Coral Reefs (2011) 30:155–165
DOI 10.1007/s00338-010-0680-8

REPORT

Isotopic signatures, foraging habitats and trophic relationships between fish and seasnakes on the coral reefs of New Caledonia

F. Brischoux · X. Bonnet · Y. Cherel · R. Shine

Received: 29 March 2010 / Accepted: 18 September 2010 / Published online: 8 October 2010

Abstract A predator's species, sex and body size can influence the types of prey that it consumes, but why? Do such dietary divergences result from differences in foraging habitats, or reflect differential ability to locate, capture or ingest different types of prey? That question is difficult to answer if foraging occurs in places that preclude direct observation. In New Caledonia, amphibious sea kraits (*Laticauda laticaudata* and *L. saintgironsi*) mostly eat eels—but the species consumed differ between snake species and vary with snake body size and sex. Because the snakes capture eels within crevices on the sea floor, it is not possible to observe snake foraging on any quantitative basis. We used stable isotopes to investigate habitat-divergence and ontogenetic shifts in feeding habits of sympatric species of sea kraits. Similarities in δ15 N ($\sim$10.5‰) values suggest that the two snake species occupy similar trophic levels in the coral-reef foodweb. However, δ13C values differed among the eight eel species consumed by snakes, as well as between the two snake species, and were linked to habitat types. Specifically, δ13C differed between soft- vs. hard-substrate eel species, and consistently differed between the soft-bottom forager *L. laticaudata* ($\sim$ −14.7‰) and the hard-bottom forager *L. saintgironsi* ($\sim$ −12.5‰). Differences in isotopic signatures within and between the two sea krait species and their prey were consistent with the hypothesis of habitat-based dietary divergence. Isotopic composition varied with body size within each of the snake species and varied with body size within some eel species, reflecting ontogenetic shifts in feeding habits of both the sea kraits and their prey. Our results support the findings of previous studies based on snake stomach contents, indicating that further studies could usefully expand these isotopic analyses to a broader range of trophic levels, fish species and spatial scales.

Keywords Anguilliform fish · Coral reefs · Sea kraits · Stable isotopes

Communicated by Biology Editor Prof. Philip Munday

Electronic supplementary material The online version of this article (doi:10.1007/s00338-010-0680-8) contains supplementary material, which is available to authorized users.

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Introduction

The diets of predators differ not only between species (Schoener 1974; Berenbaum 1996; Grant 1999; Tokeshi 1999) but also between populations of the same species (Holbrook and Schmitt 1992; Ford et al. 1998), and even between age classes and sexes within a single population (Camilleri and Shine 1990; Holbrook and Schmitt 1992; Beaudoin et al. 1999; Ford and Hampton 2009). The dietary composition of a predator has important implications not only for the biology of that animal but also for its impact on sympatric species (Schoener 1974; Holbrook and Schmitt 1992; Britt et al. 2006). Extreme dietary specialization also may increase a species' vulnerability to

extinction (Van Valkenburgh et al. 2004) or result in complex coevolution between the predator and its prey (Reid 1991). Accordingly, considerable research has explored the reasons for dietary diversity (e.g., Schoener 1971, 1974; Peckarsky 1982; Stephens and Krebs 1986; Greene 1986; Arnold 1993). That research has identified a range of ecological factors that can generate dietary divergence between any two predators: for example, a predator's body size and shape may influence the feasibility of alternative foraging modes, e.g., ambush vs. wide-foraging (Secor 1995), its ability to locate, capture, overpower and ingest prey (Mittelbach 1981) or its ability to cope with toxins in the prey (Phillips and Shine 2005). Alternatively, different predators may forage in different habitats and hence encounter a different range of prey taxa (Holbrook and Schmitt 1992). In practice, many of these factors interact; for example, a predator unable to capture or ingest a large prey type will likely forage in habitats that maximize encounter rates with smaller (and thus, potentially edible) prey instead (Schoener 1971; Stephens and Krebs 1986; Arnold 1993).

Teasing apart the determinants of diet composition in predators is a challenging task, because of these multiple potential (and non-exclusive) mechanistic pathways. The task is simplified in systems that contain a suite of sympatric predators that differ in diets but are broadly similar in morphology, ecology and behavior—such as congeneric species, or age and sex classes within a single predator species. Because all of these animals potentially are exposed to the same suite of available prey, differences in dietary composition among and within species must be due to the kinds of factors identified above. In systems that facilitate direct observation of predator–prey encounters, we can evaluate issues such as foraging habitat use and prey-handling ability. In most systems, however, encounters between predators and prey occur in situations (at night, inside shelter sites, etc.), where direct observation is impossible. Even if some encounters can be observed, they may well be a non-random sample of all predator–prey encounters. Thus, to robustly test hypotheses about niche partitioning in sympatric predators, we need objective methods to identify hard-to-measure variables, such as the habitats in which prey are captured and consumed. Stable-isotope technology offers promise in this respect.

Measuring the isotopic niche of animals provides information complementary to conventional data on foraging ecology (Peterson and Fry 1987; Newsome et al. 2007). The concept of the isotopic niche is based on the fact that an animal's chemical composition is influenced by what it consumes (Fry 2006). For example, consumers are enriched in ^{15}N relative to their food, and consequently stable nitrogen-isotope measurements ($\delta^{15}N$) can indicate trophic position (Vanderklift and Ponsard 2003). In contrast, stable carbon signatures ($\delta^{13}C$) vary little along the food chain, and in the marine environment, $\delta^{13}C$ values often depend on foraging habitats (Cherel and Hobson 2007; Cherel et al. 2007). Typically, $\delta^{13}C$ varies among specific producers, and we can use this parameter to examine differences in trophic support. Because identification and examination of the producers is often difficult, $\delta^{13}C$ of prey are used as a proxy for particular production sources and thus presumably habitats (Cherel and Hobson 2007; Cherel et al. 2007). Stable-isotope signatures can thus be used to evaluate variations in trophic positions and foraging habitats as a function of predator species, sex and size in a system where prey-type partitioning plausibly reflects habitat-type partitioning (Cherel et al. 2008; Suryan and Fischer 2010).

We used stable-isotope technology to test hypotheses that seek to explain diversity in dietary composition within and between sympatric species of amphibious seasnakes (sea kraits). Many snakes have specialized diets that differ not only between sympatric congeneric taxa but also depend on sex and body size within a species (Arnold 1993). Some of the most clear-cut examples occur in aquatic snakes (e.g., North American natricines—Mushinsky et al. 1982; Australian acrochordid filesnakes—Shine 1986, Houston and Shine 1993; Fijian sea kraits—Pernetta 1977; Shetty and Shine 2002; Neo-Caledonian sea kraits—Brischoux et al. 2007b, 2009; hydrophiine seasnakes—Voris and Moffett 1981). In all these cases, females grow larger than conspecific males and consume larger species of fishes, and within a sex, a snake's body size influences the species and size of prey that are consumed (Shine 1991). The authors who have described these patterns generally have linked habitat divergence and intraspecific niche partitioning. For example, a snake's body size may determine its ability to dive deep, for long periods (Brischoux et al. 2008)—and hence, larger snakes may take larger fishes because the latter are generally found in relatively deep water (Pernetta 1977; Shine 1986; Shine and Shetty 2001; Shetty and Shine 2002). However, predator–prey encounters are difficult to observe in such places, so the habitat-divergence hypothesis is untestable using conventional observational methods.

We aimed to test the habitat-divergence hypothesis in the Neo-Caledonian Lagoon using data on stable-isotope composition of two top-predator assemblages: sea kraits and their anguilliform fish prey. In New Caledonia, sea krait diets vary both inter-specifically (e.g., *Laticauda laticaudata* vs. *L. saintgironsi*; Brischoux et al. 2007b) and ontogenetically within species, with *L. laticaudata* experiencing a wider niche shift in its diet with age than *L. saintgironsi* (Brischoux et al. 2009). In this study, we tested whether variation in stable-isotope composition of adult sea kraits was consistent with differences in feeding

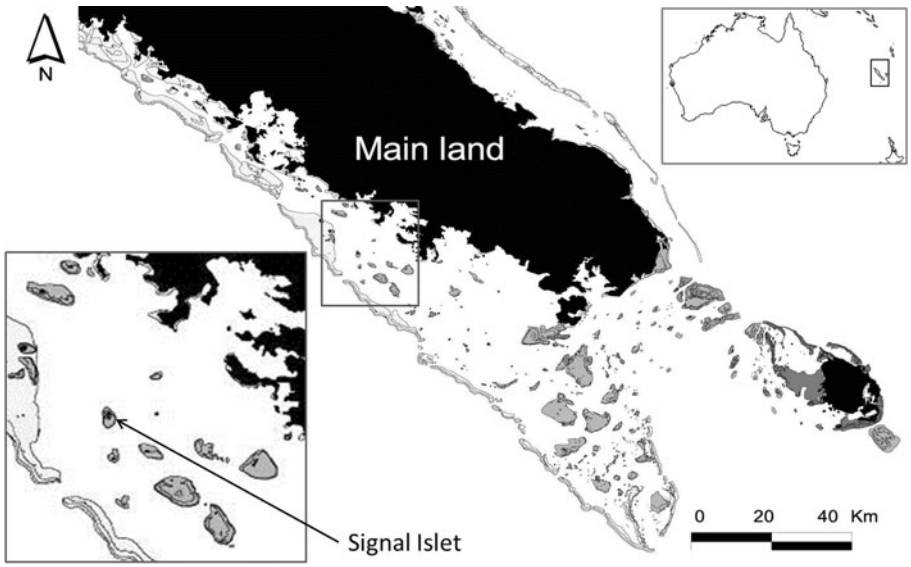

Fig. 1 Maps of our study sites. The panel in the *upper-right corner* indicates the general location of New Caledonia in the Western-Pacific. The *main panel* shows the South-West lagoon of New Caledonia. The panel in the *lower-left corner* shows the location of our sampling site for the current study (Signal Island, 22°17′45S; 166°17′34E). *Black areas* indicate emergent land (mainland and islands); *gray areas* represent coral-reef flats. The barrier reef and other fringing reefs are represented by *light gray* areas

habits between and within species that have been suggested by previous studies based on stomach contents analysis and broad prey habitat categorization (see Brischoux et al. 2007b, 2009). Our rationale was to examine an independent data set (i.e., isotopic signatures) linked to the foraging ecology of these top predators (both the sea kraits and their prey).

In the present study, we use stable isotopes to test key hypotheses suggested by earlier studies. Specifically, we predict that (1) stable carbon isotopic signatures of the predators and their prey will vary according to foraging habitats of the snakes (and thus foraging habitats and/or habitat use of their anguilliform prey), as predicted by the habitat-divergence hypothesis (Brischoux et al. 2007b) and (2) the combination of stable nitrogen (representative of the trophic level) and stable carbon (presumably reflecting foraging habitat) isotopic signatures of the predators and their prey will be related to predator body size, presumably reflecting ontogenetic shifts in feeding habits (Brischoux et al. 2009).

Materials and methods

Study species and area

Sea kraits (*Laticauda* spp.) are proteroglyphous (front-fanged) snakes (Elapidae), widely distributed through tropical reefs (Heatwole 1999). Although they resemble "true" seasnakes in specialized adaptations to marine life (e.g., flattened tail, salt-excreting glands), these snakes spend about half their time on land. They forage in the water, but return to small islets to digest their prey, slough their skins, mate and lay eggs (Heatwole 1999). Two species of sea kraits occur in New Caledonia: *Laticauda saintgironsi* (Cogger and Heatwole 2006) and *L. laticaudata* (Saint Girons 1964; Ineich and Laboute 2002). Both species forage on the sea floor, exploring cavities and burrows in search of anguilliform fish (Heatwole 1999; Brischoux and Bonnet 2009). Although we sampled 10 islets, we concentrated most of our effort on Signal Island (a 15-ha islet located in the southwest lagoon of New Caledonia; 22°17′45S; 166°17′34E, Brischoux and Bonnet 2009, Fig. 1).

Snakes were collected by hand, measured (snout-vent length [SVL], ±1 cm), weighed (±1 g) and individually marked by scale clipping (Brischoux and Bonnet 2009). The abdomen of each snake was palpated to check for the presence of prey in the stomach. Because the eels consumed by sea kraits are non-spinose, the snakes readily regurgitate their prey if gentle pressure is applied to the rear of the stomach (Brischoux and Bonnet 2009). We collected, identified, measured and preserved 1,077 regurgitated prey items (prey were identified according to the keys of Böhlke et al. 1999; Smith 1999a, b; Smith and McCosker 1999). We used allometric equations to infer eel body sizes (total length, cm) from partly digested fragments (Brischoux et al. 2007a).

Stable isotopic analyses

Blood was collected using a heparinized syringe by intracardiac puncture (~300 μL) of adult sea kraits (10 males and 10 females of each species). In endothermic vertebrates, metabolic turnover is rapid, and different blood components exhibit different protein (and hence, isotopic) turnover rates. Turnover rates for blood cells and whole blood correspond to period of a few weeks in small species

and a few months in large-bodied animals; whereas plasma typically provides information over shorter time periods (Hobson and Clark 1992, 1993; Hildebrand et al. 1996; Haramis et al. 2001; Bearhop et al. 2002). The situation is different for ectothermic vertebrates, especially snakes which exhibit very slow metabolic turnover (Pough 1980). Most snakes feed infrequently, with the cycle from prey-capture to the completion of digestion often requiring at least 2 weeks. Because it is physiologically impossible for a snake to renew its entire blood composition after a single meal, each additional prey item can affect the predator's overall isotopic signature only slightly. Between infrequent successful foraging trips, the snakes must rely on their body reserves (e.g., long-term fat and muscle stores) to buffer long starvation periods and to sustain their general metabolism (including anabolism and hence blood maintenance). Most blood components last more than a few weeks and are progressively synthesized using reserves and income. Overall, because integration times are likely to be months rather than weeks in these reptiles, whole blood isotopic signatures are likely to provide a longer-term picture of a species' foraging ecology than can direct feeding observations or stomach content analyses.

We sampled muscle (~0.5 g) from 10 individuals of each main prey species (>10% by number of the diet, Table 1) of both sea krait species. For one prey type consumed by both species of sea kraits (*Conger* sp.), the state of digestion precluded species-specific identification. Thus, we sampled five specimens of *Conger* sp. from each of the snake species. Whole blood and muscle samples were stored in 70% ethanol (this preservative does not alter isotopic composition; Hobson et al. 1997).

Before analysis, whole blood and muscle samples were dried in an oven at +60°C and ground to a fine powder. Because lipids are depleted in ^{13}C relative to proteins and carbohydrates (Post et al. 2007), lipids were extracted from muscle samples using cyclohexane. Low lipid content in blood typically renders lipid extraction unnecessary (Cherel et al. 2005a, b), but this assumption has not been tested on reptiles. We thus compared the isotopic signature of blood of *L. saintgironsi* ($N = 20$) with and without lipid removal using cyclohexane.

Relative abundances of ^{13}C and ^{15}N were determined by continuous-flow isotope-ratio mass spectrometry. We present our results in the usual δ notation relative to PDB (Pee Dee Belemnite) and atmospheric N_2 for $\delta^{13}C$ and $\delta^{15}N$, respectively (Fry 2006). Replicate measurements of internal laboratory standard (acetanilide) indicated the measurement errors of <0.13 and <0.22‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. Data were analyzed using Statistica (Statsoft Inc.). Values are mean ± SD.

Table 1 Main prey species (>10% by number in each diet) analyzed for their isotopic signatures, with their percentage by number in the diet of males and females of each sea krait species (*Laticauda laticaudata* and *L. saintgironsi* are shown as LL and LS, respectively)

Prey species	Female LL	Male LL	Female LS	Male LS
Conger sp.	68	18	12	0
Gymnothorax albimarginatus	13	32	0	0
G. chilospilus	0	3	11	65
G. eurostus	0	1	14	5
G. fimbriatus	0	0	13	2
G. moluccensis	0	15	0	1
G. pindae	0	1	11	5
Scuticaria tigrina	0	0	10	0
Other species	5 spp. (all <8%)	14 spp. (all <9%)	18 spp. (all <7%)	17 spp. (all <7%)

Minor components of each diet (<10%) are summarized in the last line of the table. This stomach content analysis was based on 40 females and 182 males in *L. laticaudata*; and 174 females and 306 males in *L. saintgironsi*

Results

Lipid extraction

Prior extraction of lipids did not affect our estimates for blood $\delta^{13}C$ or $\delta^{15}N$ (paired *t*-tests, $t = -1.27$ $P = 0.22$ for $\delta^{13}C$ and $t = 1.19$ $P = 0.24$ for $\delta^{15}N$, Electronic Supplemental Material, ESM Appendix S1), suggesting that lipid removal before isotopic measurements is not necessary (as is also the case for endotherm blood: Cherel et al. 2005a, b). Hence, we did not perform lipid extraction for the remaining blood samples, and the following analyses were done on blood isotopic values gathered prior to lipid extraction.

Isotopic niches of sea kraits

Species effect

Laticauda saintgironsi and *L. laticaudata* differed significantly in overall isotopic signatures (MANOVA, Wilk's lambda = 0.45, $F_{2,37} = 22.31$, $P < 0.001$). However, univariate analyses showed that only $\delta^{13}C$ discriminated the two species (ANOVA, $F_{1,38} = 25.04$, $P < 0.001$ for $\delta^{13}C$ and $F_{1,38} = 0.01$, $P = 0.98$ for $\delta^{15}N$, ESM Appendix S1, Fig. 2), *L. laticaudata* having lower $\delta^{13}C$ values than *L. saintgironsi*. This result suggests that the two sea kraits occupy similar trophic levels but forage on different substrates.

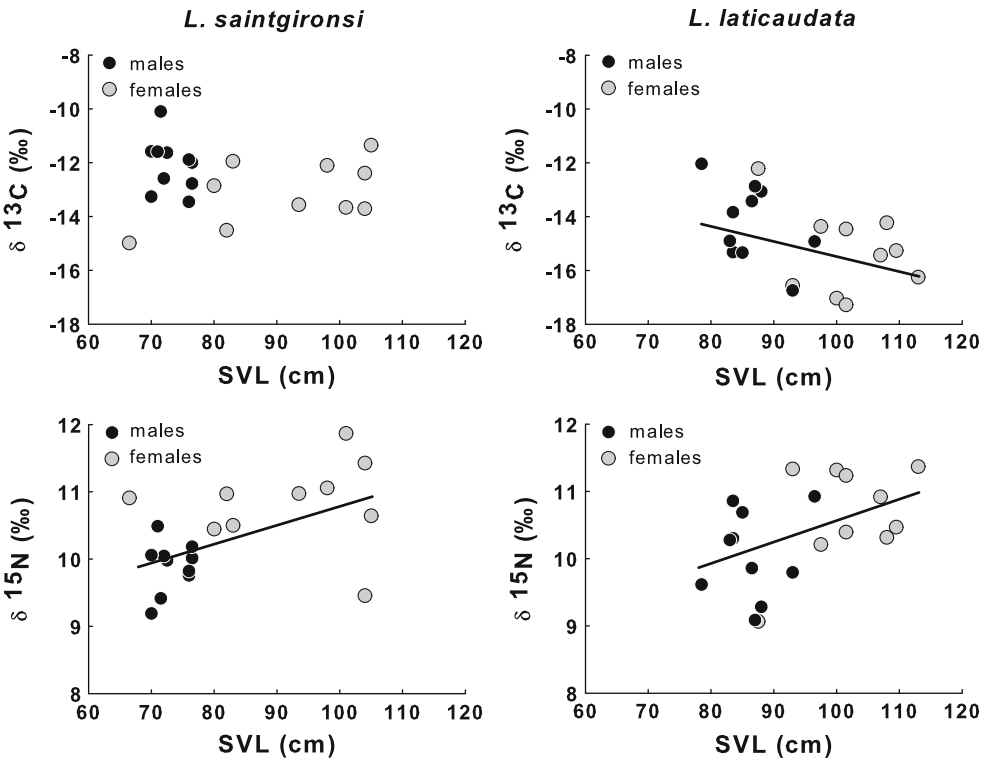

Fig. 2 Relationships between body length (snout-vent length SVL, cm) and $\delta^{13}C$ (*upper panels*) and $\delta^{15}N$ values (*lower panels*) in the sea kraits *Laticauda saintgironsi* (*left panels*) and *L. laticaudata* (*right panels*). Regression *lines* (based on pooled sexes) indicate statistically significant relationships ($P < 0.05$). *Black dots* and *gray dots* stand for adult males and adult females, respectively

Body size effect

$\delta^{15}N$ values increased with body size in both species of snakes ($F_{1,18} = 6.40$, $r^2 = 0.26$, $P = 0.02$ for *L. laticaudata* and $F_{1,18} = 6.59$, $r^2 = 0.27$, $P = 0.02$ for *L. saintgironsi*; Fig. 2), whereas $\delta^{13}C$ value was negatively correlated with body length in *L. laticaudata* alone ($F_{1,18} = 6.15$, $r^2 = 0.25$, $P = 0.02$ for *L. laticaudata* and $F_{1,18} = 0.17$, $P = 0.7$ for *L. saintgironsi*; Fig. 2). These results suggest that trophic levels increase ontogenetically in both species of sea kraits, and that foraging habitat changes ontogenetically in *L. laticaudata*.

Sex effect

In both species, adult males differed from adult females in overall isotopic signatures (MANOVA, Wilk's lambda = 0.69, $F_{2,35} = 7.59$, $P < 0.002$). This sex difference appears to be a secondary consequence of body-size dimorphism because adult females exhibit larger average body sizes than adult males in both species (ANOVA $F_{1,18} = 26.84$, $P < 0.001$ for *L. laticaudata* and $F_{1,18} = 19.03$, $P < 0.001$ for *L. saintgironsi*, Fig. 2). Correcting for body size (length), we did not detect any effect of sex on the isotopic signatures of *L. laticaudata* (ANCOVA with SVL as the covariate, $F_{1,17} = 0.09$, $P = 0.8$ for $\delta^{13}C$ and $F_{1,17} = 0.02$, $P = 0.9$ for $\delta^{15}N$). In contrast, the effect of sex on isotopic composition remained statistically significant both for $\delta^{13}C$ and for $\delta^{15}N$ values of *L. saintgironsi* (ANCOVA with SVL as the covariate, $F_{1,17} = 7.04$, $P < 0.02$ for $\delta^{13}C$ and $F_{1,17} = 6.33$, $P = 0.02$ for $\delta^{15}N$). The interaction term (SVL*Sex) was not significant for either $\delta^{13}C$ or $\delta^{15}N$ in *L. laticaudata* ($F_{1,18} = 2.55$, $P = 0.13$ and $F_{1,18} = 4.04$, $P = 0.06$ for $\delta^{13}C$ and $\delta^{15}N$, respectively), whereas in *L. saintgironsi*, the interaction between SVL and sex was significant for $\delta^{15}N$, but not for $\delta^{13}C$ ($F_{1,18} = 15.04$, $P < 0.001$ and $F_{1,18} = 2.43$, $P = 0.08$ for $\delta^{15}N$ and $\delta^{13}C$, respectively). These results suggest an ontogenetic shift (dependent of body size) in diets in *L. laticaudata*; whereas in *L. saintgironsi*, the sex differences (independent of body size) in overall isotopic signatures are more likely to reflect the divergence in dietary composition between adult males and females (Table 1).

Isotopic niches of anguilliform fish

Species effect

Anguilliform fish species were segregated by their overall isotopic signatures (MANOVA, Wilk's lambda = 0.15, $F_{2,16} = 13.67$, $P < 0.001$) and in univariate analysis, both $\delta^{13}C$ (ANOVA, $F_{1,8} = 26.54$, $P < 0.001$, post hoc tests [Fisher's LSD] having $P < 0.03$ unless otherwise stated, see Fig. 3, Appendix S1) and $\delta^{15}N$ values (ANOVA, $F_{1,38} = 7.56$, $P < 0.001$, post hoc tests [Fisher's LSD] having $P < 0.05$ unless otherwise stated, see Fig. 3, ESM

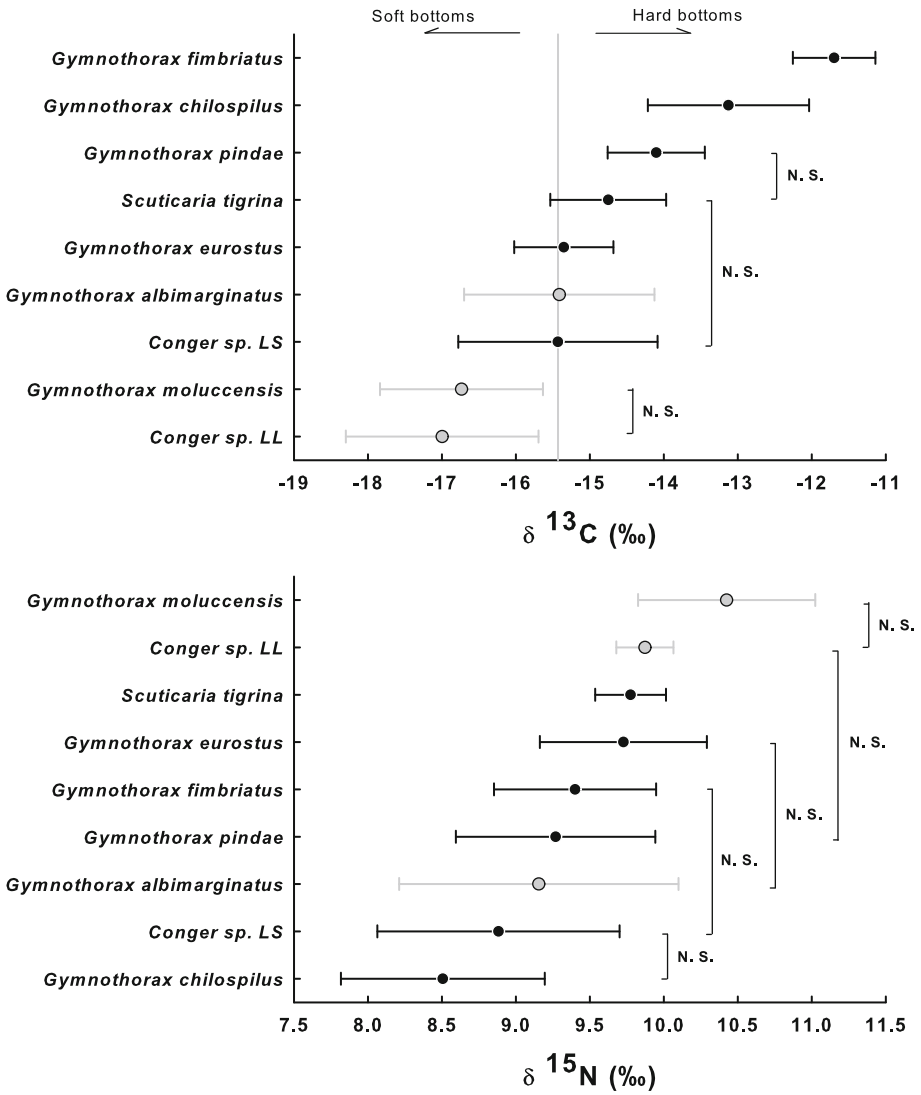

Fig. 3 Stable carbon (*upper panel*) and stable nitrogen (*lower panel*) isotope values for eight species of anguilliform fish (eels). *Gray* and *black symbols* refer to the prey of the sea kraits *Laticauda laticaudata* and *L. saintgironsi*, respectively. *Error bars* represent standard deviations. The *gray line* in the upper panel refers to the estimated boundary between soft and hard substrates (~15.4‰ δ^{13}C, see text for details). "N.S." refers to non-significant differences between species (post hoc tests having $P > 0.05$, see text for statistics). Note that the two sub-samples of *Conger* sp. differ significantly between predator species for both δ^{13}C and δ^{15}N values

Appendix S1). Overall, individual prey δ^{13}C ranged from −18.7‰ (*G. moluccensis*) to −10.9‰ (*G. fimbriatus*, Fig. 3), and individual prey δ^{15}N ranged from 7.2‰ (*G. chilospilus*) to 11.8‰ (*G. moluccensis*, Fig. 3). These results suggest that anguilliform fish form a generalist predatory assemblage that occupies a range of trophic levels and use a range of foraging habitats. However, data for each anguilliform fish species are scattered along broad trophic and habitat continua (Fig. 3).

Habitat effect

The δ^{13}C of anguilliform fish wase significantly associated with the habitats in which these species occur (the latter categories were determined from habitat data in FishBase: Froese and Pauly 2006; see Brischoux et al. 2007b, 2009 for details). Although habitats were unknown for one of the species (e.g., *G. moluccensis*, see Brischoux et al. 2007b for details), δ^{13}C signatures differed significantly among eels known to live in different habitat types (Kruskal–Wallis test, $H = 19.1$, $P < 0.001$, multiple [bilateral] comparisons showing that Hard substrates differed from Soft and Hard-plus-Soft substrates, mean δ^{13}C value = −13.6 ± 1.5‰ for Hard substrates, −15.5 ± 1.4‰ for Hard-plus-Soft substrates and −15.4 ± 1.3‰ for Soft substrates, Fig. 3). Among anguilliform fish overall, we found a significant negative relationship between δ^{13}C and δ^{15}N ($F_{1,77} = 25.62$, $r^2 = 0.25$, $P < 0.0001$, Fig. 4).

Body size effect

Within the eel *G. albimarginatus*, larger individuals had lower δ^{13}C values (Spearman rank correlation, $r_s = -0.76$, $P < 0.05$, Fig. 5). Within *G. fimbriatus* and *G. moluccensis*, larger individuals had higher δ^{15}N values (Spearman rank correlation, $r_s = 0.85$, $P < 0.05$; $r_s = 0.74$, $P < 0.05$,

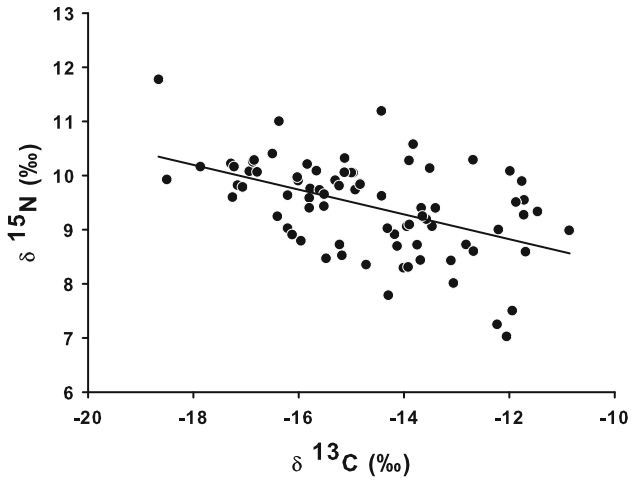

Fig. 4 Relationship between δ^{13}C and δ^{15}N for the nine species of anguilliform fish. See text for details

respectively, Fig. 5). These results suggest that some anguilliform fish species exhibit ontogenetic shifts in trophic level and/or habitat use.

Isotopic signatures of prey consumed by different sea krait species

There was a significant difference in overall isotopic signatures of the prey when they were group according to the diet of the two sea krait species (MANOVA, Wilk's lambda = 0.70, $F_{2,74} = 15.61$, $P < 0.001$). Despite some overlap, both δ^{13}C (ANOVA, $F_{1,75} = 31.51$, $P < 0.001$) and δ^{15}N values (ANOVA, $F_{1,75} = 4.77$, $P = 0.03$) differed in univariate analyses. The prey of *L. laticaudata* had lower δ^{13}C and higher δ^{15}N values than those of *L. saintgironsi* (Fig. 3). Isotopic signatures differed even between the *Conger* sp. consumed by the two species of sea kraits, in both δ^{13}C and δ^{15}N values (i.e., *Conger* sp. LL vs. *Conger* sp. LS, see Fig. 3).

Discussion

Technology can provide insights into ecological processes not amenable to direct observation. Spatial variation in isotopic signatures has allowed researchers to define the spatial ecology of foraging: for example, to distinguish whether migratory birds depend upon food intake from their summer or winter ranges to fuel reproduction (Hobson 2006). Our study takes the same approach, but on a smaller spatial scale and with a very different predator–prey system. To our knowledge, ours is the first study to use stable-isotope technology to explore the trophic ecology of seasnakes (Fisk et al. 2009; Willson et al. 2010).

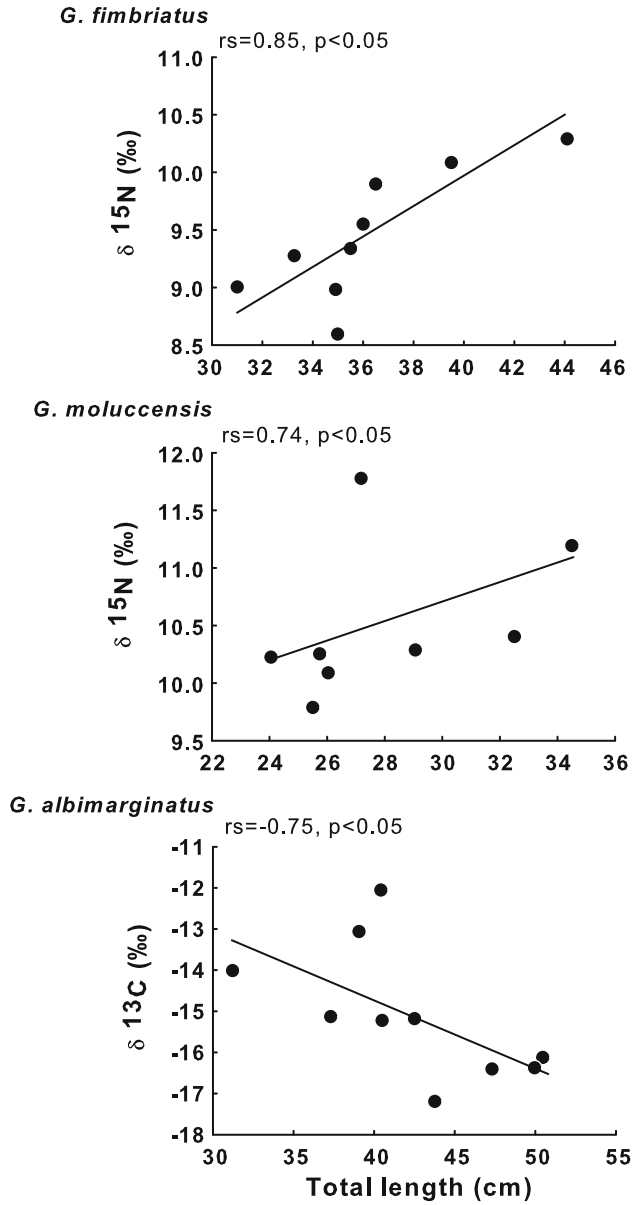

Fig. 5 Relationships between δ^{15}N values and fish total length (cm) for the anguilliform fishes *Gymnothorax fimbriatus* and *G. moluccensis*; and between δ^{13}C values and fish total length (cm) for *G. albimarginatus*

Elongate snakes that search for prey within narrow crevices in complex coralline formations on the seabed, often at considerable depth, are essentially unavailable for direct observation (but see Ineich and Laboute 2002). Even if some individuals can be seen foraging, logistically imposed limitations to the observer's ability to watch such activities inevitably bias any attempt to compare foraging microhabitats among predators of different species, sexes and body sizes. The stable-isotope approach provides an independent and objective measure that is empirically linked to prey habitat type in our data, and that varies significantly as a function of the predator's species, sex and

size. Although many factors can modify stable-isotope composition (Gannes et al. 1997; Martinez del Rio et al. 2009), the patterns in our data correspond well to a priori predictions based on the hypothesis that different species, sexes and body sizes of sea kraits differ in dietary composition according to divergence in foraging habitats.

The notion that the two species of New Caledonian sea kraits exploit distinct foraging grounds has been supported by data on diet composition, prey habitats (FishBase), duration of snake foraging trips and habitat-specific morphology of their anguilliform prey (see Brischoux et al. 2007b, 2009). Our isotopic data provide further independent evidence in support of the habitat-divergence hypothesis and extend it to intraspecific comparisons. Importantly, the stable-isotope differences that we observed suggest that these intraspecific and interspecific niche divergences are consistent through time. For example, isotopic ratios diverged not only between the types of eels consumed by the two sea krait species but also between blood samples taken from the predators themselves. However, habitat boundaries (such as those between soft-bottoms and hard-bottoms) are unlikely to be clear-cut. As a result, our data on anguilliform fish δ^{13}C show extensive variation and overlap between and within species.

Intraspecific dietary partitioning is widespread in snakes, typically based on body size and/or sex (Shine and Wall 2007). In species with strong sexual dimorphism in body size, the effects of sex and body size on dietary composition can interact in complex ways (Shine 1991). There are two likely reasons why a snake's body size influences the types of prey that it consumes. The first reason is that snakes are gape-limited predators (i.e., jaw size limits maximal ingestible prey size), and hence smaller snakes are unable to swallow the prey taken by larger conspecifics (Shine 1991; Arnold 1993). The second reason, and one that has attracted less scientific attention, is that smaller snakes may be able to enter the narrow crevices used by smaller species of prey (Shine 1991). Plausibly, both of these processes contribute to the tendency for larger sea kraits to consume larger prey items. Because the size distribution of eels differs between hard substrate and soft substrate (Brischoux et al. 2009), any shift in prey sizes is likely to involve a shift in foraging habitats as well. Similar ontogenetic (size-related) shifts in feeding habits also occur in predatory fishes (Kawakami and Tachihara 2005; Tibbetts and Carseldine 2005; Carassou et al. 2008; and present study for 3 species of anguilliform fish).

In *L. laticaudata*, larger individuals had lower δ^{13}C signatures, whereas δ^{15}N values increased with body size in both sea krait species. These results accord well with a strong ontogenetic shift of prey types within *L. laticaudata*, and a significant (albeit weaker) shift in *L. saintgironsi* detected through conventional gut content analyses (Brischoux et al. 2009). However, the convergence between results based on stable-isotope analyses and previous results based on stomach content analyses (Brischoux et al. 2007b, 2009) suggest that the ontogenetic shift in snake diets is linked to an ontogenetic shift in foraging habitats in *L. laticaudata*. In *L. saintgironsi*, the sex differences (independent of body size) in overall isotopic signature are probably linked to the divergence in dietary composition between adult males and females (e.g., males depend on 1 main prey species *versus* 6 in females: see Table 1).

Perhaps the most surprising divergence between the two sea krait species was in the isotopic signatures of *Conger* sp. Although we could not distinguish between conger eels from the two predator species on morphological criteria, these samples differed in both δ^{13}C and δ^{15}N. These differences were not linked to fish body size (both samples encompassed the same range, 30–50 cm long). We do not know whether the conger eels taken by the two sea krait taxa are different species or the same species from different habitats (e.g., hard vs. soft substrates). Regardless, the isotopic distinction between these samples provides a strong example of the power of isotopic data to tease apart superficially subtle differences in niche dimensions between sympatric predators.

Both benthic predatory communities, eels (*G. albimarginatus*, *G. moluccensis*) and sea kraits (*L. laticaudata* and *L. saintgironsi*), display δ^{15}N that sometimes exceed 11.0‰. These δ^{15}N values are similar to those found in pelagic top-predator fish species, for example, $\sim$11.5‰ for *Scomberomorus commerson* (3–7 kg) (Carassou et al. 2008). Although isotopic variance in δ^{15}N is not always synonymous with trophic diversity, the results suggest that despite their small vertical depth, reef seafloor ecosystems could contain trophic networks as complex as those occurring at a much larger spatial scale in the overlying water column. Alternatively, but not exclusively, basal δ^{15}N might vary with habitat types, with soft-bottoms having higher δ^{15}N (as suggested by the negative relationship we detected between δ^{13}C and δ^{15}N in anguilliform fish). Future studies could usefully extend our analyses to a broader range of habitat types and trophic levels.

Lastly, we note some methodological caveats to our conclusions. Most notably, we lack isotopic data on many of the steps that would unambiguously link the habitats, the fish and the snakes. Obtaining such data would require extensive isotopic analyses of complex trophic chains beginning with each substrate type and their specific biofilms, through the primary producers and all the way up to top-predators. Ideally, we would also need data on seasonal variations, water flows, individual movements and the like.

The task is prohibitive logistically. Fortunately, there is strong empirical evidence that isotopic baseline levels are indeed reflected in organisms at higher trophic levels in the marine environment (Cherel and Hobson 2007).

Additionally, the simple habitat categories we derived from FishBase are derived from scarce and anecdotal observations, reflecting a lack of knowledge on anguilliform fish ecology (e.g., Ineich et al. 2007). However, these simple habitat categories (soft-bottom vs. hard-bottom) provided a segregation of prey species that matched remarkably well with the information collected from the foraging ecology of the two species of seasnakes (Brischoux et al. 2007b). Additionally, the same simple categories successfully revealed clear habitat-specific morphologies in tropical reef anguilliform fish (Brischoux et al. 2009). Taken together, these congruent elements suggest that although crude, the habitat categories we used enabled us to detect differences in the trophic ecology of two communities of reef top-predators.

The hypothesis of differences in foraging habitats between *L. laticaudata* and *L. saintgironsi* also is supported by more subtle lines of evidence. In hard-bottom habitats, the size of the prey may not always match the size of the shelter (natural crevices). Conversely, soft-bottom species shelter in a self-made burrow that corresponds exactly to their own diameter. As expected from this situation, the relationship between snake body size and prey diameter is weaker for the hard-bottom crevice-forager (*L. saintgironsi*) than for the soft-bottom burrow-forager (*L. laticaudata*, Brischoux et al. 2009). Also, *L. saintgironsi* (the hard-bottom crevice-forager) consumes 50% of prey head first and 50% tail first, whereas the soft-bottom forager (*L. laticaudata*) ingests 85% of prey head first (Brischoux et al. 2009). Finally, our direct underwater observations (25 cases of snakes foraging) all involve *L. saintgironsi* on hard-bottom substrates versus a few observations of *L. laticaudata* foraging on soft-bottoms (Ineich and Laboute 2002; pers. obs.).

Although the totality of evidence thus supports results of the present study on isotopic signatures, further studies could usefully expand these analyses to a broader range of trophic levels, fish species and spatial scales. More generally, stable-isotope methodology has great potential to clarify niche partitioning within and among species and may be particularly valuable in systems (such as the one studied here) in which direct observation of predator–prey encounters is unlikely to be feasible.

Acknowledgments We thank P. Richard and G. Guillou for stable-isotope analysis at La Rochelle (France), and C. Chevillon and D. Ponton (DENV-Province Sud, IRD). We thank three anonymous referees as well as Aaron Fisk and Prof Philip Munday for their comments on a previous draft of the MS. T. Fauvel made the map. Rex Cambag carried the equipment. The work was supported financially by the CNRS, the Australian Research Council and the Australian Government (Endeavour Award # 2009_930). It was carried out under permits # 6024-179/DRN/ENV and # 6024-3601/DRN/ENV issued by the DENV, Province Sud, New Caledonia.

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