A STUDY OF THE GROWTH OF THE LENS IN RELATION TO AGE IN FOX SQUIRRELS

Thesis for the Dogree of M. S.
MICHIGAN STATE UNIVERSITY
Donald M. Beale
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A STUDY OF THE GROWTH OF THE LENS IN RELATION TO AGE IN FOX SQUIRRELS

bу

Donald M. Beale

AN ABSTRACT OF A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Zoology

Approved Solar Balan

ABSTRACT

A STUDY OF THE GROWTH OF THE LENS IN RELATION TO AGE IN FOX SQUIRRELS by Donald M. Beale

This thesis reports an investigation of the use of the eye-lens of the fox squirrel, Sciurus niger, to establish, if possible, a correlation between lens-weight and age. The study was based chiefly on eye lenses obtained from 167 fox squirrels taken in 1959 and 8 in 1960 by hunters on the Rose Lake Wildlife Experiment Station near Lansing, Michigan. Eye-lenses from 5 nestling fox squirrels, obtained from Michigan State University woodlots, also were studied.

Fox squirrels tagged while juveniles by biologists at the Rose Lake Wildlife Experiment Station and taken subsequently by hunters furnished eye-lenses of knownage individuals for this study. In addition, age-groups classified from X-ray photographs of forelegs were used for comparison with data obtained from lens-weight.

The fox squirrel eye-lenses were dried for 48 hours at 80°C. and then weighed to the nearest tenth of a milligram. It was found that juvenile fox squirrels

could be positively distinguished from adults by differences in lens-weight. Juvenile fox squirrels can be further differentiated into first litter or spring-born young and second litter or summer-born young. The adult fox squirrels can possibly be separated into yearly age-classes up to at least $3\frac{1}{2}$ years.

This method of determining age in fox squirrels probably has greater accuracy and wider range of use than any technique previously suggested in the literature.

The fact that classifications are determined from numerical values makes the method more exact and less subject to error than techniques which depend largely on the subjective judgment of biologists.

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Also a note of thanks to the Department of Animal Husbandry at Michigan State University for the facilities provided to carry out this research.

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INTRODUCTION

The objective of this study was to investigate the possibilities of a correlation between the weight of the lens and age in the fox squirrel, <u>Sciurus niger</u>. Such a correlation, if found, might prove highly useful in deriving a simple technique for accurately determining ages of examples of this important game species.

The ability to determine the age of individual animals is of primary importance in the management of any game species. Petrides (1949) discussed the importance of the application of data on age-ratios obtained from the hunter's kill. Some of the measurements that can be made, as stated by Petrides, are estimates of rearing success, juvenile mortality, peaks of breeding and mating, hunting pressure, average longevity, age-group size in older animals, and turnover periods. In his discussion, Petrides further stressed the need and importance of a high degree of accuracy in obtaining data on age-ratios. niques now in use for determining the age of fox squirrels during the hunting season are not sufficiently accurate. Allen (1943:123,127) and Unlig (1955:71) pointed out that fluctuations of squirrel populations are closely related to the proportions of adults and juveniles in that

population. If greater accuracy and ease could be obtained in distinguishing between age-classes of fox squirrels a substantial contribution would be made.

Lord (1959) first related eye-lens growth to age as a technique for determining age in the cottontail rabbit. Most of his information came from known-age animals raised in captivity and killed for examination at various intervals. The difficulties of obtaining and raising young fox squirrels as well as the time required made this approach prohibitive.

Most data for this investigation came from 167 fox squirrels killed by hunters in Clinton and Shiawassee counties on the Rose Lake Wildlife Experiment Station of the Michigan Department of Conservation during the 1959 small game hunting season. Eight known-age specimens were also taken during the 1960 season. Data for young squirrels also were obtained from 5 nestlings taken from nests in Michigan State University woodlots. In addition to eyelenses, a foreleg of each squirrel studied was saved, making possible a comparison of age-classification by both lensweight and degree of ossification (as shown by X-ray photographs) of the bones of the forelegs.

Evidence from this study proves that, by lensweight, juvenile fox squirrels (young-of-the-year) can be distinguished from adults. Also evidence indicates that

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spring or first-litter juveniles can be distinguished by lens weight, from summer or second-litter juveniles, and that adults probably can be segregated into yearly agegroups.

REVIEW OF LITERATURE

Determining the age of fox squirrels was first attempted by observing the development of the external sexual characteristics, chiefly the teats in females and the scrotum in males (see description in Allen, 1943:123-126). Brown and Yeager (1945:459-463) provided supplementary criteria concerning the development of the Cowper's gland in males, the size of the uterus in females, and characteristics of body length and tail pelage for both sexes. of these authors pointed out weakness of their methods and emphasized the need for a more reliable technique. mining age in males by examining the reproductive organs is difficult because of seasonal changes in the external sexual structures. After the mating season, the testes shrink and may retract into the abdomen. The dark skin of the scrotum is shed. (Allen, 1943:125). In this nonbreeding condition an adult male can easily be mistaken for a well-developed juvenile.

Mossman, Hoffman, and Charles (1955), in a study of the accessory glands and testes of males of both fox and gray squirrels, found that seasonal variations of the Cowper's gland in size and weight were more than 100 per cent. The variation of the testes in size and weight was

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more than 50 per cent. These authors (op. cit.:259) further stated that "...both the testis and accessories obtain a greater infinitive size with each recurring rut particularly the first few years of life; and the organ of a late prepubertal male may equal in size those of an adult approaching a second or third rut, or an older male in some stage of regression after the rutting season." In a study by Kirkpatrick (1955) in which observations of the testes of male fox squirrel were made at all seasons and were classified as infantile, prepubertal, functional, degenerating, and redeveloping, it was discovered from trapped specimens that individuals in the functional stage could be found in any month except August and infantile stages in all months except March. This is further indication of the difficulties that are met when attempting to separate fox squirrels into age-groups by development of sex characteristics.

A method for aging subadult squirrels has been worked out using body weight and development of the eyes, ears and teeth. Shorton (1951) used such criteria in establishing a key for aging gray squirrels from birth to seven weeks of age. Uhlig (1955) also found changes in these features with age and expanded the age-key, relying mostly on molars, from birth to about 14 weeks of age.

Brown and Yeager (1945) developed a key for aging subadult

fox squirrels up to eight weeks based on the same criteria. Probably further study of the cheek teeth of fox squirrels would provide enough information to extend the latter agekey to individuals at least as old as 14 weeks, as was done in the case of the gray squirrel by Uhlig.

In recent years, the use of X-rays to disclose the degree of ossification of the epiphyseal cartilages of the long bones have been used to determine age-groups of small mammals. Thomsen and Mostensen (1946) were first to use bone growth in cottontail rabbits as a method of separating different age categories. Hale (1949) developed this method still further and determined the approximate period of post-natal life in which the method was successful. Petrides (1951) used this method for separating young from the adults in fox squirrels, particularly those in hunters' kill. For several years biologists at the Rose Lake Experiment Station of the Michigan Department of Conservation have employed this technique for determining the age of fox squirrels taken by hunters.

Determining the age (or age-groups) of animals by studying X-rays of the long bones depends largely on the degree of cartilage displaced by bone in the epiphyseal plate. However, biologists at Rose Lake also use other characteristics such as the shape and texture of the leg bones near the epiphysis, and the "shade" of the X-ray

image which varies depending on the density of the calcium deposition in the long bones. By using these criteria, limited success has been achieved in dividing first-year fox squirrels, from the hunters' kill, into two age-groups corresponding to the major birth periods, in spring and in summer.

Examination of bones by X-ray as an aging technique has several limitations; first of all once the displacement of cartilage by bone is complete further age classifications by this method are difficult or impossible. In other words, animals can be defined as adults when ossification is complete, but cannot be segregated into yearclasses. A second limitation of the X-ray technique is the difficulty obtained when attempting to determine the approximate date of birth of first-year or juvenile animals. In Michigan fox squirrels, most young are born either in early spring or in late summer (Allen, 1943). To separate these approximate age-groups by the X-ray technique is often uncertain even with assistance of a good comparative collection of X-ray photographs of bones of known-age individuals. Two technicians might not make exactly the same age classifications; in fact, a person might not separate a group of juvenile specimens exactly the same a second time.

LENS STRUCTURE

The eye is formed in early embryonic life from both ectodermal and mesodermal tissue. Development starts with an outgrowth of neural ectoderm, the optic vesicle, which presses against the layer of embryonic surface ectoderm. The tissue that later forms the lens is the surface ectoderm (Mann, 1950).

Structurally, the lens of the mammalian eye consists of a nucleus and cortex formed from lens fibers, covered on the anterior surface by a layer of epithelium, and surrounded by a tough, elastic capsule. According to Pirie and Heyninger (1956:30), the capsule is a non-cellular membrane thought to be secreted by the lens epithelium. The growth and elongation of the epithelial cells, beginning at the lens equator and extending toward the anterior and posterior poles, result in the formation of the lens New lens fibers are laid down throughout life, fibers. and as the central portion, which corresponds to the keratin layer of the skin, cannot be shed, the lens continues to grow (Wolff, 1955). The older cells become even more compressed in the center of the lens by the young cells at the periphery which in their turn become covered with

freshly formed lens fibers and are squeezed towards the so-called nucleus of the lens. This growth of lens fibers throughout life, within the capsule, leads to a gradual hardening of the lens nucleus and an increase in density and volume of the lens.

On both the anterior and posterior surfaces, sutures are formed by the meeting lens fibers. The pattern of the sutures may vary but in the fox squirrel a three-way or star-type pattern is usually observed. The suture pattern on the anterior surface normally is turned 60° as compared to the posterior surface. These sutures are easily visible to the naked eye.

The lens is located within the eye just behind the iris. A series of fibers, the zonule of Zinn, passing from the ciliary body to the lens, holds the lens in position and enables the ciliary muscles to act on it producing accommodation (Wolff, 1955). The vitreous humour is a colorless, transparent, and gelatinous mass which is slightly adherent to the lens. However, it does not prevent the lens from being removed with its capsule.

COMPOSITION OF THE LENS

Water is a major constituent of the lens. Different investigators report that in mammals water comprises
from 60 to 75 per cent of the total lens weight. This
water exists in both free and bound states. As stated by
Bellows (1944:148), the free water but not the bound water
may be removed by normal dehydration processes. Bellows
also determined that there is no sharp line of demarcation
between free and bound water, and that only an arbitrary
separation can be made.

Work with fox squirrel lenses also indicates that a distinct separation between free and bound water cannot be made. Even after lenses were dried for 10 days, a small amount of moisture loss continued. With increasing age, according to Bellows, the percentage of water tends to decrease, the change resulting largely from the increased density of the nucleus.

METHODS

The methods used in the study reported herein are similar in many respects to those used by Lord (1959). Several changes were made, however, in order that techniques used to obtain my data could be more easily repeated and might lead to a standardized method for determining the age of fox squirrels.

The bulk of the data was obtained from fox squirrels taken by hunters at the Rose Lake Wildlife Experiment
Station during the small game hunting seasons (October and
November) of 1959 and 1960. Hunters are required to bring
to the Station for examination all game animals taken on
the area during their hunts. For each fox squirrel, one
foreleg and both eyes were removed. In addition, all animals were weighed, aged by external sex characteristics
(mainly by visual examination of teats of females and scrota
of males), measured (total length and tail length), and
examined for ear tags.

The most ideal procedure for developing an aging technique is to have a large number of animals of known age for each important age-group. When working with wild animals this is often difficult, time consuming and, of course, costly. Consequently, it was indeed fortunate

that for a number of years, fox squirrels have been livetrapped, examined for age characteristics, tagged and released at Rose Lake Experiment Station prior to the hunting
season to provide data, when taken by hunters, for population studies. Some of these animals, when handled in the
pre-hunting period could be aged, with little chance of
error, as young of the year, and in some instances the
birth period (spring or summer) in which they were born
could be determined. Six of these marked animals examined
in the hunters' kill during the 1959 season and eight during
the 1960 season provided known-age individuals which were
used to help establish a growth-rate curve.

To determine the correlation of lens-weight with age, it is important, or at least helpful, to have lenses from very young animals as well as those of the age generally killed by hunters. To obtain these lenses, five nestling fox squirrels were taken from nests in woodlots at Michigan State University. Field estimates of the ages of these young squirrels were obtained from inspecting such physical features as incisor growth, condition of eyes and ears, pelage, and body weight, following suggestions of Brown and Yeager (1945:486).

Comparative data were obtained from the X-rays taken of the forelegs collected from each specimen. These data provided an excellent opportunity to compare the results

of aging using the lenses and aging as determined by X-raying the forelegs.

The eyes of each fox squirrel were placed in a vial containing a fixative, 10 per cent formalin (as used by Lord, 1959), and labeled with the specimen's autopsy number. The forelegs were trimmed and fitted on a card for X-ray.

Eye lenses placed in the fixative become increasingly more firm and are much easier to work with than unfixed, fresh lenses. When the lens was taken from the eye, a certain amount of the vitreous humour often adhered to the lens. This material and any iris pigment which may have also adhered to the lens were removed by gently rolling the lens on a paper towel. Care was taken with each lens not to damage or peel the capsule, especially on the thin posterior surface. After cleaning, the lens was then placed in a vial for the drying process.

The vial used for drying was a blood sample tube (1/2" by 4") with a frosted label area. Each lens could be easily dried and stored separately in case further weighings would be necessary. A test tube rack provided an easy and efficient means of handling the lenses while drying. Before the vials were used, a test was conducted to determine the effect that they might have on drying of the lenses. The initial rate of moisture loss of the lens was less

in a vial than in an open dish with the result that the rate of loss with lenses in vials did not taper off so soon as did the rate for those in dishes. After 24 hours of drying lenses processed by either method had lost comparable amounts of moisture.

All of the lenses were dried in a forced hot air oven and the temperature, as suggested by Lord (1959), was maintained at 80° C. Each lens was weighed after 24, 48 and 72 hours, respectively. Two weight readings were taken for each lens. All lenses were weighed on an electrical balance to the nearest tenth of a milligram.

and, consequently, absorbs moisture from the atmosphere when taken out of the dryer, at a rate of roughly 0.1 to 0.2 milligrams per fifteen minutes. To prevent an increase in weight due to absorbed atmospheric moisture, the dried lenses, still in vials, were kept in a desiccator until weighed.

To determine the optimum drying time and rate of moisture loss, a number of lenses were placed in the oven and weighed periodically. Figure 1 shows graphically the results of this experimental drying. In the first few hours, moisture is lost rapidly and then the rate of loss tapers off. After 40 to 48 hours the rate of moisture loss is extremely low. Twelve lenses kept in the oven for

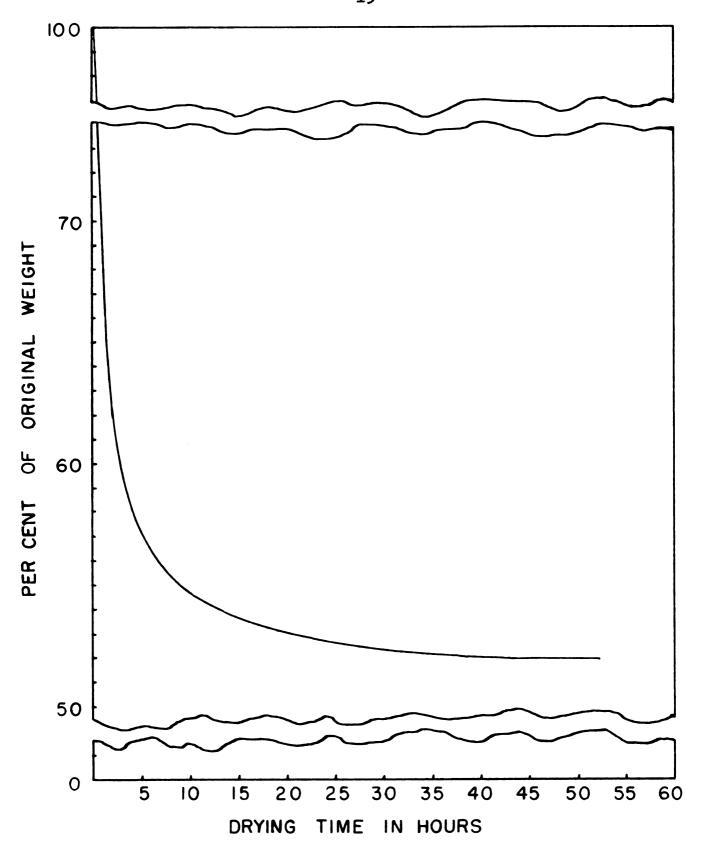


FIG.-1 Rate of weight loss of lenses when dried at 80° C.

ten days and weighed at intervals of 24 hours lost an average of 0.088 milligrams per day after the first 48 hours. Because the rate of weight loss was almost constant and the total amount of loss was slight after 48 hours, a 48-hour drying time was adopted as standard for use in this study.

Occasionally small fractures appear in lenses while they are drying. These fractures neither occur in all lenses of the same weight nor occur always in both lenses from the same animal, even though they are handled identically. When fractures do develop the loss of moisture is more rapid for a short time than in non-fractured lenses. After lenses have dried for about 40 hours, usually no additional fractures occur. Since an unfractured lens and a fractured lens from the same squirrel had approximately the same weights after the 48-hour drying period, it is believed that fracturing had no adverse effects on the final weights.

RESULTS

All of the dried weights of fox squirrel lenses obtained from 167 specimens at Rose Lake during the 1959 small-game season are listed in the Appendix along with the age classification for each squirrel as determined by X-ray photographs of the forelegs. The listing is in order of the date of kill and is by the autopsy number given to each specimen. Where both right and left lenses were available, the average weight is given. The data from known-age individuals, both from 1959 and 1960 hunting seasons, are presented in Table 1. The data from nestling squirrels are presented in Table 2.

The histogram, Figure 2, shows all of the squirrel lenses taken during the hunting season of 1959 arranged in weight-groups of 1.0 milligrams. The peaks in the histogram represent different birth periods. Lenses weighing 41 milligrams or more, presumably represent the adults. Each high in this range starting with 41 milligrams and from left to right in Figure 2 represent lenses of adults believed to have been born in 1958, 1957, and 1956 respectively. Lenses weighing less than 40 milligrams are from animals believed to be juveniles. The low points, probably, result from time-periods of few or no births; for the first

Table 1. The dry weight of lenses of known-age fox squirrels taken in the hunting seasons of 1959 and 1960.

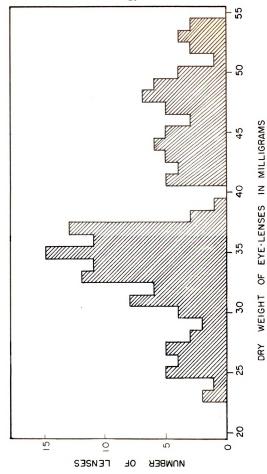
Autopsy number	Ear tag number	Age* in weeks	Lens-weight in milligrams
19243	32732	62+4	41.1
20396	19064	62 <u>+</u> 4	41.2
20642	19374	62 <u>+</u> 4	41.5
19147	32744	<u>-</u> 58+	46.8
19061	38250	82 <u>+</u> 4	46.0
20483	19379	82 <u>+</u> 4	42.7
20493	38333	82 <u>+</u> 4	47.6
20573	19392	82 <u>+</u> 4	46.6
20637	32941	134 <u>+</u> 4	48.8
19363	32840	162+	54.4
19162	32372	186 <u>+</u> 4	53.7
19392	32113	186 <u>+</u> 4	52.0
19563	32124	186 <u>+</u> 4	53.3
20619	32838	186 <u>+</u> 4	50.6

^{*}Ages of tagged squirrels were estimated as being either adult or juvenile at time of tagging and releasing by biologist at Rose Lake Experiment Station. From these data for first-year animals I have estimated as to whether they were first litter (spring-born) or second litter (summer born) chiefly by body weight.

Table 2. The dry weight of lenses of known-age nestling fox squirrels.

Number	Age in weeks	Lens-weight in milligrams
1	3	5•9
2	4	7•3
3	4	7.9
5	5	12.2
4	6	13.5





Weight distribution of Michigan fox squirrel eye-lenses collected in autumn. F16.-2

year the low represents the period between the spring and late summer breeding peaks. Subsequent low points (as modified by variations in lens growth) between adult agegroups probably represent autumn and winter periods when no births occurred.

The picture presented by the histogram in Figure 2 assumes that all squirrels were killed on exactly the same date which, of course, is not the case. The first day of the open season was October 20 and the last day was November 10. The majority of the souirrels taken were killed in the first week but some also were taken in the latter part of the season. Even in this short period of 21 days, lenses of young squirrels may grow measurable amounts and at different rates depending on age. (1959) and Bellows (1944:70) show that the growth rate of lenses in juveniles is considerably greater than that of lenses in adults. In the fox squirrel the eye-lens attains a weight of about 37 milligrams by the time the animal is 34 weeks of age. To make this gain an average daily increment of about 0.15 milligrams is required. Therefore, if the growth-rate of the lens is most rapid in the young fox squirrel one would expect an increase of more than 0.15 milligrams per day, at least for the first few weeks of life. The lens-weights from nestling fox squirrel indicate an increase during the first six or seven weeks of about 0.28 milligrams per day.

In order to present a more accurate picture of the different birth periods and the proportion of individuals making up these groups in the hunters' kill, it is necessary that a correction be used to offset the difference in dates on which the young animals examined were killed. A correction is not needed for lenses from adult animals in such a histogram, because the amount of increase in lens-weight in these older animals over the 21-day hunting season is negligible.

To show in a graphic fashion the rate of lensgrowth with age and also to assist in making the corrections for lens-weights representing young animals, an estimated growth-rate curve, Figure 3, was constructed. The
weights are those of lenses dried for 48 hours at 80° C.
Points for the curve are taken largely from the weights
of lenses of the known-age specimens; however, the point
representing the lens-weight of fox squirrels when 31 weeks
of age was estimated by other means. It is fairly well
established by Allen (1943) that the peak in litter-production of spring-born fox squirrels in Michigan is during
the second week of March. Therefore, on the first day
of the hunting season, October 20, the average juveniles
from the spring litters would be approximately 31 weeks
of age. The average of lens-weights which make up the

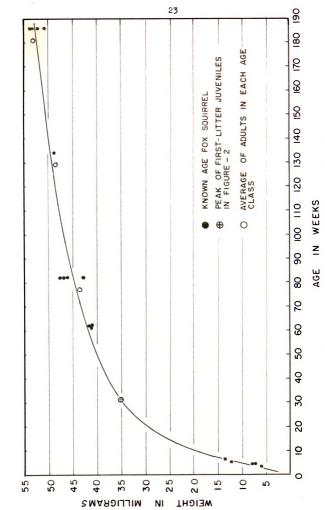
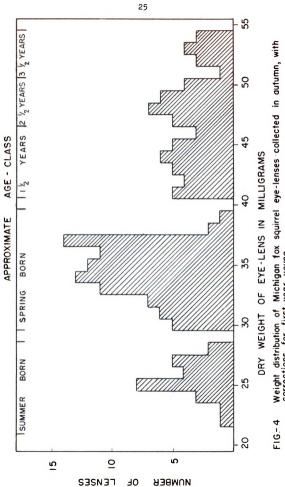


FIG.-3 Growth-rate of the eye lens of the fox squirrel.

major peak of the juvenile lenses in Figure 2 is approximately 35 milligrams. This figure was used to establish the 31-week point on the curve shown in Figure 3. Other points plotted on the curve were estimated by averaging the lens-weights for groups making up the approximate $1\frac{1}{2}$, $2\frac{1}{2}$ and $3\frac{1}{2}$ year age-classes. The average lens-weights making up these adult year-classes are undoubtedly from squirrels born in both spring and summer. In plotting these points it was assumed that the proportion of spring- and of summerborn in each of these year-classes was the same as the proportion of spring- and summer-born young making up the juveniles in the hunters' kill.

The histogram (Figure 4) represents the same specimens as shown in Figure 2; only the lens-weight of young of the year are correct in accordance with the growth curve, so that they approximate a kill on one date, namely October 20. This correction permits the first and second litters to be shown graphically with a more accurate representation in proportion of each group. The first high on the left in Figure 4 includes the lightest lenses and presumably from those animals born in summer, or second-litter juveniles. The second high represents lenses from those born in spring, or first-litter juveniles. The rest of the histogram is the same as in Figure 2 and represents the lenses of adults. The scale above the histogram in



Weight distribution of Michigan fox squirrel eye-lenses collected in autumn, with corrections for first-year young.

Figure 4 shows the approximate age-classes of juveniles and adults represented by each lens-weight. Any overlap in adult age-classes is not taken into account here.

The two sample means using eye-lenses from knownage animals in the $1\frac{1}{2}$ year age-class and in the $3\frac{1}{2}$ year age-class can be differentiated statistically at the 5 per cent level. Enough examples of known-age squirrels in the 2½ year age-class were not available to make a statistical test, but the lenses from the one that was obtained weighed exactly what would be expected. Variability of lenses of known-age fox squirrel were such that one would expect the 2½ year age-class to dovetail with the upper extreme of the $1\frac{1}{2}$ year age-class and the lower extreme of the 3½ year age-class. It is possible that squirrels more than $3\frac{1}{2}$ years of age would have lens weights only slightly heavier than those of the $3\frac{1}{2}$ year age-class and, perhaps would not be readily distinguishable. If the dry lens-weight is plotted against the log of time (a relationship very close to that observed) a weight of about 55 milligrams is intersected at the 4½ year point.

For further test of the accuracy of age and lens-weight relationships, a comparison of age classifications determined by X-rays was made with those determined by lens-weight. In this comparison there was 100 per cent agreement in separating juveniles from adults. There were

discrepancies between the two methods, in separating springand summer-born young, but all disagreements were among
borderline cases with respect to the X-ray classification.
With all of these cases the body weight of each animal
in question was much closer to that expected of a fox squirrel of the age estimated by lens-weight, than of the age
estimated by X-ray. In no case was there any disagreement
in classification, among individuals of early spring litters or late summer litters.

Another point suggesting the possible precision with which eye-lenses can be processed and, therefore, related to the accuracy that may be achieved in such a technique is the minute variation in weights of right and left lenses when dried. The mean difference and 95 per cent confidence limits between right and left lenses was 0.177 ± 0.038 milligram.

The greatest accuracy of the eye-lens method of determining age evidently is during the first 10 months after birth. The rapid increase in the weight of the eye-lens at this time is shown in Figure 3. Data obtained from nestling fox squirrels show that the rate of increase in lens-weight is sufficiently great during this time (about 0.28 milligram per day) to produce a distinct difference in weight within a week's time.

DISCUSSION

This study shows that the weight of the eye-lens apparently can be useful in determining the age of fox squirrels and seemingly permits greater precision than any other method now available. If standard procedures are followed there should be little difference in results obtained by different workers. Although the greatest accuracy using this method evidently is obtained during the first ten months of age when the rate of weight-change is highest, this method also should be useful for separating annual age-groups up to $3\frac{1}{2}$ years with reasonable accuracy.

This study did not disclose completely the extent of variation to be expected in the weights of eye-lenses of the fox squirrel of given ages. Variation observed in known-age specimens in the $1\frac{1}{2}$ and $3\frac{1}{2}$ year age classes was large enough that overlap with the $2\frac{1}{2}$ year age class would be expected. Even though there was no overlap in the known-age specimens examined probably a larger sample, particularly in the $2\frac{1}{2}$ year age-class, would show that some would exist. Absence of complete breaks between age-classes in the distribution of lens-weights of adults (Figures 2 and 4) suggests that overlap does exist.

Probably the strongest support for the use of

the eye-lens in an aging technique comes from the comparison made of X-rays of forelegs and lens-weight in an attempt to separate juveniles from adults. It seems highly improbable, if there were any significant errors in either method in making this age separation, that complete agreement would be obtained.

Correlation of lens-weight with age has contributed to the methods of determining the age of fox squirrel by providing a technique which can be used without highly specialized equipment and training and furthermore, one that can probably be applied equally well any time of the year. When used to separate juvenile fox squirrels from adults the accuracy achieved is equal to that obtained with X-rays of forelegs.

Using lens-weights in fox squirrels, it should be ultimately possible to determine age to the week or month in juveniles and to yearly age-classes in adults, rather than having to separate the animals into broad cate-gories of juveniles and adults. In order to develop further accuracy in this method, several steps are suggested. First of all more lens-weights are needed of young animals taken during the time when the lens is growing most rapidly (between birth and at least 25 weeks of age). More information here would increase the accuracy in determining the age of subadults and would help establish, more accurately,

the time of year and extent of birth periods as well as detect any yearly variation in these birth periods. Possibly the best way to accomplish this would be to raise captive young obtained from nests, and sacrifice them at different ages to obtain the lens-weights. A second area which needs further investigation concerns the amount of variation in lens-weights of adult fox squirrels of a given age, particularly in the $1\frac{1}{2}$ and $2\frac{1}{2}$ year age-classes.

SUMMARY AND CONCLUSIONS

This study provides evidence that the dry weight of the eye-lens can be used to determine age in fox squir-rels. Lenses from 180 fox squirrels from southern Michigan were oven-dried at 80°C. for 48 hours and weighed. A growth-rate curve was prepared using the lenses, which included 19 from known-age squirrels.

This method of age-determination proved to be as reliable in separating juveniles (young-of-the-year) from adults as X-ray pictures showing the degree of ossification of the forelegs. Also, the second-litter juveniles (summer-born) could be distinguished from first-litter (spring-born) juveniles by the lens-weight. Because the growth-rate of the lens is most rapid during the first 10 months after birth, age determination is most accurate at this time, although adult animals can possibly be separated into yearly age-classes up through $3\frac{1}{2}$ years of age.

Arranging lens-weights from fox squirrel taken by hunters in 1959 in a histogram revealed that lens-weights of summer-born animals ranged from 22 to 28 milligrams; and for spring-born animals, 30 to 39 milligrams. Adult age groups are not so clearly separated but without considering overlap the lens-weights for lag year-old animals ranged

from 41 to approximately 46 milligrams; for $2\frac{1}{2}$ year-old animals, approximately 47 to 50 milligrams; and for $3\frac{1}{2}$ year-old animals, approximately 51 to 54 milligrams.

This technique should provide a means of studying, with greater accuracy and scope, the population structure and dynamics of fox squirrel, thus increasing our understanding of the biology of this important game species.

LITERATURE CITED

- Allen, Durward L.
 1943. Michigan fox squirrel management. Mich.
 Dept. Cons., Game Div., Pub. 100, Lansing, 404
 pp.
- Bellows, John G.
 1944. Cataract and anomalies of the lens. The
 C. V. Mosby Company, St. Louis, 624 pp.
- Brown, Louis G., and Lee E. Yeager
 1945. Fox and gray squirrels in Illinois. Ill.
 Nat. Hist. Surv. 23 (5):449-532.
- Hale, James B.
 1949. Aging cottontail rabbits by bone growth.
 Journ. Wildl. Mgt. 13 (2):216-225.
- Kirkpatrick, Charles M.
 1955. The testis of the fox squirrel in relation to age and seasons. Am. Journ. Anatomy 97 (2): 229-255.
- Lord, Rexford D.
 1959. The lens as an indicator of age in cotton-tail rabbits. Journ. Wildl. Mgt. 23 (3):358-360.
- Mann, Ida

 1950. The development of the human eye. (Second edition) Grune and Stratton, Inc., New York, 312 pp.
- Mossman, H. W., R. A. Hoffman and C. M. Kirkpatrick 1955. The accessory genital glands of male gray and fox squirrels correlated with age and reproductive cycles. Am. Journ. Anatomy 97 (2):257-301.
- Pirie, Antoinette, and Ruth Van Heyningen 1956. Biochemistry of the eye. Charles C. Thomas, publisher, Springfield, Illinois, 323 pp.

Petrides, George A.

1949. Viewpoints on the analysis of open season sex and age ratios. Trans. N. Am. Wildl. Conf. 14:391-410.

1951. Notes on age determination in squirrels. Journ. Mammalogy 32 (1):111-112.

Shorton, Monica

1951. Some aspects of the biology of the gray squirrel (Sciurus carolinensis) in Great Britain. Proc. Zool. Soc. Lond. 121 (LLL):427-459.

Thomsen, Hans P., and Otto A. Mortensen 1946. Bone growth as an age criterion in the cottontail rabbit. Journ. Wildl. Mgt. 10 (2): 171-174.

Uhlig. Hans G.

1955. The gray squirrel, its life history, ecology, and population characteristics in West Virginia. Final report, W. Va. Federal Aid Proj. 31-R, 181 pp.

Wolff, Eugene

1955. The anatomy of the eye and orbit. (Fourth edition) The Blakiston Division, McGraw-Hill Book Company, Inc., New York, 491 pp.

Appendix. The dry weight of lenses and X-ray age classification of fox squirrels examined at Rose Lake Experiment Station in 1959.

Autopsy number	Lens- weight in mil-	X-ray classifi- cation	Autopsy number	Lens- weight in mil-	X-ray classifi- cation
	ligrams			ligrams	
19030 190331 190336 190036 190036 190048 190058 190059 190059 19005 1900	466.26.25.586.58220.521.96.31.47.56.75.47.97.32.75.2 474.33.33.43.43.25.33.33.33.33.33.33.33.33.33.33.33.33.33	adjuuadjuuadjuuadjuuadjiuuadjiuujiuujiuujiuujiuujiuu	19128 19129 19132 19133 19147 19148 19149 19154 19155 19164 19177 19179 19188 19191	98484089675975876309155975016925684 634.84089675975876309155975016925684 443333335344425323422233554	juju.addju.addju.addju.addju.addju.addju.addju.addji.u.addji.u.addji.u.addji.u.addji.u.add

19218 19218 19219 192222 192222 192222 192222 192223 192224 192224 19222	50305170990580104103493273043783765841872 710.005170990580104103493273043783765841872 733544763833344334333324233355433333254422332	juddduduudduuduuuudduuuuuddduuuuuuuuddduuuu	1936600 193366600 199373333333333333333333333333333333333	21432026306774035920461944951114770539599 4354334455354234332434232443444432323242333	audduudduudduudduuudduudddadduuuuuduuud
19308 19309 19311 19312	26.1 24.8 30.7 23.2	ju. ju. ju. ju.	19539 19540 19550 19551 19554	49.3 29.9 34.5 32.9 34.9	ad. ju. ju. ju. ju.
19316 19322 19337 19339 19340 19341	41.0 25.6 48.7 32.8 25.4 47.6	ad. ju. ad. ju. ju.	19556 19557 19559 19561 19563	34.3 35.2 44.6 27.9 53.3	ju. ju. ad. ju. ad.
19342 19349	35.1 36.8	ad. ju. ju.	19564 19565	35.3 31.3	ju. ju.

