Bowhead whale earplug reveals lifetime chemical profiles

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Abstract

The bowhead whale (*Balaena mysticetus*) is the only baleen whale to spend its entire life in and around Arctic waters, however, little is known about its chemical life history. Lifetime hormonal profiles have been reconstructed for an individual female bowhead whale using the earplug collected during subsistence harvests in 2013. This earplug (2013B1) weighed approximately 800g at a length of 53cm. An inconsistent lamina pattern was detected throughout the earplug therefore a delamination method had to be developed to separate each lamina. Previously, individual lamina (light + dark layer = 1 growth layer, GL) were used as a proxy for age with 1light/dark or 1 GL layer equaling ~1 year. There were 65 GLs identified (~65 years old) from this bowhead whale earplug. For this portion of the study, cortisol (stress) and progesterone (pregnancy) were determined for each GL. Changes in these hormone level reveal possible first pregnancy at age (GL) 19 with ~10 additional until age 65. These data will allow for a more comprehensive examination of stress, development, and pregnancy rates for these important marine sentinels.

Introduction

Over the past two decades, marine mammals and more specifically cetaceans, have been identified as health sentinels for Earths oceans. This is in part is due to their long-life spans as top-level predators with large spatial range, combined with their interaction with terrestrial/coastal systems. Current research efforts focusing on this unique group of organism are heavily reliant on blubber and muscle tissue analysis collected from opportunistic sampling making chronological datasets difficult to obtain. Chronological data serves as one of our most comprehensive approaches for identifying key knowledge gaps as well as simultaneously provide data on wildlife exposure, transport mechanisms, bioaccumulation potential, global distribution, population health, and temporal trends. This innovative and non-invasive approach of using whale earplugs as an index of chemical exposure and response offers an array of new questions to be addressed, ranging from management, conservation, physiological, and environmental chemistry. Conceptually, we are recovering valuable historical data from these marine sentinels. Because earplugs can only be extracted from beached or harvested carcasses, this non-invasive approach has the potential to provide resource managers, hunters, conservationists, and ecologists a new and powerful technique for addressing acute and chronic environmental changes.

The longevity of the bowhead whale (Keane et al., 2015, Cell Reports 10, 112–122) offers an extremely important model in which to compare past with current/future Arctic environmental events and the corresponding physiological response of the animal. This becomes more relevant in light of increased oil and gas development off Alaska's northern coast; increased contaminants, increased noise, reduced prey, or increased interactions with ships. In this study, we determined the feasibility of using bowhead whale earplugs to reconstruct lifetime chemical profiles thus providing a more historic timeframe of environmental events and the physiological response of the individual whale.

The objective of this study was to determine if lamina could be counted and extracted from a bowhead whale earplug and used for chemical analysis reconstruction .

Methods

The earplug was extracted by J.G.M. Hans Thewissen and provided to our research group through the North Slope Borough, Barrow Alaska.

Delamination

1. This earplug (length 53cm; mass ~800g) was collected from a female bowhead whale during the summer of 2013 in Barrow Alaska (Figure 1). The left plug was shipped to Baylor frozen where it was placed in a -30C until analyzed.

2. The earplug was placed in a -80C for 48 hours prior to sectioning (Figure 2). The earplug was initially bisected using a DeWALT 20" variable speed Scroll Saw (DW788) (Figure 3) and then inspected for the proper lamina orientation (Figure 4).

3. Unlike earplugs from other baleen species, the laminae of the bowhead were not deposited in a consistently linear fashion and therefore previous extracting techniques could not be employed. While there was light and dark lamina visible, the light layer could not produce enough sample mass, therefore light and dark layers were combined to form growth layers.

4. After several trials testing various delamination solutions a methodology was developed to separated lamina layers (Figure 5); 5cm X 8cm earplug sections were placed into the delaminating solution for 24-36 hours.

5. Once distinct laminae were visible (Figure 6), subsections were placed into a nitrogen filled desiccator for up to two weeks to dry (Figure 7).

6. Individual lamina were manually extracted and placed into nitrogen filled vials (Figure 8) for subsequent analyses.

Figure 1. Left earplug from Bowhead whale

Figure 2. Sectioning frozen earplug

Figure 3. Earplug half after smoothing

Figure 4. Sectioned earplug showing lamina

Figure 5. Sectioned earplug In delamination solution

Figure 6. individual lamina revealed

Figure 7. dried earplug section

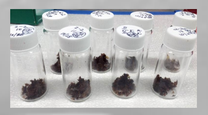
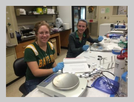


Figure 8. Stored cerumen from earplug

Hormone Assays

1. The assay solvent was modified to account for the long chain waxes and keratin of the earplug. Samples were vortexed for 30 seconds with 1 ml of PBS. 3 ml of diethyl ether was added and vortexed for 30 seconds. Samples were then centrifuged at 3000 rpm for 10 minutes and frozen at -20 for 2 hours. Ether layer was decanted and evaporated under nitrogen. 2 ml of toluene was added to the remaining waxy residue and vortexed for 45-60 seconds. Samples were then dried under nitrogen until all solvent had evaporated. Remaining residue was reconstituted with assay buffer until an appropriate dilution was reached. Cortisol and progesterone concentrations were determined using an ELISA (Enzo Life Science) for each hormone and verified using high-throughput and extremely sensitive ultra performance liquid chromatography/tandem mass spectrometry (UPLC-MS-MS) instrumentation.



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Results

1. This bowhead earplug produced 65 distinct GLs; therefore this whale aged at 65 years old.
2. Cortisol concentrations ranged from 0.21 to 38.7 ng/g (Mean = 1.87 ± 5.02 ng/g).
3. Progesterone concentrations ranged from 0.90 to 10.5 ng/g (Mean = 3.16 ± 1.96 ng/g).
4. Peak levels of both cortisol and progesterone occurred at age (GL) 19.
5. Between age (GL) 19 and age (GL) 65 there are approximately 10 progesterone peaks (Figure 9).

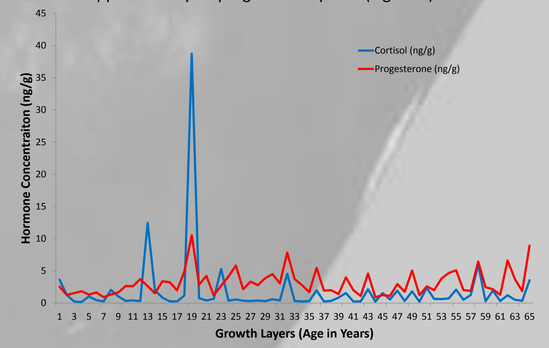

Figure 9. Cortisol and progesterone concentrations recovered from individual lamina (Growth Layers) in a female bowhead whale (2013B1). It was assumed each GL = 1 year.

Conclusion

1. New methods were developed to delaminate bowhead whale earplug.
2. First ever lifetime hormones profiles reconstructed for a bowhead whale.
3. Cortisol and progesterone concentrations recovered from individual lamina (GLs) peaked at age (GL-19) which may be indicative of age of first pregnancy. Stress hormone also peaked during GL-19 and may be indicative of stress of social interactions and breeding/birth/lactation events.
4. Contaminant concentrations are being finalized for each GL to determine changes in uptake over past 65 years and corresponding response of the whale.
5. Chemical trends and profiles that have been reconstructed from matrices such as sediment and ice cores have provided a wealth of information regarding contaminant behavior and environmental fate. Physiological or health trends can be derived from short-term indices such as serial blood samples to longer periods such as blubber. Regardless, they provide no more than a few months of historic data. Our research provides reconstructed lifetime chemical profiles for species considered marine sentinels and gives unprecedented insight on the life of these animals. This will be invaluable to managers, scientists, and communities where these species are a necessary part of tradition and subsistence.
6. Currently, estradiol (developmental sex hormone) along with a suite of contaminants (DDT/DDE, PCBs, PBDEs, PAHs) are being assayed for this earplug.
7. These data are key in identifying gaps in knowledge as well as simultaneously provide data on exposure, transport mechanisms, bioaccumulation potential, global distribution, population health, and temporal trends.