Busted! First detection of steroid hormones in Pacific walrus bones

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Introduction

The Pacific walrus (*Odobenus rosmarus divergens*) depends on the Arctic sea ice as a haulout, foraging, and reproductive platform. In recent decades, Arctic sea ice has receded significantly causing a reduction in walruses' habitat. This loss of sea ice could stress a population by limiting access to food sources and/or to resting/calving areas. This project takes a unique look at the steroid hormone levels (e.g. cortisol, progesterone, testosterone, estriol, and estradiol) of the walrus throughout various time periods within different climate regimes. This includes archaeological (3550-150 years before present (BP), historical (150-20 BP), and modern (20 BP- present) bone samples creating a hormone level baseline giving us insight into walruses resiliency to climate change. The preliminary data shown here are the first bone samples from archaeological, historical, and modern marine mammals to be extracted and analyzed for hormone detection. These results validate our method for extracting steroid hormones from marine mammal bones from these various time frames. Five of the seven archaeological samples were from Steller sea lions (*Eumetopias jubatus*); analyzed for methodological validation.

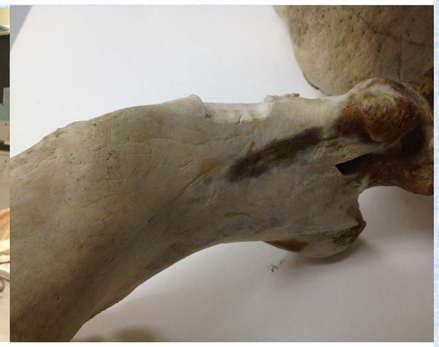
Objectives

- Validate laboratory methods for hormone extraction from marine mammal bone.
- Determine sample size needed to detect steroid hormones in prehistoric and historic bone.
- Discuss data in terms of previous blubber cortisol studies

Methods

Bone samples (1.5g) were pulverized with a Wig-L-Bug. Extraction of cortisol followed a modified procedure developed by Accorsi et al. (2008) and Bryan et al. (2013). This procedure includes extracting the lipids, and consequently the hormones bound to them, using methanol (Hunt et al. 2014). Extraction is as follows:

1. Weigh out 0.2 to 0.3g of bone powder into cryovial
2. Homogenize on vortexer
3. Add each steroid hormone standard (10 µL-total 100ng per steroid hormone) and 100% MeOH (1460 µl) to sample
4. Rotate samples for 24 hrs at room temperature
5. Centrifuge at 10,000 RPMs for 20 minutes
6. Collect supernatant and dry using nitrogen



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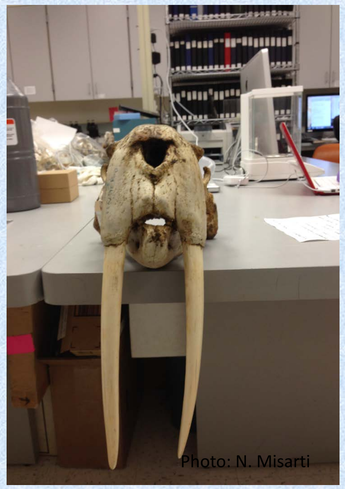

Results

Extracted Ion Chromatogram (EIC) of all internal standards in sample VL112:

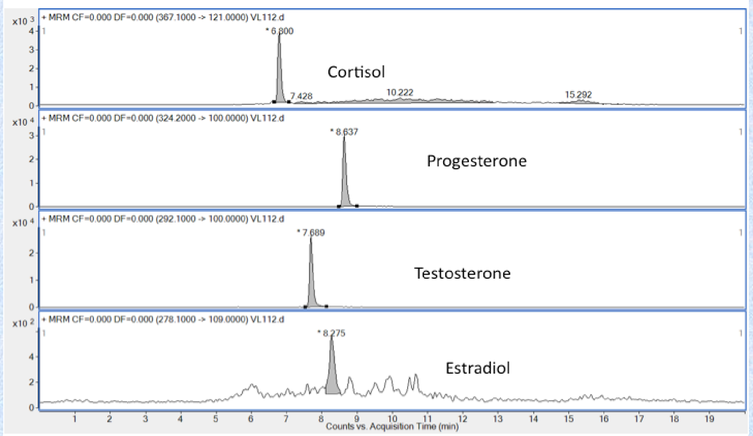

Figure 1: Sample extracts were submitted to the Purdue University Proteomics Facility and analyzed on a Agilent 6460 Triple Quadrupole mass spectrometer coupled with the Agilent 1200 Rapid Resolution high-performance liquid chromatography (HPLC) to validate the identity of the measured hormones (e.g., Lupica and Turner, 2009). The graph above, shows detectable limits of all steroid hormones.

Cortisol

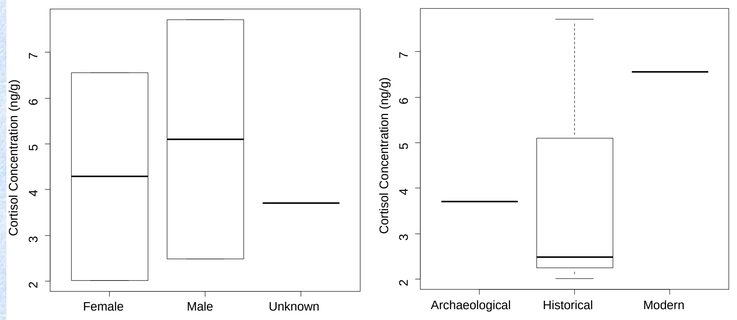

Figure 2: Mean cortisol concentrations from archaeological (n=1 of 7), historical (n=3 of 52), and modern (n=1 of 10) bone samples.

Progesterone and Testosterone

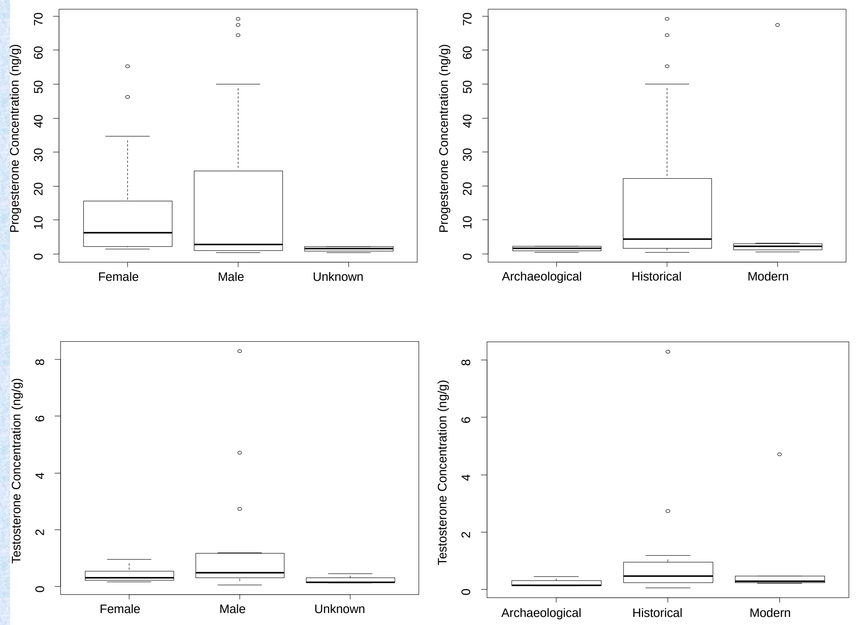

Figure 3: Mean testosterone and progesterone from archaeological (n=3 and 3 respectively of 7), historical (n=26 and 51 respectively of 52), and modern (n=6 and 8 respectively of 10) bone samples.

Discussion

- Steroid hormone extraction is possible from marine mammal bones of various ages including bones up to 3,585 years old.
- Estradiol was not detected in any samples and interestingly estriol was only detected in archaeological samples (n=7 of 7).
- The lack of detectable cortisol level s in the majority of samples leads to questions such as:
  - Are walrus resilient to stressors (e.g. climate change or disease)or were samples too small to detect cortisol ?
  - How do these steroid hormone levels compare to other tissue's steroid hormone levels (e.g. blubber and faeces)?
- The next steps are to analyze more samples, expand on our preliminary results , and hone the methodology.

Literature Cited

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