Steroid hormones in three baleen plates from the same individual

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Introduction

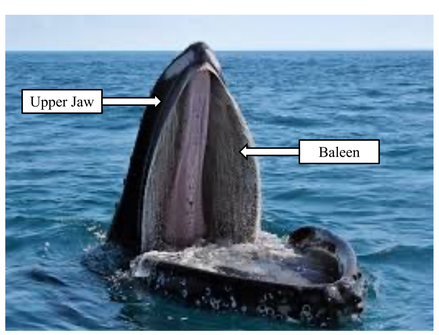
The longevity, wide-ranging migration, and prey profile of baleen whales make them exceptional models of the health of the marine ecosystem and valuable tools for measuring stress and reproductive biomarkers in marine mammals. Traditional methods of measuring stress and reproduction in cetaceans utilize serum, urine, feces and blubber (de Mello and de Oliveira, 2016). However, new methods have recently been developed that analyze accreted tissues such as earwax and baleen to reconstruct longitudinal hormone data (Trumble et al., 2013; Hunt et al., 2014). Baleen has been found to archive 1-20 years of hormone data and exhibits continuous growth over the life of the animal. However, baleen plates from the same individual have not yet been compared to one another to measure consistency in hormone deposition.

Goals

The goal of this study is to assess the consistency in hormone deposition among baleen plates from the same individual, despite sampling location within the mouth of the whale. Comparing archival trends of hormones within the baleen will assist in validating baleen hormone studies.

Materials and Methods

Three baleen plates from a single female blue whale (*Balaenoptera musculus*) were obtained from the National History Museum of London. The plates were labelled as Plate A (length: 60 cm), Plate B (length: 74 cm), and Plate C (length: 59 cm). The baleen was drilled to a powder and sampled at one cm intervals (Plate A) and two cm intervals (Plate B and C). Lipids were extracted from the baleen powder using a 2043/243 Soxtec with a modified Soxhlet method. Enzyme-linked immunosorbent assays (ELISAs) were used to measure corticosterone and progesterone concentrations in all extracted lipid samples of Plate A, B, and C. Optical density values from a plate reader were converted to hormone concentration in ng/g lipid once hormone assays were completed. Growth rates for each baleen plate were determined by periodicity in carbon and nitrogen stable isotopes, where one complete cycle would correspond with one year, as has been described previously (Hunt et al., 2017a; Vighi et al., 2019). Because different lengths of baleen represent different lengths of time, hormone concentrations for each baleen plate were truncated to represent the same time frame for comparison. Plate A hormone concentrations were averaged over two cm to match the sampling interval of Plate B and C, and Z-scores were calculated for corticosterone and progesterone in each individual plate (Crain et al., 2020)(Table 1). A mixed methods model was used to compare the three plates of baleen, controlling for autocorrelation structure due to age and assigning plate ID as the random effect: lme(CortZscores ~ 1, random = ~1 | Plate ID, correlation = corAR1(form = ~ Age | Plate ID), data=PlateHormoneConcentrations, method="REML").



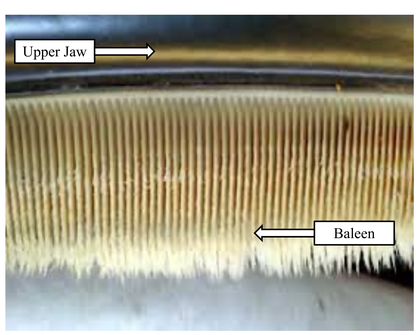


Plate ID	Corticosterone (ng/g lipid) Mean	Range	Z-score Range	Progesterone (ng/g lipid) Mean	Range	Z-score Range
Plate A	39.1 ± 12.6	20.3 - 75.9	-1.5 - 2.9	43.4 ± 43.9	9.7 - 157.6	-0.8 - 2.6
Plate B	40.9 ± 7.8	26.4 - 59.7	-1.9 - 2.4	60.6 ± 58.2	17.0 - 242.7	-0.7 - 3.1
Plate C	47.5 ± 11.9	23.5 - 74.4	-2.0 - 3.7	84.6 ± 102.5	19.9 - 461.8	-0.6 - 3.7
All	42.5 ± 11.4	20.3 - 75.9	-2.0 - 2.9	63.1 ± 74.0	9.7 - 461.8	-0.8 - 3.7

Table 1. Summary statistics of Plate A, B, and C showing mean, standard deviation, concentration range and Z-score range for corticosterone and progesterone in ng/g lipid.

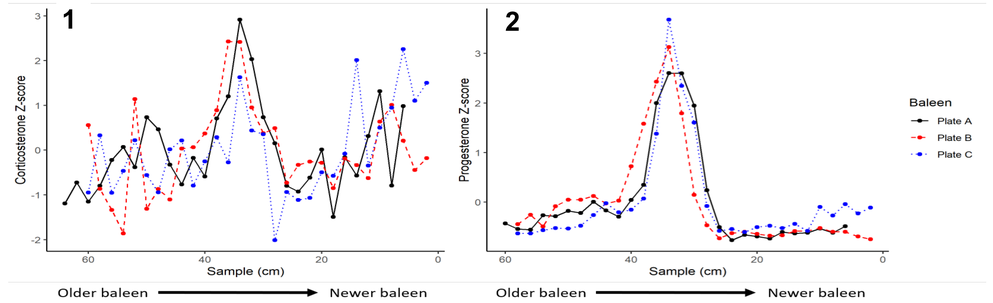


Figure 1. Older baleen extends back four years before death of this whale and newer baleen represents time closer to death of this whale. 1) Z-score values for corticosterone concentrations of Plate A, B and C. 2) Z-score values for progesterone concentration of Plate A, B and C.

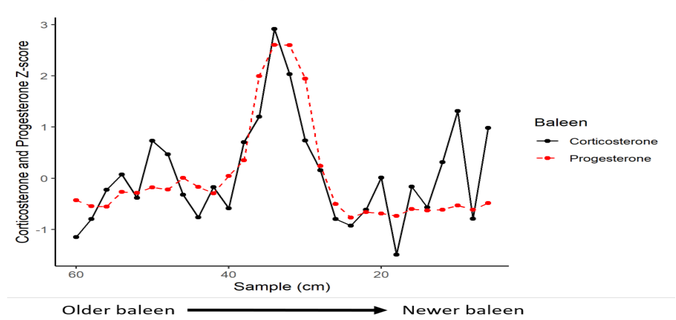


Figure 2. Corticosterone and progesterone Z-scores for Plate A over the last four years of this whale's life, where older baleen represents data from four years before the death of this whale and newer baleen represents data nearer to when the whale died.

Results

Hormone assays: The mean coefficient of variation (CV) for corticosterone assays was 9.2 ± 6.6% and for progesterone assays was 4.0 ± 2.9% (mean ± standard deviation). For lipid correction, mean lipid mass per 50 mg of baleen was 3.5 ± 0.5 mg (mean ± standard deviation). Hormone assays for corticosterone and progesterone in baleen have been validated in previous studies (Hunt et al., 2017b).

Carbon and nitrogen stable isotopes: The growth rate for Plate A, B, and C was based on the periodicity visible in the δ13C and δ15N signatures and estimated at 16 cm/year, 15 cm/year, and 15 cm/year, respectively (Hunt et al., 2017a; Vighi et al., 2019).

Comparison of hormones in three baleen plates from the same individual: Corticosterone and progesterone both had elevated levels for eight samples taken at two cm intervals, or 12 months. Corticosterone Z-scores among Plate A, B, and C were statistically no different from one another (ANOVA, $F = 0.025$, $p > 0.05$, Table 1, Fig. 1.1). Similarly, progesterone Z-scores among Plate A, B, and C were statistically no different from one another (ANOVA, $F = 0.004$, $p > 0.05$, Table 1, Fig. 1.2). Furthermore, corticosterone and progesterone were positively correlated in Plate A (Pearson's product-moment correlation, $r2 = 0.75$, $t = 5.81$, $p < 0.001$, Fig. 2).

Discussion

In this study we show that corticosterone and progesterone data collected from three different plates of the same individual blue whale were not significantly different. Additionally, we found an association between corticosterone Z-scores and progesterone Z-scores. Our results indicate that hormone analysis, and possibly other analytes including stable isotope analysis, will not depend on the specific baleen plate sampled from an individual. The use of baleen and other accreted tissues for hormone analysis have been increasing, and this study provides evidence of consistent hormone archival within baleen plates, regardless of sampling location within the mouth of the whale, as well as providing indirect evidence of consistent excretion into such tissues over time.

A period of increased progesterone Z-scores in the baleen of this individual indicate a pregnancy during the growth of her baleen plate. Gestation in baleen whales is estimated to be one year (Chittleborough, 1958). Progesterone increases with pregnancy in many mammals; therefore, by counting the number of samples that exhibit increased progesterone in the baleen, the length of gestation can be estimated for an individual, which in this case was 12 months.

The positive relationship between progesterone Z-scores and corticosterone Z-scores in the baleen of this individual may indicate a correlation between corticosterone and pregnancy in baleen whales. Cortisol and progesterone have been shown to have a positive association with one another during pregnancy as a means to support fetal development (Foley et al., 2001; Hunt et al., 2006). Further, cortisol, which has been shown to positively correlate with corticosterone in baleen (Hunt et al., 2017a), increases during pregnancy to support fetal development in African elephants, a close relative of cetaceans (Foley et al., 2001).

Additional studies are warranted to determine if these patterns of consistency are found among other baleen whale species.

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