

Life-history traits of the giant squid *Architeuthis dux* revealed from stable isotope signatures recorded in beaks

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Carbon and nitrogen isotope profiles constructed from the upper beaks of four giant squid *Architeuthis dux* from the Bay of Biscay and Namibian waters provided a time-integrated record of their diet. Values of $\delta^{15}\text{N}$ ranged from 5.5 to 13.4‰ and of $\delta^{13}\text{C}$ from -14.4 to -17.8 ‰. Nitrogen isotope profiles differed significantly in shape among the four animals analysed, but $\delta^{15}\text{N}$ increased along each profile, with lowest values around the rostral tip. The difference between the lowest and the highest $\delta^{15}\text{N}$ values was ~ 5.8 ‰, slightly less than a two-level difference between the trophic position of small and large *A. dux*. The increase in $\delta^{13}\text{C}$ values was not as pronounced as for the $\delta^{15}\text{N}$ profiles, but the changes suggest an ontogenetic shift in diet early in life from smaller prey of relatively low trophic status to larger prey of higher status. Fluctuations in $\delta^{13}\text{C}$ values observed near the rostral tip may be associated with a greater intrinsic variability in the carbon isotope composition of relatively small prey, and/or transient migratory behaviour early in life. The relative stability of the $\delta^{13}\text{C}$ profiles over the remainder of their lifespan is consistent with the hypothesis that adult giant squid inhabit relatively small, well-defined, and productive areas, where food resources have a constant carbon isotope composition.

Keywords: beak, cephalopods, giant squid, stable isotopes.

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Introduction

Traditionally, studies on the diets of cephalopods were based on the analysis of their stomach contents (Nixon, 1987; Rodhouse and Nigmatullin, 1996). However, this direct technique often provides only partial and biased results (Boyle and Rodhouse, 2005). One of the problems is the difficulty, or impossibility, of obtaining information on long-term feeding habits (Jackson *et al.*, 2007). Currently, at least two indirect techniques offer such temporal integration, fatty acid and stable isotope analyses of cephalopod tissues (Stowasser *et al.*, 2006; Jackson *et al.*, 2007).

The ratios of naturally occurring stable isotopes of nitrogen ($^{15}\text{N}/^{14}\text{N}$, $\delta^{15}\text{N}$) and carbon ($^{13}\text{C}/^{12}\text{C}$, $\delta^{13}\text{C}$) in both hard and soft tissues differ among species. Consumer tissues are enriched in $\delta^{15}\text{N}$ by around 3–5‰ relative to their food, so providing a mechanism to estimate the trophic level (Hobson, 1990; McCutchan *et al.*, 2003; Vanderklift and Ponsard, 2003; Bode *et al.*, 2004; Cherel *et al.*, 2009a). In contrast, $\delta^{13}\text{C}$ typically shows an increase of only ~ 1 ‰ per trophic level, and carbon isotope ratios are normally interpreted as providing information on the origin of the primary sources of the organic matter within the trophic web. In the marine environment, $\delta^{13}\text{C}$ values can be used to distinguish lower vs. higher latitudes, inshore vs.

offshore, or pelagic vs. benthic contributions to food intake (Rau *et al.*, 1982; Hobson *et al.*, 1994; Smith *et al.*, 1996; McCutchan *et al.*, 2003; Cherel and Hobson, 2007).

Squid beaks are hard structures composed of chitin–protein complexes (Hunt and Nixon, 1981), which resist digestion, and can be accumulated in the stomachs of predators (Clarke, 1980). Because of the incremental accretion of non-reactive biological material, they retain a molecular record of growth throughout the life cycle, which can be deciphered to better understand the trophic ecology of these animals (Cherel and Hobson, 2005).

Several studies have underlined the potential of stable isotopes for investigating trophic levels in cephalopods (Takai *et al.*, 2000; Ruiz-Cooley *et al.*, 2004; Cherel and Hobson, 2005). Controlled feeding experiments have shown that stable isotope signatures of cephalopod muscle can be used to distinguish between animals feeding on different diets both from captive studies (Hobson and Cherel, 2006; Stowasser *et al.*, 2006) and comparing stomach contents (Ruiz-Cooley *et al.*, 2006). Inter- and intra-tissue isotopic variation in wild oceanic squid *Todarodes filippovae* also suggested dietary shifts from lower to higher trophic levels during growth (Cherel *et al.*, 2009a). Parry (2008) used differences in nitrogen isotope ratios to demonstrate that adult *Ommastrephes bartramii*

are one trophic level higher than adult *Sthenoteuthis oualaniensis*. Chérel *et al.* (2009b) found that nitrogen and carbon isotopic signatures are useful tools to document the trophic structure of a deep-sea cephalopod assemblage including the giant squid.

Deagle *et al.* (2005) explored the use of polymerase chain reaction (PCR) methods for detecting DNA in the prey remains from an *Architeuthis* gut sample. The amplified DNA region varied in size, allowing a general separation between fish and squid components. The fish was identified as *Macruronus novaezelandiae*, and cannibalism was also found. However, a high frequency of PCR-generated false alleles was observed, and this can be problematic when screening for prey species represented by a low frequency of DNA.

A relatively recent innovation in stable isotope studies has been the use of recording structures such as cetacean teeth and baleen, and cephalopod statoliths to follow changes in isotopic signatures over the course of an individual animal's lifespan. As the isotopic composition of each growth layer will reflect the animal's location and feeding habits at the time the growth layer was deposited, such information may be interpreted in terms of ontogenetic variation in diet and migration patterns (Boyle and Rodhouse, 2005; Field *et al.*, 2007; Mendes *et al.*, 2007).

The aim here is to understand the long-term feeding habits and the trophic position of *Architeuthis dux* using the analysis of changes in stable isotope composition along the upper beak hood. We hypothesize that, because the anterior tip of the rostrum represents the early stages of the animal's life and the posterior edge of the hood the most recent stage, reconstruction of individual trophic histories should be achievable. This is the first study to use cephalopod beaks in this manner.

Material and methods

We analysed three maturing female *A. dux* (Table 1) that were stranded at Colunga beach (43°30'06.36"N 5°15'39.41"E; Bay of Biscay) in 2001 and 2003, and one immature female fished in 1990 by an otter trawler operating 350–390 m deep in Namibian waters (Table 1). After collection, the maturing animals were frozen at –20°C. The immature specimen was frozen on board at –40°C. Beaks were removed from defrosted animals in the laboratory at room temperature, cleaned using a solution of 5% pepsin hydrochloric acid in distilled water (pH 2) to remove the superficial soft tissue remains, then preserved in 70% ethanol. We selected the upper beak because several authors have used upper beaks for age determination (Raya and Hernández-González, 1998; Hernández-López *et al.*, 2001; Higgins *et al.*, 2009; Perales-Raya *et al.*, 2009).

Stable isotope methodology

For isotope analysis, squares of ~1 mm × 1 mm were cut from the upper beak hood of each specimen (Figure 1) every 1.43 mm from the rostral tip to the posterior edge. Subsamples (1 mg) were loaded into pre-cleaned tin cups and placed in an elemental analyser (NC 2500, CE Instruments) attached to a continuous flow isotope ratio mass spectrometer (Delta plus XL, Finnigan) for carbon and nitrogen stable isotope analysis. Stable isotope ratios (¹³C/¹²C and ¹⁵N/¹⁴N) are reported in units of per thousand (‰), using δ-notation, relative to the Vienna Pee Dee belemnite (carbon) international standard. An international certified standard (DORM-2) was analysed periodically and the results were reproducible to better than ±0.1‰ [1σ (s.d.), n = 38] for both δ¹³C and δ¹⁵N values. We also calculated the C/N ratio in each beak.

Table 1. The *Architeuthis* specimens analysed.

No.	Locality	ML	BW	S	MS	UHL	LRL	Comments and source
1932	Carrandi (Bay of Biscay)	150	104	F	Ma	60.4	15.1	Fresh stranded. Date: 23 October 2001. González <i>et al.</i> (2002) and Guerra <i>et al.</i> (2004)
1964	Carrandi	152	105	F	Ma	59.6	15.2	Fresh stranded. Date: 15 September 2003. González <i>et al.</i> (2002) and Guerra <i>et al.</i> (2004)
1963	Carrandi	153	140	F	Ma	70.2	15.2	Fresh stranded. Date: 13 September 2003. González <i>et al.</i> (2002) and Guerra <i>et al.</i> (2004)
1427	Namibia	105	47	F	Im	56.2	14.2	Caught by trawler. Date: 09 February 1990

No., specimen number at Ecobiomar Research Group's Archive (Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas, Vigo); ML, mantle length (cm); BW, body weight (kg); S, sex (F, female); MS, maturity stage (Ma, maturing; Im, immature); UHL, upper hood length (mm); LRL, lower rostral length (mm).

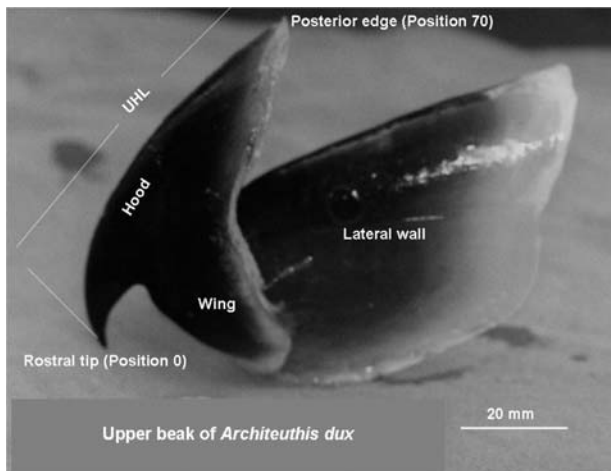


Figure 1. Upper beak of *Architeuthis*. UHL, upper hood length (after Clarke, 1980).

Statistics

As the material for isotope analysis was obtained from approximately every 1.5 mm over the 70 mm length of the hood of the largest upper beak, there were just 47 separate segments, and the other series was shorter. All dataserries were tested for autocorrelation. Autocorrelation was observed for lags of 1–3 positions, but partial autocorrelations were generally significant ($p < 0.05$) only for lag 1 (nitrogen data) or lags 1 and 2 (carbon data).

The length of the dataserries differed between animals, and for this reason, we approximated an autoregressive structure of order 1 or 2 [AR (1) or AR (2)] by including the isotope ratios for the previous one or two segments as linear predictors. Using this approach, the isotope ratios for the first one or two segments in each series (starting at the rostral tip) are excluded from the analysis because previous values are not defined. Generalized additive models (GAMs) were fitted to the isotope ratio data, the isotope ratio being the response variable. Position (segment number) was treated as a continuous explanatory variable (fitted as a smooth curve), and animal was used as a categorical explanatory variable. The isotope values were approximately normally distributed, so a Gaussian GAM with identity link function was used. We tested whether ontogenetic trends (i.e. the effect of position) varied between animals. Hence, the full model for nitrogen was

$$\begin{aligned}
 Y1 \sim & 1 + s(\text{Position, by} = \text{as.numeric(Animal}_{1427}) \\
 & + s(\text{Position, by} = \text{as.numeric(Animal}_{1932}) \\
 & + s(\text{Position, by} = \text{as.numeric(Animal}_{1963}) \\
 & + s(\text{Position, by} = \text{as.numeric(Animal}_{1964}) \\
 & + \text{as.factor(Animal)} + \text{Previous isotope ratio.}
 \end{aligned}$$

The full model for carbon includes an additional term for the effect of the penultimate isotope ratio. For both isotopes, the full model was compared, using an F -test, with the following simplified models: (i) a model with separate smoothers for the position effect for each country, using country (rather than individual) as a categorical explanatory variable; (ii) a model with a combined smoother for all animals for the position effect; (iii) a model without the previous isotope ratio(s) as predictor(s). Always, model residuals were checked for patterns. All models

were fitted using BRODGAR software (www.brodgar.com), an interface for the R statistical programming language, and procedures were based on those described in Mendes *et al.* (2007) and Zuur *et al.* (2007).

Results

The $\delta^{15}\text{N}$ values in the hood of the upper beak of the four *Architeuthis* specimens used in this study (see the Appendix for the raw data) ranged from 5.5 to 13.4‰ (Figure 2). The $\delta^{15}\text{N}$ enrichment observed between the rostral tip and the posterior edge of the hood varied between 5.2 and 7.4‰ (mean 5.8‰). Bearing in mind that the rostral tip represents the earliest stage of life and the posterior edge of the hood the adult stage of life, there was a general upward trend in $\delta^{15}\text{N}$ enrichment over time, consistent with an ontogenetic increase in the trophic level. The change in the slope of the curve suggests that the rate of increase in the trophic level is higher in the early part of life (as recorded nearer the rostral tip; see Figure 2), although it should be borne in mind that this interpretation is based on the rate of change in the isotope ratios in relation to units of beak growth rather than units of time.

The $\delta^{13}\text{C}$ values ranged from -14.3 to -17.8 ‰. They increased from -17.8 to -15.8 ‰ from the tip of the rostrum to the posterior edge of the hood in the Spanish specimens (1932, 1964, and 1963) and from -16.8 to -14.3 ‰ in the Namibian one (1427). It is notable that there was some fluctuation in $\delta^{13}\text{C}$ values close to the rostral tip, and it also appears that the isotope ratio peaks, then starts to decrease towards the end of the animal's life (Figure 3). The C/N mass ratio ranged from 1.99 ± 1.25 to 1.42 ± 0.35 , diminishing from the rostrum towards the marginal edge of the upper beak hood (Appendix).

The full GAMs for both nitrogen and carbon isotopes performed better than any of the simpler models (see Tables 2 and 3 for details of the best models), and the residuals were free of important patterns. Therefore, a model that allows for each individual to have a different ontogenetic trajectory is significantly better than one in which all animals are assumed to have the same ontogenetic trends or one in which different trends are allowed for Spain and Namibia. Nevertheless, as seen in Figures 2 and 3, the beak of the Namibian animal was more enriched in both C and N than any of the three Spanish

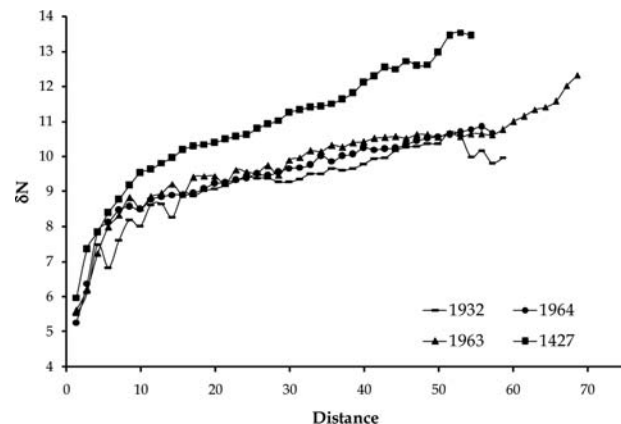


Figure 2. Nitrogen isotope profiles along the hood of the upper beak of female *Architeuthis*. Specimen numbers as in Table 1. Distance (in mm) along the hood from the rostral tip.

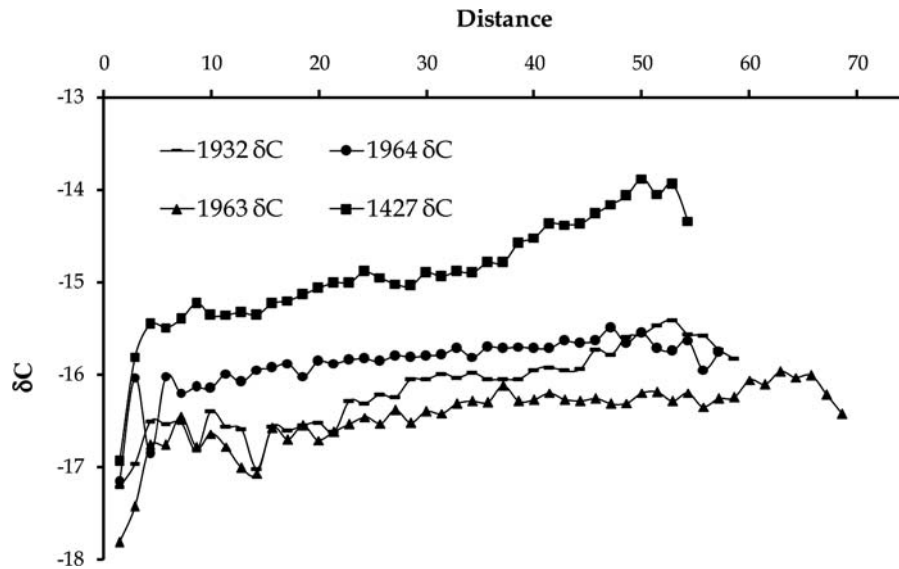


Figure 3. Carbon isotope profiles along the hood of the upper beak of females *Architeuthis*. Specimen numbers as in Table 1. Distance (in mm) along the hood from the rostral tip.

Table 2. GAM results for nitrogen isotope ratios in the hood ($n = 163$, adjusted $r^2 = 0.983$).

Parameter	edf	Coefficient	ST	Value	Probability
Previous ratio	–	0.510	<i>t</i>	13.50	<0.0001
Animal 1932	–	–1.041	<i>t</i>	–12.38	<0.0001
Animal 1963	–	–0.788	<i>t</i>	–11.32	<0.0001
Animal 1964	–	–0.851	<i>t</i>	–11.57	<0.0001
Position: animal 1427	<i>S</i> (3.26)	–	<i>F</i>	15.73	<0.0001
Position: animal 1932	<i>S</i> (4.88)	–	<i>F</i>	8.01	<0.0001
Position: animal 1963	<i>S</i> (6.28)	–	<i>F</i>	12.68	<0.0001
Position: animal 1964	<i>S</i> (1.00)	–	<i>F</i>	58.45	<0.0001

The explanatory variables were animal number (using animal 1427 as the reference animal) and position (nested within animal number). “*S*(*x*)” indicates a smoothing function with *x* degrees of freedom. An AR(1) time-series structure was approximated using the isotope ratio from the previous position on the beak as a linear predictor. Results shown are the estimated degrees of freedom for smoothers (edf), the regression coefficients for linear and categorical terms, and the results of statistical tests (ST) for individual significance of explanatory variables.

animals. The fitted smoothers for the effect of position were generally non-linear, as shown by the degrees of freedom for the smoothers usually exceeding 1.0 (except for nitrogen in animal 1964). As expected from viewing the raw data (Figures 2 and 3), all smoothers (not shown) indicated a generally upward trend in both C and N isotope ratios as the position along the beak moved farther from the rostral tip.

The consistent changes in the slopes of the relationships between isotope ratio and position along the beak were less evident from the fitted smoothers (not shown) than from the raw data, presumably because autocorrelation had been removed from the former.

Discussion

The $\delta^{15}\text{N}$ profiles verified our hypothesis of enrichment in ^{15}N in the beak with growth, which may be interpreted as evidence of an ontogenic dietary shift. This finding also confirms the ability of stable isotope analyses to track trophic changes across size

Table 3. GAM results for carbon isotope ratios in the hood ($n = 159$, r^2 (adj.) = 0.98).

Parameter	edf	Coefficient	ST	Value	<i>p</i> -value
Previous ratio	–	–0.143	<i>t</i>	–2.14	0.0343
Lag 2 ratio	–	0.222	<i>t</i>	4.04	<0.0001
Animal 1932	–	–1.090	<i>t</i>	–8.23	<0.0001
Animal 1963	–	–1.357	<i>t</i>	–8.63	<0.0001
Animal 1964	–	–0.824	<i>t</i>	–7.51	<0.0001
Position: animal 1427	<i>S</i> (7.88)	–	<i>F</i>	15.93	<0.0001
Position: animal 1932	<i>S</i> (7.07)	–	<i>F</i>	13.42	<0.0001
Position: animal 1963	<i>S</i> (7.79)	–	<i>F</i>	11.54	<0.0001
Position: animal 1964	<i>S</i> (4.96)	–	<i>F</i>	6.12	<0.0001

The explanatory variables were animal number (using animal 1427 as the reference animal) and position (nested within animal number). “*S*(*x*)” indicates a smoothing function with *x* degrees of freedom. An AR(2) time-series structure was approximated using the isotope ratios from the previous two positions on the beak as linear predictors. Results shown are the estimated degrees of freedom for smoothers (edf), the regression coefficients for linear and categorical terms, and the results of statistical tests (ST) for individual significance of explanatory variables.

ranges from paralarvae to adults, as indicated by Parry (2008). Our results are consistent with those of Hobson and Cherel (2006) and Ruiz-Cooley *et al.* (2006) for other squid species. Nevertheless, those studies used beaks of different size, whereas our approach demonstrated that the same information can be gathered using a single beak. The $\delta^{15}\text{N}$ values in the upper rostral tip correspond to the youngest phases of life of giant squid, suggesting that giant squid at early life stages feed at a lower trophic level than subadults and adults.

As expected, because $\delta^{15}\text{N}$ is a good indicator of trophic position (DeNiro and Epstein, 1981), we observed differences in the $\delta^{15}\text{N}$ values within the same upper beak, increasing from the rostrum towards the posterior edge. This finding is equivalent to those of Cherel and Hobson (2005) when comparing the values of $\delta^{15}\text{N}$ in different parts of the same beak.

The $\delta^{15}\text{N}$ enrichment observed between the rostral tip and the posterior edge of the hood varied between 5.2 and 7.4‰, slightly less than a twofold difference between the trophic position of small

and large *A. dux*. Cherel *et al.* (2009b) found that beaks of adult giant squid were more enriched in $\delta^{15}\text{N}$ than those of juveniles by 1.7‰, i.e. approximately half a trophic level. However, because the beak integrates material deposited at all stages of the animal's life, comparing whole beaks will tend to underestimate the trophic difference. An ontogenic difference of two trophic levels is within the range found in specimens of *O. bartramii* and *S. oualaniensis* (Parry, 2008) and *T. filippovae* (Cherel *et al.*, 2009a).

The $\delta^{15}\text{N}$ values found along the posterior edge of the upper beak hood of maturing giant squid (9.9–12.3‰) from the Bay of Biscay (Appendix) could suggest that that species would be the highest top predator in the mesopelagic ecosystem of the area. However, some caution is needed in interpreting the data in this manner because $\delta^{15}\text{N}$ values from the lower beaks of adult *A. dux* in the same area indicated somewhat lower values (~8.5–10.8‰), and the top squid predator in that mesopelagic ecosystem is *Taningia danae* (Cherel *et al.*, 2009b).

The upper beaks we analysed showed that $\delta^{13}\text{C}$ values increased from -17.8 to -15.8‰ from the tip of the rostrum to the posterior edge of the hood in the three Spanish specimens and from -16.8 to -14.3‰ in the Namibian one. A similar increasing trend in $\delta^{13}\text{C}$ was found by Cherel *et al.* (2009a) within the lower beak of *T. filippovae*, with the most recently formed regions (wing and lateral wall) being more enriched in $\delta^{13}\text{C}$ than the older region. The $\delta^{13}\text{C}$ values obtained by Cherel *et al.* (2009b) in the lower beak wings of adult *A. dux* preyed upon by sperm whales in the Bay of Biscay ($-16.6 \pm 0.4\%$) were comparable with those from the same area ($-16.2 \pm 0.4\%$), but were different from the Namibian specimen ($-14.9 \pm 0.6\%$).

As $\delta^{13}\text{C}$ is typically enriched by around 1‰ per trophic level, these results are consistent with those for $\delta^{15}\text{N}$, suggesting that *A. dux* rises by around two trophic levels during its lifetime; the observed differences over the animals' lives could also result from movement between different foraging areas with distinct primary sources of carbon (DeNiro and Epstein, 1978; Hobson *et al.*, 1994; Cherel *et al.*, 2000). Fluctuations in the $\delta^{13}\text{C}$ profiles near the rostral tip (Figure 3) may be associated with greater intrinsic variability in the carbon isotope composition of relatively small prey (Cabana and Rasmussen, 1996). On the other hand, the differences could be attributable to migratory behaviour during the early life of the squid.

Relative stability in the $\delta^{13}\text{C}$ profiles after ~20 mm along the beak (Figure 3) may be associated with a more constant and defined food resource later in life. The consistency of the carbon isotope values could also mean that adult giant squid live in a single area feeding on prey of similar carbon isotope composition. Sedentary behaviour would be consistent with the poor swimming abilities of *Architeuthis* (Roper and Boss, 1982; Guerra *et al.*, 2006). To maintain the high growth rate characteristic of these animals (Grist and Jackson, 2007), given a relatively low locomotion efficiency and relatively low metabolism (Seibel *et al.*, 2000), *Architeuthis* should live in the areas of relatively high productivity. The locations where *Architeuthis* were captured were relatively close to submarine canyons and water masses characterized by high productivity (Guerra *et al.*, 2005).

Variation in biochemical composition within beaks could confuse the interpretation of differences in isotopic values. Different ratios of chitin to protein were found in undarkened, darkening and darkened parts of squid beaks, with more chitin in undarkened than in darkened parts (Miserez *et al.*, 2008). As

chitin is impoverished in $\delta^{15}\text{N}$ relative to diet and has a higher C/N ratio than protein (6.9 vs. 3–4; Schimmelmann and DeNiro, 1986; Webb *et al.*, 1998), the higher the chitin content, the lower the value of $\delta^{15}\text{N}$, and the higher the C/N ratio. In our study, we observed that the values of C/N mass ratio were lower (1.99–1.42; Appendix) than those of other squid (e.g. 3.45–3.60 in *T. filippovae*; Cherel *et al.*, 2009a). This could indicate that the beaks of *Architeuthis* are darker than those of *T. filippovae*. On the other hand, the C/N ratio diminishes from the rostrum towards the marginal edge of the upper hood beak, signifying that this part is richer in chitin than the rostrum. This underscored the fact that the marginal edge of the hood tracks recent feeding.

Coinciding with the findings of Takai *et al.* (2000), the differences in $\delta^{13}\text{C}$ values between the Spanish and the Namibian specimens could indicate simply that the former inhabited different geographic areas from the latter. Valid direct comparison between the $\delta^{15}\text{N}$ values of individuals from different areas requires subtraction of baseline levels from the $\delta^{15}\text{N}$ values of predators, because the baseline varies among ecosystems.

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References

- Bode, A., Alvarez-Ossorio, M. T., Carrera, P., and Lorenzo, J. 2004. Reconstruction of trophic pathways between plankton and the North Iberian sardine (*Sardina pilchardus*) using stable isotopes. *Scientia Marina*, 68: 165–178.
- Boyle, P. R., and Rodhouse, P. G. 2005. *Cephalopods: Ecology and Fisheries*. Blackwell Science, Oxford, UK. 452 pp.
- Cabana, G., and Rasmussen, J. B. 1996. Comparison of aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Sciences of the USA*, 93: 10844–10847.
- Cherel, Y., Fontaine, C., Jackson, G. D., Jackson, C. H., and Richard, P. 2009a. Tissue, ontogenic and sex-related differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the oceanic squid *Todarodes filippovae* (Cephalopoda: Ommastrephidae). *Marine Biology*, 156: 699–708.
- Cherel, Y., and Hobson, K. A. 2005. Stable isotopes, beaks and predators: a new tool to study the trophic ecology of cephalopods, including giant and colossal squids. *Proceedings of the Royal Society of London, Series B*, 272: 1601–1607.
- Cherel, Y., and Hobson, K. A. 2007. Geographical variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. *Marine Ecology Progress Series*, 329: 281–287.
- Cherel, Y., Hobson, K. A., and Weimenerskirch, H. 2000. Using stable-isotope analysis of feathers to distinguish moulting and breeding origins of seabirds. *Oecologia*, 122: 155–162.
- Cherel, Y., Ridoux, V., Spitz, J., and Richard, P. 2009b. Stable isotopes document the trophic structure of a deep-sea cephalopod assemblage including giant octopod and giant squid. *Biological Letters*, 5: 364–367.
- Clarke, M. R. 1980. *A Handbook for the Identification of Cephalopod Beaks*. Clarendon Press, Oxford. 273 pp.
- Deagle, B. E., Jarman, S. N., Pemberton, D., and Gales, N. J. 2005. Genetic screening for prey on the gut contents from a giant squid (*Architeuthis* sp.). *Journal of Heredity*, 96: 417–423.

- DeNiro, M. J., and Epstein, S. 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, 42: 495–506.
- DeNiro, M. J., and Epstein, S. 1981. Influence of the diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta*, 45: 341–351.
- Field, I. C., Bradshaw, C. J. A., van den Hoff, J., Burton, H. R., and Hindell, M. A. 2007. Age-related shifts in the diet composition of southern elephant seals expand overall foraging niche. *Marine Biology*, 150: 1441–1452.
- González, Á. F., Guerra, Á., Rocha, F., and Gracia, J. 2002. Recent findings of the giant squid *Architeuthis* in northern Spanish waters. *Journal of the Marine Biological Association of the UK*, 82: 859–861.
- Grist, E. P. M., and Jackson, G. D. 2007. How long would it take to become a giant squid? *Reviews in Fish Biology and Fisheries*, 17: 385–399.
- Guerra, Á., González, Á. F., Dawe, E. G., and Rocha, F. 2004. A review of records of giant squid in the north-eastern Atlantic, with a note on the two first records of males *Architeuthis* sp. off the Iberian Peninsula. *Journal of the Marine Biological Association of the UK*, 84: 427–431.
- Guerra, Á., González, Á. F., Rocha, F., Laria, L., and Gracia, J. (Eds). 2006. *Enigmas de la ciencia: el calamar gigante*. Fundación Caja Rural de Asturias (Oviedo, Spain) and Instituto de Investigaciones Marinas, CSIC, Vigo, Spain. 313 pp.
- Guerra, Á., Valavanis, V., Gonzalez, Á. F., and Rocha, F. 2005. *Architeuthis* records and relations to ocean processes and bottom topography. ASLO Summer 2005 Meeting, Book of Abstracts: 65.
- Hernández-López, J. L., Castro-Hernández, J. J., and Hernández-García, V. 2001. Age determined from the daily deposition of concentric rings on common octopus (*Octopus vulgaris*) beaks. *Fishery Bulletin US*, 99: 679–684.
- Higgins, F., McKinnon, J., and Lamare, M. 2009. Age determination and growth of a small-bodied New Zealand octopus. *Cephalopod International Advisory Council Symposium*, Vigo, 3–11 September, 2009, Book of Abstracts: 76.
- Hobson, K. A. 1990. Stable isotope analysis of marbled murrelets: evidence for freshwater feeding and determination of trophic level. *The Condor*, 92: 897–903.
- Hobson, K. A., and Cherel, Y. 2006. Isotopic reconstruction of marine food webs using cephalopod beaks: new insight from captive raised *Sepia officinalis*. *Canadian Journal of Zoology*, 84: 766–770.
- Hobson, K. A., Piatt, J. F., and Pitocchelli, J. 1994. Using stable isotopes to determine seabird trophic relationships. *Journal of Animal Ecology*, 63: 786–798.
- Hunt, S., and Nixon, M. 1981. A comparative study of protein composition in the chitin–protein complexes of the beak, pen, sucker disc, radula and oesophageal cuticle of cephalopods. *Comparative Biochemistry and Physiology*, 68B: 535–546.
- Jackson, G. D., Bustamante, P., Cherel, Y., Fulton, A., Grist, E. P. M., Jackson, C. H., Nichols, P. D., et al. 2007. Applying new tools to cephalopod trophic dynamics and ecology: perspectives from the Southern Ocean Cephalopod Workshop, 2–3 February 2006. *Reviews in Fish Biology and Fisheries*, 17: 79–99.
- McCutchan, J. H., Lewis, W. M., Kendall, C., and McGrath, C. C. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulphur. *Oikos*, 102: 378–390.
- Mendes, S., Newton, J., Reid, R. J., Zuur, A. F., and Pierce, G. J. 2007. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ profiling of sperm whale teeth reveals ontogenetic movements and trophic ecology. *Oecologia*, 151: 605–615.
- Miserez, A., Schneberk, T., Sun, C., Zok, F., and Waite, J. H. 2008. The transition from stiff to compliant materials in squid beaks. *Science*, 319: 1816–1819.
- Nixon, M. 1987. Cephalopod diets. In *Cephalopod Life Cycles*. 2. Comparative Reviews, pp. 201–219. Ed. by P. R. Boyle. Academic Press, London. 441 pp.
- Parry, M. 2008. Trophic variation with length in two ommastrephid squids, *Ommastrephes bartramii* and *Sthenoteuthis oualaniensis*. *Marine Biology*, 153: 249–256.
- Perales-Raya, C., García-Santamaría, M. T., Bartolomé, A., Pascual, P., and Almansa, E. 2009. Age reading comparison between sagittal sections and lateral walls of the octopus beaks (*Octopus vulgaris* Cuvier, 1797). *Cephalopod International Advisory Council Symposium*, Vigo, 3–11 September 2009, Book of Abstracts: 55.
- Rau, G. H., Sweeney, R. E., and Kaplan, I. R. 1982. Plankton $^{13}\text{C}:^{12}\text{C}$ ratio changes with latitude: differences between northern and southern oceans. *Deep Sea Research A*, 29: 1035–1039.
- Raya, C., and Hernández-González, C. L. 1998. Growth lines within the beak microstructure of the Octopus *Octopus vulgaris* Cuvier, 1797. *Cephalopod Biodiversity, Ecology and Evolution*. South African Journal of Marine Science, 20: 135–142.
- Rodhouse, P. R., and Nigmatullin, C. h. M. 1996. Role as consumers. *Philosophical Transactions of the Royal Society of London, Series B*, 351: 1003–1022.
- Roper, C. F. E., and Boss, K. J. 1982. The giant squid. *Scientific American*, 246: 96–105.
- Ruiz-Cooley, R. I., Gendron, D., Aguinaga, S., Mesnick, S., and Carriquiry, J. D. 2004. Trophic relationships between sperm whales and jumbo squid using stable isotopes of C and N. *Marine Ecology Progress Series*, 277: 275–283.
- Ruiz-Cooley, R. I., Markaida, U., Gendron, D., and Aguinaga, S. 2006. Stable isotopes in jumbo squid (*Dosidicus gigas*) beaks to estimate its trophic position: comparison between stomach contents and stable isotopes. *Journal of the Marine Biological Association of the UK*, 86: 437–445.
- Schimmelmann, A., and DeNiro, M. J. 1986. Stable isotopic studies on chitin. 2. The $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios in arthropod chitin. *Contributions to Marine Science*, 29: 113–130.
- Seibel, B. A., Thuesen, E., and Childress, J. J. 2000. Light-limitation on predator–prey interactions: consequences for metabolism and locomotion of deep-sea cephalopods. *Biological Bulletin*, 198: 284–298.
- Smith, R. J., Hobson, K. A., Koopman, H. N., and Lavigne, D. M. 1996. Distinguishing between populations of fresh-and saltwater harbour seals (*Phoca vitulina*) using stable-isotope ratios and fatty acid profiles. *Canadian Journal of Fisheries and Aquatic Sciences*, 53: 272–279.
- Stowasser, G., Pierce, G. J., Moffat, C. F., Collins, M. A., and Forsythe, J. W. 2006. Experimental study on the effect of diet on fatty acid and stable isotope profiles of the squid *Lolliguncula brevis*. *Journal of Experimental Marine Biology and Ecology*, 333: 97–114.
- Takai, N., Onaka, S., Ikeda, Y., Yatsu, A., Kidokoro, H., and Sakamoto, W. 2000. Geographical variations in carbon and nitrogen stable isotope ratios in squid. *Journal of the Marine Biological Association of the UK*, 80: 675–684.
- Vanderklift, A., and Ponsard, S. 2003. Sources of variation in consumer-diet $\delta^{15}\text{N}$ enrichments: a meta-analysis. *Oceanologia*, 136: 32–37.
- Webb, S., Hedges, R. E. M., and Simpson, S. J. 1998. Diet quality influences the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of locusts and their biochemical components. *Journal of Experimental Biology*, 201: 2903–2911.
- Zuur, A. F., Ieno, E. N., and Smith, G. M. 2007. *Analysing Ecological Data*. Statistics for Biology and Health Series. Springer, Berlin. 685 pp.

Appendix

$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in the hood of the upper beaks of *Architeuthis* from the Bay of Biscay (Asturias, northern Spain, specimen numbers 1932, 1964, and 1963) and Namibian waters (1427). D, distance from the rostral tip to the posterior edge of the upper beak where each sample was taken; C/N ratio mean and standard deviation.

D (mm)	1932		1964		1963		1427	
	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
1.43	5.48	-17.21	5.27	-17.16	5.61	-17.81	5.98	-16.94
2.86	6.12	-16.96	6.38	-16.03	6.22	-17.43	7.35	-15.81
4.29	7.49	-16.51	7.87	-16.86	7.24	-16.76	7.84	-15.46
5.72	6.84	-16.54	8.11	-16.02	7.99	-16.76	8.39	-15.49
7.15	7.61	-16.52	8.46	-16.20	8.33	-16.45	8.77	-15.40
8.58	8.19	-16.82	8.57	-16.14	8.82	-16.79	9.17	-15.23
10.00	8.01	-16.40	8.49	-16.15	8.53	-16.64	9.53	-15.36
11.43	8.62	-16.56	8.78	-15.99	8.84	-16.78	9.63	-15.35
12.86	8.65	-16.59	8.86	-16.08	8.95	-17.01	9.82	-15.33
14.29	8.28	-17.02	8.91	-15.95	9.21	-17.07	9.96	-15.36
15.72	8.95	-16.56	8.91	-15.93	8.93	-16.58	10.19	-15.23
17.15	8.88	-16.60	8.94	-15.88	9.41	-16.70	10.28	-15.20
18.58	9.03	-16.55	9.08	-16.02	9.44	-16.55	10.35	-15.14
20.01	9.09	-16.52	9.23	-15.86	9.44	-16.71	10.39	-15.07
21.44	9.17	-16.62	9.25	-15.88	9.23	-16.62	10.49	-15.01
22.87	9.31	-16.28	9.33	-15.84	9.62	-16.54	10.57	-15.01
24.30	9.43	-16.31	9.38	-15.83	9.57	-16.46	10.61	-14.89
25.73	9.38	-16.21	9.50	-15.86	9.54	-16.53	10.79	-14.95
27.16	9.39	-16.24	9.45	-15.80	9.74	-16.39	10.92	-15.02
28.59	9.29	-16.05	9.55	-15.82	9.47	-16.52	11.03	-15.04
30.02	9.27	-16.05	9.67	-15.80	9.90	-16.40	11.25	-14.90
31.45	9.36	-15.99	9.69	-15.79	9.97	-16.42	11.33	-14.94

Continued

Continued

D (mm)	1932		1964		1963		1427	
	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
32.88	9.52	-16.03	9.75	-15.72	10.16	-16.32	11.40	-14.89
34.31	9.51	-15.98	10.01	-15.81	10.15	-16.29	11.42	-14.90
35.74	9.67	-16.05	9.87	-15.71	10.31	-16.30	11.51	-14.79
37.17	9.62	-16.05	10.01	-15.72	10.27	-16.12	11.63	-14.79
38.60	9.66	-16.05	10.06	-15.70	10.39	-16.29	11.81	-14.58
40.03	9.78	-15.95	10.24	-15.72	10.42	-16.27	12.10	-14.52
41.46	9.95	-15.92	10.20	-15.72	10.51	-16.20	12.28	-14.37
42.89	9.96	-15.96	10.21	-15.64	10.54	-16.27	12.54	-14.38
44.32	10.17	-15.94	10.23	-15.66	10.57	-16.29	12.50	-14.37
45.75	10.26	-15.73	10.34	-15.64	10.53	-16.26	12.73	-14.26
47.18	10.28	-15.78	10.44	-15.50	10.62	-16.32	12.59	-14.16
48.61	10.38	-15.59	10.52	-15.66	10.61	-16.31	12.63	-14.07
50.04	10.37	-15.57	10.58	-15.55	10.55	-16.20	12.97	-13.88
51.47	10.69	-15.47	10.63	-15.72	10.68	-16.19	13.45	-14.05
52.90	10.59	-15.41	10.69	-15.75	10.56	-16.29	13.54	-13.94
54.33	9.98	-15.56	10.78	-15.63	10.65	-16.20	13.45	-14.34
55.76	10.17	-15.58	10.84	-15.95	10.64	-16.35		
57.19	9.81	-15.73	10.68	-15.76	10.61	-16.26		
58.62	9.96	-15.83			10.76	-16.25		
60.05					10.99	-16.06		
61.48					11.14	-16.11		
62.91					11.34	-15.97		
64.34					11.41	-16.03		
65.77					11.57	-16.01		
67.20					12.02	-16.22		
68.63					12.32	-16.43		
C/N	1.99 ± 1.25		1.72 ± 0.33		1.71 ± 0.34		1.42 ± 0.35	

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