

Isotopic variation in delphinids from the subtropical western South Atlantic

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*A dual stable isotope approach ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) was used to investigate inter- and intra-specific variations in feeding ecology and habitat use of 7 delphinids from coastal/estuarine, continental shelf and offshore marine environments from southern Brazil: *Tursiops sp.*, *Orcinus orca*, *Stenella frontalis*, *Steno bredanensis*, *Delphinus delphis*, *Pseudorca crassidens* and *Lagenodelphis hosei*. Teeth from 50 specimens acquired from stranded animals were analysed in this study. *Tursiops sp.* and *O. orca* are the most coastal species, and had the highest $\delta^{13}\text{C}$ values followed by the continental shelf species *S. frontalis*, *S. bredanensis* and *D. delphis*. *Lagenodelphis hosei* showed the lowest $\delta^{13}\text{C}$ value, demonstrating its typical offshore habitat. One group of *P. crassidens* had the lowest $\delta^{15}\text{N}$ values, indicating their low trophic level feeding habit while two specimens of the same species showed the highest mean nitrogen isotope value. This first study on stable isotope values of delphinids from southern Brazil provides substantial new information about the trophic ecology, habitat use and feeding environments of these animals.*

Keywords: delphinids, stable isotopes, trophic level, habitat use, western South Atlantic

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INTRODUCTION

Delphinidae is the most successful and diverse among all Cetartiodactyla families which occupy a wide variety of ecosystems (LeDuc, 2002), including freshwater (e.g. tucuxi, *Sotalia fluviatilis*), shallow coastal (e.g. short-beaked common dolphin, *Delphinus delphis*), and deep pelagic waters (e.g. Fraser's dolphin, *Lagenodelphis hosei*) in tropical and subpolar environments. Some species have restricted distribution while others are broadly distributed. Others have evolved by adapting to specific niches, generating different ecotypes such as the coastal and offshore bottlenose dolphin (*Tursiops truncatus*) (Segura *et al.*, 2006) and killer whales (*Orcinus orca*) (Ford, 2002).

A rich diversity in delphinids can be found in waters off the coast of Brazil (Bastida *et al.*, 2007). The continental shelf and slope waters in southern Brazil are influenced by the Subtropical Convergence and represent a biogeographical transition zone between Patagonian temperate and Brazilian tropical waters. In the neritic zone, cold and warm water circulation and upwelling processes (Castello *et al.*, 1997; Garcia, 1998, Piola *et al.*, 2000; Muelbert *et al.*, 2008) influence productivity that can be considered moderate to high (Odebrecht & Garcia, 1997).

Trophic relationships and habitat use are of key importance to the understanding, management and conservation of cetacean populations and their position in complex marine food webs. In southern Brazil, studies on the feeding

ecology and habitat use of delphinids have typically focused on two coastal species: the Guiana dolphin (*Sotalia guianensis*) (e.g. Flores & Bazzalo, 2004; Oshima *et al.*, 2010) and the bottlenose dolphin (e.g. Simões-Lopes & Fabian, 1999; Fruet *et al.*, 2011). Information on the trophic ecology of the remaining delphinid species that inhabit the continental shelf or offshore waters is scarce or non-existent, mostly due to their often difficult to work in habitats.

Carcasses washed ashore provide valuable biological material from species from those habitats. Stomach content analysis, for example, can yield direct information on the diet and indirect insight on the feeding environment (Barros & Clarke, 2009). However, this information is often biased owing to different digestion rates of prey, overestimating, for example, the importance of prey with chitinous structures (e.g. cephalopod beaks and crustaceans) relative to fish, whose otoliths are rapidly digested by gastric acids (Jobling & Breiby, 1986; Santos *et al.*, 2001). Other limitations of analyses of stomach contents are due to the secondary ingestion of prey, which is prey found that was in the digestive tract of the predator's prey, as well as the short feeding time interval integrated by this kind of study, giving only information on recent feeding (Hobson *et al.*, 1994; Dehn *et al.*, 2006). These latter limitations can be reduced by increasing sample sizes and ensuring samples represent the appropriate temporal and spatial scales.

Fortunately, other complementary methods, such as naturally occurring stable isotopes of key elements and fatty acids (e.g. Herman *et al.*, 2005; Krahn *et al.*, 2007), are now available for studies on feeding ecology and habitat preference in aquatic vertebrates. Stable isotope values in animal tissues reflect those in the food webs where they feed (Rubenstein & Hobson, 2004; Graham *et al.*, 2010) and are useful particularly for determining trophic level, identifying major food sources, and

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assessing foraging habitats (e.g. Das *et al.*, 2003; Krahn *et al.*, 2007; Pinela *et al.*, 2010; Ricchialdelli *et al.*, 2010). Stable isotope ratios of nitrogen ($^{15}\text{N}/^{14}\text{N}$) and to a lesser extent carbon ($^{13}\text{C}/^{12}\text{C}$) show a stepwise enrichment with increasing trophic level in the marine environment (De Niro & Epstein, 1978, 1981). Indeed, nitrogen isotopes values change in a predictable fashion between trophic levels, owing to the preferential excretion of the light isotope (Caut *et al.*, 2009), and so reflect trophic position (De Niro & Epstein, 1981; Cabana & Rasmussen, 1996; McCutchan *et al.*, 2003). Some studies with captive (Hilderbrand *et al.*, 1996; Hobson *et al.*, 1996; Lesage *et al.*, 2002) and free-ranging (Newsome *et al.*, 2010) mammals showed that this increment varies among tissues, species, developmental stage and/or body condition. However, an approximate enrichment of 3.4‰ per trophic level is generally accepted (Post, 2002). Similar to ^{15}N , ^{13}C content also increases up trophic levels, although an increase of only roughly 1‰ is typically observed (De Niro & Epstein, 1978; Peterson & Fry, 1987). Therefore, predators' carbon isotope values are used as an indicator of the sources at the base of the food web where they feed (Hobson, 1999; Graham *et al.*, 2010).

An important aspect to be considered when using stable isotopes is that the turnover rate within a tissue is based on its metabolic rate. Therefore, diet information may be determined over a time frame that varies from a few days (e.g. blood plasma and liver), months (e.g. red blood cells or muscle) to years (e.g. whale baleen or teeth) (Walker & Macko, 1999; Kelly, 2000). Teeth are a particularly informative tissue for tracking the diet of delphinids over their lifetimes because they provide a permanent dietary record for an individual, as, under normal conditions, growth layers in teeth do not resorb or modify (Walker *et al.*, 1999; Walker & Macko, 1999; Niño-Torres *et al.*, 2006).

In this study a first estimation of carbon and nitrogen isotopes values in teeth of delphinids from southern Brazil, in the subtropical western South Atlantic, is presented. These stable isotope profiles were used to investigate inter- and intra-specific variations in feeding ecology and habitat use of seven species from coastal/estuarine, continental shelf and offshore marine environments.

MATERIALS AND METHODS

Study site and sampling

Fifty specimens of seven species (Table 1) found washed ashore during systematic beach surveys conducted along the

southern coast of Rio Grande do Sul State (RS), Brazil, (1993 to 2009) were used for this study (Figure 1). Teeth were extracted from the middle upper or lower jaw, cleaned and stored dry in the Laboratório de Tartarugas e Mamíferos Marinhos (LTMM-IO-FURG) collection.

Analysis of isotope compositions

Stable isotope analysis of teeth was performed following the protocol described in Walker & Macko (1999). Teeth were dried for 3–4 days in a 60°C oven and cleaned of outer soft tissue with a carbide burr attached to a drill. A low speed saw with a diamond-embedded blade was used to cut through the centre of the tooth in the longitudinal buccal–lingual axis in order to expose the growth layer groups (GLGs; Perrin & Myrick, 1980). Exposed dentine was sampled with a small drill bit, taking care that all GLGs were sampled so that the resulting powder would represent the entire life of the individual. The powder obtained was acidified with 30% hydrochloric acid (HCl) to remove biogenic carbonates, which could alter the organic $\delta^{13}\text{C}$ measurements, and then dried again for 1 hour in a 60°C oven. The goal of preparation is the conversion of the organic samples into gases of suitable purity that can then be analysed by the mass spectrometer. Samples of approximately 5 mg of residual acidified tooth were used for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis using an elemental analyser (EA) connected to a Micromass Optima Isotope Ratio Mass Spectrometer (IRMS; GV Instruments, Manchester, UK). Natural abundance of stable isotope ratios ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$) is expressed in a delta notation (δ) as per mil variations (‰) when compared with international standards (e.g. Pee Dee Belemnite (PDB) for carbon and atmospheric N_2 , for nitrogen). Results were expressed as:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] * 1000$$

where R_{sample} and R_{standard} are the $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ ratios of the sample and standard, respectively.

Data analysis

All results are presented as the mean \pm SD. Data were tested for normality and homogeneity of the variances using the Kolmogorov–Smirnov test and Levene's test, respectively. Comparison of isotopes values among species were conducted using 1-way analysis of variance (ANOVA) techniques followed by a Tukey's honestly significant difference (HSD) test, when a significant difference was found. The null hypothesis of no differences was rejected if $P < 0.05$. In addition,

Table 1. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values \pm SD (‰) of teeth of delphinids found washed ashore along the southern coast of Brazil.

Species	$\delta^{13}\text{C}$	Min	Max	$\delta^{15}\text{N}$	Min	Max	N
Bottlenose dolphin (BD) <i>Tursiops</i> sp.	-10.6 (\pm 0.51)	-12.0	-9.5	17.6 (\pm 0.93)	15.5	19.3	24
Killer whale (KW) <i>Orcinus orca</i>	-10.5 (\pm 0.23)	-10.6	-10.3	17.7 (\pm 0.05)	17.6	17.7	2
Short-beaked common dolphin (SBCD) <i>Delphinus delphis</i>	-11.7 (\pm 0.32)	-12.1	-11.4	16.6 (\pm 0.59)	15.7	17.1	4
Rough-toothed dolphin (RTD) <i>Steno bredanensis</i>	-12.3 (\pm 1.18)	-14.3	-11.5	16.7 (\pm 0.82)	15.6	17.8	5
Atlantic spotted dolphin (ASD) <i>Stenella frontalis</i>	-11.9 (\pm 0.36)	-12.4	-11.5	16.6 (\pm 0.91)	15.6	17.9	5
False killer whale A (FKW A) <i>Pseudorca crassidens</i>	-12.0 (\pm 1.65)	-13.1	-10.8	19.2 (\pm 0.25)	19.0	19.4	2
False killer whale B (FKW B) <i>Pseudorca crassidens</i>	-12.2 (\pm 0.20)	-12.5	-11.9	11.2 (\pm 0.62)	10.6	12.3	6
Fraser's dolphin (FD) <i>Lagenodelphis hosei</i>	-12.8	-	-	14.9	-	-	1

Min, minimum; Max, maximum.

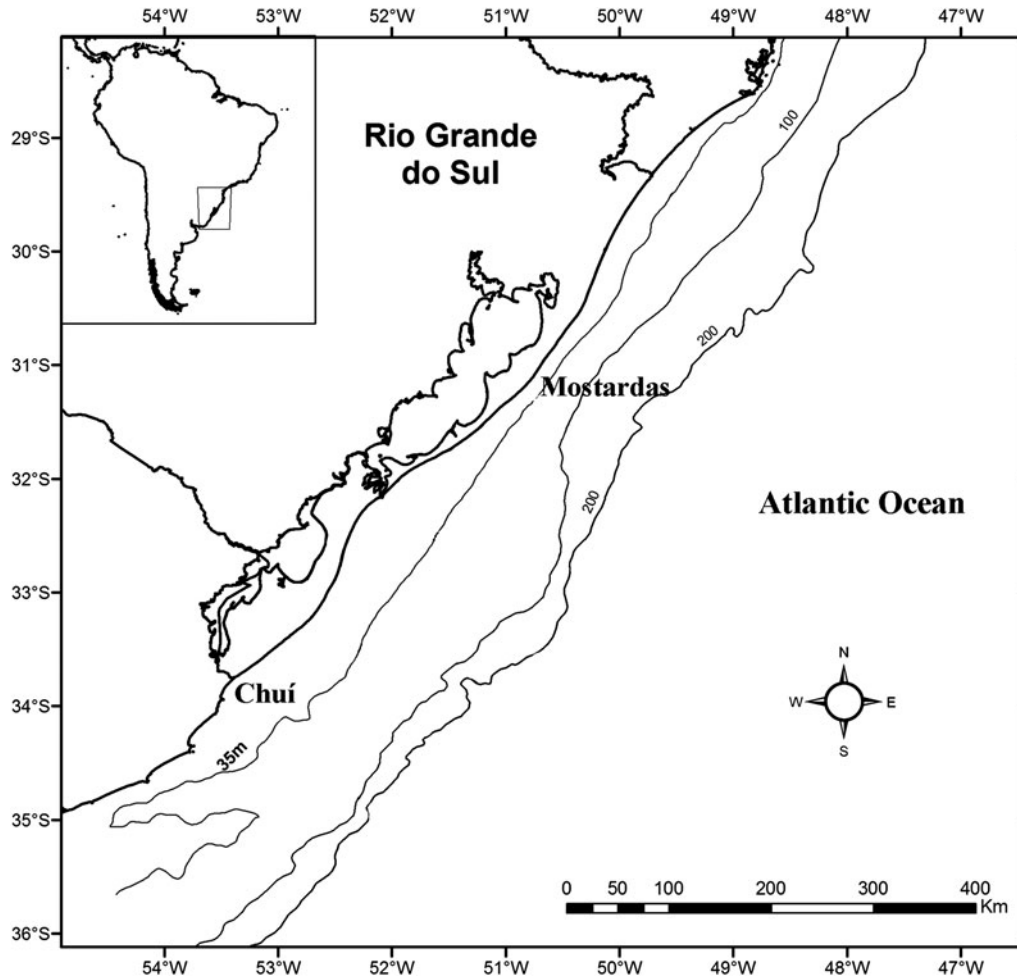


Fig. 1. Study area, western South Atlantic, southern Brazil. Northern and southern limits of the region surveyed for the collection of stranded delphinids are shown (Mostardas and Chui).

a cluster analysis (Euclidean distances, complete linkage method) based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ mean values of each species was used for the detection of isotope groupings.

RESULTS

Carbon and nitrogen isotope compositions differed significantly among species (ANOVA, $F_{6,41} = 10.78$; $P < 0.001$ and $F_{6,41} = 49.99$; $P < 0.001$, for carbon and nitrogen isotopes, respectively) (Tables 1 & 2; Figure 3). Data from the Fraser’s dolphin ($N = 1$) could not be statistically tested.

One killer whale had the lowest $\delta^{13}\text{C}$ found in our samples (-20‰) and also a low $\delta^{15}\text{N}$ value (10.5‰) (Figure 2). This 396 cm-long specimen was a very emaciated young female found stranded alive with the stomach full of oceanic salps (*Iasis zonaria*) and other unidentified planktonic invertebrates. This was considered atypical, thus, this animal was excluded from statistical analyses.

Some species showed considerable intraspecific variation, either in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$, however, in those animals presenting extreme values nothing atypical was detected when found washed ashore, as was the case of the killer whale mentioned above. Therefore, their values were considered in the range of the normal distribution of isotopic values for the species.

Carbon isotope values for rough-toothed dolphins ranged from -14.3‰ to -11.5‰ and those for false killer whales were from -13.1‰ to -10.8‰ (Table 1; Figures 2 & 3). Furthermore, values of $\delta^{15}\text{N}$ for the latter species revealed a bimodal distribution, with two specimens with high values (19.4‰ and 19.0‰) and the remainder with a mean $\delta^{15}\text{N}$ of 12.2‰ . The latter included animals from a single mass stranding while the ^{15}N -enriched individuals were found washed ashore alone. There is no stock information available

Table 2. Results of the Tukey’s honestly significant difference *post hoc* test for multiple comparisons of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from teeth of delphinids found washed ashore along the southern coast of Brazil (see Table 1 for codes). Carbon isotope ($\delta^{13}\text{C}$) P values are reported below the diagonal and those for nitrogen isotopes ($\delta^{15}\text{N}$) are reported above the diagonal. Significant P values (<0.05) are highlighted in bold.

	KW	BD	FKW A	SBCD	ASD	RTD	FKW B
KW		1.00	0.54	0.71	0.72	0.83	0.00
BD	1.00		0.14	0.29	0.24	0.41	0.00
FKW A	0.22	0.07		0.01	0.01	0.02	0.00
SBCD	0.23	0.03	1.00		1.00	1.00	0.00
ASD	0.09	0.00	1.00	1.00		1.00	0.00
RTD	0.02	0.00	1.00	0.88	0.98		0.00
FKW B	0.03	0.00	1.00	0.94	1.00	1.00	

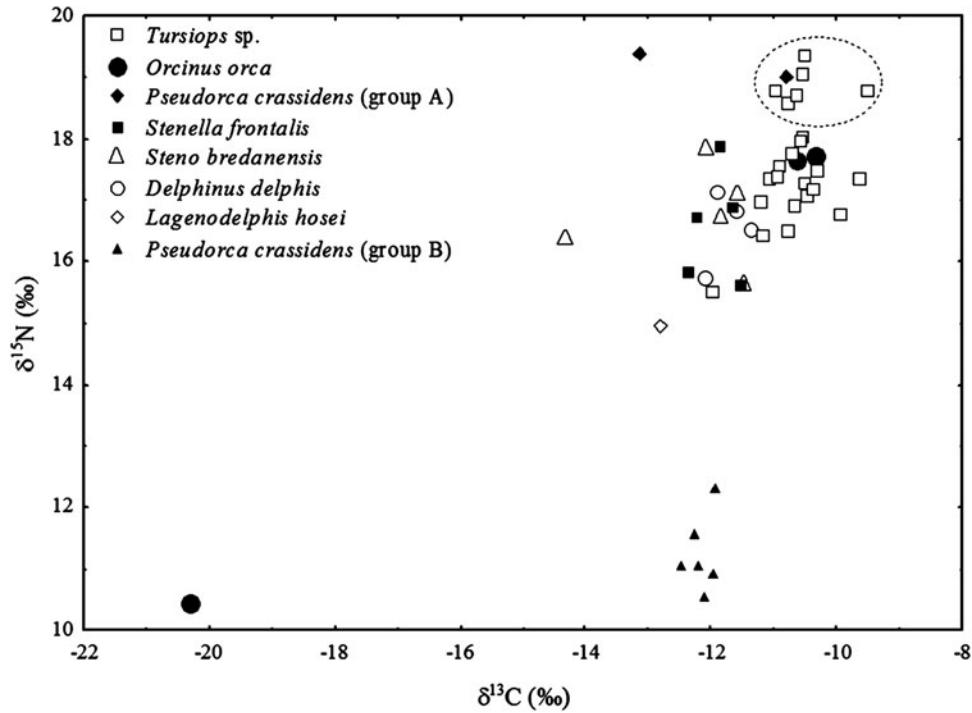


Fig. 2. Teeth $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in teeth of delphinids found stranded along the southern coast of Brazil. Dashed line circle indicates ^{15}N -enriched bottlenose dolphins, *Tursiops* sp.

for this species in the region thus we cannot infer if they belong to different ecotypes/stocks. However we treated them as two different groups (A and B), as their significantly different $\delta^{15}\text{N}$ values (Student's *t*-test, $P < 0.001$) suggest different feeding habits. The ^{15}N -enriched group (false killer whale A) was significantly different from the other species

(Tukey's HSD test, $P < 0.01$), with the exception of the bottlenose dolphin (Tukey's HSD test, $P = 0.14$) and the killer whale (Tukey's HSD test, $P = 0.54$). $\delta^{15}\text{N}$ values of the second group (false killer whale B) were significantly different from all dolphin species (Tukey's HSD test, $P < 0.001$). As for $\delta^{13}\text{C}$, group A did not significantly differ from the remainder

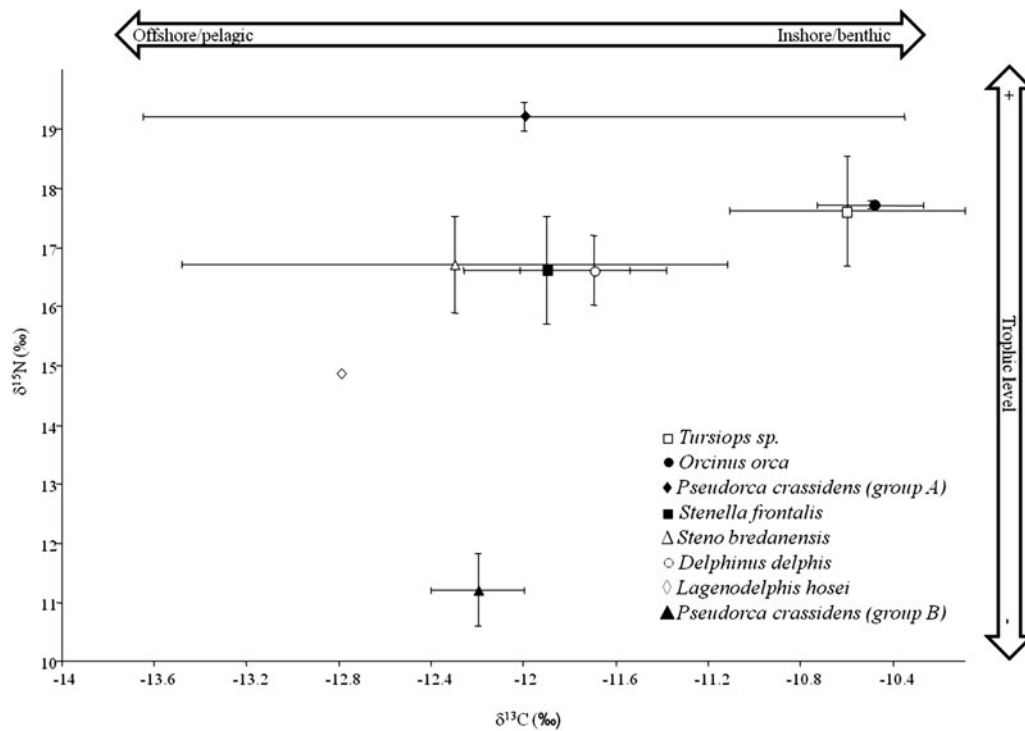


Fig. 3. Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for teeth dentine of delphinids from southern Brazil.

species (Tukey’s HSD test, $P > 0.05$), while group B was significantly different from bottlenose dolphins and killer whales (Tukey’s HSD test, $P < 0.01$).

Six bottlenose dolphins were more enriched in ^{15}N (4 males, one female and one individual of unknown sex) (Figure 2). The mean $\delta^{15}\text{N}$ of these animals was 18.9‰, being 1.6‰ more enriched than the lower $\delta^{15}\text{N}$ group (17.2‰). Their $\delta^{15}\text{N}$ values were statistically different (t -test, $P < 0.0001$) but their carbon stable isotopes values were not different (t -test, $P = 0.51$), averaging -10.5‰ for the group with enriched nitrogen signatures, and -10.6‰ for the rest of the dolphins. Finally, one bottlenose dolphin showed extremely different and depleted carbon and nitrogen signals ($\delta^{13}\text{C} = -12.0\text{‰}$ and $\delta^{15}\text{N} = 15.5\text{‰}$).

Cluster analysis of stable isotope values for carbon and nitrogen defined one group with high trophic level predators leaving the group B of false killer whales (low trophic level predator) on a separate branch. Within the cluster of high trophic level predators, the oceanic Fraser’s dolphin was separated from the continental shelf and coastal grouping, with the latter clustered together at a lower distance (Figure 4).

DISCUSSION

Carbon and nitrogen stable isotope values in teeth dentine of delphinids from southern Brazil reflected different trophic levels and/or feeding environments respectively. An offshore–inshore trend of increasing $\delta^{13}\text{C}$ values was observed, which is in agreement with the preferred habitat of the species analysed here. Indeed, carbon isotope values varied from highly ^{13}C enriched values found in coastal species, such as bottlenose dolphins ($\delta^{13}\text{C} = -10.6\text{‰}$) to more depleted signals such as the carbon isotope ratio of the

Fraser’s dolphin ($\delta^{13}\text{C} = -12.8\text{‰}$), a typical species from deep pelagic environments. Similarly, previous studies revealed a longitudinal trend in marine environments, where nearshore, benthos linked food webs are more ^{13}C enriched compared to more offshore, pelagic food webs (France, 1995; Burton & Koch, 1999; Takai *et al.*, 2000; Clementz & Koch, 2001; Lesage *et al.*, 2001; Barros *et al.*, 2010; Pinela *et al.*, 2010; Riccialdelli *et al.*, 2010) which is probably a reflection of a gradient of decreasing macrophyte influence (Hill *et al.*, 2006). Indeed, phytoplankton have lower $\delta^{13}\text{C}$ values than many inshore plants (e.g. seagrasses, kelp forests and marsh plants), making inshore carbon sources able to be distinguished from more pelagic sources (Fry & Sherr, 1984; Hobson *et al.*, 1994; Clementz & Koch, 2001).

Bottlenose dolphins and killer whales had the highest $\delta^{13}\text{C}$ values, reflecting their coastal feeding habits (Table 1). Furthermore, their $\delta^{15}\text{N}$ values also overlap, suggesting a similar trophic level for these two coastal predators. In southern Brazil, coastal bottlenose dolphins form small resident populations usually associated with estuaries and river mouths (Castello & Pinedo, 1977; Simões-Lopes & Fabian, 1999). A resident population of 84–86 animals (Dalla Rosa, 1999; Fruet *et al.*, 2011) inhabits the Patos Lagoon estuary and its adjacent coastal areas. Studies of the diet of bottlenose dolphins from RS based on stomach content analysis confirmed a coastal feeding habit with the white croaker (*Micropogonias furnieri*), the cutlass fish (*Trichiurus lepturus*) and the drum (*Paralichthys brasiliensis*) being the most important prey (Pinedo, 1982; Mehsen *et al.*, 2005). Nitrogen isotope values were also high. As noted above, due to the trophic enrichment in ^{15}N through the food chain, a high $\delta^{15}\text{N}$ is expected for this high-trophic-level predator. Two groups of bottlenose dolphins differing in their $\delta^{15}\text{N}$

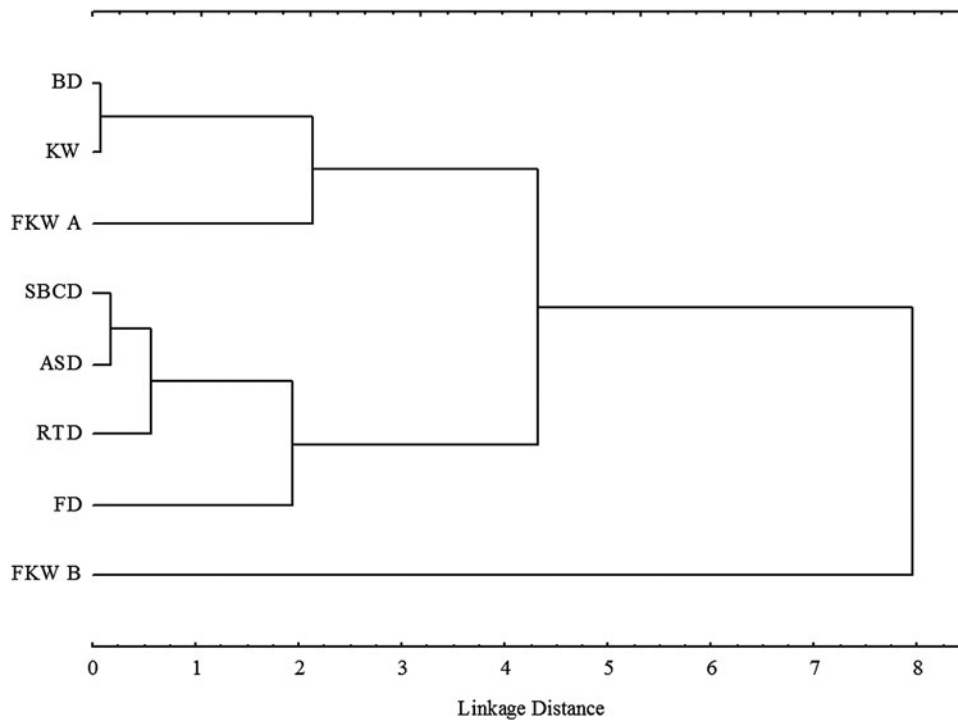


Fig. 4. Tree diagram of delphinids from southern Brazil resulted from cluster analysis of stable isotope ratios of carbon and nitrogen in teeth (see Table 1 for codes).

values were identified (Figure 2), which can be interpreted as a resource partitioning that may be occurring among bottlenose dolphins from southern Brazil. The observed mean values of the ^{15}N -enriched group ($\delta^{15}\text{N} = 18.9\text{‰}$) and the ^{15}N -depleted group (17.2‰) are higher and similar to, respectively, than values found for teeth of bottlenose dolphins from the western North Atlantic coast ($\delta^{15}\text{N} = 16.8\text{‰}$, Walker *et al.*, 1999; $\delta^{15}\text{N} = 17.6$ and 16.8‰ for the inner and outer part of the tooth dentine, Knoff *et al.*, 2008) and $\sim 5\text{--}7\text{‰}$ higher than values found by Barros *et al.* (2010) in Sarasota Bay ($\delta^{15}\text{N} = 11.9\text{‰}$) and the Gulf of Mexico ($\delta^{15}\text{N} = 12.7\text{‰}$). The latter used a different methodology for preparing the samples, centrifuging the dentine powder after demineralization, to separate collagen from non-collagenous proteins, and performing a lipid extraction. However, lipid extraction has a small influence in $\delta^{15}\text{N}$ by introducing an average fractionation of about 0.25‰ (Post *et al.*, 2007). Therefore, regional variations in diet/food web structure and/or nitrogen isotopes at the base of the food webs are likely to be the cause of the observed differences. Moreover, Abreu *et al.* (2006) reported high values of $\delta^{15}\text{N}$ inside the Patos Lagoon estuary and argued that this is probably an effect of nutrient input from domestic and industrial sewage. Nitrogen isotope content of wastewater has higher values due to ammonium volatilization and denitrification processes during sewage treatment that removes the lighter ^{14}N faster than the ^{15}N (Macko & Ostrom, 1994; McClelland *et al.*, 1997). In addition, stormwater may also be considered as an enrichment factor, due to the thermodynamically favoured volatilization of isotopically depleted $^{14}\text{NH}_3$ from stormwater as it flows across hot surfaces (Dillon & Chanton, 2007). Estuarine ^{15}N -enriched waters could influence the isotopic composition of adjacent waters; however, no data on stable isotopes of the food chain of the coastal adjacent waters of the estuary are available at this time, thus precluding further interpretations.

Additionally, a bottlenose dolphin with a clearly different isotope signal was identified, which suggests that it may have fed in another region (Figure 2). In a preliminary isotope analysis comparing bottlenose dolphins from two areas, this animal was clustered together with a northern group of dolphins found stranded along the São Paulo State, Brazil ($25^{\circ}00'\text{S}$ $47^{\circ}50'\text{W}$) (Botta *et al.*, 2010a). Three hypotheses exist for the origin of this animal: (a) this individual could be part of the northern form, as proposed by Barreto (2000); (b) it could be a disperser from a southern population of bottlenose dolphins from Uruguay, which are known to move to coastal adjacent waters of the Patos Lagoon estuary (Laporta *et al.*, 2008); or (c) it could belong to an offshore ecotype. However, based on the cranial characters proposed by Barreto (2000) to distinguish northern from southern forms (e.g. shape of the pterigoyds and their separation) we could infer that this animal was a northern form individual, which could explain its distinct isotope compositions. As stated above, carbon and nitrogen signals of the southern São Paulo population were similar to the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values found for this animal (Botta *et al.*, 2010a). Nevertheless, the possibility of this animal belonging to an offshore group cannot be discarded, as no information on cranial morphology/shape and/or isotopic signatures for this group are available so far. Isotope compositions in teeth of offshore bottlenose dolphins from other areas are similar for nitrogen, but lower for carbon to those presented by this animal

(e.g. $\delta^{15}\text{N} = 14.8\text{‰}$ and $\delta^{13}\text{C} = -13.9\text{‰}$, western North Atlantic; Walker & Macko, 1999). Indeed, the carbon isotope value for this bottlenose dolphin (-12‰) was similar to those of continental shelf species (e.g. Atlantic spotted dolphin) and higher than that presented by the offshore Fraser's dolphin.

The presence of killer whales in coastal waters of southern Brazil is seasonal, with the records from winter and spring months being more common (Dalla Rosa *et al.*, 2005). The weakfish (*Cynoscion guatucupa*), the eagle stingray (*Myliobatis* sp.) and cephalopods have been recorded as prey for killer whales in Brazilian waters (Dalla Rosa *et al.*, 2005). Although no remains of cetaceans were found in the stomach of the killer whales analysed, a franciscana dolphin (*Pontoporia blainvillei*) was reported in the stomach content of a killer whale stranded in this region (Ott & Danilewicz, 1997). Owing to differences in the period of time integrated by stomach contents and stable isotopes, these two animals may have been also eating small cetaceans, as denoted by their high $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, which are similar to values found in franciscana teeth from this area (Botta *et al.*, 2010b).

The killer whale with the lowest $\delta^{13}\text{C}$ found in our samples (-20‰) and also with a low $\delta^{15}\text{N}$ value (10.5‰) was considered atypical. The stomach contents of this individual (oceanic salps (*Iasis zonaria*) and other unidentified planktonic invertebrates) could explain the low observed stable carbon and nitrogen isotope values found. Indeed, filter-feeder salps occupy low trophic levels in the oceanic environments (Madin, 1974). Elsewhere, salps and other components of the zooplankton have $\delta^{13}\text{C}$ values around -20‰ (Hatase *et al.*, 2002; Bode *et al.*, 2004).

In the western South Atlantic, short-beaked common dolphins and Atlantic spotted dolphins inhabit shallow waters over the continental shelf and upper slope (Zerbini *et al.*, 2004; Moreno *et al.*, 2005; Tavares *et al.*, 2010), where they feed on small meso/epipelagic fish and squids (Santos & Haimovici, 2002; Melo *et al.*, 2010; E.R. Secchi, personal observation). The intermediate $\delta^{13}\text{C}$ values found in teeth of these species' individuals are presumably reflecting this pelagic phytoplankton-dependent food web. The $\delta^{13}\text{C}$ value found for common dolphins is consistent with values found in other studies ($\sim -16\text{‰}$ in muscle, Das *et al.*, 2003; $\sim -12\text{‰}$ in bone, Pinela *et al.*, 2010).

The rough-toothed dolphin is generally found in deep-offshore waters (Miyazaki & Perrin, 1994; Jefferson, 2002); however coastal sightings of this species are relatively common along Brazilian waters, mainly for the south-eastern coast (Lodi, 1992; Ott & Danilewicz, 1996; Flores & Ximenes, 1997; Lodi & Hetzel, 1998). The carbon isotope signal found for the species was similar to that of continental shelf species, indicating that a shallower water habitat is also used in the subtropical western South Atlantic. Diet reported for this species included fish and squid (Miyazaki & Perrin, 1994). Nitrogen stable isotopes for this species did not differ from the rest of the continental shelf species, thus suggesting feeding at similar trophic positions (Figure 4). Finally, a rough-toothed dolphin with a ^{13}C depleted value was identified (Figure 2). This animal had $\delta^{13}\text{C}$ even lower than the oceanic Fraser's dolphin which can reflect a more oceanic feeding habitat used by this specimen, which could belong to an offshore group of rough-toothed dolphins.

False killer whale habitats are primarily oceanic and their main prey are deep-sea cephalopods and fish (Odell &

McClune, 1999; Baird, 2002). Isotopically, two groups of divergent trophic level and/or habitats could be identified. One group of 6 false killer whales presented low carbon and nitrogen isotopes signals (false killer whale B: Figures 2 & 3; Table 1). These animals were part of a mass stranding of 14 individuals that occurred in winter of 1995. The stomach contents of four of these animals (the remainder were empty) revealed only cephalopod prey, mainly *Ommastrephes bartramii* (Andrade *et al.*, 2001). This squid is a member of the oceanic Ommastrephidae family and together with *Ilex argentinus* are common prey found in mass stranded false killer whales from Argentina too (Koen-Alonso *et al.*, 1999) and are also important in the diet of other upper slope and oceanic adjacent water predators (Santos & Haimovici, 2001, 2002). The only isotope information published for these cephalopods off RS area, revealed low carbon and nitrogen stable isotope content ($\delta^{13}\text{C} = -16.7\text{‰}$ and $\delta^{15}\text{N} = 9.3\text{‰}$, for a combined sample of *I. argentinus* and *O. bartramii*; Bugoni *et al.*, 2010). After accounting for a combination of tissue-dependent $\Delta^{13}\text{C}$ collagen-muscle ($\sim 4\text{‰}$) and a trophic discrimination factor (1‰) by subtracting a total of 5‰ from false killer whales' dentine $\delta^{13}\text{C}$ value (Koch, 2007), comparison with omastrephids data confirmed the teuthophagic feeding habit of this group of false killer whales. On the other hand, two individuals presented different isotopic values from this mass stranded group (false killer whale A: Figures 2 & 3). One of these specimens, a 333 cm-long false killer whale washed ashore in 2004, had a high ^{13}C content (-10.8‰), indicating a coastal habitat, and ^{15}N enriched dentine (19‰), indicating it was feeding at a high trophic level. Besides cephalopod prey, Sciaenidae and Serranidae fish were reported in the diet of this species in southern Brazil (Pinedo & Rosas, 1989). Other authors indicate that this species could also prey upon small cetaceans (Odell & McClune, 1999; Baird, 2002). Nevertheless, the stable isotope proxy, together with previously-reported stomach content information suggest that at least some false killer whales in southern Brazil have a more coastal piscivorous feeding habit. Indeed, this species has been observed in coastal areas off southern Brazil (LTMM-IO-FURG, unpublished data). Finally, one young specimen (total length = 165 cm) had a low $\delta^{13}\text{C}$ (-13.1‰) but a high $\delta^{15}\text{N}$ (19.4‰). This animal was found stranded alone in 2006. Higher $\delta^{15}\text{N}$ relative to the mother's signal is expected for lactate-feeding calves because mothers are catabolizing their own tissues for producing milk, which leads to a higher 'trophic level' of their offspring (Hobson & Sease, 1998; Walker & Macko, 1999; Newsome *et al.*, 2006, 2009; Knoff *et al.*, 2008). The calf's lower $\delta^{13}\text{C}$ value is probably influenced by the high lipid content in the ^{13}C depleted milk (Hobson & Sease, 1998; Newsome *et al.*, 2006, 2009). Based on its total length, this animal was probably still nursing, as false killer whales usually lactate for 18–24 months (Odell & McClune, 1999), which could be a plausible explanation for the isotopes signals found.

The carbon isotope content for the Fraser's dolphin was lower than that of typical continental shelf delphinids, which closely represents its oceanic, deep water habitat. This is a tropical species, but with unusual strandings reported for subtropical areas (e.g. Praderi *et al.*, 1992; Pinedo *et al.*, 2001; Laporta *et al.*, 2002; Moreno *et al.*, 2003). This individual was a male with a total length of 236 cm. Length at sexual maturity is about 220–230 cm (Dolar, 2002) indicating that

this animal was probably sexually mature. Mesopelagic fish, crustaceans and cephalopods are among the preferential prey for this species, which are captured mainly from near surface to at least 600 m (Robison & Craddock, 1983). However, the diet reported for individuals stranded in southern Brazil included demersal fish, the cutlass fish (*Trichiurus lepturus*), the epipelagic squid *Loligo sanpaulensis* and the demersal/pelagic cephalopod, *Argonauta nodosa*, besides some penneidae shrimp (Pinedo *et al.*, 2001; Santos & Haimovici, 2001; Moreno *et al.*, 2003; Melo *et al.*, 2010). This coastal feeding habit can be considered as unusual and likely occurred because the animals were outside their home range (Moreno *et al.*, 2003). Therefore, stomach contents may occasionally represent unusual local feeding while stable isotope values of teeth reflect the typical long-term feeding habits for this individual.

This first study on stable isotope values of delphinids from southern Brazil revealed some important information about trophic ecology and feeding environments of delphinids inhabiting coastal, shelf and oceanic environments in the subtropical western South Atlantic. Substantial information about the trophic ecology and feeding environment can be derived from biochemical analyses in teeth. Furthermore, its combination with traditional methods, such as stomach content analysis, direct observation and telemetry, and biochemical methods could be a powerful tool to investigate the natural history of marine mammals (Pauly *et al.*, 1998). However, the almost non-existent environmental isotope data for this southern region calls for additional sampling efforts and analyses involving the main components of these marine food webs which include these high trophic level predators.

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