

# Age-Related Mortality of Mediterranean Monk Seals (*Monachus monachus*) Estimated from Dental Samples

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## Abstract

Mediterranean monk seals (*Monachus monachus*) are critically endangered with a population estimated at less than 600 individuals. The population has been severely reduced by centuries of human exploitation, habitat degradation and interactions with fisheries. There are currently two main populations: (1) a northeastern group inhabiting the Ionian and Aegean Seas in Greece and Turkey, and (2) a northeastern Atlantic group inhabiting the coasts of Mauritania and Madeira. Teeth from 49 monk seals obtained from a stranding network in Greece were processed and analyzed resulting in age estimates for 43 individuals (15 females, 24 males and 4 seals of unknown sex). Ages were best determined by counting growth layer groups (GLGs) in the cementum layer along the root at the base of the tooth using untreated longitudinal or transverse sections (0.36 mm thickness) observed under polarized light. Decalcified and stained thin sections (0.008 to 0.023 mm thickness) of the cementum proved difficult to read and were inferior to the untreated sections. Ages ranged from 0.25 to 36 years for females, 0.33 to 24 years for males, and 0.33 to 25.5 years for unsexed individuals. The majority of seals (49%) were estimated to be over 5 years of age and classified as adults, followed by juveniles (22%) and sub-adults (10%). 18.4% of the aged-sample died from human-related causes, including 9 males from all age classes and 2 adult females. A single Gompertz growth curve was generated using standard length data resulting in asymptotic values of 212.2 cm for females and 220.9 cm for males. Females reached asymptotic length at approximately 9 years of age, whereas males attained it at approximately 14 years. Despite the limited sample size, this study represented the first generation of sex specific growth curves in this species and confirmed *M. monachus* lay down only one GLG annually.

## Introduction

Mediterranean monk seals reach an average length of 2.4 m and weigh between 250-300 kg (Sergeant *et al.* 1978, IUCN/UNEP 1988, Johnson *et al.* 2006). There is no extreme sexual dimorphism, though males are larger than females (Samaranch and Gonzalez 2000, Johnson *et al.* 2006). Sexual maturity is generally reached ~5-6 years of age and gestation lasts ~11 months. Mediterranean monk seals are believed to reach a maximum age of 20-30 years in the wild (Sergeant *et al.* 1978, Johnson *et al.* 2006). Although age classes can be inferred through morphology and pigmentation patterns (*e.g.*, Samaranch and Gonzalez 2000), determining the true age of monk seals by examining Growth Layer Groups (GLGs) in teeth can help assess patterns and trends in survivorship, reproductive success and nutritional status (Klevezal 1980, Scheffer and Myrick 1980, Myrick 1991). Such investigations can provide important insight into the population dynamics of the species to develop conservation and management plans.

## Project Aims and Objectives

1. Determine the best ageing method for processing and reading Mediterranean monk seal teeth – *i.e.*, how the teeth should be sectioned (type of cut), whether or not teeth should be decalcified and stained, and which part of the tooth is an optimal area for reading GLGs; and
2. Estimate the ages of wild Mediterranean monk seals from available teeth to infer population level information such as survivorship patterns (including maximum recorded ages), patterns of growth, and whether certain age/sex classes are associated with particular mortality events (*e.g.*, interactions with fisheries).

## Materials and Methods

- Teeth from 49 seals collected between 1991-2008 by MOM's Rescue and Information Network were analyzed. Seals died of natural or anthropogenic causes off the Greek Islands of the Aegean Sea.
- Teeth from only 46 seals were processed as samples from three individuals were unusable: 16 females, 25 males and 5 of unknown sex. Samples included juveniles, sub-adults and adults. Primarily, canine teeth were analyzed, though 1 incisor was also processed.
- Teeth were cut using a Buehler Isomet low speed saw to obtain 0.36 mm longitudinal (L1 to L3) and transverse (T1 to T4) sections for viewing under a polarized light microscope (see Figure 2). Larger sections (A to E) 3-4 mm in thickness were taken from a sub-sample of individuals (n=18) for decalcification and thin sectioning.
- Sections A to E were fixed in 10% neutral buffered formalin, decalcified using an acid solution, and stored in 70% ethanol.
- Ultra-thin sections 8-23 µm (0.008 to 0.023 mm) were obtained using a cryostat (-20° C) or by wax embedding the tissue and cutting using a microtome. Sections were then stained and mounted onto glass slides.
- A series of trials were conducted following Thomas (1977) to determine the best stain for reading GLGs in the dentine and cementum: (a) 0.05% Toluidine Blue, (b) Haematoxylin, (c) Haematoxylin and Lithium Carbonate mixture, and (d) Cresyl Violet Acetate at 0.1% or 2% concentrations. The optimal stain was 2% Cresyl Violet Acetate left on the tissue for 30 minutes (Figure 3).
- Ages were calculated assuming 01 October as the mean date of birth based on the prevalence of autumn births for monk seals in Greek waters (Sergeant *et al.* 1978, Pastor and Aguilar 2003, Johnson *et al.* 2006).

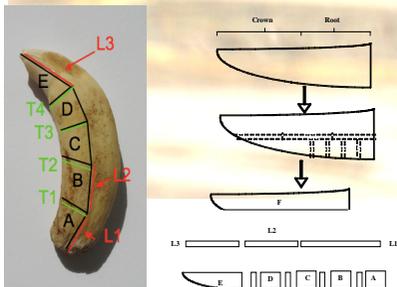


Figure 2: Canine tooth indicating sections cut with isomet saw and schematic diagram of sections cut.

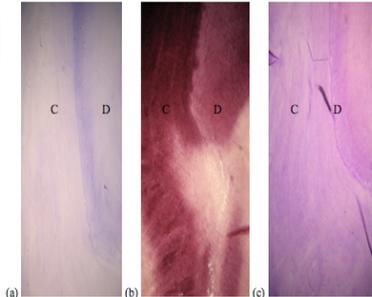


Figure 3: Comparison of section 156-A stained with (a) 0.05% Toluidine blue, (b) Haematoxylin, and (c) 2% Cresyl Violet Acetate. "C" indicates cementum and "D" indicates dentine.

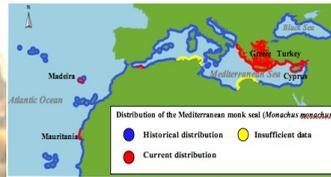


Figure 1: Historical and current distribution of Mediterranean monk seals (Johnson *et al.* 2006 and SMOm/Hellenic Society for the Study and Protection of the Monk Seal).

## Results

- Ages ranged from 0.25-36 years for females, 0.33-24 years for males, and 0.33-25.5 years for individuals of unknown sex. 49% of the sample was classified as adults (25 years of age), 22% as juveniles, and 10% as sub-adults (Figure 4).
- Ages were best determined by counting GLGs in the cementum at the base of the root using untreated longitudinal (L1) or transverse (T1) sections observed under polarized light (Figure 5).
- Decalcified and stained sections were difficult to read and inferior to untreated thicker sections (Figure 6).
- 18.4% of the aged-sample died from human-related causes, including 9 males from all age classes and 2 adult females (Figure 7).
- Although the sample size was small, a single Gompertz growth curve was generated for both sexes using standard length data. Asymptotic length was attained at 212.2 cm in females and 220.9 cm in males (Figure 8), thus producing a sexual size dimorphism ratio of 1.04 for Mediterranean monk seals. Females reached asymptotic length at c. 9 years of age, whereas males attained it at c. 14 years.

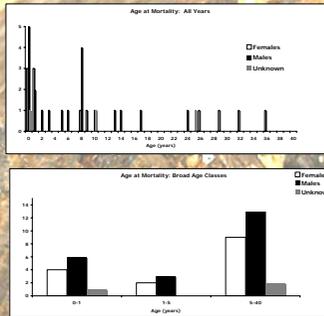


Figure 4: Age at mortality for Mediterranean monk seals (a) across all age classes, and (b) by age categories.

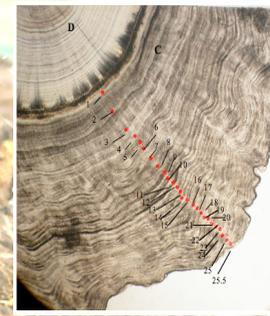


Figure 5: Photo taken under polarized light of T1 section from MOM ID No. 141. Red dots/numbers mark GLGs in the cementum, indicating 141 is 25.5 years old. "C" is cementum and "D" is dentine.

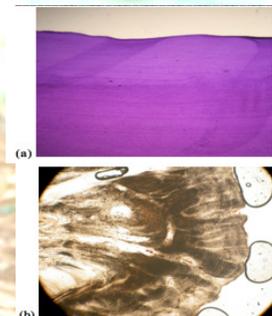


Figure 6: Comparison of MOM ID No. 90 A section stained with Cresyl Violet Acetate (a), or untreated and viewed under polarized light (b).

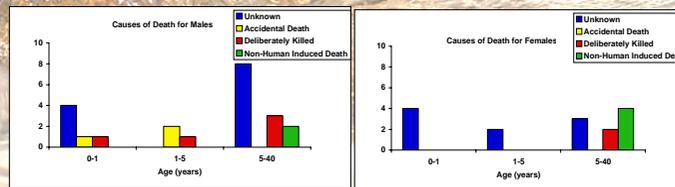


Figure 7: Causes of death attributed to each age category of females and males.

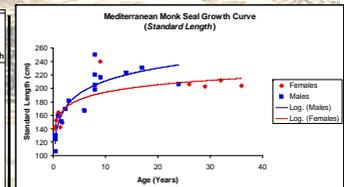


Figure 8: Gompertz growth curve using standard length data.

## Conclusions

- Untreated L1 and T1 sections (0.36 mm thickness) of the cementum layer from the base of the root viewed under polarized light were superior for reading GLGs compared to decalcified and stained ultra thin sections.
- Decalcified and stained sections were not reliable as the stains did not fix well to the monk seal dental tissue. Further research is needed to investigate the chemical composition of monk seal teeth.
- Despite the limited sample size, this study represented the first generation of sex specific growth curves in this species and confirmed that Mediterranean monk seals lay down only one GLG annually.
- Future research should strive to incorporate samples from the northeastern Atlantic group residing in waters off Mauritania/Madeira.

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