Original investigation

# Intra-specific isotope variations of franciscana dolphin *Pontoporia blainvillei* regarding biological parameters and distinct environments

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## ARTICLE INFO

## ABSTRACT

The franciscana dolphin *Pontoporia blainvillei* is the most endangered small endemic cetacean species of the southwestern South Atlantic Ocean. We assessed intra-specific variation in the diet of franciscana regarding biological parameters (sex, total body length, age class and sexual maturity) and between distinct environments (open marine area *vs.* an area adjacent to an estuary) from the Buenos Aires coast, Argentina, using both stable isotopes and stomach content analysis. We found a significant isotopic enrichment in $^{15}N$ in neonates and nursing calves, representative of the lactation period. We found differences in the diet of franciscana between dolphins from the open marine area and dolphins living in waters influenced by the estuary, by both diet estimating methods. The lower $\delta^{15}N$ values in dolphins living in waters adjacent to the estuary could be associated with a higher consumption of small sizes of the striped weakfish (*Cynoscion guatucupa*), which has lower isotope values than the larger sizes consumed in the open marine area. This trophic differentiation between locations is in agreement with the existence of two genetically distinct populations recently found in the study area. This study reveals geographical variations in the diet of franciscana dolphin, supporting the new delineation of distinct franciscana populations in Argentine waters. Considering the small home range and the limited movement pattern of the species, the population living near the estuary would likely need protection due to its low level of genetic diversity and feeding preferences. Therefore, the preservation and conservation of the mentioned habitat is recommended to ensure population viability of franciscana in the region.

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## Introduction

The franciscana dolphin *Pontoporia blainvillei* (Gervais and D'Orbigny, 1844) is the most endangered small cetacean of the southwestern South Atlantic Ocean (Reeves et al., 2012), being particularly vulnerable to anthropogenic activities (Bordino et al., 2002; Bordino et al., 2013; Di Benedito, 2003) due to its use of coastal habitats (Crespo et al., 1998). Mortality in artisanal fisheries is causing a devastating effect on its populations throughout most of its geographic distribution from Brazil to Argentina (Secchi et al., 2003; Cappozzo et al., 2007; Negri et al., 2012). The problem of bycatch species includes scientific, social and economic aspects. Therefore, the study of the behaviour and ecology of the species

involved is the first step in an attempt to reduce incidental captures (Soykan et al., 2008). In southern Buenos Aires province, Argentina, as in other areas of their distribution, entanglement of franciscana dolphins is most common early in life before sexual maturity where 69% of dead dolphins do not exceed 4 years of age (Negri et al., 2014). Mortality differences found among age classes may be related to intraspecific variations due to changes in feeding sites and/or type of prey consumed from juvenile to adulthood or may just be driven by previous experiences.

The feeding ecology of franciscana dolphin has been studied by conventional methods such as stomach content analysis (SCA) of by-caught animals (Fitch and Brownell, 1971; Praderi et al., 1989; Di Benedito and Ramos, 2001; Rodríguez et al., 2002; Bassoi, 2005; Cremer et al., 2012; Denuncio et al., 2013; Paso Viola et al., 2014). Previous studies in southern Buenos Aires coast showed that franciscana dolphins feed in coastal and shallow waters mainly on pelagic prey such as striped weakfish *Cynoscion guatucupa* and long finned squid *Loligo sanpaulensis* (Paso Viola et al., 2014).

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Stable isotope analysis (SIA) has emerged as a powerful tool that can enhance conventional dietary studies because it offers ecological information about the trophic ecology of marine mammals (*e.g.* Newsome et al., 2010; Riccialdelli et al., 2010, 2013; Botta et al., 2012), due to its ability to reflect diet over longer time periods (Newsome et al., 2010). Therefore, the combination of SCA with SIA promises to be a successful methodology for effective diet reconstruction, which is key to the understanding and management of marine mammals. While isotope ratios of nitrogen ($\delta^{15}N$) in an animal tissue is a reliable indicator of trophic level, $\delta^{13}C$ values are often used to discriminate the relative input of dietary resources and habitat use of a consumer (DeNiro and Epstein, 1976; Minagawa and Wada, 1984; Michener and Kaufman, 2007). Thus, foraging strategies in franciscana dolphins could be assess using SIA due to possible differences in feeding preferences in relation to age, sex, habitat type and prey distribution, which are expected to influence the isotopic signature of this predator directly.

Moreover, when this technique is used in conjunction with genetic and biological data, these ecological attributes may be useful in population delineation (Barros et al., 2010), as has been observed in dolphin populations from the northwestern Atlantic (Knoff et al., 2008; Barros et al., 2010). In the case of franciscana dolphin, determining stock discreteness is important in order to guide conservation and management procedures at a local level, allowing to direct limited resources to those stocks most likely in need of protection, and therefore increasing the chance of any management action to be effective (Secchi et al., 2003).

Due to the conservation problems of this coastal dolphin, Secchi et al. (2003) proposed dividing the area of distribution into four Franciscana Management Areas (FMAs) based on genetic, morphological, population and behavioral information. Franciscana dolphin range in Argentina is included in the FMA IV, where more data is necessary in order to describe stock boundaries precisely (Secchi et al., 2003). The fact that franciscana dolphin from Argentina revealed site fidelity to the study area when analyzing tracking data (Bordino et al., 2008), made SIA a suitable method to discriminate different franciscana populations. The consistency of findings across all of the tagged animals, as they limited their movements to specific areas, suggests that the current designation of a single management stock in Argentina is incorrect (Bordino et al., 2008). A genetic study of franciscana dolphins carried out by Méndez et al. (2008) suggested that ecological factors could be more relevant than geographical distance in determining population structuring and differentiation.

Given that this dolphin can be found in a variable range of marine habitats, from estuarine to open waters, differences in diet composition would be expected leading probably to local preferences. In northern Buenos Aires coast, differences in diet compositions were observed suggesting that future studies should discriminate between samples from different habitats and environmental characteristics (Rodríguez et al., 2002). Therefore, the main objective of this study was to assess intra-specific variations on the isotope composition regarding biological parameters such as sex, age class, total body length and sexual maturity of franciscana dolphin. We also aimed to determine differences in the diet of this cetacean between distinct environments through stable isotopes analysis and stomach contents, in order to characterize different habitats and local diet preferences inside the FMA IV.

## Material and methods

### Study area and sample collection

We analyzed franciscana dolphins incidentally caught in artisanal gillnet fisheries (bottom gillnets and shrimper gears) from the
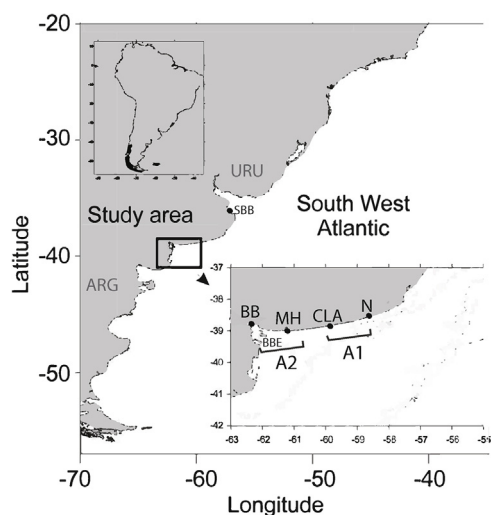


**Fig. 1.** Study area in southern coast of Buenos Aires province, Argentina (ARG). Sampling locations are shown as N = Necochea, CLA = Claromecó, MH = Monte Hermoso and BB = Bahía Blanca. BSB = Samborombón Bay and URU = Uruguay. Area 1 and Area 2 are enclosed in black lines.

Southwestern Atlantic Ocean in the Buenos Aires coast, between 2004–2010. By-caught locations are shown in Fig. 1. The sampling area, included in FMA IV, was characterized by two distinct environments: an open marine area (*Area 1*) and an area influenced by the Bahía Blanca estuary (*Area 2*). The *Area 1* (38°37′S, 58°50′W) included the localities of Necochea (N) and Claromecó (CLA) and the *Area 2* (38°44′S, 62°14′W) Monte Hermoso (MH) and Bahía Blanca (BB), adjacent to the Bahía Blanca estuary. Dolphins were collected mainly during the reproductive season (October to March) and were kept in freezers at −20 °C until necropsy was performed. Total body length (TL), weight and standardized measures of each specimen were recorded in laboratory (Norris, 1961). A total of 54 franciscana dolphins were analyzed, 23 females (41.8%) and 31 males (58.2%). Dolphins sampled ranged from 63.0 to 164.2 cm of TL (mean ± SD = 119.7 ± 21.6 cm).

### Stomach content analysis

After necropsy we recovered hard remains from the stomach contents using sieves of different mesh sizes (0.3 and 0.5 mm) and preserved in 70% ethanol. Then, prey species were identified to the species level under a binocular lens (25 x − 40x) with laboratory catalogs and references (Pineda et al., 1996 Volpedo and Echeverría, 2000). The relative importance of prey species was evaluated using the index of relative importance (IRI) calculated as IRI = (% N +%W)* % FO and then transformed as percentage (% IRI) following Pinkas et al. (1971). The frequency of occurrence (%FO) was calculated as the number of stomachs in which a prey occurred, the numerical abundance (%N) as the number of individuals of each prey type/total number of individuals of all prey types, and the reconstructed biomass (%W) as the biomass of each prey type/total biomass represented by all prey. We calculated these indexes expressed as percentage (Cortes, 1997) in each sampling location and in males and females of each locality.

Differential rates of digestion among species may bias stomach content analyses in favor of species with large and robust hard parts (Bowen, 2000). Thus, diet indexes were calculated considering teleosts, cephalopods and crustaceans separately.

## Age estimation and sexual maturity

Negri et al. (2014) estimated the age and Panebianco et al. (2012, 2015) determined the sexual maturity of franciscana dolphins from this work. Age was determined by counting growth-layer groups (GLGs) in both dentine and cementum of dolphin teeth. Although no direct validation exists for GLGs, indirect evidence supports the fact that one GLG represents one year of age (Kasuya and Brownell, 1979; Pinedo and Hohn, 2000). Sexual maturity was estimated through histological procedures following the methods of Harrison et al. (1981) and Danilewicz (2003) for females and Hohn et al. (1985) and Danilewicz et al. (2004) for males.

## Stable isotope analysis

During necropsy, a sample of dorsal muscle of each dolphin was preserved at $-20\,^{\circ}$C. Then, a subsample of $\sim 0.1$ g was dried at $60\,^{\circ}$C for 48 h. Lipids are depleted in $\delta^{13}$C in relation to proteins (Post et al., 2007; Logan et al., 2008), therefore lipid extraction was important because of the large amount present in dolphin muscle. Then, lipids were removed by repeated rinsing with a 2:1 chloroform: methanol solution. The resulting protein preparations were then rinsed several times with distilled water and dried at $60\,^{\circ}$C for $\sim 24$ h. Dried samples ($\sim 0.5$–0.6 mg) were weighed into tin capsules (3 mm $\times$ 5 mm) and analysed with a Carlo Erba Elemental Analyser interfaced with a Thermo ConFlo IV to a Finnigan MAT Delta V isotope ratio mass spectrometer at the Instituto de Geocronología y Geología Isotópica (INGEIS).

Results were expressed in delta ($\delta$) notation according to the following equation:

$$\delta = 1000 \ \frac{R_S - R_R}{R_R}$$

Where $\delta = \delta^{13}C$ or $\delta^{15}N$, $R_S$ and $R_R$ were the $^{13}$C:$^{12}$C or $^{15}$N:$^{14}$N ratios of the sample and the reference standard, respectively. The reference standards were Vienna Pee Dee Belemnite (VPDB) for carbon and atmospheric (AIR) $N_2$ for nitrogen (Gonfiantini, 1978; Coplen et al., 1992). The units were expressed as parts per mil (‰). Sample uncertainty based on repeated measures of internal standards is 0.2‰ for both $\delta^{13}$C and $\delta^{15}$N.

## Data analysis

We analysed the isotopic variability of franciscana dolphin in $\delta^{13}$C and $\delta^{15}$N regarding biological parameters such as sex (male and female), maturity stage (immature and mature), age class (1–4) and sampling localities (N, CLA and MH), performing several One-way ANOVAs and Tukey multiple comparisons for each independent variable separately. BB was excluded from this analysis due to the low number of samples collected in that locality.

We compared the sex categories using individuals of >1 year old, since we expected that nursing calves share the same food (milk) independently of sex. We categorized age classes as: (1) *neonates* or *nursing calves* including animals from 0 to <1 year old, (2) *juveniles* as animals from 1 to < 2 years and sexually inmature, (3) *subadults* animals from 2 to 4 years old and (4) *adults* as animals > 4 years old and sexually mature. We also evaluated a possible correlation between both $\delta^{13}$C and $\delta^{15}$N with age and TL of individuals using a Pearson correlation coefficient ($r$) in the whole study area and in each sampling location, respectively.

A Correspondence Analysis (CA) was performed to examine whether an association existed between different sampling locations and prey species consumed. For this analysis, we used frequencies of prey species found in the stomach contents in each sampling area.
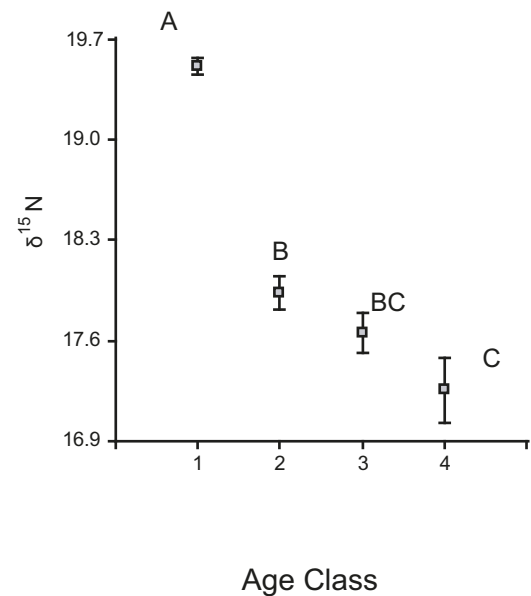


**Fig. 2.** Mean $\delta^{15}$N values ($\pm$SD) of franciscana dolphins (*Pontoporia blainvillei*) at different age classes (1–4). Different letters indicate statistical differences ($P < 0.05$).

We also estimated the relative contribution of different prey taxa to the diet of males and females in different sampling localities using the Bayesian mixing model Stable Isotope Analysis in R 3.3.2. (SIAR 4.2 Parnell et al., 2010). The main prey used in the models were defined as those with the highest% IRI (percentage of relative importance index) obtained from the stomach contents of dolphins from each sampling locality. The Trophic Enrichment Factor (TEF) was taken from Caut et al. (2011) being 1.26‰ for $\delta^{13}$C and 1.23‰ for $\delta^{15}$N. Only weaned dolphins, those with TL > 91 cm (Denuncio et al., 2013) were included in this analysis. Results are described as range percentage (%) with the 95% confidence interval (CI95).

Shapiro Wilks and Levene's tests were applied to assess data normality and homogeneity of variance. For all calculations, we tested significance at $\alpha = 0.05$ level. Statistical analyses were performed with the software InfoStat.Ink (Di Rienzo et al., 2011).

## Results

No significant differences were observed in the mean size of the animals analysed between different sampling areas (ANOVA, $F_{3,50} = 0.37$, $P = 0.78$). All age classes were represented in the sample, 10.9% of franciscana dolphins were neonates or nursing calves, 27.3% juveniles, 41.8% subadults and 20.0% adults. We did not find statistical differences between males and females in $\delta^{13}$C or $\delta^{15}$N (ANOVA, $F_{1,43} = 0.45$, $P = 0.50$; ANOVA, $F_{1,43} = 0.02$, $P = 0.89$, respectively) but we could not assess isotopic variation between sexes of each age class due to small sample size. No statistical differences were observed among age classes in $\delta^{13}$C (ANOVA, $F_{3,46} = 0.53$, $P = 0.66$) and $\delta^{13}$C was not correlated with age ($r = -0.03$, $P = 0.82$) or TL ($r = 0.11$, $P = 0.42$). However, $\delta^{15}$N values were negatively correlated with age ($r = -0.58$, $P < 0.0001$) and TL ($r = -0.50$, $P < 0.0001$). Moreover, $\delta^{15}$N were significantly different between age classes (ANOVA, $F_{3,46} = 9.31$, $P < 0.0001$). Specifically, nursing calves had higher $\delta^{15}$N values than juveniles, subadult and adult individuals, with differences of $\sim 2.0$‰, 1.5‰ and 1.3‰, respectively (Fig. 2). Summary of the isotope data can be found in Table 1.

Considering SCA in different sampling localities, the long finned squid was an important prey together with the striped weakfish in N and CLA, unlike in MH where the squid had a very low contribution to the diet and even being absent in BB. When IRI%

**Table 1**

Mean $\delta^{13}$C and $\delta^{15}$N values ($\pm$SD) of muscle of franciscana dolphin (*Pontoporia blainvillei)* from southern Buenos Aires coast. Isotope values of males, females, age classes and distinct sampling locations n = number of individuals, TL (cm) = range of total length and mean TL = mean of total length (in cm).

| Category | n | TL (cm) | Mean TL | $\delta^{13}$C $\pm$ SD | $\delta^{15}$N $\pm$ SD |
|---|---|---|---|---|---|
| Franciscana dolphin | 48 | 93.3–164.2 | 124.1 $\pm$ 17.4 | −15.8 $\pm$ 0.6 | 17.7 $\pm$ 0.8 |
| Females | 21 | 93.3–164.2 | 127.4 $\pm$ 22.2 | −15.9 $\pm$ 0.5 | 17.7 $\pm$ 0.8 |
| Males | 27 | 94.0–141.5 | 121.6 $\pm$ 12.3 | −15.8 $\pm$ 0.6 | 17.6 $\pm$ 0.8 |
| Neonates | 6 | 63.0–87.7 | 80.6 $\pm$ 9.3 | −16.1 $\pm$ 0.5 | 19.2 $\pm$ 0.8 |
| Juveniles | 14 | 93.3.0–116.0 | 105.5 $\pm$ 7.2 | −15.9 $\pm$ 0.5 | 18.1 $\pm$ 0.4 |
| Subadults | 21 | 109.2–147.0 | 127.4 $\pm$ 11.9 | −15.8 $\pm$ 0.6 | 17.7 $\pm$ 0.7 |
| Adults | 13 | 119.3–164.2 | 139.6 $\pm$ 15.5 | −15.9 $\pm$ 0.5 | 17.3 $\pm$ 0.9 |
| Necochea | 21 | 100.0–158.5 | 126.6 $\pm$ 16.5 | −15.7 $\pm$ 0.4 | 17.9 $\pm$ 0.4 |
| Claromecó | 14 | 94.0–160.5 | 122.2 $\pm$ 17.6 | −16.2 $\pm$ 0.4 | 17.8 $\pm$ 0.5 |
| Monte Hermoso | 10 | 98.3–164.2 | 123.8 $\pm$ 19.7 | −15.9 $\pm$ 0.5 | 17.1 $\pm$ 0.9 |
| Bahía Blanca | 3 | 93.3–129.7 | 116.8 $\pm$ 20.4 | −14.6 $\pm$ 0.5 | 16.2 $\pm$ 0.7 |

**Table 2**

FO (%) and IRI (%) of the main prey species found in stomach contents of franciscana dolphin (*Pontoporia blainvillei)* from different sampling locations of southern Buenos Aires coast.

| Prey species | Necochea | | Claromecó | | Monte Hermoso | | Bahía Blanca | |
|---|---|---|---|---|---|---|---|---|
| | %FO | %IRI | %FO | %IRI | %FO | %IRI | %FO | %IRI |
| Teleosts | | | | | | | | |
| *Trachurus lathami* | 46.7 | 26.5 | 33.3 | 5.2 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Engraulis anchoita* | 26.7 | 6.3 | 45.5 | 10.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Cynoscion guatucupa* | 73.3 | 65.2 | 72.7 | 84.7 | 57.1 | 99.1 | 100.0 | 93.7 |
| *Micropogonias furnieri* | 0.0 | 0.0 | 0.0 | 0.0 | 14.3 | 0.9 | 50.0 | 6.3 |
| Cephalopods | | | | | | | | |
| *Loligo sanpaulensis* | 100.0 | – | 97.0 | 99.9 | 85.7 | – | 0.0 | – |
| *Octopus tehuelchus* | 0.0 | – | 3.0 | 0.01 | 0.0 | – | 0.0 | – |
| Crustaceans | | | | | | | | |
| *Artemesia longinaris* | 0.0 | – | 9.1 | 39.7 | 14.3 | – | 25.0 | 81.6 |
| *Pleoticus muelleri* | 0.0 | – | 3.0 | 60.3 | 0.0 | – | 25.0 | 18.4 |
| Penaeidae | | | | | | | | |
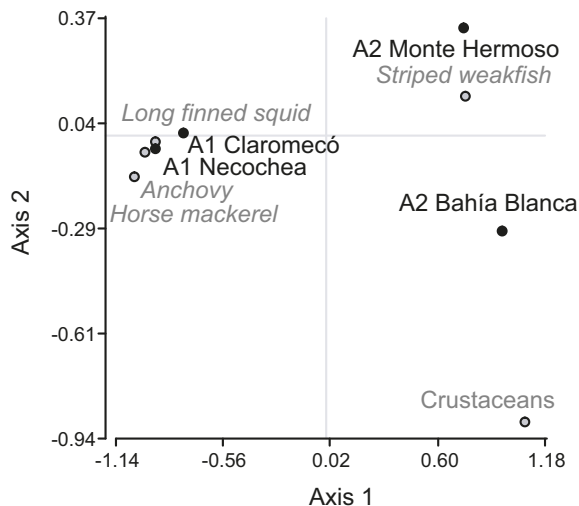


**Fig. 3.** Correspondence Analysis of franciscana dolphin (*Pontoporia blainvillei*) from different sampling locations of southern Buenos Aires province, Argentina. Variables: Sampling locations (black circles) and prey species (grey circles). Area 1 and Area 2 are indicated in respective sampling locations.

was calculated among fish species, the most important prey was the stripped weakfish in all sampling localities (Table 2). The CA showed a strong association between prey frequency and distinct environments. *Area 1* was highly associated with long finned squid and two species of pelagic fish, the anchovy *Engraulis anchoita* and the horse mackerel *Trachurus lathami;* while in *Area 2* the association was with striped weakfish and crustaceans (Fig. 3).

When analyzing SIA in different localities, we observed an increase of $\delta^{13}$C values from east to west (N to BB). Mean values of

$\delta^{13}$C and $\delta^{15}$N ($\pm$ SD) of distinct localities are presented in Table 1. We observed significantly more enriched values of $\delta^{13}$C in N than CLA (ANOVA, $F_{2,42} = 6.56$, $P = 0.0033$), while dolphins from MH did not differ from either of these locations (Fig. 4a). For $\delta^{15}$N, we found values less enriched in western than eastern areas. Dolphins from MH had significantly lower $\delta^{15}$N values than N and CLA (ANOVA, $F_{2,42} = 7.53$, $P = 0.0016$) (Fig. 4b). In addition, a higher variance in $\delta^{15}$N was observed in MH than in other sampling areas (Fig. 4). Although the $\delta^{15}$N values of franciscana dolphins were negatively correlated with body length and age, we considered analyzing each locality separately, due to the differences observed in both $\delta^{13}$C and $\delta^{15}$N among distinct localities within the study area.

The models (SIAR) revealed a greater importance of horse mackerel in the diet of females in N (Fig. 5a), showing significantly lower $\delta^{15}$N values than males (ANOVA, $F_{1,19} = 4.85$, $P = 0.04$, n = 21) (Fig. 5b). In the case of $\delta^{13}$C, values did not differ between sexes. SCA in N revealed the same difference in fish consumption with a much higher contribution (IRI%) of horse mackerel in females (~50%) than males (~2%). In the other sampling localities, we did not find significant differences in both $\delta^{13}$C and $\delta^{15}$N with sex. However, males from MH had lower $\delta^{15}$N values with a difference of 0.8‰, as well as mature dolphins which reached a difference of more than 1.0‰ in $\delta^{15}$N with immature dolphins (ANOVA, $F_{1,8} = 5.27$, $P = 0.05$). Moreover, the $\delta^{15}$N was significantly negatively correlated with age in MH ($r = 0.52$, $P = 0.018$) (Fig. 6).

## Discussion

We identified intra-specific variations in the isotopic composition of franciscana dolphins from southern Buenos Aires coast in Argentina.
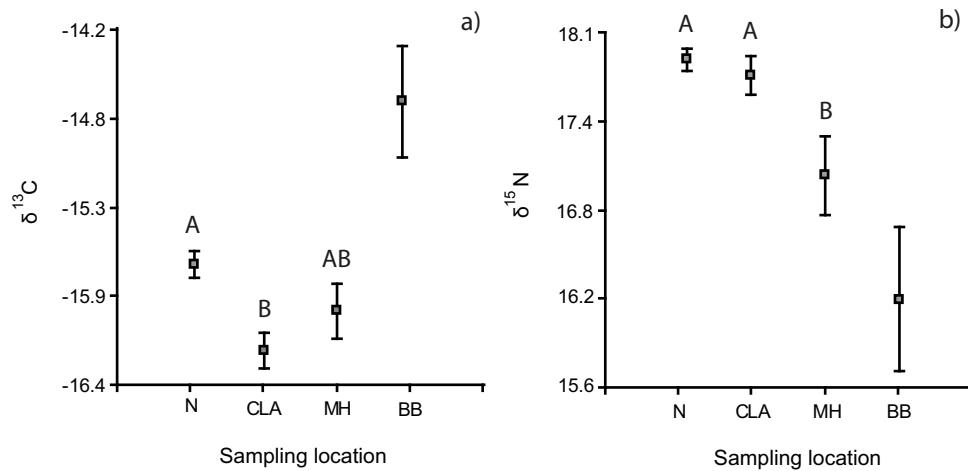
**Fig. 4.** a) Mean $\delta^{13}C$ and b) $\delta^{15}N$ values ($\pm$SD) of franciscana dolphins (*Pontoporia blainvillei*) at different sampling locations in Buenos Aires province, Argentina. N = Necochea, CLA = Claromecó, MH = Monte Hermoso, BB = Bahía Blanca. Different letters indicate statistical differences ($P < 0.05$).
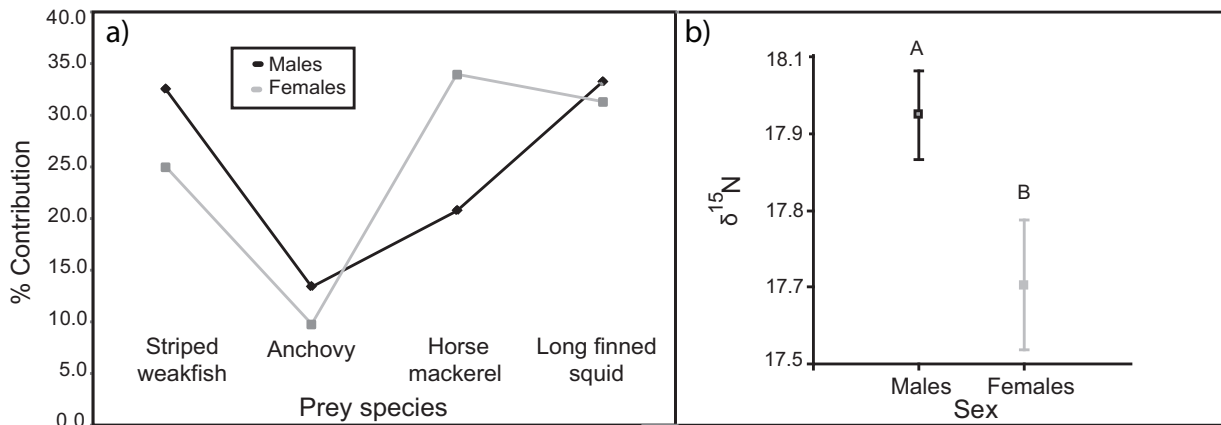


**Fig. 5.** a) Mean contribution values (%) obtained by SIAR of each prey species to the diet of male and female franciscana dolphins (*Pontoporia blainvillei*) from Necochea, Buenos Aires, Argentina. b) Mean $\delta^{15}N$ values ($\pm$SD) of different sexes of franciscana dolphins (*Pontoporia blainvillei*) from Necochea, Buenos Aires province. Different letters indicate statistical differences ($P < 0.05$).

## Effect of lactation and age class

In previous studies of marine mammals, depleted $^{13}C$ values in younger animals were reported, associated with the influence of high lipids content in the milk consumed by nursing calves (Hobson et al., 2004; Riccialdelli and Goodall, 2015; Troina et al., 2016). In tooth dentin of franciscana from Brazil, age explained a fraction of the pattern in the carbon isotope ratios (Troina et al., 2016). However, we did not observe this pattern in muscle of franciscana dolphins from the study area. In this work, we analyzed muscle, as this tissue facilitates the comparison between lactating *vs.* adult individuals due to the fact it has an isotope turnover rate between 3 and 6 months, depending on the age and size of the individuals (Vander Zanden et al., 2015). However, it is possible that other factors such as the duration of the lactation period, the incorporation of solid food at a very early age and the lipid constitution of the milk, among others, could be contributing to a decrease in the isotopic differences observed in $\delta^{13}C$ between lactating *vs.* adults analyzed. On the other hand, the greater differences expected in $\delta^{15}N$ ($\sim$3‰), ensure that these mentioned factors would not completely mask differences in $\delta^{15}N$ between lactating *vs.* adult individuals. Thus, we have found a significant isotopic enrichment of $\sim$2‰ in $^{15}N$ between neonates or nursing calves and adults, representing the isotopic effect of lactation. The enrichment in $^{15}N$ indicates that calves are feeding at a higher trophic level, as

was expected if they were theoretically 'feeding' on their mother's tissues (Jenkins et al., 2001). After a short period of exclusive lactation of $\sim$40 days, nursing franciscana calves start to complement milk with solid food indicating that weaning is gradual, occurring at approximately 7 months of age (Denuncio et al., 2013). Thus, feeding independence starts when calves are less than one year old and exceed 91 cm in length and 11 kg in weight (Denuncio et al., 2013). After weaning, we observed a decrease in the difference of the nitrogen isotopic signatures as juveniles increase the consumption of solid food, reaching gradually the adult isotope value. A similar pattern was found in tooth dentin of franciscana from southern Brazil (Troina et al., 2016) and in other cetacean species such as right whales (Valenzuela et al., 2010), killer whales (Newsome et al., 2009), Commerson's dolphins (Riccialdelli et al., 2013) and false killer whales (Riccialdelli and Goodall, 2015). Moreover, in the case of bottlenose dolphins, Knoff et al. (2008) concluded that the ontogenetic dietary shift likely reflects a shift from a sole reliance on milk to a combination of milk and prey species during the first year of life.

## Effect of sex

We only found sex differences in isotope composition of franciscana dolphins from N, showing significantly lower $\delta^{15}N$ values in females. Moreover, mixing models (SIAR) revealed a greater
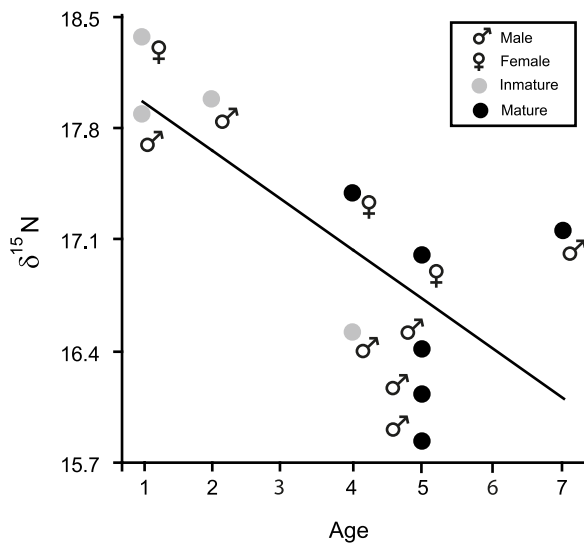
**Fig. 6.** Regresion of mean $\delta^{15}$N values with different ages (years) of franciscana dolphins (*Pontoporia blainvillei*) from Monte Hermoso, Buenos Aires province, $R^2 = 0.52$, $P = 0.0186$, $Y = 18.30 - 0.32.x$. Maturity stage and sex are indicated in the graph.

importance of horse mackerel in the diet of females as well as stomach contents analysis that showed the same difference with a high contribution of that prey item in females. Additionally, Perez (2005) found a similar result in dolphins collected in the same area but more than a decade before, where mature females presented higher IRI percentage of horse mackerel. Females seem to prefer horse mackerel when this fish species is available in the environment as happens in N. This preference could be due to the high caloric value of horse mackerel, being the prey species with the highest energy and lipid content (Argenfood, 2010), necessary for the energy requirement of female dolphins to deal with the high-energy costs related to reproduction such as pregnancy, lactation or taking care their calves (Kastelein et al., 1993; Oftedal, 1997).

*Habitat, geographic area and conventional diet*

By studying the SCA, we observed differences between *Area 1* and *Area 2* (open marine habitat and habitat influenced by the estuary), finding a strong association between the occurrence of prey species and distinct environments. Rodríguez et al., (2002) obtained the same result in northern Buenos Aires coast where diet differed between two distinct environments. However, a strong association in the occurrence of a prey species in a particular area does not mean that this prey species is absent in the others. Precisely, the striped weakfish was the most important fish in all sampling localities observed by IRI%, although it was particularly more consumed in *Area 2*. Paso Viola et al. (2014) described an opportunistic behavior in the diet of this dolphin in the study area feeding on the most abundant species of the Buenos Aires coast. Therefore, the differences in prey consumption in distinct *Areas* would be related to the abundance and habits of the prey species in each region. The striped weakfish is an important prey item in the whole study area, being a typical inhabitant of estuaries and coastal areas, tolerating a wide range of environmental conditions (Ruarte et al., 2004). Instead, the long finned squid presented a very low frequency of occurrence in *Area 2*, probably due to the estenohaline habits of this cephalopod species (Barón and Ré, 2002).

*Isotope differences and sampling locations*

Geographic variations in the diet were also observed through the isotopic compositions among distinct localities from the study

area. Although the $\delta^{15}$N values of franciscana dolphins were negatively correlated with TL and age, we cannot concluded that there is an ontogenetic variation in diet in the whole study area, as we found differences in both $\delta^{13}$C and $\delta^{15}$N among sampling locations. Then, we analyzed each locality separately and concluded that the decrease observed in $\delta^{15}$N with age or TL would be in fact related to the significantly lower $\delta^{15}$N values in dolphins from *Area 2* (MH) compared to *Area 1* (N or CLA). In addition, dolphins from MH presented a high variance in the isotope composition in contrast with the other localities. The lowest values in MH corresponded to sexually mature males, although the low number of animals recovered from this locality did not allow us to determine if the decrement was related to sex, sexual maturity or both. In franciscana dolphins from southern Brazil, models for $\delta^{15}$N suggested an effect of total body length and sex, with males being more depleted in $^{15}$N than females (Troina et al., 2016). However, the differences in $\delta^{15}$N were attributed to ontogenetic variations due to this species being sexualy dimorphic and the models compared females and males within the same size range but from different age categories (Troina et al., 2016). In this study, we observed a similar pattern in MH with mature males (older ages) having significantly lower $\delta^{15}$N values than females. Due to the fact that both young and adult franciscana from MH share the same foraging area, the differences in isotope values of MH must be driven by differences in the types of prey consumed which may be due to different foraging strategies or experience.

The stripped weakfish was the main prey found in stomach contents of animals from MH. Paso Viola et al. (in press) found that isotopic values of striped weakfish from the study area increase as the animals grow in size. This increment is due to a shift in the diet of this species observed also by stomach contents in the area (Sardiña and Lopez Cazorla, 2005). Franciscana consumed significantly smaller striped weakfish near the estuarine area (*Area 2*, MH and BB) than in the open marine area (*Area 1*, N and CLA) (Paso Viola et al., 2014). Then, the significantly lower $\delta^{15}$N values found in MH may be a consequence of a major consumption of significantly smaller sizes of striped weakfish (TL < 7 cm) in comparison with dolphins from N or CLA (TL ∼14 cm and ∼10 cm, respectively), as observed by Paso Viola et al. (2014). In conclusion, differences observed among localities would be due to the consumption of different sizes of striped weakfish, the most important prey in whole study area (Paso Viola et al., 2014). Moreover, *Area 2* is an important nursery ground for stripped weakfish (Lopez Cazorla, 2000), where small juveniles are an easily available prey for franciscana dolphins (Paso Viola et al., 2014).

It is important to mention a spatial proximity observed in the isotopic bi-plot between individuals from BB and MH, adjacent areas. However, lower $\delta^{15}$N values were observed in BB that may be due to a higher consumption of crustaceans as was observed by stomach contents. However, more studies with a larger amount of animals from BB would be necessary to enlarge the knowledge of this species in that particular place.

**Conclusion**

In our study, we found differences in the diet of franciscana dolphins inside FMA IV, estimated by two methods. Dolphins from the open marine area (N and CLA) have a different diet to dolphins living in waters influenced by the estuary (BB and MH). This geographic differentiation, detected with both isotope and stomach content analysis, is in agreement with the existence of two genetically distinct populations recently found by Gariboldi et al. (2015) in the study area. We found isotopic differences that can suggest a little evidence of differences among sexes and/or sexual maturity within localities (N and MH). However, more studies would be necessary to

analyze the existence of sex-related resource partitioning of franciscana dolphins in the study area. This is the first study that reveals new ecological data on feeding preferences, ontogenetic trends and geographical variations in muscle of franciscana dolphins through the analysis of stable isotopes, integrating this information with distinct franciscana populations inside FMA IV in Argentine waters. Considering the small home range and the limited movement pattern of the species (Bordino et al., 2008; Crespo et al., 2010), MH population would likely need protection due to the low level of genetic diversity found there (Gariboldi et al., 2015) and the local feeding preferences on small fishes. Therefore, the preservation and conservation of that habitat is recommended to ensure the population viability of franciscana dolphin in that region. The combination of conventional methods (*e.g.* stomach contents analysis) with stable isotope analysis allowed a more appropriate assessment of the diet of this dolphin in distinct areas, highlighting the need for multimethod approaches in trophic reconstructions and population assignment.

## Acknowledgements

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