

# Aging live New Zealand sea lions using post-canine teeth

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# 1. Introduction

Estimating the age of individuals within a population provides important information about population structure, age-specific survival and reproductive rates, longevity and growth rates. The most common method used to estimate age in pinnipeds is to count annual growth layers in the teeth (eg. Scheffer 1950; Laws 1953 and 1962; Carrick and Ingham 1962; Klevezal and Kleinenburg 1967; McCann 1993; Rosas et al. 1993; Stewart et al. 1996). Most pinniped ageing techniques use either upper or lower canine teeth taken from dead animals. However, it is often desirable to know the age of living seals for scientific purposes. Recently, post-canines and incisors have been used to age species such as Antarctic fur seals (*Arctocephalus gazella*), southern elephant seals (*Mirounga leonina*) (Arnbom et al. 1992) and grey seals (*Halichoerus grypus*; Bernt et al. 1996).

The purpose of this work was to develop a technique to estimate the age of New Zealand sea lions using post-canine teeth extracted from live animals, and to provide age estimates using the technique for known age specimens.

# 2. Methods

The method was adapted from Stewart et al. (1996). The teeth were stored in 70% ethanol prior to preparation. The ethanol was removed from the teeth by soaking them in distilled water for at least 24 hours before further treatment. They were decalcified in 5% nitric acid for 24 hours, rinsed and trimmed to expose the plane of the section. Decalcification continued for 48 - 65 hours in a solution of 10 parts formic acid to 90 parts 10% formalin, and the teeth were then rinsed for several hours in water.

The teeth were embedded in O.C.T. (Tissue-Tek) embedding compound, frozen, and sectioned on a cryostat at approximately  $-20^{\circ}\text{C}$ , to produce  $12\mu\text{m}$  thick longitudinal sections from the centre of the tooth. Sections were floated on slightly basic water (pH 8.5) for several minutes, mounted on gelatin coated glass slides and air dried. The slides were stained for 4 - 5 minutes in a filtered 0.032% aqueous solution of toluidine blue made with slightly basic water (pH 8.5). They were thoroughly air dried, moistened with xylene and mounted under a glass cover slip using DPX.

The slides were examined using a compound microscope at 40-100X magnification. Light and dark bands were seen which correspond to incremental growth layers deposited during the year. Each pair of light and dark bands was interpreted as equivalent to one year's growth (Perrin and Myrick 1980).

### 3. Results and Discussion

Growth layers could only be counted in the dentine. No layering was seen in the cementum, either with toluidine blue or cresyl violet as the stain. The teeth were read a number of times over the course of about 10 days, to improve the consistency of the estimates, and consensus readings are presented (Table 1). The results were reasonably accurate, but a number of separate readings were required to improve the consistency of the estimates. However, consistency among readers should improve with time and practice. Three independent readers will help improve the precision of the estimates, which is particularly important when the animals are not of known age.

Canine teeth are both easier to read and easier to prepare than post-canines, but the technique used for the post-canine teeth shows promise. The major problems with the method are the possible limitations in estimating the age of older animals using dentine layers. These layers are formed on the inside of the tooth in the pulp cavity which fills and closes with age (McCann 1993). The age at which dentine layers are no longer reliable is unknown in New Zealand sea lions. Cementum layers are deposited on the outside of the tooth, but are generally much narrower and more difficult to read (McCann 1993). Cementum layers do not appear to be readable in thin sections of teeth in New Zealand sea lions, but the reason for this is unknown and may need to be the subject of future research.

### 4. Acknowledgements

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Table 1. Age estimates of known age New Zealand sea lions from stained thin sections.

Sample Number	Age Estimate (years)	Known Age (years)
E42	6	6
E68	4	4
E69	6	6
E70	4	4
E71	4	4
E72	section unreadable	5
E73	8	10
E74	10	12
E75	10	10
E76	11	10
E77 not stained	5	