



## Potential biases in dietary interpretation derived from stable isotope analysis of small dolphin teeth

Genyffer C. Troina<sup>a,b,c,\*</sup>, Clarissa R. Teixeira<sup>d</sup>, Frank Dehairs<sup>b</sup>, Eduardo R. Secchi<sup>a</sup>,  
Silvina Botta<sup>a</sup>

<sup>a</sup> Laboratório de Ecologia e Conservação da Megafauna Marinha, Instituto de Oceanografia, Universidade Federal do Rio Grande (FURG), Av. Itália, km 8, Rio Grande, RS, 96203-900, Brazil

<sup>b</sup> Analytical, Environmental and Geo-Chemistry Department (AMGC), Vrije Universiteit Brussel (VUB), B-1050, Brussels, Belgium

<sup>c</sup> Institute for the Oceans and Fisheries, University of British Columbia (UBC), V6T 1Z4, Vancouver, BC, Canada

<sup>d</sup> Whale Habitat, Ecology and Telemetry Laboratory, Marine Mammal Institute, Oregon State University, Newport, OR, USA

### ARTICLE INFO

#### Keywords:

Small cetaceans  
Franciscanas  
*Pontoporia blainvillei*  
Stable isotopes  
Growth layer groups  
Mixing models

### ABSTRACT

We analyzed  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in different tooth portions (Growth Layer Groups, GLGs) of franciscanas, *Pontoporia blainvillei*, to investigate their effect on whole tooth (WT) isotopic values and the implications for dietary estimates. Tooth portions included the dentin deposited during the prenatal development (PND), the first year of life (GLG<sub>1</sub>) deposited during the nursing period and the central part of the tooth with no distinction amongst subsequent GLGs (Center). Isotopic mixing models estimating the contribution of PND, GLG<sub>1</sub> and Center to WT showed that GLG<sub>1</sub> has a strong effect on WT isotope values in juveniles, while Center only starts to affect WT isotopic values from age four. Isotopic mixing models estimating prey contribution to the diet of juveniles using WT vs Center tooth portions significantly differed in dietary outputs, demonstrating that GLG<sub>1</sub> influence on WT isotope values affects dietary estimates in young franciscanas. As the small tooth size and narrowness of the last GLGs hinder the analysis of individual layers, we recommend excluding GLG<sub>1</sub> in studies based on teeth isotope composition in franciscanas and caution when interpreting isotopic values from the WT of other small cetaceans.

### 1. Introduction

Stable isotope analysis (SIA) is commonly applied in studies investigating the feeding habits, foraging areas, and movement patterns of mobile and elusive organisms like marine mammals (e.g., Kiszka et al., 2014; Troina et al., 2020, 2021; Busquets-Vass et al., 2021). This method is based on the principle that the naturally occurring variation in the stable isotope composition observed in consumer tissues reflects the isotopic composition of their assimilated prey. Variation in carbon stable isotopes ( $\delta^{13}\text{C}$ ) reflects the sources of primary production (e.g., phytoplankton vs macroalgae) that fuel the habitats where consumers forage (e.g., inshore vs offshore) (Trueman and Glew 2019), whereas nitrogen stable isotopes ( $\delta^{15}\text{N}$ ) are commonly used as proxies for their trophic positions (Minagawa and Wada, 1984; Post, 2002).

The analysis of the isotopic composition of metabolically inert but continuously growing tissues (e.g., baleen plates, tooth dentin, vibrissae) provides temporally ordered ecological information over

different timescales (Newsome et al., 2009; Busquets-Vass et al., 2017; Botta et al., 2018). The dentin in odontocetes, for example, comprises most of the tooth and is chronologically deposited in annual layers, known as Growth Layer Groups (GLGs; Perrin and Myrick, 1980), that fill in the pulp cavity. Temporal and ontogenetic dietary shifts at the individual level can be recorded by subsampling these sequential layers (e.g., Mendes et al., 2007; Knoff et al., 2008; Feyrer et al., 2020), while sampling an entire tooth provides an integrated and unweighted average of the diet through their lifetime (e.g., Walker and Macko 1999; Troina et al., 2016).

Whole tooth stable isotope values have been applied to assess ontogenetic variation in prey consumption by franciscanas (*Pontoporia blainvillei*) from southern Brazil (Troina et al., 2016). Nursing calves have higher  $\delta^{15}\text{N}$  values equivalent to feeding at one trophic level higher than their mothers, and hence are often used as an indicator of weaning in marine mammals (Newsome et al., 2006; Matthews and Ferguson 2015; Evacitas et al., 2017). Nevertheless, relatively little variation in

\* Corresponding author. Institute for the Oceans and Fisheries, University of British Columbia (UBC), V6T 1Z4, Vancouver, BC, Canada.

E-mail address: [g.troina@oceans.ubc.ca](mailto:g.troina@oceans.ubc.ca) (G.C. Troina).

<https://doi.org/10.1016/j.marenvres.2022.105857>

Received 26 July 2022; Received in revised form 23 October 2022; Accepted 18 December 2022

Available online 22 December 2022

0141-1136/© 2022 Elsevier Ltd. All rights reserved.

$\delta^{15}\text{N}$  values were found between nursing calves and weaned juveniles (with high  $\delta^{15}\text{N}$  values), while a major shift in  $\delta^{15}\text{N}$  values was only observed between three- and four-year-old individuals (Troina et al., 2016). The authors suggested that this “late” shift might be related to the significantly wider first GLG (GLG<sub>1</sub>) (Pinedo and Hohn 2000) that outweighs the isotopic signal of the subsequent (narrower) GLGs in young franciscanas. Although subsampling the different GLGs provides a finer resolution of the feeding patterns throughout the animals’ lifetime, it is not always feasible for species with small-sized teeth as it may be impossible to individually sample each layer and would likely yield insufficient material for isotopic analysis of that specific GLG. Therefore, understanding how individual GLGs may affect whole tooth isotopic values is critical for studies using this methodology to investigate the dietary habits of small odontocetes such as the franciscana dolphin, as well as potential implications on the assessment of ontogenetic dietary shifts.

Because of their coastal distribution, franciscanas are particularly vulnerable to human activities, and interactions with fisheries are frequent in different locations along their geographic range (e.g., Secchi et al., 1997, 2021; Di Benedetto et al., 2011; Franco-Trecu et al., 2009; Negri et al., 2016). The incidental capture of non-target species during fishing operations is the most widespread threat to cetaceans (Reeves et al., 2013; Brownell et al., 2019). Bycatch of small cetaceans often involve young and sexually immature individuals (e.g., Slooten 1991; Read and Hohn 1995; Hohn et al., 1996; Brown et al., 2014). High capture of young individuals has also been reported for franciscana dolphins, with 70–80% of the total fishing-related mortality events represented by individuals younger than four-years-old, i.e., before they have reached sexual maturity (e.g., Secchi et al., 1997, 2003a; Ramos et al., 2000; Rosas et al., 2002; Franco-Trecu et al., 2009; Negri et al., 2016). Given the high proportion of young franciscanas by-caught in fisheries, accurate assessment of their foraging behavior may help to understand this interaction with human activities. For instance, information on the foraging strategies of young franciscanas may help to assess whether important prey species or size ranges consumed are also targeted and/or overexploited by local fisheries. Knowledge on the feeding ecology of young franciscanas may not only inform on population dynamics, but also help to elucidate how fishing activities can influence their diet, supporting the elaboration and implementation of effective actions for the conservation of this threatened coastal species and their ecosystems.

Here, we analyzed the isotopic values in different portions of franciscanas’ teeth to investigate why high  $\delta^{15}\text{N}$  values that represent the nursing period of calves are still observed in juveniles (between 1 and 3-years-old), long after they had been weaned (Troina et al., 2016). Our aim was to evaluate the isotopic composition in each portion of franciscanas’ teeth to assess their potential influence on data interpretation using this type of samples in trophic ecology studies. For this, we compared the whole tooth, the GLG deposited during the prenatal development (PND), the first year of life (GLG<sub>1</sub>) and the central portion of the tooth of franciscanas to 1) determine the influence of the GLG<sub>1</sub> to the whole tooth (WT) isotopic values of juveniles vs. adults; and 2) assess the influence of the GLG<sub>1</sub> isotopic values on their dietary estimates. Understanding the effect of the GLG<sub>1</sub> in WT stable isotopes is critical for the correct assessment of juveniles’ feeding habits using this methodology, and could be extended to dietary studies using stable isotope analysis in tooth samples of other small odontocete species.

## 2. Material and methods

### 2.1. Sample collection

Teeth from the central portion of the mandible or maxilla were collected from franciscanas that were by-caught by the fishing fleet operating in Rio Grande, southern Brazil (32°S, 52°W) between 1994 and 2004. Each tooth was cleaned with distilled water and stored dry to

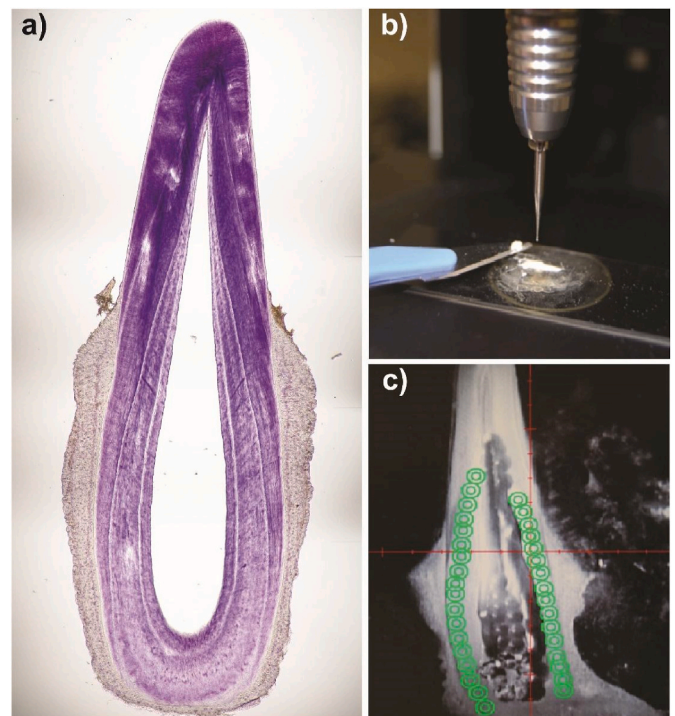
be used for age estimation and for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measurements.

### 2.2. Age estimation

We estimated the age of individuals by counting the GLGs in the dentin and cement of thin, mid-longitudinal sections of teeth. The method described by Pinedo and Hohn (2000) includes the fixation of the whole tooth in 10% formalin, and decalcification in a rapid decalcifier solution (RDO®, Apex Engineering Products Corporation, Aurora, IL). Once teeth were flexible and transparent, they were washed with running water, and then cut into 25  $\mu\text{m}$  thin sections using a freezing microtome. We stained the resulting sections in Mayer’s hematoxylin, submerged them in a weak ammonia solution for a few seconds to intensify the coloration, and mounted them on microscope slides with 100% glycerin (Fig. 1A). Each GLG represents one year of age (Kasuya and Brownell 1979; Pinedo and Hohn 2000). We then selected samples to carry out stable isotope analysis in specimens of different age categories representing different stages of sexual maturity: Age class 1, which includes juveniles (one-to three-years-old individuals) and age class 2, which includes adults (individuals older than four years-old).

### 2.3. Stable isotope analysis

To prepare teeth for stable isotope analysis, we grounded the whole tooth (WT) of 21 franciscanas to a fine homogeneous powder, exposed to a vaporous 30% hydrochloric acid (HCl) overnight inside a desiccator to remove the inorganic carbon, and dried at 60 °C for 4 h. As different teeth within a single individual have similar isotopic values (Walker et al., 1999), we glued another tooth from each dolphin to a glass slide and polished with successive sheets of sandpaper with decreasing grit sizes (from medium coarse - 100/150 to ultra-fine - 320) to expose the central portion and collect within each dentin GLG. We then sampled dentin powder by using a high-resolution drilling system (Merchantek®



**Fig. 1.** a) Stained 25  $\mu\text{m}$  thin section of a two-year-old franciscana, *Pontoporia blainvillei*, for age estimation; b) collecting tooth powder samples from a franciscana tooth using the MicroMill System; and c) first Growth Layer Group (GLG<sub>1</sub>) of a franciscana tooth marked for sample collection, after tooth powder from the Center tooth portion had been extracted.

MicroMill System) (Fig. 1B–C) fitted with a 300  $\mu\text{m}$  drill bit at a depth of approximately 500  $\mu\text{m}$ . We milled tracks in dentin deposited during the prenatal development (PND), the first year of life (GLG<sub>1</sub>) and the central portion of the tooth (excluding the PND and GLG<sub>1</sub>) (Center), which included different number of GLGs, according to the age of the individual. We placed  $\sim 2$  mg of dentin powder into silver cups for acidification (30% vaporous HCl) and oven drying. Additionally, a portion ( $n = 12$ ) of tooth samples (GLG<sub>1</sub>, Center and WT) was double analyzed, acidified and non-acidified, to assess the effect of acidification on stable isotope values.

Isotopic measurements were carried out at the Analytical, Environmental and Geo-Chemistry laboratory at the Vrije Universiteit Brussel (AMGC, VUB, Brussels) using an Elemental Analyzer (EuroEA 3000, EuroVector, Milano, Italy) coupled to an Isotope Ratio Mass Spectrometer (Delta V Plus, Thermo Electron Corporation). Results are expressed in the delta notation ( $\delta$ ) as part per mil (‰) using the equation:  $\delta_{\text{sample}} = [\text{R}_{\text{sample}}/\text{R}_{\text{standard}} - 1] * 1000$ , where  $\text{R}_{\text{sample}}$  and  $\text{R}_{\text{standard}}$  are the  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  ratios of the sample and standard, respectively (Peterson and Fry 1987). The standards are Vienna Pee Dee Belemnite limestone (VPDB) for  $\delta^{13}\text{C}$  and atmospheric  $\text{N}_2$  for  $\delta^{15}\text{N}$ . The within-run analytical precision, measured as the standard deviation of two in house lab standards, analyzed after every 10 samples was less than 0.1‰ for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .

#### 2.4. Statistical analysis

We applied linear mixed-effects models to assess isotopic differences among the different tooth portions (WT, PND, GLG<sub>1</sub> and Center), allowing to account for both random (individual franciscanas) and fixed (tooth portion) effects. We used the lmerTest package to fit the models in R (Kuznetsova et al., 2017). Additionally, to compare the isotopic values in tooth portions, we estimated smooth curves of the distribution of isotopic values using the Kernel density method. All statistical analyses were carried out separately for age classes 1 (one-to three-years-old) and 2 (older individuals). Lastly, to assess how the acid-treatment affected the stable isotope values of GLGs and whole tooth samples, we applied a pair-wise Student's t-test comparing the isotopic values between acidified and non-treated samples. We used the non-parametric Wilcoxon-Mann-Whitney test when data were not fit for parametric tests.

#### 2.5. Isotope mixing models

We estimated the proportional contribution of different tooth portions (PND, GLG<sub>1</sub> and Center) to WT isotopic composition using the Stable Isotope Mixing Models in R package (SIMMR; Parnell, 2016). Isotope mixing models use the isotopic mean  $\pm$  SD values of the sources (tooth portion) to estimate their proportional contribution to a mixture (WT). The source data for this Bayesian model included the mean  $\pm$  SD  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in PND ( $n = 13$ ), GLG<sub>1</sub> ( $n = 21$ ) and Center ( $n = 14$ ) portions of franciscanas' tooth dentin. We have not collected PND in adult franciscanas. However, as PND is deposited during fetal development phase, we assumed that it would have similar isotopic values for both juveniles and adults. We ran separate mixing models for each age class.

To estimate the proportional contribution of prey items to the diet of young franciscanas (two- and three-year-old) we applied isotopic mixing models using only the isotopic values of the central tooth portion, and compared these with estimates based on the isotopic values of the whole tooth for the same individuals. We used the isotopic values of prey items from Troina et al. (2016) and ran the mixing models with a diet-to-tissue discrimination factor of 4.0‰ for  $\delta^{13}\text{C}$ , based on muscle-to-collagen fractionation (Koch 2007), and 3.4‰ for  $\delta^{15}\text{N}$  (Post, 2002). These discrimination factors were selected after testing a range of different values in mixing models to estimate the diets of franciscanas from southern Brazil (Troina et al., 2016).

### 3. Results

#### 3.1. Effect of acidification on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values

Both  $\delta^{13}\text{C}$  ( $t = 2.99$ ,  $df = 11$ ,  $p < 0.05$ ) and  $\delta^{15}\text{N}$  ( $t = -6.03$ ,  $df = 11$ ,  $p < 0.0001$ ) values were significantly different between acidified and non-treated samples. Acidified tooth samples had  $\delta^{13}\text{C}$  values 1.3‰ higher, on average, than non-acidified samples (see supplementary data S1). This was an unexpected outcome that will be discussed in section 4.1. In contrast,  $\delta^{15}\text{N}$  values showed an opposite trend and were  $\sim 0.5$ ‰ higher in the non-treated samples. The C:N ratio was significantly higher ( $p < 0.001$ ) in non-treated samples ( $3.8 \pm 0.5$ ) than in treated samples ( $3.2 \pm 0.06$ , supplementary data S1). When considering only dentin tooth portions (PND, GLG<sub>1</sub> and Center), treated samples were 1.8‰ higher than acidified samples, whereas WT samples barely differed (supplementary data S1). This indicated that WT sample decalcification was incomplete, which was supported by the  $\delta^{13}\text{C}$  values in the overall WT samples that were  $\sim 1$ ‰ higher than in the GLG<sub>1</sub> and the Center tooth portions (See supplementary data S2). Therefore, we applied a correction to WT carbon isotopes, using a linear regression equation derived from our paired analysis of treated and untreated samples. To model this relationship, we used only the dentin samples (PND, GLG<sub>1</sub> and Center), that we assume have undergone complete decalcification (details in the supplementary information S3). We present measured and corrected WT  $\delta^{13}\text{C}$  values in the supplementary data S2.

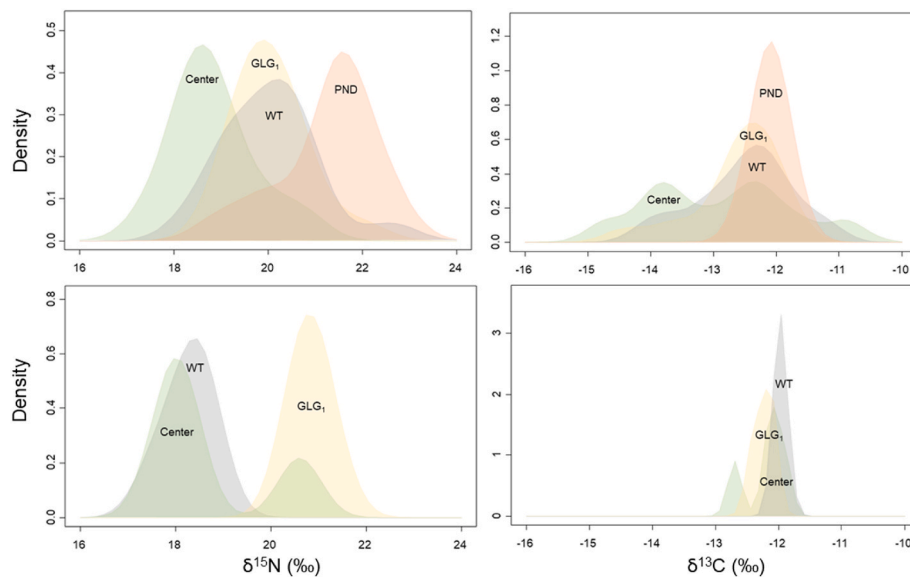
#### 3.2. Isotopic patterns of the different tooth portions

The total number of samples, with mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for each tooth portion (PND, GLG<sub>1</sub> and Center) and for the whole tooth are presented in Table 1 (details are provided in the supplementary data S2). The mixed-effect model in age class 1 (one-to three-years-old) showed significant differences in both  $\delta^{13}\text{C}$  ( $F(3, 38.8) = 4.59$ ,  $p < 0.01$ ) and  $\delta^{15}\text{N}$  ( $F(3, 39.1) = 18.83$ ,  $p < 0.001$ ) values among tooth portions. In this age class,  $\delta^{13}\text{C}$  values in WT were 0.8‰ higher than in the center, but differences between the other tooth portions were not significant. Significant differences in  $\delta^{15}\text{N}$  values were observed among tooth portions, with  $\delta^{15}\text{N}$  in the WT (20.0‰), GLG<sub>1</sub> (20.0‰), and PND (21.3‰) being between 1 and 2‰ higher than those in the Center (18.8‰). In age class 2 (i.e., > four years of age), models also showed significant differences in  $\delta^{15}\text{N}$  among tooth portions. However, in this age class GLG<sub>1</sub> (20.8‰) had higher  $\delta^{15}\text{N}$  than both WT (18.7‰) and Center (18.3‰), whereas the latter two had similar  $\delta^{15}\text{N}$  values (Table 1). The patterns in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values among the different tooth portions for each age class are shown in the Kernel density estimation plots (Fig. 2).

**Table 1**

Sample size (N) and average ( $\pm$  Standard Deviation – SD)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (‰) in each tooth portion: prenatal development (PND), Growth Layer Group 1 (GLG<sub>1</sub>), Center, and in the whole tooth (WT) of individuals for each age class: group 1 (one-to three-years old) and group 2 (>four-years old), franciscana dolphin, *Pontoporia blainvillei*, from southern Brazil.

	N	$\delta^{13}\text{C}$ ( $\pm$ SD)	$\delta^{15}\text{N}$ ( $\pm$ SD)
<b>Age Class 1 (1-to-3-years old)</b>			
PND	13	-12.5 (0.7)	21.3 (1.0)
GLG <sub>1</sub>	17	-12.6 (0.7)	20.0 (0.7)
Center	10	-12.9 (1.1)	18.8 (0.8)
WT	17	-12.1 (0.1)	20.0 (1.0)
<b>Age Class 2 (&gt; 4-years old)</b>			
PND	–	–	–
GLG <sub>1</sub>	4	-12.2 (0.1)	20.8 (0.2)
Center	4	-12.2 (0.3)	18.3 (0.4)
WT	4	-12.0 (0.1)	18.7 (1.1)



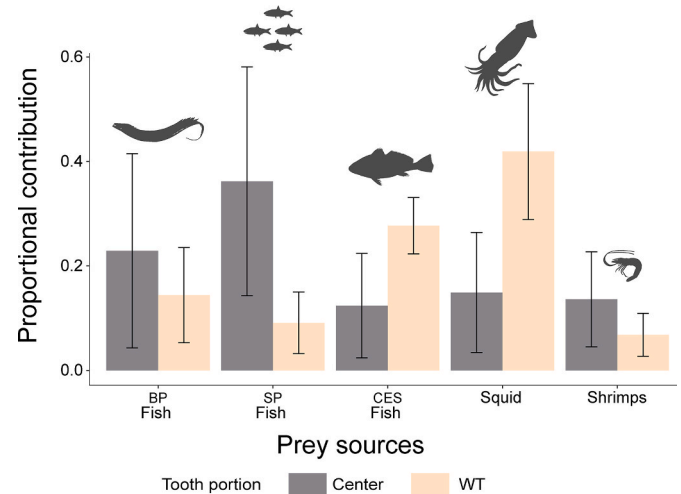
**Fig. 2.** Kernel density estimates of a)  $\delta^{15}\text{N}$  and b)  $\delta^{13}\text{C}$  values for each tooth portion (PND: prenatal dentin;  $\text{GLG}_1$ : Growth Layer Group 1; Center; WT: whole tooth) in age class 1 (one-to three-years-old); and c)  $\delta^{15}\text{N}$  and d)  $\delta^{13}\text{C}$  values in age class 2 (older than 4 yrs old individuals) of franciscanas; No PND was collected for franciscanas from age class 2.

### 3.3. Proportional contribution of tooth portions to whole tooth isotopic values

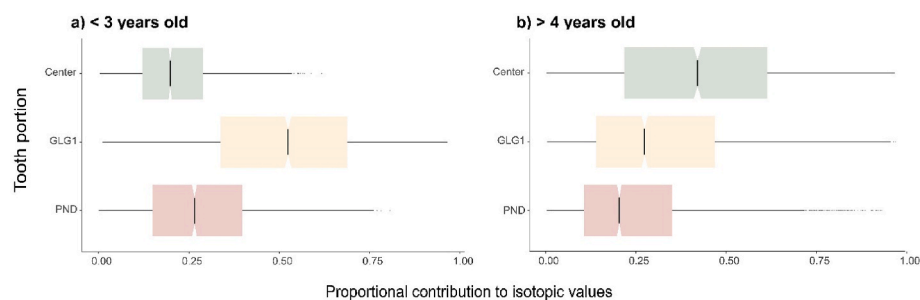
In age class 1, the  $\text{GLG}_1$  contributes up to 51% ( $\pm 0.2$ ) of the isotopic composition of the whole tooth (Fig. 3a). The combined proportional contribution of PND and  $\text{GLG}_1$  was 79%, while the Center portion had a minor contribution of 21% ( $\pm 0.1$ ). In age class 2, the contribution of the Center portion to the whole tooth increased to 42% ( $\pm 0.2$ ), while the contribution of the PND and  $\text{GLG}_1$  decreased to 26% ( $\pm 0.2$ ) and 32% ( $\pm 0.2$ ), respectively (Fig. 3b).

### 3.4. Dietary estimates

We found remarkable differences in the proportional contribution of prey species when models used the isotopic values of the whole tooth in comparison to models based on the isotopic values of the Central tooth portion in juveniles (Fig. 4). The contribution of benthopelagic fish (*Cynoscion guatucupa* and *Trichiurus lepturus*) and shelf-pelagic fish (*Engraulis anchoita* and *Anchoa marmorata*) increased respectively, from 14% and 9%, to 23% and 36%. The importance of squids decreased from 42% to only 15%. The importance of coastal-estuarine fish (*Paralichthys brasiliensis*) decreased from 28% to 12%, and shrimps' contribution to juveniles' diet increased from 6% to 14% in the analysis excluding  $\text{GLG}_1$  isotopic values.



**Fig. 4.** Estimated proportional contribution of prey sources to the diet of juvenile franciscanas (*Pontoporia blainvillei*) based on carbon and nitrogen stable isotope values from the whole tooth (WT) and the Center. Prey groups: BP Fish (*C. guatucupa* and *T. lepturus*) = benthopelagic fish; SP Fish (*E. anchoita* and *A. marmorata*) = shelf-pelagic fish; CES Fish (*P. brasiliensis*) = coastal-estuarine fish; Squids (*D. sanpaulensis*) and Shrimps (*P. muelleri* and *A. longinaris*).



**Fig. 3.** Estimated proportional contribution of the different tooth portions, prenatal development (PND), Growth Layer Group 1 ( $\text{GLG}_1$ ) and Center to the whole tooth isotopic values in a) age class 1 (<math>< 3</math>-years-old) and b) age class 2 (>4-years-old) franciscanas, *Pontoporia blainvillei*. Boxplot values represent the 25%, 50% and 75% credible intervals.

#### 4. Discussion

Our isotopic data and mixing model analysis have helped to confirm that GLG<sub>1</sub> has a major contribution to the isotopic composition of the whole tooth in juveniles, whereas its contribution to whole tooth isotopic values decreases in older individuals. Additionally, we found differences in the estimated contributions of prey sources when juvenile franciscana isotopic values include the whole tooth or only the central portion (thus removing the influence of GLG<sub>1</sub>). These findings have important implications for studies using whole tooth isotopic values to assess the feeding habits of this small cetacean species, especially affecting dietary estimates for juveniles.

##### 4.1. The effect of acidification and $\delta^{13}\text{C}$ patterns among tooth portions

The lower C:N ratios observed in treated samples suggest that inorganic carbon (i.e., carbonates) have been removed. When comparing the effects of the acidification treatment in the different tooth portions,  $\delta^{13}\text{C}$  values in untreated samples were on average 1.3‰ lower than in acidified samples. Controlled feeding experiments have shown that, within the same individual, carbonates have higher  $\delta^{13}\text{C}$  values than collagen (e.g., DeNiro and Epstein 1978; Krueger and Sullivan 1984; Lee-Thorp et al., 1989). Therefore, we would expect to see lower  $\delta^{13}\text{C}$  values after acid treatment, which is the opposite to what we observed. The differences in  $\delta^{13}\text{C}$  between collagen and carbonates depend on the amount of dietary protein vs. dietary energy (e.g., lipids and carbohydrates) available for the consumer (Ambrose and Norr 1993). The organic portion of tooth dentin is mainly composed of protein collagen (Linde 1989; Marshall et al., 1997), with a small fraction of lipids and other elements (Wentrup-Byrne et al., 1997; Loch et al., 2014). Carbon isotopes in dentin collagen are mostly derived from dietary protein, while the inorganic portion (i.e., biological apatite) reflects the carbon derived not only from protein, but also from lipids and carbohydrates in the consumed diet (Ambrose and Norr 1993; Tieszen and Fagre 1993; Sponheimer and Lee-Thorp 2015). As a result, carbon derived from a lipid rich diet may be underrepresented when analyzing only the organic portion of teeth. During the conversion of pyruvate to acetyl coenzymeA in lipid synthesis, the high discrimination against  $^{13}\text{C}$  results in lipids being more  $^{13}\text{C}$ -depleted in comparison to other macromolecular components (DeNiro and Epstein 1977). Thus, the relatively higher  $\delta^{13}\text{C}$  values observed in pure collagen (acidified samples) in comparison to the untreated samples containing the inorganic portion of the tooth may indicate the removal of carbon derived from dietary lipids.

As GLG<sub>1</sub> is synthesized during the nursing period, we would expect to see lower  $\delta^{13}\text{C}$  values reflecting the high lipid content in the milk consumed by the nursing calves (e.g., Hobson and Sease 1998; Elorriaga-Verplancken et al., 2013; Evacitas et al., 2017). Nevertheless, our data showed no differences in  $\delta^{13}\text{C}$  between GLG<sub>1</sub> and Center. The analysis of stable isotopes in the whole tooth of franciscanas between zero and 12-years-old showed that the small variations (<1‰) in  $\delta^{13}\text{C}$  values were not explained by age or ontogenetic classes (Troina et al., 2016). This lack of  $^{13}\text{C}/^{12}\text{C}$  isotopic effect from the nursing period was observed in different odontocete species (Knoff et al., 2008; Newsome et al., 2009; Matthews and Ferguson 2015), and may be due to the lower lipid content in their milk, as discussed by Matthews and Ferguson (2015). The fat content in the milk of franciscanas from southern Brazil during summer and winter months ranges between 8% and 19%, respectively (Caon et al., 2008). As franciscanas' prey include species with highly variable lipid contents (Denuncio et al., 2017), the lack of differences in  $\delta^{13}\text{C}$  between GLG<sub>1</sub> and Center may imply that the fat in milk consumed by nursing calves and in prey consumed in adulthood have comparable concentrations.

##### 4.2. Influence of the different tooth portions on juveniles' whole tooth $\delta^{15}\text{N}$ values

We demonstrate that  $\delta^{15}\text{N}$  values in dentin deposited during the prenatal development and in the first year of franciscanas' life (GLG<sub>1</sub>) were remarkably higher than those of the Center tooth portion. Relatively higher  $\delta^{15}\text{N}$  values in the prenatal dentin in comparison to the dentin deposited later in life has also been observed in Risso's dolphins (*Grampus griseus*) (Evacitas et al., 2017). Such patterns have been attributed to nutritional demands during fetal development that require amino acids derived from the rerouting of maternal tissues and dietary protein. Similarly, as milk is produced using lipids and proteins from the mother's tissues, nursing calves have  $\delta^{15}\text{N}$  values that suggest feeding at a relatively higher trophic level than their mothers (Fogel et al., 1989; Jenkins et al., 2001). In several marine mammal species, juveniles' tissues that are synthesized during the nursing period (e.g., GLG<sub>1</sub>) have significantly higher  $\delta^{15}\text{N}$  values than those of adult females (or tissues that are synthesized later in life) (e.g., Hobson et al., 1997; Niño-Torres et al., 2006; Knoff et al., 2008; Newsome et al., 2006, 2009; Ricciardelli and Goodall 2015; de Albernaz et al., 2017; Evacitas et al., 2017; Zhao et al., 2021). Therefore, a decrease in  $\delta^{15}\text{N}$  values from the early-ages (GLG<sub>1</sub>) to adulthood (Center) is usually associated with changes from a milk-based diet to a diet based on solid food.

In franciscanas, data obtained from stomach contents have shown that calves start the transition from milk to solid food between 2.5 and 3 months of age, and those completely weaned (no trace of milk in their stomachs) were less than nine-months-old (Rodríguez et al., 2002). Accordingly, decreased  $\delta^{15}\text{N}$  values were expected after the calves' first anniversary. Nevertheless,  $\delta^{15}\text{N}$  values measured in the whole tooth of franciscanas from southern Brazil were higher not only in nursing calves but also in juveniles (~20‰), decreasing to approximately 18‰ only in individuals older than four years of age (Troina et al., 2016). By using  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in different tooth portions of franciscanas, we have demonstrated that the isotopic values of both PND and GLG<sub>1</sub> have a great influence on the whole tooth isotope values of juveniles. The proportional contribution of GLG<sub>1</sub> alone was 51% in this age group (Fig. 3). Thus, WT average  $\delta^{15}\text{N}$  values still represent the signal of the nursing period, even long after individuals have been weaned. Our data showed that GLG<sub>1</sub> and central tooth portions affect differently the isotopic values of the whole tooth, and that their influence depends on the individuals' age. In young individuals (Age class 1), the GLG<sub>1</sub> comprises a major portion of the whole tooth, and its  $\delta^{15}\text{N}$  signal predominates over the remaining tooth portions, resulting in similar isotopic values between the whole tooth and GLG<sub>1</sub> (Fig. 2A). However, the influence of this tooth portion in the whole tooth isotopic values decreases in older individuals (Age class 2), when the Center portion dominates over the signals of the PND and the GLG<sub>1</sub> (Fig. 2C). Consequently, the isotopic measurements of juveniles' whole teeth may result in misleading dietary information due to the large contribution of GLG<sub>1</sub> deposited in the first year of life during the nursing period (Troina et al., 2016). The results presented here highlight the importance of excluding the GLG<sub>1</sub> to minimize the influence of signals derived from the nursing period in the  $\delta^{15}\text{N}$  values of juvenile franciscanas' whole tooth. When the whole tooth is the only option for stable isotope measurements, researchers are advised to exclude the juveniles (and not only nursing calves) from analyses.

##### 4.3. Implications for dietary assessments

We applied the same prey isotopic data and trophic discrimination factors in the isotopic mixing models to estimate prey consumption by juvenile franciscana dolphins. However, the proportional contribution of the main prey differed considerably when applying WT isotopic values in comparison to models using only the central tooth portion. For example, the mixing model using WT estimated a diet dominated by squids followed by coastal-estuarine fish (*P. brasiliensis*), whereas the

model using the central tooth portion estimated increased importance of shelf-pelagic and benthic-pelagic fish, followed by a more balanced proportional contribution of the other prey groups (Fig. 4). Dietary estimates using Center tooth portion agree with a more diverse and omnivorous feeding habit for juvenile franciscanas in southern Brazil, as data from previous studies indicate that the species is a generalist opportunistic predator (e.g., Paso-Viola et al., 2014; Campos et al., 2020; Bassoi et al., 2020, 2021; Botta et al., 2022). Additionally, excluding the influence of the isotopic values of GLG<sub>1</sub> in juveniles resulted in increased contribution of the benthic-pelagic prey *C. guatucupa* and *T. lepturus*, which is more aligned with the reported diets based on stomach contents for this species in southern Brazil (Bassoi et al., 2021).

The most important prey type estimated using WT stable isotopes was the squids. This pattern is similar to those observed in a much larger dataset to assess the ontogenetic feeding habits of franciscanas from southern Brazil using the isotopic values of the whole tooth, which have estimated a proportional contribution of >80% of squids in the diet of juvenile franciscanas (Troina et al., 2016). Dietary studies based on stomach content analysis of franciscanas from southern Brazil have identified that adults consume more cephalopods (e.g., *Doryteuthis sanpaulensis*) than juveniles, whereas juveniles consume small fish prey that are more abundant and easier to catch (Bassoi et al., 2021). Unlike soft bodied prey or fish otoliths that have fast digestibility rates (Sekiguchi and Best 1997; Bassoi et al., 2019), squid beaks take longer to be digested, and may actually be overestimated in studies based on stomach content analysis (Bowen and Iverson, 2013). Thus, it is unlikely that dietary studies based on stomach content data would be missing this type of prey for franciscanas from southern Brazil, thereby resulting in an underestimation of squids. This inconsistency between these two approaches to assess juveniles' diet (Troina et al., 2016) could only be noticed due to the large number of stomachs that have been analyzed from individuals of this population (e.g., Botta et al., 2022). The franciscanas analyzed in the present study correspond to the Franciscana Management Area (FMA) III (Secchi et al., 2003b), a stock whose feeding ecology has been extensively studied using stomach contents (Bassoi et al., 2020, 2021; Botta et al., 2022). Conversely, there are only a few dietary studies using stomach contents of franciscanas from other subpopulations, especially those from the northern portion of the species distributional range (i.e., FMAs Ia and Ib) (Botta et al., 2022). Therefore, the assessment of the feeding ecology of these franciscanas' stocks may rely more strongly on isotopic data from teeth of dead animals.

The significant difference in the estimated diet for juvenile franciscanas using mixing models with isotope data from WT versus the Center tooth portion demonstrates the relevance of our study. In the case of this endangered species, mainly threatened by fisheries bycatch, isotopic data pointing to a diet dominated by squids and the coastal estuarine fish *P. brasiliensis* could imply different conservation management strategies, as these prey types are not targeted by the fisheries in southern Brazil. On the other hand, the benthic-pelagic prey *C. guatucupa* is amongst the most fished sciaenid species in southern Brazil, which represent a great proportion of the total fish landings and the species is currently considered overexploited (Haimovici and Cardoso 2017). The correct understanding of franciscanas' feeding habits and prey preference is important to quantify their interactions with fisheries, especially when it concerns juvenile franciscanas that are more vulnerable to bycatch (Rosas et al., 2002; Secchi et al., 2003a, 2021). This information is key to improve conservation efforts and to inform management actions at local scales, such as creating no fishing zones (e.g., Prado et al., 2021), regulating the size range of fish targeted or the type of gear to be used. In this regard, our findings have strong implications for dietary studies using stable isotopes in juvenile franciscanas, and might be extended to other odontocete species with small tooth size.

## 5. Concluding remarks

Our isotopic approach allowed us to demonstrate that the GLG<sub>1</sub>, which is the dentin layer deposited during the nursing period, influences the average isotope values in the whole tooth affecting dietary interpretations of young franciscana dolphins. The effect of GLG<sub>1</sub>  $\delta^{15}\text{N}$  values on the whole tooth only starts to decrease in older individuals (> than 3 years), as more dentin from the subsequent GLGs is deposited. As the small tooth size and narrowness of the last GLGs hinder the analysis of each layer alone, we recommend that future studies aiming to assess the foraging strategies of young franciscanas by using isotopic analysis avoid the use of the whole tooth. Additionally, we call the attention of researchers that use this methodology to study the feeding ecology of other small odontocetes, such as porpoises or small delphinids, as their small tooth size could result in similar isotopic biases. Instead, researchers should exclude the GLG<sub>1</sub>, especially in the case of young individuals or, alternatively, use soft tissues with faster turnover rates such as skin or muscle (Teixeira et al., 2022).

## Authors' contribution

Genyffer C. Troina: Conceptualization, Funding acquisition, Methodology, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review and editing.

Clarissa R. Teixeira: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review and editing.

Frank Dehairs: Conceptualization, Funding acquisition, Investigation, Writing – review and editing.

Eduardo R. Secchi: Conceptualization, Funding acquisition, Investigation, Writing – review and editing.

Silvina Botta: Conceptualization, Funding acquisition, Investigation, Writing – review and editing.

## Funding

This work was supported by Cetacean Society International, and the National Council for Scientific and Technological Development, CNPq (Edital Universal – grant no.425890/2018–0; 315365/2020–0; PQ 310597/2018–8, PQ 315365/2020–0).

## Availability of data and material

All data generated and analyzed during this study are included in this published article and its supplementary information files.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

All data generated and analyzed during this study are included in this published article and its supplementary information files

## Acknowledgements

We thank the students and researchers from the Laboratory of Ecology and Conservation of Marine Megafauna (Laboratório de Ecologia e Conservação da Megafauna Marinha, EcoMega), Universidade Federal do Rio Grande. We are grateful to Yaqu Pacha NGO (Germany) for its long-term financial support. We also thank the researchers at the Analytical, Environmental and Geochemistry Department, Vrije Universiteit Brussel, especially Dr. Debany F. Batista, Dr. Arnout Roukaerts, and David Verstraeten, for their assistance during lab analysis. This

research was supported by a grant from Cetacean Society International awarded to GCT. The National Council for Scientific and Technological Development (CNPq) provided funds for this study (Edital Universal –grant no. 425890/2018–0), and research grants awarded to SB (315365/2020–0) and ERS (PQ 310597/2018–8). This study is a contribution of the Research Group “Ecologia e Conservação da Megafauna Marinha-ECOMEGA/CNPq”.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2022.105857>.

## References

- Ambrose, S.H., Norr, L., 1993. Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate. In: *Prehistoric Human Bone*. Springer, Berlin, Heidelberg, pp. 1–37.
- Bassoi, M., Shepherd, J.G., Secchi, E.R., 2019. Opportunistic feeding experiment in a captive franciscana (*Pontoporia blainvillei*): assessing gastrointestinal transit time and gastric digestion. *Lat. Am. J. Aquatic Mamm.* 13, 15–22. <https://doi.org/10.5597/lajam00244>.
- Bassoi, M., Shepherd, J.G., Secchi, E.R., Moreno, I.B., Danilewicz, D., 2020. Oceanographic processes driving the feeding ecology of franciscana dolphin off Southern Brazilian coast. *Continental Shelf Res.* 201, 104124. <https://doi.org/10.1016/j.csr.2020.104124>.
- Bassoi, M., Secchi, E.R., Danilewicz, D., Moreno, I.B., Santos, R.A., Shepherd, J.G., 2021. Intrapopulation variation in the diet of franciscana dolphin (*Pontoporia blainvillei*) off southern Brazil. *J. Mar. Biol. Assoc. U. K.* 101, 621–637. <https://doi.org/10.1017/S0025315421000436>.
- Botta, S., Secchi, E.R., Rogers, T.L., Prado, J.H.F., Lima, R.C., Carlini, P., Negrete, J., 2018. Isotopic niche overlap and partition among three Antarctic seals from the Western Antarctic Peninsula. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 149, 240–249. <https://doi.org/10.1016/j.dsr2.2017.11.005>.
- Botta, S., Bassoi, M., Troina, G.C., 2022. Overview of franciscana diet. In: Simoes-Lopes, P.C., Cremer, M. (Eds.), *The Franciscana Dolphin: on the Edge of Survival*, pp. 15–48. <https://doi.org/10.1016/B978-0-323-90974-7.00003-3>.
- Bowen, W.D., Iverson, S.J., 2013. Methods of estimating marine mammal diets: a review of validation experiments and sources of bias and uncertainty. *Mar. Mamm. Sci.* 29, 719–754. <https://doi.org/10.1111/j.1748-7692.2012.00604.x>.
- Brown, S., Reid, D., Rogan, E., 2014. Characteristics of fishing operations, environment and life history contributing to small cetacean bycatch in the northeast atlantic. *PLoS One* 9, e104468. <https://doi.org/10.1371/journal.pone.0104468>.
- Brownell Jr., R.L., Reeves, R.R., Read, A.J., Smith, B.D., others, 2019. Bycatch in gillnet fisheries threatens Critically Endangered small cetaceans and other aquatic megafauna. *Endanger. Species Res.* 40, 285–296. <https://doi.org/10.3354/esr00994>.
- Busquets-Vass, G., Newsome, S.D., Pardo, M.A., Calambokidis, J., Serra-Valente, G., Jacobsen, J.K., Aguñiga-García, S., Gendron, D., 2017. Estimating blue whale skin isotopic incorporation rates and baleen growth rates: implications for assessing diet and movement patterns in mysticetes. *PLoS One* 12, e0177880. <https://doi.org/10.1371/journal.pone.0177880>.
- Busquets-Vass, G., Newsome, S.D., Pardo, M.A., Calambokidis, J., Aguñiga, S., Páez-Rosas, D., Gomez-Gutiérrez, J., Enríquez-Paredes, L.M., Gendron, D., 2021. Isotope-based inferences of the seasonal foraging and migratory strategies of blue whales in the eastern Pacific Ocean. *Mar. Environ. Res.* 163, 105201. <https://doi.org/10.1016/j.marenvres.2020.105201>.
- Campos, L.B., Lopes, X.M., da Silva, E., Santos, M.C.O., 2020. Feeding habits of the franciscana dolphin (*Pontoporia blainvillei*) in south-eastern Brazil. *J. Mar. Biol. Assoc. U. K.* 100, 301–313. <https://doi.org/10.1017/S0025315420000120>.
- Caon, G., Secchi, E.R., Capp, E., Kucharski, L., 2008. Milk composition of franciscana dolphin (*Pontoporia blainvillei*) from Rio Grande do Sul, southern Brazil. *J. Mar. Biol. Assoc. U. K.* 88, 1099–1101. <https://doi.org/10.1017/S0025315408000283>.
- de Albernaz, T.L., Secchi, E.R., de Oliveira, L.R., Botta, S., 2017. Ontogenetic and gender-related variation in the isotopic niche within and between three species of Fur seals (genus *Arctocephalus*). *Hydrobiologia* 787, 123–139.
- DeNiro, M.J., Epstein, S., 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science* 197, 261–263.
- DeNiro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochem. Cosmochim. Acta* 42, 495–506.
- Denuncio, P., Paso Viola, M.N., Machovsky-Capuska, G.E., Raubenheimer, D., Blasina, G., Machado, R., Polizzi, P., Gerpe, M., Cappozzo, H.L., Rodriguez, D.H., 2017. Population variance in prey, diets and their macronutrient composition in an endangered marine predator, the Franciscana dolphin. *J. Sea Res.* 129, 70–79. <https://doi.org/10.1016/j.seares.2017.05.008>.
- Di Benedetto, A.P.M., de 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. <https://doi.org/10.1007/s00227-011-1726-3>.
- Elorriaga-Verplancken, F., Auriolos-Gamboa, D., Newsome, S.D., Martínez-Díaz, S.F., 2013.  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in dental collagen as a proxy for age- and sex-related variation in foraging strategies of California sea lions. *Mar. Biol.* 160, 641–652. <https://doi.org/10.1007/s00227-012-2119-y>.
- Evacitas, F.C., Kao, W.-Y., Worthy, G.A.J., Chou, L.-S., 2017. Annual variability in dentin  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  reveal sex differences in weaning age and feeding habits in Risso's dolphins (*Grampus griseus*). *Mar. Mamm. Sci.* 33, 748–770. <https://doi.org/10.1111/mms.12396>.
- Feyrer, L.J., Zhao, St, Whitehead, H., Matthews, C.J.D., 2020. Prolonged maternal investment in northern bottlenose whales alters our understanding of beaked whale reproductive life history. *PLoS One* 15, e0235114. <https://doi.org/10.1371/journal.pone.0235114>.
- Fogel, M.L., Tuross, N., Owsley, D.W., 1989. Nitrogen Isotope Tracers of Human Lactation in Modern and Archaeological Populations, vol. 88. Carnegie Institution of Washington Yearbook, pp. 111–117.
- Franco-Trecu, V., Costa, P., Abud, C., Dimitriadis, C., Laporta, P., Passadore, C., Szephegyi, M.N., 2009. By-catch of franciscana *Pontoporia blainvillei* in Uruguayan artisanal gillnet fisheries: an evaluation after a twelve-year gap in data collection. *Lat. Am. J. Aquatic Mamm.* 7, 11–22.
- Haimovici, M., Cardoso, L.C., 2017. Long-term changes in the fisheries in the Patos Lagoon estuary and adjacent coastal waters in Southern Brazil. *Mar. Biol. Res.* <https://doi.org/10.1080/17451000.2016.1228978>.
- Hobson, K.A., Sease, J.L., 1998. Stable isotope analyses of tooth annuli reveal temporal dietary records: an example using Steller sea lions. *Mar. Mamm. Sci.* 14, 116–129. <https://doi.org/10.1111/j.1748-7692.1998.tb00694.x>.
- Hobson, K.A., Sease, J.L., Merrick, R.L., Piatt, J.F., 1997. Investigating trophic relationships of pinnipeds in Alaska and Washington using stable isotope ratios of nitrogen and carbon. *Mar. Mamm. Sci.* 13, 114–132. <https://doi.org/10.1111/j.1748-7692.1997.tb00615.x>.
- Hohn, A.A., Read, A.J., Fernandez, S., Vidal, O., Findley, L.T., 1996. Life history of the vaquita, *Phocoena sinus* (phocoenidae, cetacea). *J. Zool.* 239, 235–251.
- Jenkins, S.G., Prtridge, S.T., Stephenson, T.R., Farley, S.D., Robbins, C.T., 2001. Nitrogen and carbon isotope fractionation between mothers, neonates, and nursing offspring. *Oecologia* 129, 336–341. <https://doi.org/10.1007/s004420100755>.
- Kasuya, T., Brownell, R.L., 1979. Age Determination, Reproduction, and Growth of the Franciscana Dolphin, *Pontoporia blainvillei*, vol. 31. Scientific Reports of the Whales Research Institute, pp. 45–67.
- Kiszka, J.J., Méndez-Fernandez, P., Heithaus, M.R., Ridoux, V., 2014. The foraging ecology of coastal bottlenose dolphins based on stable isotope mixing models and behavioral sampling. *Mar. Biol.* 161, 953–961. <https://doi.org/10.1007/s00227-014-2395-9>.
- Knoff, A., Hohn, A., Macko, S., 2008. Ontogenetic diet changes in bottlenose dolphins (*Tursiops truncatus*) reflected through stable isotopes. *Mar. Mamm. Sci.* 24, 128–137. <https://doi.org/10.1111/j.1748-7692.2007.00174.x>.
- Koch, P.L., 2007. Isotopic study of the biology of modern and fossil vertebrates. In: Michener, R., Latja, K. (Eds.), *Stable Isotopes in Ecology and Environmental Science*. Blackwell Publishing, Oxford, U.K, pp. 99–154.
- Krueger, H.W., Sullivan, C.H., 1984. Models for carbon isotope fractionation between diet and bone. In: Turnlund, J.E., Johnson, P.E. (Eds.), *Stable Isotopes in Nutrition*, ACS Symposium Series, vol. 258. American Chemical Society, pp. 205–222.
- Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmerTest package: tests in linear mixed effects models. *J. Stat. Software* 82, 1–26. <https://doi.org/10.18637/jss.v082.i13>.
- Lee-Thorp, J.A., Sealy, J.C., van der Merwe, N.J., 1989. Stable carbon isotope ratio differences between bone collagen and bone apatite, and their relationship to diet. *J. Archaeol. Sci.* 16, 585–599. [https://doi.org/10.1016/0305-4403\(89\)90024-1](https://doi.org/10.1016/0305-4403(89)90024-1).
- Linde, A., 1989. Dentin matrix proteins: composition and possible functions in calcification. *Anat. Rec.* 224, 154–166. <https://doi.org/10.1002/ar.1092240206>.
- Loch, C., Swain, M.V., Fraser, S.J., Gordon, K.C., Kieser, J.A., Fordyce, R.E., 2014. Elemental and chemical characterization of dolphin enamel and dentine using X-ray and Raman microanalyzes (Cetacea: delphinoidae and Inioidea). *J. Struct. Biol.* 185, 58–68. <https://doi.org/10.1016/j.jsb.2013.11.006>.
- Marshall, G.W., Marshall, S.J., Kinney, J.H., Balooch, M., 1997. The dentin substrate: structure and properties related to bonding. *J. Dent.* 25, 441–458. [https://doi.org/10.1016/S0300-5712\(96\)00065-6](https://doi.org/10.1016/S0300-5712(96)00065-6).
- Matthews, C.J.D., Ferguson, S.H., 2015. Weaning age variation in beluga whales (*Delphinapterus leucas*). *J. Mammal.* 96, 425–437. <https://doi.org/10.1093/jmammal/gyv046>.
- Mendes, S., Newton, J., Reid, R., Frantzis, A., Pierce, G., 2007. Stable isotope profiles in sperm whale teeth: variations between areas and sexes. *J. Mar. Biol. Assoc. U. K.* 87, 621–627. <https://doi.org/10.1017/S0025315407056019>.
- Minagawa, M., Wada, E., 1984. Stepwise enrichment of  $^{15}\text{N}$  along food chains: further evidence and the relation between  $\delta^{15}\text{N}$  and animal age. *Geochem. Cosmochim. Acta* 48, 1135–1140. [https://doi.org/10.1016/0016-7037\(84\)90204-7](https://doi.org/10.1016/0016-7037(84)90204-7).
- Negri, M.F., Panebianco, M.V., Denuncio, P., Viola, M.N.P., Rodríguez, D., Cappozzo, H. L., 2016. Biological parameters of franciscana dolphins, *Pontoporia blainvillei*, by-caught in artisanal fisheries off southern Buenos Aires, Argentina. *J. Mar. Biol. Assoc. U. K.* 96, 821–829. [https://doi.org/10.1016/0016-7037\(84\)90204-7](https://doi.org/10.1016/0016-7037(84)90204-7).
- Newsome, S.D., Koch, P.L., Etnier, M.A., Auriolos-Gamboa, D., 2006. Using carbon and nitrogen isotope values to investigate maternal strategies in Northeast Pacific otariids. *Mar. Mamm. Sci.* 22, 556–572. <https://doi.org/10.1111/j.1748-7692.2006.00043.x>.
- Newsome, S.D., Etnier, M.A., Monson, D.H., Fogel, M.L., 2009. Retrospective characterization of ontogenetic shifts in killer whale diets via  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analysis of teeth. *Mar. Ecol. Prog. Ser.* 374, 229–242. <https://doi.org/10.3354/meps07747>.
- Niño-Torres, C.A., Gallo-Reynoso, J.P., Galván-Magaña, F., Escobar-Briones, E., Macko, S.A., 2006. Isotopic analysis of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  “a feeding tale” in teeth of the longbeaked common dolphin, *Delphinus capensis*. *Mar. Mamm. Sci.* 22, 831–846. <https://doi.org/10.1111/j.1748-7692.2006.00065.x>.

- Parnell, A., 2016. Simmr-package A stable isotope mixing model. Version 0.3. <https://cran.r-project.org/web/packages/simmr/>.
- Paso-Viola, M.N., Denuncio, P., Negri, M.F., Rodríguez, D., Bastida, R., Cappozzo, H.L., 2014. Diet composition of franciscana dolphin *Pontoporia blainvillei* from southern Buenos Aires, Argentina and its interaction with fisheries. *Rev. Biol. Mar. Oceanogr.* 49, 393–400.
- Report of the workshop on determination age of Odontocete Cetaceans. In: Perrin, W.F., Myrick Jr., A.C. (Eds.), 1980. Age Determination of Toothed Whales and Sireniacs, *Reports Of the International Whaling Commission*, Special Issue, vol. 3, p. 229.
- Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. *Annu. Rev. Ecol. Evol. Syst.* 18, 293–320.
- Pinedo, M.C., Hohn, A.A., 2000. Growth layer patterns in teeth from the franciscana, *Pontoporia blainvillei*: developing a model for precision in age estimation. *Mar. Mamm. Sci.* 16, 1–27. <https://doi.org/10.1111/j.1748-7692.2000.tb00901.x>.
- Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83, 703–718. [https://doi.org/10.1890/0012-9658\(2002\)083\[0703:USITET\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2).
- Prado, J.H.F., Kinan, P.G., Pennino, M.G., Seyboth, E., Silveira, F.R., Ferreira, E.C., Secchi, E.R., 2021. Definition of no-fishing zones and fishing effort limits to reduce franciscana bycatch to sustainable levels in southern Brazil. *Anim. Conserv.* <https://doi.org/10.1111/acv.12679>.
- Ramos, R.M.A., Di Benedetto, A.P.M., Lima, N.R.W., 2000. Growth parameters of *Pontoporia blainvillei* and *Sotalia fluviatilis* (cetacea) in northern Rio de Janeiro, Brazil. *Aquat. Mamm.* 26, 65–75.
- Read, A.J., Hohn, A.A., 1995. Life in the fast lane: the life history of harbor porpoises from the Gulf of Maine. *Mar. Mamm. Sci.* 11, 423–440. <https://doi.org/10.1111/j.1748-7692.1995.tb00667.x>.
- Reeves, R.R., McClellan, K., Werner, T.B., 2013. Marine mammal bycatch in gillnet and other entangling net fisheries, 1990 to 2011. *Endanger. Species Res.* 20, 71–97. <https://doi.org/10.3354/esr00481>.
- Riccialdelli, L., Goodall, N., 2015. Intra-specific trophic variation in false killer whales (*Pseudorca crassidens*) from the southwestern South Atlantic Ocean through stable isotopes analysis. *Mamm. Biol.* 80, 298–302.
- Rodríguez, D., Rivero, L., Bastida, R., 2002. Feeding ecology of the franciscana (*Pontoporia blainvillei*) in marine and estuarine waters of Argentina. *Lat. Am. J. Aquatic Mamm.* 1, 77–94.
- Rosas, F.C.W., Monteiro-Filho, E.L.A., Oliveira, M.R., 2002. Incidental catches of franciscana (*Pontoporia blainvillei*) on the southern coast of sao paulo state and the coast of paraná state, Brazil. *The Latin American Journal of Aquatic Mammals*, Special Issue 161–168.
- Secchi, E.R., Zerbini, A.N., Bassoi, M., Dalla Rosa, L., Möller, L.M., Rocha-Campos, C.C., 1997. Mortality of franciscanas, *Pontoporia blainvillei*, in coastal gillnetting in southern Brazil: 1994–1995. *Rep. Int. Whal. Comm.* 47, 653–658.
- Secchi, E.R., Ott, P.H., Danilewicz, D., 2003a. Effects of fishing by-catch and conservation status of the franciscana dolphin, *Pontoporia blainvillei*. *Marine mammals: Fisheries, tourism and management issues* 174–191.
- Secchi, E.R., Danilewicz, D., Ott, P.H., 2003b. Applying the phylogeographic concept to identify franciscana dolphin stocks: implications to meet management objectives. *J. Cetacean Res. Manag.* 5, 61–68.
- Secchi, E.R., Cremer, M.J., Danilewicz, D., Lailson-Brito, J., 2021. Synthesis of the ecology, human-related threats and conservation perspectives for the endangered franciscana dolphin. *Front. Mar. Sci.* <https://doi.org/10.3389/fmars.2021.617956>.
- Sekiguchi, K., Best, P., 1997. In vitro digestibility of some prey species of dolphins. *Fish. Bull.* 95, 386–393.
- Slooten, F., 1991. Age, growth and reproduction of Hectorís dolphin. *Can. J. Zool.* 60, 1689–1700. <https://doi.org/10.1139/z91-234>.
- Sponheimer, M., Lee-Thorp, J., 2015. Hominin paleodiets: the contribution of stable isotopes. In: Henke, W., Tattersall, I. (Eds.), *Handbook of Paleoanthropology*. Springer, Berlin, Heidelberg. [https://doi.org/10.1007/978-3-642-39979-4\\_18](https://doi.org/10.1007/978-3-642-39979-4_18).
- Teixeira, C.R., Troina, G.C., Daura-Jorge, F.G., Simões-Lopes, P.C., Botta, S., 2022. A practical guide on stable isotope analysis for cetacean research. *Mar. Mamm. Sci.* 38, 1200–1228. <https://doi.org/10.1111/mms.12911>.
- Tieszen, L.L., Fagre, T., 1993. Effect of diet quality and composition on the isotopic composition of respiratory CO<sub>2</sub>, bone collagen, bioapatite, and soft tissues. In: Lambert, J.B., Grupe, G. (Eds.), *Prehistoric Human Bone*. Springer, Berlin, Heidelberg. [https://doi.org/10.1007/978-3-662-02894-0\\_5](https://doi.org/10.1007/978-3-662-02894-0_5).
- Troina, G., Botta, S., Secchi, E.R., Dehairs, F., 2016. Ontogenetic and sexual characterization of the feeding habits of franciscanas, *Pontoporia blainvillei*, based on tooth dentin carbon and nitrogen stable isotopes. *Mar. Mamm. Sci.* 32, 1115–1137. <https://doi.org/10.1111/mms.12316>.
- Troina, G.C., Botta, S., Dehairs, F., Di Tullio, J.C., Elskens, M., Secchi, E.R., 2020. Skin  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  reveal spatial and temporal patterns of habitat and resource use by free-ranging odontocetes from the southwestern Atlantic Ocean. *Mar. Biol.* 167, 1–19. <https://doi.org/10.1007/s00227-020-03805-8>.
- Troina, G.C., Riekenberg, P., van der Meer, M.T., Botta, S., Dehairs, F., Secchi, E.R., 2021. Combining isotopic analysis of bulk-skin and individual amino acids to investigate the trophic position and foraging areas of multiple cetacean species in the western South Atlantic. *Environ. Res.* 111610 <https://doi.org/10.1016/j.envres.2021.111610>.
- Trueman, C.N., Glew, K.S.J., 2019. Isotopic tracking of marine animal movement. In: Hobson, K.A., Wassenaar, L.I. (Eds.), *Tracking Animal Migration with Stable Isotopes*, pp. 137–172.
- Walker, J.L., Macko, S.A., 1999. Dietary studies of marine mammals using stable carbon and nitrogen isotopic ratios of teeth. *Mar. Mamm. Sci.* 15, 314–334.
- Walker, J.L., Potter, C.W., Macko, S.A., 1999. The diets of modern and historic bottlenose dolphin populations reflected through stable isotopes. *Mar. Mamm. Sci.* 15, 335–350.
- Wentrup-Byrne, E., Armstrong, C.A., Armstrong, R.S., Collins, B.M., 1997. Fourier transform Raman microscopic mapping of the molecular components in a human tooth. *J. Raman Spectrosc.* 28, 151–158.
- Zhao, S.T., Matthews, C.J.D., Davoren, G.K., Ferguson, S.H., Watt, C.A., 2021. Ontogenetic profiles of dentine isotopes ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) reveal variable narwhal *Monodon monoceros* nursing duration. *Mar. Ecol. Prog. Ser.* 668, 163–175. <https://doi.org/10.3354/meps13738>.