Determining the Pattern of Cementum Annuli and Relationship to Reproduction in Male Sea Otters

Student Researcher: Josh Proper, Wasilla High School; Anchorage, Alaska
Mentors: Vanessa R. von Biela; Jennifer M. Burns, PhD, Department of Biological Sciences, University of Alaska Anchorage; Anchorage, Alaska

Since the early 1990s, the southwestern Alaskan sea otter (*Enhydra lutris*) population has declined dramatically and the cause has yet to be determined. Population trajectories of large mammals are determined by three factors: survival rate, reproduction rate, and age of first reproduction (AFR). Of these three, AFR should respond first to environmental change. Life history theory predicts that AFR will be older with bottom-up causes (ie, food limitation) and younger when the cause of the decline is top-down (ie, predation), as there is usually abundant resources in this scenario. Traditionally, determining AFR required lethal sampling, which may not always be possible. Work on many mammalian species suggests that the width of annual cementum layers in teeth may decline when breeding begins. If so, examining teeth annuli may provide a non-lethal alternative for determining AFR. Ongoing research has shown this relationship in female sea otters, but male sea otters have not been tested. Sea otter testes and premolar teeth slides were collected by subsistence hunters working with the US Fish and Wildlife Service and the Alaska Sea Otter and Steller Sea Lion Commission from Alaska (1994–2005). We determined the pattern in cementum annuli thickness for male sea otters across age by measuring annuli at three sites on each of the two slide sections available. We found that cementum annuli layers decreased with age, but found no correlation between cementum annuli and sexual maturity in male sea otters. This lack of correlation may be due to sampling error or different energy expenditures during reproduction for each sex. Since females expend large amounts of energy through gestation and lactation, we hypothesize that the width of female cementum annuli decreases at a much sharper rate when they reach AFR.

Introduction

The southwest Alaskan sea otter population has plummeted up to 90% since the early 1990s and the reason is unknown.[1] Declines may be due to a bottom-up source caused by lack of food and habitat that occurs when populations are dense, in which case we would expect reproduction rates to decrease and age of first reproduction (AFR) to increase. However, circumstantial evidence suggests that the decline may be due to a top-down source, ie, predation.[2] In this case, we would expect reproduction rates to increase and AFR to decrease due to lowered densities that coincide with top-down declines.[3]

An ongoing study of female sea otters has shown that past reproduction can be determined by analysis of cementum annuli in the teeth, and used to calculate AFR.[4] Cementum annuli are annual deposits of minerals that occur on the root of a tooth that correspond to the growth of the animal (Figure 1). During summer and spring, growth is fast which accounts for the white area, but during winter, growth slows down and the cementum is compressed which accounts for a darkened line. In females, the relationship between cementum width declines and reproduction likely reflects the large expenditure of energy that is required during gestation and lactation. However, male and female mammals expend energy for reproduction in very distinct ways,[3] and the same pattern of cementum decline may not be present in males. Since shifts in the life history strategy of males are poorly understood, we are interested in testing the relationship between cementum declines and reproduction in male sea otters using cementum analysis.

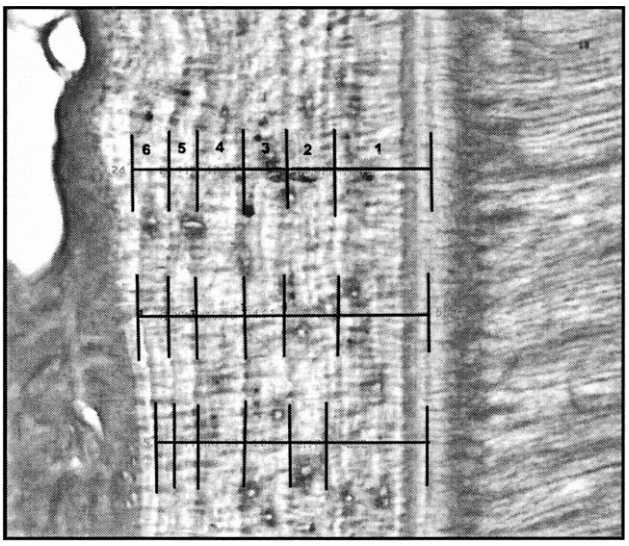

Fig 1. Section of a male sea otter premolar tooth. Age is determined by cementum annuli, a yearly deposit of minerals on the root of the tooth

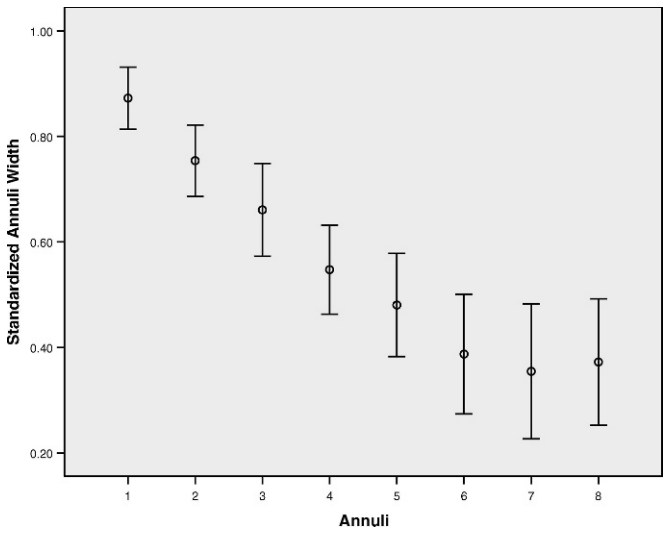

Fig 2. Change in the mean (± 2 SD) width of annuli with age in male sea otter premolars. Similar superscripts indicate that the widths were similar among those age classes

METHODS

Sea otter premolar teeth slides were obtained from subsistence hunted samples that were made available through collaboration with the US Fish and Wildlife Service (USFWS) and the Alaska Sea Otter and Steller Sea Lion Commission (TASSC) (N=37). For each otter, annuli were measured at three locations on each of two tooth sections prepared by Matson's Lab (Milltown, MT) using a Nikon eclipse E400 microscope with 100× magnification. We took a digital picture of the slide using a MicroPublisher 3.3 camera (Qimaging, Burnaby, BC) and measured the width of each annual cementum deposit using MetaVue 6.1 (Molecular Devices Sunnyvale, CA). Widths were standardized by dividing each cementum width by the largest cementum annuli at each measurement site. Data were normalized using the arcsine transformation. A repeated measure two-way ANOVA was used to test for difference by site and section. If differences were not found, then the average cementum annuli width was calculated for each annuli per animal. The minimum annuli width (MAW) was used for further analysis. In order to describe the pattern of cementum annuli a one-way ANOVA was used to determine the age at which annuli measurements became consistent.

The testicle weight for each male sea otter was recorded by the subsistence hunter and used to determine if male otters were reproductive. When the testes weight is >14 g, spermatozoa is present indicating that the sea otter was capable of reproduction.[5] Next, our objective was to determine a threshold value for MAW. To find the optimal threshold value, the proportion of animals correctly classified as reproductive or not, the false positive and false negative rates were calculated for each threshold value between 0 and 1 at 0.01 intervals. We then picked the spot that the false positive and false negative results overlapped as the MAW threshold.

RESULTS

We found no significant difference (P>.05) in cementum annuli width due to measurement site or tooth section, so we were able to calculate the width of annuli and compare these widths to successive annuli. Annuli width declined with age, for ages ranging from 1–4 years, and were similar for years >4 (Figure 2). Twenty seven percent of the sea otters were judged to be reproductive based on testes mass, but overall there was no link between age and testes mass, or between season and testes mass. The optimal threshold for MAW was 0.56, but this value only correctly identified 47% of the otters as being sexually reproductive as determined by testicle weight. False positive and negative error rates were 26.5% each.

DISCUSSION

Cementum annuli width did decrease with age (Fig. 1), but there appeared to be no correlation between the male otter's cementum layer width and the ability to reproduce, as assessed through testes mass. However, concurrent research supports a correlation between decreases in cementum annuli width and past reproduction in female sea otters.[4] Some of the possible explanations for the lack of correlation between cementum annuli and reproduction in male sea otters include seasonal changes in testes mass, and / or inaccurate measurements of testicle mass. While we are not aware of research showing seasonal change in testicle mass, and sea otters are known to reproduce throughout the year,[5] if seasonal changes occurred, this might cause a reduction in testes mass. Another possible explanation is that many of these measurements were performed in the field by untrained personnel and measurement errors may account for the lack of correlation between otter age and testes mass. Finally, there may not be a correlation between cementum annuli and reproductive history in male sea otters. Female sea otters use large amounts of energy during reproduction because of gestation and lactation.[6] These energy demands may be related to the large decrease in cementum annuli in female sea otters. Because males do not expend as much energy as reproductively active female sea otters, there may be no

relationship between declines in cementum annuli width and reproduction in male sea otters.

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