



Trophic relationships in apex predators in an estuary system: A multiple-method approximation



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ABSTRACT

The trophic level and ecological role of species in the ecosystem are determined by their feeding habits. While the trophic level and niche width are characteristic at population level, trophic overlap indicates the interaction between populations of a community. Three apex predators coexist in Uruguayan waters: Franciscana dolphin (*Pontoporia blainvillei*), South American sea lion (SASL, *Otaria flavescens*) and South American fur seal (SAFS, *Arctocephalus australis*). The two otariids consume different main prey species and have different isotopic niche, whereas feeding habits of Franciscana dolphin in Uruguayan waters remain practically unknown. This study aimed to determine Franciscana diet composition by stomach content and stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) analyses, and to assess trophic overlap in diet composition as well as in isotopic niche space among the three apex predators. Diet composition of each species was estimated by Bayesian mixing models with prior information on the diet of predators. Moreover, Bayesian ellipses were used to define the isotopic niche space and overlap among the three species. Diets among predators varied: diet of SAFS was composed mainly by a few pelagic prey species, diet of SASL was more diverse and included pelagic and benthic preys, and for Franciscana dolphin the diet was mainly composed by coastal prey species. The SAFS had the largest isotopic niche area compared to the Franciscana and the SASL. The isotopic niche of the SASL did not overlap with those of the other two species. Although the Franciscana and the SAFS overlap in isotopic space, they do not overlap in feeding areas; this is because particulate organic matter $\delta^{13}\text{C}$ values increase from inner Rio de la Plata to the estuary and the Atlantic Coast. Here, the relevance of using different sources of information to reach strong conclusions about feeding habits of marine predators and about co-use of resources is shown, mainly in environments where variation in $\delta^{13}\text{C}$ can be related to diverse reasons.

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1. Introduction

Feeding habits are a major determinant of population interactions as well as an indicator of the species role in the community structure and ecosystem functioning (Arim et al., 2010; Pauly et al., 1998a). It has been widely demonstrated that apex predators play an important role in the structure and dynamics of their communities either through direct effects on their prey or via indirect impact on species at other trophic levels (Estes et al., 1998; Shurin et al., 2002). The ecological niche is defined as an n-dimensional hypervolume containing the relevant variables that explain the persistence of a species or a local population (Hutchinson, 1957). Sympatric species that require similar

trophic resources usually minimize potential food competition by using different preys or foraging in different areas or times (Pimm, 2002). In this context, niche differentiation among species is an evolutionary process by which different forms of trophic behavior have arisen, and has been used to explain the co-occurrence of similar species (Levins, 1968; McArthur, 1972). While population level approaches frequently use trophic position and niche width as measures of niche dimensions, community approaches use trophic overlap as a guesstimate of the amount of resources shared among populations (Bearhop et al., 2004).

There are no “universal methods” allowing reliable determination of diet composition of predators. In marine mammals, feeding habits have been typically studied using traditional methods such as scat or stomach content analysis (e.g. Koen Alonso et al., 1998; Naya et al., 2002). These traditional methods provide high-resolution information on prey taxonomy and features such as body length and biomass; however, they have intrinsic biases mostly due to differential digestion, retention and recovery of prey hard items (Arim and Naya, 2003; Trites and Joy,

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2005). Furthermore, these methods provide only a snapshot of the diet of each individual just before sampling. Since stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) analysis provides information on assimilated food and, thus, avoids biases associated with traditional methods, it has become a standard tool in the study of foraging ecology of vertebrates, even when it offers less detailed information on dietary composition than scat or stomach content analysis (Newsome et al., 2012). While $\delta^{15}\text{N}$ increases in a predictable way along the trophic web, allowing comparisons of consumer trophic positions (Post, 2002), $\delta^{13}\text{C}$ signature is a mirror of baseline ecosystem and provides information on the origin of feeding sources used by predators (Bearhop et al., 2004; DeNiro and Epstein, 1978). Moreover, stable isotopes reveal useful information for a given time depending on the tissue from which the sample was taken, since their turnover rates vary as a function of tissue metabolic rate (Dalerum and Angerbjörn, 2005).

Three apex predators coexist in Uruguayan waters: Franciscana dolphin (*Pontoporia blainvillei*), South American sea lion (SASL, *Otaria flavescens*) and South American fur seal (SAFS, *Arctocephalus australis*). While the Uruguayan population of SAFS has steadily increased over the last 60 years, with a current estimated size of about 130,000 individuals, the SASL population has declined in the same period and its current population size is about 9000 individuals (Franco-Trecu, 2015). The underlying reasons for these contrasting trends, that are unique in the geographic ranges of both species (Crespo et al., 2015; Dans et al., 2009), remain unknown. Franciscana dolphin is an endemic species that inhabits coastal waters ranging from Itaúnas (18°25'S) in Espírito Santo State, Brazil, to Península Valdés (42°30'S) in Chubut Province, Argentina (Crespo et al., 1998; Praderi et al., 1989). The Franciscana is the most threatened small cetacean of the Southwestern Atlantic Ocean due to bycatch-caused mortality in gillnets throughout its geographic range and is considered *Vulnerable* by the IUCN (Reeves et al., 2012). Indeed, Franciscana dolphins and SASL frequently interact with coastal fisheries in Uruguay but in different ways. One of the major conflicts between SASL and fishermen occurs when sea lions prey on the catch of fishing vessels and damage the fishing gear (Szteren and Páez, 2002). Meanwhile, interaction of Franciscana dolphins with fisheries does not imply an economic loss for fishermen but a threat to the species (incidental capture) (Franco-Trecu et al., 2009).

Studies of diet composition based on scat and stable isotopes analyses showed that the two otariid species, considered central place foragers (Pyke, 1984; Stephens and Krebs, 1986), consume different main prey species (Franco-Trecu et al., 2013). As for the Franciscana dolphin, the only species among the five species of river dolphins worldwide to inhabit marine and estuarine waters, little is known about its feeding habits in Uruguayan estuary waters, and available information comes from studies performed in the 80's (Praderi, 1985; Praderi et al., 1989).

Studying the feeding habits of apex predators and trophic relationships among species is important for understanding the functioning and structure of a community (Arim et al., 2010; Pauly et al., 1998a), and, thus, for being able to predict modifications arisen from changes in resource abundance (Pauly et al., 2000, 1998b). In the vulnerable conservation context of Franciscana dolphins and Uruguayan SASL population, this study aimed to: i) determine Franciscana dolphins diet composition by traditional methods and stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and ii) to assess the trophic overlap among the three apex predators, both in diet composition and in isotopic niche space. Regarding known diet composition, here the stomach contents of Franciscana and published information of both otariid species (Franco-Trecu et al., 2013) is analysed. Also, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ is analysed in skin samples, a tissue with mean turnover rates (Alves-Stanley and Worthy, 2009; Gimenez et al., 2016) which provides information on diet assimilated from 1 to 2 months (Dalerum and Angerbjörn, 2005; Hobson et al., 1996). Such data allows complementing the determination of diet composition, and estimating isotopic niche width and overlap among these three apex predators in order to understand their trophic relationships in Uruguayan waters.

2. Materials and methods

2.1. Study area

The Río de la Plata (34°00'–36°10'S, 55°00'–58°10'W) is the second largest estuary basin of South America with a large and highly dynamic mixing of seawater and freshwater (Ortega and Martínez, 2007). In the adjacent Atlantic Ocean, wind regimes, river discharge and the displacement of the Brazil-Malvinas Confluence generate seasonal variability, with cold and nutrient-rich sub-Antarctic waters dominating in winter, and warm, nutrient-poor sub-tropical waters during summer (Ortega and Martínez, 2007; Piola et al., 2000). This whole system constitutes one of the most productive regions in the world, supporting large fisheries (Guerrero et al., 1997; Lopes et al., 2006; Ortega and Martínez, 2007). The Franciscana dolphin inhabits Río de la Plata estuary (RPE) and adjacent Atlantic Ocean waters, and the main breeding rookery for the SAFS and the SASL is Isla de Lobos (35°01'S, 54°50'W), located in the area considered as the outer RPE (between the line Montevideo – Punta Piedras and the line Punta del Este – Punta Rasa).

2.2. Sample collection

A total of 38 stomachs were collected from Franciscana dolphins incidentally caught in artisanal fisheries and stranded dead individuals along the Uruguayan coast in 2009. Sixty-five percent of individuals were males, whereas 35% were females. Mean size for females was 1.25 m (SD = 0.22), and for males it was 1.17 m (SD = 0.23). Skin samples of Franciscana dolphin (n = 13) were obtained from individuals incidentally caught by fishermen and fresh stranded dead individuals along the Uruguayan coast during summer 2009. While females (n = 6) presented a mean size of 1.33 m (SD = 0.24), for males (n = 7) it was 1.07 m (SD = 0.08).

Skin samples of both species of otariids were collected from randomly chosen, physically mature females at Isla de Lobos during the 2009 breeding season (summer). Female capture and manipulation was done as described in Riet Sapriza et al. (2013) and Franco-Trecu et al. (2013). A small skin sample from the caudal flippers of each female (n = 10 SASL; n = 33 SAFS) was collected using a biopsy collection kit. All skin samples were stored at –20 °C until stable isotope analysis was conducted. All procedures of animal manipulation were submitted and approved by the Ethics Committee in Animal Experimentation of the University of the Republic (Uruguay) as valid according to the national laws on animal welfare.

2.3. Stable isotope analysis

Skin samples were thawed, dried in a stove at 60 °C for 36 h, and ground into a fine powder using a mortar and pestle. Lipids were removed from each sample using chloroform: methanol (2:1) solution (Bligh and Dyer, 1959) because lipids are depleted in ^{13}C compared with other molecules and variability in lipid content of samples may result in undesirable variability in $\delta^{13}\text{C}$ values (DeNiro and Epstein, 1978). Approximately 0.3 mg of each powdered and processed sample of skin was weighed, using a microanalytical balance, into tin capsules (3.3 × 5 mm) and analysed using a continuous-flow isotope ratio mass spectrometer (CF-IRMS) coupled with an elemental analyser (PDZ Europe ANCA-GSL). All analyses were performed at the Stable Isotope Laboratory of the University of California at Davis.

Stable isotope abundances, expressed in delta (δ) notation, where the relative variations of stable isotope ratios are expressed in per thousand (‰) deviations from predefined international standards, were calculated as:

$$\delta^i\text{X} = \left[\left(\frac{{}^i\text{X}/{}^i\text{X}}{\text{sample}} \right) / \left(\frac{{}^i\text{X}/{}^i\text{X}}{\text{standard}} \right) \right] - 1$$

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