understand its role in the food webs. For instance, cephalopods can significantly impact marine food webs due to their high metabolic requirements, voracious predatory behavior and inter-annual variation in recruitment (Rodhouse & Nigmatullin 1996). If migratory, the predatory pressure over prey populations is spatially and temporally variable (Rodhouse & Nigmatullin 1996). Therefore, investigating aspects of trophic ecology and migration simultaneously is crucial to having an integrative picture of their role in the marine ecosystem.

The jumbo squid is considered highly migratory (Nigmatullin et al. 2001). Research into *Dosicus*

gigas migration includes: analyses of landing statistics and commercial catch data (e.g. Ehrhardt et al. 1983), ultrasonic telemetry (Yatsu et al. 1999), mark and recapture methods (Markaida et al. 2005), and electronic tagging (Gilly et al. 2006). Although these methods have provided valuable information, efforts are limited to regional areas and are therefore inconclusive at the population level. Stomach content analyses have yielded important and detailed information on dietary aspects of *D. gigas* (e.g. Markaida & Sosa-Nishizaki 2003), but these data only reflect recent diet, and underestimate the role of jumbo squid in food webs due to detection problems for some prey and interpretation of data (Rodhouse & Nigmatullin 1996). Moreover, the 'snapshot' nature of data derived from this approach is a poor indicator of long-term trophic status of the species.

In marine biogeochemistry, $\delta^{13}C$ and $\delta^{15}N$ are effective tracers of element cycling and the source/sink relationship of organic and inorganic matter; they are commonly used to investigate spatial and temporal variability throughout the water column (e.g. Benson & Parker 1961, Richards & Benson 1961, Libes & Deuser 1988) and marine sediments (Peters et al. 1978, Sweeney & Kaplan 1980, Altabet et al. 1999). These biochemical tracers have not only been used in marine ecology to describe food webs and trophic relationships (Rau 1982, Minagawa & Wada 1984, Hobson & Welch 1992, Kelly 2000), but also to investigate movements of animals (Fry 1981, Schell et al. 1989a, Best & Schell 1996, Hobson 1999). In jumbo squid, $^{13}C/^{12}C$ and $^{15}N/^{14}N$ from muscle and beak wing, including beaks recovered from a sperm whale's stomach, have been used to provide a relative trophic position and to evaluate trophic relationships between sperm whale and squid (Ruiz-Cooley et al. 2004, 2006). The $\delta^{13}C$ and $\delta^{15}N$ values in jumbo squid muscle represent a trophic position that changes mainly as a function of growth rates, whereas isotopic ratios in beak wing reflect a mean trophic position for the period of time in which the entire beak wing is synthesized (Ruiz-Cooley et al. 2006). Therefore, the biochemical composition and metabolism of a given tissue determine the period of time that isotopic ratios reflect an average diet composition (Hobson et al. 1996). Hence, animals moving between isotopically distinct food webs incorporate signatures from their diet as metabolic inactive tissues grow (Schell et al. 1989a, Hobson & Clark 1992, Hobson et al. 1996). In bowhead whales *Balaena mysticetus*, C and N stable isotope ratios (in subsamples taken along keratinous baleen plates) revealed temporal changes as a result of feeding in winter versus summer areas (Schell et al. 1989a,b). These variations were explained by the geographically distinct isotopic composition of zooplankton along the migratory routes of bowhead whales (Saupe et al. 1989, Schell et al. 1989a,b).

In the present study, we measured isotopic ratios of C and N along the gladius to examine trophic shifts. The gladius is a chitinous structure that is synthesized throughout the life of a squid, and it grows by adding chitin to the anterior ventral side of the gladius plate (Bizikov 1991). Therefore, information from a squid's assimilated diet would be integrated along the proostracum (a morphological part of the gladius; Bizikov 1991) by the addition of chitin with no turnover after synthesis as it grows. Our objectives were to investigate the isotopic variability along the proostracum as a function of size, and to evaluate differences in proostracum isotopic composition among 6 locations. We tested the hypothesis that stable isotopic values tend to increase as squid grow, and secondly, that squid have different isotopic signatures in their gladii among sites. We suggest that $\delta^{13}C$ and $\delta^{15}N$ values along the proostracum represent an integrated dietary chronology of a squid's life that can be used to make inferences about squid trophic variation and distinguish between subpopulations.

MATERIALS AND METHODS

Sampling. During the fall of 2006, jumbo squid (n = 39) were collected at night in the Gulf of California, USA (Site 1), and inshore (Sites 2, 3 and 4) and offshore (Site 5) waters of the central eastern Pacific and Fort Bragg, California (Site 6: Fig. 1), using jigs and fishing rods onboard National Oceanic and Atmospheric Administration (NOAA) research vessels. Specimens were frozen immediately at –20°C. From all sites, individuals with mantle length (ML) between 20 to 40 cm were selected for comparison (except for large sized-squid collected at Fort Bragg). Mantle lengths were measured and gladii were removed. Total gladius length (GL) was measured in the lab to the nearest 1 mm. Squids had GLs ranging from 19.8 to 40.3 cm (Table 1), but an individual of 13.3 cm GL was also included for Site 2 to increase sample size. For Site 6, we included large individuals from 45.5 to 73 cm GL; size ranges that were commonly found in this area (J. Field pers. comm.).

The gladius has 3 morphological parts: proostracum, conus and rostrum (Fig. 2, Bizikov 1991). Material is added to these layers as the squid grows and previous studies have used this gladius for age determination (e.g. Bizikov 1991). In the present study, part of the proostracum was used while the rostrum or conus were not included for stable isotope analysis (Fig. 2). The proostracum was cleaned carefully with double-distilled water and KimWipes (Kimberly-Clark), mea-
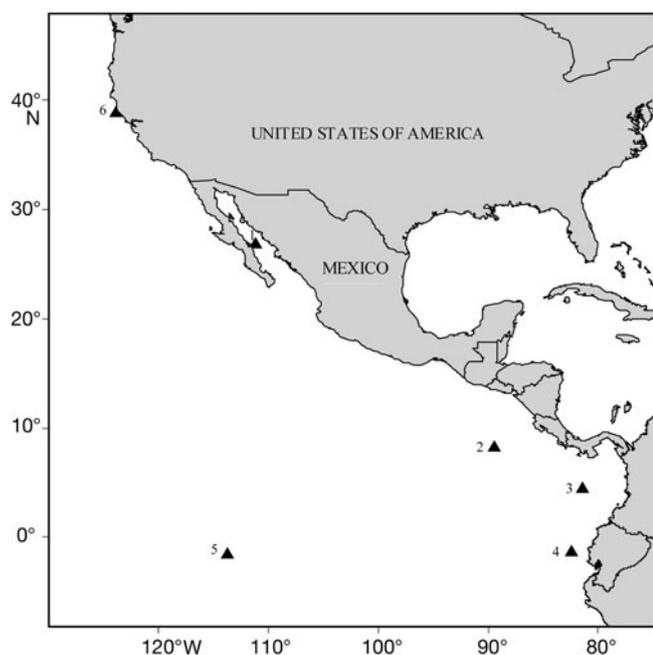
Fig. 1. Sampling sites where *Dosidicus gigas* were collected aboard NOAA research vessels in 2006

sured and marked every 3 cm beginning from the anterior edge of the gladius to the posterior narrower section (Fig. 2). Sections were cut with fine scissors following the 'V' shape of the growth lines.

Stable isotope analysis (SIA). Proostracum sections were freeze-dried and superficial lipid remains from attached tissue were removed by sonication using a 2:1 mixture of chloroform and methanol (modified from Bligh & Dyer 1959). Dry sections were ground to a fine powder following procedures of Alexander et al. (2007), but liquid nitrogen was not used. Isotope ratios of C and N were measured using a mass spectrometer (Thermo Finnigan) coupled with an Elemental Analyzer at the University of New Mexico. The ^{13}C and ^{15}N values were measured relative to Vienna Pee Dee Belemnite (VPDB) and atmospheric nitrogen (N$_2$). The international reference materials for carbon were NBS-21, NBS-22 and USGS-24 and USGS RSIL-N11, IAEA N1 and IAEA-N3 for nitrogen. The relative differences (δ) of ^{13}C and ^{15}N of the samples were calculated using the equation: $\delta^{15}N$ or $\delta^{13}C = [(R_{sample}/R_{standard}) - 1] \times 1000$. Where R_{sample} and $R_{standard}$ are the ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$ of the sample and the standard, respectively. Repeated measurements of internal lab standards every 10 gladii samples had a standard deviation of ±0.12‰ for $\delta^{15}N$ and ±0.07‰ for $\delta^{13}C$.

Statistical analysis. Regression analysis was used to evaluate the linear relationship between ML and GL. Along the proostracum, each isotopic signature represents a mean isotopic value for a given 3 cm section. Therefore, $\delta^{13}C$ and $\delta^{15}N$ from each section were matched according to their mean section length. For each site, we used random coefficient models (RCM, Longford 1993) using SAS software (version 9.1) to examine the relationship between GL and the $\delta^{13}C$ and the $\delta^{15}N$ values for each squid; for Site 6 (Fort Bragg), we only considered isotopic values from sections of gladius <41 cm in length. RCMs allow one to estimate the average relationship across individuals by incorporating individual squid effects as random (intercepts and slopes). The approach recognizes the hierarchical structure of the data and estimates site level regression
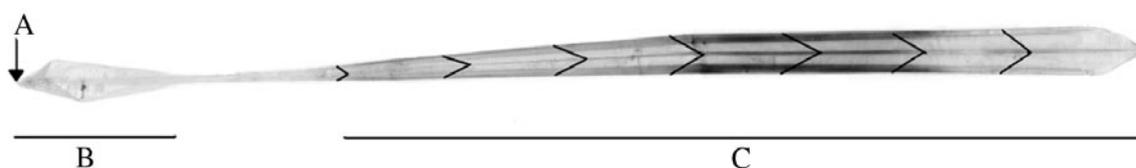

Fig. 2. Gladius of *Dosidicus gigas*. (A) Rostrum, (B) conus and (C) part of the proostracum (adapted from Bizikov 1991). The indicated proostracum was used in this study for sub-sampling of 3 cm sections (as shown by the v-shaped lines) following the direction of growth lines

Table 1. *Dosidicus gigas.* Mean gladius length (cm, ±95% CI) and isotopic ratios of carbon (C) and nitrogen (N) (mean ± 2 SE) from all squid by site using average values from all proostracum sections of a given squid

Site	n	Date	Gladius length	No. of sections	Proostracum isotopic values (‰)	
					$\delta^{13}C$	$\delta^{15}N$
1	8	11 Aug	34.92 (±3.27)	7 to 10	−17.52 ± 0.17	14.14 ± 0.35
2	7	21 Sep	23.22 (±4.85)	4 to 7	−17.57 ± 0.18	8.90 ± 0.74
3	7	10 Sep	28.50 (±3.32)	6 to 8	−17.50 ± 0.17	9.20 ± 0.16
4	6	28 Sep	28.00 (±6.38)	5 to 9	−17.33 ± 0.26	7.01 ± 0.14
5	8	17 Sep	33.90 (±4.57)	7 to 11	−18.10 ± 0.22	4.87 ± 0.41
6	3	14 Sep	56.00 (±36.83)	12 to 19	−18.40 ± 0.52	10.53 ± 0.59

coefficients that are compared by analysis of covariance (ANCOVA). We used the estimated regression models to examine changes in $\delta^{13}C$ or $\delta^{15}N$ as related to GL. ANCOVA was used to examine site differences for $\delta^{13}C$ or $\delta^{15}N$ values. Therefore, individual linear regression models were fit for each site with length along the gladius operating as the explanatory variable or covariate. Slopes (β) different from zero would indicate that $\delta^{13}C$ or $\delta^{15}N$ values increase or decrease along the GL. Different intercepts (α) would indicate differences in $\delta^{13}C$ or $\delta^{15}N$ values among sites. Testing for interaction would determine if slopes differ among sites. Spearman's rank correlation (r_S) was used to correlate $\delta^{13}C$ or $\delta^{15}N$ values to latitude and longitude to consider the degree to which any observed variations are linear.

RESULTS

A strong positive linear relationship was detected between GL and ML ($r^2 = 0.938$; GL = $0.994 \times$ ML + 0.968). We obtained 4 to 19 sections of 3 cm each along the proostracum on each squid depending on GL (Table 1). A total of 312 stable isotopic values for each element were obtained.

Trophic variation

The average $\delta^{13}C$ and $\delta^{15}N$ isotopic values of each section were matched with its corresponding position along the length, beginning from the anterior end to the posterior narrower end of the proostracum (Fig. 2). The $\delta^{13}C$ and $\delta^{15}N$ values varied throughout proostracum sections for all individuals (Figs. 3 & 4) with overall increasing N-isotopic values as squids became larger (RCM, $F_{1,13.9} = 53.85$, p < 0.0001). The degree to which $\delta^{15}N$ values increased by length did not differ among sites (ANCOVA, $F_{5,86} = 1.44$, p = 0.2186). Thus, a common slopes model was adopted, and this is reflected in the estimated slope (β, Fig. 3). Similarly, a positive relationship was detected between $\delta^{13}C$ and GL (RCM, $F_{1,19.3} = 17.23$, p = 0.0005), which also led to the adoption of a common slopes model (Fig. 4) given non-detectable interaction term effects using ANCOVA ($F_{5,102} = 0.82$, p > 0.5351). The estimated slope was nearly 3 times larger for N than for C.

Geographic variation

The $\delta^{15}N$ isotopic values differed significantly among sites (ANCOVA, $F_{5,110} = 120.67$, p < 0.0001) as indicated by the different regression model intercepts

(Fig. 3). The $\delta^{13}C$ isotopic values also detectably differed among sites (ANCOVA, $F_{5,124} = 10.75$, p < 0.0001, Fig. 4). The $\delta^{15}N$ (Fig. 3) and $\delta^{13}C$ (Fig. 4) along proostracum amongst all squid and sites did not follow a specific pattern. The highest mean $\delta^{15}N$ values in proostracum were observed for squid from the Gulf of California (Site 1) and it was followed by Site 6 > Site 3 > Site 2 > Site 4 > Site 5 (Table 1, Fig. 5). The isotopic difference in $\delta^{15}N$ mean values between Site 1 and 5 was 9.27 ‰, whereas Site 4 and 5 differed only by 2.17‰. In contrast, the isotopic difference between the mean values from the site with the highest $\delta^{13}C$ (Site 4) versus the lowest $\delta^{13}C$ (Site 6) values was 1.07 ‰ (Table 1, Fig. 5). The site with squid having GLs of >45 cm (Site 6) exhibited the highest isotopic variability in $\delta^{13}C$. However, the site with the fewest overlapping lines among individuals (Fig. 3, Site 2) had the highest standard deviation in $\delta^{15}N$, which was followed by Site 6 (Fig. 5). The r_S correlation between $\delta^{15}N$ and latitude demonstrated a strong positive association ($r_S = 0.82$, p < 0.001). The $\delta^{13}C$ also exhibited a positive relationship with latitude, but the strength of the association was weak ($r_S = 0.15$, p = 0.011). The observed relationships with $\delta^{15}N$ and $\delta^{13}C$ and longitude were best described as moderate negative associations ($r_S = -0.14$, p = 0.014 and $r_S = -0.57$, p < 0.001, respectively).

DISCUSSION

Trophic variation

This research demonstrates the use of C and N SIA in proostracum to reconstruct trophic variations of the jumbo Humboldt squid *Dosidicus gigas*. Since growth rates in jumbo squid vary by size, season and location (see Argüelles et al. 2001, Markaida et al. 2004), each 3 cm section is likely to represent different time periods within a squid and also among individuals and areas. Therefore, each isotopic signature by proostracum section was related with a measurement that is common for all squid: length along gladius (Figs. 3 & 4). The strong positive linear relationship between ML and GL agree with previous studies (e.g. Jackson et al. 1993, Perez et al. 1996). The $\delta^{13}C$ and $\delta^{15}N$ values varied along the proostracum for all squid and sites with increasing $\delta^{15}N$ values as a function of size (Figs. 3 & 4). The positive relationship of size was weaker for $\delta^{13}C$ values. These results were expected since $\delta^{13}C$ signatures undergo only slight enrichment in consumers over their diet (0.5 to 1.5 ‰: DeNiro & Epstein 1978, Tieszen et al. 1983), whereas $\delta^{15}N$ can discriminate between trophic levels due to higher isotopic shifts between predator tissues relative to their diet (DeNiro & Epstein 1981, Minagawa & Wada 1984). A

previous study in the Gulf of California showed the $\delta^{13}C$ and $\delta^{15}N$ values of both muscle and beak wing tissues increased continuously as jumbo squid increased in size, indicating an increase in trophic position (Ruiz-Cooley et al. 2004, 2006). In our study, we did not

observe these continuously increasing isotopic values, but observed increasing variable isotopic shifts along the proostracum. Previous studies analyzed squid that ranged from 20 to 85 cm ML with signatures obtained from different squid, whereas the present study mea-
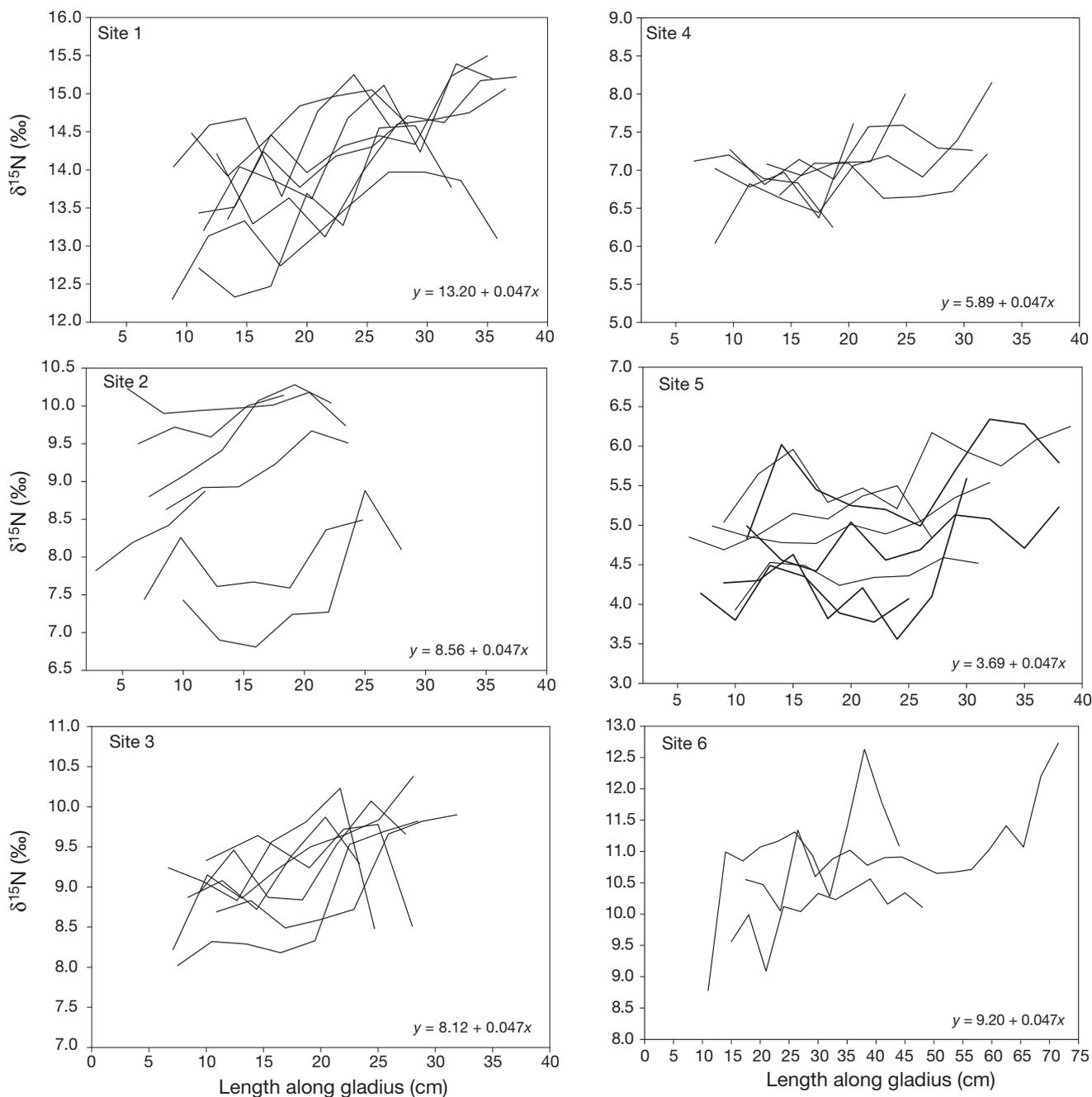

Fig. 3. *Dosidicus gigas*. Relationship between $\delta^{15}N$ values (‰) and length along gladius from all proostracum sections by squid and site: (Site 1) Gulf of California, (Sites 2, 3, 4) inshore and (Site 5) offshore central eastern Pacific, and (Site 6) Fort Brag. Note that the scale for Site 6 is different. Estimated regression models describing the relationship between isotopic signatures (y) of nitrogen (N) and gladius section (x) by site; we only considered isotopic values from sections at gladius length <41 cm for Site 6. The estimated intercepts (α) represent differences in isotopic signatures among sites. A common slope (β) model for the effect of gladius was used given non-detectable interaction term effects in the analysis of covariance ($F_{5, 86} = 1.44$, p = 0.2186). The slope for the model in $\delta^{15}N$ had an SE of 0.006
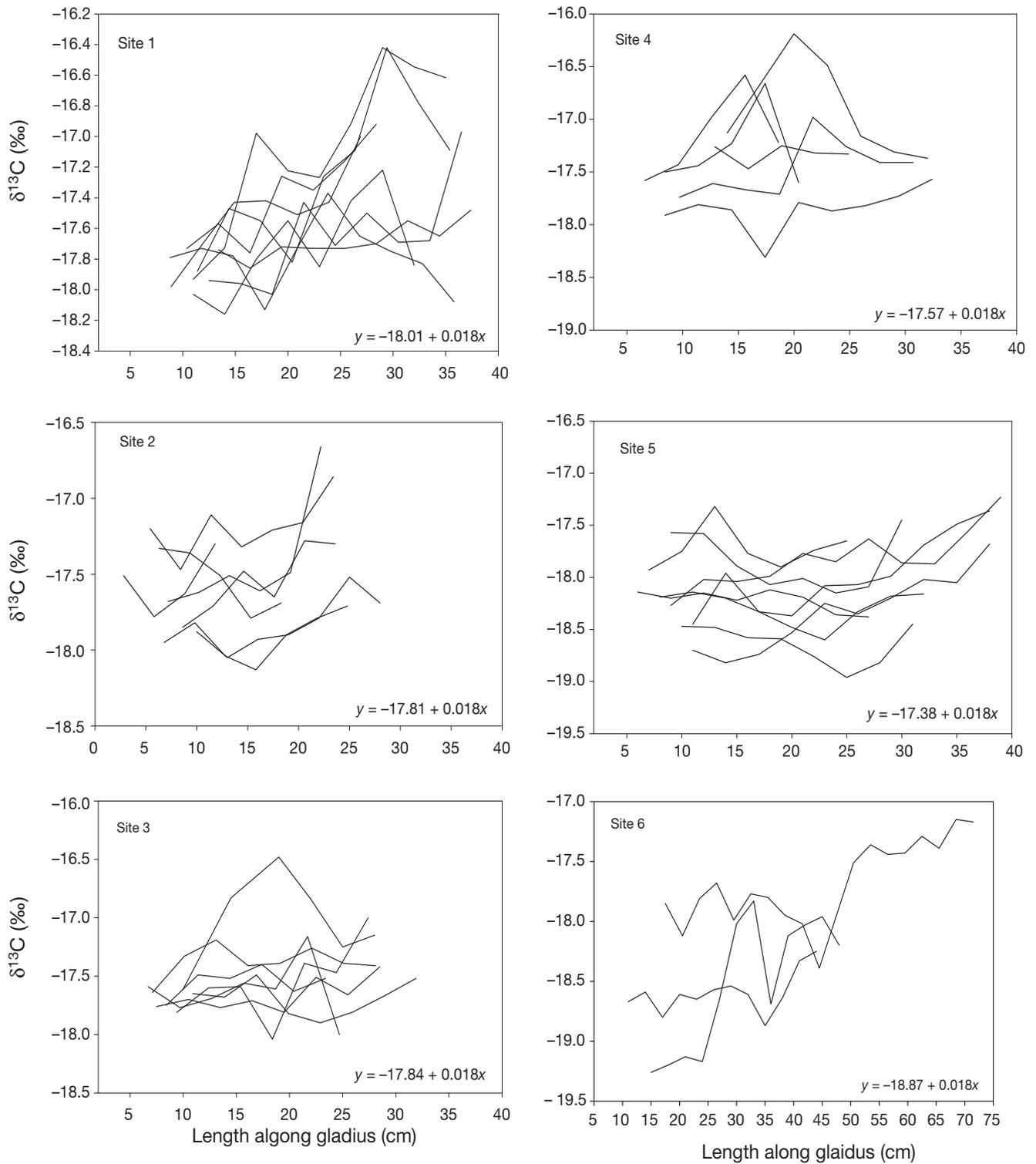
Fig. 4. *Dosidicus gigas*. Relationship between $\delta^{13}C$ values (‰) and length along gladius from all proostracum sections by squid and site: (Site 1) Gulf of California, (Sites 2, 3, 4) inshore and (Site 5) offshore central eastern Pacific, and (Site 6) Fort Brag. Note that the scale for Site 6 is different. Estimated regression models describing the relationship between isotopic signatures (*y*) of carbon (C) and gladius section (*x*) by site; we only considered isotopic values from sections at gladius length <41 cm for Site 6. The estimated intercepts (α) represent differences in isotopic signatures among sites. A common slope (β) model for the effect of gladius was used given non detectable interaction term effects in the analysis of covariance ($F_{5,102} = 0.82$, p > 0.5351). The slope for model for $\delta^{13}C$ had an SE of 0.004
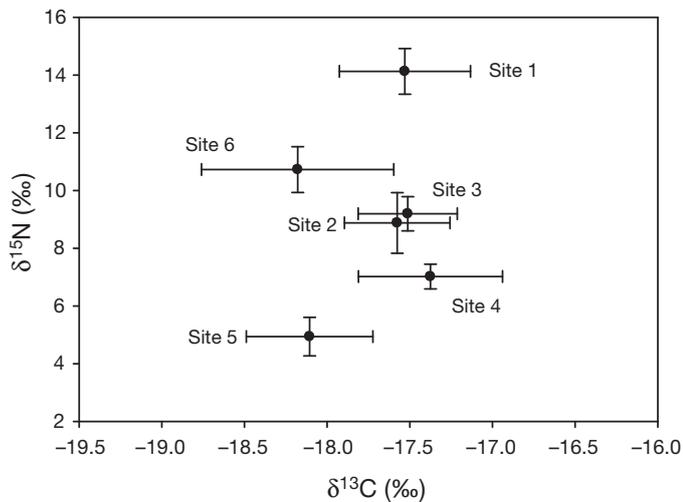
Fig. 5. *Dosidicus gigas*. Relationship between mean stable isotope ratios of carbon and nitrogen (‰; ±1 SD) from all proostracum sections by site: (Site 1) Gulf of California, (Sites 2, 3, 4) inshore and (Site 5) offshore central eastern Pacific and (Site 6) Fort Bragg

sured isotopic ratios along proostracum sections from specimens of similar lengths, except for Site 6.

These variations in methodology reveal different information: (1) the individual isotopic variability along the proostracum throughout the life of a given squid as a function of size (present study) versus (2) isotopic ratios in muscle from different squid of a given site (previous study from Ruiz-Cooley et al. 2004). Using our estimated regression models (Figs. 3 & 4), the isotopic difference between an old (e.g. at 6 cm) and more recent section (e.g. at 40 cm) along the proostracum was 1.6 ‰ for $\delta^{15}N$ and 0.6‰ for $\delta^{13}C$ for an increment of 34 cm. The largest squid analyzed in our study (75 cm ML, Site 6) had an isotopic difference of 2.18 ‰ for $\delta^{15}N$ and 0.68‰ for $\delta^{13}C$, with a difference of 54 cm between the oldest and most recent section. All these values were lower than the isotopic difference documented in both *Dosidicus gigas* beak (6.8 ‰ for $\delta^{15}N$ and 2.81 ‰ for $\delta^{13}C$) and muscle (3.96 ‰ for $\delta^{15}N$ and 2.69 ‰ for $\delta^{13}C$) for a shift in MLs of 64 cm (calculated or estimated using maximum and minimum values published in Ruiz-Castro 2002). Our values were also lower than the variation in $\delta^{15}N$ from muscle (>5 ‰) between squid with a difference in MLs >35 cm from both *Ommastrephes bartramii* and *Sthenoteuthis oualaniensis* (Parry 2008). As previously explained, these inconsistencies derived from the approach taken. For instance, variable $\delta^{15}N$ values between some parts of the beak (e.g. 3.2‰ between the rostrum and wing) and gladius (e.g. 1.9‰ between the conus and middle part or 5.4 ‰ between the middle and anterior piece) were also observed in *Todarodes filippova* because these parts integrate diet at different periods of time

(Cherel et al. 2009). Unfortunately, since this previous study did not relate isotopic values from hard tissues with length, we were unable to compare our results.

In our study, we suggest that C- and N-isotopic ratios derived from jumbo squid assimilated diet are incorporated along proostracum as chitin is added during growth. Hence, the isotopic variability along the proostracum (Figs. 3 & 4) represents a record of *Dosidicus gigas* dietary life, possibly due to temporal variation in diet consumption. Isotopic ratios for a given 3 cm proostracum section reflect shifts in *D. gigas* feeding activity at a particular length interval. Low shifts may reflect a diet composed mainly by invertebrates (e.g. crustaceans) whereas higher values may indicate a diet based on vertebrates (e.g. fish). Control dietary experiments revealed that higher trophic shifts in $\delta^{15}N$ were observed in consumers fed with high protein diets while low shifts were recorded if predators were fed with diets composed of invertebrates (McCutchan et al. 2003). In the Gulf of California, both stomach content and SIA showed that medium size squid fed at lower trophic positions than large jumbo squid (Ruiz-Cooley et al. 2006). Hence, the isotopic compositions of these shifts in diet are likely to be incorporated in proostracum as squid grow. Control dietary experiments and SIA in the lower beak of *Sepia officinalis* linked low $\delta^{15}N$ values from the rostral tip to a diet based on mysids during juvenile stages while higher values in wing were associated with a more recent diet composed of shrimps (Hobson & Cherel 2006). Perhaps, the high $\delta^{15}N$ values observed at Site 6 (Fig. 3) may correspond to a high consumption of hake for the squid larger than 50 cm ML as stomach content analysis revealed (Field et al. 2007). Another source of variation in stable isotopes along the proostracum could derive from squid moving and feeding in areas with distinct isotopic values as they grow. This could be particularly important for large squid since they are expected to be highly mobile.

The increasing but variable $\delta^{13}C$ and $\delta^{15}N$ along proostracum (Figs. 3 & 4) suggest that *Dosidicus gigas* opportunistically feeds on available prey (Nigmatullin et al. 2001) but tends to consume prey from higher trophic positions as it grows, perhaps to maximize feeding success. Based on prey frequency of occurrence, Field et al. (2007) suggested that larger prey items are likely to make a greater contribution to jumbo squid diet. Optimal foraging theory predicts that a predator foraging decision should increase if the resulting gain in time spent per unit food exceeds the loss (MacArthur & Pianka 1966). Hence, the cost/benefit of *D. gigas* foraging behavior in a given period of time and site may be associated with prey attributes (e.g. size, density, biodiversity, abundance), which are likely to change through time in response to environ-

mental stochasticity. For instance, $\delta^{15}N$ in muscle from *Ommastrephes bartramii* of the same overall area were significantly different among 4 yr of study, possibly due to changes in prey consumption (Parry 2008).

The variability in isotopic signatures along proostracum and possible changes in prey composition through time may explain the apparently contrasting evidence of jumbo squid diet described by stomach content analysis. For example, some studies have indicated that *Dosidicus gigas* mainly fed on myctophids (e.g. >60%; Markaida & Sosa-Nishizaki 2003), while other studies have reported different taxa or fish of commercial importance as the main prey (e.g. Sato 1976, Ehrhardt et al. 1983, Nixon 1987, Olson et al. 2006, Field et al. 2007). Even though ontogenic shifts in diet have been observed in some locations using analysis of stomach contents (e.g. Nesis 1970, Shchetinnikov 1989), *D. gigas* did not exhibit these shifts in the Gulf of California since squid from 14.5 to 87.5 cm ML consumed prey of a similar size (Markaida & Sosa-Nishizaki 2003). Additionally, the diet composition of this species seemed to vary at temporal scales (monthly and annually) before the 1980s (e.g. Sato 1976, Ehrhardt et al. 1983, 1991), but a stabilized diet of myctophids (mainly *Benthosema panamense* and *Triphoturus mexicanus*) was documented between 1995 and 2000, possibly resulting from the high abundance of these fish (Markaida & Sosa-Nishizaki 2003, Markaida 2006).

SIA using beak wing, muscle (Ruiz-Cooley et al. 2006) and now proostracum support jumbo squid onto-genic shifts in diet. These shifts have been documented by also measuring isotopic ratios in soft and hard tissues from other squid species collected in the wild (Cherel & Hobson 2005, Parry 2008, Cherel et al. 2009) and in individuals raised in controlled experiments (Hobson & Cherel 2006). Recent evidence based on electronically tagged squid indicates that *Dosidicus gigas* forages during the day at depths >200 m (Gilly et al. 2006). Therefore, it is likely that fresh prey ingested by jumbo squid at these depths may be partially digested or excreted before the squid begin foraging in the upper water column. Consequently, stomach content analysis may partially record the presence of prey caught at depth. Since stomach content and isotopic analysis reveal different dietary aspects of *D. gigas* (Ruiz-Cooley et al. 2006), a study based on SIA along the proostracum, and in muscle combined with stomach content analysis, may elucidate the feeding habits of jumbo squid as a function of time, area and size. This is of particular interest since the current range expansion (Pearcy 2002) and predatory pressure of *D. gigas* in new colonized areas seem to have a negative impact (increase in mortality and changes in prey behavior) on fish stocks of commercial importance (i.e. Pacific hake; Field et al. 2007, Holmes et al. 2008).

Geographic variation

Significant differences in the $\delta^{15}N$ values were found among sites of contrasting latitude (>18°), but were not observed between Sites 2 and 3 (distance of 4° latitude). A significant geographic variation in the $\delta^{15}N$ values was detected in muscle tissues of the squid *Sthenoteuthis oualaniensis* (Takai et al. 2000), and also in the tissues of sperm whales (Marcoux et al. 2007) and leatherback turtles (Wallace et al. 2006). Spatial variation in $\delta^{15}N$ has been well documented in marine phytoplankton and particulate organic and inorganic nitrogen (Miyake & Wada 1967, Wada & Hattori 1978, 1991, Montoya & McCarthy 1995, Waser et al. 1998). For instance, denitrification (conversion of NO_3^- and NO_2^- to N_2O by microbes) produces high isotopic values in suboxic subsurface waters such as in the Carmen and Guaymas basin of the Gulf of California (Altabet et al. 1999), while undetectable changes in $\delta^{15}N$ exist during N_2 fixation (Hoering & Ford 1960). Because N is an essential nutrient that can limit primary production (Naqvi et al. 2008), the interoceanic and intraoceanic variation in $\delta^{15}N$ from animal tissues may relate to differences in the metabolism and cycling of N, which in turn is driven by variability in the physical and biochemical properties between oceanic basins (Takai et al. 2000, Wallace et al. 2006). Our samples were collected in sites from various systems such as the Gulf of California, Costa Rica Dome, the eastern equatorial current, and the California Current. Therefore, our results agree with these geographic isotopic variations in N and suggest that $\delta^{15}N$ in jumbo squid proostracum varied as a function of location, perhaps due to differences in the assimilated diet, but also due to variability in the organic pools as well as physical and biochemical properties among areas. Because the isotopic pattern along the proostracum represents a dietary record throughout the life of a squid, the resultant $\delta^{15}N$-isotopic differences among areas provides the strongest evidence in geographic variation in squid. Hence, $^{15}N/^{14}N$ not only discriminates between trophic levels (DeNiro & Epstein 1981) but also provides a means to differentiate geographically between squid subpopulations.

Variations in the $\delta^{13}C$ in the proostracum as a function of location were expected since $\delta^{13}C$ values trace the origin of C sources (DeNiro & Epstein 1978, Rau et al. 1982, Peterson & Fry 1987) and vary among latitudes, possibly due to variable water temperature and CO_2 concentrations (Sackett et al. 1965, Rau et al. 1982). The isotopic ratios of other squid species collected between different oceanic areas across a wider latitudinal range (>60°) showed a strong relationship between $\delta^{13}C$ and latitude (Takai et al. 2000). The results of this study partially agree with this $\delta^{13}C$ latitudi-

nal variation because the values varied among sites but the relationship between $\delta^{13}C$ and latitude was weak; perhaps the narrower latitudinal range (~40°) where squids were sampled can explain this weak association. In contrast, the positive relationship between $\delta^{15}N$ and latitude was strong and different between sites. A latitudinal $\delta^{15}N$ relationship has also been observed using the skin of sperm whales from the southeast Pacific (r = 0.89 from 25° S to 0°; Marcoux et al. 2007), but the relationship was negative. In turn, a moderate positive association was found in muscle tissues from yellowfin tuna from the western Indian Ocean ($r^2 = 0.25$, from 20° S to 8° N; Ménard et al. 2007) and from the central eastern Pacific (from 10° S to 25° N, Popp et al. 2007). From the latter area, $\delta^{15}N$ from amino acids and meso-zooplankton paralleled the latitudinal relationship recorded in yellowfin tuna muscle (Popp et al. 2007). This latitudinal variation might be associated with the effect of latitudinal gradients in physical variables over primary producers (Sackett et al. 1965, Rau et al. 1982, Wada & Hattori 1991).

Implications of the geographic heterogeneous isotopic values in gladii

Although isotopic values along the proostracum were highly variable for all individuals (Figs. 3 & 4), they grouped by site and did not overlap between areas except for those of Sites 2 and 3 (Table 1, Fig. 5). These results were unexpected because *Dosidicus gigas* is considered highly migratory (Nigmatullin et al. 2001) and foraging across large areas would homogenize isotopic ratios. In particular, for the squid size ranges, year and sites analyzed in our study, our results suggest that these squid did not move at latitudes >18° (e.g. between the central East Pacific and Gulf of California) but could have possibly moved within narrower latitudinal ranges: perhaps at distances <4° latitude (~442 km) and <8° longitude (890.5 km) based on the distance between Sites 2 and 3; this distance is 2 to 4.5 times longer than the maximum displacement movement (i.e. 200 km over 7 d) recorded for an electronically tagged jumbo squid (Gilly et al. 2006). Although *D. gigas* is believed to migrate for long distances over its entire range (Nesis 1983) or between the Gulf of California and Pacific Ocean (Ehrhardt et al. 1983), these migrations have not been observed (Morales-Bojórquez et al. 2001). Instead, conventional mark-recapture methods revealed that jumbo squid exhibited seasonal movements between the east and west central Gulf of California (Markaida et al. 2005), while electronic tagging showed that squid >75 cm ML moved in various directions but stayed in the central Gulf (Gilly et al. 2006). Due to the current lack of evidence to sup-port long-distance migration between oceanic currents or different marine systems, along with the observed significant geographic differences in $\delta^{15}N$ proostracum among sites, it is possible that passive dispersal of eggs could favor considerable colonization of areas. This could occur because *D. gigas* has the highest potential for fecundity amongst cephalopods (Nigmatullin et al. 2001, Nigmatullin & Markaida 2008) with egg mass occurring near the sea surface (e.g. 16 m depth; Staaf et al. 2008); it is likely that oceanic currents passively transport eggs over long distances (Nigmatullin et al. 2001, Rodhouse 2008). Another mechanism for passive dispersal may be attributed to the strength and pathway of hurricanes. This phenomenon deepens mixed layers (Dickey & Simpson 1983, Sanford et al. 1987), changes the biogeochemistry of superficial waters (e.g. Bates et al. 1998), and produces phytoplankton blooms (Babin et al. 2004); its role in the biology of marine species is relatively unknown, but may have important implications in the ecology of jumbo squid.

CONCLUSIONS

This study presents an innovative method to investigate the trophic ecology of pelagic squid such as *Dosidicus gigas*. The isotopic variability can be traced throughout squid life at temporal and spatial scales by measuring $\delta^{13}C$ and $\delta^{15}N$ through systematic subsampling along the proostracum. Further research is needed to relate high or low isotopic values along proostracum to changes in diet (e.g. from a diet composed mainly of invertebrates to vertebrates or shifts in diet from myctophids to sardine or hake). We recommend incorporating an analysis of age determination using gladius with the approach presented in this work to provide a period of time for each proostracum section. Using analysis of stable isotopes along proostracum in combination with other methods (e.g. stomach content analysis) can help scientists to integrate and understand the function of *D. gigas* in food webs at the current geographic distribution. This is particularly important since changes in marine ecosystems due to climate change continue. To improve management strategies, there is a need to better understand the role of cephalopods in the dynamics of marine food webs, as well as pelagic communities and their impact on fish stocks of commercial importance. Measuring $^{13}C/^{12}C$ and $^{15}N/^{14}N$ along the proostracum provides a way to trace squid feeding variability as a function of size and to differentiate subpopulations geographically, based on what and where they eat. Studies investigating trophic relationships in marine ecosystem and assigning trophic levels based on SIA should consider this spatial geographic variation in the $\delta^{15}N$ values from primary producers and consumers.

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