ALASKA DEPARTMENT OF FISH AND GAME JUNEAU, ALASKA

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AGE DETERMINATION OF SEA OTTER

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FINAL REPORT (RESEARCH)

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Cooperator:	Karl B. Schneider		
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Job No.:	<u>8.10R</u>	Job Title:	Age Determination of Sea Otter
Period Covered:	July 1, 1971 to June	30, 1973	

SUMMARY

Various techniques for sectioning and staining teeth were tested to develop a method for estimating the age of sea otters from cementum layers. A technique of staining longitudinal sections of decalcified premolars with Giemsa's stain was selected. Approximately 2,000 teeth were prepared in this manner. Interpretation of cementum layers was based on information from a known-age female, comparison with other potential indicators of age, and an analysis of the time and frequency of layer formation.

Tooth eruption and suture closure were more reliable than cementum layers as indicators of the ages of pup and subadult sea otters. Lensink's (1962) nine pup and subadult age classes were used to group animals on the basis of these parameters. Approximate ages were assigned to each class, making it possible to estimate the ages of young animals on the basis of tooth eruption and skull changes, and the ages of older animals on the basis of cementum deposition.

Total curvilinear lengths and weights of pup and subadult sea otters were plotted against estimated ages to provide a means of roughly estimating the ages of young sea otters during field operations.

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BACKGROUND

Intensive sea otter (Enhydra lutris) studies were initiated in the mid-1950's after certain Aleutian Island populations had completely recovered from the exploitation of the 18th and 19th centuries. These studies required some method of determining a sea otter's age. Initially, rough grouping of animals into pups, subadults and adults on the basis of body size and dentition was adequate for most purposes. Lensink (1962) discussed various criteria for identifying these age groups in the field and investigated the possibility of more precise age determination from skull measurements. He found that, because sea otters may be born at any time of the year, polymodal distributions of measurements did not occur, although there was a correlation between size and age. He devised a system of nine age classes for pups and subadults based on skull conformation, suture closure and tooth eruption. While this system proved useful for certain purposes, he could only guess at what ages these classes represented.

As studies progressed, the opportunity to handle more animals arose. Detailed studies of reproduction, growth, mortality and sex and age segregation were hampered by the lack of adequate knowledge of ages. In 1962, the Alaska Department of Fish and Game initiated a program which included attempts to manipulate sea otter populations through the harvest and transplant of large numbers of animals. This required a greater knowledge of population dynamics and population composition which in turn required an accurate method of determining the ages of sea otters.

Most recent efforts to develop an age determination technique for sea otters have been directed at looking for annuli in teeth. Kenyon (1969) described attempts by V. B. Scheffer and by members of the Research Division of the American Dental Association to locate such annuli. They employed a number of techniques including x-ray photography and ultraviolet microscopy, but failed to find any annual growth layers. Kenyon (1969) also reported that cementum layers had been found in the early stages of the present study.

Klevezal' and Kleinenberg (1967) reported that the age of sea otters could be determined from cementum layers in sections of canines stained with hematoxylin. They did not elaborate on the technique or the interpretation of layering and I have been unable to obtain the original paper (Klevezal' and Marakov, 1966).

Although the present study was not formally documented as a research effort until July 1, 1971, it was initiated in 1967 (Vania, et al, 1968 and 1969). In order to bring all of the pertinent information together, I will cover all efforts between 1967 and 1973 in this report.

OBJECTIVES

To develop a technique for determining the age of sea otters.

PROCEDURES

Teeth were collected from approximately 2,000 sea otters that were taken during harvests in 1967, 1968 and 1970 or that died during transplants in 1968, 1969 and 1970.

Initially, transverse sections of decalcified canines were stained with Harris' hematoxylin. Once the existence of annuli in the cementum had been established by this method, all incisors, premolars and molars were treated similarly. The upper and lower PM_1^* and upper I_3 were selected as having the most consistently distinct annuli. A number of these teeth were prepared using different techniques in order to select the procedures that would make the annuli most distinct. Teeth extracted from raw skulls were compared with those from steam cleaned or boiled skulls. Those stored dry were compared with those stored in formalin and Loess' solution. Formic, nitric and hydrochloric acids were compared as decalcifying agents. Sections cut at different angles and thicknesses were compared, as were a variety of stains and staining techniques.

Undecalcified tooth sections were examined by ultraviolet microscopy and mandibles were decalcified, sectioned and stained with Harris' hematoxylin. While both of these techniques showed some layering, the results were not encouraging and neither was pursued.

Once a technique had been selected, teeth from all available animals were prepared. Several hundred were examined under a variety of magnifications and light conditions until I was familiar with the variations in layering and could achieve relatively consistent counts of annuli.

*Fisher (1941) correctly identifies these teeth as PM_2 . Permanent PM_1 rarely develops in sea otters. The notation PM_1 is used here to avoid confusion by laboratory workers, as it is usually the first existing premolar.

Karl Kenyon of the U. S. Bureau of Sport Fisheries and Wildlife made the skull of "Suzy", (KWK 61-2), a known-age sea otter, available. A lower PM_1 from her skull was sectioned and stained.

"Cementum ages" were compared with other possible indicators of age such as body measurements, skull measurements, suture closure, tooth eruption, numbers of corpora albicantia, etc. When inconsistencies were found, tooth sections were reexamined to determine if another interpretation would be more consistent. The stage of eruption of each tooth in all available pup and subadult skulls was recorded in tabular form using the following format:

 \mathbf{I}_1 \mathbf{I}_2 \mathbf{I}_3 \mathbf{C}_1 \mathbf{PM}_1 \mathbf{PM}_2 \mathbf{PM}_3 \mathbf{M}_1 \mathbf{M}_2

Deciduous

Upper

Permanent

Deciduous

Lower

Permanent

The right and left sides could also have been broken down, however teeth usually erupt at the same time on both sides and it was sufficient to make a special notation when they did not.

The stage of eruption of each tooth was recorded by the following notations:

NE - Not erupted through bone.

E/1 - Erupted through bone but not through the gum.

E/2 - Just through the gum.

E/3 - Eruption 1/4 to 3/4 complete.

E - Fully erupted.

0 - Deciduous tooth shed.

This system was easily applied to unfleshed heads as well as cleaned skulls. It was necessary to cut into the gum on some specimens, but complete cleaning of the skull was neither necessary nor desirable.

When possible each animal was assigned to one of Lensink's (1962) nine age classes. Broken or uncleaned skulls could be assigned to classes I through VI on the basis of tooth eruption alone. Older classes are more dependent on suture closure and skull shape, requiring that the skull be intact and clean.

The "cementum ages" of pups and subadults which had been assigned to one of Lensink's classes were recorded. Those pups that had no annuli

were subjectively classified into the following categories:

NE - Tooth not erupted.

A - Tooth erupted, no visible cementum.

- B Small amount of cementum at root tip.
- C Thin layer of cementum on sides.
- D Amount of cementum approximately equal to that normally deposited before the first line.

The age classes and "cementum ages" of these pups and subadults were compared to provide a basis for the transition from one system to the other. The age classes were related to actual age by comparing the proportions of sea otters in each age class collected at different times of year.

Weights and curvilinear lengths of pups and subadults were plotted against estimated ages to provide a rough means of estimating age in the field.

FINDINGS

Preparation of Tooth Sections

All sea otter teeth have cementum annuli, but some consistently have more regular and distinct annuli than others. Lower PM_1 was the best choice with upper I_3 and upper PM_1 being second and third choices, respectively. How the tooth was handled before processing affected the quality of the finished slide. Boiling or steaming during skull cleaning adversly affected the sharpness of annuli when tooth sections were later stained, and the resulting clean edge of the section often made detection of outer layers difficult. It was best to remove teeth from raw skulls. Skulls were frozen, salted or even allowed to decay somewhat without adverse effects on the teeth. Extracted teeth should be stored in alcohol, preferably Loess' solution. Teeth stored in a l percent formalin solution did not accept the stain well. Teeth that were allowed to dry developed dark edges which made differentiation of outer annuli difficult.

Longitudinal sagital sections were superior to transverse sections as they provided a greater variety of areas to examine. Sections cut from the center of the tooth, so that the angle of cut was perpendicular to the layers, had the sharpest annuli.

Several stains, including Harris' hematoxylin and Paragon Multiple Stain for Frozen Sections, produced usable results; however, Giemsa's stain proved to be best.

The following technique was selected for mass processing of sea otter teeth:

Extract teeth (preferably lower PM_1) from raw skulls whenever possible. Extraction is easier if the skulls have been allowed to decompose slightly. A "bulb" of cementum forms at the root tip of the PM_1 in older animals, and it is necessary to chip the bone away from the tooth to free this portion. This bulb is important and care should be taken not to break it off. Any debris clinging to the root should be left intact, as this protects the outer layers of cementum. Teeth should be stored in Loess' solution.

Decalcify the tooth in 3 percent HCl until soft. The time required for sufficient decalcification depends on the volume of acid used, the number of teeth decalcified in this volume, temperature, and the size of the tooth. When 50 teeth are placed in individual, perforated capsules and decalcified in approximately one liter of acid solution, incisors soften after 18 to 24 hours and premolars after 8 to 24 hours. Rinse teeth for a minimum of half a day in a constant exchange of tap water and then store in water in a refrigerator.

An attempt should be made to section teeth immediately after the water rinse. If teeth must be stored for longer than a week before sectioning, Loess' solution is the most acceptable preservative. Teeth stored in Loess' solution must be soaked in water prior to sectioning to avoid incomplete freezing in the cryostat.

Cut frozen longitudinal, sagital sections, 32 microns thick, on a cryostat at -14 C. Sections should be taken as close to the center of the pulp cavity as possible. These sections should be stained immediately or placed in Loess' solution for storage. Incisor crowns should be trimmed before sectioning, but premolars may be sliced untrimmed.

Prepare a working solution of Giemsa's stain in small amounts and discard it when signs of heavy precipitation occur (usually after 30 min.). For 25cc of working stain use:

23.5cc distilled HOH

1.0cc Giemsa buffer solution, pH 6.5 (Paragon) (PO₄ buffer, pH 6.8 is also usable)

0.5cc Giemsa stock stain (Paragon)

Pour a small amount of stain (approximately 5cc) into a small Petrie dish. Place tooth sections in the covered staining dish for 10 to 12 minutes. Sections that have been stored in Loess' solution should first be rinsed in water. Take the sections out of the stain, rinse quickly in water and place them on a slide for viewing. If the degree of staining is acceptable, air dry the slide in a vertical position for 16 to 18 hours, then place it in a Copeland Jar containing xylene for final dehydration and clearing. Dry the slide with Kim Wipes, and mount with Permount and a coverslip. It is often helpful to have several sections stained to varying degrees. Usually it is better to understain than overstain.

Viewing Tooth Sections

A variable power, stereo microscope was found to be best for viewing tooth sections. A flourescent illuminator produced a soft, uniform light and made reading easier than an incandescent light source. The sections were usually viewed under 40 to 60X then the magnification was increased to 100 to 120X to examine areas where cementum layers were most distinct.

Sea otter teeth are difficult to read. Cementum layers tend to be irregular, the first few layers are often indistinct, occasionally double lines are formed, secondary dark lines may be difficult to distinguish from the normal dark annuli, and very little cementum may be laid down in older animals. Fig. 1 shows the variability between animals. Precise age estimation from any single tooth is difficult, and even an experienced worker will make errors. In order to reduce this error, it is necessary to carefully look over the entire area of cementum of several sections and to count layers at several different places.

Near the gum line (Fig. 2, Area A), the cementum appears dense and layers tend to be very even and regular. However, on some teeth, there are many secondary lines in this area which may be impossible to distinguish from true annuli. The first one or two layers may blend with the dento-cementum interphase. On older animals (e.g. 8+ years), the outer layers may not be laid down this high on the tooth. However, this area often provides a good check to compare with other areas. On some teeth it is the only place where distinct layers can be seen.

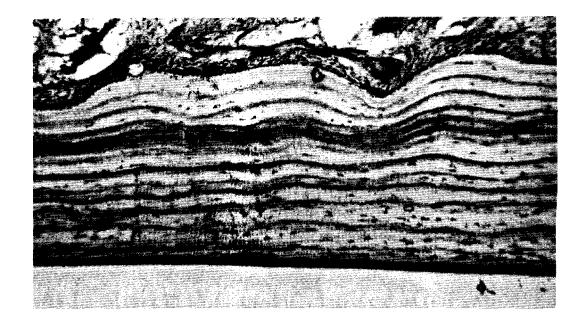
Layering on the sides of the root (Fig. 2, Area B) is highly variable (Fig. 1). Generally one portion of this area will be clearer than the others and many teeth are read from this area.

The best area to count is usually near the tip of the dentine where the tooth curves in above the bulb of cementum at the tip, particularly on the anterior side of the tooth, directly below the cusp (Fig. 2, Area C). At this point, the layers fan out and are easier to distinguish. It is the best, and often the only, point at which the first two or three layers can be sorted out, and is often the only area where outer layers on animals over 10 years of age can be found.

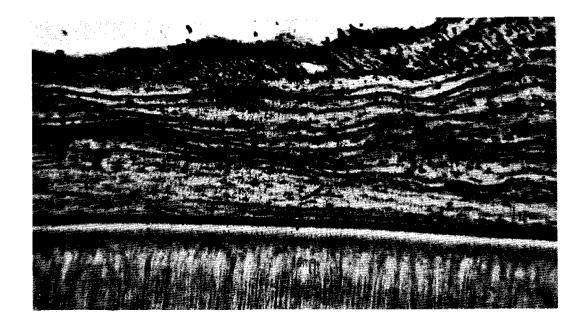
The layers in the bulb at the root tip (Fig. 2, Area D) are usually not distinct. However, broad bands are laid down and it is often possible to check the count from another area against the alternating lighter and darker bands found here.

Counting Cementum Layers

With light transmitted through the section from below, cementum layers appear as fine, dark lines separated by broad, light bands. For the purpose of counting, each dark line was considered to be one year.

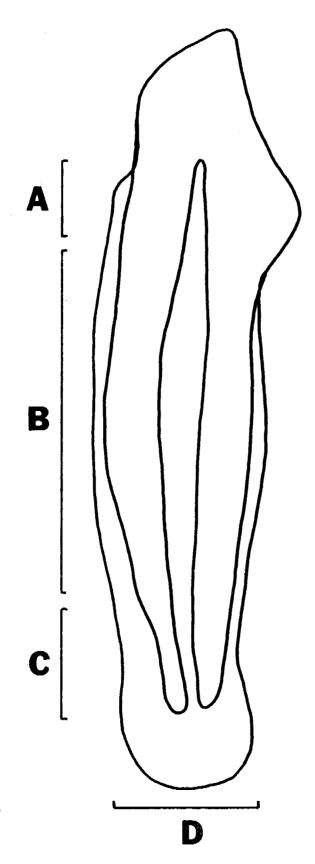


S068-613, 14+ years old. Collected October 20, 1968. Unusually regular and distinct lines. Note outer light band without a subsequent dark line, typical of fall animals.



S068-251, 13+ years old. Collected October 13, 1968. Very irregular lines. Second and third lines indistinct.

Figure 1. Photomicrographs of stained sections of sea otter premolars demonstrating variability in cementum layers.



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Figure 2. Longitudinal section of lower PM showing areas of cementum described in text.

The following is a description of each line and how it has been interpreted in the teeth read to date. This description is meant only to point out some of the more common patterns. Individual variation is great and few individuals have all of the characteristics described.

- Line 1: This line may blend with the dento-cementum interphase on the sides of the tooth (Fig. 2, Areas A and B). It is easiest to distinguish around the tip of the root. A considerable amount of cementum is laid down in the "bulb" (Area D) and the first line is sharpest just above the bulb (Area C). This line is usually easily distinguished on lower PM₁, but difficult to locate on other teeth. In some cases, there is no sharp line, only a slight change in coloration (density?) of the cementum.
- Line 2: The band between lines 1 and 2 is usually broader than the band between the interphase and line 1. Line 2 is seldom sharp and is often visible in only one or two places (Fig. 1). In some cases, it may be necessary to assume the existence of line 2 on the basis of the pattern of other lines and the width of the light band after line 1.
- Line 3: The band between lines 2 and 3 is usually about the same width as that between lines 1 and 2. Line 3 is often diffuse or indistinct, but is usually more distinct than line 2.
- Line 4: Often this is the first sharp line. Its formation appears to coincide with sexual maturity in females. The band of light cementum between lines 3 and 4 is usually similar in width to subsequent bands and thinner than the previous two light bands. The cementum often appears more dense beyond line 3. The spacing between lines from 4 to 8 or 10 (occasionally farther) is fairly regular and the lines are usually sharp. However, some lines will be sharper than others, some will run together in places and some may be double lines.
- Line 8 to 10+: Around line 10 the lines may become irregular and visible at only a few points. Apparently less cementum is deposited after this. In some cases the 10th line may actually represent several years, causing the age to be underestimated. There are exceptions when there is an actual increase in cementum deposition and broad irregular bands are formed. Most of the teeth from animals which have been estimated to be 15 or more years old are of this type.

Time of Line Formation

The dark line, which is assumed to represent a period during which the rate of cementum deposition was low, appears to be laid down at a particular time of year and is not necessarily related to the animals' birthdate. Variation in rates of cementum deposition, between individuals and between different years in the same individual, makes it difficult to precisely determine the time of line formation. However, it appears to be in the winter months.

Teeth from subadult sea otters, collected at different times of the year in the Aleutian Islands, were compared. The rate of cementum deposition in subadults is high and the lines tend to be well separated making it easier to tell when a line is formed. Most animals collected in May, had deposited a thin layer of light colored cementum after the line. The line was so close to the edge that it often was not visible on teeth that had been cleaned by cooking. Animals collected in June and July had a wider band of light cementum; however, the line often appeared very close to the edge on cooked teeth. September and October animals had a much broader, but still incomplete, band at the edge. It would appear that the dark line is laid down in the January to March period in Aleutian otters.

One otter (SO 68-2) collected in Prince William Sound in February had deposited a small amount of light cementum after a line, indicating that the line was formed in late fall and early winter. Individual variation is so great that it is impossible to determine if this reflects a difference between areas without further collections.

Frequency of Line Formation

Cementum layers, one light band and one dark line, are laid down annually. The first line may be laid down when the animal is somewhat more or less than 1 year old depending on what time of year it is born. After the first line is formed, a new line should be formed each subsequent winter.

The only available known-age sea otter, over 1 year old, was Suzy (KWK 61-2). She weighed 28 pounds when captured on September 4, 1955, at an estimated age of one year. She died on October 27, 1961, after six years in captivity at the Woodland Park Zoo in Seattle (Kenyon, 1969). Even though a 28-pound sea otter may be somewhat younger or older than one year, the timing of Suzy's capture and death were such that she must have lived through seven winters and should have had seven dark cementum lines. The effects of captivity cannot be ignored, as Suzy did not develop normally in some respects (Kenyon, 1969). Sections made from her teeth were more difficult to read than average but were no worse than those from many wild otters.

There were seven dark lines in the cementum of Suzy's teeth (Fig. 3). The first layer was quite thin along the sides of the root, but formed the normal "bulb" at the root tip. The second dark line, which was widely separated from the first and third, was distinct in places but,

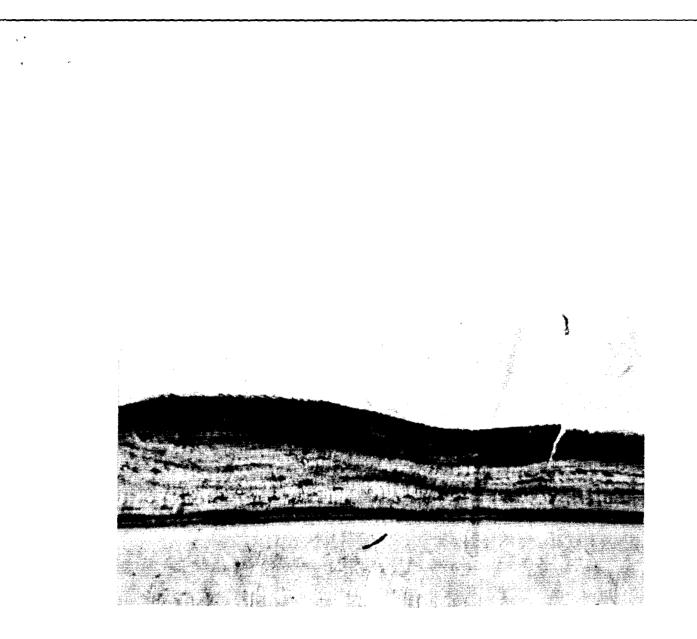


Figure 3. KWK-61-2, 7-year-old known-age sea otter. Note incomplete two-year line, and intermediate lines between third and fourth and fifth and sixth lines.

typically, became diffuse and indistinct in many areas. After the third line, the spacing was more uniform. The seventh line was very near the outer edge of the sections. There were a few fine intermediate lines, which might be interpreted as annuli, but these did not persist for any distance and were not visible in lightly stained sections.

The pattern of cementum layers in Suzy's teeth fits the interpretation described above. The only discrepancy is that there should have been a light band representing the last summer's growth. This was not readily visible because the tooth had been allowed to dry after cleaning. The outer layer tends to disappear on teeth handled in this manner. Suzy's cementum layers were relatively thin after the third year. The fact that a line was visible at the edge indicates that a band of light cementum existed beyond the last line.

Because sea otters may be born at any time of year, no polymodal distribution of skull or body measurements which could be correlated with the number of cementum layers was found. There was only a general correlation of numbers of layers with size. Similarly the number of corpora albicantia tended to increase with the number of cementum layers (Schneider, 1972).

Aging Pups and Subadults

The need for precise age determination is greatest for younger animals. Analyses of growth, age of sexual maturity, early survival, etc., require a relatively accurate and precise knowledge of age. Errors in age estimation of a year or two for older animals are not a serious problem for most purposes. Unfortunately the cementum aging technique is weakest for young animals. Because the second and third dark lines are often faint, it may be difficult to separate secondary "false annuli" from true annuli. Ironically, this appears to have less influence on the estimation of ages of animals over 4 years old. Once the darker lines begin to be laid down around that age, it becomes easier to distinguish the pattern of cementum deposition for that tooth and to correctly identify annuli. Familiarity with the normal pattern and amounts of cementum laid down in the first years will reduce errors in estimating the ages of young animals.

A major source of age determination error in younger animals is caused by the fact sea otters may be born at any time of year. When the first line is laid down, the animal may be more or less than a year old. The first line cannot be laid down until the tooth has erupted and a certain amount of light colored cementum has been deposited. The fact that there is always a large bulb of cementum before the first line near the root tip indicates that animals below a certain age will not develop a dark line. Based on the analysis of early cementum deposition presented later in this report, it appears that an otter may have to be around 8 months old before the first line can be laid down. Animals that are younger during their first winter would not form a line for another year. Therefore it is possible that the animal is anywhere from 8 to 19 months old when the first line forms. The limitations of the cementum deposition technique for estimating the ages of young sea otters are such that an alternative technique would be of value for some studies. Lensink's (1962) age classes (reprinted in Table 1) are based on more positive criteria for at least the first year. If his classes could be assigned to a relatively accurate time frame, it would be possible to use his system to age subadults and counts of cementum layers to age adults. This would overcome some of the shortcomings of each system.

The age class of each otter for which easily readible tooth sections were available was plotted against the stage of cementum deposition (Fig. 4). The correlation between the two systems is quite good, considering that both rely heavily on subjective criteria. The means of classes III, IV and V roughly coincide with the means of classes A, B and C, respectively. If we look only at the means, we can assume that the "average" sea otter classified as "1" is approximately 1 year old and that subsequent dark cementum lines coincide with birthdates. The majority of sea otters are born between early April and mid-June (Schneider, 1972) and would be about a year old when the first line becomes visible in April or May (the actual line is formed when they are 8 to 10 months old, but is not readily visible on stained tooth sections until April or May).

The mode of pup births which occurs in May is evident in the distribution of pup ages at least through the fall (Table 2). If most pups are born between April 1 and June 15 (Schneider, 1972), those pups born in early April would be in the early stages of Class III by June 25. This means that a pup enters Class III before it is 3 months old but does does not reach the midpoint of the class until it is over 3 months old. Similarly, those pups born in mid-June are in Class III by mid-October, when 4 months old.

Pups born in early April are 7 months old in mid-October and are in Class V. Those in Class VI in October would have been born at the very beginning of pupping or just before the peak pupping period, perhaps in March. The Class VI pups in Table 2 were virtually the same size as the Class V pups, and the amount of cementum on their teeth was comparable (Fig. 4). This indicates that these animals had either just entered Class VI or that there is little difference between the classes. The boundary between the classes is very subjective. However, Class VI must begin shortly after 7 months. Sea otters born during the peak pupping period the previous year would be between 12 and 15 months old in June. These animals are beyond Class VI by that age. Class VII animals almost always have a visible dark line in the cementum and should be over 12 months old.

There is probably considerable overlap between Classes VII, VIII and IX because of the subjectivity of the criteria separating the classes. However, it is possible to assign some rough ages to these groups from the data in Fig. 4. For example, animals born in the peak pupping period two years before would be 25 to 27 months old, would have two dark cementum lines and be in Class VIII by June. Those born three years before would be 37 to 39 months old, have three dark lines and be in Class IX.

Age	Class	Probable Age	Skull and Dental Characteristics
Pup	<u>s</u> 2/		
	I	Newborn	Dental formula: I 3/3, C 1/1, PM 1/2 = 22; all deciduous. In the upper jaw I 1 and I 2, and on the lower jaw I 1 are weak and probably non- functional. C 1/1 are only partially exposed. Cranium inflated and protruding over the zygo- matic process of the squamosal, the mastoid process and the occipital condyles. Postorbital processes distinct. Facial region short and jaws extremely swollen.
	II	1 month	Dental formula: I 3/3, C 1/1, PM 2/3 = 26. All teeth on lower jaw are deciduous but on the upper jaws pI 1 and occasionally pI 2 have erupted. Cranium becoming triangular anteriorly, lengthening the facial region. Postorbital processes distinct, but breadth at postorbital constriction much greater than the intraorbital breadth. Jaws still appear swollen.
	III	2 - 3 months	Dental formula: I 3/2, C 1/1, PM 3/3 = 28. I 1-3 and PM 1 are permanent teeth although dPM 1 may persist anteriorly to pPM 1 in a separate alveolus. The deciduous canine on the upper jaw may be loose in its alveolus due to the growth and near eruption of the permanent canine. Facial region much lengthened, and the postorbital processes are pronounced. The breadth at the postorbital constriction is now approximately equal the intraorbital breadth, and the swelling of the upper jaw no longer constricts the eye sockets. Mastoid processes are easily visible when the skull is viewed from above, thus accentuating the triangular appearance of the cranium. Bones may be taking on a purple coloration from a dye injested with sea urchins.
	IV	4 - 5 months	Dental formula: I 3/2, C 1/1, PM 3/3 = 28. Permanent canines erupting and milk canines have usually been lost. Milk premolars may have small cavities on crests. All (Amchitka) skulls with definite purple coloration. Mandibles at location of molars still swollen.

Table 1. Age Classification of Sea Otters^{1/}. Reprinted from Lensink (1962)

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Age	Class			Probable Age	Skull and Dental Characteristics
	V	5		8 months	Dental formula: I 3/2, C 1/1, PM 3/3, M 1/2 = 34. Molars erupting and permanent canines nearly to their final position. Milk premolars (2, 3) may have deep cavities. Lower jaws depressed and still appearing swollen.
Suba	dults				
	VI	· 9		15 months	Dental formula as in Age Class V. Permanent canines appear fully erupted in most specimens and the molars have reached the approximate level of the milk premolars. On skulls with flesh removed, the milk premolars appear as caps on the erupting permanent premolars. Breadth at the postorbital constriction visibly less than the intraorbital breadth on all specimens. Exoccipital-basioccipital suture usually fused, but still evident.
V	II	10	6 –	24 months	Dental formula: I 3/2, C 1/1, PM 3/3, M 1/2 = 34. All permanent teeth in place. Insertion of temporal muscles on parietal about 1/4 inch from sagittal plane. Evidence of exoccipital- basioccipital suture nearly obliterated.
VI	ΓĨ	25	5 -	34 months	Insertion of temporal muscles on parietal bones has advanced to the sagittal plane. Lambdoidal crest forming. A slight ridge is forming on maxilla just above alveolus of canines.
	ĹX	32	3	36 months	Sagital crest forming between temporal muscles. Lamboidal crest prominent. Basioccipital- basisphenoid suture fusing.
Adu	ılt	3	ye	ars or older	Sagital and lamboidal crests prominent. The latter extending to the mastoid process. Basioccipital-basisphenoid suture fused. Surface of skull smooth and glossy. In older animals teeth may have become extremely worn and pitted from abrasion and cavities. Exostoses common on maxilla and premaxilla above alveoli of incisors and canines. Purple color of bones may decrease with advance in age.

Table 1. Age Classification of Sea Otters (cont.).

 $\frac{17}{2}$ Ages listed are estimates which may be quite imprecise. Animals listed as pups are those believed to be still at

Animals listed as pups are those believed to be still at least partially dependent on their mother. Subadults include all other sexually immature animals.

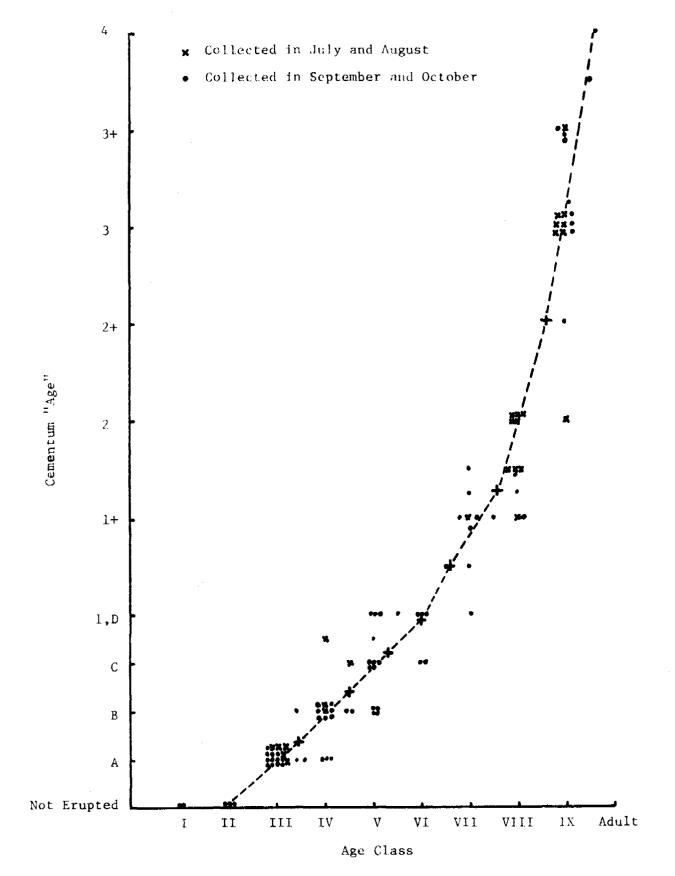


Figure 4. Correlation of cementum deposited on lower PM₁ with Lensink's (1962) age classes.

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Лде	_June,]	1971 ^{1/}	October, 19682/		
Class	N	%	N	%	
I	7	28	4	5	
11	7	28	4	5	
111	9 <u>3</u> /	36	24	32	
IV	· _	,	17	23	
v	1	4	18	24	
VI	1	4	8	11	
Total	25	100	75	100	

Table 2. Distribution of Age Classes of Pup Sea Otters Collected in June and October.

 $\frac{1}{1}$ Average June 26.

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 $\frac{2}{}$ Average October 15.

 $\underline{3'}$ Seven early Class III, two mid Class III.

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Using the above information, I have assigned the following rough ages to the various age classes. In some cases the dividing lines are subjectively assigned on the basis of proportions of animals collected in each class. The boundaries between Class I and II and between IV and V are particularly arbitrary.

	Age in Months		
Age Class	Range	Midpoint	
I	0 - 1.5	0.75	
II	1.5 - 2.5	2	
III	2.5 - 5	4	
IV	5 - 6.5	5.5	
v	6.5 - 7.5	7	
VI	7.5 - 12	10	
VII	12 - 18	16	
VIII	18 - 29	24	
IX	29 - 42	38	

These estimates differ somewhat from Lensink's (Table 1); however, his overall time span was realistic.

The best method for estimating the age of sea otters is to assign pups to age Classes I through VI on the basis of tooth eruption. If fleshed whole skulls are available, assign older subadults to Classes VII through IX. If the skulls are not available or if the animal is an adult beyond Class IX, estimate the age from cementum layers.

Investigators using this system should keep its limitations in mind. The ages assigned to the nine age classes are approximate, and errors may be caused by individual variation. The accuracy of counting cementum layers is limited by the irregularity of the layers. Variation in the time of deposition of the first line caused by different birth dates may also cause errors. Therefore the estimated age of any one animal may be in error by one or more years.

With experience and caution, an investigator can achieve sufficient accuracy in estimating ages for most purposes, particularly if he deals with large numbers of animals and avoids basing concrete conclusions on the estimated ages of one or two animals.

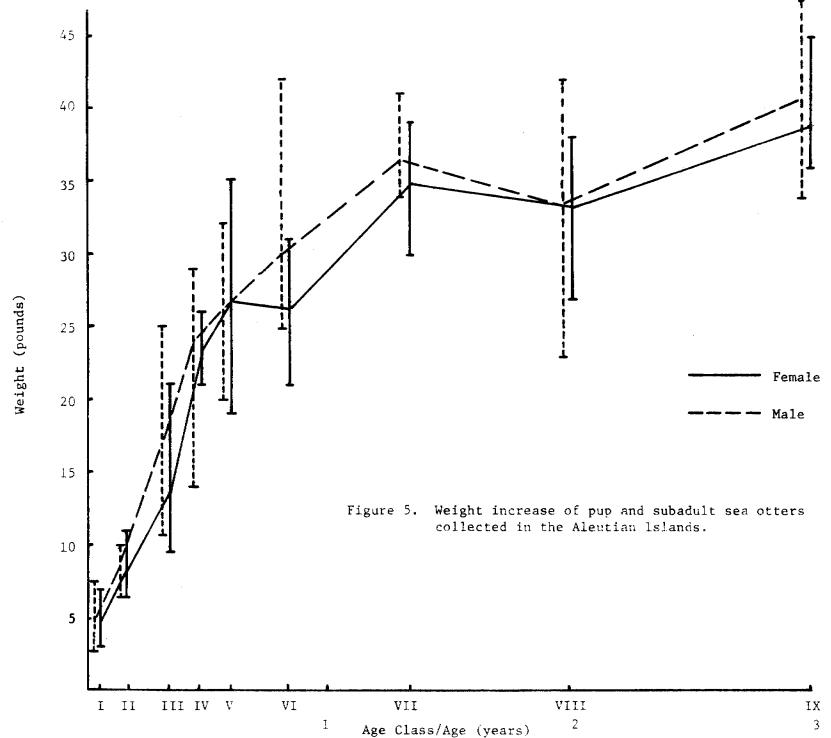
Estimating Age From Body Size

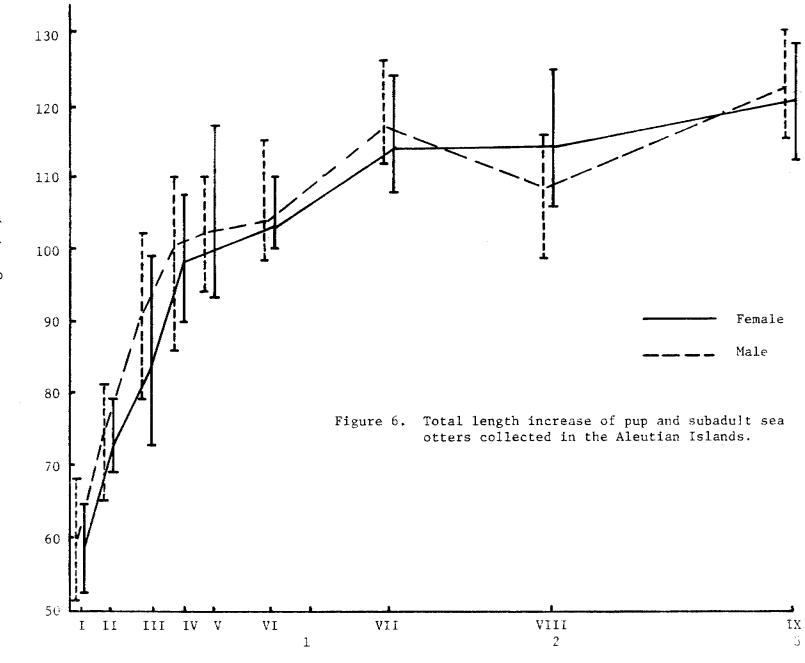
A method for roughly separating live pups, subadults and adults would be useful during transplants, tagging operations and physiological studies. In some cases it might be possible to record tooth eruption or to extract a tooth; however, this would require an undesirable amount of handling of the animal. Body measurements, particularly weight, have proved most useful for this purpose in the past. Weight and total curvilinear length measurements of sea otters in the various age classes are presented in Table 3 and plotted against estimated age in Figs. 5 and 6.

Age			Weight in Pounds		Total Length in cm			
Class	Sex	N	Mean	Max.	Min.	Mean	Max.	Min.
1	F	12	4.8	3	6.75	58.5	65	52
	М	14	5.0	2	7.5	59.7	68	51
II	F	8	8.5	11	6.5	73.6	79	69
	М	8	8.6	10	6.5	74.9	81	65
III	F	20	13.6	21	9.5	84.0	99	73
	М	28	17.1	25	10.75	91.1	102	79
IV	F	9	23.2	26	21	98.6	107	90
	М	12	24.2	29	14	100.9	110	86
v	F	14	26.8	35	19	103.7	117	93
	М	5	26.2	32	20	102.8	110	94
VI	F	4	26.3	31	21	103.5	110	100
	М	5	30.2	42	25	104.4	115	99
VII	F	9	35.4	39	30	114.8	124	108
	М	3	37.0	41	34	117.3	126	112
VIII	F	17	33.6	38	27	114.5	125	106
	М	6	33.5	42	23	109.3	116	99
IX	F	9	39. 2	45	36	121.6	129	113
	М	9	41.0	48	34	123.7	131	116

Table 3. Mean, Maximum and Minimum Weights and Total Curvilinear Lengths of Sea Otter Pups Collected in the Western Aleutian Islands Between 1967 and 1971.

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Age Class/Age (years)

Total Curvilinear Length (cm)

21

These data are valid only for Aleutian Island populations which are limited by their food supply. Otters from other areas, where food is more abundant, grow at a faster rate and may achieve a larger body size.

It is evident that precise age determination from Figs. 5 and 6 is impossible. However, growth is sufficiently fast for very rough estimates.

RECOMMENDATIONS

More known-age sea otters should be obtained either by encouraging attempts to breed otters in zoos or by using a tagging program.

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Jeanette Earnest assisted with early sectioning and staining attempts. Mary Schneider experimented with staining techniques and developed the final technique. She also processed the teeth collected in 1967 and 1968, developed a tooth eruption notation system and recorded the stage of tooth eruption on many of the pup skulls. Nicholas Steen processed the teeth collected in 1969 and 1970 with the help of Ken Pitcher. Dorothy Pettison took the photographs in Figs. 1 and 3.

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