Mammalian Biology 80 (2015) 298–302

Contents lists available at ScienceDirect

Mammalian Biology

journal homepage: www.elsevier.com/locate/mambio

Short Communication

Intra-specific trophic variation in false killer whales (*Pseudorca crassidens*) from the southwestern South Atlantic Ocean through stable isotopes analysis

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ARTICLE INFO

Article history:
Received 16 June 2014
Accepted 9 January 2015
Handled by Heiko G. Rödel
Available online 15 January 2015

Keywords:
Pseudorca crassidens
False killer whale
Trophic habits
δ^{13}C
δ^{15}N
Tierra del Fuego

ABSTRACT

The false killer whale (*Pseudorca crassidens*) is a highly social top predator difficult to study in the wild because of its wide-ranging oceanic habits. Mass strandings offer good opportunities to gather ecological information about cetacean species. In this study we analyze a possible isotopic (δ^{13}C and δ^{15}N) variation in bone collagen among individual false killer whales that mass stranded on the south shore of the Strait of Magellan, Chile, in 1989, in relation to their ontogenetic classes, sex and total body length. We found an isotopic enrichment in δ^{13}C and δ^{15}N values from young and smaller to older and larger individuals, respectively. These isotopic shifts may reflect differences in foraging and diving capabilities, enabling older and larger individuals to feed more deeply in the water column and/or on larger prey. The lack of isotopic differences between females and males may suggests little evidence for sex-related resource partitioning in this group, as result of a possible coordination during feeding activities, e.g. food sharing as previous studies have found. We also found an important isotopic effect of lactation in both the carbon and nitrogen isotope composition of nursing calves, probably due to a prolonged nursing period (from 19 month to two years). This study integrate ecological information from several years to lifetime in relation to sex and total body length, obtaining information on possible changes linked to the development of foraging capabilities in false killer whale.

The false killer whale, *Pseudorca crassidens*, can be found worldwide in tropical, subtropical and warm temperate oceans (Baird, 2002). Despite its known distribution, this species usually does not range beyond 50° of latitude in both hemispheres (Stacey and Baird, 1991). In the southwestern South Atlantic Ocean its presence has been reported (as strandings) from Brazil (33°00′S, 52°40′W) to the Strait of Magellan (52°27′S, 69°31′W), Falkland (Malvinas) Islands and the Beagle Channel (54°53′S, 67°18′W) (Koen Alonso et al., 1999; Andrade et al., 2001; Zylber et al., 2002; Goodall et al., 2008).

A major problem in studying this species in the wild is because of its wide-ranging oceanic habits; thus little is known about false killer whales in the southwestern South Atlantic Ocean. Because of their extremely social behavior, false killer whales frequently strand in large numbers (Odell et al., 1980; Stacey and Baird, 1991), offering good opportunities to gather biological and ecological information on this cetacean species.

False killer whales are known to occur in socially cohesive herds of ~20–50 animals, in which both sexes are often equally represented, but larger aggregations may also occur, associated with foraging events (Stacey et al., 1994; Baird, 2002). Stomach content analyses performed on individuals that mass-stranded along the western and southwestern South Atlantic Ocean suggest that this species may have an adaptable foraging behavior. Depending on location, stomach contents have included coastal fish (e.g. Serranidae and Scianidae, Pinedo and Rosas, 1989); epipelagic squids (e.g. *Ommastrephes batramii*, Andrade et al., 2001); oceanic-neritic squids (*Illex argentinus*, *Martialia hyadesi*) and demersal-benthic fish *Macruronus magellanicus* (Koen Alonso et al., 1999).

Based on these stomach content studies, Andrade et al. (2001) suggested a possible sex-related resource partitioning (in feeding grounds or in prey selection) between males and females. Despite that gut contents are the best snapshot of what an individual have been eating recently, is not always a good reflection of truly

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http://dx.doi.org/10.1016/j.mambio.2015.01.003
1616-5047/

individual, sexual or ontogenetic trophic differences due to widely recognized biases of this method when applied to cetaceans. Gut content can vary greatly among stranded individuals and provide data only from recent meals. In many cases prey items are regurgitated during the stranding process, and the stomach appears empty (Pierce et al., 2004). Also, differential digestion of different types of prey can result in under-representation of some common item such as soft body prey (Sheffield et al., 2001; McLeod et al., 2003). Thus, to evaluate intra-specific trophic differences, a good collection of prey items from a broad range of individuals is essential.

Nevertheless, studies on captive individuals and measurements taken at mass stranding events have shown that males became more robust than females at the same age, with higher annual food consumption (Kastelein et al., 2000; Ferreira et al., 2014). Little is known about the diving behavior of this species in the wild (Baird, 2002), but the age and size of individuals are known to influence diving capability in marine mammals. Larger bodies (larger animals) have a higher oxygen storage capacity in blood and muscle, allowing better diving capabilities, enabling them to search for larger prey in deeper water and/or spend more time in apnea during diving in the wild (Noren et al., 2001). Also, the limited diving capacity of young dolphins may influence their foraging behavior, not only of newly weaned calves but also of females accompanied by calves (Noren et al., 2002). Thus, differences in physiology, energetic requirements and hunting experiences within different age and sex classes may result in resource partitioning, e.g. different patterns of diet and/or habitat use, as has been observed in other species of marine mammals (e.g. Drago et al., 2010; Orr et al., 2011; Riccialdelli et al., 2013; among others).

Stable isotope analysis (SIA) of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) has proven to be a valuable tool for determining a wide variety of trophic aspects of the ecology of animal taxa along present and historical contexts (e.g. Kelly, 2000; Koch, 2007; Michener and Kaufman, 2007). Indeed, intraspecific trophic differences among age and sex classes have been successfully studied through SIA (e.g. Knoff et al., 2008; Valenzuela et al., 2010; Riccialdelli et al., 2013, among others). Isotopic composition of consumer tissues reflects the composition of their food (e.g. milk for purely lactating and prey for weaning individuals) plus a trophic discrimination factor (~0–2‰ for $\delta^{13}C$ and ~2–5‰ for $\delta^{15}N$) (DeNiro and Epstein, 1978, 1981; Minagawa and Wada, 1984; Fogel et al., 1989). Therefore, by analyzing the isotopic composition of false killer whale tissues from stranded individuals, we can evaluate possible dietary shifts that may occur as animals mature, e.g. from nursing periods to independent foraging, as well as diet differences attributed to physiology or morphology (Newsome et al., 2010; Orr et al., 2011). Bone collagen has a slow (multi-year) turnover rate and represents a long-term integrator of ecological information, which has its advantages in determining long-term resource and habitat use in marine mammals. Thus, its isotope values are not influenced by periodic (seasonal) or sporadic fluctuations in resource or habitat use (Koch 2007). Because of this, bone collagen is a useful tissue in order to compare the main trophic behavior between sex and ontogenetic classes.

In order to better understand the trophic ecology of this species in this area, we investigated a possible isotopic ($\delta^{13}C$ and $\delta^{15}N$) variation among individual false killer whales that mass stranded along the southern shore of the Strait of Magellan in 1989, in relation to ontogenetic classes, sex and total body length. We hypothesize that (1) isotopic differences between nursing calves and older classes are related to the isotopic effect of lactation. We expected to measure low $\delta^{13}C$ values in calves due to the high lipid content in the ^{13}C depleted milk and high $\delta^{15}N$ values in respect to adult individuals, since the calf is actually feeding on breast milk with similar isotopic compositions to their mother's tissues (Fogel et al., 1989), and (2) isotopic differences between age and sex classes are related to differences in foraging habits that result in resource partitioning, probably due to body size and diving capabilities. In general, body size and isotope values are positively correlated for several prey species (e.g. squid, Cherel and Hobson, 2005); thus an individual false killer whale that is feeding deeper in the water column and/or on larger prey is expected to have enriched ^{13}C and ^{15}N compositions in its tissues.

We analyzed bone cranial samples of 29 individual false killer whales from the mass-stranding event that occurred along the Strait of Magellan in 1989 (Goodall et al., 2008). The specimens sampled are held in the Museo Acatushun de Aves y Mamiferos Marinos Australes (RNP Goodall collection), Estancia Harberton, Tierra del Fuego (Argentina).

The data on sex and total body lengths (TBLs) were determined by external examination during the field necropsy. Sexual maturity was determined by TBL or the presence of fetuses. For southern stocks, body lengths at birth are about 155 cm, and both sexes appear to reach sexual maturity between 8 and 14 years, with a body length of 325 cm in females and >360 cm in males (Ferreira et al., 2014). Although weaning was not recorded, calves may begin eating solid food between six to eight months of age and lactate for 19–24 months (Clark and Odell, 1999).

Based on the average TBLs at attainment of sexual maturity of southern stock animals, two individuals analyzed that measured 332 and 360 cm were classified as subadult males, and 24 were classified as adults. One pregnant female lacked a TBL measurement, but for its gestational stage it was obviously sexually mature. The other adult females ranged from 404 to 448 cm ($n = 13$) and adult males ranged from 491 to 548 cm ($n = 10$). Three of the individuals analyzed were classified as nursing calves, based on a combination of (a) their TBLs, that ranged from 207 to 253 cm; (b) the state of physical maturity for two of this individuals (PhM = 0, fetus or neonates, with at least some neural spines unfused to the centra, according to Goodall et al., 1988) and lastly (c) the estimated age for the larger one (0–1 year old, age estimated by ACM Schiavini and EA Crespo, pers. comm.). The individuals classified in this study as subadult were probably maturing. However, the isotopic composition of bone collagen is derived from food assimilated over a period of time that depends on the turnover rate of the tissue. Bone collagen, due to its slow isotopic turnover, integrates ecological information over several years, five to ten years depending on the ontogenetic stage of the animal (Hirons et al., 2001; Koch, 2007), thus SIA on subadult individuals gives us information on their foraging behavior prior to this stage. Also, the superior preservation qualities of bone in comparison to soft tissues (skin, muscle) allow for a post-mortem assessment of the ecological characteristics at the individual level (Hare et al., 1991; Newsome et al., 2010).

Bone samples were treated according to Riccialdelli et al. (2010); during this procedure each sample was demineralized in an 0.2 N HCl solution for ~96 h and then lipid extracted with a 2:1 chloroform:methanol solution. The collagen extraction was dried in an oven at 60 °C for ~48 h. Results are expressed in delta notation (δ) using the equation:

$$\delta^{13}\mathrm{C} \quad \text{or} \quad \delta^{15}\mathrm{N} = \left[\left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}}\right) - 1\right] \times 1000 \qquad (1)$$

where R_{sample} and R_{standard} are the ^{13}C:^{12}C or ^{15}N:^{14}N ratios of the sample and standard, respectively. The standards are Vienna-Pee Dee Belemnite limestone (V-PDB) for carbon and atmospheric N_2 for nitrogen. The units are expressed as parts per thousand or per mil (‰). The within-run standard deviation (SD) of an acetanilide standard was $\leq$0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ values.

We assessed isotopic variation for false killer whales in relation to ontogenetic class (calves, subadult and adult individuals), sex and TBLs. Due to the small sample size of our data set we use a