NOTE / NOTE

Isotopic reconstruction of marine food webs using cephalopod beaks: new insight from captively raised *Sepia officinalis*

K.A. Hobson and Y. Cherel

Abstract: Cephalopod beaks are a valuable source of material for stable isotope analysis to reconstruct the feeding ecology of cephalopods and their predators. We conducted a controlled captive-rearing experiment on the cuttlefish *Sepia officinalis* L., 1758 to 10 months of age. During the first and second months of life, animals were fed mysids (*Mysidopsis* sp.) and shrimp (*Palaemonetes pugio* Holthuis, 1949), but after 2 months, they were fed a homogeneous diet of shrimp (*Farfantepenaeus aztecus* (Ives, 1891) and *Litopenaeus setiferus* (L., 1767)) until sampling. Soft tissues such as buccal mass, arm, and mantle were enriched in ^{15}N over the shrimp diet by about 3.3‰, with little change in ^{13}C, a result in keeping with previous findings for other marine predators. However, beaks showed little isotopic enrichment over the diets at the time of beak formation, with the beak rostral tips representing the neonatal diet and the remaining beak material representing the adult (shrimp) diet. Additionally, for four wild cephalopod species (*Todaropsis eblanae* (Ball, 1841), *Illex coindetii* (Verany, 1839), *Loligo vulgaris* (Lamarck, 1798), and *S. officinalis*) obtained commercially in France, we measured $\delta^{13}C$ and $\delta^{15}N$ values in lower beaks and soft tissues. Similar to the results of our captive study, soft tissues were consistently enriched in ^{15}N over beaks by an average of 4.8‰ (range 3.9‰–6.1‰) but were slightly depleted in ^{13}C by 0.8‰ (0.7‰–1.2‰). The isotopic measurement of cephalopod beaks can thus be used to directly trace the isotopic composition of cephalopod diets and will be a powerful tool in the reconstruction of higher-trophic-level predators of cephalopods, since beaks are often the only material remaining for analysis from field samples.

Résumé : Les becs des céphalopodes représentent une source importante de tissu pour les analyses d'isotopes stables qui visent à reconstituer l'écologie de l'alimentation chez les céphalopodes et leurs prédateurs. Nous avons mené une expérience contrôlée d'élevage en captivité de la seiche commune, *Sepia officinalis* L., 1758, jusqu'à l'âge de 10 mois. Durant les deux premiers mois de leur vie, les animaux se sont nourris de mysidés (*Mysidopsis* sp.) et de crevettes (*Palaemonetes pugio* Holthuis, 1949), mais après 2 mois, ils ont reçu un régime alimentaire homogène de crevettes (*Farfantepenaeus aztecus* (Ives, 1891) et *Litopenaeus setiferus* (L., 1767)) jusqu'à leur prélèvement. Les tissus mous, tels que la masse buccale, les bras et le manteau, s'enrichissent en ^{15}N d'environ 3,3 ‰ avec le régime de crevettes, mais les concentrations de ^{13}C changent peu, ce qui est en accord avec les observations faites chez d'autres prédateurs marins. En revanche, les becs subissent peu d'enrichissement isotopique durant leur formation sous les divers régimes; le bout du bec représente le régime du nouveau-né et le reste du tissu du bec le régime (de crevettes) de l'adulte. De plus, nous avons mesuré les valeurs de $\delta^{13}C$ et de $\delta^{15}N$ dans les parties inférieures du bec et dans les tissus mous chez quatre espèces sauvages de céphalopodes (*Todaropsis eblanae* (Ball, 1841), *Illex coindetii* (Verany, 1839), *Loligo vulgaris* (Lamarck, 1798) et *S. officinalis*) obtenus commercialement en France. Comme dans notre étude en captivité, les tissus mous sont uniformément enrichis en ^{15}N de 4,8 ‰ (étendue 3,9 ‰ – 6,1 ‰) par rapport aux becs, mais légèrement appauvris en ^{13}C de 0,8 ‰ (0,7 ‰ – 1,2 ‰). Les dosages des isotopes dans les becs de céphalopodes peuvent donc servir à retracer directement la composition isotopique des régimes alimentaires de céphalopodes; ils pourraient aussi devenir des outils puissants pour la reconstitution des régimes alimentaires chez les prédateurs de niveau élevé des céphalopodes, puisque les becs sont souvent les seuls tissus disponibles pour analyse dans les prélèvements de terrain.

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Introduction

The measurement of stable carbon and nitrogen isotope ratios of consumer tissues in marine food webs has provided important insights into the ecology of individuals and species and the trophic interactions within complex species communities (Michener and Schell 1994; Hobson et al. 2002). This approach is based on the fact that stable isotopes of elements enter food webs during primary production from inorganic substrates, and their behaviour through subsequent trophic transfers can be predicted (DeNiro and Epstein 1978, 1981). Thus, by knowing how isotope ratios ($^{13}C/^{12}C$, $^{15}N/^{14}N$) change during such processes, it is often possible to forensically delineate the source of feeding and its trophic position (Schell et al. 1989; Hobson and Welch 1992; Hobson et al. 1994).

The success of the stable isotope approach depends upon how well we know the way in which stable isotope ratios change between one trophic level and another, the so-called isotopic discrimination factor. These factors are best approximated using captive-rearing experiments, where the isotopic composition of the diet can be carefully controlled. To date, most of these experiments have been conducted on terrestrial vertebrates such as gerbils (Tieszen et al. 1983), birds (Hobson and Clark 1992; Bearhop et al. 2002; Evans-Ogden et al. 2004; Cherel et al. 2005), bats (Leticia Mirón et al. 2006), and fox (Roth and Hobson 2000), but some information exists for marine invertebrates and fish (Fry 1981; Hesslein et al. 1993). Importantly, once these tissue-dependent factors are established, it becomes possible to use them to provide quantitative inputs of various dietary components of consumers.

Ecologists have made use of a variety of tissue types for isotopic analysis. Fortunately, for a number of applications, nondestructive sampling can be done using whole or cellular and plasma fractions of blood (Hobson and Clark 1993) and metabolically inactive tissues such as hair, feathers, and nails (Mizutani et al. 1990; Bearhop et al. 2003; Mazerolle and Hobson 2005). For marine mammals, skin and fat biopsies from wild animals are also possible (Todd et al. 1997; Hooker et al. 2001). However, while several aspects of the stable isotope approach can remove the necessity of sacrificing animals for stomach sampling, there are numerous situations where stomach contents, obtained from salvaged or collected specimens or through nondestructive techniques (Cherel et al. 2004), can assist dietary and trophic reconstructions, especially for marine birds and mammals (Hobson 1993; Hobson et al. 2004).

For higher-order consumers in marine food webs, beaks, the chitinous structure used by cephalopods for feeding, are often found in stomach samples and often represent the only identifiable material remaining from cephalopod prey (Rodhouse and Nigmatullin 1996). Moreover, the feeding ecology of cephalopods is often poorly understood, despite their crucial role as predators and prey in a number of marine food webs (Clarke 1996). Thus, the isotopic measurement of cephalopod beaks could be an important tool in reconstructing marine food webs and the role of cephalopod taxa in diets of upper-trophic-level organisms. Recently, Cherel and Hobson (2005) used isotopic measurements of beaks from wild cephalopods from the Southern Ocean to reconstruct trophic relationships among several species. However, no previous studies have examined the relationship between isotope ratios in cephalopod beaks and those in their diet, and few studies have reported on isotope ratios in the soft tissues of cephalopods that provide nutrition to consumers of cephalopods (Ruiz-Cooley et al. 2004). Here, we describe the results of a controlled experiment to establish diet–tissue isotopic discrimination factors for various cephalopod tissues, especially the beak. Our motivation was to provide a tool for isotopic investigations of the ecology of cephalopods and their role in marine food webs involving sharks, bony fishes, birds, and mammals.

Materials and methods

We obtained five cuttlefish, *Sepia officinalis* L., 1758, that had been raised in captivity on known diets to the age of 10 months, when they were sacrificed under permit (National Resource Center for Cephalopods, University of Texas Medical Branch) for analysis. From birth, the animals were fed mysids (*Mysidopsis* sp.) for the first 30 days, grass shrimp and penaeids (*Palaemonetes pugio* Holthius, 1949) from day 30 to day 60, and frozen shrimp (*Farfantepanaeus aztecus* (Ives, 1891) and *Litopenaeus setiferus* (L., 1767)) for the remainder of their lives. All prey items were collected from the Galveston Bay Estuary at different times throughout the year. Subsamples of these diets were taken throughout the rearing period.

We sampled soft tissue from the buccal mass, arm, and mantle. We dissected out the complete squid beak, including the upper and lower mandibles. From the lower mandible we subsampled beak material from the left wing, right wing, rostral tip, and remaining material. From the upper mandible we subsampled from the rostral tip and the remaining material. Beak material was cleaned in distilled water, dried, and powdered using an analytical mill. Soft tissues of all samples (including dietary samples) were washed, freeze-dried, and powdered, then soaked in 2:1 chloroform:methanol for 2 h and rinsed with fresh solvent to remove lipid. These samples were then air-dried in a fume hood.

In a separate component of the study, we obtained a variety of cephalopods from a local market in Niort, France. Here, we were simply interested in contrasting the isotopic values of the soft materials with those of the beak, since diets of these wild cephalopods were unknown. We reasoned that if similar differences in isotopic discrimination were seen between soft tissues and the chitinous beak material across individuals and species, this would tend to confirm the results of our captive study. We measured lower beak, mantle, and arm material from several specimens of each of the following taxa: two oegopsid squids (the ommastrephids *Todaropsis eblanae* (Ball, 1841) and *Illex coindetii* (Verany, 1839)), one myopsid squid (the loliginid *Loligo vulgaris* (Lamarck, 1798)), and the cuttlefish *S. officinalis*. Tissue samples for these specimens were handled identically to those of the captively raised animals, except that lower beaks were not subsampled but were powdered whole.

Crustaceans were treated with 0.5 mol/L HCl to remove carbonates and then washed to neutrality in distilled water, dried, and powdered. All powdered tissue was loaded into tin cups for continuous-flow isotope ratio mass spectrometry

Table 1. Results of stable isotope analyses (mean ± SD) of squid (*Sepia officinalis*) tissues and diet.

Tissue	*n*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C/N mass ratio
Cuttlefish soft tissue				
Buccal mass	5	–18.4±0.2	14.8±0.2	3.4
Arm	5	–18.4±0.4	14.8±0.5	3.3
Mantle	5	–18.1±0.3	15.0±0.4	3.2
Cuttlefish beak				
Upper beak rostral tip	5	–17.5±0.1	9.7±0.6	3.4
Upper beak remaining	5	–18.2±0.2	11.5±0.3	3.4
Lower beak rostral tip	5	–17.4±0.1	8.8±0.5	3.4
Lower beak left wing	5	–17.9±0.6	11.6±0.8	3.4
Lower beak right wing	5	–18.1±0.3	12.5±0.7	3.4
Lower beak remaining	5	–17.9±0.2	11.1±0.5	3.3
Cuttlefish diet				
Month 1: *Mysidopsis* sp.	3*	–17.3±0.02	9.8±0.1	2.8
Month 2: *Palaemonetes pugio*	3*	–17.5±0.2	7.8±0.4	3.4
Months 3–10: *Farfantepenaeus aztecus* and *Litopenaeus setiferus*	10†	–18.1±0.9	11.5±1.5	3.8

*Each sample represents a homogenate of several individuals.
†Each sample represents a homogenate of small and large individuals.

Table 2. Results of stable isotope analyses (mean ± SD) of cephalopod tissues from wild animals available commercially in Niort, France.

Species	Tissue	*n*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C/N mass ratio
Todaropsis eblanae	Lower beak	10	–17.0±0.3	8.8±0.4	3.7
	Buccal mass	10	–18.2±0.3	12.7±0.4	3.7
	Mantle	10	–17.8±0.3	13.2±0.4	3.9
Illex coindetii	Lower beak	7	–17.0±0.2	7.5±0.4	3.7
	Buccal mass	7	–18.2±0.4	12.2±0.6	3.8
	Mantle	7	–17.8±0.1	12.7±0.2	3.7
Loligo vulgaris	Lower beak	10	–15.9±0.3	8.1±0.4	3.6
	Buccal mass	10	–17.1±0.3	13.1±0.3	4.1
	Mantle	10	–16.6±0.4	14.2±0.2	3.9
Sepia officinalis	Lower beak	10	–15.5±0.3	8.4±0.3	3.3
	Buccal mass	10	–16.6±0.2	12.6±0.2	3.7
	Mantle	10	–16.4±0.2	13.2±0.2	3.5

(CFIRMS), yielding the relative abundance of stable isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$). Results are presented in the usual δ notation relative to Pee Dee Belemnite and atmospheric N_2 (air) for $\delta^{13}C$ and $\delta^{15}N$, respectively. Replicate measurements of internal laboratory standards indicated measurement errors of ±0.1‰ and ±0.3‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. Further information on the mass spectrometric analysis can be found in Evans-Ogden et al. (2004).

Results

In growing cephalopods, there are high rates of protein synthesis, high efficiencies of retention of synthesized proteins, and, therefore, little protein degradation (Houlihan et al. 1990). Owing to differential mass accretion over time, we anticipated that the soft tissues of the captive cuttlefish would be isotopically enriched over the diet corresponding to the last several months of their lives. This was indeed the case. Soft tissue δ values did not differ among arm, buccal mass, and mantle for both isotopes (^{15}N: $F_{[2,12]} = 0.20$, $p = 0.823$; ^{13}C: $F_{[2,12]} = 1.19$, $p = 0.34$), but these tissues were enriched in ^{15}N by 3.3‰, on average, over the *F. aztecus* and *P. setiferus* diet corresponding to the last 8 months of the animals' lives ($t = 17.4$, $p < 0.001$); there was little change in ^{13}C content. Cuttlefish beaks were expected to show ontogenetic changes in their stable isotope values corresponding to the isotopic diet shift animals were subjected to and the regions of analyses. This again was the case, as the upper and lower rostral tips did not differ from each other (Table 1) but were depleted in ^{15}N ($t = 7.3$, $p < 0.0001$) and slightly enriched in ^{13}C ($t = 3.93$, $p < 0.01$) compared with the wings and the combined remaining materials. Known patterns of mass accretion in growing beaks allowed us to best associate the isotopic enrichment factors with formation of the rostral tip (juvenile diet, mysids) and the wings of the lower beak (most recent diet, shrimps). The grass shrimp diet, corresponding to day 30 to day 60, was not considered to be a major factor in the formation of the rostral tip (formed earlier) or remaining beak area (formed later). Thus, we found little overall isotopic discrimination between beak material and diet during the formation of both parts of the beak for both ^{13}C and ^{15}N (tip: ^{13}C, $t = 0.8$, $p = 0.78$; ^{15}N, $t = 1.1$, $p = 0.56$; wings: ^{13}C, $t = 0.2$, $p = 0.83$; ^{15}N, $t = 0.89$, $p = 0.70$).

In general, our analysis of the wild specimens provided results similar to those obtained for the captively raised cephalopods (Table 2). Overall, there was a significant effect of species (^{13}C: $F_{[3,111]} = 193.9$, $p < 0.001$; ^{15}N: $F_{[3,111]} = 38.6$, $p < 0.001$) and tissue (^{13}C: $F_{[2,111]} = 26.8$, $p < 0.001$; ^{15}N: $F_{[2,111]} = 560$, $p < 0.001$) for both isotopes. There was also a significant interaction between species and tissue for ^{15}N ($F_{[6,111]} = 7.82$, $p < 0.001$) but not for ^{13}C ($F_{[6,111]} = 0.33$, $p = 0.918$). Tukey's post hoc analyses revealed that *I. coindetii* differed from all other species in $\delta^{15}N$ values and *T. eblanae* and *I. coindetii*, *L. vulgaris*, and *S. officinalis* formed three homogeneous subsets for $\delta^{13}C$ values. For both isotopes, tissues differed significantly from each other. For each species, buccal mass was depleted in ^{13}C (0.3‰–0.5‰) and ^{15}N (0.4‰–1.1‰) when compared with mantle, and, importantly, lower beaks were consistently slightly enriched in ^{13}C (0.7‰–1.2‰) and highly depleted in ^{15}N (3.9‰–6.1‰) when compared with soft tissues (Table 2).

Discussion

Our study provides the first estimates of isotopic discrimination between diet and the hard and soft tissues of cephalopods. For the buccal mass, arms, and mantle, the isotopic discrimination we measured, namely 3.3‰ in ^{15}N, is similar to other discrimination factors found in marine food webs (Michener and Schell 1994). However, we were surprised to discover that there appeared to be little, if any, isotopic discrimination between diet and the cuttlefish beak. Results from our analyses of tissues from wild cephalopods generally confirmed results from our studies of captively raised animals. Here, across species, we found soft tissues to be substantially enriched in ^{15}N over beak material. Our wild cephalopods encompassed most of the major cephalopod taxa (oegopsid and myopsid squids together with cuttlefish), thus suggesting that the observed differences probably apply to most living cephalopods. However, more information is needed on other major groups of living cephalopods, such as octopuses. These results, moreover, confirm the recent observations of Cherel and Hobson (2005), who found similar isotopic differences between muscle tissues and beaks of the Antarctic squid *Psychroteuthis glacialis* Thiele, 1920. However, we did find species effects for both isotopes and a tissue by species interaction for ^{15}N. These results undoubtedly are related to lack of control over diet and foraging location among wild species, which are expected to reflect trophic level, especially for $\delta^{15}N$ measurements (Cherel and Hobson 2005).

Previous work on delineating trophic levels in marine food webs through the measurement of $\delta^{15}N$ values of consumer tissues has effectively monitored protein pathways. According to Hunt and Nixon (1981), cephalopod beaks contain both protein (~90%) and chitin (~10%), a polymer of N-acetyl-β-D-glucosamine (Merzendorfer and Zimoch 2003). Palaeoecologists using chitin from marine sediments or other sources have typically relied on isotopic information derived from purified chitin or nitrated chitin (Miller 1991). That the cephalopod beak material we analysed is formed from the diet with little discrimination in ^{13}C and ^{15}N suggests that carbon and nitrogen in these forms are transferred trophically with little biochemical change. For $\delta^{15}N$ measurements, an expected enrichment in ^{15}N between diet and beak proteins may thus be countered by a depletion in ^{15}N between diet and chitin, but further biochemical studies are required to investigate this. Schimmelmann and DeNiro (1988) found chitin to be depleted in ^{15}N relative to diet but observed little difference in $\delta^{13}C$ values between diet and chitin (see also Miller 1991).

Researchers concerned with estimating trophic relationships among marine organisms, especially among cephalopods and higher-order predators such as marine birds and mammals, are encouraged to measure stable isotope ratios of cephalopod beaks. Such measurements should allow a direct estimate of the isotopic signature of the prey of the cephalopod for the period of beak formation. Moreover, analysis of the tip of the beak allows estimation of the early diet of the cephalopod. Whole beaks or portions of the lateral walls and wings, which are the last to become fully chitinized, likely represent the most recent (adult) diet of the animal (see also Cherel and Hobson 2005). Future studies could more clearly define these temporal periods of dietary integration (see figure in Clarke 1965).

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