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

Running head: Stable isotopes in dorsal and ventral whale skin

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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.

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., 2015; 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 1980). 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, such as in standings where the specimens were upside down or when the dorsal part had been predated, or even in dead animals floating in the water.

Materials and Methods

Skin samples from 2 body positions (dorsal and ventral; Figure 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 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 (Figure 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.

Results

Boxplots of the obtained $\delta^{13}C$ and $\delta^{15}N$ values by position (ventral and dorsal) are presented in Figure 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‰).

Paired-samples *t*-tests did not indicate significant differences in any of the two variables tested (p>0.05) (Table 1). Moreover, the dorsal and ventral $\delta^{15}N$, $\delta^{13}C$ values showed a significant positive relationship (Figure 3). The regression slope between $\delta^{15}N$ values was not significantly different from 1 and the intercepts from 0 (p>0.05), whereas the regression slope and the intercepts of $\delta^{13}C$ values were different from 1 and 0 respectively (p<0.05)

Table 1: Results of paired t-tests between dorsal and ventral skin positions for $\delta^{13}C$ and $\delta^{15}N$ 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.

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

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. 2016; 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; Gomez-Campos et al., 2016). 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 balaneopterids (Lockyer et al. 1985; Víkingsson 1995).

Regarding the isotopic composition of the skin, the current study did not show any significant difference between the two skin positions, although the variation between paired samples was higher for $\delta^{13}C$ than for $\delta^{15}N$values. We did not find any explanation for these results, but we hypothesize that differences in the proportions of proteins present in the ventral and dorsal zones may have a stronger effect on $\delta^{13}C$ than on $\delta^{15}N$ values. Nevertheless, additional studies will be needed to explain this difference. Despite this observation, the values for both $\delta^{15}N$ and $\delta^{13}C$ were significantly correlated at the two positions (Figure 3). These correlations, together with the fact that no significant differences were found between paired samples, might suggest that the skin is sufficiently homogeneous to allow comparisons of samples taken from diverse positions of the epidermis in the same study.

Two previous studies have analysed the isotopic differences between different skin positions in three species of dolphins: bottlenose, striped and common dolphins (Williams et al. 2008, Arregui et al. 2017). Both studies evaluated a greater number of positions but considered a smaller number of individuals per species than the current study. In bottlenose dolphins, Williams et al. (2008) compared the isotopic ratios among four skin positions (the dorsal fin, a mid-thoracic site parallel to the dorsal fin, the leading edge of the fluke, and the dorsal surface of the fluke) in two individuals. In common and striped dolphins, Arregui et al. (2017) studied the differences between 11 skin positions: four dorsal positions, three lateral positions, and four ventral positions. The results of these two studies did not indicate significant differences in isotope ratios between positions, and the authors concluded that isotopic homogeneity existed throughout the body in these three dolphin species, in agreement with the results obtained in the current study.

Conclusions

Homogeneity of isotope ratios throughout tissues is a potential concern. We examined two sites of fin whale skin and did not find a difference between ventral and dorsal skin so, maybe homogeneity of skin tissue is not a huge concern within the bounds of normal sampling protocols which at least attempt to sample within a relatively confined area of the animal.

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Figure captions

Fig. 1. Sampling positions. D=dorsal and V=ventral

Fig. 2. Boxplot distribution of $\delta^{13}C$ and $\delta^{15}N$ 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

Fig. 3. Paired comparisons between dorsal and ventral positions for the variables $\delta^{15}N$, $\delta^{13}C$.. The red diagonal line indicates a 1:1 relationship. The black trend line for $\delta^{15}N$ is modelled with the equation y=0.93x+0.63 (r^2=0.787, p<0.001; n=27), while for $\delta^{13}C$, y=0.60x-8.48 (r^2=0.364, p<0.01; n=27).