

Contribution of prey to Humboldt squid *Dosidicus gigas* in the northern California Current, revealed by stable isotope analyses

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ABSTRACT: Diet studies have shown Humboldt squid *Dosidicus gigas* to be aggressive opportunistic predators, yet this approach has provided only a limited and potentially biased view of their trophic feeding behavior. As an alternative, we measured the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *D. gigas* and their prey from the northern California Current ecosystem (NCC) and applied stable isotope Bayesian mixing models (Stable Isotope Analysis in R [SIAR]) to assess if *D. gigas* isotopically matched NCC or southern California Current (SCC) migratory end-members and to examine the proportional trophic contributions of prey groups from the NCC to their diet. For the trophic SIAR model, cluster analysis of prey taxa by their respective $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values was first applied to consolidate prey into groups, which were then incorporated into the model as source groups to the diet mixture. Model results from examination of NCC and SCC migratory end-members indicated greatest contributions from the NCC system, indicating *D. gigas* was more integrated with the regional NCC isotopic signature. From the trophic SIAR model, the results indicated mixed but lower trophic-level feeding by *D. gigas* relative to previous diet-based studies, with greatest contributions from macrozooplankton, ichthyoplankton, and nekton such as juvenile rockfish, market squid, sand lance, and juvenile Pacific hake. Sensitivity analyses of the SIAR model based on varying isotopic fractionation factors of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ showed that proportional contributions of prey to squid diets were resilient to change.

KEY WORDS: Humboldt squid · *Dosidicus gigas* · California Current · Stable isotopes · Bayesian mixing model · Trophic analysis · Prey

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INTRODUCTION

Humboldt squid *Dosidicus gigas* exhibit major population shifts in abundance, which have led to a number of range expansions into the northeast Pacific Ocean (Zeidberg & Robison 2007, Keyl et al.

2008) and as far north as the Gulf of Alaska (Wing 2006). Members of this large ommastrephid species, also known as the jumbo squid, attain a maximum weight of 30 to 50 kg and live between 1 and 2 yr (Markaida et al. 2004, Mejía-Rebollo et al. 2008), reaching much of their final length within the first

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year (Markaida et al. 2004). This level of growth undoubtedly requires substantial energetic inputs, and *D. gigas* has been the focus of numerous trophic studies applying diet analysis and examining relational shifts in abundance of *D. gigas* and their prey resources. The seasonal migration of *D. gigas* into the central and northern California Current has been of particular concern because of its potential to alter the food web and prey upon commercially utilized and threatened species of fishes (Field et al. 2007, 2012). Although diet studies provide very useful information on trophic behavior, there are substantial biases and limitations in their interpretation. Trophic analysis using stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) can provide a different approach that can help resolve ambiguities in diet, especially in species that are highly opportunistic and not amenable to stomach content analysis (Miller et al. 2010). However, stable isotopes are limited in that they only provide values that are evidential of trophic links or behavior, while isotopic shifts associated with trophic level, and the duration isotopes are represented of past feeding, are not well resolved (Gannes et al. 1997, Post 2002).

From previous diet studies, *Dosidicus gigas* has been shown to feed predominantly on mesopelagic fishes and euphausiids but also on a diverse range of prey including other crustaceans, pteropods, other squid (Markaida & Sosa-Nishizaki 2003, Markaida 2006, Field et al. 2007, Markaida et al. 2008), and commercially important species of fish, such as Pacific hake *Merluccius productus*, rockfishes *Sebastes* spp., Pacific sardine *Sardinops sagax*, and northern anchovy *Engraulis mordax* (Ehrhardt 1991, Markaida & Sosa-Nishizaki 2003, Field et al. 2007, 2012, Braid et al. 2011). Decreases in abundances of Chilean hake *Merluccius gayi* (Alarcón-Muñoz et al. 2008, Arancibia & Neira 2008) and sardine (Ehrhardt 1991) have been attributed in part to *D. gigas* predation. Within the California Current, Litz et al. (2011) observed reductions in survey catches of juvenile Pacific hake, a species found to be an important component of *D. gigas*'s diet (Field et al. 2007), that were coincident with the arrival of *D. gigas* off Oregon and Washington. However, inference of trophic interactions based on shifts in prey abundance is circumstantial, and although diet studies provide invaluable information on feeding behavior, they are limited in only providing a snapshot of feeding activity, and there are inherent biases in interpretation of results (Cortés 1997). This is particularly so for stomach analysis of cephalopods because prey are often ingested piecemeal to allow passage from the

restricted esophagus to the stomach. Also, dietary studies of *D. gigas* have shown strong biases associated with fishing gear type (Ibáñez et al. 2008) as this species has been shown to exhibit net feeding, which has been presented as a possible reason for the importance of certain prey in diet-based studies (Field et al. 2007, Ibáñez et al. 2008, Olson et al. 2010).

Stable isotope analyses of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are additional tools in trophic studies that can help to determine an organism's respective source production and relative trophic position (Fry 2006). Stable isotopes are measured as a delta (δ) value in units of parts per million (‰) and are calculated as $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$, where X is ^{15}N or ^{13}C , and R is the ratio of the heavy (^{15}N or ^{13}C) to the light (^{14}N or ^{12}C) isotope. The $\delta^{15}\text{N}$ value is a measure of an organism's relative trophic position in which the consumer retains the $\delta^{15}\text{N}$ of its diet plus a trophic fractionation factor ($\Delta\delta^{15}\text{N}$) of $\sim 3.4\text{‰}$ (SD = 1.0; Post 2002). For $\delta^{13}\text{C}$, trophic fractionation ($\Delta\delta^{13}\text{C}$) is less pronounced ($\sim 0.4\text{‰}$, SD = 1.3, Post 2002), with greater differences appearing to occur between the type and location of primary production. Within marine systems, the difference in the type and location of primary production is most pronounced between nearshore and offshore waters, with a higher occurrence of ^{13}C -enriched diatom production nearshore that is expressed throughout the food web (Miller et al. 2008). Values of $\delta^{15}\text{N}$ can also vary substantially among habitats at the level of primary production and be expressed throughout the food web, and this variation is often marked between coastal and more oceanic waters that may vary in nutrient sources (Hobson 1999). In the case of migratory species, which may move between variable baselines of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, a comparison of a migrant's isotopic signature to the isotopic signatures of its food or a similar baseline trophic level can provide insight regarding whether a species is a migrant or has integrated with the local food web (Hansson et al. 1997).

Humboldt squid are seasonal migrants along the California Current, arriving between early and mid-summer months in the northern California Current (NCC) (Litz et al. 2011), potentially from either offshore of south-central California or subtropical slope-offshore waters off Baja Mexico (Staaf et al. 2011). Their occurrence in the NCC poses questions as to what prey species or groups are contributing to their diet and ultimately growth. Although stable isotope analysis has been proposed as a potential tool for examining trophic dynamics in *Dosidicus gigas* (Ibáñez et al. 2008), it has only been applied to exam-

ine ontogenetic shifts and trophic position relative to a limited number of their prey (Ruiz-Cooley et al. 2006, Drazen et al. 2008, Lorrain et al. 2011) and as a means to evaluate the importance of *D. gigas* as prey for marine mammals (Ruiz-Cooley et al. 2004).

In the present study, we measured the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *Dosidicus gigas* and a diverse range of their potential prey from the NCC ecosystem to examine the proportional contributions of these prey contributing to their diet. This was accomplished by utilizing a Bayesian mixing model, termed SIAR (Stable Isotope Analysis in R; Parnell et al. 2010), that incorporates the means and standard deviations of predator and prey and their trophic fractionation factors of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Prior to this analysis, we confirmed the feeding and isotopic integration of *D. gigas* within the NCC by use of a SIAR model that compared *D. gigas* isotope values to baseline migratory end-members from the NCC and southern California Current (SCC) systems. From *D. gigas* measured for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, we also examined size-specific differences in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and the C:N ratio that may be indicative of ontogenetic shifts in feeding. The results of the present study address important questions pertaining to *D. gigas*'s trophic status relative to a diverse range of prey that has thus far only been viewed through diet analyses.

MATERIALS AND METHODS

Field sampling

Collections of samples for isotope analysis covered the NCC ecosystem. For *Dosidicus gigas*, collections were made during August 2007 and July, August, and September 2009 by both hook-and-line using baited jigs and from midwater trawls during monthly fisheries surveys conducted along 4 transects ranging from Heceta Head, OR ($43^{\circ}59'49''\text{N}$), to Willapa Bay, WA ($46^{\circ}38'57''\text{N}$) (see sampling details in Phillips et al. 2009 and Litz et al. 2011) (Fig. 1). A total of 11 prey types (species and life history stages) of nekton and macrozooplankton from shelf ($\leq 150\text{ m}$ isobath) and slope ($>150\text{ m}$ isobath) waters were collected and analyzed from midwater trawls from the same region and time period as the above collections. However, we expanded our trophic analysis by obtaining additional prey isotope values from the same region but different time periods: June to July 2001 from Bosley et al. (2004), June and August 2000 and 2002 from Miller et al. (2010), and collections off northern California (Año Nuevo and Monterey Bay)

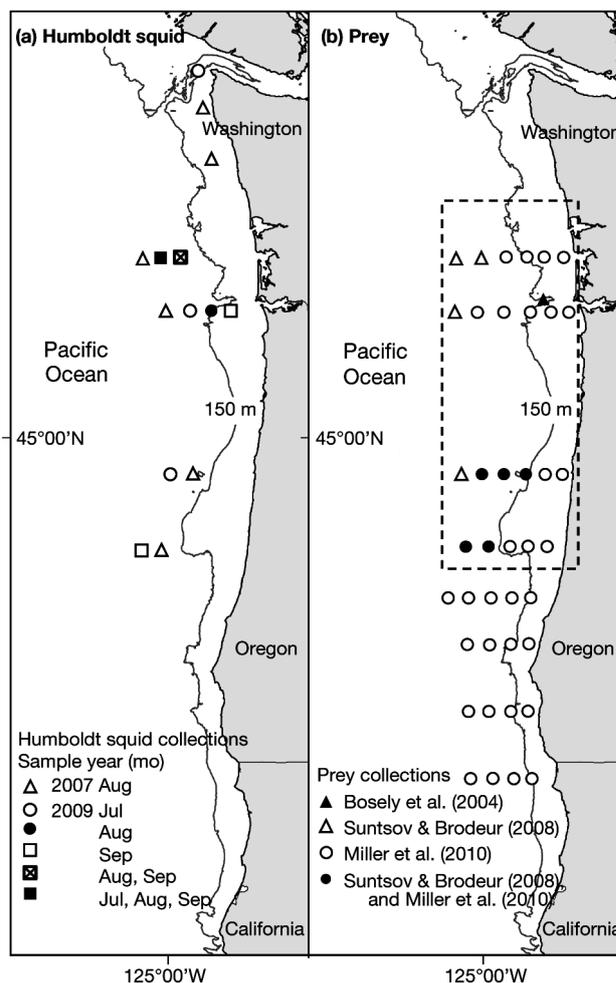


Fig. 1. (a) Collection sites of Humboldt squid *Dosidicus gigas* by year and month within the northern California Current. (b) Collection region of *D. gigas* prey from specific studies as noted by reference and from 2007 (August) and 2009 (July to September) from the present study (boxed region)

from 1996 to 2001 (season and year unspecified; Becker et al. 2007). All stable isotope values were obtained from analyses that did not use chemical preservation or lipid extraction methods on the tissue.

Isotope analysis

All organisms were either frozen (-20°C) whole at sea for eventual processing in the laboratory or, as in the case of *Dosidicus gigas*, were measured in the field for dorsal mantle length (cm), and a section of mantle tissue was removed and frozen for stable isotope analysis in the laboratory. For other organisms brought to the laboratory for processing and analy-

ses, we applied the procedure described by Miller et al. (2010). Briefly, fish were measured for standard length (mm), and a portion of anterior dorsal muscle was removed for isotope analysis. For small post-larval fishes, such as sardine, the head, gut, and caudal fin were removed, and the remaining whole body was subjected to isotope analysis. Micronekton, such as pandalid shrimp, had abdominal tissue removed for isotope analysis, whereas euphausiids (*Thysanoessa spinifera* and *Euphausia pacifica*) were analyzed whole. All tissue samples were initially lightly rinsed with distilled water and subsequently dried in a drying oven at 60°C for 48 h. After drying, the samples were pulverized to a fine powder using a mortar and pestle. For mesopelagic fish species, such as California headlight fish *Diaphus theta*, broadfin lampfish *Nannobranchium ritteri*, northern lampfish *Stenobranchius leucopsarus*, bigfin lanternfish *Symbolophorus californiensis*, and blue lanternfish *Tarletonbeania crenularis*, stable isotope analysis was carried out using a Costech elemental analyzer coupled to a Thermo Finnegan stable isotope ratio mass spectrometer (IRMS) in continuous flow mode. In-house standards were L-histidine and aspartic acid, with instrument error (SD) of 0.3 for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. The remaining organisms were analyzed using a Carlo Erba Elemental Analyzer 2500 coupled to a Finnigan MAT Delta Plus IRMS via a ConFlo-III continuous flow interface. The in-house standard was L-histidine, with instrument error (SD) of 0.05 for C and 0.08 for N. Upon analysis, samples with atomic C:N ratios >3.5 were lipid corrected using the following equation by Post et al. (2007) for aquatic organisms:

$$\delta^{13}\text{C}_{\text{normalized}} = \delta^{13}\text{C}_{\text{untreated}} - 3.32 + 0.99 \times \text{C:N} \quad (1)$$

where $\delta^{13}\text{C}_{\text{normalized}}$ and $\delta^{13}\text{C}_{\text{untreated}}$ are the lipid-corrected and uncorrected $\delta^{13}\text{C}$ values, respectively. This was done to remove the lipid depletion effect in which high levels of lipids can cause significant depletion in ^{13}C irrespective of the source $\delta^{13}\text{C}$ (McConnaughey & McRoy 1979).

Data analysis

Examination of between-year (2007 and 2009) and sex-specific (from 2009 only) differences in $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and C:N ratios of *Dosidicus gigas* were performed using *t*-tests ($\alpha = 0.05$), and regression analysis was applied to examine the relationship between *D. gigas* size and $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and C:N ratio. For prey species that were collected across months and years, we applied a *t*-test to examine potential temporal dif-

ferences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. All analyses were run using the statistical package R (v.2.15.1; R Development Core Team 2010).

To first assess whether migratory *Dosidicus gigas* isotopically matched $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ baselines of the NCC system relative to their southern migratory end-point region off Baja Mexico, we examined the proportional contribution of copepod-based baselines from 4 migratory end-point regions using the Bayesian isotope mixing model SIAR (Parnell et al. 2010). The SIAR model is useful in situations in which the number of sources exceeds the number of isotopes used as tracers, and it incorporates isotopic variation in the source and mixture and in the trophic fractionation factors ($\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$). Results from SIAR provide a relative proportional contribution of sources to the predator, in which variability is expressed as a 95, 75, and 50% credible interval of the estimates. The 4 migratory end-members used in the SIAR model consisted of the nearshore and offshore NCC and the nearshore and offshore SCC. For the NCC, we used data from Miller et al. (2008, 2010), with the limitation of using nearshore ($\delta^{15}\text{N} = 9.1 \pm 0.7\text{‰}$ [mean \pm SD], and $\delta^{13}\text{C} = -17.7 \pm 1.1\text{‰}$, $n = 15$) and offshore ($\delta^{15}\text{N} = 9.8 \pm 0.7\text{‰}$, and $\delta^{13}\text{C} = -20.3 \pm 0.6\text{‰}$, $n = 6$) copepod values within 7 km and >50 km off the coast, respectively. Isotope end-points from the SCC region were obtained from Olson et al. (2010), with northern-most sites selected nearshore (latitude 20 to 25° N, longitude 110 to 125° W; $\delta^{15}\text{N} = 10.7 \pm 1.4\text{‰}$ and $\delta^{13}\text{C} = -21.4 \pm 1.3\text{‰}$, $n = 7$) and offshore (latitude 10 to 19° N, longitude 110 to 125° W; $\delta^{15}\text{N} = 8.9 \pm 1.2\text{‰}$, and $\delta^{13}\text{C} = -21.5 \pm 0.6\text{‰}$, $n = 15$) of the Baja Peninsula. The sampling area of Olson et al. (2010) was very large, and we selected only the northern-most points (excluding samples within the Gulf of California) that represented the SCC system. The SIAR model was run using a mean (± 1 SD) $\Delta\delta^{15}\text{N}$ and $\Delta\delta^{13}\text{C}$ of $3.4 \pm 1.0\text{‰}$ and $0.4 \pm 1.3\text{‰}$, respectively, set at 500000 iterations with the initial 50000 iterations discarded.

To examine the proportion of prey sources in the *Dosidicus gigas* diet, we employed a separate SIAR trophic model. We first compiled a list of *D. gigas* prey from diet studies within the NCC (Field et al. 2007) and other regions (Humboldt Current and Gulf of California). The overall list consisted of 46 species/life history stages for which there were isotope data available from within the NCC or the Central California Current region off Monterey, CA (from Drazen et al. 2008). The total number of potential prey sources far exceeded what could reasonably be applied to the SIAR model. As a result, we grouped

prey into isotopic-based trophic groups based on agglomerative hierarchical cluster analysis (AHCA) of the 46 species/life history types (rows) and their respective $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (columns). For the AHCA, we applied a Sørensen (Bray-Curtis) distance and flexible beta ($\beta = -0.25$) linkage function using the software package PC-ORD v.4.25 (McCune & Mefford 1999). The cutoff level for establishing trophic groups was based on a scree plot and the choice of the maximum decrease in dissimilarity observed with increasing number of cluster groups (McCune & Grace 2002); however, we also balanced this with maintaining groups that had ecological significance, such as zooplankton, zooplanktivores, and piscivores. From within each of the trophic groups, we used the average and pooled SD of the organisms to be applied to the SIAR model. Source groups were excluded from the SIAR if they were substantially too distant in isotope space from *D. gigas* because their inclusion in the model would have only resulted in low proportional contributions while reducing model inference. We excluded groups that had mean $\delta^{15}\text{N}$ differences from *D. gigas* $>4.0\text{‰}$, after already subtracting the trophic enrichment factor ($\Delta\delta^{15}\text{N}$) of 3.4‰ from mean $\delta^{15}\text{N}$ of *D. gigas*. This still allowed for a substantial $\delta^{15}\text{N}$ range of prey that were >1 trophic level above *D. gigas* to be included in the model. The main SIAR model was run using the same fractionation values and iterations as the previous migratory end-member SIAR model. We examined the sensitivity of our source group contribution results to changes in isotopic fractionation factor by running the SIAR model with the following paired combinations of fractionation factors for $\Delta\delta^{15}\text{N}$ (2.2, 2.6, 3.0, and 3.4‰) and $\Delta\delta^{13}\text{C}$ (0.0, 0.39, 0.8, and 1.2‰). All runs of the model maintained an SD of 1.0 and 1.3 (from Post 2002) for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, respectively.

RESULTS

Dosidicus gigas expressed a mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of -19.1 and 13.9‰ , respectively (Table 1). No significant differences were observed between male and female *D. gigas* with respect to $\delta^{15}\text{N}$ ($p = 0.12$) and C:N ratio ($p = 0.72$); however, a small difference (0.16‰) was observed in $\delta^{13}\text{C}$ ($p = 0.05$), with females higher (less negative) relative to males. The between-year comparison of *D. gigas* showed a statistically significant difference in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N ratio, with individuals from 2007 on average expressing a 0.4‰ higher $\delta^{15}\text{N}$ ($p = 0.01$) but a lower $\delta^{13}\text{C}$ (by

0.3‰) and C:N ratio (by 0.1‰) relative to the 2009 squid. When samples were compared among months of collection, only September 2009 was significantly different, with slightly lower (0.3 to 0.6‰) mean values (Table 2).

There was also a weak but significant negative linear relationship between size and $\delta^{15}\text{N}$ ($p = 0.01$, $r^2 = 0.07$, $df = 75$) and a significant positive relationship with $\delta^{13}\text{C}$ ($p = 0.01$, $r^2 = 0.08$, $df = 75$) but marginal non-significance with the C:N ratio ($p = 0.06$). The between-year comparison of *D. gigas* mantle length showed that 2009 individuals were significantly larger (mean DML = 600 ± 53 mm) than those collected in 2007 (533 ± 34 mm). For other species of nekton for which we had multiple collections across months and years, the $\delta^{15}\text{N}$ values of northern anchovy were significantly higher in August 2007 (mean 14.1‰), with no difference between the other collections in June to July 2007 and August and September 2009 (mean of 13.7 to 13.8‰) (Table 2). For $\delta^{13}\text{C}$, northern anchovy values were significantly ^{13}C -enriched in August and September of 2007 and 2009, respectively, relative to June to July of 2007 and August 2009 (Table 2).

Migratory end-members

Results from the 4-source SIAR model examining the isotopic match of *Dosidicus gigas* collected in the NCC to migratory end-members nearshore and offshore of the NCC and SCC systems showed highest contributions from waters offshore of the NCC (proportion 0.49), followed by nearshore SCC (0.27), nearshore NCC (0.26), and offshore SCC (<0.01) (Figs. 2 & 3). The combined high contribution of NCC nearshore and offshore end-members (0.74) allowed for trophic-based SIAR model analysis of the NCC prey contributing to *D. gigas* from the NCC system.

Trophic contributions of prey groups

Agglomerative hierarchical cluster analysis of prey $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values resulted in 11 trophic groups based on their distribution in isotope space (groups labeled A to K; Table 1, Figs. 4 & 5). In establishing groups, we chose a cutoff level that was at the first to second node of the dendrogram, which was established by a scree plot, and by choosing the number of groups at the point of maximum decreasing dissimilarity. The 11 trophic cluster groups represented zooplankton, larval-adult fishes, and other nekton. For

Table 1. Summary data (mean \pm SD) of Humboldt squid *Dosidicus gigas* and prey (by cluster group) utilized in the trophic SIAR model. Length measurements for some taxa with larval-juvenile-yearling stages are in mm (SD) as total length. The $\delta^{13}\text{C}$ values applied to the SIAR model are listed under $\delta^{13}\text{C}_{\text{SIAR}}$ and consist of either original $\delta^{13}\text{C}$ values or lipid-normalized values (following Post et al. 2007). Original $\delta^{13}\text{C}$ values for lipid-normalized taxa are provided under $\delta^{13}\text{C}_x$. Length type: mantle length (ML), total length (TL), standard length (SL), fork length (FL), orbit-to-telson length (OT), no data (nd). Other the present study, literature sources for isotope data of additional taxa used in the model are as follows: (1) Bosley et al. (2004), (2) Miller et al. (2010), (3) Becker et al. (2007)

Cluster group	Common and scientific name, Length type = length mm (SD)	n	Mean (SD)				Source
			$\delta^{15}\text{N}$	$\delta^{13}\text{C}_{\text{SIAR}}$	C:N	$\delta^{13}\text{C}_x$	
	Humboldt squid <i>Dosidicus gigas</i> , ML = 590 (56)	76	13.9 (0.5)	-19.1 (0.2)	3.1		
A	Abraliopsis squid <i>Abraliopsis felis</i> , nd	4	14.5 (0.7)	-18.9 (0.5)	5.1	-20.6	
	Broadfin lampfish <i>Nannobranchium ritteri</i> , SL = 74	1	13.6	-19.4	3.4		
	California headlightfish <i>Diaphus theta</i> , SL = 52 (8)	35	14.7 (0.8)	-19.7 (1.4)	7.5	-21.5	
	California Smoothtongue <i>Bathylagus stilbius</i> , nd	3	14.8 (0.3)	-20.3 (0.2)	3.5		1
	Giant grenadier <i>Albatrossia pectoralis</i> , nd	1	14.1	-19.5	3.2		1
	Northern lampfish <i>Stenobranchius leucopsarus</i> , SL = 49 (10)	32	14.4 (0.7)	-19.0 (0.9)	6.6	-21.5	
	Pacific viperfish <i>Chauliodus macouni</i> , nd	3	14.0 (0.3)	-19.7 (0.2)	3.9	-20.3	1
	Spiny dogfish <i>Squalus acanthias</i> , TL = 509	1	14.6	-19.1	2.9		
	Group average (SD):	14.3 (0.7)	-19.4 (1.1)				
B	Albacore tuna <i>Thunnus alalunga</i> , FL = 694 (66)	40	13.5 (1.0)	-19.9 (0.5)	6.5	-23.4	
	Blue lanternfish <i>Tarletonbeania crenularis</i> , SL = 55 (9)	45	13.2 (0.6)	-20.1 (0.5)	4.1	-20.8	
	Clubhook squid <i>Onychoteuthis boreali-japonicus</i> , ML = 196 (30)	14	13.2 (0.6)	-20.1 (0.4)	3.2		
	Group average (SD):	13.3 (0.6)	-20.2 (0.4)				
C	Northern anchovy <i>Engraulis mordax</i> , FL = 151 (8)	60	13.8 (0.4)	-18.3 (0.5)	3.0		
	Chinook salmon <i>Oncorhynchus tshawytscha</i> , subyearling, nd	7	13.7 (0.7)	-18.8 (1.6)	3.7	-18.8	2
	Pacific hake <i>Merluccius productus</i> , SL = 417 (70)	67	13.5 (0.6)	-17.6 (0.4)	3.1		
	Jack mackerel <i>Trachurus symmetricus</i> , FL = 534 (38)	4	13.6 (0.6)	-17.8 (0.9)	3.2		
	Ocean shrimp <i>Pandalus jordani</i> , OT = 64 (6)	5	13.3 (0.2)	-17.3 (0.4)	3.2		
	Sablefish <i>Anoplopoma fimbria</i> , juvenile, nd	14	13.5	-18.0	3.7	-19.8	2
	Group average (SD):	13.5 (0.6)	-17.9 (0.5)				
D	Northern anchovy <i>Engraulis mordax</i> , larvae, SL = 32 (2)	5	12.2 (0.2)	-19.7 (0.4)	5.1	-23.4	
	Pacific sardine <i>Sardinops sagax</i> , larvae, SL = 31.0 (8.0)	12	11.5 (0.6)	-19.6 (0.7)	3.8	-20.4	
	Group average (SD):	11.8 (0.5)	-19.6 (0.6)				
E	Bigfin lanternfish <i>Symbolophorus californiensis</i> , SL = 105 (8)	9	14.4 (0.3)	-17.0 (2.4)	9.1	-21.2	
	Bocaccio <i>Sebastes paucispinis</i> , nd	9	15.5 (0.3)	-16.9 (0.2)	3.2		1
	Canary rockfish <i>Sebastes pinniger</i> , nd	4	14.9 (0.6)	-17.0 (0.4)	3.3		1
	Chinook salmon <i>O. tshawytscha</i> , yearling, nd	76	14.3 (0.9)	-17.5 (2.2)	5.3	-18.5	2
	Darkblotched rockfish <i>Sebastes crameri</i> , nd	2	14.8 (0.1)	-17.6 (0.1)	3.1		1
	Group average (SD):	14.7 (0.8)	-17.2 (2.0)				
F	Blue rockfish <i>Sebastes mystinus</i> , yearling, nd	3	11.5 (0.4)	-16.1 (0.2)			3
	Pacific hake <i>M. productus</i> , juvenile, SL = 31 (9)	6	12.1 (0.2)	-17.1 (0.2)	3.5		
	Market squid <i>Loligo opalecens</i> , nd	58	12.8 (0.8)	-16.3 (0.8)	3.4		2
	Sand lance <i>Ammodytes hexapterus</i> , nd	34	11.6 (0.6)	-17.5 (1.2)	3.4		2
	Shortbelly rockfish, <i>Sebastes jordani</i> , yearling, nd	10	11.6 (0.6)	-17.1 (0.3)			3
	Widow rockfish, yearling <i>Sebastes entomelas</i> , nd	2	11.7 (0.4)	-17.0 (0.1)			3
	Group average (SD):	11.8 (0.1)	-16.8 (0.1)				
G	Dungeness crab <i>Cancer magister</i> , megalopae, nd	6	10.9 (0.5)	-20.2 (0.5)	4.5	-21.4	1
	Shelf euphausiid <i>Thysanoessa spinifera</i> , nd	6	10.2 (0.3)	-19.6 (0.4)	3.9	-20.1	1
	Group average (SD):	10.5 (0.4)	-19.9 (0.4)				
H	Dover sole, larvae <i>Microstomus pacificus</i> , SL = 32 (8)	3	11.7 (0.2)	-21.2 (0.3)	7.2	-25.0	
	Rockfish <i>Sebastes</i> sp., pelagic juvenile, SL = 45.0 (13.0)	24	12.3 (0.5)	-21.2 (1.0)	3.4		2
	Group average (SD):	12.0 (0.4)	-21.2 (0.7)				
I	Slope euphausiid <i>Euphausia pacifica</i> , nd	36	9.8 (0.7)	-21.0 (0.8)	4.0	-22.7	
	Rex sole <i>Glyptocephalus zachirus</i> , larva, SL = 61 (5)	4	10.6 (0.3)	-21.4 (0.7)	5.7	-23.7	
	Group average (SD):	10.2 (0.6)	-21.2 (0.7)				
J	Clawed armhook squid <i>Gonatus onyx</i> , nd	6	12.7 (0.3)	-19.3 (0.3)	4.0	-20.0	1
	Pacific hake, yearling <i>M. productus</i> , SL = 167 (15)	10	12.7 (0.1)	-18.6 (0.1)	3.1		
	Pacific sardine <i>S. sagax</i> , FL = 233 (24)	66	12.6 (0.6)	-19.1 (0.8)	3.3		
	Pacific saury <i>Cololabis saira</i> , nd	41	12.8 (0.6)	-18.5 (1.5)	3.4		2
	Group average (SD):	12.7 (0.5)	-18.8 (0.1)				
K	Black eelpout <i>Lycodes diapterus</i> , nd	5	16.7 (0.3)	-18.3 (0.4)	4.2	-17.4	1
	Chub mackerel <i>Scomber japonicus</i> , FL = 332 (10)	2	15.4 (0.6)	-18.4 (0.9)	3.6	-18.9	
	Deepsea sole <i>Embassichthys bathybius</i> , nd	1	17.2	-18.5	3.3		1
	Longspine thornyhead <i>Sebastolobus altivelis</i> , nd	1	16.9	-18.5	3.5		1
	Pacific flatnose <i>Antimora microlepis</i> , nd	4	15.7 (0.4)	-18.3 (0.3)	3.2		1
	Group average (SD):	16.3 (0.2)	-18.2 (0.3)				

Table 2. Mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (SD) (as well as sample size) of Humboldt squid *Dosidicus gigas* and major prey by year and month collected from the northern California Current ecosystem. Statistically significant (*t*-test) differences between periods are denoted by superscript letters. Species scientific names are provided in Table 1

Taxon	2007			2009		
	May	June–July	August	July	August	September
Humboldt squid	n		11	2	7	56
	$\delta^{15}\text{N}$		14.3 (0.3) ^a	14.3 (-) [*]	14.0 (0.3) ^a	13.7 (0.5) ^b
	$\delta^{13}\text{C}$		-19.3 (0.1)	-19.1 (-) [*]	-18.9 (0.1)	-19.0 (0.2)
Hake, adult	n	30	20	17		
	$\delta^{15}\text{N}$	13.4 (0.6)	13.4 (0.5)	13.6 (0.4)		
	$\delta^{13}\text{C}$	-17.6 (0.4)	-17.6 (0.4)	-17.4 (0.3)		
Northern anchovy	n		35	11	10	10
	$\delta^{13}\text{C}$		13.8 (0.4) ^a	14.1 (0.4) ^b	13.7 (0.3) ^a	13.8 (0.3) ^a
	$\delta^{13}\text{C}$		-18.4 (0.3) ^a	-17.7 (0.4) ^b	-18.1 (0.5) ^a	-17.7 (0.2) ^b

*Insufficient data for statistical analysis

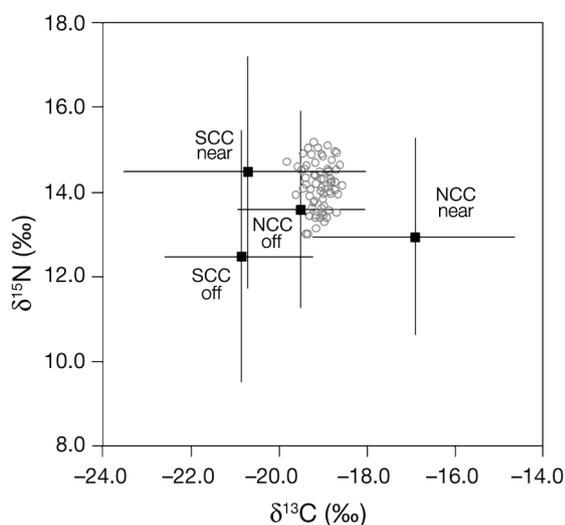


Fig. 2. Mean (bars are SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of copepod baseline values (post-adjusted for trophic fractionation $\Delta\delta^{15}\text{N} = 3.4\%$ and $\Delta\delta^{13}\text{C} = 0.8\%$) from the Northern California Current (NCC) and Southern California Current (SCC) systems from nearshore (near) and offshore (off) areas. (○) Individual values of Humboldt squid *Dosidicus gigas* from the NCC. Copepod values were obtained from Miller et al. (2008, 2010) for the NCC and from Olson et al. (2010) for the SCC (see 'Materials and methods' for details)

the SIAR model, one group was excluded because of its substantial distance in isotopic space from *Dosidicus gigas*; this group consisted of black eelpout, chub mackerel, deepsea sole, longspine thornyhead, and Pacific flatnose (Group K; Table 1). A plot of *D. gigas* and the 11 prey groups in $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ space, after being corrected for trophic fractionation, indicated that *D. gigas* were more oriented toward feeding on prey groups consisting of macrozooplankton, ichthyoplankton, and juvenile stages of rockfishes, com-

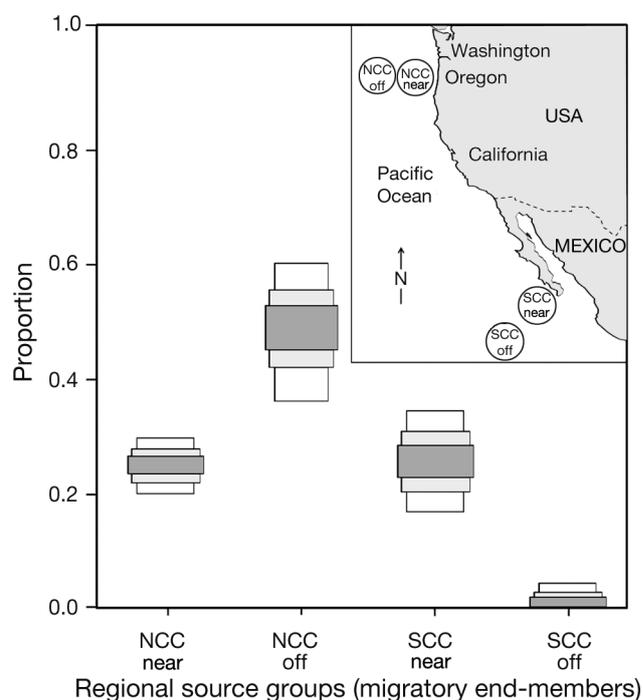


Fig. 3. *Dosidicus gigas*. Proportional contributions of regional isotopic baselines (regional source groups) to Humboldt squid from the migratory end-member SIAR model (fractionation factors applied, $\Delta^{13}\text{C} = 0.80$, $\Delta^{15}\text{N} = 3.4$). Boxes denote the 95, 75, and 50% credible intervals of the estimates. Sources from the Northern California Current (NCC) and Southern California Current (SCC) nearshore (near) and offshore (off) waters were based on copepod values from Miller et al. (2008, 2010) for the NCC and Olson et al. (2010) for the SCC regions

pared to larger adult stages of fishes (Fig. 4). This was reflected in the SIAR model, which showed highest contributions from crustaceans including Dungeness crab *Cancer magister* megalopae and the

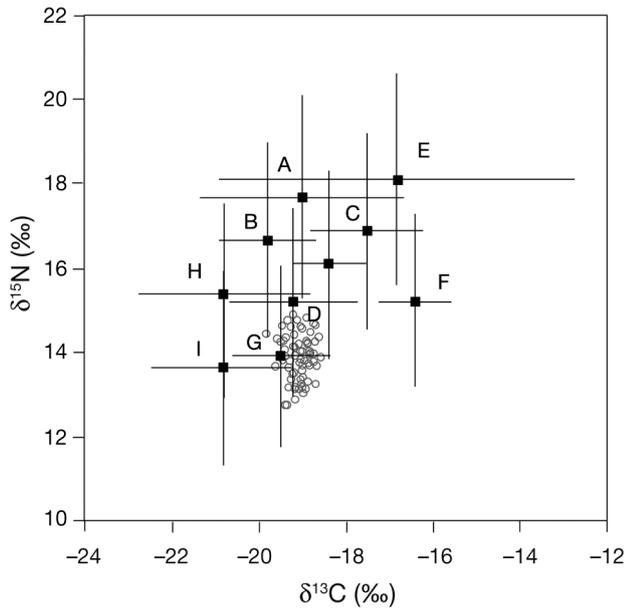


Fig. 4. Mean (SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of Humboldt squid *Dosidicus gigas* prey groups (A to J) after adjustment for trophic fractionation ($\Delta\delta^{15}\text{N} = 3.4\text{‰}$ and $\Delta\delta^{13}\text{C} = 0.8\text{‰}$) and (○) individual values of *D. gigas* applied to the SIAR model. Prey taxa composition of each group and specific isotope values (non-adjusted) are provided in Table 1. Group K was excluded from the trophic SIAR model

shelf euphausiid *Thysanoessa spinifera* (Group G, proportional contribution 0.43), rex sole larvae, and the slope euphausiid *Euphausia pacifica* (Group I, 0.26) and from nekton such as juvenile hake, yearling rockfish species, market squid, and sand lance (Group F, 0.22) (Fig. 5). The next highest contributors were post-larvae of northern anchovy and Pacific sardine (Group D, 0.01). The lowest contributions were from groups with notably higher $\delta^{15}\text{N}$ values relative to *D. gigas*, such as adult rockfishes, myctophids, and pelagic species including mackerel, albacore tuna, adult hake, and other nekton (Groups A–C, E, H and J).

Analysis of the sensitivity of the SIAR model to differences in the trophic fractionation factors ($\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$) showed largest fractionation-dependent shifts in group contribution as a result of $\Delta\delta^{15}\text{N}$. The lower trophic level groups of F, G, and I collectively expressed greater contributions with increasing $\Delta\delta^{15}\text{N}$ (Fig. 6). Even at the lowest $\Delta\delta^{15}\text{N}$ of 2.6‰, contributions by the same groups (F, G, and I) remained high at combined levels between 0.7 to >0.8. Shifts in contribution attributed to $\Delta\delta^{13}\text{C}$ were most pronounced between Groups F, G, and I, and the remaining group contributions never exceeded 0.1.

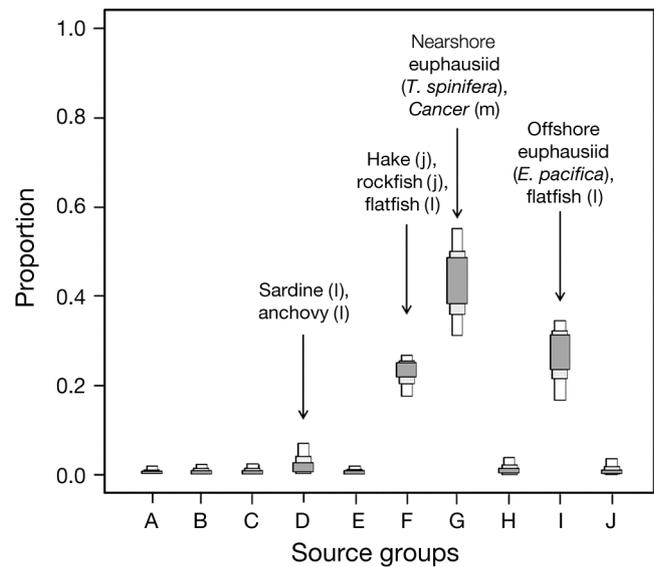


Fig. 5. Distribution of proportional contributions to Humboldt squid *Dosidicus gigas* diet by prey source groups (A to J) from the trophic SIAR model (fractionation factors applied, $\Delta^{13}\text{C} = 0.40$, $\Delta^{15}\text{N} = 3.4$). Boxes denote the 95, 75, and 50% credible intervals of the estimates. Major prey of groups with relatively high proportional contributions are listed, with prey being adults or otherwise noted as larvae (l), crab megalopae (m), or juveniles (j). *T.*: *Thysanoessa*; *E.*: *Euphausia*

DISCUSSION

Our initial migratory end-member SIAR model showed that *Dosidicus gigas* predominantly matched the isotopic baseline values of the NCC offshore and to a lesser extent the nearshore system. The relative importance of the SCC onshore contribution (0.26) partly matches the known distribution of *D. gigas* as it seasonally migrates along the shelf-slope waters and northward to the NCC (Field et al. 2012), and this would support the view that *D. gigas* from our study possibly migrated from along the shelf-slope offshore waters from the SCC. Despite the isotopic contribution from SCC waters, the estimated combined contribution of NCC nearshore and offshore waters (0.74) suggests that *D. gigas* values from our study were largely indicative of feeding from the NCC system. Our measurements of *D. gigas* predominantly occurred in the later summer months of August and September, whereas *D. gigas* has been shown to arrive in waters off Oregon and Washington in early to mid-July (Litz et al. 2011). As a result, the window of isotopic temporal integration of *D. gigas* to the NCC within our study may have been as long as 2 to 3 mo (~60 to 90 d). From modeling the age and growth-dependent dilution of C and N in *D. gigas*,

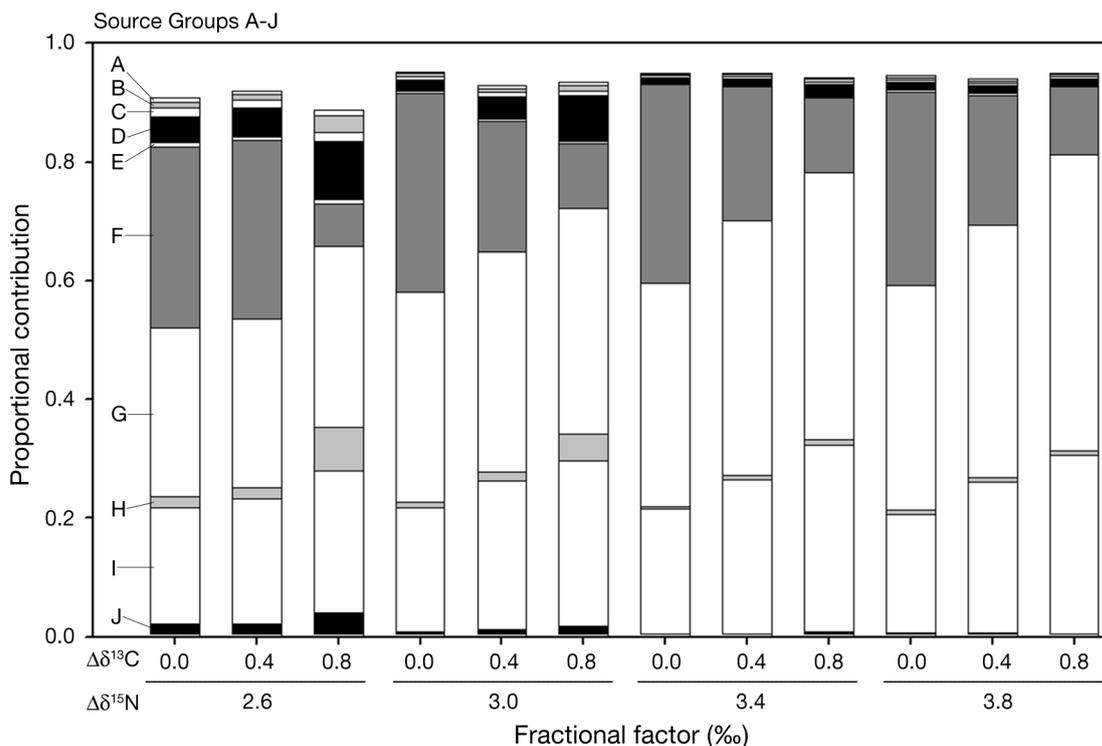


Fig. 6. Proportional contributions of prey groups (A to J) to Humboldt squid *Dosidicus gigas* from runs of the SAIR model with adjustment in trophic fractionation factor ($\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$). Proportional contributions represent only the median 50% credible interval. Taxonomic compositions of each group are provided in Table 1

Ruiz-Cooley et al. (2006) observed ~80% dilution from approximately day-at-age 205 to Day 315 and 90% after Day 400. From Markaida et al.'s (2004) mantle length-age relationships, the *D. gigas* from our study (~40 to 80 cm ML) would represent ~200 to 400 d old individuals. Assuming the dilution rate used by Ruiz-Cooley et al. (2006), animals of ~300 d of age measured in our study would express most (~80%) of their feeding within 100 d (~3 mo) prior to collection. The 80% dilution of *D. gigas* feeding is reasonably close to the combined proportional contributions of the NCC nearshore and offshore signature (0.74).

Results from stable isotope measures of *Dosidicus gigas* and their known prey, and application to the trophic SIAR model, showed substantial trophic contributions of prey groups consisting of macrozooplankton (euphausiids and decapod larvae), juvenile or young-of-year groundfish species (*Sebastes* spp. and *Merluccius productus*), *Loligo* squid, and to a lesser degree post-larval clupeoids and engraulids. We found no indication isotopically of mesopelagic species of fishes or adult species of hake or other groundfish contributing to the diets of *D. gigas*. Within the NCC ecosystem, the stable isotope values of *D. gigas* appears to indicate a greater contribution

from lower trophic level prey than what has previously been implied based on diet analysis alone and studies examining relational population shifts between *D. gigas* and certain prey (e.g. adults of Pacific hake, other groundfish, and Pacific sardine). Our overall isotope results and relative trophic position of *D. gigas* to lower trophic level prey is similar to the results of Drazen et al. (2008; mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ~14.0 and ~-18.2‰, respectively) collected off Point Conception, CA (August 2006). They estimated a trophic level of 2.7, which is indicative of greater zooplanktivory. From the Gulf of California (GOC), Ruiz-Cooley et al. (2006) compared isotope values of *D. gigas* to 2 dominant prey species, the euphausiid *Nyctiphanes simplex* and the myctophid *Benthosema panamense*; however, their mean $\delta^{15}\text{N}$ values for *D. gigas* were as much as 2.9‰ above those observed here and by Drazen et al. (2008). To what extent higher $\delta^{15}\text{N}$ values from the GOC are indicative of higher trophic level feeding is difficult to ascertain because no baseline $\delta^{15}\text{N}$ was established by Ruiz-Cooley et al. (2006). Further study is needed to better establish regional differences in $\delta^{15}\text{N}$ baseline values and to understand the importance of regional differences in production and community structure in shaping trophic behavior in *D. gigas*.

Comparison of our SIAR-based results to *Dosidicus gigas* diet-based studies showed some similarities but also major differences. The importance of euphausiids and other macrozooplankton and juvenile fish species determined from SIAR has also been observed in diet studies (Field et al. 2007, Rosas-Luis et al. 2011). Size distributions of many nekton prey species as estimated by Field et al. (2007) from otoliths appear to match the importance of sub-yearling and yearling individuals as shown in our study. Although macrozooplankton have been acknowledged as important prey for *D. gigas*, in nearly all diet studies, macrozooplankton were lower than nekton in terms of weight contribution to *D. gigas* diet. Differences in results are to be expected given how differently diet and stable isotope analyses measure and estimate contributions of various prey. However, a major difference was in the SIAR's exclusion of mesopelagics as an important contributor to *D. gigas*, whereas these are almost always among the top prey observed in diet studies from the NCC (Field et al. 2007), Gulf of California (Markaida 2006, Markaida et al. 2008), and off Peru (Rosas-Luis et al. 2011). This discrepancy may be due to some extent to issues associated with net feeding by *D. gigas* (Field et al. 2007, Ibáñez et al. 2008, Olson et al. 2010) and with differential digestion rates of various prey and the retention of hard parts. A recent study by Braid et al. (2011) using DNA to examine prey contents in stranded *D. gigas* from Vancouver Island, Canada, suggested that *D. gigas* is an active feeder of nekton such as (in order of importance) other *D. gigas*, Pacific sardine *Sardinops sagax*, Pacific herring *Clupea pallasii*, and whitebait smelt *Allosmerus elongatus*.

It is possible that our isotope values of *Dosidicus gigas* were not representative of the population or that our isotope values of mesopelagics and other groundfish were not isotopically representative of the prey field consumed by *D. gigas*. However, our values of *D. gigas* matched those of Drazen et al. (2008) collected in August 2006, 1 yr prior to our study. The isotope values for mesopelagics reported here and from Bosley et al. (2004) were predominantly above or at the same value as *D. gigas*, and these values matched well with those reported by Choy et al. (2012), which showed high values in the myctophid *Stenobrachius leucopsaurus* ($\delta^{15}\text{N} = 13.5 \pm 0.9$) and stomiid *Chauliodus sloani* ($\delta^{15}\text{N} = 16.0 \pm 0.8$) from the NCC. Similarly, Ruiz-Cooley et al. (2006) also observed $\delta^{15}\text{N}$ values of the myctophid *Benthosema panamense* to be within the mid-range of $\delta^{15}\text{N}$ observed in their large size class of *D. gigas*.

Unless *D. gigas* consumes mesopelagics with substantially lower $\delta^{15}\text{N}$ values than those reported here and in other studies, this evidence suggests that mesopelagics are not as important as implied through diet studies.

Sensitivity analyses of the SIAR model showed that major shifts in the trophic fractionation factors of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ did not markedly change the outcome of the model. Despite major shifts along $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$, prey contributions of lower trophic level organisms were always a substantial proportion of the SIAR total prey contributions (Fig. 6). This resilience is because of the distribution of *Dosidicus gigas* in isotope space relative to macrozooplankton and several zooplanktivorous nekton prey used in the present study. For major contributions of larger nekton, such as adult hake or mesopelagic fishes of myctophids or stomiids, to occur, the trophic fractionation factor would have to be $<2.0\%$ for $\Delta\delta^{15}\text{N}$, which is nearly nonexistent in organisms that feed on high-protein diets (Vander Zanden & Rasmussen 2001, McCutchan et al. 2003). Although sensitivities in models such as the present one must be considered and choosing the correct isotopic fractionation is important in diet reconstruction (Bond & Diamond 2011), the resiliency of our SIAR model to major shifts in fractionation factors points to the importance of lower trophic level prey to *D. gigas* within the NCC ecosystem.

Size-specific isotope trends in *Dosidicus gigas* from our study indicated a statistically significant negative relationship with $\delta^{15}\text{N}$ and a positive relationship with $\delta^{13}\text{C}$, but neither relationship was strong ($r^2 = 0.07$ and 0.08 , respectively). This contrasts with the findings of Ruiz-Cooley et al. (2006) from the GOC, who noted a strong positive shift in muscle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with increasing mantle length from individuals between 20 to ~85 cm, including the range of ~40 to 80 cm that we looked at in our study. Ruiz-Cooley et al. (2010) also observed shifts in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with length measured along the gladius from specimens collected throughout the central eastern Pacific, including the NCC ecosystem (northern California). Although general positive relationships were observed from all sites, it is difficult to determine if these shifts were because of size-related changes in the isotope baseline (geographic or depth-related), trophic level changes in feeding, or some combination thereof.

Our analysis of *Dosidicus gigas* and a number of its prey assumes we captured most of the possible range of isotope values comprising their diet. This is unlikely given the mobility of organisms, the vast

range of prey available to *D. gigas*, and the variable baseline sources of carbon (benthic-pelagic and oceanic-neritic) and nitrogen (upwelled and N_2 -fixed contributions) across the northeastern Pacific Ocean. Geographic, seasonal, and interannual variations in baseline $\delta^{13}C$ and $\delta^{15}N$ can be particularly problematic for studying large marine ecosystems because changes can occur irrespective of whether an organism changes the taxonomic composition of its diet. However, our isotope values showed reasonable similarities between months and years (Table 2), and comparisons between our values for *D. gigas* and those of Drazen et al. (2008), the slope euphausiid *Euphausia pacifica* (Miller et al. 2010) and myctophids (Choy et al. 2012) indicate relative stability in the $\delta^{15}N$ baseline at the time of our study.

The region-specific differences in isotope-size relationships between Ruiz-Cooley et al. (2006) and our study indicate that regional differences in trophic dynamics are at play. Regional differences in overall levels of primary and secondary production and the community structure of macrozooplankton and nekton could plausibly drive *Dosidicus gigas* feeding preferences and therefore shape regional differences in overall relative trophic position, along with diminishing (isotopically) the observable effects of ontogenetic shifts in feeding. The very high abundance and importance of euphausiids and other macrozooplankton within the NCC has been suggested as a reason for the relatively lower trophic levels of many nekton species (Miller et al. 2010). Higher reliance on lower trophic level prey would also dilute size-specific isotopic shifts from feeding on higher trophic levels, as possibly observed here in *D. gigas* from the NCC ecosystem. Further analyses involving size-specific shifts in stable isotopes of *D. gigas* between regions of variable production in zooplankton and prey community structure would better clarify these relationships.

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