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Short Communication

An evaluation of whale skin differences and its suitability as a tissue for stable isotope analysis

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ABSTRACT

Stable isotope analysis of whale skin has been recurrently used to assess diet and movement patterns. Such studies rely on the untested assumption that the stable isotope ratios in the small skin biopsies analysed are representative of those throughout the skin. In balaenopterids, the ventral skin looks notably different from that of the dorsal region, which is smoother and darker. To investigate possible differences in isotopic ratios throughout the skin, we collected and analysed samples from dorsal and ventral positions in 28 fin whales (*Balaenoptera physalus*). No significant differences were found between these two skin positions, which might suggest that whale skin is likely a homogeneous tissue. Thus, the isotopic ratios determined at a specific point may be representative of the whole skin in whales.

1. Introduction

Stable isotope ratios have been widely used to investigate the trophic ecology, habitat use, migration patterns and physiological events of marine mammal populations (e.g., Lesage et al. 2001; Borrell et al. 2006; Drago et al. 2009; Vighi et al. 2014; Borrell et al., 2016; Pinela et al. 2015). However, while the applicability of stable isotopes has been repeatedly tested and analyses of these isotopes are commonly performed, some authors highlighted the need to control factors that can lead to errors in the interpretation of the results (e.g., Barrow et al. 2008; Mill et al. 2008, Ryan et al. 2012; Payo et al. 2013; Yurkowsky et al. 2014). Among the factors that deserve more attention are the suitability and homogeneity of the analysed tissues, due to the limited existing information on this topic (Williams et al. 2008; Tod et al. 2010; Hussey et al. 2011; Arregui et al. 2017).

The collection of tissues from free-ranging cetaceans is not easy, and biopsy techniques have been developed for this reason (Aguilar and Nadal, 1984). The biopsy obtained using darts equipped with a small head-shaped drill usually consists of a small section of the skin that frequently is not sampled in the same site.

The skin is not a uniform tissue but, instead, presents variations in different locations of the body. In balaenopterids, the appearance of the ventral skin is very different to that in the dorsal region, which is smoother, thinner and darker. Despite this obvious variability, the site-specific variation in the skin isotopic ratios of whales has never been analysed. However, knowledge of this variation is central to studies using this tissue.

Hence, the objective of this study was to investigate the possible differences in stable isotope ratios in the skin of fin whales between the two positions reflecting the greatest differences in skin characteristics: the dorsal and ventral regions. We hypothesized that if skin isotope ratios were compared among sites across the body, then ventral and dorsal skin should be the most different. For this reason, we chose these two positions, even though it is not easy to get skin biopsies from the ventral side of the whales. However, in some special circumstances, only the ventral part of the animal might be accessible to sample the skin. This is the case of strandings, where the specimens are upside down, or when the dorsal part has been predated, or even in dead animals floating in the water.

2. Materials and methods

Skin samples from 2 body positions (dorsal and ventral; Fig. 1) were obtained from 28 fin whales caught off of W Iceland and processed by legal commercial whaling operations at the Hvalur H/F whaling station (Hvalfjörður, Iceland) in 2015.

All samples were preserved frozen. Prior to the analyses, skin samples weighing approximately 250 mg were dried at 40 °C for 24 h

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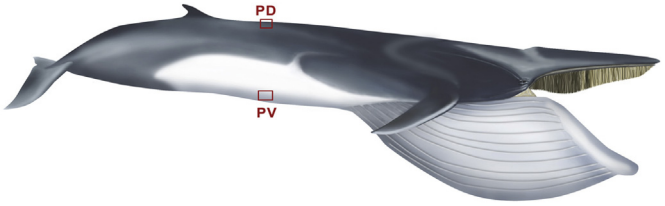

Fig. 1. Sampling positions. PD = dorsal and PV = ventral.

and then ground to a powder with a mortar and pestle. Since lipids may bias the analysis by decreasing $\delta^{13}C$ values (Yukowski et al. 2014), they were removed from the samples by rinsing the powdered tissue several times with a chloroform/methanol (2:1) solution.

The preparation for isotope analysis followed that of Borrell et al. (2012). After pre-treatment, approximately 0.3 mg of each powdered sample was weighed into tin capsules and combusted at 900 °C. Isotopic analyses were carried out by means of analyser/isotope ratio mass spectrometry (EA-IRMS) using a Thermo Finnigan Flash 1112 (CE Elantech, Lakewood, NJ, USA) elemental analyser, coupled to a Delta C isotope ratio mass spectrometer via a ConFlo III interface (both from Thermo Finnigan, Bremen, Germany).

Carbon isotope ratios are reported relative to Vienna Pee Dee Belemnite limestone (VPDB) and nitrogen relative to AIR. The accuracy for $\delta^{13}C$ and $\delta^{15}N$ measurements were 0.1‰ and 0.3‰, respectively.

The distribution of the isotope ratios and the presence of outliers were tested graphically through boxplots. Two outliers (one each for the $\delta^{15}N$ and $\delta^{13}C$ values) were removed from the posterior statistical analysis (Fig. 2). The normality and homoscedasticity of the data were checked using Lilliefors' and Levene's tests, respectively. Differences in $\delta^{15}N$ and $\delta^{13}C$ mean values between the dorsal and ventral skin were tested by Pairwise Student's *t*-tests; The relationship between dorsal and ventral skin was calculated using linear regressions. All statistical analyses were conducted with the IBM SPSS 23 software package.

3. Results

Boxplots of the obtained $\delta^{15}N$ and $\delta^{13}C$ values by position (ventral and dorsal) are presented in Fig. 2. Descriptive statistics were calculated for the ventral and dorsal positions together to summarize the data, with the following results: $\delta^{13}C$ values ranged from −20.24‰ to −18.92‰ (mean ± SD = −19.41 ± 0.30‰) and $\delta^{15}N$ values, ranged from 8.27‰ to 10.50‰ (mean ± SD = 9.40 ± 0.58‰).

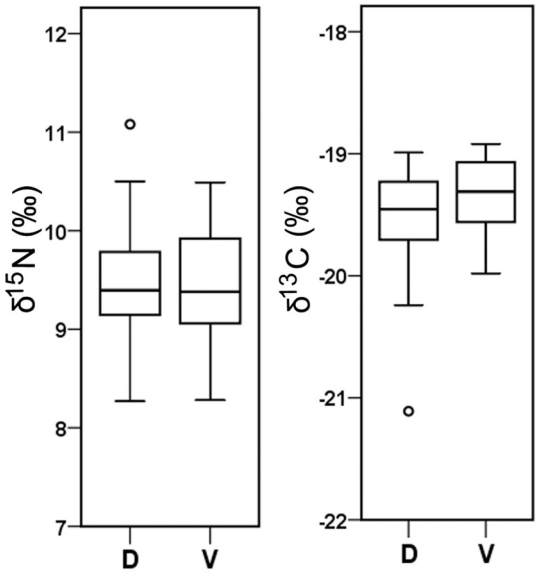

Fig. 2. Boxplot distribution of $\delta^{15}N$ and $\delta^{13}C$ values determined in the dorsal (D) and ventral (V) skin of fin whales. The top and bottom boundaries of each box indicate the 75th and 25th quartile values, respectively, and lines within each box represent the 50th.

Table 1
Results of paired *t*-tests between dorsal and ventral skin positions for $\delta^{15}N$ and $\delta^{13}C$ values. Abbreviations: **mean**: average difference between the two positions, **S.D.**: standard deviation of the difference between the two positions, **S.E. mean**: standard error of the mean, **95% C. I. D.**: confidence interval of the difference and the upper and lower boundaries of the confidence interval, **t**: paired t-test statistic, **df**: degrees of freedom.

Variables	Paired differences					t	df	Sig.
	Mean	S.D.	S. E. mean	95% C. I. D.				
				Lower	Upper			
$\delta^{15}N$ dorsal - $\delta^{15}N$ ventral	−0.02	0.28	0.05	−0.13	0.10	−0.29	26	0.77
$\delta^{13}C$ dorsal - $\delta^{13}C$ ventral	−0.11	0.28	0.05	−0.23	0.01	−1.93	26	0.06

Paired-samples *t*-tests did not indicate significant differences in any of the two variables tested ($p > .05$) (Table 1). Moreover, the dorsal and ventral $\delta^{15}N$ and $\delta^{13}C$ values showed a significant positive relationship (Fig. 3). The regression slope between $\delta^{15}N$ values was not significantly different from 1 and the intercepts from 0 ($p > .05$), whereas the regression slope and the intercepts of $\delta^{13}C$ values were different from 1 and 0 respectively ($p < .05$).

4. Discussion

Stable isotope analysis of mysticete skin has been repeatedly used to assess diet and movement patterns. In most studies, the skin was collected from free-ranging individuals through biopsies (e.g., Gavrilchuk et al. 2014; Wright et al. 2015; Dehn et al., 2006; Das et al. 2017) taken at variable positions (usually dorsally or laterally) on the whales' bodies. Many factors can bias the collection procedure, such as the skill of the collector, the sampling equipment or sampling platform employed, and external variables such as weather or animal movements.

Within an individual, skin coloration varies due to local differences in the concentration of melanocytes (Berta et al. 2015; Perrin 2017). In fin whales, the dorsal skin is black or dark brownish grey, while the ventral skin is white (Aguilar and García-Vernet 2017). Additionally, the epidermis exhibits differences in thickness depending on the position due to differences in dermal papilla height (Jones and Pfeiffer 1994). In fin whales specifically, the epidermis is quite thick across the general body surface, with a thickness varying from a maximum of 3.0 mm over the ventral surface to 2.5 mm on the back (Giacometti 1967). Studies conducted on dolphins indicate the importance of the thickness of the skin when calculating the turnover time of the epidermal cells (Brown et al. 1983; Hicks et al. 1985). Therefore, variations in the thickness of the dorsal and ventral skin in fin whales could result in differences in the renewal rates of distinct skin positions, leading to dissimilar isotope values.

Moreover, in balaenopterids, the anterior ventral blubber forms semi-elastic feeding grooves, which permit distension of the mouth and throat while feeding (Shadwick et al. 2013; Gómez-Campos et al., 2015). This morphology implies that blubber from this region is composed of abundant structural collagen and has a lower lipid content than that of the dorsal posterior region in fin, sei (*B. borealis*), and common minke whales (*B. acutorostrata*) (Watanabe and Suzuki 1950; Lockyer et al. 1984, 1985; Kvadsheim et al. 1996). These observations indicate that the dorsal posterior region is the main body location for energy storage in balaenopterids (Lockyer et al. 1985; Víkingsson 1995).

Regarding the isotopic composition of the skin, the current study