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# Fatty acids and stable isotopes ($\delta^{13}C$, $\delta^{15}N$) in southern right whale *Eubalaena australis* calves in relation to age and mortality at Península Valdés, Argentina

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ABSTRACT: Baleen whales accumulate fat reserves during the summer to sustain reproduction while fasting in the winter. The southern right whale *Eubalaena australis* population that calves off Península Valdés, Argentina, experienced high calf mortality events from 2003 to 2013 and poor nutritional states of mothers could be a contributing cause. Previous studies found that the population's reproductive success is influenced by prey availability. Mothers unable to build sufficient fat reserves or feeding on prey with different nutritional value may fail to meet the demands of lactation. Milk is the only source of nutrients and energy for calves at Valdés, so their fatty acids (FAs) and stable isotopes should reflect their mother's diet and feeding-ground locations. Here, we compared FA profiles and C and N stable isotopes of dead calves with those of living calves to evaluate the potential impact of maternal nutrition on calf survival. We found no differences in the FA composition of blubber in dead and living calves, indicating similar maternal diets. Likewise, the isotopic values of living and dead calves imply that their mothers had similar foraging ranges. However, FA composition was greatly affected by calf length, indicating effects of calf age and duration of nursing. These findings suggest that mothers of dead calves did not feed on different diets or feeding grounds compared to mothers of living calves. Future research should further assess the overall health and body condition of the Valdés southern right whale calves.

KEY WORDS: Fatty acids · Calves · Diet · Blubber · Mortality · Carbon-13 · Nitrogen-15 · Nutritional status · Growth

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## 1. INTRODUCTION

Diet provides essential nutrients and energy to support self-maintenance and reproduction in all living organisms. Capital breeders have the capacity to accumulate energy reserves in their bodies that are later used to support reproduction during the breeding season (Stearns 1989). This is the case for baleen whales that increase their fat and protein energy reserves by feasting on prey in high-latitude feeding

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grounds during the summer, and then migrating to low-latitude calving grounds (where food is scarce) to calve during the winter. This strategy could become problematic when prey are scarce on the whales' feeding grounds. If whales are unable to accumulate sufficient fat reserves or are feeding on prey items with different nutritional value, then they may not meet the energy demands of reproduction, migration or even self-maintenance.

The southern right whale *Eubalaena australis* population that calves at Península Valdés, Argentina, increased at an average annual rate of 6.8% for several decades, but then slowed to 5.1% in 2000–2010 (Cooke & Rowntree 2003, Cooke et al. 2015). In 2003, large numbers of calves began dying on the Valdés calving ground, with 607 calf deaths recorded from 2003 through 2013 (Rowntree et al. 2013, Sironi et al. 2018). The causes of the increased calf mortality remain unknown and differential intrinsic or extrinsic variables may influence survival depending on calf age, maternal body condition and calving season (Rowntree et al. 2013). For instance, newborn mortality could be linked to maternal inexperience (Best & Rüther 1992), and older calf mortality to harmful algal blooms (Wilson et al. 2016) or kelp gull harassment (Marón et al. 2015a, Fernández Ajó et al. 2018). Moreover, a suite of local or regional environmental disturbances may have affected deaths in calves of all ages at Valdés over the years (Rowntree et al. 2013).

Poor nutritional state of mothers (IWC 2011, 2018, Thomas et al. 2013) has been proposed as a contributing factor to calf deaths since declines in the abundance of Antarctic krill *Euphausia superba*, a primary prey for the whales (Tormosov et al. 1998), have been correlated with reproductive failures. The Valdés females had fewer calves than expected following El Niño events (Leaper et al. 2006), when sea surface temperatures are higher than normal and Antarctic krill abundance declines (Trathan et al. 2003). A similar response was reported for the southern right whale population off Brazil (Seyboth et al. 2016). These reproductive failures suggest that mothers suffering from nutritional stress are unable to sustain their pregnancy or experience the death of newborn calves, as observed in other mammals (Frisch 1997). When the whales migrate north of 40° S, they feed predominantly on calanoid copepods (Tormosov et al. 1998, Hoffmeyer et al. 2010); these prey are much smaller in size and biomass compared to Antarctic krill (Brierley et al. 1999, Atkinson et al. 2001), but it is unknown if reductions in the abundance of calanoid copepods affect southern right whale reproduction. If variations in prey abundance and type affect female reproductive success in the Valdés whales, then assessment of the nutritional status of calves and its relationship to calf survival would be essential for understanding whether differences in maternal diet are contributing to the recent high calf mortality events. In the present study, we used fatty acid (FA) and stable isotope analyses to evaluate differences in maternal diet and feeding ground locations between dead and living calves.

FAs of blubber and stable carbon and nitrogen isotopes of skin have been used as biomarkers to study diet composition and feeding locations of marine mammals (Hooker et al. 2001, Tucker et al. 2008, Valenzuela et al. 2018). Both FAs and stable isotopes are transferred throughout the food web and incorporated into animal tissues with predictable modifications (Wada et al. 1991, Iverson et al. 1995, 2004, Budge et al. 2006). These markers are particularly useful for studying the diets of species whose feeding cannot be observed directly. FA profiles (relative concentrations of all FAs identified in a sample) in the blubber of right whale females should reflect their diet composition since the FAs obtained from their prey (zooplankton) are allocated with little biochemical change into their blubber (Iverson 2009). Females usually fast while nursing at their calving grounds; thus, milk FAs are expected to derive from blubber mobilization, as documented for other fasting marine mammals (Iverson 1993, Iverson et al. 1995). FA profiles in the blubber of right whale calves should then reflect their mother's diet since the maternal milk that delivers the FA is the only source of energy and nutrients for calves during their first 3 mo of life at Península Valdés (Thomas & Taber 1984).

Stable isotopes of nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$) in the skin of the Valdés whales reflect their feeding sources. Valenzuela et al. (2009, 2018) found distinct isotopic groups that suggest that the southern right whales of Península Valdés may use at least 3 different feeding grounds: one group with the lowest isotope values feeds potentially south of the Polar Front and around South Georgia, another group with the highest isotope values feeds in the Patagonian Sea and northerly waters, while a third group uses both previous areas or a third unknown site. Females may feed on different prey according to their feeding ground choice. For example, the whales feeding north of 40° S in the Patagonian Sea may primarily consume calanoid copepods (Tormosov et al. 1998, Hoffmeyer et al. 2010, R. Payne pers. comm.), and females feeding south of 50° S (including South Geor-

gia and the Polar Front) may primarily consume euphausiids (Hamner et al. 1988, Tormosov et al. 1998). Since there is little difference in nitrogen and carbon isotopes between female right whales and their calves, isotope values in the skin of the Valdés' calves can be used to infer their mothers' main feeding locations (Valenzuela et al. 2010).

Here, we ask: (1) whether the FA profiles of dead calves indicate different maternal diets than those of living calves; and (2) whether the isotopic values of dead calves indicate different maternal foraging locations than those of living calves. We also evaluate changes in the FA profiles of dead calves with growth (length), stranding location, time of death, sex and carcass decomposition.

## 2. MATERIALS AND METHODS

### 2.1. Sample collection from dead calves

Blubber and skin samples were collected from southern right whale calves that died from June to December at Península Valdés (Golfo Nuevo and Golfo San José) in 2003–2011. Samples were used if they were taken from whales in states of decay 'code 2' (fresh) and 'code 3' (moderate decomposition) (Geraci & Lounsbury 2005). Even though body sampling location did vary, 71% of samples were taken along the dorsal region of the body. FA composition is uniform across most body sites that share the same function (i.e. FA composition is similar regardless of body site in the fat that serves as energy stores) (Koopman et al. 1996, Thiemann et al. 2006, Budge et al. 2008). A full blubber sample (5 × 5 to 15 × 15 cm) with skin attached was removed from each whale and stored frozen at −20°C and/or at −80°C in an airtight plastic bag until analysis. For each frozen sample, a vertical core (0.8 cm in diameter) was drilled from the skin to the end of the inner blubber layer. We removed the skin and the blubber from the extremes of the core (which were in contact with the air and thus more prone to oxidation) and used an ~3 cm blubber sample taken from the outer blubber layer (close to the skin) for lipid analysis. Blubber samples were only taken from the outer blubber layer and not from the deeper layers to make them comparable to blubber biopsy samples collected from living whales. Skin samples were either frozen or preserved in ethanol for isotope analysis. Length (straight line from snout–tip to fluke notch), carcass decomposition, date and location of necropsy were also recorded for each dead calf (Table S1 in the Supplement at www-int-res.com/articles/suppl/m646p189_supp.pdf). Most necropsied calves died during high calf mortality years (2003, 2005, 2007–2011) when the number of calf deaths was significantly greater than expected based on the population's long-term growth rate (Marón et al. 2015b). Fewer calves were available for sampling during low mortality years (2004 and 2006). Most necropsied calves were newborns, neonates or nursing calves less than 4–6 mo old (McAloose et al. 2016). Considering that calves are, on average, 5 to 5.5 m long at birth (Whitehead & Payne 1981, McAloose et al. 2016), we defined small dead calves as those shorter than 6 m and large dead calves as those longer than 6 m.

### 2.2. Sample collection from living calves

Biopsy samples of skin and blubber from living calves were collected from 21 September to 2 October 2011 in Golfo San José, Península Valdés, using darts propelled by a crossbow (Brown et al. 1991) for stable isotope and FA analyses, respectively. Samples were taken dorsally, mainly at the mid-section of the body. Small calves (shorter than 6 m) were not biopsied, hence all living calves that were biopsied were considered to be large (>6 m). Calf length and callosity development (McAloose et al. 2016) indicated that most of the sampled calves were less than 4–6 mo old and likely nursing (calves at Península Valdés nurse for at least their first 4 mo of life; Thomas & Taber 1984). The biopsy darts were fitted with tips 0.5 cm in diameter and 4 cm long, and removed small samples of skin and blubber approximately 3–4 cm deep in calves. Skin and blubber biopsies were preserved for up to 3 mo in an insulated Dewar flask filled with liquid nitrogen, and were later transferred to freezers at −79°C. Similar methods were followed by Valenzuela when collecting the skin biopsy samples from living calves in 2003–2005 used in this study for isotope analysis.

Importantly, we cannot assume that all the living calves used in this study (13 for fatty acid and 89 for stable isotope analysis) did not die later in the season. For instance, considering mortality records for large calves in 2011 (n = 23; Rowntree et al. 2013), 74% (n = 17) of that year's deaths occurred after the biopsy samples were collected. Thus, some or all of these deaths could have included the biopsied calves. Conversely, in 2004, only one calf died after biopsy sampling; thus, almost all calves may have survived the season. We consistently biopsy-sampled calves around the peak of whale abundance at

Península Valdés in September (Payne 1986, Crespo et al. 2019). Individual calves were not followed throughout the season after biopsying due to ethical, logistical and technical limitations inherent to that task, which would have involved tagging or other invasive procedures.

### 2.3. Lipid analysis

Lipids were extracted from 1 g blubber samples using a 2:1 chloroform:methanol ratio following a modified Folch et al. (1957) procedure (Budge et al. 2006) in 13 living and 51 dead calves. Fatty acid methyl esters (FAMEs) were prepared from 1–10 mg of the extracted lipid, using $H_2SO_4$ in methanol (Budge et al. 2006). FAMEs were extracted into hexane, concentrated and brought up to a concentration of 50 mg $ml^{-1}$ with high-purity hexane. Analyses of FAMEs were performed according to Budge et al. (2006) by gas liquid chromatography using a GC2010 gas chromatograph (Shimadzu Scientific Instruments) fitted with a silica capillary column (30 m × 0.25 mm ID) coated with 50% cyanopropyl polysiloxane (0.25 µm film thickness; Agilent Technologies, DB-23). A total of 58 FAs were identified using the commercial calibration standard GLC-463 (Nu-Chek Prep). Each FA was expressed as mass percent of total FAs and described using the nomenclature of carbon chain length:number of double bonds and location (n-x) of the double bond closest to the terminal methyl group. The total percentage of saturated FAs (SFAs), monounsaturated FAs (MUFAs) and polyunsaturated FAs (PUFAs) was also calculated.

### 2.4. Stable isotope analysis

To determine whether the mothers of dead and living calves fed in different locations, we compared the stable isotope values in the skin of 58 dead calves and 89 living calves (47 of which were previously analyzed by Valenzuela et al. 2010; Table S2 in the Supplement). For dead calves, samples were collected in 2003–2006 and 2011 in both Golfo San José (26%, n = 15) and Golfo Nuevo (72%, n = 43). For living calves, collections were made in 2003–2005 and 2011 but only in Golfo San José. Stable nitrogen and carbon isotopes were analyzed in the SIRFER Laboratory (Stable Isotope Ratio Facility for Environmental Research, University of Utah). Skin samples were lyophilized and ground to a fine powder before lipid extraction following Todd et al. (1997). Skin samples (~1 mg) were analyzed using a Carlo Erba 1108 elemental analyzer coupled to a Thermo Finnigan Delta S Isotope Ratio Mass Spectrometer. Isotope values are expressed as $\delta^{13}C$ or $\delta^{15}N$ (‰) = $[(R_{sample}/R_{standard}) - 1] \times 1000$, where $R$ is $^{13}C/^{12}C$ or $^{15}N/^{14}N$, for $\delta^{13}C$ or $\delta^{15}N$, respectively. Standards of glutamic acid were referenced to Vienna-Pee Dee Belemnite (VPDB) for carbon and to atmospheric air for nitrogen. Isotope values of standards used were Primary Laboratory Reference Material (PLRM)-1: $\delta^{13}C$ = 23.96‰, $\delta^{15}N$ = 49.63‰; and PLRM-2: $\delta^{13}C$ = −29.18‰, $\delta^{15}N$ = −4.56‰. Measurement precision (within-run replicate analysis of PLRMs) was better than 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$. C:N ratios varied from 3.1 to 3.6 for living calves (n = 42) and from 2.9 to 3.9 for dead calves (n = 58; Table S2). C:N ratios were not available for the 47 living calves previously analyzed by Valenzuela et al. (2010).

### 2.5. Statistical analyses

To achieve normality, whale blubber FA data were transformed using the following function (Aitchison 1983): $x_t = \ln (x_i/c_r)$, where $x_t$ represents the transformed FA data, $x_i$ is a given FA expressed as percent of total FAs and $c_r$ is the percentage of a reference FA. We used 18:0 as the reference FA. To assess variation in FA composition between dead and living calves (n = 13), a principal components analysis (PCA) was conducted on a reduced set of 30 transformed FAs that have a dietary origin (according to Iverson et al. 2004; see Table 1) using the FA profiles of all calves. Multivariate analysis of variance (MANOVA) was carried out on the PC1 and PC2 scores with calf length (small or large) and calf condition (dead or alive) as factors. To assess variation in the FA composition of calves with growth, dead calf state of decay, time of death (month of the season in which the calf was found dead), sex and stranding location, PCA was performed on all 58 transformed FAs using the FA profiles of dead calves (small, n = 40; large, n = 11). The entire suite of FAs was used since FAs with endogenous sources may vary with growth. MANOVA was then carried out on the PC1 and PC2 scores with dead calf state of decay, time of death, sex and location as factors and body length (as a proxy for age) as a covariate.

To determine whether the feeding grounds of mothers of living and dead calves differed, Kruskal-Wallis ANOVA was used to test for differences of isotopic values between living and dead calves (living versus all dead calves, and living versus large dead

Table 1. Proportions of fatty acids in the external blubber layer of dead and living southern right whale calves. Small and large dead calves indicate individuals smaller or longer than 6 m long, respectively

| Fatty acid | Dead small calves n = 40 | | Dead large calves n = 11 | | Living large calves n = 13 | |
|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE |
| **Saturated** | | | | | | |
| 12:0 | 2.48 | 0.82 | 0.12 | 0.02 | 0.09 | 0.00 |
| 13:0 | 0.15 | 0.02 | 0.05 | 0.01 | 0.04 | 0.00 |
| 14:0 | 5.54 | 0.38 | 6.04 | 0.24 | 5.92 | 0.19 |
| *i*-15:0 | 0.25 | 0.03 | 0.22 | 0.02 | 0.23 | 0.05 |
| *ai*-15:0 | 0.06 | 0.01 | 0.07 | 0.00 | 0.07 | 0.00 |
| 15:0 | 0.33 | 0.01 | 0.35 | 0.02 | 0.34 | 0.01 |
| *i*-16:0 | 0.63 | 0.12 | 0.22 | 0.12 | 0.10 | 0.01 |
| 16:0 | 16.07 | 0.84 | 10.83 | 0.48 | 10.61 | 0.26 |
| *i*-17:0 | 0.23 | 0.01 | 0.24 | 0.01 | 0.27 | 0.01 |
| *ai*-17:0 | 0.23 | 0.04 | 0.13 | 0.01 | 0.13 | 0.01 |
| 17:0 | 0.23 | 0.01 | 0.22 | 0.02 | 0.21 | 0.01 |
| 18:0 | 5.72 | 0.47 | 2.65 | 0.34 | 2.66 | 0.16 |
| 20:0 | 0.23 | 0.02 | 0.10 | 0.02 | 0.09 | 0.01 |
| Subtotal | 32.17 | 1.49 | 21.24 | 0.75 | 20.75 | 0.47 |
| **Monounsaturated** | | | | | | |
| 14:1n-9 | 0.14 | 0.01 | 0.12 | 0.01 | 0.13 | 0.01 |
| 14:1n-7 | 0.10 | 0.01 | 0.06 | 0.00 | 0.07 | 0.01 |
| 14:1n-5 | 0.85 | 0.04 | 1.07 | 0.06 | 1.11 | 0.06 |
| 16:1n-11 | 0.29 | 0.04 | 0.38 | 0.03 | 0.46 | 0.09 |
| 16:1n-9 | 1.10 | 0.13 | 0.36 | 0.07 | 0.39 | 0.07 |
| 16:1n-7 | 12.71 | 0.52 | 15.63 | 0.90 | 15.83 | 0.69 |
| 16:1n-5 | 0.29 | 0.01 | 0.29 | 0.01 | 0.28 | 0.01 |
| 17:1 | 0.07 | 0.00 | 0.06 | 0.00 | 0.07 | 0.00 |
| 18:1n-11 | 0.64 | 0.10 | 0.51 | 0.12 | 0.38 | 0.07 |
| 18:1n-9 | 17.62 | 0.41 | 19.17 | 0.33 | 19.98 | 0.37 |
| 18:1n-7 | 7.08 | 0.34 | 5.66 | 0.57 | 5.86 | 0.35 |
| 18:1n-5 | 0.79 | 0.05 | 0.56 | 0.06 | 0.50 | 0.04 |
| 20:1n-15 | 0.05 | 0.01 | 0.05 | 0.00 | 0.07 | 0.01 |
| 20:1n-11[a] | 0.22 | 0.01 | 0.32 | 0.07 | 0.24 | 0.02 |
| 20:1n-9[a] | 0.92 | 0.10 | 2.12 | 0.46 | 1.51 | 0.21 |
| 20:1n-7[a] | 0.50 | 0.04 | 0.25 | 0.04 | 0.24 | 0.02 |
| 22:1n-11[a] | 0.27 | 0.03 | 0.61 | 0.18 | 0.31 | 0.05 |
| 22:1n-9[a] | 0.23 | 0.02 | 0.24 | 0.03 | 0.20 | 0.02 |
| 22:1n-7[a] | 0.09 | 0.01 | 0.04 | 0.01 | 0.06 | 0.01 |
| 24:1 | 0.18 | 0.03 | 0.12 | 0.03 | 0.17 | 0.02 |
| Subtotal | 44.14 | 0.77 | 47.64 | 0.45 | 47.86 | 0.60 |
| **Polyunsaturated** | | | | | | |
| 16:2n-6[a] | 0.08 | 0.01 | 0.05 | 0.00 | 0.05 | 0.00 |
| 16:2n-4[a] | 0.29 | 0.03 | 0.50 | 0.04 | 0.48 | 0.02 |
| 16:3n-4[a] | 0.27 | 0.02 | 0.41 | 0.02 | 0.42 | 0.01 |
| 16:4n-3[a] | 0.21 | 0.02 | 0.29 | 0.02 | 0.35 | 0.02 |
| 16:4n-1[a] | 0.09 | 0.01 | 0.12 | 0.01 | 0.11 | 0.01 |
| 18:2n-6[a] | 1.35 | 0.12 | 1.80 | 0.07 | 1.94 | 0.05 |
| 18:2n-4[a] | 0.14 | 0.01 | 0.12 | 0.01 | 0.11 | 0.01 |
| 18:3n-6[a] | 0.09 | 0.01 | 0.11 | 0.01 | 0.11 | 0.01 |
| 18:3n-4[a] | 0.17 | 0.01 | 0.20 | 0.02 | 0.17 | 0.01 |
| 18:3n-3[a] | 0.54 | 0.07 | 0.93 | 0.08 | 0.88 | 0.04 |
| 18:3n-1[a] | 0.10 | 0.01 | 0.06 | 0.01 | 0.06 | 0.00 |
| 18:4n-3[a] | 0.51 | 0.08 | 1.08 | 0.19 | 1.14 | 0.13 |
| 18:4n-1[a] | 0.33 | 0.03 | 0.49 | 0.03 | 0.46 | 0.03 |
| 20:2n-6[a] | 1.00 | 0.12 | 0.34 | 0.14 | 0.28 | 0.03 |
| 20:3n-6[a] | 0.41 | 0.04 | 0.23 | 0.02 | 0.23 | 0.01 |
| 20:4n-6[a] | 1.10 | 0.16 | 0.70 | 0.17 | 0.65 | 0.03 |
| 20:3n-3[a] | 0.07 | 0.01 | 0.13 | 0.02 | 0.13 | 0.01 |
| 20:4n-3[a] | 1.19 | 0.16 | 2.31 | 0.22 | 2.25 | 0.12 |
| 20:5n-3[a] | 4.54 | 0.44 | 8.95 | 0.74 | 9.09 | 0.45 |
| 21:5n-3[a] | 0.53 | 0.03 | 0.41 | 0.03 | 0.42 | 0.02 |
| 22:4n-6[a] | 0.28 | 0.03 | 0.13 | 0.02 | 0.17 | 0.03 |
| 22:5n-6[a] | 0.17 | 0.02 | 0.14 | 0.02 | 0.21 | 0.04 |
| 22:4n-3[a] | 0.21 | 0.02 | 0.21 | 0.03 | 0.27 | 0.04 |
| 22:5n-3 | 5.50 | 0.23 | 4.70 | 0.31 | 4.76 | 0.22 |
| 22:6n-3[a] | 4.54 | 0.31 | 6.71 | 0.46 | 6.66 | 0.34 |
| Subtotal | 23.69 | 1.05 | 31.12 | 0.63 | 31.39 | 0.53 |
| **Total** | 100 | | 100 | | 100 | |

[a]FAs which have a dietary origin (Iverson et al. 2004), and were included in a reduced set for statistical analyses

calves). Additionally, and according to their stable carbon and nitrogen values (Valenzuela et al. 2018), we placed living and dead calves into 2 distinctive foraging locations, the Patagonian Shelf (highest isotopic values) and the Southern Ocean (lowest isotopic values; see Table S1 in the Supplement), and compared the 2 frequencies with a Chi-squared test. Finally, we tested whether the stable carbon and nitrogen values differed between years (2003, 2004, 2005 and 2011) using Kruskal-Wallis ANOVA and the Wilcoxon rank sum test adjusted by Bonferroni for pairwise comparisons. Data from 2006 were excluded from these comparisons because of the small sample size (n = 2). All statistical analyses were conducted in R 3.5.1 (R Core Team 2018).

## 3. RESULTS

### 3.1. FA composition in southern right whale calves

A total of 58 FAs with 12–24 carbon atoms were identified in the blubber of 64 dead and living southern right whale calves at average concentrations of 0.04% or more (Table 1). Five major FA accounted for around 60% of the whale blubber in all calves: 16:0, 16:1n-7, 18:1n-9, 20:5n-3 and 22:6n-3. Other major FA components (1% or more of total FAs) were 14:0, 18:0, 18:1n-7, 18:2n6, 20:4n3 and 22:5n-3 in all calves. The FA composition of small dead calves (<6 m, n = 40) showed 3 other major (>1%) FAs: 12:0, 16:1n-9 and 20:4n-6. The percentage of total MUFAs was the highest in larger calves, followed by % PUFA and % SFA, but in small dead calves the relative abundances were MUFAs>SFAs>PUFAs.

### 3.2. FA profiles do not differ between large dead and living calves

PCA of all calves (living, n = 13; dead, n = 51; Table 2) using just the suite of 30 dietary FAs generated 2 PCs that explained 70% of the variance (PC1: 59%, PC2: 11%; Fig. 1). Large dead and living calves were clustered together but small dead calves showed a wider range of loadings. MANOVA was carried out using the PC1 and PC2 scores and a significant effect of length (shorter or longer than 6 m) was found when comparing all calves (n = 64, Wilks' $\lambda = 0.46$, $p < 0.00001$). The

factor dead or alive also had an effect when considering all calves (Wilks' $\lambda$ = 0.78, p = 0.0005), but had no effect when analyzing only large dead and living calves (Wilks' $\lambda$ = 0.96, p = 0.67).

### 3.3. FA composition of calves changes with growth

To assess variation in the FA composition of calves with growth, a PCA of dead calves (large and small) was carried out on all 58 identified FAs. Only dead calves were selected since their exact length could be measured (range 3.64 to 8.05 m, 78% smaller than 6 m; Table 1). The first 2 PCs explained 68% of the variance (PC1: 55%, PC2: 13%). Length was the only factor that had a detectable effect on the FA composition of dead calves (Wilks' $\lambda$ = 0.45, p < 0.00001). All other factors (time of death, sex, fresh versus moderate state of decomposition, and stranding location) had no detectable effects on the FA profiles of dead calves. Percentages of total SFAs, total MUFAs, total PUFAs, the SFA 16:0, and the PUFAs 20:5n-3 and 22:6n-3 were compared with dead calf length (Fig. 2). Both total SFAs and the FA 16:0 decreased with calf length (regression, % SFA p < 0.00001, FA 16:0 p < 0.00001). However, total MUFAs, total PUFAs and the FAs 20:5n-3 and 22:6n-3 increased with length (regression, % MUFA p = 0.01, % PUFA p < 0.00001, FA 20:5n3 p < 0.00001, FA 20:5n3 p < 0.00001). The levels of total MUFAs, total PUFAs and the FA 22:6n-3 showed a slight plateau in calves longer than 6 m. However, the levels of 20:5n-3 increased even in calves longer than 6 m.

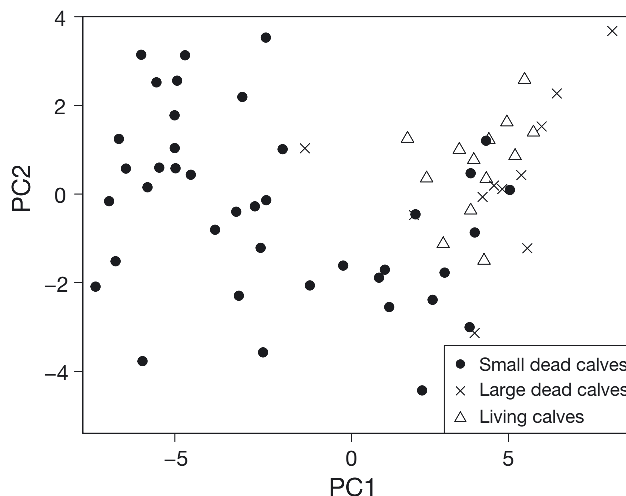


Fig. 1. Plot of scores of principal components 1 (PC1) and 2 (PC2), representing the influence of length on fatty acid composition of dead calves. Large dead and living calves (approximately >6 m in length) are clustered together, while small dead calves (<6 m) are more dispersed

Table 2. Number of dead and living calves included in this study for fatty acid and stable isotope analyses ($\delta^{13}C$, $\delta^{15}N$). Summaries of each analysis are provided in **bold**

| Type of analysis | Year | Dead | Living |
|---|---|---|---|
| Fatty acids | 2003 | 4 | |
| | 2004 | 2 | |
| | 2005 | 4 | |
| | 2006 | 1 | |
| | 2007 | 3 | |
| | 2008 | 3 | |
| | 2009 | 10 | |
| | 2010 | 13 | |
| | 2011 | 11 | 13 |
| | **9 years** | **51** | **13** |
| Stable isotopes | 2003 | 11 | 12 |
| | 2004 | 6 | 25 |
| | 2005 | 17 | 35 |
| | 2006 | 2 | |
| | 2011 | 22 | 17 |
| | **5 years** | **58** | **89** |

### 3.4. Feeding grounds do not differ between mothers of living and dead calves

Stable carbon and nitrogen isotopes were analyzed in the skin of 89 living and 58 dead calves, showing a wide range of values (Fig. 3, Table 2). $\delta^{13}C$ values ranged from −23.8 to −16.9‰ (mean = −21.6‰, SD = 1.5‰) in living calves and from −23.7 to −16.9‰ (mean = −21.8‰, SD = 1.6‰) in dead calves. $\delta^{15}N$ values ranged from 6.7 to 14.1‰ (mean = 8.3‰, SD = 1.6‰) in living calves and from 5.9 to 14.1‰ (mean = 8.3‰, SD = 2.0‰) in dead calves. Carbon and nitrogen isotope values varied among years for living calves (Kruskal-Wallis $\delta^{13}C$: $\chi^2$ = 15.39, p = 0.002; $\delta^{15}N$: $\chi^2$ = 12.39, p = 0.01), but this variation was only observed in carbon values for dead calves (Kruskal-Wallis: $\delta^{13}C$: $\chi^2$ = 14.13, p = 0.01; $\delta^{15}N$: $\chi^2$ = 7.26, p = 0.12). $\delta^{13}C$ was the highest in 2003 compared to 2004, 2005 and 2011 in living calves (Wilcoxon all p ≤ 0.01, Fig. S1), and the highest in 2005 compared to 2011 in dead calves (Wilcoxon p = 0.04). $\delta^{15}N$ was significantly low in 2004 compared to 2003 and 2005 in living calves (Wilcoxon all p = 0.01), but showed no significant differences in dead calves (Kruskal-Wallis $\delta^{15}N$: $\chi^2$ = 7.26, p = 0.12).

Neither $\delta^{13}C$ nor $\delta^{15}N$ values differed between living and dead calves (Kruskal-Wallis $\delta^{13}C$: $\chi^2$ = 1.08, p = 0.3;

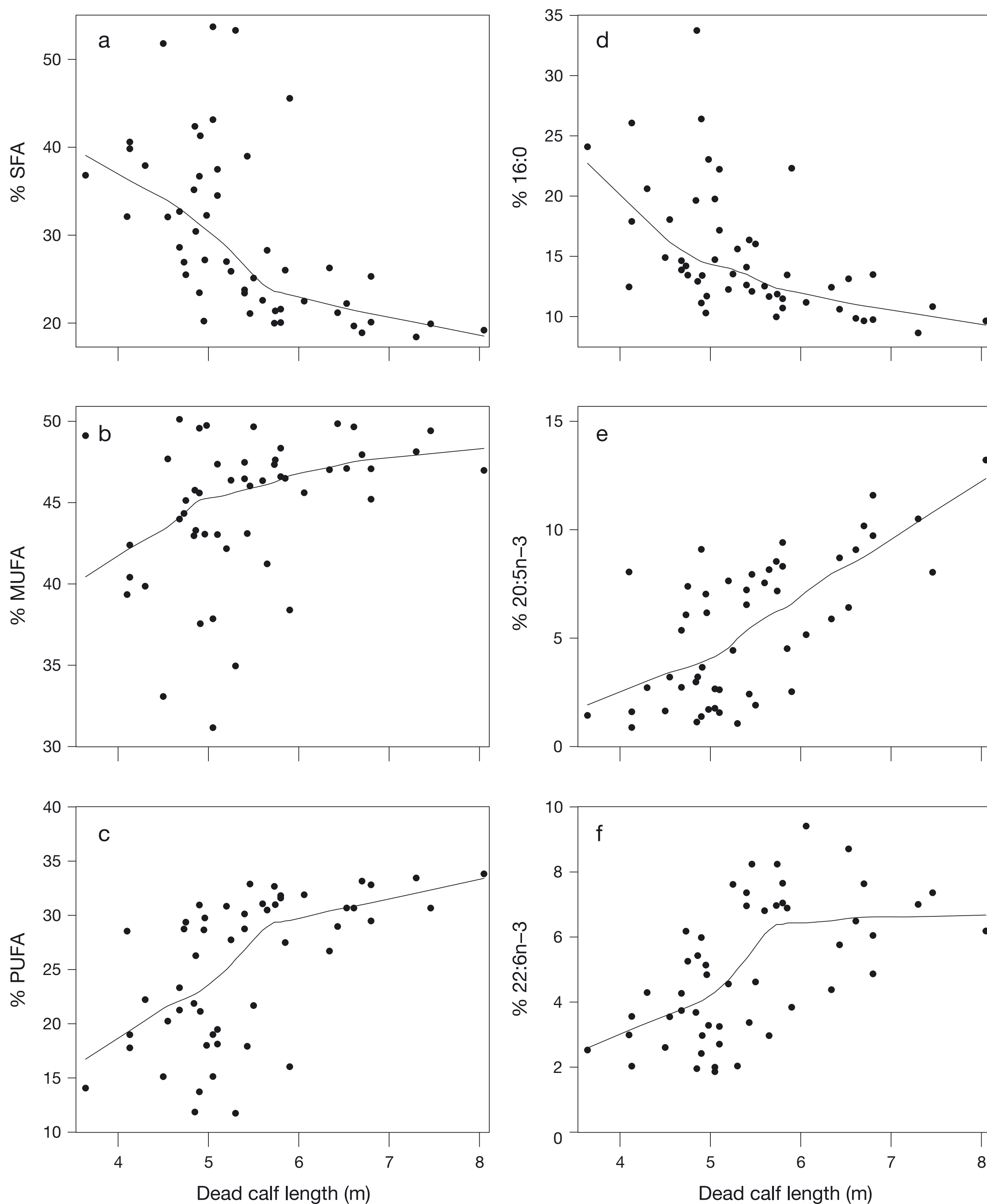


Fig. 2. Changes in percent of different fatty acids in the blubber of dead southern right whale calves with body length. (a) Total saturated fatty acids (SFAs) and (d) SFA 16:0 decrease with calf length. (b) Total monounsaturated fatty acids (MUFAs), (c) total polyunsaturated fatty acids (PUFAs), and the PUFAs (e) 20:5n-3 and (f) 22:6n-3 increase with calf length. Length measurements were taken as a straight line from snout-tip to fluke notch. Smoothed trend lines were estimated by locally weighted polynomial regression using the lowess() function in R. Note that each panel has its own *y*-axis scaling

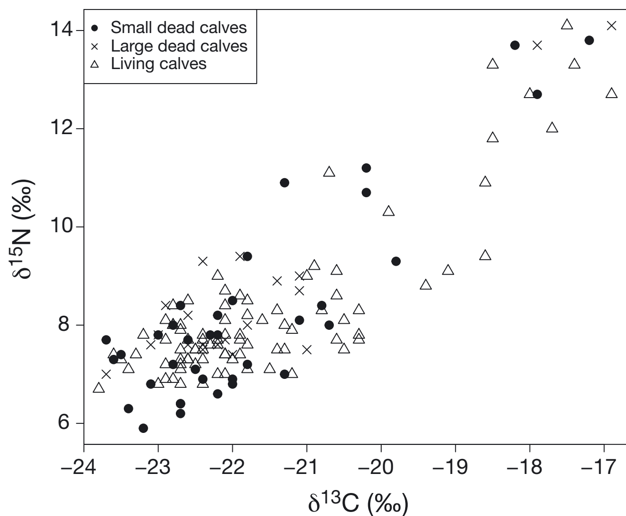


Fig. 3. Stable carbon and nitrogen isotope values in the skin of southern right whale living (n = 89) and dead calves (n = 58). Statistical analyses indicate no significant differences between these 2 groups

$\delta^{15}$N: $\chi^2$ = 0.6, p = 0.8), nor between large living and large dead calves (Kruskal-Wallis $\delta^{13}$C: $\chi^2$ = 0.46, p = 0.5; $\delta^{15}$N: $\chi^2$ = 0.65, p = 0.42). Similar results were found between large and small dead calves (Kruskal-Wallis: $\delta^{13}$C: $\chi^2$ = 0.1, p = 0.75; $\delta^{15}$N: $\chi^2$ = 2.04, p = 0.15). Seven living and 5 dead calves were assigned to the Patagonian shelf feeding ground and 82 living and 53 dead calves were assigned to the Southern Ocean feeding ground. Chi-squared results indicated no difference between the proportion of living and dead calves in each specific feeding ground ($\chi^2$ = 0.33, p = 0.56).

## 4. DISCUSSION

This study combines FA and stable isotope analyses to better understand the nutritional status of the southern right whale calves off Península Valdés that experienced unusually high mortality from 2003 through 2013. Our findings suggest that mothers of dead calves fed on similar diets and feeding grounds compared to mothers of living calves, and that dead calves did not suffer from an inefficient transfer of milk FAs.

The FA composition of southern right whale calves is typical for marine mammals. Dominant FAs in the whale calf blubber, including 16:0, 16:1n-7, C18:1n-9, 20:5n-3 and 22:6n-3, resemble those found in the blubber of bowhead whales *Balaena mysticetus* (Budge et al. 2008), minke whales *Balaenoptera acutorostrata* (Olsen & Grahl-Nielsen 2003), harbor porpoises *Phocoena phocoena* (Koopman et al. 1996) and humpback whales *Megaptera novaeangliae* (Waugh et al. 2014). However, the FA proportions were substantially different from those of southern right whales off South Africa (Reeb 2001). For instance, in neonates off South Africa, SFAs were the highest (~60%) compared to the Valdés neonates, where MUFAs showed the highest proportions (44%). Furthermore, in the whales off South Africa, PUFAs were only 10% but 24% in the Valdés small calves. In addition, the essential FA 20:5n-3, found in all marine mammals, was not identified in the whales off South Africa. These differences likely result from applying different protocols for FA analysis. Given these uncertainties, more data is needed before further comparisons can be made between whales from other southern right whale populations, including the whales off South Africa.

No significant differences were found among the FA profiles of dead and living calves of similar size (>6 m). Important FAs such as 20:5n-3 and 22:6n-3, which are required for the normal development of young in many animals (Neuringer et al. 1984, Cunnane et al. 2000), showed almost identical values in both living and large dead calves on the Valdés calving ground. We were unable to compare small (<6 m) dead and living calves since we only biopsied large living calves. However, the FA profiles of small dead calves were similar to the profiles found in newborns of other marine mammals (Iverson et al. 1995, Grahl-Nielsen et al. 2000, Birkeland et al. 2005, Wheatley et al. 2008), and essential FAs, such as 18:2n-6, 18:3n-3, 20:5n-3 and 22:6n-3, were not depleted in their blubber, indicating that they might have nursed for a short period of time. This finding suggests that maternal transfer of FAs was not limited during the years assessed in our study.

The FA composition of calves changes with growth. In small calves, the total percentage of SFAs was higher than in larger calves. The proportion of 16:0 was particularly high in small calves (<6 m in length) as is typical of newborn marine mammals (Iverson et al. 1995, Birkeland et al. 2005). This SFA is thought to be produced by the offspring during gestation through the action of FA synthetase (Iverson et al. 1995, Birkeland et al. 2005). The MUFA 18:1n-7 also showed higher concentrations in small calves and it is known to be highly concentrated in the blubber of newborns (Iverson et al. 1995, Birkeland et al. 2005, Wheatley et al. 2008).

In large calves, the total percentages of MUFAs and PUFAs increased with length, particularly the

proportions of the PUFAs 20:5n-3 and 22:6n-3 when compared to the levels found in small calves. There is evidence of selective mobilization of 20:5n-3 and 22:6n-3 through maternal milk, which results in accumulation of these FAs in the blubber of the offspring throughout lactation (Iverson et al. 1995, Grahl-Nielsen et al. 2000, Wheatley et al. 2008). Both FAs play a role in the development of the circulatory and neurological systems in many animals (Neuringer et al. 1984, Bell et al. 1995, McCann & Ames 2005), making it likely that higher concentrations of these FAs in the calves' blubber may be required for enhancing calf growth.

It has been hypothesized that differences in the FA profiles of small and large calves are due to differential times of nursing (Reeb 2001). Small (younger) calves will not have nursed as long as large (older) calves, and their FAs will reflect a profile more similar to those of gestational stages (with prevalence of SFAs) than to the profiles influenced by milk consumption (with prevalence of 20:5n-3, 22:6n-3, other PUFAs, and MUFAs) typical of large calves. The distribution of FA profiles in small calves (Fig. 1) may also reflect a differential nursing period among individuals within this group. Small dead calves that clustered much closer to the profiles of living calves and large dead calves may have been nursing for a longer period of time than small calves that were distributed away from those profiles. Thus, FA analysis may indeed serve as a useful tool to determine age in calves. Although length can be used as a proxy for age, it cannot be used to differentiate the age of calves that actually have the same lengths but were born at different times (Best & Rüther 1992). Primiparous females usually give birth to smaller calves compared to multiparous females. FA analysis has been successfully used to determine the age of juvenile and adult humpback whales *Megaptera novaeangliae* and killer whales *Orcinus orca* (Herman et al. 2008, 2009). In this study, we provide some evidence that FAs may be used as a more accurate indicator of calf age even in a very small window of time (months) since the profiles of small calves were very different from large calves, which were no more than 4 mo old (mothers stay at Valdés up to 4 mo; Thomas & Taber 1984). This FA indicator along with new photogrammetry methods currently in use (Christiansen et al. 2018) could help monitor early calf development and growth during the breeding season and improve our knowledge about the specific ages that might be affected by calf mortality events in large species of baleen whales such as right whales.

Stable isotope values in the skin of living and dead calves support the FA data, suggesting that their mothers used similar foraging areas and fed on similar prey. The isotopic ranges of the calves corresponds to the 3 isotopic sources suggested by Valenzuela et al. (2018) that may represent the whales' feeding grounds: South Georgia and the Polar Front, the Patagonian Sea, and an unknown area or a combination of the previous two. In addition, differences in carbon and nitrogen stable isotopes were detected between different calving years in living calves coincidentally with results from Valenzuela et al. (2010). Only variation in carbon was detected in dead calves. In living calves, higher $\delta^{13}C$ values in 2003 and $\delta^{15}N$ values in 2003 and 2005 may reflect changes in maternal diet throughout pregnancy (Valenzuela et al. 2010). Results found in the present study also support previous findings about little variation in nitrogen and carbon isotopes ratios between female right whales and their calves. Stable isotope values of living and dead calves reported here differed in less than a trophic level to the values found by Valenzuela et al. (2010, 2018) for adult female right whales. This confirms that stable isotopes in the skin of young calves can be used to infer maternal feeding grounds. Although we found no evidence to support the hypothesis that mothers of dead calves had fed on different diets than mothers of living calves or that dead calves suffered from an inefficient transfer of milk FAs compared to living calves, a nutritional factor cannot be excluded as a potential contributor to the calf deaths. Other ongoing studies, such as the evaluation of anatomical, physiological and behavioral conditions of calves, are needed to evaluate the overall health of the Valdés right whale calf population. Further research should examine how blubber FA composition changes with the whale's body location, age, sex and reproductive status. Our findings show that data for such studies can be collected from living whales or from dead whales in fresh or moderate states of decay.

The main limitations of this study are the low sample size of living calves for FA analysis and our inability to ensure that all living calves sampled survived the calving season. Samples from living calves for FA analysis (n = 13) were obtained in only one calving season (2011) due to resource limitations. Future studies should broaden the sample size to include several years. In addition, we categorized as 'living calves' those whales that were biopsied when they were alive, but we did not tag or follow each individual throughout the season to ensure survival. Hence, some of the living calves biopsied may have

died later in the season and may be included in both the living and dead data sets. Ethical, technical and logistical challenges hinder individual calf monitoring throughout the season (Andrews et al. 2019); however, new drone-based methods (Christiansen et al. 2018) may allow pairing biopsy sampling with individual follow-up.

Further research should examine how blubber FA composition changes with blubber depth. In the present study, we used a superficial biopsy technique to sample blubber from the outer layer of living calves. However, in adult whales, the outer layer is less rapidly influenced by changes in diet, and the use of a more active inner layer (close to the muscle) is preferable for evaluation of diet (Cooper 2004, Iverson et al. 2004). Other techniques, such as a biopsy pole, can be used to collect deep core blubber samples from the inner, middle and outer layers (Reeb & Best 2006). However, limitations related to the proximity to the whales and the whales' reaction and safety should be considered further. More research is needed to understand the dynamics of FA deposition and mobilization in the outer and inner layers of calf blubber.

If southern right whales are dependent on Antarctic krill for successful reproduction (Leaper et al. 2006, Seyboth et al. 2016), and krill is affected by sea surface temperature increments, changes in maternal diets and feeding grounds could be monitored using FA and stable isotope data. This study provides a useful baseline to evaluate changes in the feeding ecology of the southern right whales under future environmental scenarios driven by climate change.

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