Stable isotopes document the trophic structure of a deep-sea cephalopod assemblage including giant octopod and giant squid

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Although deep-sea cephalopods are key marine organisms, their feeding ecology remains essentially unknown. Here, we report for the first time the trophic structure of an assemblage of these animals (19 species) by measuring the isotopic signature of wings of their lower beaks, which accumulated in stomachs of stranded sperm whales. Overall, the species encompassed a narrow range in δ13C values (1.7‰), indicating that they lived in closely related and overlapping habitats. δ15N values can be interpreted in terms of distribution with the more 13C-depleted species (e.g. Stigmatooteuthis arcturi, Vampyroteuthis infernalis) having a more pelagic habitat than the more 13C-enriched, bathyal species (e.g. Todarodes sagittatus and the giant squid Architeuthis dux). The cephalopods sampled had δ15N values ranging 4.6‰, which is consistent with the species spanning approximately 1.5 trophic levels. Neither the giant octopod (Haliphron atlanticus) nor the giant squid reached the highest trophic position. Species δ15N was independent of body size, with large squids having both the highest (Taniaha danae) and lowest (Lepidoteuthis grimaldii) δ15N values. Their trophic position indicates that some species share the top of the food web, together with other megacarnivores such as the sperm whale.

Keywords: marine predator; North Atlantic; pelagic ecosystem; sperm whale; trophic level

1. INTRODUCTION

Cephalopods play a major role in the marine ecosytems, as underlined by their global standing stock and annual consumption of resources (Rodhouse & Nigmatullin 1996). Squids and octopods dominate the cephalopod biomass in the deep sea, and determining their trophic relationships is a key issue in understanding the structure of this poorly known environment. The role of cephalopods as prey is demonstrated by their importance in the diet of predators (Clarke 1996), but knowledge of their diet is limited by lack of data (Rodhouse & Nigmatullin 1996). A new tool to investigate cephalopod feeding ecology has recently been developed, by combining the use of their predators as biological samplers together with measurements of the stable isotopic signature of their chitinized beaks (Cherel & Hobson 2005). The basic concept is that an animal’s isotopic composition is directly influenced by what it consumes. In the marine environment, stable isotope ratios of carbon (13C/12C, δ13C) and nitrogen (15N/14N, δ15N) are indicators of the foraging areas and trophic levels of consumers, respectively (e.g. Cherel & Hobson 2005).

Here, we report the trophic structure of an assemblage of deep-sea cephalopods by measuring the isotopic signature of beaks that accumulated in the stomach of sperm whales. We used whales that stranded together as samplers, because it is the only way to collect large numbers of coexisting cephalopod species. Stable isotopes were analysed in wings of lower beaks, because they retain the most recent growth bands, thus indicating the isotopic signal of the more recently assimilated food (Cherel & Hobson 2005; Hobson & Cherel 2006). The assemblage is made of 19 species that are representative of the large taxonomic, size range and lifestyle diversity of deep-sea cephalopods worldwide. It includes 17 oceanic squids, one octopod and the only species of the order Vampyromorpha. Fast-swimming muscular cephalopods are represented by the ommastrephid Todarodes sagittatus and the onychoteuthid Ancistroteuthis lichtensteini, but the assemblage is mainly composed of ammonial and gelatinous species (including the giant octopod Haliphron atlanticus; see the electronic supplementary material).

2. MATERIAL AND METHODS

Beaks were collected from three immature male sperm whales that stranded in the Bay of Biscay (44°13’ N, 01°18’ W) in December 2001. The three whales fed on the same cephalopod assemblage, as indicated by the overall similar percentages by number of the different beaks found in their stomachs (J. Spitz, Y. Cherel & V. Ridoux 2009, unpublished data). Cephalopods were identified from the morphology of their beaks. Lower rostral length of squid beaks and lower hood length of octopod beaks were measured, and allometric equations were used to estimate their dorsal mantle length (Clarke 1986; Lu & Ickeringill 2002). Lower beaks were cut into small pieces. Relative abundance of stable isotopes of carbon (δ13C) and nitrogen (δ15N) was determined with an elemental analyzer connected online to an isotope ratio mass spectrometer. Results are presented in the usual δ notation relative to PeeDee belemnite marine fossil limestone and atmospheric N2 for δ13C and δ15N, respectively. To help interpret the isotopic signature of cephalopods, the muscle δ15N values of top consumers living in the Bay of Biscay/eastern North Atlantic were used (Das et al. 2000, 2003). Since beaks were depleted in δ15N when compared with soft tissues, their signatures were corrected by adding 3.5‰ at their δ15N values (Cherel et al. 2009). Values were mean±s.d. Data were statistically analysed using SYSTAT v. 9 for Windows.
3. RESULTS

Cephalopods were segregated by the δ¹⁵N and δ¹³C values of their beak wings (Kruskal–Wallis, $H=146$ and 111, respectively, both $p<0.0001$; see the electronic supplementary material). Species were deliberately placed in trophic sequence according to their nitrogen signatures to illustrate the trophic structure of the assemblage (figure 1). As indicated by their δ¹⁵N values, coexisting deep-sea cephalopods from the North Atlantic fed along a continuum of less than two trophic levels (4.6‰) from T. danae (7.1±0.5‰) to T. sagittatus (11.7±0.8‰) (T. danae). They had a more restricted range (1.7‰) in δ¹³C values, with all the 19 species showing a gradual enrichment in ¹³C from S. arcturi (K=16.6±0.4‰) to T. pavo (K=11.1±0.8‰). The δ¹³C values of cephalopods eaten by sperm whales were impoverished in ¹³C when compared with two control species living in neritic waters, the myopsid Loligo vulgaris and the cuttlefish Sepia officinalis (figure 1). Overall, estimated mantle lengths were different between cephalopod species ($H=152, p<0.0001$). Mantle length encompassed a large range within the assemblage (approx. 1.3 m), from the small H. b. bonnellii to the giant squid (A. dux (adults) 5.8±0.3 cm and 134±3 cm, respectively). Values of δ¹⁵N did not correlate with size ($F_{1,15}=0.21, p=0.656$), but δ¹³C values increased linearly with mantle length ($F_{1,15}=10.70, p=0.005$; figure 2).

The two gigantic forms were segregated by their stable isotope signatures, with beak wings of the giant octopod having lower δ¹³C and δ¹⁵N values than those of the giant squid (Mann–Whitney, both $U=30$ and $p=0.01$). Juvenile and adult giant squids were segregated by their stable isotope signatures, with juveniles having lower δ¹⁵N and δ¹³C values than adults ($U=30$ and 27 $p=0.011$ and 0.043, respectively). Finally, the three histioteuthids were

**Figure 1.** (a) δ¹⁵N and (b) δ¹³C values of lower beak wings of cephalopods eaten by sperm whales (filled circles). Open squares refer to control species living in neritic waters. Values are mean ± s.d.
significantly different multiple comparison tests, all teuthids had different V ampyroteuthis infernalis to G sperm whales. Values are mean versus estimated mantle length of cephalopods eaten by sperm whales that stranded together, thus focusing on the recent feeding habits of coexisting cephalopods (Gartner et al. 2008). Finally, the small and continuous δ13C gradient indicates that the species lived in closely related and overlapping isotopic habitats. This allows the comparison of their nitrogen signatures as reflecting their relative trophic position, with no deleterial effect linked to different δ15N baseline levels.

The trophic structure of the assemblage showed a continuum of δ15N values that amounted to 4.6‰, corresponding to approximately 1.5 trophic levels (e.g. Hobson & Cherel 2006). Interestingly, neither the giant octopus nor the giant squid reached the highest δ15N values, and large species occupied both the highest (T. danae) and lowest (L. grimaldii) trophic positions. That between-species differences in δ15N were independent of body size is consistent with previous studies on fishes and contradicts species size-based trophic structuring in communities (Layman et al. 2005). This does not preclude a structuring effect of size within species, however, as the nitrogen signature of cephalopod beaks increase during ontogeny (Cherel et al. 2009). Indeed, beaks of adult giant squids were more 15N-enriched than those of juveniles by 1.7‰, i.e. approximately half a trophic level. Their δ15N values nevertheless indicated that giant squids are not positioned at the top of the food web, either in the Southern Ocean (Cherel & Hobson 2005) or in the North Atlantic, where, surprisingly, some fragile smaller forms (e.g. Chiroteuthis spp.) have a similar or even higher trophic position.

Comparison of corrected isotopic signatures of cephalopods with the δ15N values of other top consumers (Das et al. 2000, 2003) shed a new light on the trophic structure of the oceanic ecosystem. The nitrogen signature of T. danae is close to that of the sperm whale, indicating that the species has a trophic position similar to that of some top marine consumers. This is in agreement with the recent finding that T. danae is an aggressive and tenacious predator rather than a sluggish, inactive squid, as previously thought (Kubodera et al. 2007). Histiotethids are one of the main prey of sperm whales worldwide (Clarke 1996) and, accordingly, their δ15N values were lower than the nitrogen signature of their predator. Finally, the cephalopods with the lowest trophic positions (L. grimaldii and V. infernalis) are comparable with common and striped dolphins (Delphinus delphis and Stenella coeruleoalba), showing that the assemblage of deep-sea cephalopods overall encompassed the same range of trophic positions than the most common oceanic mammals in the area. Isotopic niches of cephalopods illustrate the different mechanisms allowing species coexistence. The three histiotethids segregate by their δ13C values, suggesting a habitat gradient from more bathyal. Indeed, large specimens of the three species seem to be associated with, but not restricted to, the bottom (Nesis 1987), indicating that some cephalopods adopt a benthopelagic or demersal lifestyle as they age. There, the fast-growing cephalopods would benefit from enhanced food supplies, due to the persistent near-bottom aggregations of mesopelagic organisms and of benthopelagic and demersal predators feeding on them (Gartner et al. 2008). Moreover, impoverished in 13C when compared with the more 13C-depleted species (e.g. T. sagittatus) having a more pelagic lifestyle than, for example, T. sagittatus that lives primarily above the slope and at the bottom near the slope (Nesis 1987). Interestingly, δ13C values were positively related to cephalopod size, suggesting that giant squid, T. danae and L. grimaldii, lived in the

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\delta^{13}C_{\text{mollusk}} = y = 0.010x - 17.92, \quad r = 0.65, \quad p = 0.005.
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Figure 2. (a) δ15N and (b) δ13C values of lower beak wings versus estimated mantle length of cephalopods eaten by sperm whales. Values are mean ± s.d. Open diamond refers to Vampyroteuthis infernalis and filled circles refer to squids (Y. Cherel et al. 2007). Histiotethids segregate by their habitats (δ13C values; ANOVA, F_{2, 27} = 36.34, p < 0.0001, post hoc Tukey’s honestly significantly different multiple comparison tests, all p ≤ 0.012) but not their trophic positions (δ15N values; F_{2, 27} = 1.84, p = 0.178), while the two octopoteuthids had different δ15N values but identical δ13C values (two-sample t-test, t = 3.19 and 0.38, p = 0.005 and 0.712, respectively).

4. DISCUSSION

Here, we report for the first time the δ15N and δ13C values of beak wings of adult/subadult squids eaten by sperm whales that stranded together, thus focusing on the recent feeding habits of coexisting cephalopods that characterize the deep-sea teuthofauna. All the 19 cephalopod species encompassed a narrow range, with a small variance in their δ13C values. They were, moreover, impoverished in 13C when compared with the signatures of the neritic loliginid and cuttlefish, thus indicating that neither squids nor octopods recently foraged over the shelf but, instead, that they lived in oceanic waters. The small but significant δ13C gradient between species can nevertheless be interpreted in terms of horizontal/vertical distribution, with the more 13C-depleted species (e.g. S. arcturi, Vampyroteuthis infernalis) having a more pelagic lifestyle than, for example, T. sagittatus that lives primarily above the slope and at the bottom near the slope (Nesis 1987). Interestingly, δ13C values were positively related to cephalopod size, suggesting that giant squid, T. danae and L. grimaldii, lived in the...
pelagic *S. arcturi* to more bathyal *Histioteuthis bonnellii bonnellii*, while octopoteuthids segregate by different δ¹⁵N values, with *T. danae* occupying a higher trophic position than *Octopoteuthis* sp., as would be expected from its much greater size. It is also noteworthy that the two gigantic forms of the assemblage segregate by both their habitat and trophic position. Their δ¹³C and δ¹⁵N values suggest that the giant octopod is a more pelagic species, feeding on low-trophic-level prey, and the giant squid a more bathyal species feeding at a higher trophic position.

In summary, beak δ¹³C and δ¹⁵N values of squids and octopods eaten by sperm whales reveal new information about the trophic relationships of deep-sea cephalopods. Importantly, the stable isotopic signature of beaks accumulated in predators’ stomachs is one of the few tools available to gather meaningful biological information on oceanic cephalopods that are rarely collected in the field. In this way, a further promising step should be to look at trace elements in beaks to better characterize cephalopod habitats, and to sample sequentially along the beak growth increments to produce a chronological record of trace elements and isotopic information, allowing for reconstruction of an individual’s trophic history.

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