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Feeding ecology of striped dolphins, *Stenella coeruleoalba*, in the north-western Mediterranean Sea based on stable isotope analyses

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The feeding ecology of striped dolphin, Stenella coeruleoalba, *in the north-western Mediterranean Sea was studied using stable isotope analyses. Carbon and nitrogen stable isotope ratios were measured in skin and muscle tissues of stranded and by-caught dolphins from six geographical areas in the Mediterranean Sea and Atlantic Ocean. Variation in* $\delta^{15}N$ *in relation to dolphin size is attributed to changes in diet. Nursing calves have a higher trophic level than weaned animals and their* $\delta^{15}N$ *values decrease progressively until weaning, estimated to be at a body length of around 155 cm.* $\delta^{15}N$ *values then increased for larger individuals which suggests changes in diet for mature dolphins. Geographical differences in diet were apparent between the Atlantic and the Mediterranean, although no clear differences were apparent between the five Mediterranean areas. Comparisons of the nitrogen isotope ratios of skin and muscle highlighted a higher fractionation in skin compared to the muscle tissue. Values of* $\delta^{13}C$ *also increased with body length although it appears that this is not only driven by trophic level enrichment.* $\delta^{13}C$ *increases before weaning and the difference in trophic level between newly-weaned and mature dolphins was twice as high for carbon as for nitrogen. Ontogenetic changes in carbon isotope composition may therefore be driven by feeding on deep water prey and dolphin movements outside the coastal feeding grounds. Indeed, seasonal variations in* $\delta^{13}C$ *are suspected to be driven by migration within the Mediterranean basin.*

Keywords: striped dolphin, *Stenella coeruleoalba*, feeding ecology, nitrogen and carbon stable isotopes, skin, muscle, north-western Mediterranean Sea

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INTRODUCTION

The striped dolphin, *Stenella coeruleoalba* (Meyen, 1833), is a world-wide cetacean species, distributed across the temperate to tropical waters of the world (Perrin *et al.*, 1994; Archer, 2000). The species inhabits deep waters beyond the continental shelf, typically over the continental slope out to oceanic waters (Notarbartolo di Sciara *et al.*, 1993; Forcada *et al.*, 1994; Perrin *et al.*, 1994; Marini *et al.*, 1996; Gannier, 1998, 2005; Aguilar, 2000; Archer, 2000; Gordon *et al.*, 2000; Cañadas *et al.*, 2002; Reeves & Notarbartolo di Sciara, 2006). Although no separate subspecies are recognized (Archer & Perrin, 1999), geographical variation has been found in body size throughout the species' range (Perrin *et al.*, 1994; Archer, 2000) suggesting distinct populations. A number of studies suggest that there is limited gene flow between Mediterranean and Atlantic populations (Calzada & Aguilar, 1995; Archer, 1996; Garcia-Martinez *et al.*, 1999). Moreover, morphological (Calzada & Aguilar, 1995), toxicological (Monaci *et al.*, 1998) and genetic differences (Gaspari, 2004) suggest limited dispersal range between areas within the Mediterranean.

Based on stomach content analyses, the feeding ecology of striped dolphins has been investigated in the Mediterranean Sea, the Atlantic Ocean (Bay of Biscay) and in Japan (Miyazaki *et al.*, 1973; Desportes, 1985; Würtz & Marrale, 1993; Astruc, 2005 unpublished results; Ringelstein *et al.*, 2006; Spitz *et al.*, 2006). The diet of striped dolphin was found to be primarily composed of cephalopods and fish, and secondarily of crustaceans. Prey are typically small-sized (up to 200–300 mm length), pelagic, schooling and vertically migrating organisms. Seasonal or inter-annual variability in environmental conditions and prey resources are suspected to generate shifts in the distribution of striped dolphin (Gannier, 1999; Laran & Drouot-Dulau, 2007; Azzelino *et al.*, 2008). However, the species may be less mobile than other cetacean species as a consequence of its foraging plasticity (Azzelino *et al.*, 2008). Differences in feeding ecology could then drive population structure of striped dolphins, a hypothesis supported by different stomach contents

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according to the habitat (Astruc, 2005 unpublished results; Spitz *et al.*, 2006).

However, it is widely recognized that stomach contents analyses have a number of limitations. In particular, they are strongly influenced by the most recent feeding event. Partially digested prey are not always identifiable, limiting the conclusions that can be made about the overall diet, and the varying time of digestion of different prey species can result in an overestimation of some species (Aguiar dos Santos & Haimovici, 2001; Santos *et al.*, 2001). Stable isotope analysis offers a powerful complementary method to study marine mammal feeding ecology (Smith *et al.*, 1996; Hobson *et al.*, 1997; Burns *et al.*, 1998; Das *et al.*, 2000). The method is based on the fact that stable isotope ratios of carbon ($^{13}C/^{12}C$, normally expressed as $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$ or $\delta^{15}N$) of a consumer are the weighted average of the isotopic composition of the ingested prey animals (DeNiro & Epstein, 1978, 1981), modified by some enrichment associated with metabolic activities within the individual (Mook & de Vries, 1989). Lighter isotopes (^{14}N and ^{12}C) are preferentially mobilized in biochemical reactions and are therefore more likely to be excreted (Minawaga & Wada, 1984; Peterson & Fry, 1987). This results in heavier isotopes (^{15}N and ^{13}C) being preferentially retained in the consumer (Das *et al.*, 2000). Thus consumer tissues exhibit a higher isotopic value than those of their prey.

Generally, the nitrogen stable isotope ratio of a consumer is enriched on average by 3.4‰ over that of its diet (Minawaga & Wada, 1984; Vander Zanden & Rasmussen, 2001), although considerable variation can be found in the amount of enrichment between one trophic level and the next (Minawaga & Wada, 1984; Wada *et al.*, 1987; Hobson & Welch, 1992; Burns *et al.*, 1998). Nitrogen isotope ratios ($\delta^{15}N$) can thus give insights into consumer trophic level (Hobson & Clark, 1992; Vander Zanden *et al.*, 1997; Burns *et al.*, 1998; Das *et al.*, 2000, 2003b; Witteveen *et al.*, 2011) (assuming that there is information available on $\delta^{15}N$ at the base of the food web to allow calibration)—although the resolution provided in relation to diet composition is coarse—and can potentially allow reconstitution of trophic food webs (Wada *et al.*, 1987; Fry, 1988; Hobson & Welch, 1992; Hobson *et al.*, 1994). In contrast, a small increase (typically 1‰) of carbon isotope ratios ($\delta^{13}C$) between prey and predator is often reported (DeNiro & Epstein, 1978; Fry & Sherr, 1984; Peterson & Fry, 1987; Vander Zanden & Rasmussen, 2001), thus providing information about the origin of the prey, since $\delta^{13}C$ values at the base of a trophic web are often characteristic of the ecosystem (Schell *et al.*, 1989; Smith *et al.*, 1996; Marcoux *et al.*, 2007). For example, in the marine environment, $\delta^{13}C$ can allow discrimination between inshore and offshore (Rau *et al.*, 1983; Schell *et al.*, 1989; Hobson *et al.*, 1994; Walker *et al.*, 1999; Das *et al.*, 2003a, b) or pelagic and benthic foraging areas (France, 1995; Burns *et al.*, 1998; Das *et al.*, 2000).

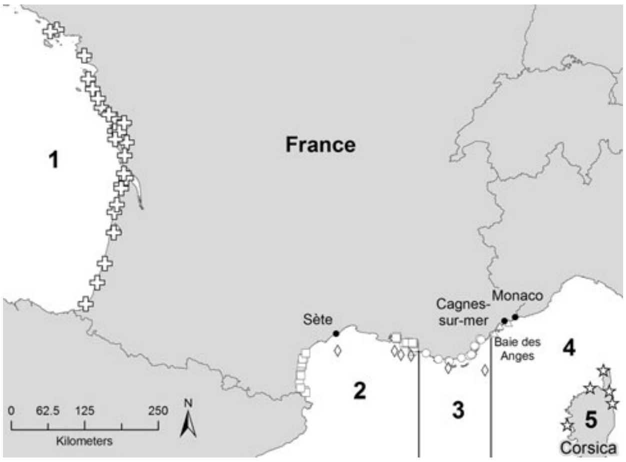

Fig. 1. Striped dolphin samplings localities in the Atlantic (area 1, indicated by crosses) and in the north-western Mediterranean Sea: Gulf of Lion (area 2, squares), Provençal Basin (area 3, circles), Ligurian Sea (area 4, triangles), Corsica (area 5, stars) and offshore area (across areas 2 and 3, diamonds). Preys were collected off Sète (area 2), Cagnes-sur-mer, Monaco, and Baie des Anges area (area 4).

As stable isotopes ratios derive from assimilated food, the isotopic composition of an animal's tissue reflects the dietary inputs over the turn-over time of the analysed tissue (DeNiro & Epstein, 1978, 1981). Stable isotope measurements can therefore provide time-integrated dietary information, over a series of time-scales if analyses are performed on tissues with different turnover rates (DeNiro & Epstein, 1978, 1981; Tieszen *et al.*, 1983; Das *et al.*, 2000; Knoff *et al.*, 2008). However, caution is needed due to differences in the biochemistry of different tissues, which could also affect fractionation (DeNiro & Epstein, 1978; Tieszen *et al.*, 1983; Hobson & Clark, 1992; Hobson *et al.*, 1996; Pinnegar & Polunin, 1999).

In this study, we use stable isotope analyses to explore a number of aspects of the feeding ecology of striped dolphin in the north-western Mediterranean Sea. Specifically we look at whether there is evidence of geographical and seasonal variation in diet, and how it varies ontogenetically. We analysed ratios of stable nitrogen and carbon isotopes in tissue samples of dolphins from six geographical areas. $\delta^{15}N$ was used to assess the trophic levels at which the animals fed, while $\delta^{13}C$ was used as an indicator of the source of food resources and thus of habitat differences. Analysis of tissues with slow and fast protein turnover rates can in theory identify animals that are switching to an isotopically novel diet (Tieszen *et al.*, 1983). We measured isotope values in skin and muscle. The turnovers of both tissues are unknown for the striped dolphin. But, based on studies on beluga *Delphinapterus leucas* (St Aubin *et al.*, 1990) and bottlenose dolphin *Tursiops truncatus* (Hicks *et al.*, 1985; Knoff *et al.*, 2008), we expected the isotopic composition of striped dolphin skin to reflect the input of recent diet (over the last two months). According to the body size of the species, and relying on previous studies (Tieszen *et al.*, 1983; Hobson & Welch, 1992; Sponheimer *et al.*, 2006), we estimated a turnover rate for striped dolphin muscle of approximately several months. Finally, we also analysed the isotope composition of muscle and mantle tissues in some fish and cephalopod species in order to allow us to put dolphin isotope signatures in an ecosystem context (Hobson & Welch, 1992; Hobson *et al.*, 1997; Burns *et al.*, 1998; Das *et al.*, 2003b).

MATERIALS AND METHODS

Sample origin and preparation

Skin and muscle samples derived from stranded and by-caught dolphins collected between 2001 and 2008 along the French Mediterranean coast and between 2001 and 2007

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from the French Atlantic coast, by the members of the French Stranding Network. Samples were frozen (−20°C) immediately after collection.

Samples of a variety of potential prey species were also analysed (Table 3). The chosen species were those commonly reported in the diet of striped dolphins and available from markets, fishers or research surveys. These included six fish species (*Boops boops*, *Engraulis encrasicolus*, *Merluccius merluccius*, *Micromesistius poutassou*, *Sardinella aurita* and *Sardina pilchardus*), two squid genera (*Illex coindetii* and *Loligo vulgaris*), and two cuttlefish species (*Sepia officinalis* and *S. orbignyana*) for the Gulf of Lion and Ligurian Sea. Cephalopod and fish samples were obtained from a local fish market in Sète (Gulf of Lion), directly from fishers in Cagnes-sur-mer (Ligurian Sea) and during trawls surveys off Monaco (Ligurian Sea) (Figure 1). Mantle tissue of cephalopod and muscle tissue from the dorsal region of fish were used for analyses.

All samples were dried at 50°C or freeze-dried for at least 48 hours prior to analysis and ground to a fine powder. Lipids are depleted in ^{13}C compared to the whole body or proteins, such that any material which is rich in lipids will have relatively lower δ^{13}C (DeNiro & Epstein, 1978; Tieszen *et al.*, 1983). Therefore, lipids were extracted from all samples using cyclohexane (Cherel *et al.*, 2010). About 100 mg of sample were put in a screw-capped tube along with 4 ml of cyclohexane p.a., left under rotary agitation for 1 hour at room temperature, and centrifuged (1000 g, 10 minutes). After centrifugation, the pellet was rinsed with 2 ml of cyclohexane, centrifuged again and dried using a dry bath at 45°C under a hood. For very fatty samples, when the supernatant appeared coloured, the 4 ml extraction was repeated. Carbonates were removed from fish muscles with dilute acid (HCl 0.1 N), since inorganic carbonates tend to be enriched in ^{13}C compared to other body fractions (DeNiro & Epstein, 1978).

Stable isotope analyses

Stables isotope analyses were completed at the University of La Rochelle, France, using an isotope ratio mass spectrometer (Thermo Scientific Delta V Advantage, Bremen, Germany) coupled to an elemental analyser (Thermo Scientific Flash EA1112, Milan, Italy). Stable carbon and nitrogen isotope ratios are expressed in delta (δ) notation, defined as parts per thousand (‰, or per mil) deviations from a universal standard, according to the following formula:

$$\delta X = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1000$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio ^{13}C/^{12}C or ^{15}N/^{14}N. The standards are atmospheric nitrogen for δ^{15}N and Pee Dee Belemnite carbonate (PDB) for δ^{13}C and the reference materials used for calibration were IAEA CH-6 and IAEA-N1. Replicate measurements of a laboratory standard (acetanilide, Thermo Scientific) analysed with the samples indicated that analytical precision of the measurements was <0.1‰ for δ^{13}C and <0.15‰ for δ^{15}N. Results on elemental composition (%C and %N) of tissues obtained during isotope analyses were used to calculate the sample C:N ratio, which indicates good lipid removal efficiency when C:N <4.

Data analysis

For the statistical analyses, dolphin samples were classified according to the location of the stranding or the by-catch. Samples derived from strandings from Brittany to the Bay of Biscay along the French Atlantic coast (area 1), were all grouped in the same (Atlantic) pool. Samples derived from strandings in the Mediterranean were separated into four areas, i.e. the Gulf of Lion (area 2), Provençal Basin (area 3), Ligurian Sea (area 3), and Corsica (area 5). By-catches collected in the Mediterranean Sea were grouped as 'offshore' samples (Figure 1).

Previous studies based on stomach content analysis showed evidence of ontogenetic, seasonal, and geographical patterns in the diet of striped dolphins (Astruc, 2005 unpublished results). Thus, we focused our analyses to determine relationships (Anderson *et al.*, 2000) between isotopic values in dolphin tissues and the explanatory variables body length, sex, year, month, and area. Data exploration has been undertaken following Zuur *et al.* (2010). Specifically, pair plots of the explanatory variables have been examined to avoid nonlinear or bivariate collinearity and variance inflation factors (VIF) have been calculated to check for multivariate collinearity. Generalized additive models (GAMs) (Hastie & Tibshirani, 1990) were fitted to C and N isotope ratio data. Body length was fitted as a continuous variable while year, month, sex and area were fitted as nominal variables. In order to take account of the effect of trophic level on carbon values, we added nitrogen as a continuous explanatory variable in the carbon model. A Gaussian distribution with identity link was applied since isotope ratios appeared to be approximately normally distributed. GAMs were fitted using a backward selection procedure and the optimal model was identified by Akaike's information criterion (AIC). Generally, the best model is that with the lowest value for the AIC, in which all remaining explanatory variables have significant effects. We started with a full model which included all variables, and at each step the least significant variable was excluded from the model, the process continuing until no further fall in the AIC values was obtained. Stepwise procedures have recently received considerable criticism (Whittingham *et al.*, 2006; Mundry & Nunn, 2009) even if compared to other methods of variable selection they generate similar models (Murtaugh, 2009). To avoid the inclusion of non-significant variables in models, which could be indicative of a Type I error (Mundry & Nunn, 2009), extra model selection steps (F-tests) were applied to test differences between nested models with and without the non-significant variable. A significant difference implies that the model is improved by the additional terms, which can therefore be retained, otherwise it is dropped (Zuur *et al.*, 2007). Once final models were obtained, they were checked to ensure that there were no obvious patterns in the residuals and residuals appeared to be approximately normally distributed. All analyses were performed using the statistical software R (R Development Core Team, 2008). For both additive models (for N and C respectively), we present both the smoothing function and a plot of the predicted values of the ratios against the relevant explanatory variable. Predicted values were calculated using the final models for nitrogen and

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carbon. Assumption has been made on length values: we used the range from 80 to 220 cm, with an interval of 5 cm.

Following the literature, an average increase of 3.4‰ in $\delta^{15}N$ (Minawaga & Wada, 1984; Vander Zanden *et al.*, 1997; Vander Zanden & Rasmussen, 2001) and 1‰ in $\delta^{13}C$ is typically observed between a consumer and its diet (DeNiro & Epstein, 1978; Fry & Sherr, 1984; Minawaga & Wada, 1984; Peterson & Fry, 1987; Vander Zanden *et al.*, 1997; Vander Zanden & Rasmussen, 2001). We estimated the isotopic enrichment (IE) between newly weaned dolphins (155 cm length) and mature dolphins (200 cm length). We calculated this enrichment using nitrogen and carbon ratios from fitted GAM, according to the following equation:

$$IE = \frac{(\delta X_{200cm} - \delta X_{155cm})}{E}$$

where δX is $\delta^{15}N$ or $\delta^{13}C$ of mature and newly weaned dolphins, E is the enrichment of 3.4‰ for nitrogen and 1‰ for carbon assumed for a difference of one trophic level.

Analysis of tissues that have slower and faster turnover rates can identify whether an individual changed its diet or its feeding area over time (Tieszen *et al.*, 1983; Das *et al.*, 2000). Temporal variation was investigated by comparing skin and muscle isotopic compositions in animals for which both tissues were available. If the isotope ratio of skin was consistently higher or lower than muscle across all individuals within a season, size-class or area, this would suggest a change in the diet or feeding area over time. Chi-squared tests were used to assess whether there were any such patterns between the stable isotope ratios of skin and muscle. For this analysis, comparisons were conducted between seasons, areas and body size-classes. The reported body size of striped dolphins in the western Mediterranean is 90–120 cm for calves, 120–160 cm for juveniles, and 160–190 cm for sub-adults (Calzada *et al.*, 1997). Accordingly, and examining the smoothers resulting from the GAMs, size-classes were chosen as 80–130 cm for calves, 130–155 cm for juveniles, 155–200 cm for subadults and above 200 cm for adults. For each grouping, if a significant difference was found between both ratios, a second test was performed to investigate if the pattern was related to season, area and length.

Comparisons between prey and dolphin isotopic compositions were made within the same area (Gulf of Lion or Ligurian Sea). We used isotopic ratios measured in mature dolphins (>200 cm length). We calculated their isotopic composition and compared them to prey isotopic composition.

RESULTS

In the Mediterranean, adult and sub-adult striped dolphins (i.e. those larger than 155 cm length) exhibited an average $\delta^{13}C$ of −17.7 ± 0.6 in skin and −17.4 ± 0.4 in muscle, and an average $\delta^{15}N$ of 10.0 ± 0.9 in skin and 9.6 ± 0.7 in muscle (Table 1). In the Atlantic, the average $\delta^{13}C$ in skin and muscle were −17.5 ± 0.7 and −17.5 ± 0.3 respectively, and an average $\delta^{15}N$ of 12.1 ± 0.8 in skin and 10.9 ± 0.6 in muscle (Table 1).

Isotopic signatures of striped dolphins: statistical modelling results

VARIATION IN $\delta^{15}N$ VALUES

Of the five variables tested, only dolphin length and geographical area showed significant relationships with the nitrogen signature (length: $t = 8.86$, $P < 0.0001$; area: $t = 9.93$, $P < 0.0001$). The final model explained 59.2% of deviance in $\delta^{15}N$ values. In terms of the relationship with length, nitrogen isotope ratios decreased with increasing length for dolphins between 80 and 155 cm (Figure 2a). The curve inflects at 155 cm and $\delta^{15}N$ shows an increasing pattern for larger individuals, although this increase may reach an asymptote for dolphins larger than 200 cm. In terms of actual nitrogen isotope ratios, they decreased on average by 3.50‰ between dolphins of 80 and 155 cm, while they increase by 0.57‰ between 155 and 200 cm (Figure 2b).

In terms of areas of origin, Atlantic dolphin samples had a higher $\delta^{15}N$ (a general enrichment of more than 1‰) than dolphin samples from the Mediterranean Sea (Table 2; Figure 2b). There were no significant differences found amongst the five Mediterranean areas.

VARIATION IN $\delta^{13}C$ VALUES

Of the six variables included in the original model, only length had a significant effect on carbon isotope composition ($t = 8.48$, $P < 0.0001$). The final model explained 31.1% of deviance in $\delta^{13}C$. In terms of the relationship with body length, carbon isotope ratios increased for dolphins of lengths between 130 and 200 cm (Figure 3a), and the actual enrichment was 0.56‰ (Figure 3b).

Isotopic enrichment according to length

Based on the model, we estimated that dolphins of 155 cm typically have a $\delta^{15}N$ of 10.55‰ and a $\delta^{13}C$ of −17.83‰. Dolphins of 200 cm would exhibit a $\delta^{15}N$ of 11.12‰ and a $\delta^{13}C$ of −17.41‰. Mature dolphins would exhibit, therefore, an isotopic enrichment of 0.17 when inferred from nitrogen isotope ratios and an inferred enrichment of 0.41 for carbon, compared to smaller dolphins. The difference of enrichment between newly-weaned and mature dolphins is therefore twice as high for carbon as it is for nitrogen.

Variation in isotopic composition over time

VARIATION IN NITROGEN COMPOSITION

Nitrogen isotope ratios were higher in skin than in muscle. This did not vary with season ($\chi^2_3 = 0.87$, $P = 0.83$) (Table 3). However, it did vary with body length and, while the nitrogen isotope ratios were lower in skin than in muscle in dolphins between 80 and 130 cm, they were higher in skin than in muscle in dolphins over 130 cm ($\chi^2_3 = 11.73$, $P = 0.01$) (Table 3). In relation to area, nitrogen isotopes in skin were higher than in muscle in all areas except Corsica ($\chi^2_5 = 14.66$, $P = 0.01$) (Table 3).

VARIATION IN CARBON COMPOSITION

Carbon isotope ratios tended to be lower in skin than in muscle in all seasons except winter when the ratio was

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Table 1. Skin and muscle mean isotopic values (± SD) of adult and sub-adult striped dolphins (>155 cm length) from the Atlantic and the Mediterranean (with the five regions grouped and separated).

Area	**Skin**			**Muscle**		
	N	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**	**N**	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**
Atlantic	13	−17.5 ± 0.7	12.1 ± 0.8	21	−17.5 ± 0.3	10.9 ± 0.6
Mediterranean	24	−17.7 ± 0.6	10.0 ± 0.9	26	−17.4 ± 0.4	9.6 ± 0.7
Gulf of Lion	15	−17.5 ± 0.5	10.1 ± 1.1	16	−17.4 ± 0.5	9.8 ± 0.7
Provençal Basin	4	−17.7 ± 0.4	9.4 ± 0.4	6	−17.3 ± 0.4	9.5 ± 0.9
Ligurian Sea	1	−18.9	9.7	1	−17.8	9.1
Corsica	2	−18.1 ± 1.1	10.0 ± 0.3	1	−17.8	8.8
Offshore	2	−17.8 ± 0.5	10.6 ± 0.6	2	−17.6 ± 0.2	9.5 ± 0.2

higher in skin. This pattern was however not significant ($\chi^2_3 = 7.18$, $P = 0.07$) (Table 3). Carbon isotope ratios between skin and muscle did not vary significantly according to length-class ($\chi^2_3 = 1.37$, $P = 0.71$) (Table 3) nor according to area ($\chi^2_5 = 9.43$, $P = 0.09$) (Table 3).

Comparison with isotopic compositions of potential prey species

The average carbon isotopic ratio was −18.99 ± 0.57‰ for fish and −17.96 ± 0.45‰ for cephalopods (Table 4). The nitrogen isotopic ratios were 7.55 ± 0.71‰ and 8.87 ± 1.13‰ respectively. The nitrogen isotopic ratio seemed higher for larger prey, and this pattern was more obvious for cephalopods (Figure 4). For example, specimens of *Illex coindetii* measuring 27.5cm length were enriched by 1.6‰ in $\delta^{15}N$ compared to specimens half their length (14.9 cm). The average carbon and nitrogen signatures of mature dolphins were −17.22 ± 0.47‰ and 9.94 ± 0.94‰ respectively (N = 8). Nitrogen fractionation was 2.39‰ from fish to dolphins and 1.07‰ from cephalopods to dolphins. On average, carbon isotope ratios in dolphins were 1.77‰ greater than fish, and 0.74‰ greater than cephalopods (Figure 5).

DISCUSSION

Isotopic signatures of striped dolphins

VARIATION IN $\delta^{15}N$ VALUES: EFFECT OF BODY LENGTH

Variation in $\delta^{15}N$ suggests ontogenetic changes in striped dolphin diet according to their size. The decrease in nitrogen isotope signatures up to a body length of 155 cm is likely to be associated with animals changing from primarily feeding on milk to primarily feeding on live prey. Feeding on milk is known to increase N ratios above those typically found in mature animals of the same species (Steele & Daniel, 1978; Hobson *et al.*, 1997; Das *et al.*, 2003b; Knoff *et al.*, 2008; Fernandez *et al.*, 2011) and thus explains the higher nitrogen ratios found in small individuals. Our results suggest that weaning occurs at around 155 cm, as indicated by the point of inflection in the smoother for effect of length on nitrogen isotope ratio. In animals with body lengths >155 cm, nitrogen isotope ratios increased with body length. This pattern could be explained by larger individuals feeding on larger prey and/or changing the composition of their diet. Indeed, comparisons between the diets of striped dolphins of different size-groups in the Atlantic indicated that larger dolphins fed on larger prey (Ringelstein *et al.*, 2006). Moreover, higher

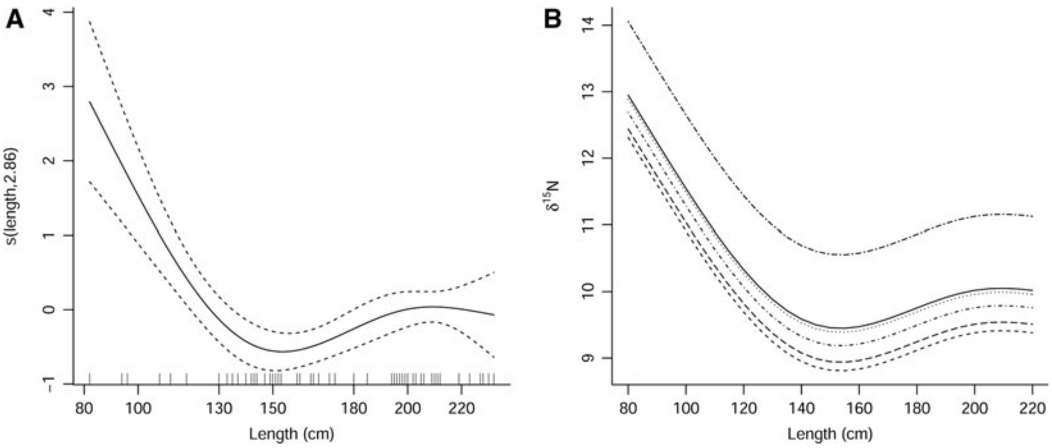

Fig. 2. Plots for nitrogen signature. (a) Effect of dolphin length on nitrogen signature. The solid line is the estimated smoother, the dashed line is the 95% confidence interval; (b) predicted nitrogen signature for muscle according to length and area, using a fitted generalized additive model. From top to bottom, the lines indicate samples from the Atlantic, Corsica, Ligurian Sea, Gulf of Lion, Offshore and Provençal Basin, respectively.

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Table 2. Comparison between nitrogen isotope ratios for dolphins from the Atlantic and Mediterranean: estimated parameters, standard error (SE), values of Student's *t* and probability (*P*)

	Estimate	SE	*t* value	*P* value
Atlantic—Gulf of Lion	−1.37	0.23	−5.82	<0.0001
Atlantic—Provençal Basin	−1.74	0.32	−5.46	<0.0001
Atlantic—Ligurian Sea	−1.16	0.56	−2.10	0.04
Atlantic—Corsica	−1.11	0.56	−1.97	0.05
Atlantic—Offshore	−1.61	0.40	−4.06	0.01

Table 3. Percentages of striped dolphins for which skin had higher or lower isotope value than muscle.

		N	δ^{15}N		δ^{13}C	
			$\delta_{skin} > \delta_{muscle}$	$\delta_{skin} < \delta_{muscle}$	$\delta_{skin} > \delta_{muscle}$	$\delta_{skin} < \delta_{muscle}$
Season	Spring	6	83.4	16.6	0	100
	Summer	15	80	20	46.7	53.3
	Autumn	21	85.7	14.3	33.3	66.7
	Winter	13	92.3	7.7	61.5	38.5
Size (cm)	80–130	7	42.9	57.1	42.9	57.1
	130–155	11	100	0	45.5	54.5
	155–200	16	87.5	12.5	43.8	56.2
	> 200	18	88.9	11.1	38.9	61.1
Area	Atlantic	17	94.1	5.9	58.8	41.2
	Gulf of Lion	21	90.5	9.5	33.3	66.7
	Provençal Basin	7	85.7	14.3	14.3	85.7
	Ligurian Sea	4	75	25	25	75
	Corsica	4	25	75	25	75
	Offshore	4	100	0	75	25

nitrogen values are typically found in larger prey, and this pattern has been shown for fish and cephalopods (Hooker *et al.*, 2001; Jennings *et al.*, 2002; Ruiz-Cooley *et al.*, 2004, 2010; Fernandez *et al.*, 2011). In the north-western Mediterranean, the composition of the diet was found to change according to the maturity of striped dolphins, with a higher proportion of fish in the diet of immatures (Astruc, 2005 unpublished results). As cephalopods were found to have consistently higher isotopic values than fish species (Figures 4 & 5; Table 4), higher nitrogen values are expected to be found in adult compared to younger individuals. Both suggestions are consistent with previous stomach contents analysis (Astruc, 2005 unpublished results; Ringelstein *et al.*, 2006) and are not necessarily mutually exclusive.

VARIATION IN δ^{13}C VALUES: EFFECT OF BODY LENGTH

Carbon isotope ratio increases (+0.56‰) with dolphin body length (between 130 and 200 cm length) and this enrichment could be explained by dolphins feeding at higher trophic levels or on larger prey, as suggested for the increase in nitrogen isotope ratios. However, the observed increase in the carbon isotope ratio before weaning (155 cm) is in apparent contradiction with the decreasing pattern for nitrogen. We suggest that a change in mother's rearing behaviour occurs during this period. Marine mammal calves are known not to follow the adults in their foraging dives, because of lower physiological capacity constraining their dive depth and duration (Slip, 1995; Noren *et al.*, 2002; Noren & Edwards, 2007). According to the species, the degree of dependence of the calves can also be relatively strong in the first months but decreases with time (Würsig & Clark, 1993; Noren & Edwards, 2007). If striped dolphins exhibit a similar pattern, the observed increase in carbon composition may be explained by a change in mothers' feeding behaviour: once calves reach around 130 cm length they can be left at the surface allowing adults to undertake longer and deeper dives to feed on prey in food webs with higher basal carbon isotope ratios but that do not differ in nitrogen isotope ratios.

VARIATION IN δ^{15}N VALUES: EFFECT OF AREA

Nitrogen isotopic composition revealed some geographical differences in diet according to the study areas. Atlantic dolphins had nitrogen isotope ratios that were on average 1‰ higher than recorded in Mediterranean dolphins. Intrinsic dietary differences (Astruc, 2005 unpublished results; Ringelstein *et al.*, 2006) could explain the differences observed in cetacean tissues between the two regions. Geographical variation in the δ^{15}N values has been shown for various marine consumers (Walker *et al.*, 1999; Takai *et al.*, 2000; Ruiz-Cooley *et al.*, 2004, 2010; Marcoux *et al.*, 2007;

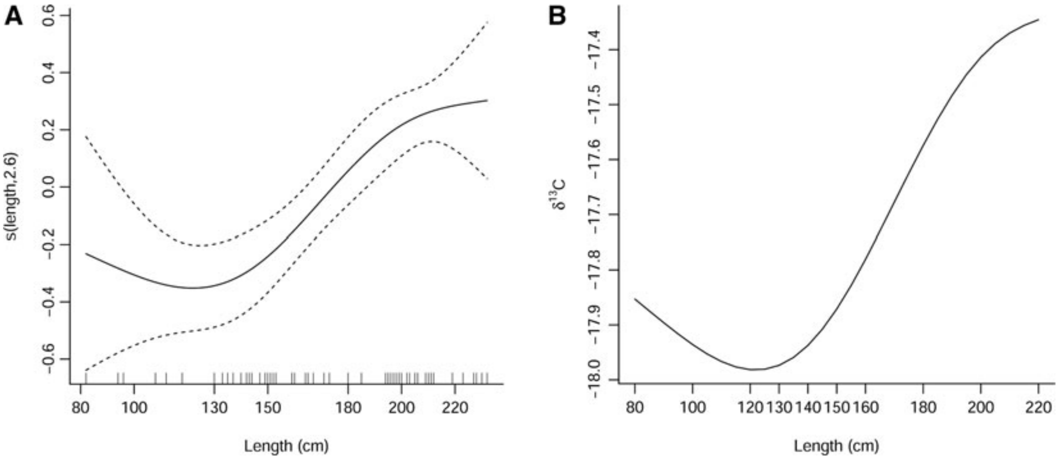

Fig. 3. Plots for carbon signature. (a) Effect of dolphin length on carbon signature. The solid line is the estimated smoother, the dashed line is the 95% confidence interval; (b) predicted carbon signature for muscle according to length using a fitted generalized additive model.

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Table 4. Prey samples: species, area of origin (LS, Ligurian Sea; BA, Baie des Anges; M, Monaco; GL, Gulf of Lion; S, Sète; na, unknown location), number of items (N), carbon and nitrogen isotopic ratios (mean ± SD). The length from the tip of the snout to the tip of the longer lobe of the caudal fin was measured for fish and the dorsal mantle length was measured for squids.

Species	Area of origin	Date of capture	N	Length (cm)	δ^{13}C (‰)	δ^{15}N (‰)
Clupeidae						
Sardina pilchardus	GL, S	2008	3	16.8 ± 1.1	−18.4 ± 0.1	8.0 ± 0.1
	GL, na	2004	2	na	−19.0 ± 0.0	8.0 ± 0.3
	GL, S + na	2004/2008	5	na	−18.6 ± 0.4	8.0 ± 0.2
Sardinella aurita	LS, BA	2007	2	24.3 ± 2.5	−18.7 ± 0.3	7.4 ± 0.4
Engraulidae						
Engraulis encrasicolus	GL, S	2008	4	12.5 ± 0.6	−18.7 ± 0.3	8.0 ± 0.5
	GL, na	2004	3	na	−20.4 ± 0.2	6.4 ± 0.2
	GL, S + na	2004/2008	7	na	−19.4 ± 0.9	7.3 ± 0.9
Gadidae						
Micromesistius poutassou	GL, na	2004	2	na	−18.8 ± 0.1	6.2 ± 0.8
Merlucciidae						
Merluccius merluccius	GL, S	2008	3	15.9 ± 1.5	−19.1 ± 0.1	7.4 ± 0.1
Sparidae						
Boops boops	LS, BA	2007	7	16.8 ± 1.4 †	−18.9 ± 0.2	7.9 ± 0.2
Loliginidae						
Loligo vulgaris	GL, na	2004	4	15.8 ± 3.8	−17.7 ± 0.2	9.9 ± 0.5
Ommastrephidae						
Illex coindetii	GL, S	2008	2	27.5 ± 2.8	−18.7 ± 0.0	8.3 ± 0.2
	GL, na	2004	2	14.9 ± 1.8	−18.1 ± 0.1	9.9 ± 0.2
	GL, S + na	2004/2008	4	21.2 ± 7.6	−18.4 ± 0.3	9.1 ± 1.0
Sepiidae						
Sepia officinalis	LS, M	1999	3	11.6 ± 0.8	−17.7 ± 0.6	8.6 ± 0.8
Sepia orbignyana	LS, M	1999	3	5.6 ± 0.7	−18.0 ± 0.1	7.4 ± 0.2

†, mean and standard deviation on 6 items.

Bentaleb *et al.*, unpublished results). Difference in the baseline δ^{15}N signatures of the Mediterranean and Atlantic food webs could therefore account for the difference found in dolphin tissues. Isotope data from dolphins revealed no population structuring within the north-western Mediterranean, suggesting that striped dolphin diet might be isotopically homogeneous across this area. This may reflect a wide foraging range: if individual dolphins move between several study areas this would tend to mask any geographical differences in isotopic composition. Absence of differences could also be observed if dolphins fed in distinct feeding grounds but on prey with similar isotopic values, therefore exhibiting a similar overall isotopic composition. Regional differences in diets are reported from stomach content analysis (Astruc, 2005 unpublished results) and would therefore support the second hypothesis. Differences in isotopic signature could exist between stranded and by-caught dolphins, as suggested for other cetaceans (Gannes *et al.*, 1998;

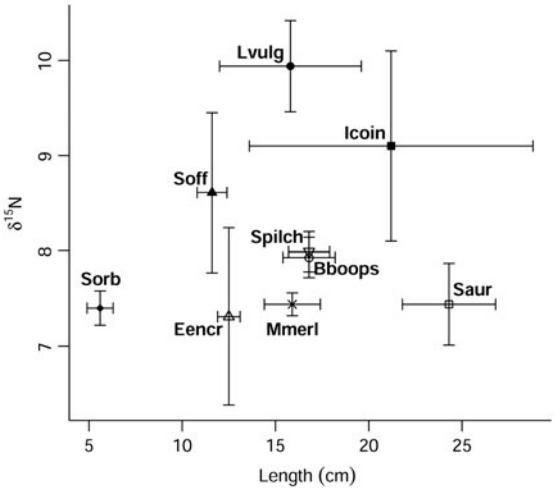

Fig. 4. Nitrogen isotopic ratios according to the length (cm) of fish (open symbols: *Boops boops* (Bboops), *Engraulis encrasicolus* (Eencr), *Merluccius merluccius* (Mmerl), *Sardinella aurita* (Saur), *Sardina pilchardus* (Spilch)) and cephalopods (filled symbols: *S. orbignyana* (Sorb), *Sepia officinalis* (Soff), *Loligo vulgaris* (Lvulg), *Illex coindetii* (Icoin)) from Gulf of Lion and Ligurian Sea. Mean ± standard deviation.

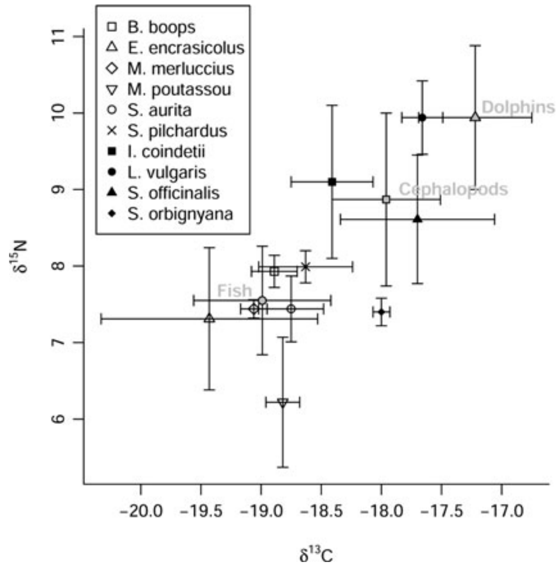

Fig. 5. Carbon and nitrogen isotopic ratios for fish, cephalopods, and mature striped dolphins from the Gulf of Lion and Ligurian Sea. Mean ± standard deviation.

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Ruiz-Cooley *et al.*, 2004). Starvation or disease can affect dolphin metabolism before the stranding (Markussen, 1995; Boily & Lavigne, 1997). But again, the absence of differences within the five Mediterranean areas, suggest that by-caught dolphins analysed in the present study exhibit a similar isotopic composition to stranded dolphins.

Isotopic enrichment according to the length

Mature dolphins present higher isotopic values compared to newly-weaned dolphins. However, carbon ratios increased more rapidly than nitrogen ratios, suggesting that the increase in carbon isotope ratios is not driven by solely trophic level enrichment. Polunin *et al.* (2001) found that $\delta^{13}C$ of some fish species increases from depths of 200 to 1800 m. Striped dolphins are believed to dive to depths of up to 700 m (Archer, 2000). Although this range reflects physiological diving capacity rather than the usual foraging depth, striped dolphins are known to feed on deep water prey (especially cephalopods) (Astruc, 2005 unpublished results). The increase in $\delta^{13}C$ with body length could then be explained by increased feeding on cephalopods in older animals as suggested by stomach content analyses (Astruc, 2005 unpublished results).

Variation in isotopic composition over time

VARIATION IN NITROGEN COMPOSITION

Enrichment in the composition of skin relative to muscle could indicate that the most recent diets are always more enriched than the less recent ones. However, this pattern is observed for weaned dolphins in all seasons and most areas, which makes the explanation highly implausible. The difference in nitrogen isotope ratios between tissues may therefore be caused by a higher fractionation in skin compared to the muscle, as such differences are reported for other tissues (DeNiro & Epstein, 1978; Tieszen *et al.*, 1983; Hobson *et al.*, 1996; Pinnegar & Polunin, 1999). Therefore, to answer ecological questions, comparisons of isotopic composition should be undertaken on the same tissue. Unfortunately this means that analyses of isotope ratios in different tissues of the same animal cannot provide a reliable indication of diet over different time scales, unless biases due to differences in fractionation between tissues are known.

VARIATION IN CARBON COMPOSITION

Enrichment and depletion in carbon isotopic composition of skin relative to muscle has been observed according to the season. Skin showed lower carbon isotope ratios than muscle from spring to autumn, while it showed higher ratios in winter. Although a larger sample size is needed to confirm this pattern, this observation suggests some possible seasonal movements outside the study areas. Migration of the population between the central Spanish Mediterranean Sea (end of autumn–winter) to the Tyrrhenian Sea (summer) has been suggested by Laran & Drouot-Dulau (2007) and may explain the changes in the composition of the diet observed in our results.

Comparison with isotopic composition of potential prey species

Without a sufficient amount of prey species, covering each study area and a wide spectrum of size-classes, we could not examine the variation of isotopic signatures for prey as we did for dolphins. However, comparison of isotopic composition in dolphins and some prey species enable us to give some insights into prey–predator enrichment. We found a general low enrichment rate: nitrogen fractionation was 2.39‰ from fish to dolphins and 1.07‰ from cephalopods to dolphins, and carbon fractionation was 1.77‰ from fish to dolphins and 0.74‰ from cephalopods to dolphins. The reported enrichment values of 3.4‰ for nitrogen (DeNiro & Epstein, 1981; Minawaga & Wada, 1984; Pinnegar *et al.*, 2003) and 1‰ for carbon (DeNiro & Epstein, 1978) from one trophic level to the next are averages and values from different predator–prey pairings can vary very widely (Minawaga & Wada, 1984; Wada *et al.*, 1987; Hobson & Welch, 1992; Burns *et al.*, 1998; Ruiz-Cooley *et al.*, 2004). Lower isotopic fractionations have often been found (Ostrom *et al.*, 1993; Abend & Smith, 1997; Vander Zanden & Rasmussen, 2001). The enrichment found between putative prey species and dolphins in our study falls within the range of values reported in the literature. However, it could also suggest that dolphins feed on prey species other than those sampled in this study (Bode *et al.*, 2006; Bentaleb *et al.*, unpublished results) as our sampling of potential prey species was not exhaustive. With an apparently opportunistic species like striped dolphins, we can expect a large range of isotopic signatures to occur.

CONCLUSION

This study is the first to explore feeding ecology of striped dolphins in the north-western Mediterranean Sea through stable isotope analyses. Our results give insights that are complementary to previous work based on stomach contents analysis.

Variations in nitrogen isotope ratios allowed confirmation that weaning of calves occur around 155 cm length. Enrichment in nitrogen ratio occurs post-weaning, probably because larger dolphins feed on larger and on higher trophic level prey. The carbon isotope ratio increases with length as well, but this seems not to be driven solely by trophic level enrichment. Dolphins are suspected to forage on deep water prey and in offshore waters.

Comparisons of the nitrogen isotope ratios of skin and muscle revealed that skin presents a higher fractionation, and highlights the need to carefully interpret results of comparisons between samples from different tissues. It cannot be assumed that differences reflect only the timescale of turnover, so inferences about recent feeding versus feeding over a long timescale may not be possible.

A geographical difference in isotopic signatures of striped dolphins between the Atlantic and the Mediterranean was observed, probably because of fundamentally different diets and different isotopic compositions at the base of the food chain. However, no regional population structure based on feeding ecology emerged within the Mediterranean. The small sample size may explain the absence of difference. Another possible hypothesis is that dolphins move between

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the study areas and therefore exhibit a homogeneous isotopic composition. However, absence of differences in isotopic composition could also be explained if dolphins forage on similar trophic level prey in distinct feeding grounds. Regional differences in diets are reported from stomach content analysis (Astruc, 2005 unpublished results). Stable isotope analyses have their own limitations, and studies on feeding ecology should ideally integrate both stomach content and stable isotope analyses.

Seasonal differences in carbon isotope signatures could suggest seasonal movements, and this hypothesis would be consistent with previous results from analysis of distribution. However, more samples are required to confirm our results. Finally, comparisons between potential prey and striped dolphins suggest a low enrichment rate.

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