Use of carbon and nitrogen stable isotopes to study the feeding ecology of small coastal cetacean populations in southern Brazil

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Abstract: Samples from individuals of the populations of *Sotalia guianensis* (Guiana) and *Pontoporia blainvillei* (Franciscana) dolphins living in the Babitonga Bay estuary (26° 28' S/48° 50' W), and samples from individuals of a second population of *P. blainvillei* from a nearshore area (26° 38' S/48° 41' W), were collected and analyzed along with their prey between 2000 and 2006, to determine the carbon and nitrogen stable isotope ratios and to verify differences in their feeding ecology. No differences were found in the median $\delta^{15}N$ values of Guiana (15.2‰) and Franciscana (15.9‰) dolphins living in Babitonga Bay, nor of nearshore Franciscana (15.0‰) individuals, suggesting no variation in the trophic level of these populations. However, the lack of more information on the isotopic compositions of their putative prey in the nearshore areas prevents the ability to draw definitive conclusions on this issue. The estuarine Franciscana and Guiana dolphin populations presented mean $\delta^{13}C$ values of approximately –15.7‰, which were not statistically different from nearshore Franciscana individuals (–14.8‰). Based on stomach content analyses of these species from a previous study, it was reported that there was little overlap in the diet of estuarine Franciscanas and Guiana dolphins. However, based on the similarity of the $\delta^{13}C$ values between these two species and of their putative prey, it appears that in fact there is an overlap in the diet of these two species. Based solely on stable isotope analysis, it was not possible to differentiate between estuarine and nearshore Franciscana populations, making it difficult to conclude whether captured nearshore specimens were indeed yearlong residents of these areas. Finally, this study suggests that Franciscana and Guiana dolphin populations are sharing the same resources, mostly *L. brevis*, *D. rhombeus*, and *S. rastrifer*. Therefore, the combination of resource sharing and commercial exploitation of their prey makes these two cetacean species vulnerable.

Keywords: *Pontoporia blainvillei, Sotalia guianensis, Babitonga Bay, feeding ecology, trophic level.*

Resumo: Amostras de tecido de indivíduos pertencentes às populações de *Sotalia guianensis* (boto-cinza) e *Pontoporia blainvillei* (toninha) que vivem no estuário da Baía da Babitonga (26° 28' S/48° 50' W) e de uma segunda população de *P. blainvillei* da área costeira (26° 38' S/48° 41' W) foram coletadas entre os anos 2000 e 2006 para determinar as composições de isótopos estáveis de carbono e nitrogênio, assim como de suas presas para analisar diferenças na ecologia alimentar. Não foram detectadas diferenças nos valores médios de $\delta^{15}N$ entre os botos-cinza (15.2‰) e as toninhas (15.9‰) que vivem na Baía da Babitonga, e entre indivíduos de toninhas da área costeira (15.0‰), sugerindo que não existe variação no nível trófico destas populações. Contudo, a ausência

de informações mais completas sobre a composição isotópica das presas na área costeira limita a proposição de conclusões mais definitivas sobre esta temática. As populações estuarinas de toninhas e botos-cinza apresentaram valores médios de δ^{13}C de aproximadamente –15.7‰, que não apresentaram diferença estatística com relação às toninhas da área costeira (–14.8‰). Baseado na análise de conteúdos estomacais destas espécies num estudo anterior sugere-se que não há sobreposição na dieta das toninhas e botos-cinza do estuário. Contudo, baseado na similaridade dos valores de δ^{13}C entre estas espécies e nos distintos valores de δ^{13}C de suas presas, há indícios de que de fato existe uma sobreposição na dieta destas duas populações. Com base apenas na análise de isótopos estáveis não foi possível diferenciar a população estuarina e costeira de toninhas, tornando-se difícil concluir se os indivíduos capturados acidentalmente na área costeira eram residentes de longo prazo. Finalmente, este estudo sugere que toninhas e botos-cinza estão compartilhando os mesmos recursos, principalmente *L. brevis* , *D. rhombeus* e *S. rastrifer* . Portanto, a exploração comercial dessas espécies pode ameaçar a sobrevivência do boto-cinza e das toninhas na Baía da Babitonga.

Palavras-chave: *Pontoporia blainvillei, Sotalia guianensis, Baia da Babitonga, ecologia alimentar, nível trófico.*

Introduction

Babitonga Bay, an estuarine sub-tropical system located on the north coast of Santa Catarina State in southern Brazil, is home to two small cetacean populations: Guiana *Sotalia guianensis* (Van Bénéden 1864) and Franciscana dolphins *Pontoporia blainvillei* (Gervais & d'Orbigny 1844) (Cremer & Simões-Lopes 2005, 2008, Hardt et al. 2010, Cremer et al. 2011). *Sotalia guianensis* is considered "data deficient" by the International Union for Conservation of Nature (IUCN), and *Pontoporia blainvillei* is considered "vulnerable" (Reeves et al. 2008, 2012). In fact, Franciscana dolphins are considered to be the most threatened small cetacean species in the southwestern Atlantic Ocean (Secchi et al. 2003, Danilewicz et al. 2010).

Based on stomach content analysis of individuals collected in the Babitonga Bay area, Cremer et al. (2012) concluded that both species exhibit a generalist diet that includes several teleost fish and one cephalopod species, which are important food sources for both species. Correspondingly, similar results have been found in other regions of the Brazilian coast (Santos et al. 2002, Di Beneditto & Siciliano 2007, Di Beneditto et al. 2009, Daura-Jorge et al. 2011). Aside from this cephalopod, there is not much overlap in the diet of these two cetacean species in the Babitonga Bay area. Additionally, these authors also concluded that the diet of Guiana dolphins exhibited a higher diversity of fish species than Franciscana dolphins, leading to a broader feeding niche of the former compared to the latter.

As both species of cetaceans are composed of small populations foraging in Babitonga Bay and are threatened by several anthropogenic impacts (Cremer 2007, Danilewicz et al. 2010, Lailson-Brito et al. 2010, Yogui et al. 2010), detailed information about the feeding ecology of these species and other species linked to them is important in order to establish effective conservation initiatives. In previous studies on the population distribution of Franciscana dolphins in the bay, Cremer & Simões-Lopes (2005, 2008) suggested that the population is resident, which has increased the level of concern for their conservation. However, further evidence is still needed to confirm whether they are indeed resident year-round in the bay area.

One approach for studying animal feeding behavior is to examine stomach contents, as performed by Cremer et al. (2012). This technique is very useful, although stomach content analyses represent just a "snapshot" of a brief period in the lifecycle of animals like cetaceans. Additionally, some items in the stomach are not recognizable, due to advanced decomposition, and there is no guarantee that identifiable stomach contents will be digested and incorporated in animal tissues. Analysis of carbon and nitrogen stable isotopes represent a longer period in the animal's lifecycle and have been recognized as an effective method to complement feeding

ecology studies based on stomach contents of marine mammals (Knoff et al. 2008, Gross et al. 2009, Barros et al. 2010, Kiszka et al. 2010, Newsome et al. 2010, Caut et al. 2011). The basic principle of this technique is that the isotopic composition of consumers is derived from the isotopic composition of their food (*e.g.,* Kelly 2000, Cherel et al. 2010). In marine systems, several studies have shown that there are consistent differences between the isotopic composition of animals living in pelagic *vs.* benthic environments, and among estuarine, nearshore and offshore environments (France 1995, Kurle & Gudmundson 2007, Cherel & Hobson 2007, Newsome et al. 2010, Ohizumi & Nobuyuki 2010). Therefore, the use of stable isotopes can help identify the utilization of different habitats by species (Das et al. 2003, Capelli et al. 2008, Gross et al. 2009, Newsome et al. 2010, Pinela et al. 2010). Additionally, by using this technique, it can be possible to determine if sympatric species explore the same resources and occupy the same trophic level (Cherel et al. 2008, Newsome et al. 2010).

The main objective of this study was to investigate the feeding ecology of Guiana and Franciscana cetacean populations in Babitonga Bay using carbon and nitrogen stable isotopes. Understanding the feeding ecology of specific species is considered essential in order to implement effective conservation measures (Owen et al. 2011). Franciscana individuals from a separate population found in the nearshore area, located outside of the bay, were also included in the analysis in order to check for variation in feeding strategies of the same species in relation to habitat differences (Ford et al. 1998). The diet of these populations was characterized to complement the findings of a recent study on their feeding strategies, which analyzed the stomach contents of specimens collected in Babitonga Bay (Cremer et al. 2012). Additionally, a second objective of this study was to investigate whether the use of stable isotopes could effectively distinguish nearshore Franciscana specimens from estuarine individuals.

Materials and Methods

1. Samples collection

Tissue samples from Guiana and Franciscana dolphins were collected from dead, stranded animals found in the Babitonga Bay estuary and surrounding beaches located on the north coast of Santa Catarina State in southern Brazil (26° 28' S/48° 50' W). Tissue samples of Franciscana dolphins were also collected outside of the bay in a nearshore area from animals incidentally caught in nets used in an artisanal fishery at Barra Velha (26° 38' S/48° 41' W) (Figure 1). Whenever possible, total animal length was measured and sex was

Figure 1. Study area, showing the map of Brazil and in the insert the location of Babitonga Bay estuarine area and Barra Velha beach, on the north coast of Santa Catarina State, southern Brazil.

visually determined. Samples of muscle tissue were collected from the dorsal area of the dolphins and frozen immediately.

In this study, it was presumed that Franciscana dolphins found in estuarine and nearshore areas, respectively, are from distinct populations, and that the estuarine population is probably a resident one (Cremer & Simões-Lopes 2005, 2008). In the estuarine area, the Franciscana population was estimated to of approximately fifty individuals (Cremer & Simões-Lopes 2008), while the Guiana dolphin population was estimated to be 208 individuals (Cremer et al. 2011). There is no available data on the abundance of the nearshore Franciscana population.

The individuals of each population were collected during the study period, between 2000 and 2006. At Barra Velha beach, a small artisanal fishery was monitored between 2002 and 2005 and reports indicate that the incidental capture of the species in this area is low (A. S. Barreto, unpublished data.). Twelve Guiana and sixteen

Franciscana dolphins were recovered in the estuarine area, while seven Franciscana individuals were recovered in the nearshore area, obtained directly from fishermen.

Sex could not be determined in all cases, mainly because of genital mutilation by avian predators after they washed up on the beach. Therefore, there was not enough data available to test for differences in stable isotopic composition in relation to gender. Although not well established, differences in stable isotopic composition in females may arise due to pregnancy or nursing (Newsome et al. 2010). Also, a few studies have found important ontogenic differences in isotopic composition, mainly in the isotopic fractionation between young and adult specimens (Newsome et al. 2009, 2010). Based on total length, most of the specimens analyzed in this study were adults, apart from one dead Guiana calf collected in the estuarine area.

Based on the stomach content study of Cremer et al. (2012), potential food sources were selected for isotopical analysis, based

on their index of relative importance. The cephalopod *Lolliguncula brevis* (Blainville 1823) was selected as an important prey item for both species and three teleost fish were selected for Guiana dolphins: *Mugil curema* (Valenciennes 1836); *Micropogonias furnieri* (Desmarest 1823); and *Diapterus rhombeus* (Cuvier 1829). For Franciscana dolphins, two teleost fish were selected: *Stellifer rastrifer* (Jordan 1889) and *Cetengraulis edentulus* (Cuvier 1829). All fish samples were captured from the estuary using drift nets inside the bay. The cephalopods were collected only in nearshore areas by trawl nets used in the shrimp fishery. *Lolliguncula brevis* is the only eurihaline cephalopod species, which moves back and forth between bays and estuaries in response to changes in water temperature (Bartol et al. 2002). Although relatively important for both species, it was not possible to capture *Gobionellus oceanicus* individuals for analysis. Prey samples were selected based on the specific total length that both dolphins consume (Cremer et al. 2012). Samples of muscle tissue were collected from the dorsal area of each specimen and frozen immediately.

2. Isotopic analysis

Samples of muscle tissue and three samples of Franciscana fat were lyophilized in preparation for carbon and nitrogen stable isotope analysis. Samples were first dried in a 60 °C oven for at least 48 hours and then were grounded into a fine powder using a mortar and pestle. Because of possible samples heterogeneity the samples were well mixed. Isotopic measurements were determined by a ThermoElectron Delta Plus mass spectrometer interfaced with an Elemental Analyzer (Carlo Erba model 1110, Milan, Italy). Stable isotope ratios were registered relative to internationally recognized standards, included in every run and expressed in δ notation (‰). For carbon the standard used was Peedee Belemnite (PDB) and for nitrogen atmospheric air. The precision of the isotope ratio measurements was ± 0.3‰ and ± 0.4‰ for δ¹³C and δ¹⁵N, respectively. All isotope analyses were conducted at the Centro de Energia Nuclear na Agricultura – CENA, University of São Paulo, in Piracicaba, SP, Brasil.

3. Lipid interference on isotope analysis

Lipids generally have 6‰ lower δ¹³C values than proteins, which can interfere with accurate δ¹³C readings of muscle tissues, leading to erroneous interpretation (Newsome et al. 2010, Caut et al. 2011). In this study, three samples of cetacean fat were analyzed, with an average δ¹³C value of −20.3 ± 1.1‰, which is approximately 4.3‰ lower than the average δ¹³C of all muscle tissue samples (−16.0 ± 1.3‰). After analysis of the C:N muscle tissue ratios, approximately 60% had ratios lower than 3.5, but the other 40% had C:N ratios higher than 3.5, suggesting high lipid content (Post et al. 2007, Newsome et al. 2010).

There is no consensus in the literature on lipid extraction, however, it has been recommended not to extract lipids when lipid-rich prey could be part of a consumer's diet (Newsome et al. 2010). Otherwise, lipid extraction is recommended (Newsome et al. 2010). All the C:N muscle-tissue ratios of prey specimens were lower than 3.5, indicating that they were lipid-poor. Due to these results, it was decided to correct δ¹³C ratios for lipid content only in cetacean samples with a C:N ratio greater than 3.5 (Table 1).

There are several equations or mass balance approaches that have been developed to correct for lipid content (Fry et al. 2003, Post et al. 2007, Sweeting et al. 2006, Abrantes et al. 2012). However, the equation developed by Post et al. (2007) is simple because it depends exclusively on the C:N ratios of tissues, having shown to be very applicable when tested in lipid-rich salmonids (Abrantes et al. 2012). Therefore, it was decided to use the equation here in this study, with δ¹³C values corrected for lipid contents being referred to as $\delta^{13}C_{corr}$.

4. Statistical analyses

Due to $\delta^{13}C_{corr}$ and δ¹⁵N values did not present normal distribution, and could not be normalized, the non-parametric Kruskal-Wallis ANOVA was used to test for differences between the populations (two populations of Franciscana and one population of Guiana dolphins). In this case, the different populations and habitats (estuary and nearshore) were the independent variables and the δ¹³C and δ¹⁵N values the dependent variables. A probability value of less than 0.05 was considered to indicate statistical significance. All statistical analyses were performed with specific software's.

Results

Table 1 summarizes the δ¹³C values of non-lipid extracted samples, $\delta^{13}C_{corr}$ and δ¹⁵N values of each specimen, as well as C:N muscle-tissue ratios, with gender and total length measurements stated when available.

A plot of δ¹³C values of non-lipid extracted samples in relation to δ¹³C values corrected for lipid content ($\delta^{13}C_{corr}$) showed that most corrections were made for Guiana dolphin specimens and less for Franciscana specimens (Figure 2).

The average $\delta^{13}C_{corr}$ ratio of nearshore Franciscanas was almost 1‰ higher than the average $\delta^{13}C_{corr}$ values of estuarine Franciscana and Guiana dolphins (Figure 3a). However, differences among populations were not statistically significant according to the Kruskal-Wallis test (Figure 3a).

In terms of average δ¹⁵N values, there was a slight increase (0.4‰) between nearshore and estuarine Franciscana dolphins and a 0.2‰ increase between the estuarine Franciscana and Guiana dolphins. Consequently, the Guiana individuals presented δ¹⁵N values 0.6‰ higher than nearshore Franciscanas (Figure 3b). However, these differences were not statistically significant according to the Kruskal-Wallis test (Figure 3b).

In order to position putative prey and predators in the demoninated δ-space (after Newsome et al. 2007), the stable isotope ratios of cetaceans and potential prey were plotted in Figure 4. Carbon and nitrogen stable isotope values demonstrated that *L. brevis, M. furnieri, D. rhombeus, S. rastrifer,* and *C. edentulus* are most likely important prey for estuarine Franciscana and Guiana dolphins, while *M. curema* appeared to be less important, especially for Franciscana specimens (Figure 4).
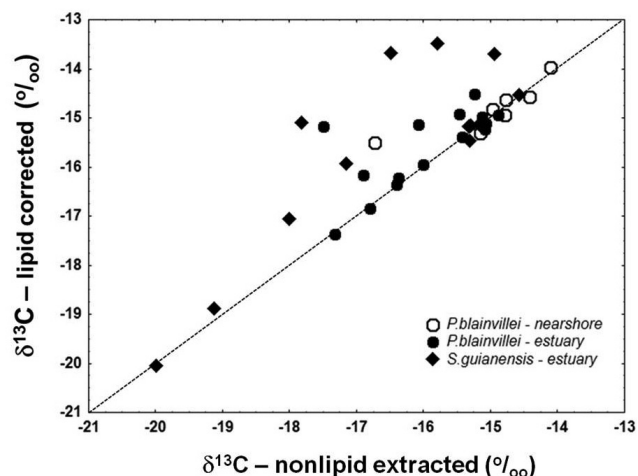
Figure 2. Comparison of non-lipid extracted δ¹³C values and corrected δ¹³C values for lipids according to the equation proposed by Post et al. (2007). The dashed line represents a 1:1 line.

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Table 1. $\delta^{13}C$ values ($\delta^{13}C$) and $\delta^{13}C$ values corrected for lipid content ($\delta^{13}C_{corr}$) both expressed as ‰, $\delta^{15}N$ values ($\delta^{15}N$), C:N ratio, sex, and total length (cm) of specimens of *Pontoporia blainvillei* (Franciscana) and *Sotalia guianensis* (Guiana dolphin).

| Species	Site	$\delta^{13}C$	$\delta^{13}C_{corr}$	$\delta^{15}N$	C:N	Sex	Length
P. blainvillei Franciscana	Estuary	−16.9	−16.2	17.1	4.1	-	Calf
		−16.1	−15.1	17.9	4.3	M	62
		−15.1	−15.1	16.6	3.3	M	109
		−16.4	−16.2	15.6	3.5	F	-
		−16.8	−16.8	15.9	3.3	-	-
		−16.4	−16.4	14.1	3.4	-	-
		−16.0	−16.0	14.3	3.4	-	-
		−15.1	−15.1	14.7	3.2	-	-
		−15.1	−15.0	15.2	3.5	-	-
		−15.5	−14.9	15.2	3.9	-	-
		−17.5	−15.2	15.4	5.7	-	-
		−17.3	−17.3	16.0	3.3	-	-
		−14.9	−14.9	15.0	3.3	-	-
		−15.4	−15.4	14.9	3.4	-	-
		−15.2	−14.5	14.8	4.1	-	-
		−15.3	−15.2	17.8	3.5	-	-
S. guianensis Guiana dolphin	Estuary	−20.0	−20.0	16.0	3.3	F	145
		−16.5	−13.7	15.2	6.2	-	187
		−17.8	−15.1	16.4	6.1	M	202
		−19.1	−18.9	14.8	3.6	M	182
		−14.6	−14.5	15.7	3.4	M	-
				14.9	3.9	-	-
		−18.0	−17.1	15.1	4.3	-	-
		−15.2	−15.1	15.0	3.4	-	-
		−15.3	−15.2	17.8	3.5	-	-
		−15.3	−15.3	14.4	3.2	-	-
		−15.8	−13.5	14.9	5.7	-	-
		−14.9	−13.7	16.0	4.6	-	-
		−17.2	−15.9	16.4	4.6	-	-
P. blainvillei Franciscana	Nearshore	−14.8	−14.8	15.9	3.2	-	-
		−15.2	−15.2	15.8	3.2	F	103
		−14.1	−14.0	14.9	3.5	M	103
		−14.4	−14.4	15.0	3.2	F	136
		−16.7	−15.5	14.4	4.6	F	131
		−15.0	−14.8	15.1	3.5	F	108
		−14.8	−14.6	15.0	3.5	F	114

Discussion

Although the study involves a small number of samples, any available information on the foraging habits of these species is beneficial for establishing conservation measures (Cremer et al. 2012, Owen et al. 2011). Nonetheless, results found in this study may not represent the diet behaviour of a large population and conclusions should be drawn with caution. Also, we are aware that it is not ideal to collect samples from dead animals due to two factors: the possibility of sickness causing their mortality; and the decomposition process which can inter with the results (Barros & Odell 1990).

In addition, animals experiencing nutritional stress may have increased muscle $\delta^{15}N$ values due to preferential loss of ^{14}N-enriched wastes (Newsome et al. 2010). However, the animals collected in this study did not exhibit signs of disease and most likely died as a consequence of entanglement in fishing nets, which has been a problem in Babitonga Bay, as described by Pinheiro & Cremer (2003).

According to Payo-Payo et al. (2013), analyzing decomposition on stable isotope signatures of striped dolphins *Stenella coeruleoalba*, no statistical change in $\delta^{13}C$ or $\delta^{15}N$ over period of 62 days in muscle and skin samples from carcasses decomposing was found, showing that they can be used as reliable material for stable isotope analysis in probably other marine mammal species.

For the nearshore Franciscana population, the $\delta^{13}C$ values of most of the specimens were higher than their putative prey (Figure 4). Di Beneditto et al. (2011), working with the same species in the coastal area of Rio de Janeiro State, also observed higher $\delta^{13}C$ values than in their potential prey. This is probably due to carbon trophic fractionation, which occurs between $\delta^{13}C$ values of prey and predator, the latter becoming enriched in ^{13}C relative to its prey. However, carbon isotope trophic fractionations can vary between tissues (Newsome et al. 2010), body size (Caut et al. 2011), and diet type (Vander Zanden & Rasmussen 2001), among other causes, but they are generally considered smaller than nitrogen isotope trophic
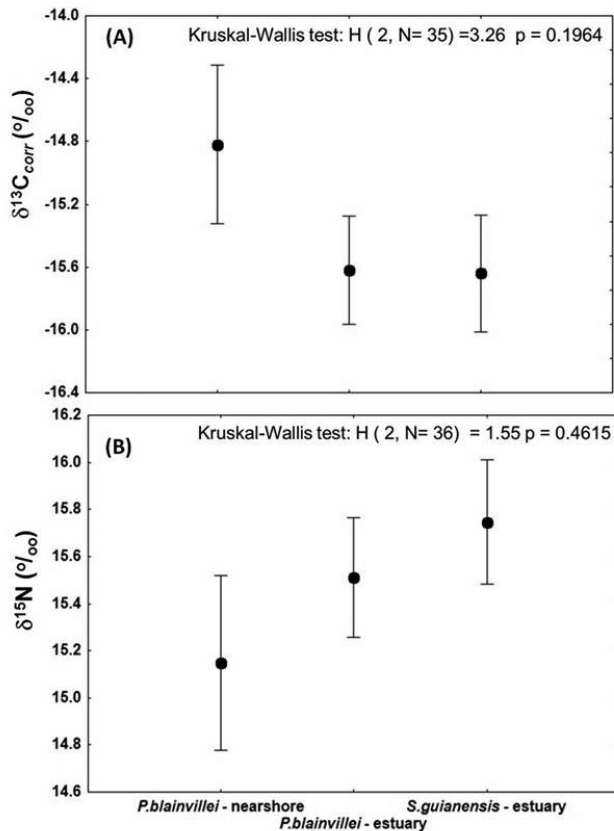
Figure 3. (A) Average $\delta^{13}C$ values corrected for lipids ($\delta^{13}C_{corr}$) and (B) average $\delta^{15}N$ values followed by standard-errors (bars) of cetacean populations grouped according to habitat (estuary and nearshore) of samples acquisition.

fractionation rates (Post 2002, Owen et al. 2011). For instance, McCutchan et al. (2003) found an average carbon trophic fractionation of only 0.4‰ between aquatic animals and their respective diets. Caut et al. (2011), analyzing blood plasma of captive bottlenose dolphins (*Tursiops truncatus*), encountered a similar carbon trophic fractionation of 0.6‰, while Vander Zanden & Rasmussen (2001) measured trophic fractionation in aquatic systems to be on average 0.8‰. It appears that most nearshore Franciscana individuals present $\delta^{13}C$ values more than 1‰ enriched relative to most of their prey (Figure 4).

Based on stomach content analyses performed by Cremer (2007), prey listed in Figure 4 are most likely contributing to the diet of these individuals, although a large variation in $\delta^{13}C$ values was observed among nearshore Franciscanas. However, it is also reasonable to consider the existence of prey ^{13}C-enriched in relation to nearshore Franciscanas, which were not included in this study.

The $\delta^{15}N$ values of animals have been used extensively to determine their trophic level based on the assumption of a considerable ^{15}N enrichment between each trophic level (DeNiro & Epstein 1981). The main reason for this trophic fractionation seems to be due to internal N metabolism, leading to ^{15}N enrichment in body proteins coupled with excretion of ^{15}N-depleted ammonia via urine (Balter et al. 2006, Martínez del Rio et al. 2009). There have been several recent revisions on differences between $\delta^{15}N$ values of diet and consumers (Post 2002, McCutchan et al. 2003, Vanderklift & Ponsard 2003, Vander Zanden & Fetzer 2007, Martínez del Rio et al. 2009). Based on these revisions, it has been observed that nitrogen

trophic fractionation depends on several aspects of both diet and the consumer. With regards to the former, the quantity and the quality of the protein is a key factor (Robbins et al. 2005, 2010, Martínez del Rio et al. 2009, Caut et al. 2011). In the latter, the magnitude of nitrogen trophic fractionation depends on the type of tissue in question, as there are variations in turnover rates for different tissues (Newsome et al. 2010).

Based on the revisions cited above, it seems reasonable to consider that nitrogen trophic fractionation varies between 2 to 4‰, with the value of 3‰ being the most commonly used (Vander Zanden & Fetzer 2007). This latter value of trophic fractionation is similar to the value of 2.6‰ found in blood plasma of captive bottlenose dolphins by Caut et al. (2011). In this study, it proved difficult to estimate the nitrogen trophic fractionation of cetaceans under field conditions based on a small number of consumer samples and a limited number of prey.

Trophic fractionation in this study ($\delta^{15}N_{consumer} - \delta^{15}N_{diet}$) was estimated using average $\delta^{15}N$ values of cetaceans (15.2‰, n=20) and fish samples (12.3‰, n=15). Consequently, the estimated average trophic fractionation was 2.9‰, which is close to the value found by Caut et al. (2011) for blood plasma of captive bottlenose dolphins, and also close to 3‰, the most commonly used nitrogen trophic fractionation value (Vander Zanden & Fetzer 2007).

No significant differences were found among the average $\delta^{15}N$ values of the three cetacean populations (Figure 3b). Based on the discussion above regarding nitrogen trophic fractionation, it is tempting to conclude from the results that the cetacean populations are feeding at the same trophic level. However, as only a few prey samples were collected from nearshore areas (only *L. brevis* was sampled in coastal areas), we cannot exclude the possibility that these food sources may possess distinct $\delta^{15}N$ values from the estuarine prey. If this is the case, although the $\delta^{15}N$ values were similar among the cetacean populations inside and outside of the bay, this does not necessarily mean that they were feeding at the same trophic level. This kind of situation has already been shown in Victoria Bay (Australia), where two cryptic species of cetaceans living inside and outside of the bay were occupying different trophic levels (Owen et al. 2011).

As this study involved a small number of specimens, future research should increase the sampling size and further investigate trophic interactions among cetacean species of the Babitonga Bay area. The constraint of estimating nitrogen trophic fractionation based on a small number of samples has been illustrated by Di Beneditto et al. (2011), where lower $\delta^{15}N$ values were found in several Guiana and Fransiscana dolphins, in comparison to their putative prey.

A secondary objective of this study was to test whether it was possible to differentiate specimens of estuarine Franciscanas from nearshore individuals using carbon stable isotopes. In the literature, there are several studies where differences were found in feeding habits of populations of the same species living in distinct habitats: killer whales (*Orcinus orca*) in the Pacific Northwest on the border between the US and Canada (Ford et al. 1998); sea lions (*Eumetopias jubatus*) in Alaska (Kurle & Gudmundson 2007); and dolphin species in Australia (Owen et al. 2011).

Although the average $\delta^{13}C$ value of nearshore Franciscanas was higher than that of the estuarine group, no statistically significant difference was found (Figure 3a). Therefore, due to the similarity in stable isotope composition, it is tempting to consider that these two distinct groups could belong to the same population. But, this type of comparison is further complicated by seasonal differences in their diet (Kiszka et al. 2010, Olin et al. 2012), as well as a time lag between food ingestion and carbon assimilation in the muscle tissues (Newsome et al. 2010). Additionally, it has been reported that $\delta^{13}C$ values of several marine species tend to decrease from

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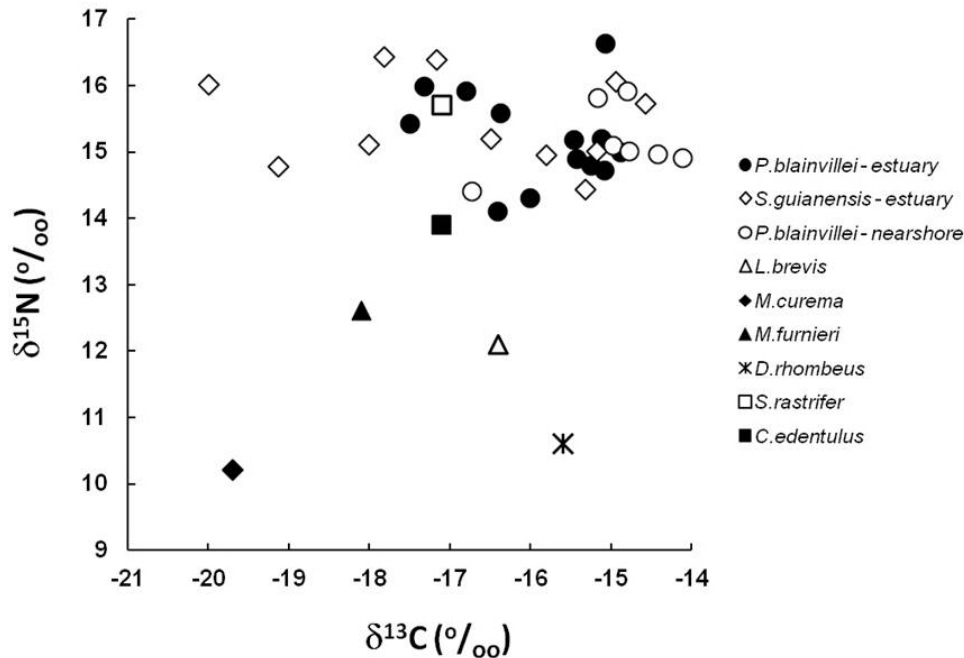
Figure 4. Plot of $\delta^{13}C_{corr}$ in relation to $\delta^{15}N$ (δ-space) of cetacean specimens and potential prey. Stable carbon isotope composition of cetacean species was corrected for lipid content, but not for prey.

inshore to offshore areas (France 1995, Clementz & Koch 2001, Kurle & Gudmundson 2007, Cherel & Hobson 2007, Newsome et al. 2010, Ohizumi & Nobuyuki 2010, Gibbs et al. 2011, Méndez-Fernandez et al. 2012). Based on these constraints, a future line of investigation could apply molecular tracers to more accurately identify the ecological niches of these animals (Fernández et al. 2011).

Although the conclusions drawn from the current study have a limited scope, the results suggest that these threatened species of cetaceans are sharing similar resources. Additionally, previous studies have shown that food sources harvested by Guiana and Fransiscana dolphins are also being threatened by shrimp trawling in coastal areas of Santa Catarina (Zaleski 2005, Souza & Chavez 2007). Therefore, a decrease in stocks of these prey items by predatory fishing may have a significant impact on the conservation of these two species.

Based on stomach content analyses (Cremer 2007), it appeared that there was not much similarity in the diet of estuarine Franciscana and Guiana dolphins. However, stable isotope results here suggests that there is an overlap in the diet of these two species, especially with respect to the following prey: *L. brevis*, *D. rhombeus*, and *S. rastrifer*. The similarity of $\delta^{15}N$ values among all populations suggest that they are feeding at the same trophic level; however, the absence of fish prey items collected from nearshore coastal areas prevents definitive confirmation of this conclusion. Additionally, carbon stable isotope results might indicate the presence of only one population of Franciscana dolphins, which occupy both estuarine and nearshore habitats. Coupled with the already threatened conservation status of Fransiscana and Guiana dolphins, the fact that these species are apparently sharing limited resources in a relatively small area increases even more the necessity of urgent action by government authorities to implement effective conservation measures to protect them.

Acknowledgements

References

ABRANTES, K.G., SEMMENS, J.M., LYLE, J.M. & NICHOLS, P.D. 2012. Normalisation models for accounting for fat content in stable isotope measurements in salmonid muscle tissue. Mar. Biol. 159(1):57-64.

BALTER, V., SIMON, L., FOUILLET, H. & L'ECUYER, C. 2006. Box-modeling of 15N/14N in mammals. Oecologia 147:212-222.

BARTOL, I.K., MANN, R. & VECCHIONE, M. 2002. Distribution of the euryhaline squid *Lolliguncula brevis* in Chesapeake Bay: effects of selected abiotic factors. Ecol. Progr. Ser. 226:235-247. http://dx.doi.org/10.3354/meps226235

BARROS, N.B. & ODELL, D.K. 1990. Food habits of bottlenose dolphins in the southeastern United States. In The bottlenose dolphin (Leatherwood & R.R. Reeves, eds). Academic Press, San Diego, p.309-328.

BARROS, N.B., OSTROM, P.H., STRICKER, C.A. & WELLS, R.S. 2010. Stable isotopes differentiate bottlenose dolphins off west-central Florida. Mar. Mamm. Sci. 26:324-336. http://dx.doi.org/10.1111/j.1748-7692.2009.00315.x

CAPELLI, R., DAS, K., PELLEGRINI, R.D., DRAVA, G., LEPOINT, G. & MIGLIO, C. 2008. Distribution of trace elements in organs of six species of cetaceans from the Ligurian Sea (Mediterranean), and the relationship with stable carbon and nitrogen ratios. Sci. Total. Environ. 390:569-78. PMid:18035400. http://dx.doi.org/10.1016/j.scitotenv.2007.10.036

CAUT, S., LARAN, S., GARCIA-HARTMANN E. & DAS, K. 2011. Stable isotopes of captive cetaceans (killer whales and bottlenose dolphins). J. Exp. Biol. 214:538-545. PMid:21270301. http://dx.doi.org/10.1242/jeb.045104

CHEREL, Y. & HOBSON, K.A. 2007. Geographical variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. Mar. Ecol. Progr. Ser 329:281-287. http://dx.doi.org/10.3354/meps329281

CHEREL, Y., CORRE, M., JAQUEMET, S., MÉNARD, F., RICHARD, P. & WEIMERSKIRCH, H. 2008. Resource partitioning within a tropical seabird community: new information from stable isotopes. Mar. Ecol. Progr. Ser: 366281-366291.

CHEREL, Y., FONTAINE, C., RICHARD, P. & LABAT, J.P. 2010. Isotopic niches and trophic levels of myctophid fishes and their predators in the Southern Ocean. Limnol. Oceanogr. 55:324-332. http://dx.doi.org/10.4319/lo.2010.55.1.0324

CLEMENTZ, M.T., & KOCH, P.L. 2001. Monitoring habitat and foraging ecology in aquatic mammal with stable isotopes in the tooth enamel. Oecologia 129:461-472.

CREMER, M.J. 2007. Ecologia de populações simpátricas de pequenos cetáceos em ambiente estuarino no sul do Brasil. Tese de doutorado, Universidade Federal do Paraná, Curitiba.

CREMER, M.J. & SIMÕES-LOPES, P.C. 2005. The occurrence of *Pontoporia blainvillei* (Gervais & d'Orbigny) (Cetacea, Pontoporiidae) in an estuarine area in southern Brazil. Rev. Bras. Zool. 22:717-723. http://dx.doi.org/10.1590/S0101-81752005000300032

CREMER, M.J. & SIMÕES-LOPES, P.C. 2008. Distribution, abundance and density estimates of Franciscanas, *Pontoporia blainvillei* (Cetacea: Pontoporiidade) in Babitonga bay, southern Brazil. Rev. Bras. Zool. 25:397-402. http://dx.doi.org/10.1590/S0101-81752008000300003

CREMER, M.J., HARDT, F.A.S., TONELLO-JÚNIOR, A.J. & SIMÕES-LOPES, P.C. 2011. Distribution and status of the Guiana dolphin *Sotalia guianensis* (Cetacea, Delphinidae) population in Babitonga bay, Southern Brazil. Zool. Stud. 50:327-337.

CREMER, M.J., PINHEIRO, P.C. & SIMÕES-LOPES, P.C. 2012. Prey consumed by Guina dolphin *Sotalia guianensis* (Cetacea, Delphinidae) and franciscana dolphin *Pontoporia blainvillei* (Cetacea, Pontoporiidae) in an estuarine environment in southern Brazil. Iheringia, Sér. Zool. 102: 131-137.

DANILEWICZ, D., MORENO, I.B., OTT, P.H., TAVARES, M., AZEVEDO, A.F., SECCHI, E.R. & ANDRIOLO, A.A. 2010. Abundance estimate for a threatened population of Franciscana dolphins in a southern coastal Brazil: uncertainties and management implications. J. Mar. Biol. Assoc. UK. 90:1649-1657. http://dx.doi.org/10.1017/S0025315409991482

DAS, K., LEPOINT, G., LEROY, Y. & BOUQUEGNEAU, J.M. 2003. Marine mammals from the Southern North Sea: feeding ecology data from δ13C and δ15N measurements. Mar. Ecol. Progr. Ser. 263:287-298. http://dx.doi.org/10.3354/meps263287

DAURA-JORGE, F.G., WEDEKIN, L.L. & SIMÕES-LOPES, P.C. 2011. Feeding habits of the Guiana dolphin, *Sotalia guianensis* (Cetacea: Delphinidae), in Norte Bay, southern Brazil. Sci. Ma. 75:163-169. http://dx.doi.org/10.3989/scimar.2011.75n1163

DENIRO, M.J. & EPSTEIN, S. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochim. Ac. 45:341-351.

DI BENEDITTO, A.P.M. & SICILIANO, S. 2007. Stomach contents of the marine tucuxi dolphin (*Sotalia guianensis*) from Rio de Janeiro, south-eastern Brazil. J. Mar. Biol. Assoc. UK 87:253-254. http://dx.doi.org/10.1017/S0025315407053647

DI BENEDITTO, A.P.M., SANTOS, M.V.B. & VIDAL, M.V. 2009. Comparison between the diet of two dolphins from south-eastern Brazil: proximate-composition and caloric value of prey species. J. Mar. Biol. Assoc. UK. 89:903-905. http://dx.doi.org/10.1017/S0025315409001519

DI BENEDITTO, A.P.M., SOUZA, C.M.M., KEHRIG, H.A. & REZENDE, C.E. 2011. Use of multiple tools to assess the feeding preference of coastal dolphins. Mar. Biol. 158:2209-2217.

FERNANDEZ, R., GARCIA-TISCAR, S., SANTOS, M.B., LOPEZ, A., MARTINEZ-CEDEIRA, J.A., NEWTON, J. & PIERCE, G.J. 2011. Stable isotope analysis in two sympatric populations of bottlenose dolphins *Tursiops truncatus*: Evidence of resource partitioning? Mar. Biol. 158(5):1043-1055. http://dx.doi.org/10.1007/s00227-011-1629-3

FORD, J.K.B., ELLIS, G.M., BARRETT-LENNARD, L.G., MORTON, A.B., PALM, R.S. & BALCOMB III, K.C. 1998. Dietary specialization in two sympatric populations of killer whales (*Orcinus orca*) in coastal British Columbia and adjacent waters. Can. J. Zool. 76:1456-1471.

FRANCE, R. 1995. Carbon-13 enrichment in benthic compared to planktonic algae: food web implications. Mar. Ecol. Progr. Ser. 124:307-312. http://dx.doi.org/10.3354/meps124307

FRY, B., BALTZ D.M., BENFIELD, M.C., FLEEGER, J.W., GACE, A., HAAS, H.L. & QUIÑONES-RIVERA, Z.J. 2003. Stable isotope indicators of movement and residency for brown shrimp (*Farfantepenaeus aztecus*) in coastal Lousiana marshscapes. Estuaries 26:82-97.

GIBBS, S.E., HARCOURT, R.G. & KEMER, C.M. 2011. Niche differentiation of bottlenose dolphin species in South Australia revealed by stable isotopes and stomach contents. Wildlife Res. 38:261-270.

GROSS, A., KISZKA, J., VAN CANNEYT, O., RICHARD, P. & RIDOUX, V. 2009. A preliminary study of habitat and resource partitioning among co-occurring tropical dolphins around Mayotte, southwest Indian Ocean. Estuar. Coast. Shelf Sci. 84:367-374. http://dx.doi.org/10.1016/j.ecss.2009.05.017

HARDT, F.A.S., CREMER, M.J., TONELLO JÚNIOR, A.J. & SIMÕES-LOPES, P.C. 2010. Residence patterns of the Guiana dolphin *Sotalia guianensis* in Babitonga Bay, South coast of Brazil. LAJAM 8:117-121. http://dx.doi.org/10.5597/lajam00160

KELLY, J.F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Can. J. Zool. 78:1-27. http://dx.doi.org/10.1139/z99-165

KISZKA, J., OREMUS, M., RICHARD, P., POOLE, M. & RIDOUX, V. 2010. The use of stable isotope analyses from skin biopsy samples to assess trophic relationships of sympatric delphinids off Moorea (French Polynesia). J. Exp. Mar. Biol. Ecol. 395:48-54. http://dx.doi.org/10.1016/j.jembe.2010.08.010

KNOFF, A., HOHN, A. & MACKO, S. 2008. Ontogenetic diet changes in bottlenose dolphins (*Tursiops truncatus*) reflected through stable isotopes. Mar. Mamm. Sci. 24:128-37. http://dx.doi.org/10.1111/j.1748-7692.2007.00174.x

KURLE, C. & GUDMUNDSON, C. 2007. Regional differences in foraging of young-of-the-year Steller sea lions *Eumetopias jubatus* in Alaska: stable carbon and nitrogen isotope ratios in blood. Mar. Ecol. Progr. Ser. 342:303-310. http://dx.doi.org/10.3354/meps342303

LAILSON-BRITO, J., DORNELES, P.R., AZEVEDO-SILVA, C.E., AZEVEDO, A.F., VIDAL, L.G., ZANELATTO, R.C., LOZINSKI, C.P., AZEREDO, A., FRAGOSO, A.B., CUNHA, H.A., TORRES, J.P. & MALM, O. 2010. High organochlorine accumulation in blubber of Guiana dolphin, *Sotalia guianensis*, from Brazilian coast and its use to establish geographical differences among populations. Environ. Pollut. 158:1800-1808. PMid:19932538. http://dx.doi.org/10.1016/j.envpol.2009.11.002

MARTÍNEZ DEL RIO, C., WOLF, N., CARLETON, S.A. & GANNES, L.Z. 2009. Isotopic ecology tem years after a call for more laboratory experiments. Biol. Rev. 84:91-111.

McCUTCHAN, J.H., LEWIS, W.M., KENDALL, C. & McGRATH, C.C. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102:378-390.

MÉNDEZ-FERNANDEZ, P., BUSTAMANTE, P., BODE, A., CHOUVELON, T., FERREIRA, M., LÓPEZ, A., PIERCE, G.J., SANTOS, B., SPITZ, J., VINGADA, J. & CAURANT, F. 2012. Foraging ecology of five toothed whale species in the Northwest Iberian Peninsula, inferred using δ13C and δ15N isotopic signatures. J. Exp. Mar. Biol. Ecol. 413:150-158. http://dx.doi.org/10.1016/j.jembe.2011.12.007

NEWSOME, S.D., MARTINEZ DEL RIO, C., BEARHOP, S. & PHILLIPS, D.L. 2007. A niche for isotopic ecology. Front. Ecol. Environ. 5:429-436.

NEWSOME, S.D., CLEMENTZ, M.T. & KOCH, P.L. 2010. Using stable isotope biogeochemistry to study marine mammal ecology. Mar. Mamm. Sci. 26:509-572.

NEWSOME, S., ETNIER, M., MONSON, D. & FOGEL, M. 2009. Retrospective Characterization of 606 Ontogenic Shifts in Killer Whale Diets via d 13c and d 15n Analysis of Teeth. Mar. Ecol. Progr. Ser. 374: 229–242.

OHIZUMI, H. & M. NOBUYUKI, P.L. 2010. Differences in stable isotope ratios of Dall's porpoises (*Phocoenoides dalli*) between coastal and oceanic areas of the North Pacific. Fish. Oceanogr. 19:257-261. http://dx.doi.org/10.1111/j.1365-2419.2010.00547.x

OLIVEIRA, M.R. 2003. Ecologia alimentar de *Sotalia guianensis* e *Pontoporia blainvillei* (Cetacea, Delphinidade e Pontoporiidae) no litoral sul do Estado de São Paulo e litoral do Paraná. Dissertação de Mestrado, Universidade Federal do Paraná, Curitiba.

OLIN, J.A., FAIR, P.A., RECKS, M.A., ZOLMAN, E., ADAMS, J. & FISK, A.T. Unique seasonal forage bases within a local population of bottlenose dolphin (Tursiops truncatus). 2012. Mar. Mamm. Sci. 28:28-40. http://dx.doi.org/10.1111/j.1748-7692.2011.00470.x

OWEN, K., CHARLTON-ROBB, K. & THOMPSON, R. 2011. Resolving the trophic relations of cryptic species: An example using stable isotope analysis of dolphin teeth. PLoS ONE6:e16457. PMid:21364748 PMCid:PMC3041760. http://dx.doi.org/10.1371/journal.pone.0016457

PAYO-PAYO, A., RUIZ, B., CARDONA, L. & BORRELL, A. 2013. Effect of tissue decomposition on stable isotope signatures of striped dolphins *Stenella coeruleoalba* and loggerhead sea turtles *Caretta caretta*. Aquat Biol 18:141-147. http://dx.doi.org/10.3354/ab00497

PINELA, A., BORRELL, A., CARDONA, L. & AGUILAR, A. 2010. Stable isotope analysis reveals habitat partitioning among marine mammals off the NW African coast and unique trophic niches for two globally threatened species. Mar. Ecol. Progr. Ser. 416:295-306. http://dx.doi.org/10.3354/meps08790

PINHEIRO, L. & CREMER, M.J. 2003. Etnoecologia e captura acidental de golfinhos (Cetacea: Pontoporidae e Delphinidae) na Baía de Babitonga, Santa Catarina. Desenvolvimento e Meio Ambiente 8:69-75.

POST, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703-718.

POST, D.M., LAYMAN, C.A., ARRINGTON, D.A., TAKIMOTO, G., QUATTROCHI, J. & MONTAÑA, C.G. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152(2):179-189.

REEVES, R.R., CRESPO, E.A., DANS, JEFFERSON, T.A., KARCZMARSKI, L., LAIDRE, K., O'CORRY-CROWE, G., PEDRAZA, S., ROJAS-BRACHO, L., SECCHI, E.R., SLOOTEN, E., SMITH, B.D., WANG, J.Y. & ZHOU, K. 2008. *Sotalia fluviatilis*. IUCN Red List of Threatened Species 2009. http://www.iucnredlist.org (ultimo acesso em 03/12/2012).

REEVES, R., DALEBOUT, M., JEFFERSON, T.A., KARKZMARSKI, L., LAIDRE, K., O'CORRY-CROWE, G., ROJAS-BRACHO, L., SECCHI, E., SLOOTEN, E., SMITH, B.D., WANG, J.Y., ZERBINI, A.N. & ZHOU, K. 2012. *Pontoporia blainvillei*. In IUCN Red List of Threatened Species (International Union for Conservation of Nature). version 2012.2. www.iucnredlist.org. (ultimo acesso em 03/12/2012).

ROBBINS, C.T., FELICETTI, L.A. & SPONHEIMER, M. 2005. The effects dietary protein quality on nitrogen isotope discrimination in mammals and birds. Oecologia 144:534-540.

ROBBINS, C.T., FELICETTI, L.A. & FLORIN, S.T. 2010. The impact of protein quality on stable nitrogen isotope ratio discrimination and assimilated diet estimation. Oecologia 162:571-579

SANTOS, M.C.O., ROSSO, S., SANTOS, R.A., LUCATO, S.H.B. & BASSOI, M. 2002. Insights on small cetacean feeding habits in southeastern Brazil. Aquatic Mammals 28:38-45.

SECCHI, E.R., OTT, P.H. & DANILEWICZ, D. 2003. Effects of fishing bycatch and the conservation status of the Franciscana dolphin, *Pontoporia blainvillei*. In Marine Mammals: Fisheries, Tourism and Management Issues Pages. (Gales, M. Hindell & R. Kirkwood, eds) N. SCIRO Publishing, Collingwood, p.174-191.

SOUZA, L.M. & CHAVES, P.T. 2007. Reproductive activity of fish (Teleostei) and closed season to shrimp trawling off the northern coast of Santa Catarina, Brazil. Brazil. Braz. J. Zool. 24:1113-1121. http://dx.doi.org/10.1590/S0101-81752007000400031

SWEETING, C.J., POLUNIN, N.V.C. & JENNINGS, S. 2006. Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. Rapid Comm. Mass Spectrom. 20:595-601.

VANDERKLIFT, M.A., & PONSARD, S. 2003. Sources of variation in consumer-diet delta ^{15}N enrichment: a meta-analysis. Oecologia 136:169-182. PMid:12802678. http://dx.doi.org/10.1007/s00442-003-1270-z

VANDER ZANDEN, M.J., & RASMUSSEN, J.B. 2001. Variation in d15N and d13C trophic fractionation: Implications for aquatic food web studies. Limnol. Oceanogr. 46: 2061-2066. http://dx.doi.org/10.4319/lo.2001.46.8.2061

VANDER ZANDEN, M.J., & FETZER, W.W. 2007. Global patterns of aquatic food chain length. Oikos 116:1378-1388. http://dx.doi.org/10.1111/j.0030-1299.2007.16036.x

YOGUI, G.T., SANTOS, M.C.O., BERTOZZI, C.P. & MONTONE, R.C. 2010. Levels of persistent organic pollutants and residual pattern of DDTs in small cetaceans from the coast of São Paulo, Brazil. Marine Poll. Bull. 60:1862-1867. PMid:20699191. http://dx.doi.org/10.1016/j.marpolbul.2010.07.022

ZALESKI, T. 2005. Posição sistemática e dinâmica populacional da lula *Lolliguncula brevis* (Mollusca: Cephalopoda). Dissertação de Mestrado, Universidade Federal do Paraná, Curitiba.