EVALUATION OF THE LENS WEIGHT AS AN AGING TECHNIQUE FOR THE COTTON RAT, *Sigmodon hispidus* Say and Ord.

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Submitted to the Faculty of the Graduate School of
the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
DOCTOR OF PHILOSOPHY
August, 1964

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ACKNOWLEDGMENTS

This investigation was supported by the Zoology Department, Oklahoma State University and the Oklahoma Academy of Science.

The project was carried out under the direction of my major advisor, Dr. B.P. Glass. Members of my advisory committee, Drs. F.M. Baumgartner, D.D. Dwyer, R.W. Jones, R.D. Morrison, and A.M. Stebler, gave aid throughout the study.

Many other persons assisted me. These include Dr. J.J. Crockett, Messrs. C. Bottrell, D.M.J. Mueller, J. Hart, J.W. Echols, L.J. Croy, and the late C. "Andy" Kinkead. Miss C. Diane Walker and Miss Sharon A. Lamb kindly typed early drafts of this dissertation. H. W. Reno aided in the preparation of figures.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

The cotton rat, Sigmodon hispidus Say and Ord, is found throughout the southern United States. According to several investigators, its populations are subject to rather large fluctuation in numbers. During periods of abundance, it may destroy enough eggs of the Bob-white Quail, Colinus virginianus, and other ground-nesting birds to seriously affect the population of these birds (Stoddard, 1931; Komarek, 1937). This mammal is a potential reservoir of diseases such as plague and typhus (Meyer and Meyer, 1944b). It also damages agricultural crops such as sugar cane (Dunaway and Kay, 1961 and 1964).

Because of the availability of cotton rats, several investigators have used this species in studies on the population dynamics of small mammals (Dunaway and Kaye, 1961 and 1962; Goertz, 1962; Odum, 1955; Sealander and Walker, 1955).

The age composition of wild populations of Sigmodon living in various cover types at different levels of abundance are not adequately known due to the lack of a valid aging technique which will apply to adults as well as to young animals.

Investigators studying Sigmodon have attempted to age them according to weight and sexual maturity. Meyer and Meyer (1944a and 1944b) found the rate of growth of captive animals to be fairly constant during the first 50 days of life. The rate of growth decreased somewhat between the ages of 50 and 100 days, then slowed greatly thereafter. Most of the weight gain was due to fat deposition. The age of sexual maturity (first descent of testes in male, breeding in female) was observed to be variable and often had been reached at the age of 50 days. Dunaway and Kaye (1964) concluded that body weight is not a useful index to cotton rat age, especially during cold weather. From these studies it can be seen that using weight and sexual maturity as criteria, the age of a wild individual cannot be determined with any degree of confidence after about 50 days. This leaves a large gap in the knowledge of the age structure of a cotton rat population. This gap assumes greater importance when it is noted that breeding adults may persist in wild populations until at least the age of eight to thirteen months (Goertz, 1962; Dunaway and Kaye, 1961).

In the absence of a better method for determining age, Sigmodon populations studied by Sealander and Walker (1955) and Odum (1955) were arbitrarily divided into three age classes on the basis of body weight. Age classes erected in the two studies did not coincide, however. Odum's age classes were non-breeding juveniles, less than 60 gm.; young adults, 60 to 110 gm.; and old adults, more than 110 gm. Odum felt that females over 100 gm. and males over 110 gm. were probably five months old or older. Sealander and Walker's age classes were: subadults of both sexes, 12 to 46 gm. (ten to 29 days old); adult males, 47 to 138 gm. (30 to 50 days old); adult females, 47 to 111 gm. (30 to 50 days old); old adult males, 139 to 258 gm. (51 to 250 or more days old); old adult females, 112 to 230 gm. (51 to 250 or more days old).

Komarek (1937) noticed a regular yearly drop in numbers in cotton rats in the springs of years 1934, 1935, 1936, and 1937. It is not known if this annual reduction was the result of reduced reproduction, an increased mortality in certain age classes, or a combination of these two factors.

Howell (1954) found heavier females (over 110 gm.) occupied territories in the best habitat while smaller females ranged in the marginal habitats. It is not known if the best territories were occupied by heavier females because these were mature, vigorous females in the prime of life who kept out the very young and very old; or if the better food supply allowed greater weights to be gained and maintained.

Odum (1955) observed that individual size tended to increase when total numbers were high. It is not known if this was because of nutritional factors or because of the relative numbers of rats in mature age classes associated with the different population levels.

Questions raised by the studies of Komarek, Howell, and Odum cannot be fully resolved until a valid aging technique has been developed. Sealander and Walker (1955) recorded a sex ratio of 120 males: 100 females for samples from wild Sigmodon population. This type of numerical imbalance, if real, will be better understood when the adult population can be aged as well as sexed.

A search of literature pertaining to aging techniques applicable to birds and mammals suggested that the weight of the eye lens might be directly correlated to age in several mammals even after they reach sexual maturity and attain adult body weight (Cane, 1962).

Priestly Smith (1883) found that the weight of the human lens increased even after sexual maturity was attained. Burdon-Cooper (1914) recorded the total weight of human lenses as about 100 mgm. in the newborn infant, about 160 mgm. in a ten-year old child, 220 mgm. in a 25 year old, 250 mgm. in a 65 year old, and about 260 mgm. in an 80 year old adult. He also discovered that the dry weight of the human lens is about one-third of the total weight of the undried lens.

Dr. S. Hatai in 1923 (Donaldson, 1924) found a direct correlation between age and lens weight in the albino laboratory rat, Rattus norvegicus albinus. The aqueous humor was removed by lightly rolling the lens over filter paper. Each pair of lenses were weighed together without first being dried. At birth the two lenses of a rat averaged 5.2 mgm. in weight. A 664 day old male rat weighing 460.8 gm. had lenses that weighed 130.3 mgm. each.

Krause (1934) determined the lens weights of known-age laboratory rabbits, Oryctolagus cuniculus. A positive correlation was found between age and lens weight. His study also indicated that the average weights of the lenses of the male and female of the same strain and age were approximately equal, although the average body weight of the female rabbit was greater than that of the male.

Lord (1959) developed this technique for aging wild cottontail rabbits, Sylvilagus floridanus. According to Sanderson (1961), Lord "found that lenses in wild cotton-tails grow at a slower rate than they do in captives." This finding is of importance since several aging techniques for use on wild populations have been developed on captive animals under the assumption that captivity did not alter the rate of growth or development of the aging characters (Kirkpatrick and Sowls, 1962; Severinghaus, 1949). The literature does contain other references to differences in the rate of development of wild and captive animals however, Foster and Peterson (1961) found that the skulls of laboratory-raised voles, Phenacomys ungava, do not develop exactly as do those raised in the wild.

Lord (1961) used lens weight as an indicator of age in the gray fox, Urocyon cinereoargenteus, and Sanderson (1961) used this technique to indicate age in the raccoon, Procyon lotor. The latter investigator found the lens growth curve of the raccoon to be similar to those of the gray fox and cottontail. For all three species the rate of lens-weight gain was rather constant with little variation among individuals of the same age until adult body size was reached. The rate of lens-weight increase slowed but did not stop after adulthood. This aging technique is apparently useful for adults of both the cottontail and the gray fox, but is not in the adult raccoon since in this species the rate of lens-weight increase is very low and the individual variation is too great to permit valid aging.

Sanderson (1961) castrated two female and three male raccoons. They were later sacrificed at various ages and their lenses were weighed. All lens-weights were typical of those of uncastrated animals. This study suggests that lens weight may be relatively independent of other factors acting on raccoons.

Martinson, et al (1961) obtained lens weights of swamp rabbits, Sylvilagus aquaticus, in Missouri. They concluded that lens weight was "... a valid criterion for distinguishing adult from first-year swamp rabbits." They also felt that the lens weight data tended to agree with results obtained from the use of the epiphyseal closure aging technique.

The Australian wild rabbit, Oryctolagus cuniculus, can be aged by the lens weight technique up to about 150 days after birth (Dudzinski and Mykytowycz, 1961). This aging technique gives more reliable results than does the technique using body weight that was developed by Southern (1940) and Dunnet (1956).

Lens weight as an aging technique was applied to the fox squirrel, *Sciurus niger*, by Beale (1962). He concluded that this technique is reliable for separating young-of-the-year from adults and speculated that adults possibly can be separated into yearly age classes up to at least the age of 2½ years.

Kolenosky and Miller (1962) determined the lens weight of hunter-killed pronghorn antelope, *Antilocapra americana*. The study animals were aged by an examination of their teeth. From their data they developed a growth curve for the lens.

Lens weights from 2,876 hunter-killed cottontail rabbits were collected in Ohio by Edwards (1962). He used Lord's findings (1959) to assign the collected rabbits to age classes, concluding that this was the best aging technique available.

Wight and Conaway (1962), in studying cottontail rabbit populations in Missouri "...concluded that the lens-weight age-determination technique is clearly superior to the X-ray technique." The X-ray technique was developed by Thomsen and Mortensen (1946).

The lens weight curve is not known to be valid for aging any bird except the House Sparrow, *Passer domesticus*, according to Payne (1961). He found it to be valid only up to the age of two months in this species. Payne cited the following investigators who tried this technique on three other avian species. R. D. Lord worked with the Ring-necked Pheasant, *Phasianus colchicus*; Howard Campbell worked with the Scaled Quail, *Callipepla squamata*; and Lois I. Bear worked with the Red-winged Blackbird, *Agelaius phoeniceus*. The lens weight curve was not useful in aging these latter species because the lens in these birds is developed and grows rapidly in very young birds, then almost stops growing. Further growth is masked by the wide individual variations in lens weight.

Lord (1962) felt that the lens technique might work with Sigmodon, since he had successfully applied this technique to the deer mouse, Peromyscus maniculatus.

It was decided to use captive, known-age cotton rats to determine a lens-weight growth curve for this species. The validity of applying such a curve, derived from captive animals, to animals from wild populations was to be checked by comparing the empirically-determined curve with data taken from known-age cotton rats raised in an outdoor enclosure under natural conditions. Samples from wild populations were to be taken, sexed, measured and aged by the lens-weight technique to determine any correlations of lens weight to body weight and/or body measurements.

CHAPTER II

METHODS AND MATERIALS

Captive Animals

Live wild *Sigmodon hispidus texianus* were trapped from three localities in Payne County, Oklahoma. These were on the south side of Lake Carl Blackwell, along the west edge of Boomer Lake, and at an old homesite five miles north and two miles west of Stillwater. None of these three localities were on or near the study areas where wild cotton rat populations were sampled.

Cotton rats were caught in Havahart metal livetraps using dry rolled oats for bait. Captured animals were sexed and toe-clipped to insure later identification of individuals. They were held in wire mesh cages that measured 8 x 8 x 11 inches. The caged animals were kept in an animal room in the basement of the Health Research Building on the campus of Oklahoma State University. The room was totally dark from between 8 and 10 P.M. until 7 A.M. Room temperatures varied from 72 degrees F. to 86 degrees F. Relative humidity varied from 12 to 50 percent. Temperature and relative humidity were variable because of the presence of a large autoclave in the animal room. While in use, usually during the day, the autoclave raised the room temperature and relative humidity appreciably.

Water, laboratory rat chow, and blocks of unpainted wood were provided in each cage. Captive rats spent much time, at night, gnawing the wood into shavings which fell through the bottom of the cages. Waste sweet corn, carrots, lettuce, cabbage, and other vegetables were obtained from a grocery store and used to supplement the diet for several months. Any benefits derived from this practice were not discernable.

Cotton rats are relatively easy to raise in captivity. Captive animals apparently adjusted to their caged existence since they grew, reproduced, and cared for their young. Their culture in captivity presented but two major problems. First, the animals continued to be very nervous even after several generations in captivity. Meyer and Meyer (1944b) also noted this in their work with the subspecies S. h. hispidus. In the present study, feeding and handling techniques were adapted to lessen the effects of this condition. Cages were only partially opened during feeding since rats in a completely opened cage often jumped out. Rats being handled for any reason were firmly held using heavy gloves. They were grasped by the skin on their shoulders. Occasionally an animal would be docile while it was being handled. It was hoped that this might be a heritable trait which could be selected for, but it was soon noted that an individual which was very docile one day would be quite nervous and apparently panic-stricken the next day. No individuals were found to be docile at all or even most of the time.

The second major problem encountered in raising cotton rats involved their strife with one another. Young rats from the same or different litters could be held in a cage until long after they had attained sexual maturity without their harming each other. However, newly-paired adult rats often fought until the male or female was killed. Meyer and Meyer (1944b) found that it was generally the female who killed the male, but the present study did not indicate that the female was any more prone to kill than she was to be killed.

Captive adults were mated by placing a male in a cage with a female. This cage was new to both animals since strife was greatest when an animal of either sex was placed in a cage familiar to its mate. One animal, either male or female, would occasionally kill its mate soon after they were paired, even when this precaution was taken.

Meyer and Meyer (1944b) insisted that a nest box was a necessity. Rats in the present study would not nest in a box but rather defecated and urinated in the boxes thereby creating an unsanitary condition in their cages. Therefore, nest boxes were removed.

Paired animals were left together until the female gave birth to a litter. The male was then removed from the cage and shredded paper towels were placed in a cage. The female further shredded this paper and used it to construct the nest.

Occasionally some or all of the young in newborn litters were killed by the sire and/or dam, or by the dam after the sire had been removed from the cage. Apparently the losses were greatest if the parent or parents happened to be exceptionally nervous at the time the litter was born.

Young cotton rats were weaned when three to five weeks of age. They were toe-clipped at the time of weaning and their dam was placed in a new cage with a male at this time.

Known-age cotton rats were sacrificed by being placed in a killing jar containing chloroform. Immediately after death, they were weighed, body measurements were taken, and the eyes were removed from the body.

The number of known-age cotton rats produced was limited only by shortages of cages, funds for the purchase of feed, and time for caring for the study animals.

Release and Recapture of Known-Age Sigmodon

Known-age Sigmodon were released in an enclosure (see Chapter III) in an attempt to determine if their lens weights increased at the same rate as the lens weights of captive animals. After being in the enclosure for a period of time, the rats were livetrapped, killed, and processed as were the captive rats.

Five known-age cotton rats were released in the enclosure on January 21, 1963. The release included a 111 day old male, a 110 day old male, a 101 day old female, a 96 day old female, and a 63 day old male. They were released into a pile of baled hay where grain sorghum and shelled corn had been placed. Livetraps baited with rolled oats were set in the enclosure during the period of June 18 to June 25, 1963. A total of 90 trapnights yielded no cotton rats. The cotton rats apparently died prior to June 18, disappearing before reproduction occurred.

Eight, 30 day old cotton rats (four females, four males) were released in the enclosure on June 28, 1963. Twenty livetraps were set in the enclosure during the period of August 31 through September 4, 1963. The released animals had apparently reproduced since 20 immature (six females, 14 males) cotton rats were captured. They were destroyed. Three pregnant female cotton rats from the June 28 release were captured, sacrificed, and processed.

Ten cotton rats were released in the enclosure on September 5, 1963. Nine (five females, four males) were 46 days old and one female was 45 days old. Livetraps were set in the enclosure during the period of October 20 through October 29, 1963. A total of 143 trapnights yielded 31 subadult unmarked cotton rats, one adult unmarked female cotton rat, and four (three pregnant females, one male) cotton rats from the September release. All trapped animals were removed from the enclosure.

The number of known-age cotton rats released in the enclosure was limited by the lack of sufficient study animals. Due to the low recovery rate of released animals and the need for adequate samples of captive animals, it was felt that additional known-age animals could not be expended in this phase of the study.

Processing the Eye Lenses

Eyeballs were removed, slit and placed in ten per cent formalin for a period of at least two weeks. Scissors were then used to open the eyeball and the lens was gently squeezed out into a Syracuse watch glass filled with water. The lens surface was then cleaned with a wet camel-hair brush. The latter operation was carried out with the aid of a binocular dissecting microscope.

The cleaned lenses were placed in 95 per cent ethyl alcohol for 24 hours. The alcohol was then poured off and the lenses were allowed to air-dry in an open vial. The reason for placing the wet lenses in alcohol was to reduce the incidence of splitting and flaking off of the surface layers as they dried. It was felt, that the alcohol would displace the formalin solution in the lenses. Exposed to the air, the wet lenses would give off the alcohol more quickly than they would have given off the formalin solution. This would tend to reduce the opportunity for the lens surface to dry and shrink while the interior portion was still distended by moisture.

The air-dried lenses were then placed in Exax (Kimble 15146) weighing bottles and oven-dried for 48 hours at 67 to 70 degrees centigrade. The oven was a Thelco Model 2 manufactured by the Precision Scientific

Company. The weighing bottles were capped inside the oven, then weighed on a Mettler Balance Type H5 or H16. After recording the gross weight, the tare weight was obtained by removing the lens from the bottle and reweighing the bottle. The net lens weight was then determined by subtracting the tare weight from the gross weight.

Only one lens from each animal was weighed on the assumption that there was no significant difference between the weight of the two lenses. This procedure allowed the selection of the best-preserved and cleanest lenses for weighing.

Weights and Measurements of Sigmodon

Body weight in grams was determined. A Toledo Scale, Style 4606AU or an Ohaus Triple Beam Balance was used to obtain body weight. Standard body measurements were taken in millimeters. These measurements included total length, tail length, hind foot length, and length of ear from bottom of notch to top of pinna.

Age Classes of Sigmodon

Captive animals were sacrificed at the proper time to yield samples for each of ten age classes. Age classes erected included: animals killed at birth - 1; 30 days of age - 2; 60 days of age - 3; 90 days of age - 4; 120 days of age - 5; 180 days of age - 6; 240 days of age - 7; 300 days of age - 8; 360 days of age - 9; 500 or more days of age - 10.

Samples of cotton rats from these ten age classes were used to secure the data listed in Tables I through VI and to construct Figure 1. Animals in the first nine age-class samples were born and raised in captivity. Animals making up the Age Class 10 sample were born in the wild and were not made captive until after they had attained sexual maturity. Their minimum ages are known to be more than 500 days and the maximum ages may be considerably more than 500 days.

Sampling of Wild Sigmodon Populations

Samples were taken on the Airport Pasture and Baumgartner Study Areas (see Chapter III) in November 1962 using the standardized sampling technique developed by Calhoun (1956). Twenty trapping stations were established in a straight line. Three rat snaptraps were placed at each station and the stations were placed at 50-foot intervals. The traps were baited with peanut butter. Traplines were run morning and evening for five consecutive days. This sampling procedure does not reveal total population numbers and may not even be a suitable index if the ratio of the relative abundance of one species to another changes between sampling periods (Calhoun, 1959), but a better, feasible sampling technique was not available.

The Mueller Study Area (see Chapter III) was sampled two times (November 1963, March 1964) using a straight line of 25 livetraps baited with dry rolled oats. One livetrap was placed at each station and the stations were spaced five steps apart. This change in the sampling technique was brought about by the publication of a paper by Montgomery (1963) who found that freezing and/or decomposition of raccoon lenses resulted in a loss of lens weight. Snaptrapping cotton rats during the winter allows the lenses of the animals trapped several hours before the trapline is run to freeze. Decomposition of lenses may also begin if the air temperature is well above freezing. Lens decomposition probably would not be a major factor, however, since at least two days at room temperature were required before raccoon lenses lost weight.

Animals caught on the Mueller Study Area were taken to a laboratory while still alive, then were chloroformed and processed in the manner described for known-age cotton rats. It was assumed that livetrapping the cotton rats did not appreciably alter the body weight even though some of the animals were in the traps several hours prior to being sacrificed. Dunaway and Kaye (1964), while livetrapping S. h. komareki, found weight losses due to trap stress were slight. Trapped animals, in the present study, ate all the available bait, thus indicating that they probably were not experiencing undue stress.

The body weight of rats from the two samples was considered to be a measurement that was valid although the two samples were obtained by different methods. This assumption allowed the samples to be combined to evaluate the value of the lens weight as an aging tool.

CHAPTER III

DESCRIPTION OF THE STUDY AREAS

Outdoor Enclosure

An enclosure was constructed on Oklahoma State University property near the main campus. Corrugated metal roofing was used to enclose the 90 x 100 foot area. The sheets of metal were attached to steel fence posts and the lower edge of the fence was buried six inches in the ground. Construction took place in September, 1962. Due to a misunderstanding with the workmen, the vegetation on the area was completely removed by burning on or about September 1, 1962. The area was then disked and wheat was sown prior to the area being enclosed by the fencing.

Twenty bales of low-quality hay were stacked in the enclosure during the first week of November to provide additional cover.

Vegetative cover in the enclosure was dominated, in November 1963, by Bermuda grass, *Cynodon dactylon*, interspersed by Johnson grass, *Sorghum halepense*, and forbs.

Natural Study Areas

The cotton rat populations on the three study areas in Payne County, Oklahoma, were sampled by trapping. The average annual precipitation for Payne County is 32.2 inches, with approximately 70 per cent of this total occurring from April to October. The springs tend to be cool and the summers are hot and often dry. The autumns are mild and the winters are mild to cold. (Sims, 1962). Topography is gently rolling to level and the soils are loamy. The soils have slow to medium permeability and are neutral to slightly acid (U.S.D.A. Soil Conservation Service, 1961).

Airport Pasture Study Area

This study area was Sim's (1962) study area III. He reported the 105 acre pasture to be in excellent range condition when he made his observations. At the time the area was trapped for cotton rats in the present study, the forage had been overutilized by cattle, however.

The Airport Pasture Study Area was sampled from November 20, through November 25, 1962. No cotton rats or other small mammals were trapped during the 300 trapnights. Old cotton rat runways were present but the cotton rat population was apparently at a very low level possibly because the area was heavily overgrazed. The absence of fresh grass cuttings in the runways and the fact that no cotton rats were observed while the traps were being run were further evidences of the presence of an extremely low cotton rat population.

Baumgartner Study Area

This five acre native hay meadow is across the road from the home of Dr. Fred Baumgartner and is designated by Sims (1962) as his study area IV. Sims found the range condition to be excellent.

Snaptraps were set on this area on the evening of November 20, 1962, and were picked up on the evening of November 25, 1962. No precipitation occurred during the trapping period and temperatures ranged from a low of 33 degrees F. on the night of November 21 to a high of 65 degrees F. during the day of November 21. Vegetation had been mowed and removed as hay where trapping stations 1, 2, 3, 4, 14, 15, 16, and 20 were located. Apparently cotton rats did not venture out into the mowed area since no animals were caught in traps set at these stations. Of the 25 cotton rats trapped during the 300 trapnights, 16 were females and nine were males. None of the females were pregnant or lactating. The only other small mammal trapped with this sample was an adult male short-tailed shrew, *Blarina brevicauda*. At the time the sample was taken, the cotton rat population on the study area was considered to be at a moderate level. Sign, in the form of runways, fresh grass cuttings, and scats, was plentiful but animals were not observed in the runways while the traps were being run.

Mueller Study Area

This three-acre study area was near the south side of Lake Carl Blackwell and was used by Mueller (1964) as his unmowed, tallgrass prairie study area. It had been undisturbed by grazing or burning for eight years. This small study area was surrounded by several hundred areas of comparable rangeland. Thus, the cotton rat population was not restricted to the study area by any barriers.

Twenty-five livetraps were set on the Mueller Study Area on the evening of November 25, 1963 and were picked up on the evening of November 28, 1963. No precipitation occurred during the trapping period. Of the 42 cotton rats trapped during the 75 trapnights, 28 were females and 14 were males. None of the females were pregnant or lactating. The only other small mammal taken was an adult least shrew, *Cryptotis parva*. The cotton rat population was considered to be high at the time the sample was taken. Fresh sign was abundant and cotton rats were occasionally observed when the traps were being run. Green (1964) was trapping, marking, releasing, and retrapping cotton rats on a twenty-acre grid 2½ miles west of the Mueller Study Area. Cover was quite similar on the two areas. Green estimated a cotton rat population of 35.25 animals per acre on his study area in November, 1963.

The Mueller Study Area was again sampled in the same manner during the period of March 28 through March 31, 1964. No cotton rats [illegible] other small mammals were captured during 75 trapnights. No precipitation occurred during the trapping period. Green (1964), still working on his nearby study area, estimated a cotton rat population of 4.05 animals per acre during March, 1964. Breeding had stopped prior to the taking of the sample in November, 1963, as evidenced by the absence of pregnant females. The March, 1964, population reflected the results of natural attrition on a non-breeding population.

CHAPTER IV

RESULTS AND CONCLUSIONS

Gestation and Litter Size

Captive animals were checked two to several times each day for, among other things, the birth of young. Since the male was removed from the female as soon as a new litter was discovered, the males were with the females only a few minutes to a few hours after the females gave birth. Post-partum breeding occurred at least three times during the course of this study since three females had litters 27 days after the birth of their last previous litter and after the removal of the male from their cage. These observations tend to confirm the 27-day gestation period which has been reported by previous investigators (Asdell, 1946).

A total of 70 litters was born in captivity. The number of young per litter was determined for 60 litters. Litter size ranged from two to eleven and averaged 5.47. Meyer and Meyer (1944b) and Svihla (1929) published litter size data for S. h. hispidus trapped in Louisiana. The former investigator found that the number of young in 44 litters born in captivity varied from two to ten and averaged 5.6 per litter. The latter investigator noted three to six young per litter with an average of 4.75 young. Litter size of captive animals in these two subspecies of cotton rats is apparently similar whether the animals are from Oklahoma or Louisiana.

Measurements of Captive Sigmodon

Sigmodon of known age were divided into ten age classes. Individuals were measured for length of body plus tail (total length), tail, hind foot, ear, and weight of eye lens and body (see Tables I through VI). Due to differences in diet, amounts of exercise and stress, pregnancy, and other factors, captive cotton rat measurements are probably poor guides for the aging of wild animals particularly after they have attained sexual maturity.

It is of interest, however, to compare the sample means of the various measurements of all age classes of captive Sigmodon.

Sample means for each measurement were ranked from least value to greatest value and the means were then compared at the 95 per cent level of significance by the use of "Duncan's new multiple-range test" (Steel and Torrie, 1960).

Most hind-foot growth appears to occur during the first 30 days of life. This is seen in Table I. Mean hind-foot length was significantly different between age classes 1 and 2, and between 2 and 10 (the next Age Class in the order of ranked means). No significant difference was shown between the mean of Age Class 10 and the means of age classes having larger values. Hind-foot length is of little value in estimating age according to these comparisons and Table I. Similar conclusions could be drawn for tail length and ear length. This can be seen in Tables II and III, respectively.

TABLE I

Comparison of Hind-Foot Lengths (in millimeters) of Captive *Sigmodon* in Ten Age Classes

Age Class	Number in Sample	Mean Hind Foot Length	Standard Deviation
1 (killed at birth)	3	13.33	1.16
2 (30 days old)	19	28.53	1.17
3 (60 days old)	23	31.26	0.45
4 (90 days old)	26	31.12	0.77
5 (120 days old)	22	31.45	0.91
6 (180 days old)	9	31.67	0.50
7 (240 days old)	15	31.20	1.42
8 (300 days old)	14	31.86	1.23
9 (360 days old)	13	31.69	1.25
10 (500 or more days old)	19	30.95	1.13

TABLE II

Comparison of Tail Lengths (in millimeters) of Captive *Sigmodon* in Ten Age Classes

Age Class	Number in Sample	Mean Tail Length	Standard Deviation
1 (killed at birth)	3	27.67	2.52
2 (30 days old)	19	80.47	7.01
3 (60 days old)	22	97.73	5.65
4 (90 days old)	26	99.12	5.90
5 (120 days old)	21	106.24	5.12
6 (180 days old)	9	109.11	9.09
7 (240 days old)	12	103.33	6.93
8 (300 days old)	12	110.08	5.35
9 (360 days old)	12	103.92	7.72
10 (500 or more days old)	18	101.06	4.70

TABLE III

Comparison of Mean Ear Lengths (in millimeters) of Captive *Sigmodon* in Ten Age Classes

Age Class	Number in Sample	Mean Ear Length	Standard Deviation
1 (killed at birth)	3	5.00	1.73
2 (30 days old)	19	16.68	1.11
3 (60 days old)	23	17.78	0.60
4 (90 days old)	26	17.58	0.90
5 (120 days old)	22	18.14	0.56
6 (180 days old)	9	18.44	0.88
7 (240 days old)	15	18.53	0.99
8 (300 days old)	14	19.79	0.80
9 (360 days old)	13	19.23	0.93
10 (500 or more days old)	19	19.84	0.50

Sample mean tail lengths were significantly different between Age Classes 1 and 2, and 2 and 3, but not between 3 and older age classes.

Sample mean ear lengths were significantly different between Age Classes 1 and 2 but Age Class 2 was not significantly different from any mean smaller than that of Age Class 7.

Total length may be of value in estimating age up to 60 days after birth (Table IV). Total length estimates were 79.33 mm. at birth, 196.58 mm. 30 days after birth, and 238.27 mm. at 60 days of age. After this age, the sample means fluctuate without marked increases. Differences among sample means of the first three age classes were significant at the 95 per cent level. There was no significant difference between Age Classes 3 and 4 but significant differences were found between Age Class 4 and older age classes. No significant differences were found between Age Class 5 and older age classes.

Body weight samples (Table V) did not include pregnant females. Differences in sample means were not significant between Age Classes 1 and 2, 3 and 4, 3 and 5, 4 and 5, 5 and 6, 6 and 7, 6 and 9, 7 and 8, 7 and 10, and 9 and 10. Significant differences were noted between Age Classes 2 and 3, however.

Sample mean lens weights (Table VI) were significantly different, at the 95 per cent level, between Age Classes 1 and 2, 2 and 4, 3 and 5, 4 and 6, 5 and 7, and 6 and 10. Differences were not significant between Age Classes 2 and 3, 3 and 4, 4 and 5, 5 and 6, 6 and 7 or any older age classes.

A growth curve of the lens of the captive cotton rat (see Fig. 1) was determined by passing a curve through the mean lens weight value for each age class (see Table VI). A broken curve, on each side of the growth curve, passes through points that were obtained by using twice the

TABLE IV

Comparison of Total Lengths (in millimeters) of Captive *Sigmodon* in Ten Age Classes

Age Class	Number in Sample	Mean Total Length	Standard Deviation
1 (killed at birth)	3	79.33	4.73
2 (30 days old)	19	196.58	14.33
3 (60 days old)	22	238.27	7.55
4 (90 days old)	26	239.35	13.62
5 (120 days old)	21	259.90	10.91
6 (180 days old)	9	275.78	18.62
7 (240 days old)	12	268.25	14.44
8 (300 days old)	12	282.33	13.22
9 (360 days old)	12	270.75	14.62
10 (500 or more days old)	18	271.89	13.58

TABLE V

Comparison of Mean Body Weight (in grams) of Captive *Sigmodon* in Ten Age Classes

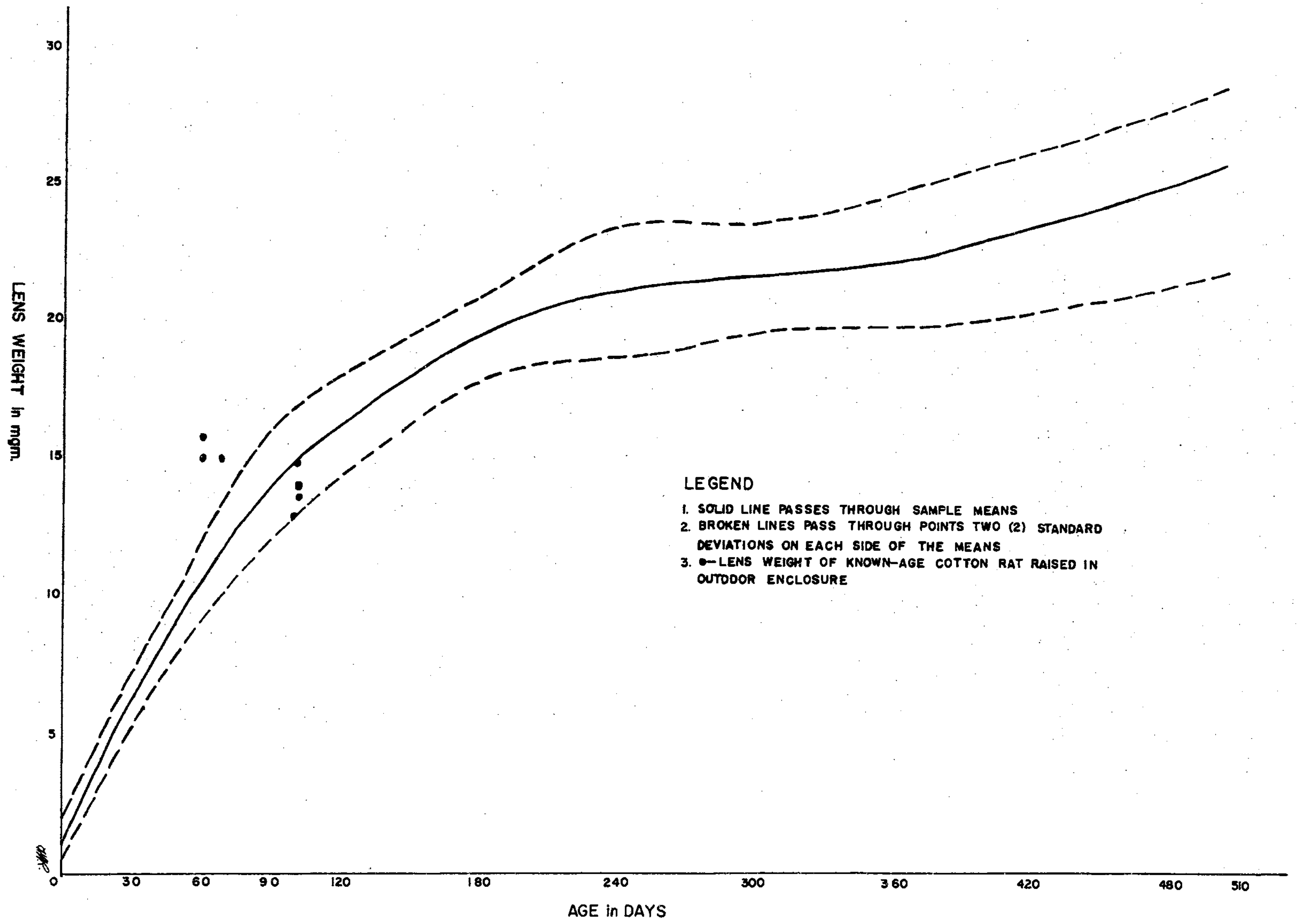
Age Class	Number in Sample	Mean Body Weights	Standard Deviation
1 (killed at birth)	3	5.93	0.01
2 (30 days old)	19	51.79	9.36
3 (60 days old)	23	100.43	14.25
4 (90 days old)	31	101.52	15.42
5 (120 days old)	22	122.59	25.18
6 (180 days old)	13	156.31	25.03
7 (240 days old)	15	168.33	29.65
8 (300 days old)	14	206.36	31.57
9 (360 days old)	13	200.08	31.75
10 (500 or more days old)	19	208.16	40.63

TABLE VI

Comparison of Mean Lens Weight (in milligrams) of Captive *Sigmodon* in Ten Age Classes

Age Class	Number in Sample	Mean Lens Weight	Standard Deviation
1 (killed at birth)	13	1.195	0.389
2 (30 days old)	19	6.326	0.504
3 (60 days old)	23	10.600	0.759
4 (90 days old)	31	14.135	1.024
5 (120 days old)	23	16.160	0.951
6 (180 days old)	13	19.415	0.662
7 (240 days old)	15	21.213	1.289
8 (300 days old)	15	21.806	0.957
9 (360 days old)	13	22.276	1.264
10 (500 or more days old)	26	25.784	1.941

FIGURE I. GROWTH CURVE of THE LENS of CAPTIVE COTTON RATS BASED on THE MEAN VALUE FOR EACH AGE CLASS



estimated standard deviations above and below the mean. The procedure for deriving these intervals is similar to that used by other investigators (Dudzinski and Mykytowycz, 1961) except they made transformations on the data to stabilize variances and then their confidence intervals were set on the lens weights. In this study no transformations were made and the intervals for a given age were obtained by using only animals of that age.

The literature contains severals papers, including Dudzinski and Mykytowycz (1961) and Kolenosky and Miller (1962), which list various algebraic equations purported to describe curves that approximate the lens weight data from various mammalian species. The curve illustrated in Figure 1 was passed through the sample means because no theoretical assumptions could be found which revealed the type of curve that could be fitted to the data.

Sample ranges are not included in Figure 1 since they approximated the intervals for all age classes. It was found that in Age Class 2 the sample range and interval almost coincide. The sample size, mean, and standard deviation for each age class are listed in Table VI.

The growth curve of the cotton rat lens continues to ascend through all age classes. This type of curve agrees with the lens growth curve in humans (Smith, 1883; Burdon-Cooper, 1914), the albino laboratory rat (Donaldson, 1924), the laboratory rabbit (Krause, 1934), the cottontail rabbit (Lord, 1959), the grey fox (Lord, 1961), the raccoon (Sanderson, 1961), the Australian rabbit (Dudzinski and Mykytowycz, 1961), the fox squirrel (Beale, 1962), the fur seal (Bauer et al, 1964), the pronghorn (Kolenosky and Miller, 1962), and the House Sparrow (Payne, 1961). The lens growth curve of the species in the present study is not identical to the lens growth curve of any of the above cited species, but it is similar to each of them inasmuch as the rate of increase slows at approximately the time adult body size is attained.

Mortality of known-age cotton rats released in the enclosure was high. This agrees with the findings of Green (1964) who released cotton rats in enclosures adjacent to the present study enclosure and Goertz (1962) who was trapping, marking, and trying to recapture cotton rats in a wild population. Goertz found that approximately 50 per cent of his marked animals disappeared each 30 days. In this present study of the eleven males released in the enclosure, only one was recaptured, while only six of the twelve females released were recaptured. The sole unmarked cotton rat that did not enter a trap during the August 31 to September 4 trapping period, but was trapped on October 23, was a female. Green observed comparable selective mortality in his enclosures. The reasons for the males being selected against in the enclosures are not known.

The lens weights of the seven known-age cotton rats which were liberated in the outdoor enclosure and later recaptured are indicated in Figure 1. Only one of the seven cotton rats had a lens weight that approximated the empirically-derived lens growth curve. Three of the remaining six had lens weights that fell below the average growth curve but within the interval. The other three had heavier lenses that were above the interval. This sample size of seven recaptured rats appears to be too small to indicate the validity of the lens growth curve as applied to wild cotton rats. It should be noted also that even though the seven animals had lived a part of their lives in the enclosure, they had all been born in captivity and had remained there until after they were weaned. The early stages of their development while in captivity were not unlike those of the animals from which the growth curve was developed.

The lens growth curve (Fig. 1) may be a suitable aging tool for captive animals of this subspecies up to the age of approximately 180 days. After this age has been attained, the rate of lens weight increase has slowed and the estimates of the variances have increased to a point where the value of this technique is greatly reduced.

The lens weight may be a good aging technique for captive cotton rats but it is of no value to persons studying wild cotton rat populations unless it can be shown that the lens weight curve of wild cotton rats coincides with the curve for captive cotton rats. A large sample of known-age cotton rats born and raised in the wild is needed to test the validity of the lens growth curve as illustrated in Figure 1. In the absence of such a sample, other evidence must be examined in drawing conclusions concerning the value of the lens growth curve in Figure 1.

The animals in Age Class 10 (500 or more days old) were all born in the wild. After they had attained adult body size and weight, they were livetrapped and held in captivity until sacrificed. The lens weight growth curve (Fig. 1) shows a shift upward between Age Class 9 and 10 indicating that the mean lens weight for Age Class 10 is disproportionately heavy. This observation may be explained in one or more ways. First, the sample of 26 animals, although larger than most of the samples for other age classes, may give a distorted impression of the actual population mean due to sampling variation. Second, the lenses of old cotton rats may actually be accelerated in their rate of weight increase after the age of 360 days (Age Class 9). This possibility is improbable since such a phenomenon has not been reported in any other mammalian or avian species.. Third, most of the animals in the Age Class 10 sample may have been considerably older than 500 days. This explanation is unacceptable inasmuch as the animals in Age Class 10 would have needed to have been at least 1,000 days old at the time they were sacrificed in order to follow the indicated trend. Not even one cotton rat is known to have lived, in the wild, for as long as 500 days. Goertz (1962) concluded that population turnover was complete in from five to as long as 12 to 15 months. Population turnover was virtually complete in six months on Goertz' study areas since 98 percent of a marked population of over 1,000 cotton rats had disappeared by the sixth month. Odum (1955) assumed population turnover to be complete approximately each six months, and only reported one female which lived at least as long as 159 days. Hays (1958) reported one animal, of unknown sex, which lived at least five months and one day. McCulloch (1959) mentioned one female which lived at least seven months. Fourth, the birth and growth of the animals in this sample while in the wild may have an accelerated growth of their eye lenses. Papers by Sanderson (1961) and Foster and Peterson (1961), quoted earlier, recorded instances of wild animals developing at a different rate than captive animals. Sanderson's paper, however, reported that the lenses of wild cottontail rabbits grew slower than those of captives, instead of faster as the present study could be interpreted. The possibility that the birth and growth of these 26 animals in the wild somehow accelerated the growth of their eye lenses is the most acceptable explanation, particularly in light of the following observations and conclusions.

The Baumgartner Study Area sample of 25 cotton rats was combined with the Mueller Study Area sample of 42 animals. The combination of these samples was considered to be feasible and justifiable since the populations from which the samples were drawn differed mainly in density. Both populations had stopped reproducing prior to being sampled, since no pregnant or lactating females were captured in either sample. The sample sex ratios were similar, being 55 males: 100 females in the Baumgartner sample and 50 males: 100 females in the Mueller samples. Each of the 67 animals was sexed, measured, and an age was assigned to it, using its lens weight as the aging criterion. An age was determined for each animal by the use of Figure 1. A horizontal line was drawn from the animal's lens weight as plotted on the Y-axis. At the point where the horizontal line intersected the growth curve, a vertical line was drawn to the X-axis and the probable age of the animal was recorded.

A high degree of correlation between the assigned age and the age as determined by body measurements would tend to substantiate the validity of the lens weight as an aging technique for wild cotton rats. Figure 2 compares the total body length of 65 wild cotton rats with the total body length of known-age captive animals. Figure 3 compares body weights of the captive animals and wild animals. The body weight sample includes 67 individuals. Comparisons were not made between tail lengths, hind foot lengths, and ear lengths of wild and captive samples because of the problems inherent in the use of these body measurements (see page 21).

Figure 2 shows that total length of 60-day old wild animals (Age Class 3) and 60-day old captive animals do not fall in the same interval. Total length of wild animals in other age classes was usually less than the mean total length of captive animals of comparable age. Body weight (Fig. 3) of 60-day old wild animals (Age Class 3) was appreciably less than that of captive animals of the same age. In other age classes, wild animal body weight was generally less than the body weight of captive animals of the same age.

The differences between wild and captive cotton rats in these body measurements (Figs. 2 and 3) can be accounted for in one or more of several ways. First, this discrepancy may be due to sampling variation. Second, captive animals, due to better nutrition, restricted activity, and other factors, may be heavier and larger than wild cotton rats of comparable age, especially early in life. It is not known if this is true or untrue. Third, the lenses of wild cotton rats may increase in weight more rapidly than the lenses of captive animals, particularly during the early weeks or months following birth. This choice is more acceptable, since it best explains the observations. A more rapid weight increase of wild cotton rat lenses would result in the wild animals being assigned an older age than was warranted.

The sample of 67 wild cotton rats offered an opportunity to check for similarity between Sealander and Walker's (1955) aging method (see p. 2) and the lens weight aging technique. Their age classes were derived from data taken from body weights of known-age laboratory-raised cotton rats which were published by Meyer and Meyer (1944b). Using the body weights of the wild sample and their approximate ages as determined by lens weight, a comparison was made with the body weights of known-age study animals. Using the lens weight curve (Fig. 1) on the 67 wild animals, Sealander and Walker's aging method would have underestimated the probable age for 62 of the animals. Only five of the 35 adult females in the sample would have been properly placed in their Old Adult Female age class. Since Sealander and Walker did not know the age of

FIGURE 2. TOTAL LENGTH of a SAMPLE of WILD SIGMODON COMPARED WITH TOTAL LENGTH of CAPTIVE ANIMALS

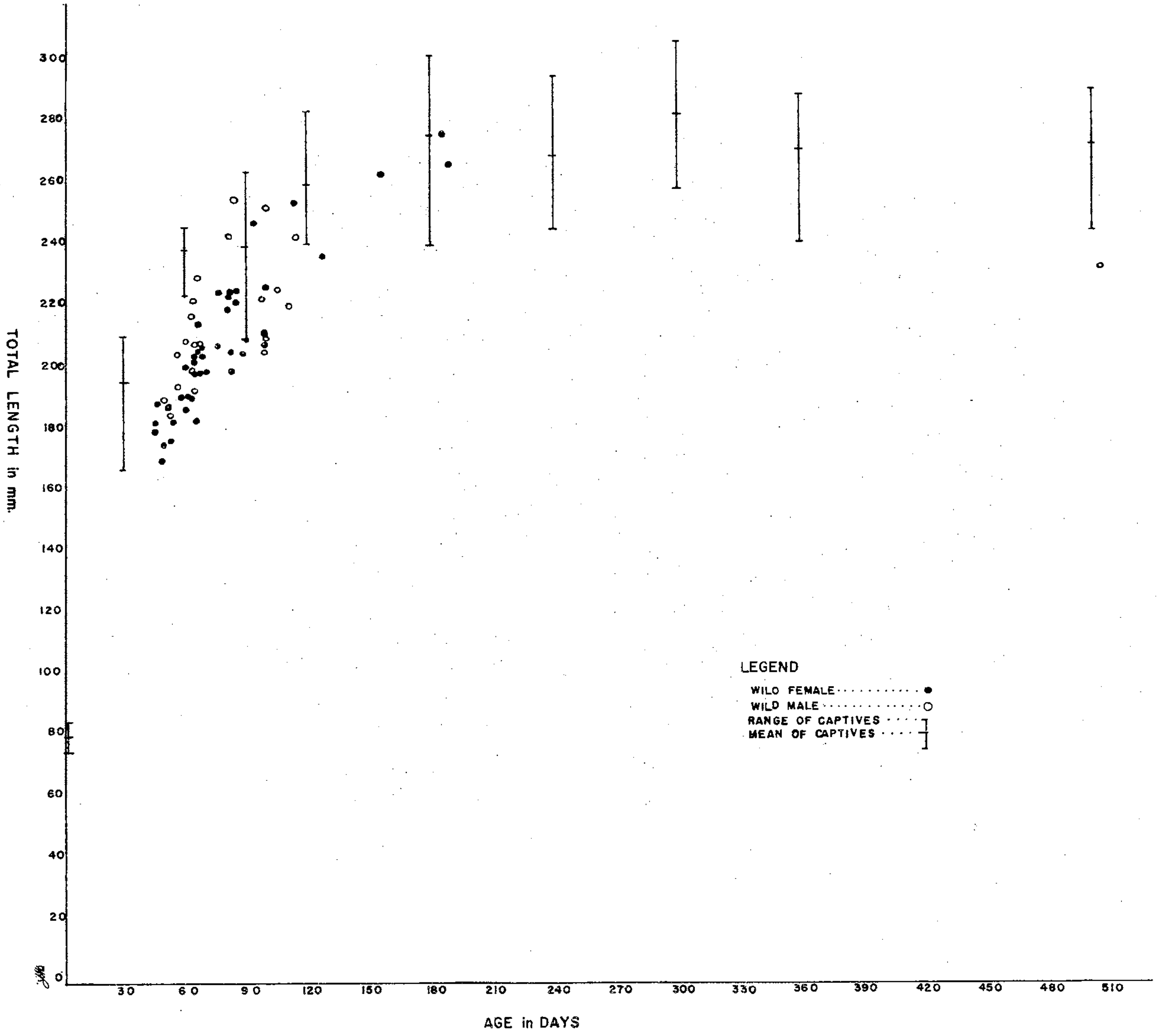
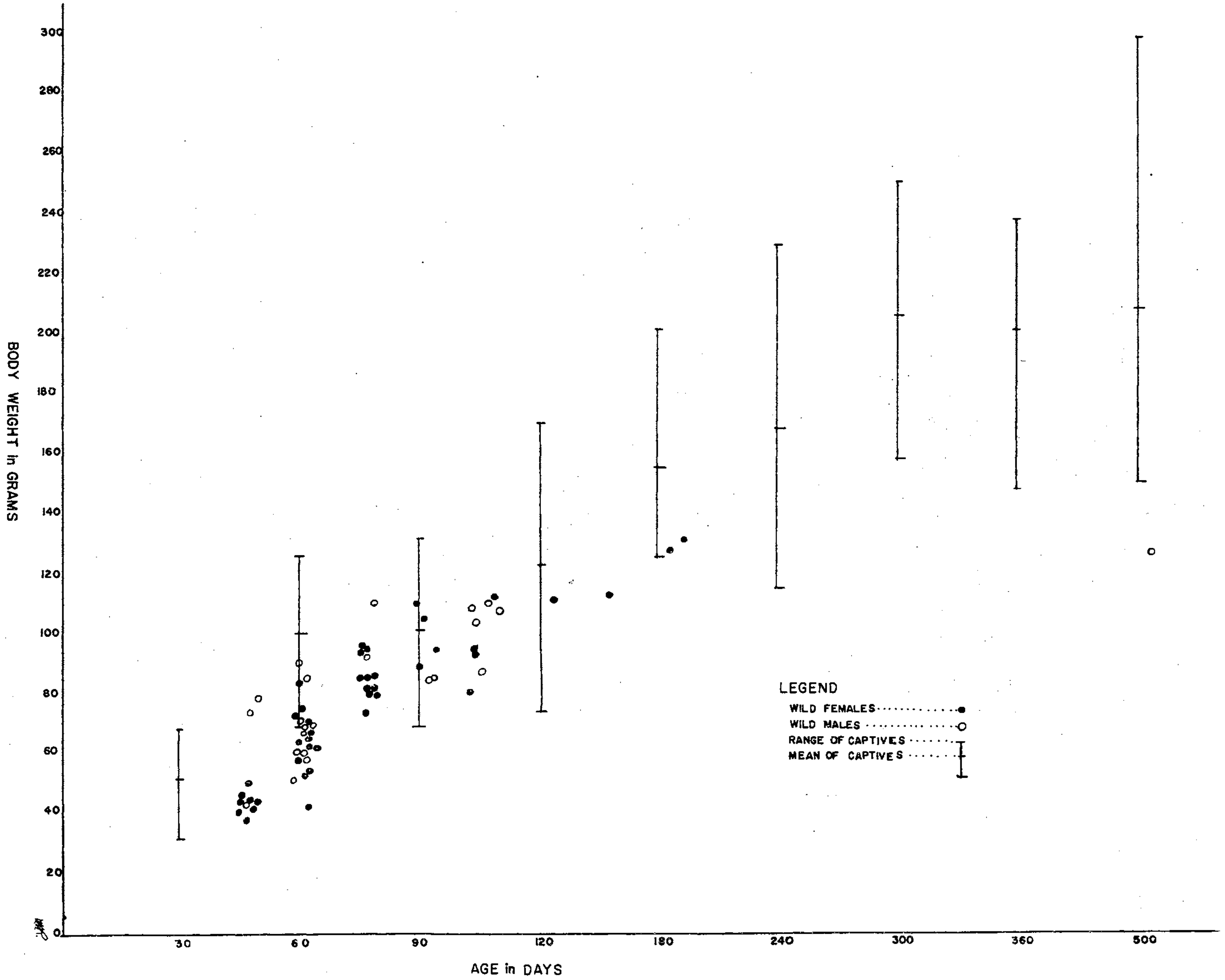


FIGURE 3. BODY WEIGHT of a SAMPLE of WILD *SIGMODON* COMPARED WITH BODY WEIGHT in CAPTIVE ANIMALS



their wild rats and the age of the wild rats is not known in this study, one cannot state which aging technique is preferable.

It is concluded that the lens weight curve (Fig. 1) is not suitable for aging samples from populations of wild cotton rats because it probably does not coincide with the actual lens growth curve as found in wild populations. The latter curve has not been determined, however.

A valid technique for determining the age of wild cotton rats up to the age of 180 days is not known.

Results of this study indicate that wild cotton rats will need to be found and marked at birth, later being trapped and sacrificed to determine the lens weight growth curve of wild cotton rats. Such a project would demand a tremendous amount of effort on the part of the investigator since many nests containing young would need to be found to insure the recapture of adequate numbers of known-age wild animals.

CHAPTER V

SUMMARY

This study was undertaken to determine the value of the lens weight as an aging technique in the cotton rat.

Samples of known-age animals were raised in captivity and later sacrificed to gather data on various body measurements including total length, tail length, length of hind foot and ear, and weight of body and lens.

Cotton rats of known-age were released in an enclosure in an attempt to find out if they developed at a different rate in the wild.

Mortality of released animals was found to be very high.

It was concluded that all body measurements, with the possible exception of lens weight, were inadequate for aging cotton rats beyond the age of approximately 60 days.

A lens weight growth curve was determined for captive animals. It was concluded that this curve probably is unlike the lens weight growth curve of wild cotton rats.

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