

Whiskers as a novel tissue for assessing reproductive histories in phocid and otariid species: laboratory and biological validations



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Introduction

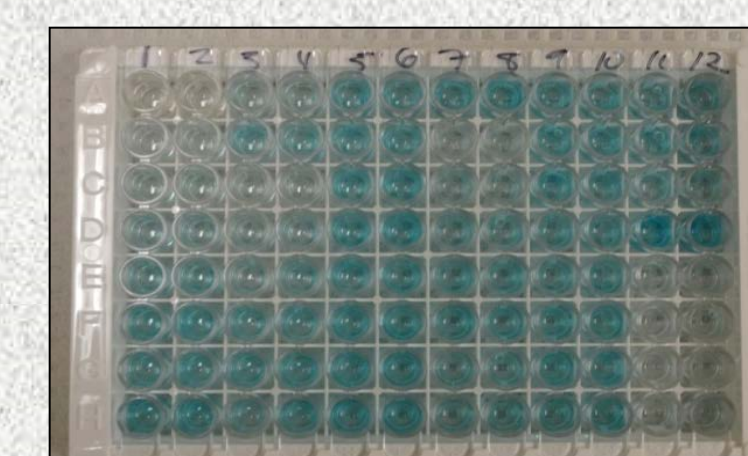
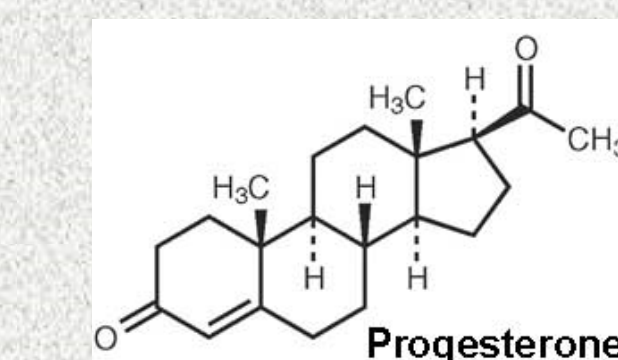
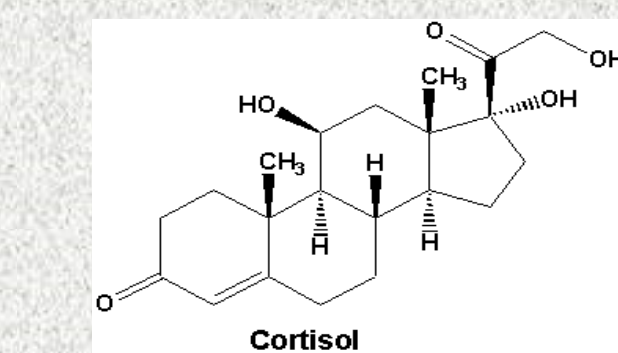
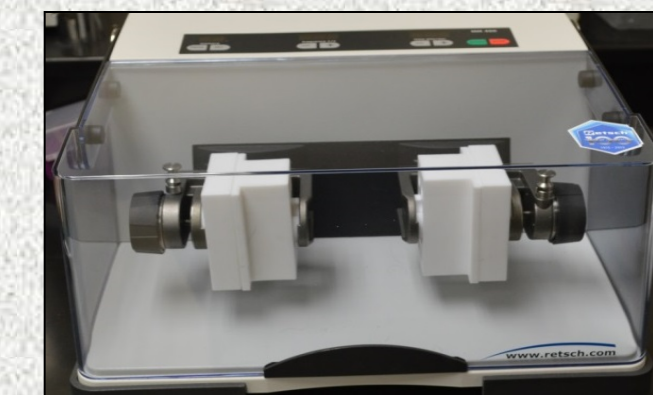
Several North Pacific pinnipeds are listed as depleted, endangered or with unknown status, and information concerning reproductive success is lacking or incomplete, highlighting the need for new methods to assess reproductive rates. Keratinized tissues including baleen and whiskers have already proven an ideal tissue for acquiring a temporal record of physiological parameters, such as stable isotope signatures. Unlike other tissues currently used for determining reproductive status (e.g. serum, reproductive tracts), whiskers do not require special storage or handling and have been routinely collected from both live-captured pinnipeds and bio-sampled carcasses, providing tissues necessary for this study. More importantly, unlike current methods which provide a snap shot of reproductive status, hormone signatures are expected to be laid down sequentially along the length of the whiskers, allowing for measurements of hormone concentrations longitudinally over the course of time the whiskers are retained: one year in phocids and multiple years for otariids. Therefore, this study proposes to validate the methods for extraction and measurement of steroid hormones from pinniped whiskers.

Objectives

1. Laboratory validation: standard methods including recovery of added mass, parallelism and dilution linearity for four North Pacific pinniped species: 2 phocids and 2 otariids
2. Biological validation: For all four species, whiskers from females with known reproductive histories will be used to determine the concentration and/or pattern of progesterone in whiskers to classify reproductive states as non-reproductive, diapause/ pseudo-pregnancy, or pup produced

Material and Methods

Archived whiskers were provided by the Alaska Dept. Fish & Game's Marine Mammal Program (NOAA permit 18528, 18537, 17410, 358-1787), the Marine Mammal Laboratory and the Alaska SeaLife Center (881-1724).



- Whiskers were sectioned with a hand chisel (0.5 - 1cm sections), cut into smaller pieces and transferred and weighed in a Sarstedt tubes (type I)
- Two stainless steel balls (5 mm) were added to each tube and ground in 4-5 min intervals at 30 Hz in a Retsch Mixer Mill MM 400 with adapters for 10 vials
- 1 ml methanol was added and rotated for 24hr, centrifuged, and supernatant removed and frozen at -80°C
- Methanol extracts were centrifuged again and methanol removed
- Negative controls (grinding balls and methanol only) were included
- Pools of methanol extracts were made for females of each species, dried under force air and reconstituted in assay buffer
- Commercially available cortisol and progesterone EIA kits (Arbor Assays) were validated through standard practices including recovery of added mass, parallelism and dilution linearity (Fig 1)
- To date, progesterone and cortisol have been measured in serial sections of whiskers from two species, Steller sea lions and harbor seals

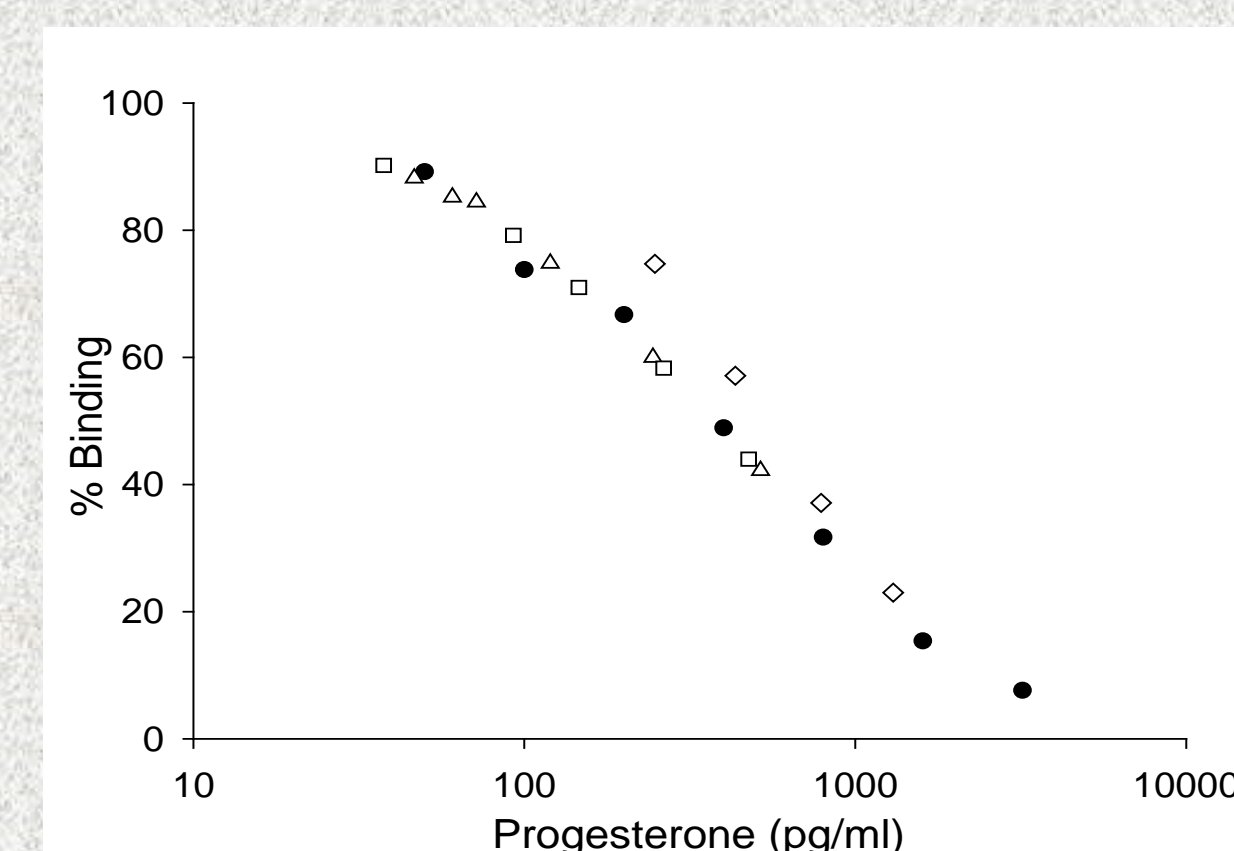


Fig 1. Pools of extracted hormones were validated through standard practices including (A) parallelism and dilution linearity for Steller sea lion (Δ), ringed seal (\diamond), and harbor seal (\square) and recover of added progesterone (50 – 1,600 pg/ml).



Results

- Progesterone was extracted from serial sections of whiskers from Steller sea lions and harbor seals (Fig 2 A, B)
- Progesterone concentrations along the length of one Steller sea lion whiskers showed variation and a cyclical pattern approximately every 9 months
- Progesterone was higher in a known pregnant harbor seal compared to three non-pregnant harbor seals (Fig 2B)
- Cortisol was extracted from serial sections of whiskers from Steller sea lions and harbor seals (Fig 3 & 4)
- Cortisol was greater near the root and rapidly decreased towards the tip of the whiskers in both Steller sea lions (Fig 3) and harbor seals (Fig 4)
- Progesterone (\blacksquare) did not show a decrease towards the tip as found with cortisol (\square) in a harbor seal whisker (Fig 4)

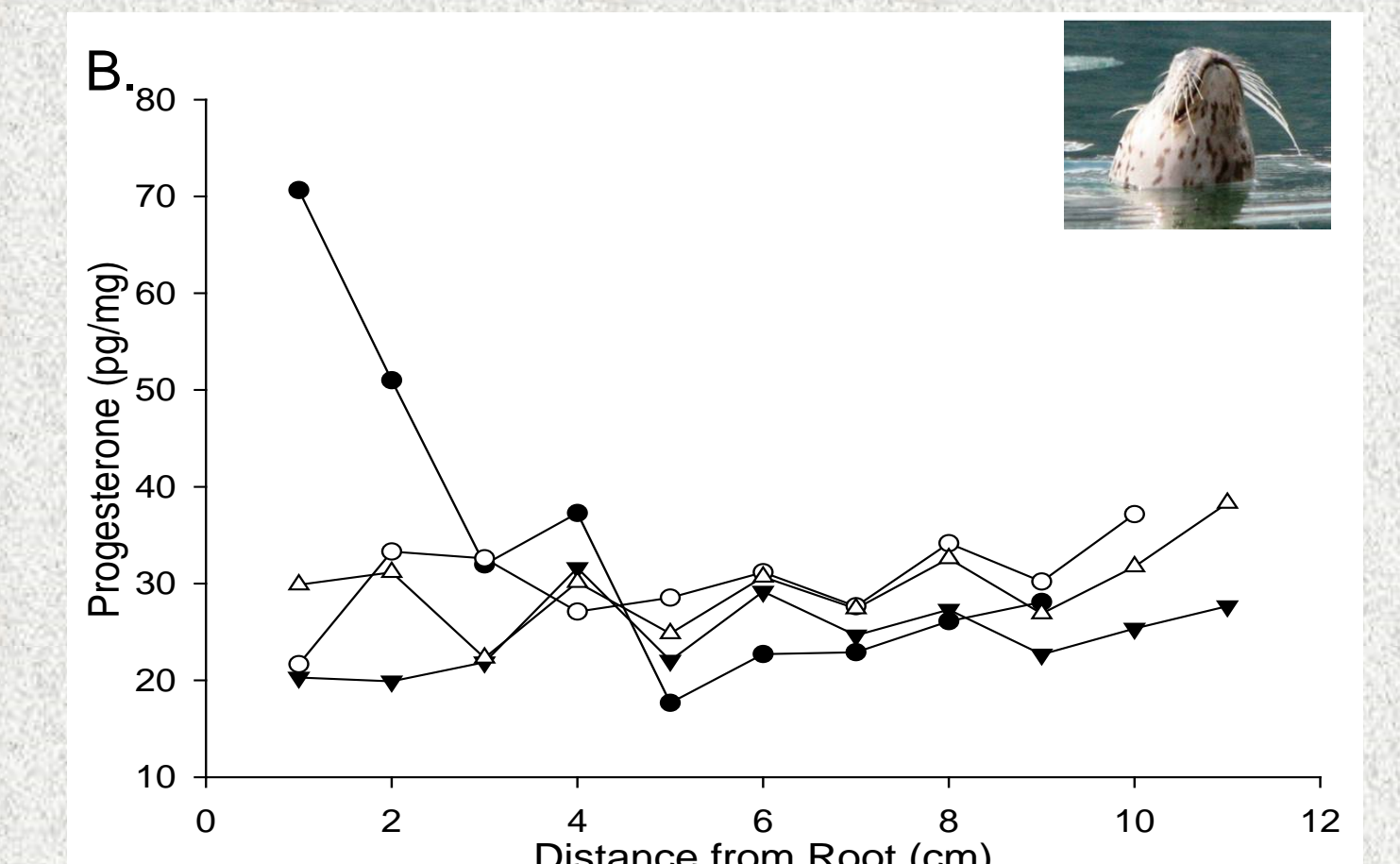
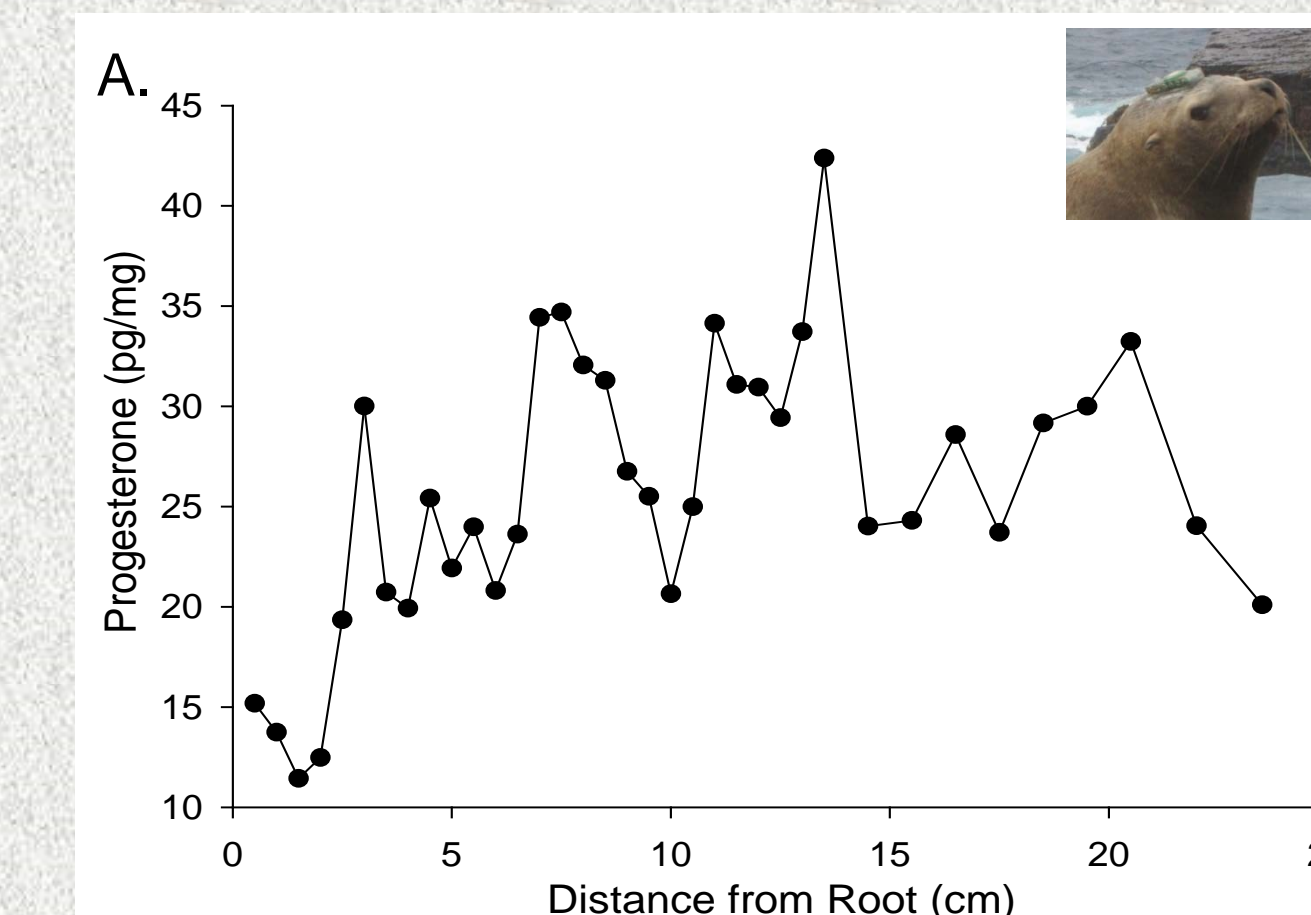


Fig. 2. Progesterone concentrations in (A) a whisker from an adult female SSL collected in March 2016 (0 cm) near Ketchikan AK (no fetus present) 1 cm ~ 2 months growth; and (B) whiskers from four adult female harbor seals sampled in May 2014 in Tracy Endicott, AK. Three females (\square 028; \blacktriangledown 016; \triangle 015) were noted as "large enough to be pregnant but no visible lump of fetus" while the fourth female (\bullet 020) was noted as "distended nipples, visible lump of fetus".

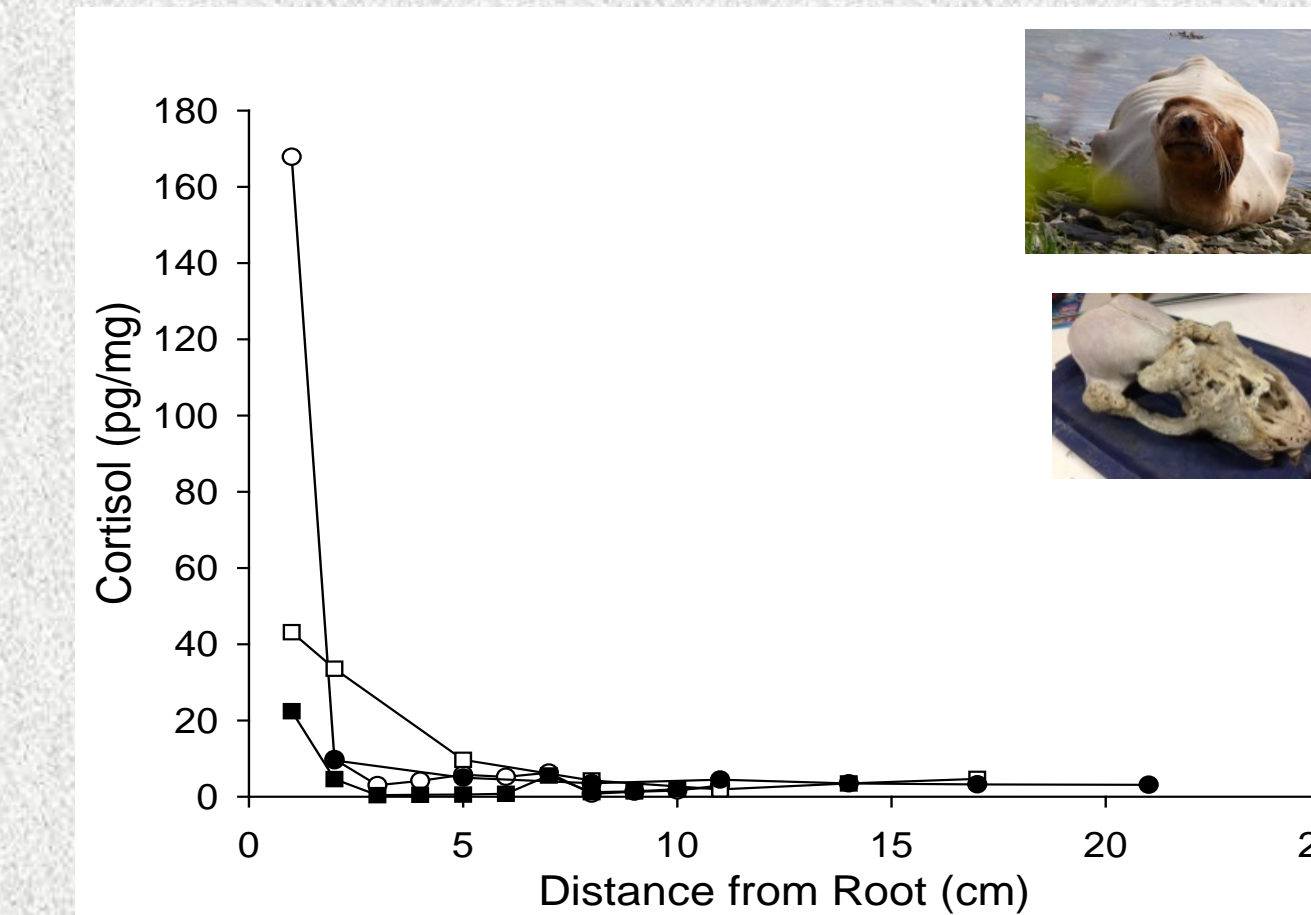


Fig 3. Cortisol profiles along whiskers from four Steller sea lions: (\blacksquare) Homer1 Unknown sex, (\square) 93007 an adult male, (\square) 2015021 a starving male with deformed skull (photos), (\bullet) 93003 an adult female.

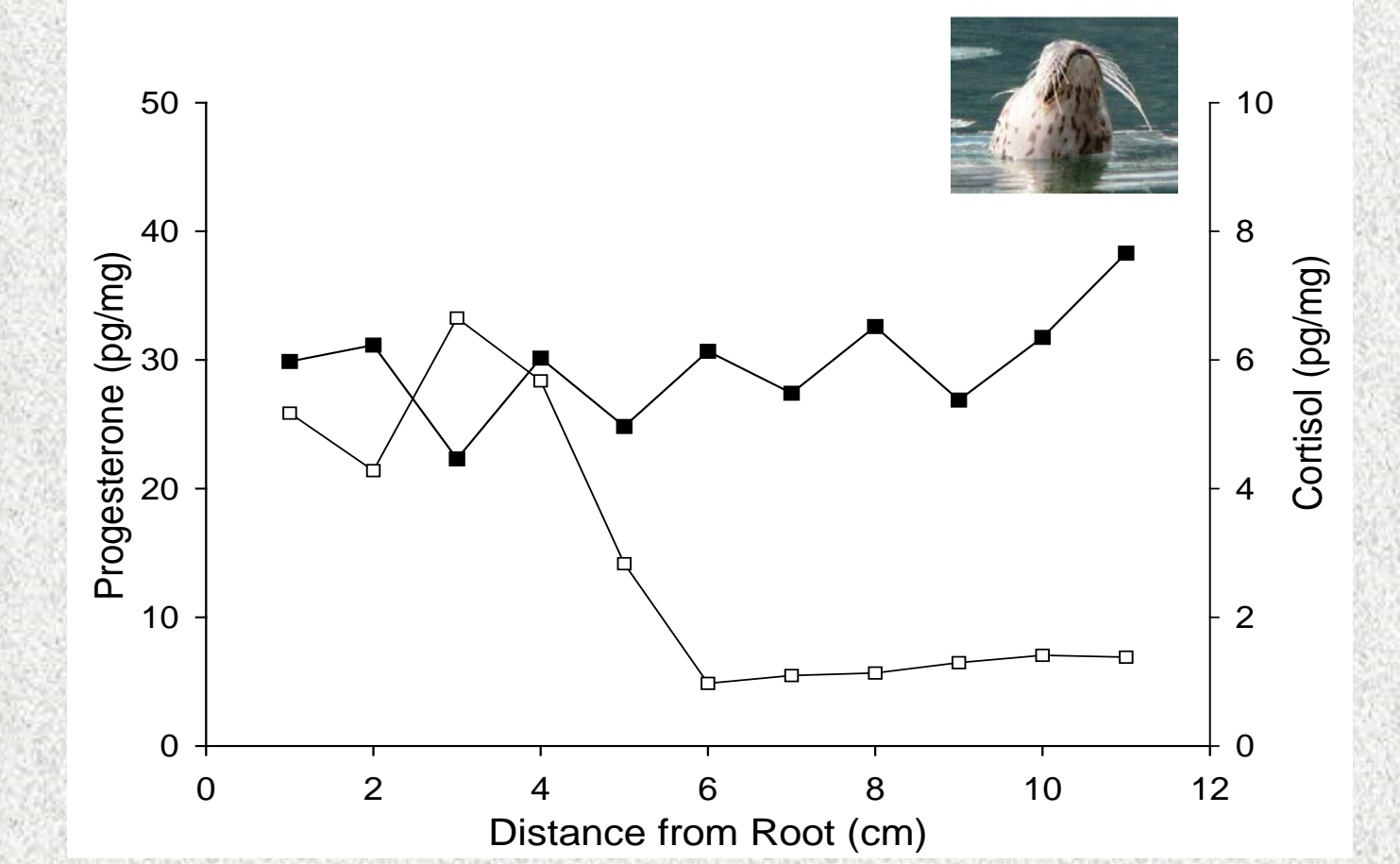


Fig 4. Progesterone (\blacksquare) and cortisol (\square) profiles along one whisker from an adult female harbor seal (015) noted as "large enough to be pregnant but no visible lump of fetus".

Future Work

- Validate progesterone EIA kit for northern fur seals
- Validate estradiol EIA kits
- Biological Validation: Analyze whiskers from females with known reproductive histories
- IHC staining for cortisol and progesterone in whiskers



Phocid seal whiskers contain up to one year of reproductive information providing far less information than otariid whiskers. Whereas ice seal claws contain up to 10 years of stable isotope signatures. As part of a recently funded NOAA proposal we will investigate ice seal claws as a novel tissue for tracking stable isotopes, stress and reproductive hormones.



Steller sea lion
Eumetopias jubatus



Harbor seal
Phoca vitulina



Northern fur seal
Callorhinus ursinus



Ringed seal
Phoca hispida

Acknowledgements

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