COMPARISON AND EVALUATION OF SOME METHODS

USED TO AGE COLUMBIAN BLACK-TAILED DEER

(*Odocoileus hemionus columbianus*, Richardson)

COLLECTED IN THE VICINITY OF COWICHAN LAKE, B. C.

by

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ABSTRACT

The age of 232 deer was determined by three methods. Of these, that by tooth eruption and wear was found to be fast and cheap, but limited in accuracy. Use of lens weights, because it dealt with a continuous variable, permitted separation of fawns and adults only. Use of tooth annuli appears to give results that are exact to any age, but the time involved in the preparation of teeth for examination is prohibitive. A new technique, involving the use of a cryostat, was developed for tooth preparation, that shortens the time of preparation to one tenth that required by other tooth sectioning methods.

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TABLE OF CONTENTS

	PAGE
Abstract	i
List of Tables	iii
List of Figures	iv
Acknowledgements	v
Introduction	1
Methods and Materials	4
I. Molar Eruption and Wear	4
II. Incisor Annuli	5
III. Eye Lenses	7
Results	8
I. Molar Eruption and Wear	8
II. Incisor Annuli	9
III. Eye Lenses	13
Discussion	19
Summary	31
Literature Cited	33
Appendix	37
Vita	

LIST OF TABLES

TABLE	TITLE	PAGE
I.	Lens weight intervals arbitrarily assigned to age-classes within the population	18
II.	Comparison of age determination of 232 black-tailed deer by molar eruption and wear characteristics with counts of incisor annuli from 464 incisors	23
III.	Comparison of age determination of 232 black-tailed deer by molar eruption and wear and jaw-board. Actual age shown for deer by counts of annuli from 464 incisors.	25

LIST OF FIGURES

FIGURE		PAGE
1.	Age structure for the black-tailed deer population determined by (a) molar eruption and wear, (b) jaw-board, (c) counts of incisor annuli, and (d) weight of the eye lens	10
2.	Cross-section of incisor roots of deer age (a) 6 1/2 years, and (b) 9 1/2 years to illustrate the increase of cementum annulations with age within the 'boss' of the root	12
3.	Increase in lens weight with age of black-tailed deer. Horizontal lines represent the range of lens weights for each age-class about the mean lens weight (circled). The small rectangles represent the 95 percent confidence intervals about the calculated mean weights. The small numbers above the curve represent the sample sizes within each age-class	15
4.	Regressions of lens weight on age for black-tailed deer. Age is estimated from counts of cementum annuli and includes the seven month correction for the period of gestation	16

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INTRODUCTION

Assigning animals to age-classes is essential for demographic analysis of wildlife populations. For valid assessments of age structure, there should be little, if any, variation between the results of aging an animal by different methods. The work reported here consists of a comparison and evaluation of three methods that can be used to age black-tailed deer (Odocoileus hemionus columbianus, Richardson): (1) molar eruption and wear, (2) counts of annuli within cementum of roots of the incisor teeth, and (3) weights of the eye lens.

The tooth eruption and wear method (Severinghaus, 1949) has been used extensively by biologists as a rapid field technique to assess the annual age structure of ungulate populations from hunter kills. This method is considered to give reasonably accurate results for studies in game management (Mosby, 1966).

Scheffer (1950) and Laws (1952) in contemporaneous studies described annular layers of dentine and cementum in canine teeth of marine mammals. In the Cervidae, studies of tooth development have shown similar formations of annulations which correlate exactly with the age of the animal

(Sergeant and Pimlott, 1959; McEwan, 1962; Low and Cowan, 1963; Mitchell, 1963; Gilbert, 1966; Ransom, 1966; Keiss, 1969; Wolfe, 1969). Even though small counting errors do occur in older age groups (Low and Cowan, 1963; Novakowski, 1965; Reimers and Nordby, 1969), the use of the tooth annuli method for age determination has been applied to many mammal species (Klevezal' and Kleinenberg, 1968). However, due to the inconvenience of preparing large quantities of teeth for population studies, use of the method has been limited.

Weight of the eye lens of cottontail rabbits (Sylvilagus floridanus) was shown by Lord (1959) to increase with age and promised to provide another method of determining the age of game animals. Subsequently, however, the range of lens weights between age-classes was found to be so great to make accurate age determinations difficult (Lord, 1962; Kolenosky and Miller, 1962; Longhurst, 1964; Novakowski, 1965; Nellis, 1966; Simkin, 1967).

Comparisons of age estimates obtained by these methods have been made using elk, Cervus canadensis, (Keiss, 1969); white-tailed deer, Odocoileus virginianus, (Lueth, 1963); bison, Bison bison, (Novakowski, 1964); and moose, Alces alces, (Simkin, 1967). Results were similar in all cases;

age determination by tooth annuli, although time consuming, is most accurate.

It was believed that a comparative study of the accuracy and efficiency of three different methods available to age black-tailed deer would be useful in game management. With this information, the reliability of final inferences on the age distribution in a species population as determined by one or more of these methods would be known.

METHODS AND MATERIALS

The specimens used (232 deer of both sexes) were shot by hunters during the 1967-68 seasons in the Cowichan Lake Watershed on Vancouver Island, B. C. Disarticulated lower mandibles were removed from each deer, and the main musculature was excised. Jaws were labelled and subsequently air-dried, and the teeth removed. The eyeballs were collected in the manner described by Friend (1967:1968), injected with 10 percent formalin until turgid and left for 90 days.

I. Molar Eruption and Wear:

Deer jaws were first aged, using the method of Severinghaus (1949), by personnel of the B. C. Fish and Wildlife Branch at Nanaimo, B. C. Subsequently, I repeated the process using the same method, but getting somewhat different results. A reference set of jaws was constructed to be used as a standard for further aging. Each 'type-jaw', possessing characteristics of tooth eruption and erosional patterns specific to an age-class, was cleaned, polished and labelled. These jaws were dipped in Gelva skeletal compound (in methanol) to prevent bone and tooth fracture, dried, and then fitted in chronological sequence to serve as a reference "jaw-board".

II. Incisor Annuli:

Standard petrological methods (cutting and grinding) were attempted to examine incisor teeth for cementum annulations (Fisher and Mackenzie, 1954; Sergeant, 1959; Sergeant and Pimlott, 1959; Kenyon and Fiscus, 1963; Hewer, 1964; Mundy and Fuller, 1964; Can Nostrand, 1964; Ransom, 1966; Mitchell, 1967; Dow and Wright, 1968; McCutchen, 1968; Wolfe, 1969). Incisor roots were sectioned longitudinally with a jeweller's circular saw blade, 3 inches in diameter, 0.20 inches thick with 24 teeth to the inch and 1/2 inch central hole (obtained from the Thurston Manufacturing Company, 45 Borden Street, Providence, Rhode Island) mounted on a 5/8 inch sleeve adapter on a 1/3 hp electric motor (1725 rpm). The blades were water cooled by a dampened sponge held with a plastic reservoir beneath the blade. The roots were held in an Eclipse Instrument Vice No. 180 (obtained from Thomas Skinner & Sons, Ltd., 51 Cordova Street, Vancouver) and hand fed into the rotating blade. The sections were ground and polished to translucency with carborundum powders starting with grade 220, passing to 280 and then to 400. The sections were then washed and mounted on microscope slides with Canada Balsam. The slides were examined under transmitted light on a compound microscope at 10X and 40X but annulations were not recognizable for counting purposes.

Alternatively, standard histological techniques used for tooth examination were tried but without success (Sergeant and Pimlott, 1959; McEwan, 1962; Low and Cowan, 1963; Finnegan, 1965; Gilbert, 1966; Stoneberg and Jonkel, 1966; Adams and Watkins, 1967; Thomas, 1968). As a consequence, a new technique making use of a cryostat was devised.

Similarly aged mandibles, with intact incisors, were placed in one litre beakers. An eighty percent solution of 30 percent formic acid in 4 percent formalin was added so as to immerse the anterior portions of the jaws. As decalcification progressed, softened bone tissue was removed to expose the roots, until the teeth could be removed. During and preceding removal of the incisors care was taken not to damage either the periodontal membranes or the hypercementotic regions ('boss') about the roots. Once extracted, the crowns were removed, the roots placed in 3 dram shell vials, acid solution added, and decalcification continued. Every 10 hours, the acid solution was changed and the completeness of decalcification determined (Humason, 1966, p. 27). Decalcified roots were handcut into 1 - 2 mm sections, placed in Tissue-Tek Paks (Canlab), and washed under running water for 12 hours.

Sections were stained with Delafield's hematoxylin for 42 hours.

The stained sections were placed on cryostat plates, covered with "Cryoform", and frozen at -15°C for 6 minutes. Cross-sections were cut at 12 - 16 microns in the cryostat and the sections immediately transferred to water baths. Once thawed, the sections were placed on microscope slides, dried, and examined at 40X under transmitted light. For permanent slides, the root sections were covered with Histoclad and a cover slip.

III. Eye Lenses:

One week prior to the end of the formalin treatment, the hardened lenses were removed from the eyeballs (Friend, 1967) and all ciliary muscle removed by rolling in lens tissues. The lenses were then placed in 3 dram shell vials containing 10 percent formalin. At the end of formalin treatment, the lenses were wiped with lens tissues. All discoloured and damaged lenses were discarded (Friend, 1968). Lenses were then weighed wet to the nearest milligram on a Mettler analytical balance (Lueth, 1963; Downing and Whittington, 1964).

RESULTS

I. Molar Eruption and Wear:

The deer jaws were aged by personnel of the B. C. Fish and Wildlife Branch at Nanaimo, B. C. Using the method of Severinghaus (1949), the mandibles were subjectively grouped into six wear-classes by time of premolar replacement and subsequent wear, the sequence for eruption of the first, second, and third molar teeth, progressive wear and reduction of buccal and lingual heights of the molar teeth, increased exposure of the dentine with decreasing width of the enamel, and loss of the infundibular cavities on the molars. Results of this grouping are shown in Fig. 1 (a). Those jaws showing excessive wear on the teeth were assigned to a 5 1/2+ wear-class; such jaws cannot be fitted into a more definite wear-class.

I re-aged the jaws using the same criteria. Because the work was done by one individual working on all jaws simultaneously, the results were likely considerably more accurate. From each group of jaws, I selected one jaw that best typified the wear characteristics of that wear-class and constructed a "jaw-board" with these chosen standards. Jaws were aged against these 'type-jaws'. The results are shown in Fig. 1 (b). Discrimination of older wear-classes was improved by this analysis, since older wear-classes could be identified by subjective characteristics (Appendix A).

II. Incisor Annuli:

The counting of cementum annuli for age determination of game species of mammals has received world-wide recognition and use (Klevezal' and Kleinenberg, 1968). The precision of this method for aging black-tailed deer has been previously established from studies of incisors from 23 known-age deer (Low and Cowan, 1963). When the month of death was known it was possible to determine the actual age of the animal to the nearest month but when no record of death was available, ages could only be determined to the nearest six months. Also, Thomas (pers. comm.) has stated the method is 100 percent accurate for known-age (tagged) wild deer on Vancouver Island. Errors in aging have in general been found to be few in number. For older groups, age can be estimated to plus or minus one year of the actual age of the animal. However, rut lines and absorption lacunae with secondary formation of cementum in the root can complicate the count (Sergeant and Pimlott, 1959; Low and Cowan, 1963; Reimers and Nordby, 1968).

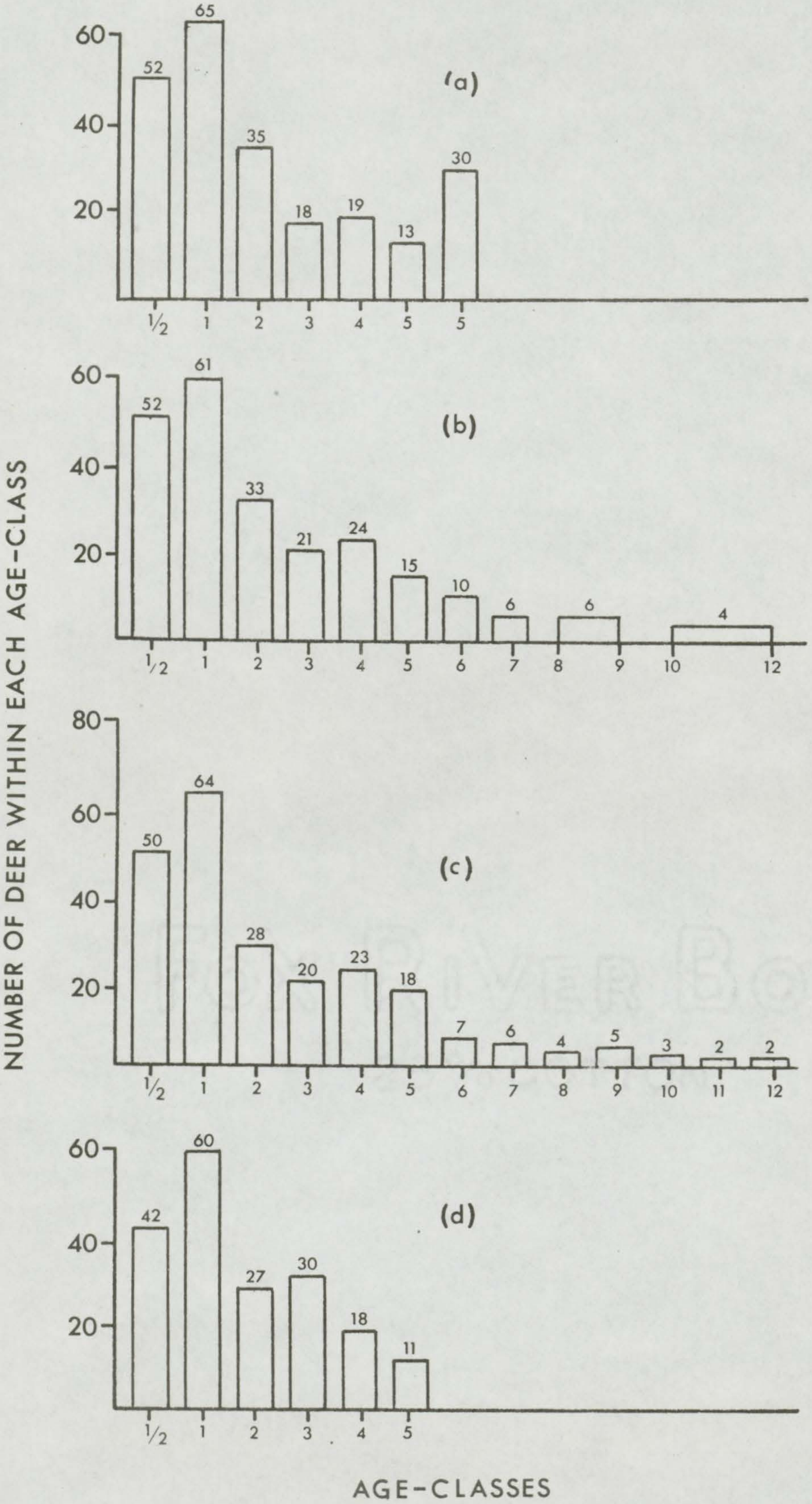
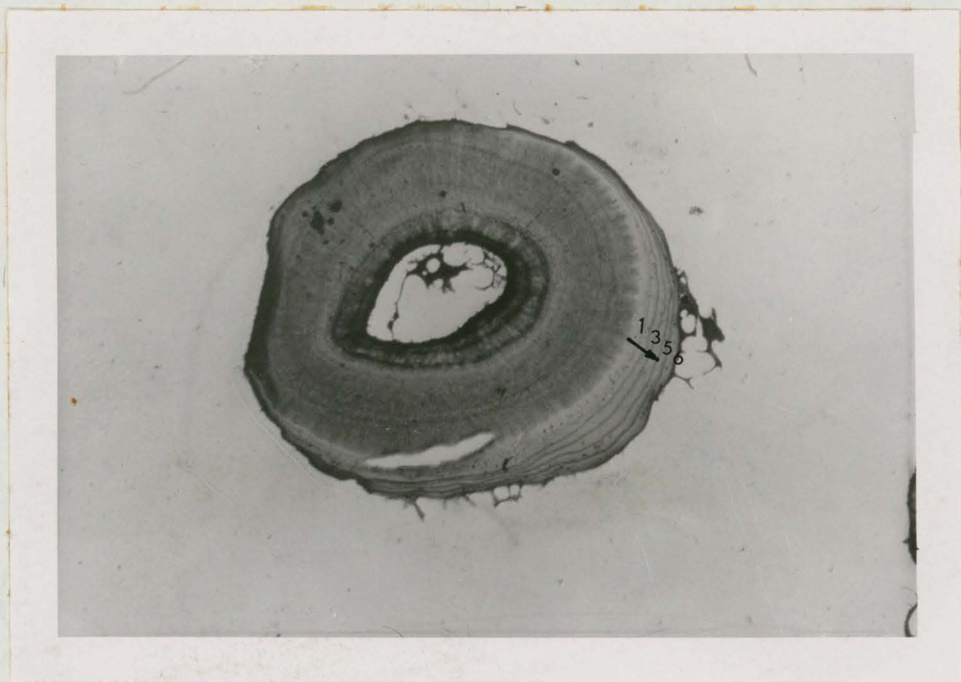

NUMBER OF DEER WITHIN EACH AGE-CLASS
(a)
60
40
20
52
65
35
18
19
13
30
½
1
2
3
4
5
5
(b)
60
40
20
52
61
33
21
24
15
10
6
6
4
½
1
2
3
4
5
6
7
8
9
10
12
(c)
80
60
40
20
50
64
28
20
23
18
7
6
4
5
3
2
2
½
1
2
3
4
5
6
7
8
9
10
11
12
(d)
60
40
20
42
60
27
30
18
11
½
1
2
3
4
5
AGE-CLASSES

Figure 1 - Age structure for the black-tailed deer population determined by (a) molar eruption and wear; (b) jaw-board; (c) counts of incisor annuli; and (d) weight of the eye lens.

Annuli were easily recognized from the stained sections of the teeth (Fig. 2). Counts of annuli were compared between incisors and between sections of the same incisor for each of one hundred and twenty-three (123) deer. No counting errors were found. Subsequently, the 464 incisors were examined and by annuli counts, the deer were grouped into age-classes (Fig. 1). Because of its established accuracy for age determination, the incisor annuli technique is used here as the absolute standard against which comparisons with the results of the other aging methods are made.



(a)

(b)

Figure 2 - Cross-sections of incisor roots of deer aged (a) 6 1/2 years, and (b) 9 1/2 years to illustrate the increase of cementum annulations with age within the 'boss' of the root.

III. Eye Lenses:

The lens weights were divided into annual age groups based on counts of cementum annuli (see preceding paragraphs). For individual deer, a difference in the weights of right and left lenses was found but neither lens was significantly heavier. The right lens was heavier in 50 percent of the males and 49 percent of the females sampled. The left lens, on the other hand, was found heavier in 43 percent of the males and 51 percent of the females sampled. Of the total sample, only 6 percent of the deer had paired lenses of equal weight.

For deer of the same sex and age class, the mean weights of the right and left lenses were non-significantly different. Deer of the opposite sex but of the same age group also showed non-significant differences in mean weight of the lens. However, between consecutive age-classes, mean weights were significantly different and when plotted against age, a curvilinear relationship was described (Fig. 3).

Nine common logarithmic and arithmetic regression models were fitted to the data. Ages were increased by a factor of seven months (the approximate gestation period) for the regression calculations to avoid problems of negative values. This procedure was used by Kolenosky and Miller (1962) for pronghorn antelope and Longhurst (1964) for black-tailed deer. In both instances, the adjustment had no effect on the correlation coefficients calculated. Of the models the two equations

$$Y = 0.095 + 0.675 (\text{Log } (X+7))$$

and

$$\text{Log } Y = 2.644 + 0.257 (\text{Log } (X+7))$$

gave what appeared to be the best representation of the lens weight-age relationship, where X+7 represents the age of deer in months following birth and Y is the weight of individual lenses in milligrams (Fig. 4).

From inspection of the graphs, a better fit of the data was deemed possible. Using the IBM System/360 Scientific Subroutine package, higher level polynomials were fitted to the data. Individual lens weights were found to be so variable within age-classes that the analysis failed. When, however, mean weights by age-class were used the best fits were obtained by the fourth degree polynomial,

$$Y = -111.55 + 340.89X - 335.82X^2 + 109.29X^3 - 0.45X^4$$

with significant reduction in deviations from the linear and simpler polynomial regressions (Fig. 3).

Figure 3 - Increase in lens weight with age of black-tailed deer. Horizontal lines represent the range of lens weights for each age-class about the mean lens weight (circled). The small rectangles represent the 95 percent confidence intervals about the calculated mean weights. The small numbers above the curve represent the sample sizes within each age-class.

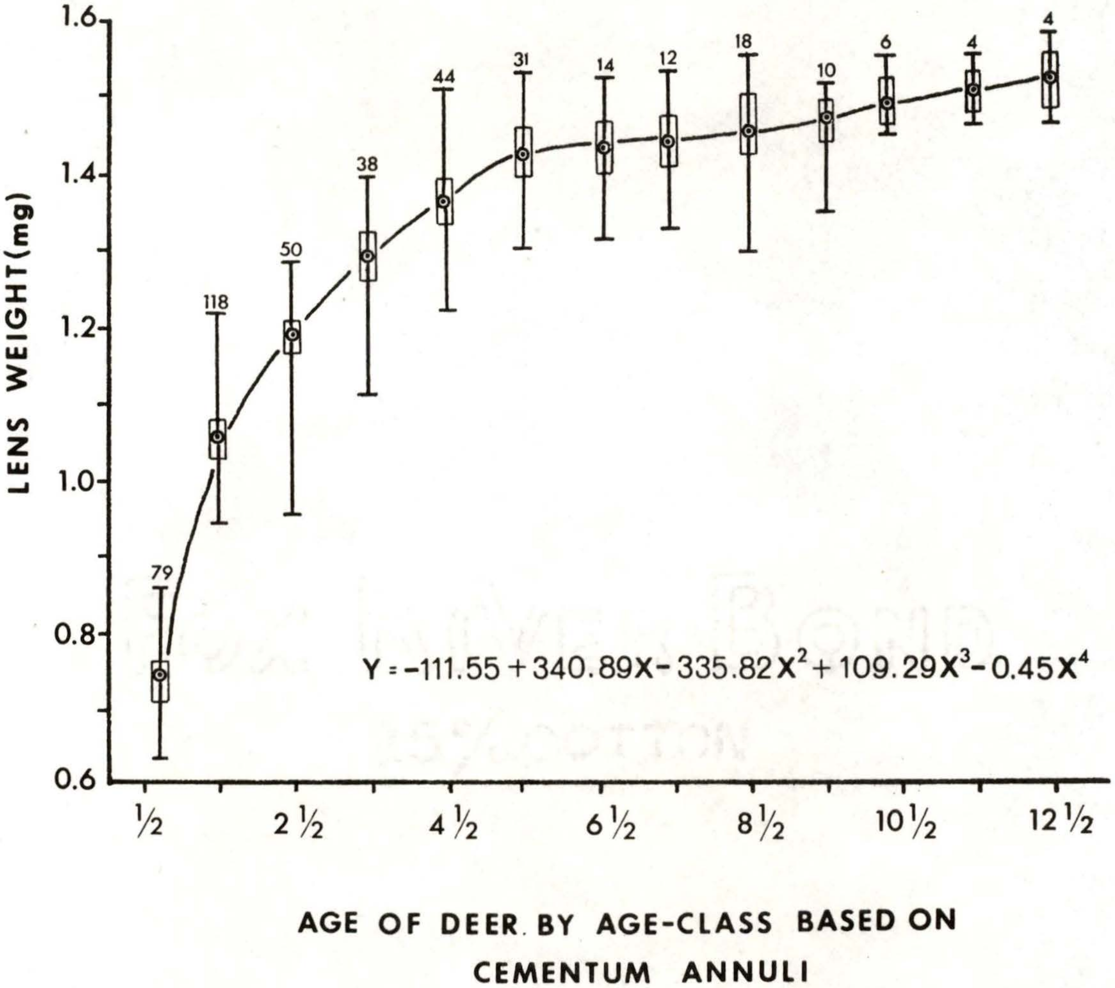
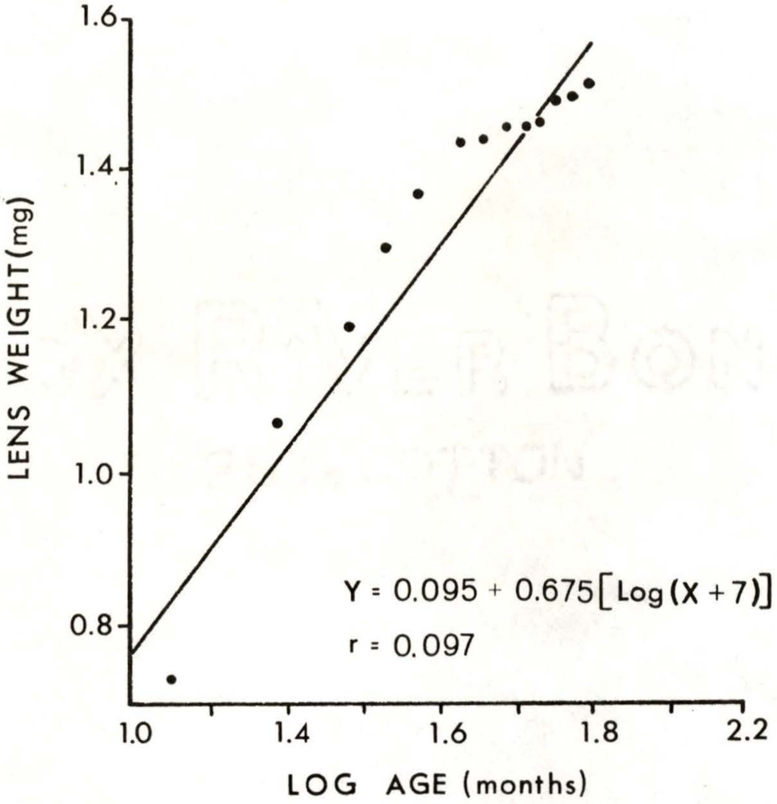
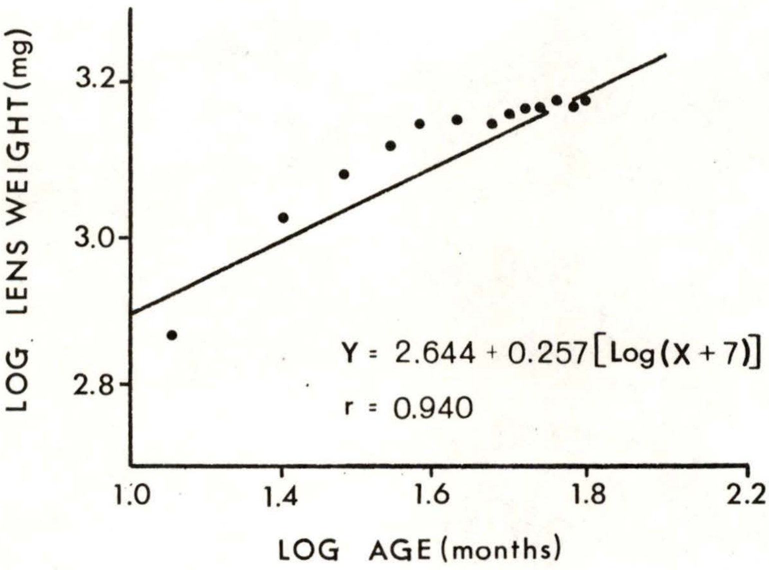

LENS WEIGHT (mg)
1.6
1.4
1.2
1.0
0.8
0.6
79
118
50
38
44
31
14
12
18
10
6
4
4
Y = -111.55 + 340.89X - 335.82X² + 109.29X³ - 0.45X⁴
½
2 ½
4 ½
6 ½
8 ½
10 ½
12 ½
AGE OF DEER BY AGE-CLASS BASED ON
CEMENTUM ANNULI

Figure 4 - Regressions of lens weight on age for black-tailed deer. Age is estimated from counts of cementum annuli and includes the seven month correction for the period of gestation.



1.6
1.4
1.2
1.0
0.8
LENS WEIGHT (mg)
Y = 0.095 + 0.675[Log (X + 7)]
r = 0.097
1.0
1.4
1.6
1.8
2.2
LOG AGE (months)



3.2
3.0
2.8
LOG LENS WEIGHT (mg)
Y = 2.644 + 0.257[Log (X + 7)]
r = 0.940
1.0
1.4
1.6
1.8
2.2
LOG AGE (months)

The inability to discriminate between age-classes of deer beyond 3 1/2 years, due to increased overlap of lens weights in the older age groups, made further use of regression analysis impractical. However, Simkin (1967) concluded that by arbitrary intervals of lens weights, moose could be assigned to age-classes when the upper 95 percent confidence limit of a younger age-class and lower probability limit of the next higher adjacent age-class were averaged. The results of this method, in the present study, are given in Table I. Since 23 specimens had discoloured and damaged lenses and 21 had lens weights greater than the upper limit calculated for the oldest age group, only 188 specimens were considered in this analysis. The results of grouping specimens into age-classes according to these arbitrary weight intervals are shown in Fig. 1 (d).

TABLE I. LENS WEIGHT INTERVALS ARBITRARILY ASSIGNED TO AGE-CLASSES WITHIN THE POPULATION

AGE-CLASS* (years)	MEAN LENS WEIGHTS FOR AGE-CLASSES	WEIGHT-INTERVALS** CALCULATED
1/2	0.733	0.724 - 0.896
1 1/2	1.060	0.897 - 1.124
2 1/2	1.197	1.125 - 1.240
3 1/2	1.292	1.241 - 1.331
4 1/2	1.370	1.332 - 1.399
5 1/2	1.432	1.400 - 1.434

* Age-classes calculated to the nearest year by counts of cementum annuli within the incisor teeth.

** Weight intervals are calculated by averaging the upper and lower 95 percent confidence limits of adjacent age-classes.

DISCUSSION

I. Accuracy of Methods:

The accuracy of most single methods of age determination has been investigated with known-age specimens (Lord, 1959:1963; Low and Cowan, 1963; Longhurst, 1964; Marks and Erickson, 1966; Stoneberg and Jonkel, 1966; Wolfe, 1969). Comparisons of two different methods have been conducted as well to give some indication of the variation to be expected in the final age estimates (Larson and Van Nostrand, 1968; Reimers and Nordby, 1968; Simpson and Elder, 1968; Keiss, 1969). Methods have been compared for bison, (Novakowski, 1964) and moose, (Simkin, 1967) but for an important game species as deer, no comparative work has been done for more than two methods (Lueth, 1963; Ryel, Fay, and Van Etten, 1961). Since the formation of annuli within the cementum tissues of the teeth has been shown to be an annular phenomenon in deer of known-age (Low and Cowan, 1963; Gilbert, 1966; Ransom, 1966), it seems reasonable to expect that counts of annuli would give accurate estimates of age. On this assumption, the tooth annuli method is chosen as the standard for comparison against which the results of the other methods are evaluated for accuracy.

In the early fifties, Scheffer (1950) and Laws (1952) described layered patterns within the dentine and cementum tissues of the permanent canine teeth for northern fur seals (Callorhinus ursinus cynocephalus) and elephant seals (Mirounga leonina) respectively. These authors demonstrated with known-age seals that one alternation of an opaque (dark) and a translucent (light) layer constituted an annual increment of growth. Subsequent examinations have shown a close correlation between the number of tooth annulations and increasing age for many North American cervids including: mule and black-tailed deer (Low and Cowan, 1963); barren ground caribou, Rangifer tarandus, (McEwan, 1962); moose, (Sergeant and Pimlott, 1959; Wolfe, 1969); white-tailed deer, (Gilbert, 1966, Ransom, 1966); pronghorn antelope, Antilocapra americana, (McCutchen, 1969); and elk, (Keiss, 1969).

There is almost no doubt amongst biologists that passage of a year in a deer's life usually results in a distinctive pattern of lamellar increments to the cementum of the teeth. From histological studies, cross-sections of teeth show an alternating pattern of concentric light and dark bands (Fig. 2). The wider pale staining zones are indicative of spring and summer growth and the narrower darker staining zones, the winter growth. It is believed that the formation of these annulations is due to either seasonal changes in the animal's food intake or to hormonal disturbances (Low and Cowan, 1963).

Since the permanent incisors of this species are fully erupted by the seventh month and cementum deposition begins before eruption, the first visible dark band in the cementum is that of the first fall and winter of the deer's life. Therefore, the age of deer can be determined by using this band as Year 1. In this way, age estimates of deer killed during the hunting season will be to the next half year, that is, from 1 1/2 to 2 1/2. In figure 2 (b), nine dark winter lines can be distinguished as shown, indicating the animal was in its ninth winter and is estimated to be 9 1/2 years old.

Counts of tooth annuli are believed to give absolutely accurate age determinations where the method has been used. Low and Cowan (1963) concluded the estimates of age for mule and black-tailed deer of known-age were accurate to within two months for animals older than one year. Further these authors concluded that age could be estimated to the nearest month when the month of death was known. Otherwise estimates of age were possible only to the nearest half year. Assuming the approximate date of birth, Ransom (1961) similarly concluded that the age of deer could be estimated in months by tooth annuli. Also Thomas (pers. comm.) considers the annuli method 100 percent accurate for determining the age of known-age wild deer on Vancouver Island. Because of its accuracy and objectivity, the tooth annuli method can be used as a standard for comparative studies of age determination.

The method based on molar eruption and wear is limited in accuracy. Because of indefinite dental characteristics on the jaws, eruption and wear-classes for deer older than 5 1/2 years were not assigned to the population by personnel of the B. C. Fish and Wildlife Branch. Although a simple, fast and inexpensive method, wear characteristics become so generalized with progressive age that considerable overlap between adjacent wear-classes occurs. In Table II, a comparison of age determination of deer by molar eruption and wear and cementum annuli methods is shown to illustrate the amount of overlap of age within wear-classes. Normally, however, with careful scrutiny one can separate the fawns and yearlings from the 2 1/2 year olds. But intermediate

TABLE II. COMPARISON OF AGE DETERMINATION OF 232 BLACK-TAILED DEER BY MOLAR ERUPTION AND WEAR CHARACTERISTICS WITH COUNTS OF ANNULI FROM 464 INCISORS.

Molar Eruption and Wear			Age-classes based on Counts of Annuli within Incisor Teeth												
Wear Class	No. in Sample	Age-Class	0	1	2	3	4	5	6	7	8	9	10	11	12
I	52	1/2	49	3	-	-	-	-	-	-	-	-	-	-	-
II	65	1 1/2	1	58	2	-	3	1	-	-	-	-	-	-	-
III	35	2 1/2	-	1	25	6	2	-	-	-	-	1	-	-	-
IV	18	3 1/2	-	-	1	11	4	2	-	-	-	-	-	-	-
V	19	4 1/2	-	-	-	2	14	2	1	-	-	-	-	-	-
VI	13	5 1/2	-	1	-	-	-	7	1	1	-	-	2	-	1
VI+	30	5 1/2	-	1	-	1	-	6	5	5	4	4	1	2	1

stages of tooth eruption and wear can make class distinction difficult. Consequently, three yearlings were included in the fawn class; 10 percent of the jaws aged as yearlings (wear-class II) were from older deer; and wear-classes III, IV, V, VI, and VI+ contained many jaws that were incorrectly aged by subjective analysis of tooth wear (Table II). Ryel et al (1963) illustrated this same tendency of overlap to occur between adjacent wear-classes when 50 known-age jaws were assigned to wear-classes by 22 biologists. The variation was explained by the level of experience of each biologist with the Severinghaus method and by individual preferences for certain eruption and wear characteristics chosen to typify a wear-class. Lueth (1963) on the other hand attributes this variation to the "error of the intermediates". This source of error, he explains, occurs when the pattern of tooth eruption and wear on a jaw has characteristics of two adjoining wear-classes such that the jaw will be placed within one of two adjacent wear-classes. The jaw-board offers some compensation for this source of error as more wear-classes are assigned to the population thereby reducing intermediate characteristics. In Table III, the amount of overlap is shown to have been reduced after comparison of jaws to the chosen standards. In this way,

TABLE III. COMPARISON OF AGE DETERMINATION OF 232 BLACK-TAILED DEER BY MOLAR ERUPTION AND WEAR AND JAW-BOARD. ACTUAL AGE SHOWN FOR DEER BY COUNTS OF ANNULI FROM 464 INCISORS.

Jaw-board Inference			Age-classes based on Counts of Annuli within Incisor Teeth												
Wear Class	No. in Sample	Age-Class	0	1	2	3	4	5	6	7	8	9	10	11	12
I	52	1/2	50	2	-	-	-	-	-	-	-	-	-	-	-
II	61	1 1/2	-	59	2	-	-	-	-	-	-	-	-	-	-
III	33	2 1/2	-	3	25	5	-	-	-	-	-	-	-	-	-
IV	21	3 1/2	-	-	1	15	4	1	-	-	-	-	-	-	-
V	24	4 1/2	-	-	-	-	18	4	2	-	-	-	-	-	-
VI	15	5 1/2	-	-	-	-	1	13	1	-	-	-	-	-	-
VII	10	6 1/2	-	-	-	-	-	-	3	3	2	2	-	-	-
VIII	6	7 1/2	-	-	-	-	-	-	1	1	1	1	2	-	-
IX	6	8 1/2 to 9 1/2	-	-	-	-	-	-	-	2	1	2	-	1	-
X	4	10 1/2 to 12 1/2	-	-	-	-	-	-	-	-	-	-	1	1	2

the error of placing a specimen in an adjoining wear-class is minimized.

Lord (1959) concluded that cottontail rabbits could be aged to 30 months by dry weight of the eye lens and considered the technique applicable to most other mammals. Subsequently, the growth of the eye lens and its use for age determination has been investigated for over one hundred species and subspecies of mammals (Friend, 1968). Included among these were members of the Cervidae: pronghorn antelope, (Kolenosky and Miller, 1962); white-tailed deer, (Lord, 1962; Lueth, 1963; Friend and Severinghaus, 1967); mule and black-tailed deer (Longhurst, 1964; Nellis, 1966); and moose, (Simkin, 1967).

Although there was shown an increase of lens weight with age for all species, the use of lens weight to determine the age of mammals has been found to be difficult because of individual variation amonst age-classes. However, Longhurst (1964) for black-tailed deer, and Kolenosky and Miller (1962) for pronghorn antelope suggested these species could be aged by linear regression of lens weight to 5 years and 9 1/2 years, respectively. Lueth (1963) felt that due to extensive overlap of lens weights in the older age-classes, white-tailed deer could not be aged beyond the fawn class by this means. Also, Novakowski (1964) was only able to separate calves, yearlings and adult bison by lens weight. Simkin (1967) ignored the variability and calculated a discontinuous series of intervals of lens weights which he assigned to each of sixteen age-classes of moose. The age of individual moose was determined to 9 1/2 years by objectively assigning each lens weight to a particular weight interval of an age-class.

Because of individual variation of lens weight, linear regression models of weight with age, although significant, were impractical for reliable estimates of the age of black-tailed deer. When the data are graphed after logarithmic transformation, an obvious relationship of curvilinearity between lens weight and age is described (Fig. 4). Fitting the data to higher degree polynomials on an individual lens weight basis, the computer would not generate a mathematical expression because of the wide variation of lens weights and overlap amongst the age-classes. However, when the variation is minimized by using the mean weights of lenses for each age-class in the analysis, a fourth degree polynomial was generated. But to use this expression for age determination is impractical since no level of confidence can be placed on the final estimates where variance has been ignored.

If one calculates weight intervals for age-classes following the method of Simkin (1967), deer can be objectively assigned to 5 1/2 years of age by individual lens weights (Fig. 1 (d)). However, variation has again been ignored and this destroys the user's confidence in the age estimates obtained. At best, therefore, it seems that lens weights can only allow separation of fawns from older age-classes for this species (Fig. 3).

II. Efficiency of Methods

Even though molar eruption and wear gives inaccurate estimates of an animal's age, the method is used by game biologists. Its simplicity encourages its continued use in field studies. As a rapid technique for age determination, it gives the type of data desired by game management from which rapid assessments of a species' population can be made. Much of the subjective inaccuracies can be minimized if standards for each wear-class are chosen for the population and a "jaw-board" constructed. The lens because of its weight variability probably does not serve as a reliable method for age determination. Arbitrary analytic methods may allow some inferences to be made on age, but more work is needed to make the procedure useful.

The growth of cementum, because it is discontinuous, is by far the most accurate method for determining the age of game mammals; but because of the inconvenience and time required for preparation and examination of material, its use has been limited for management studies. However, if the cryostat procedure, developed in this study, is used the tooth annuli method would become more efficient for field studies.

The requirement for examination of large quantities of teeth for population studies necessitates the development or improvement of methods for decalcification, staining, and sectioning. General histological methods were attempted without much success due mainly to poor decalcification and infiltration of paraffin within the cellular matrix of the tooth. Grinding and cutting did not give uniform tooth sections making annuli counts unreliable. Unaware of the work of Reimers and Nordby (1968), then published, I devised a rapid method whereby large quantities of teeth could be decalcified, stained, and sectioned within a relatively short period of time. Whereas Reimers and Nordby used a freezing microtome for their work, I used a cryostat for all phases of the tooth study. The simplicity of operation is the major advantage of this technique. Large quantities of teeth are simultaneously decalcified and stained. Each stained tooth is cut into several sections with a razorblade then frozen at -15°C. Once sectioned on the cryostat immediate records of age are available. There is no need for numerous chemicals except for decalcifying acid solutions, Cryoform embedding medium, water and mounting medium for slide preparations. Reimers and Nordby could only process 40 teeth per day, but with this method, after preliminary preparations of the teeth, 40 - 50 incisors could be simultaneously frozen and sectioned within one hour. When permanent slides are desired, this rate of operation is reduced but with practice, one's rate improves accordingly.

The data available from this study was unsuited for any extensive inferences on the age structure of the deer population in the Cowichan Lake Watershed. Restricted by sample size, no valid judgements could be presented on the status of the population, although the kill-curve resulting is one of the same general pattern as that found by other workers (Fig. 1).

SUMMARY

1. Lower mandibles and eyeballs were collected from 232 black-tailed deer of both sexes and unknown-age in the Cowichan Lake Watershed, on Vancouver Island, during the 1967-68 hunting season.

2. Deer were subsequently aged by three methods of age determination: molar eruption and wear, counts of annuli within cementum of the incisor teeth, and weights of the eye lens. The results were compared and evaluated for their accuracy, and the efficiency of each method was described.

3. The tooth eruption and wear method, at best subjective, is limited in accuracy for age determination. If standards are chosen for each wear-class and a jaw-board constructed, the accuracy of the age estimates is improved. Because of its simplicity, the method serves as a rapid field method.

4. Counts of annuli within the cementum of incisor teeth gives accurate estimates of age of deer to the nearest half year. However, because it is time consuming, its use for population studies is discouraged.

5. Lens weights are too variable for age determination by

regression analysis. At best, the lens allows separation of fawns from older age-classes. Consequently, its use for determining age is limited. However, when arbitrary intervals of lens weights are assigned to age-classes, age determination of deer by individual lens weights appears promising in the younger age-classes.

6. In order to process larger quantities of teeth for population study, a new preparatory technique was developed, using a cryostat. With this, decalcified and stained incisors were sectioned at a rate of 40 - 50 teeth per hour. It is concluded that the use of the cryostat should widen the use of the tooth annuli method of age determination considerably.

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APPENDIX

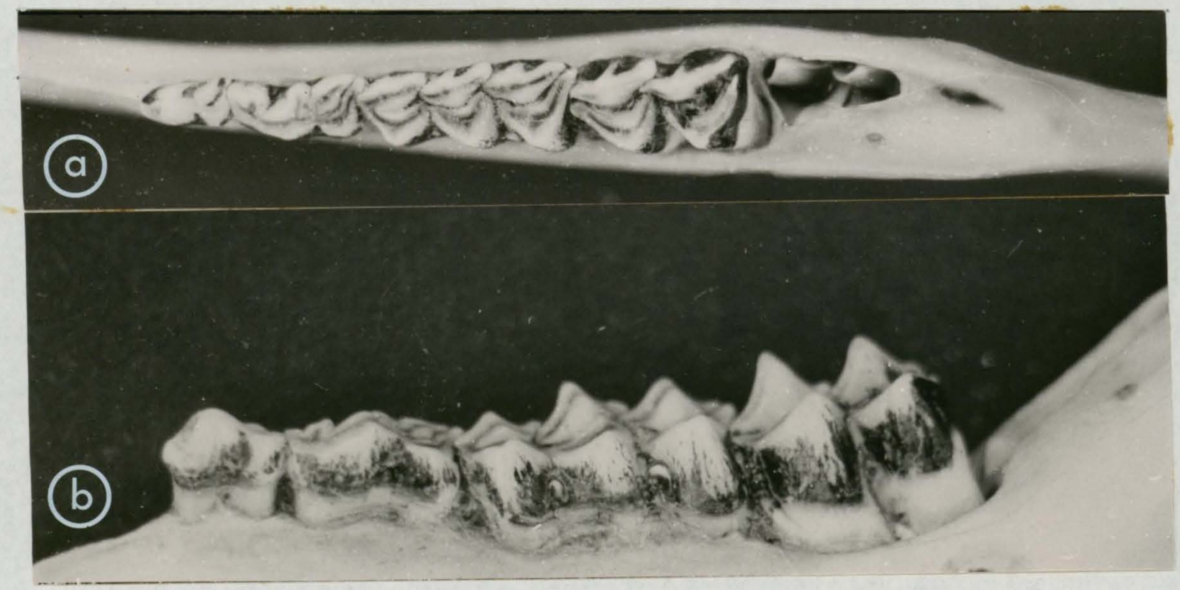
Jaw-board standards used to

determine the age of black-tailed deer by

molar eruption and wear.

PLATE 1 - Occlusal (a) and buccal (b) aspects of the mandible. First molar usually fully erupted. Between the 7th and 8th month, the second molar starts to erupt through the gum. Three temporary (milk) premolars present. Age: 6-10 months. Age-class: 1/2.

PLATE 2 - Milk premolars moderate to heavily worn. Lingual and buccal crests of molars sharp. Third premolar lost replaced by two cuspid permanent premolars which might already show some evidence of slight wear. Third permanent molar appears (16 months); becomes fully erupted (20-24 months); may show slight wear but dentine is not exposed on the crests. Age: 16-24 months. Age-class: 1 1/2.


a
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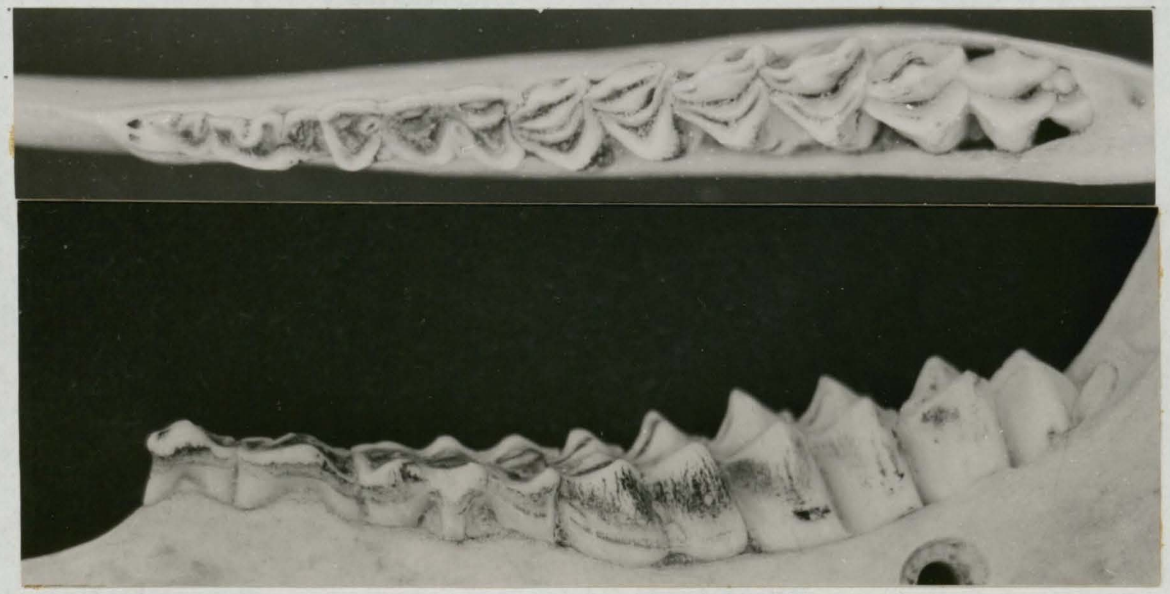
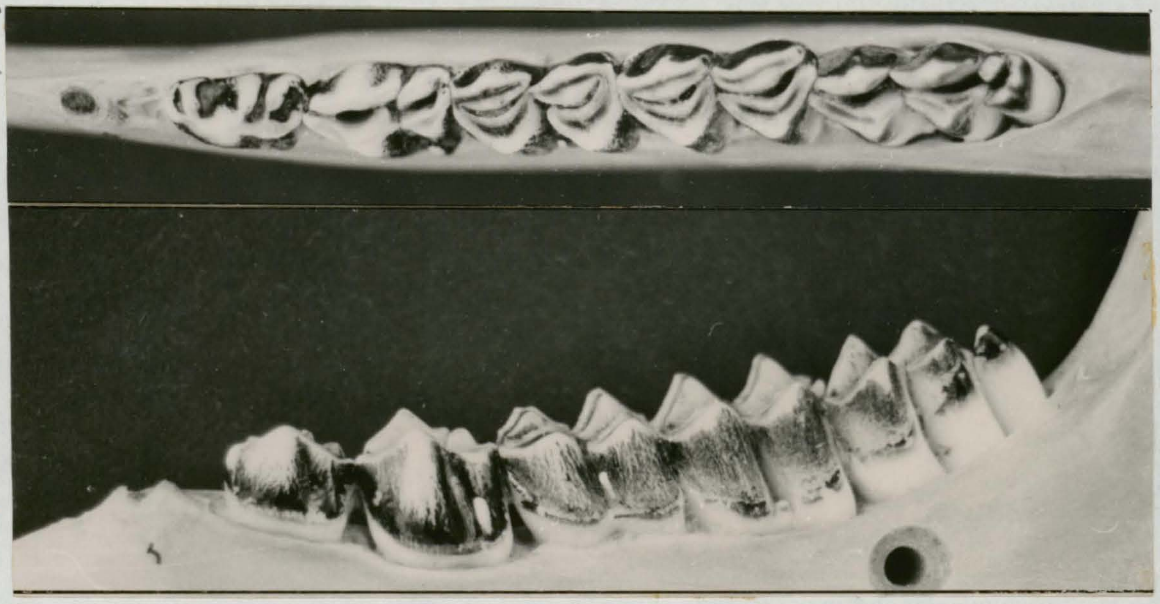


PLATE 3 - Slight wear on second and third premolars, very little wear on the first. Lingual crests of first molar sharp; enamel well above narrow dentine of the crest and both equal in width. Enamel of buccal crest of second and third molars also extends above dentine. Slight wear on posterior cusp of the third molar.

PLATE 4 - Lingual crests of first molar blunt, dentine line wider than enamel. Dentine begins to assume greater width, particularly on buccal cusps, where it is generally wider than enamel on first and second molars. Posterior cusp of third molar flattened by wear. Little wear on first premolar, slight on second, moderate on third. Age: 41-53 months. Age-class: 3 1/2.



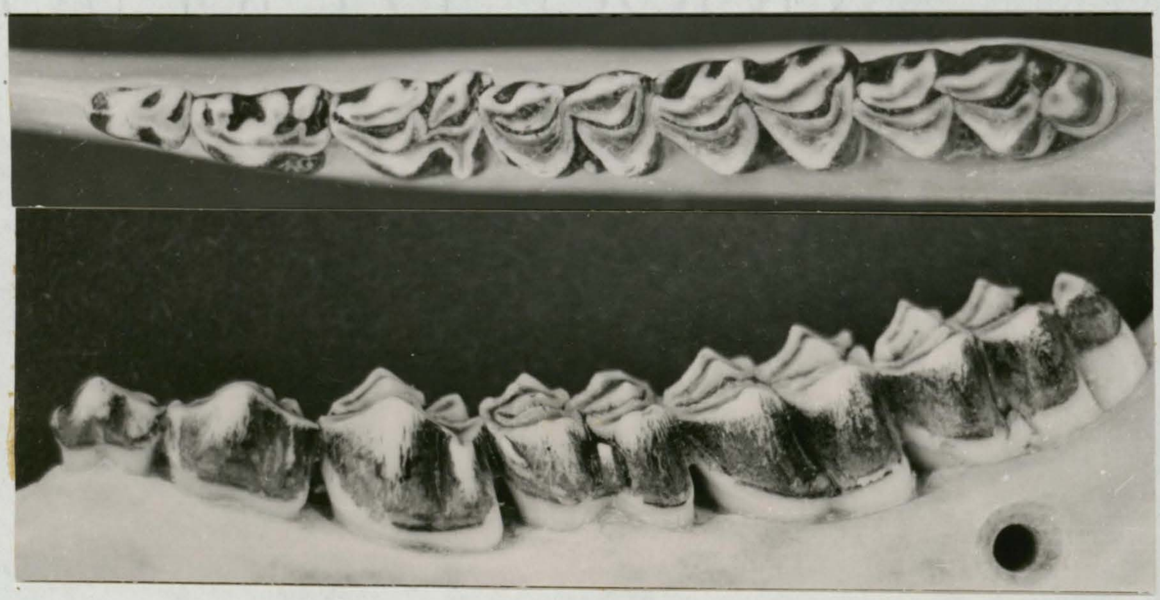


PLATE 5 - Lingual crests of first molar almost worn away, dentine almost twice width of the enamel. Lingual crests of the second molar blunt. Dentine of second molar wider than enamel, on third molar about as wide. The infundibulum begins to disappear in some molars. Wear on first premolar very slight, moderate on second, moderate to heavy on third. Age: 54-64 months. Age-class: 4 1/2.

PLATE 6 - Moderate to heavy wear on second and third premolars, slight on first. Original lingual crests of first and second molars worn away, blunt on third. Dentine of crests on all molars much broader than enamel on most teeth. Infundibulum of first molar greatly reduced. Age: 65-76 months. Age-class: 5 1/2.

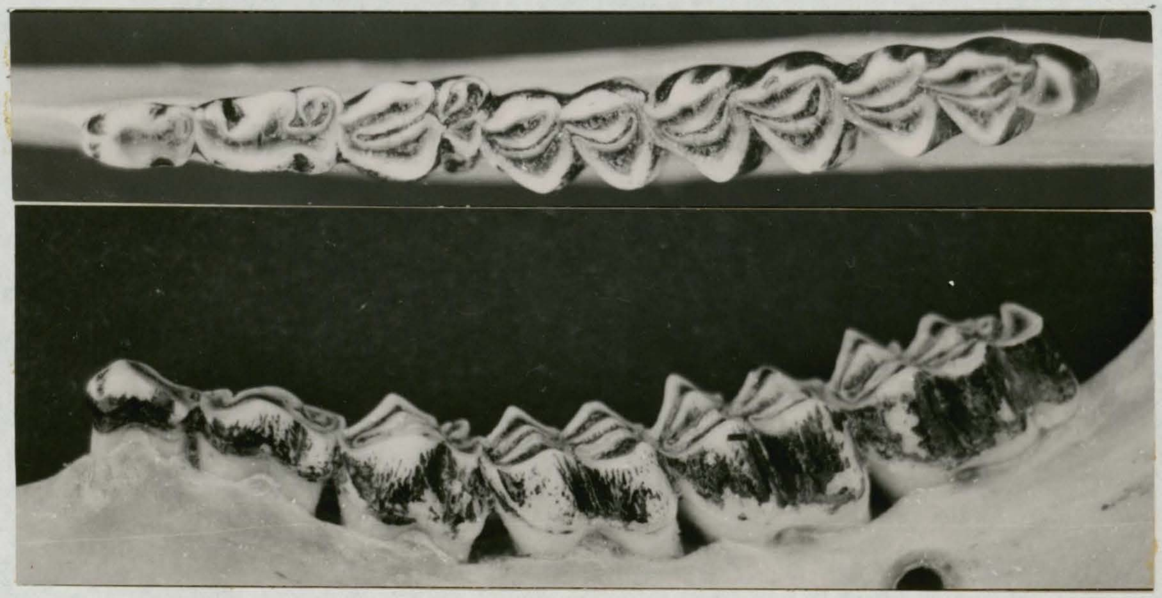


PLATE 7 - Wear moderate on first premolar, heavy on second, and third. Infundibulum of the first molar reduced. Dentine joined and broadens in the second and third molars. Grinding surfaces smooth, especially on the first molar. Age: 77-89 months. Age-class: 6 1/2.

PLATE 8 - Wear on first and second premolars heavy. Infundibulum reduced on third premolar; gone in the first molar but usually present although much reduced in the second and third molars. No lingual crest on third molar and grinding surfaces rough. Second molar almost worn smooth. Age: 90-101 months. Age-class: 7 1/2.

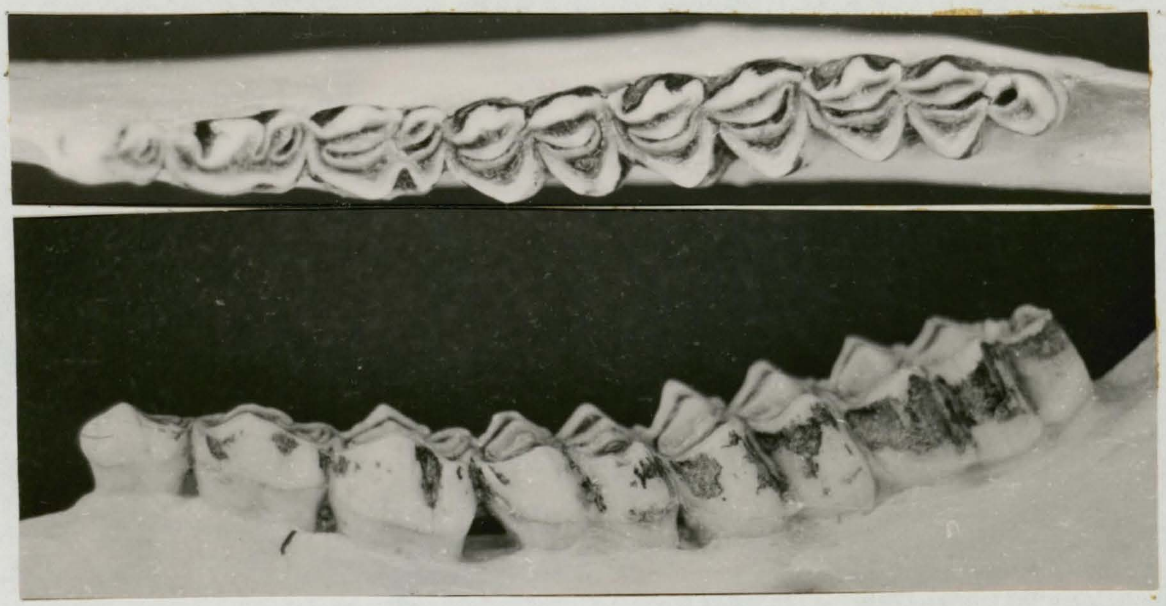
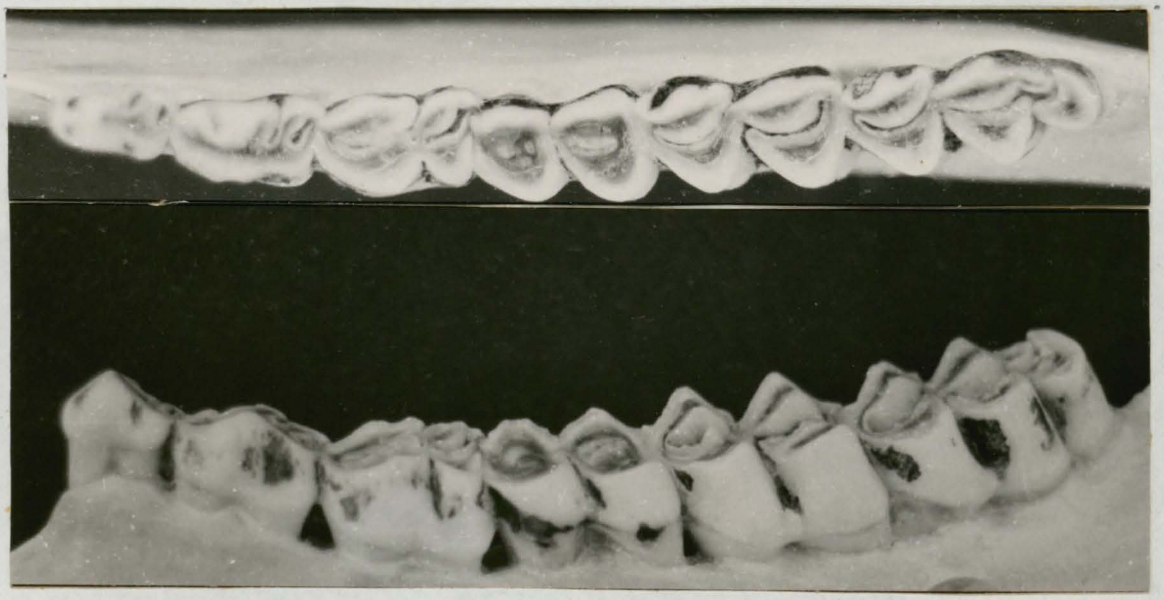
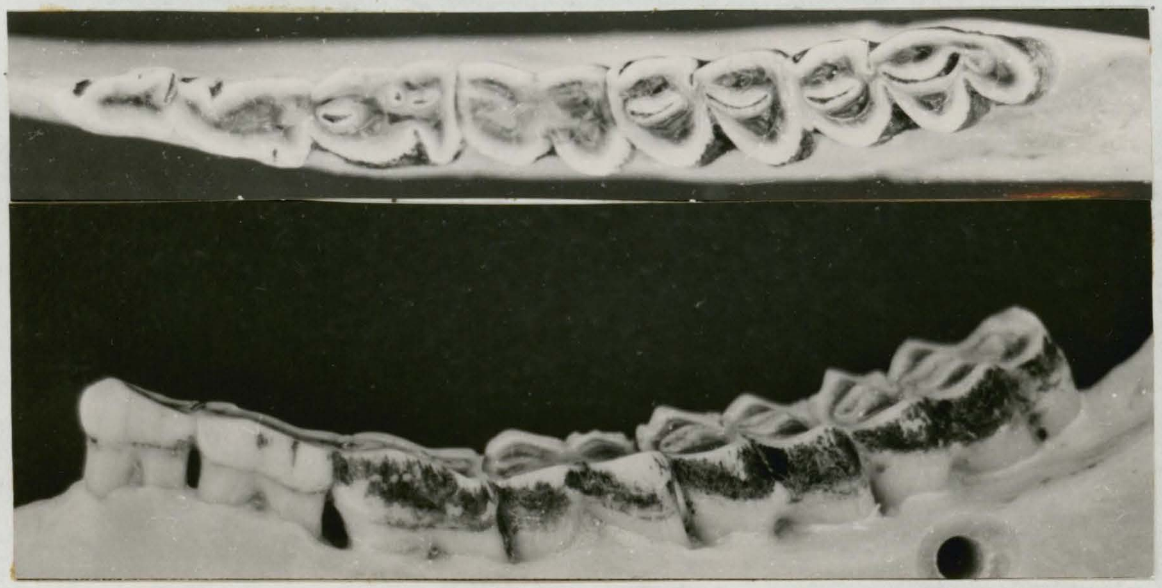


PLATE 9 - Infundibulum greatly reduced on third premolar and reduced on all molars. Third premolar and first molar usually flat and smooth above. Dentine usually joined in all teeth. Pulp cavities may in some cases begin to become exposed. Lingual crests worn, sometimes missing in second molar but present in third molar. Wear on all premolars and molar teeth has worn buccal and lingual crests smooth. Age-class: 8 1/2 to 9 1/2.

PLATE 10 - Heavy wear characteristic of all teeth. No lingual crest present although there may be some evidence of their presence on the third molar. Molar teeth frequently hollowed-out or entirely flat. Some shrinkage of bone around the roots of the teeth may have occurred. Pulp cavities exposed and the infundibulum is missing from most teeth. Age-class: 10 1/2 to 12 1/2.





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