A C T A T H E R I O L O G I C A

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STUDIES ON THE EUROPEAN HARE. XXVII.

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Methods for Determination of Age in the European Hare

[With 4 Figs]

It was found in relation to a series of 2277 hares killed in one shooting area in four shooting seasons (1966/67 — 1969/70) that compared with estimating age on the basis of lens weight, Stroh's character underestimates the percentage of young hares by about 10% at the beginning of November and by 40% in January; on an average for the shooting season by 30%. This disqualifies Stroh's method for studies on the age structure of a population during the shooting season. The epiphyseal cartilage of the ulnar bone begins to disappear when lens weight is from 155—200 mg (in 2—10% of the individuals) and is absent in 98% of hares in the 275 mg class. The dry mass of lens gives the most accurate picture of the age structure of a population, on condition that the recommended method of fixing is used, that lenses are dried at 100°C and extreme values are established each time from the frequency distribution.

1. INTRODUCTION

The development in recent years of ecological studies on the European hare has stimulated efforts to find increasingly accurate methods of defining the age of individuals and in consequence the age structure of populations. From the point of view of game management the possibility of distinguishing »current year's« hares from »older« hares, and by this means determining the extent of annual growth of young hares, is of prime importance. Such estimates are most often made on material collected during the shooting season, when it is impossible to distinguish all young hares on the basis of their body dimensions and weight. Among various ways of distinguishing young hares the one which has achieved the greatest popularity in Europe is the method described by Stroh (1931), consisting in establishing the presence or otherwise of the epiphyseal protrusion of the ulna. The advantages of this method are its simplicity, the ease with which it can be applied under field conditions and on living animals. Attention has, however, been drawn to certain of its defects when applied to population studies (Bujalska *et al.* 1965; Walhovd, 1966).

Lord (1959) used the weight of the dry crystalline lenses to define the age of *Sylvilagus floridanus*, and this method is now successfully used for many species of vertebrates (Friend, 1968). The ranges of lens weight in the hare (*Lepus europaeus* Pallas, 1778) of a known absolute age are not as yet known. There are, however, two distinct peaks in the distribution of lens weight in autumn samples, with a zone lying in the vicinity of 270 mg separating young hares of the latest reproduction season from the group of older animals (Rieck, 1962; Walhovd, 1966; Caboń-Raczyńska & Raczyński, 1968; Möller, 1969; Wandeler & Huber, 1969).

The purpose of the present studies is to review the technique at present in use for dealing with lens and to assess the practical usefulness of Stroh's and Lord's methods in population studies.

2. MATERIAL AND METHODS

The studies were carried out on a series of 2277 hares coming from one population used for shooting purposes over a period of four consecutive years, from 1966—1970. The whole material was obtained solely from the shooting season *i.e.* the period from November 1st to January 10th. The study area was a shoot of 20,000 ha situated in the east part of Poland (23°30′ E, 52°35′ N) in the Białystok voivodship. Every hare killed during shooting was inspected under field conditions, before *rigor mortis* set in.

The age of the hares was determined in two ways: (1) by palpation of the ulna to discover the presence or otherwise of the epiphyseal protrusion near the carpal joint, and by dissection. The breadth of this protrusion was also measured. If the protrusion could not be felt through the skin a detailed examination was made of this region on the exposed bones of the forearm. Individuals with a visible band of cartilage between the shaft and distal base of the ulna were allocated to the group of hares in the first year of life — »young«. Individuals with a negligible protrusion and absence of traces of cartilage were allocated to the group of animals more than one year old — »adult«. (2) The crystalline lenses were taken from hares within 1—2 hours after death and kept in 10% formalin for one week. The procedure used formed a convenient modification under field conditions of the method of excising and fixing the whole eyeball. It consists in incising the sclera of the eye with scissors on the periphery of the eyeball and squeezing the lens out, together with part of the vitreous body into a labelled test tube containing formalin. Care must be taken during this operation to ensure that the incision on the eyeball is large enough, as squeezing a lens through too small a slit may damage it and lead to superficial losses. The lenses were dried in an oven in the laboratory at a temperature of 60—70°C until there was no decrease in weight, and weighed with accuracy to 1 mg, after which drying was continued at a temperature of 100°C. The effect of drying methods on results is discussed in the further part of this study.

3. RESULTS AND DISCUSSION

3.1. Technique for Drying Lenses

An essential condition for obtaining correct results is to remain aware throughout the whole of the time taken from preparation to drying of the consequences of errors made in the technique used for preparing lenses for measurement. The established procedure for dealing with lenses has been exactly adhered to in the present study. The lenses were fixed in a 10% formalin solution (ratio for mixing: 1 part 40% formaldehyde + 3 parts of distilled water) for 7 days. Before drying the lenses were soaked in water in a Petri dish to remove the solution from their surface, but were not rinsed in running water. The hygroscopic nature of lenses after drying was also taken into consideration and on this account weighing was carried out within 1—2 hours after removal from the drying chamber, having previously checked to see that this period does not cause any perceptible changes in weight.

During the studies it was found that hares' lenses dried in an oven at a temperature of 60—70°C to constant weight (drying time 10 days) may next lose weight if they are kept under dry conditions of a laboratory room. Accordingly this problem was examined using a series of 74 lenses dried at a temperature of 70°C until weight loss ceased. Half of these lenses were next dried for a further period at a temperature of 100°C in a vacuum oven, with 20 mm Hg pressure and with the presence of $CaCl_2$ (calcium chloride dehydr.) as a hygroscopic substance. The second group of lenses was dried at 100°C only, continuing drying until minimum weight decrease was obtained. Decreases in the weight of the lenses was recorded individually for both experimental groups at established intervals (Fig. 1).

It was found that under conditions of reduced pressure and water absorption the material can be treated as finally dried after 48 hours. Under the effect of temperature only stabilization of weight of the lenses did not take place until after 96—120 hours of drying. Considerable decreases in lens weight were observed in both groups, some of the lenses losing in weight from 0 to 16% of mass, with an average 7%.

Decreases in lens weight as the result of continued drying at a temperature of 100°C were investigated in the whole experimental series in six classes of lens weight: up to 200 mg, 201—250 mg, 251—300, 301—350, 351—400 and over 400 mg. The average weight loss in the various classes was similar and also varied within limits of 6.2—7.6% of dry mass. This shows that under continued drying conditions loss of water is proportional only in relation to the lens mass and factors connected with the structure of the lens in young and old hares do not enter into the question here.

The first drying of lenses at a temperature of 60—70°C, despite the apparent stabilization in weight loss, does not thus cause total evaporation of water from the lenses. Drying at a temperature of 100°C intensifies evaporation and probably affects the protein mass of the lens to a minimum degree. Acceptance of this temperature encourages standardization of the drying process. Drying for a varying period at lower temperatures leads in consequence to erroneous segregation of the material into age classes in cases when correct segregation is the chief purpose of this method. The class of oldest hares among those born in a given year is

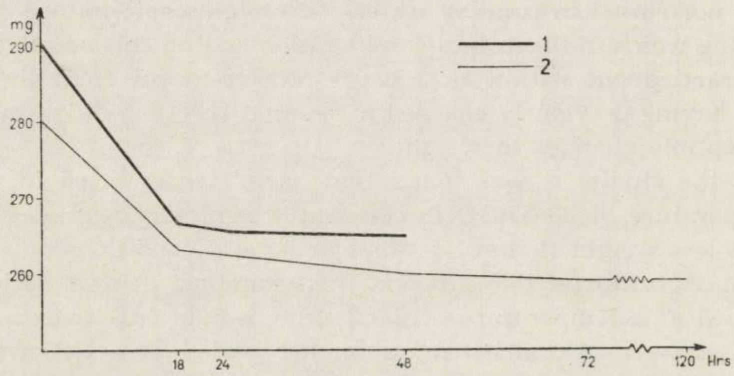

Fig. 1. Curves of weight loss in two groups of lenses during drying to constant mass at 100°C. 1 — Drying in oven, 2 — Drying in an oven under partial vacuum with calcium chloride dehydr. present.

particularly exposed to error. In our material, for instance, we encountered a female weighing 5.20 with a trace of the epiphyseal protrusion, the lens of which was 284 mg at the first weighing, and was thus allocated to the »adult« group. After continued drying the lens weighed 238 mg, confirming the diagnosis reached on the basis of ossification of the skeleton.

Friend (1968) discusses in detail factors connected with the technique for dealing with lenses which are responsible for undesirable variations in lens weight. Our experiments show that in addition to the method and time of fixing, the main critical moment in preparation of lenses for weighing is drying them. Lord (1959) applied drying at a temperature of 80°C with air flow for 24—36 hours, which he considered sufficient. Many authors modify drying depending on the apparatus available, continuing drying only until the time that weight loss ceases. Möller (1969) was the first to point out the necessity for using a temperature of 100°C when drying hare lenses. The weight loss curve which he presents shows that after only 24 hours lens mass is stabilized on an unvarying level.

It is clear from the above discussion that it is only the observance of identical and correct methods which can ensure that results obtained by different authors are comparable and permits of avoiding misunderstandings, an example of which is formed by the data given by Hell (1967). The widespread application of Lord's method in studies on the age structure of different populations of *Lepus europaeus* therefore makes it necessary to accept strict unification of methods for fixing and drying lenses. On the basis of the experiments we have so far made and those of other authors we recommend the following procedure:

(1) Removal of the eyeball or lens immediately after the animal is killed or within a few hours *post mortem* (unless temperature conditions hasten rapid decomposition or freezing of the eye). Preparation carried out 1—2 days *post mortem*, when there are no changes in the tissue, is possible, but the fact must be noted in the methodical part of the study as it leads to reduction of several per cent in dry mass (Andersen & Jensen, 1972).

(2) Fixing the eyeball in 10% formalin solution for one week.

(3) Drying the lens in two phases: preliminary, not very intensive drying at a temperature of 40—80°C under normal pressure, so as to avoid deformation and destruction of the lenses, and continued drying at 100°C to constant mass. In view of the time saved it is recommended that this process is carried out under reduced pressure and with hygroscopic substances present. Drying to constant mass is also possible after a long period of storage of lenses in the dry state.

3.2. Weight of Lenses

Division of the material into age groups is in practice carried out on the basis of frequency distribution of lens weight. In a sample of varying ages in autumn there is always a distinct decrease in numbers in the age class corresponding to the pause in reproduction between the first and second reproduction season. Under the conditions prevailing in east Poland this pause covers the period from October to the beginning of March of the following calendar year, that is, about 5 months. Hence it follows that at the start of the shooting season in November the oldest hares from the last reproduction season are 7—8 months old, and the youngest animals from the »adult« group — from 15 to 16 months old. Therefore

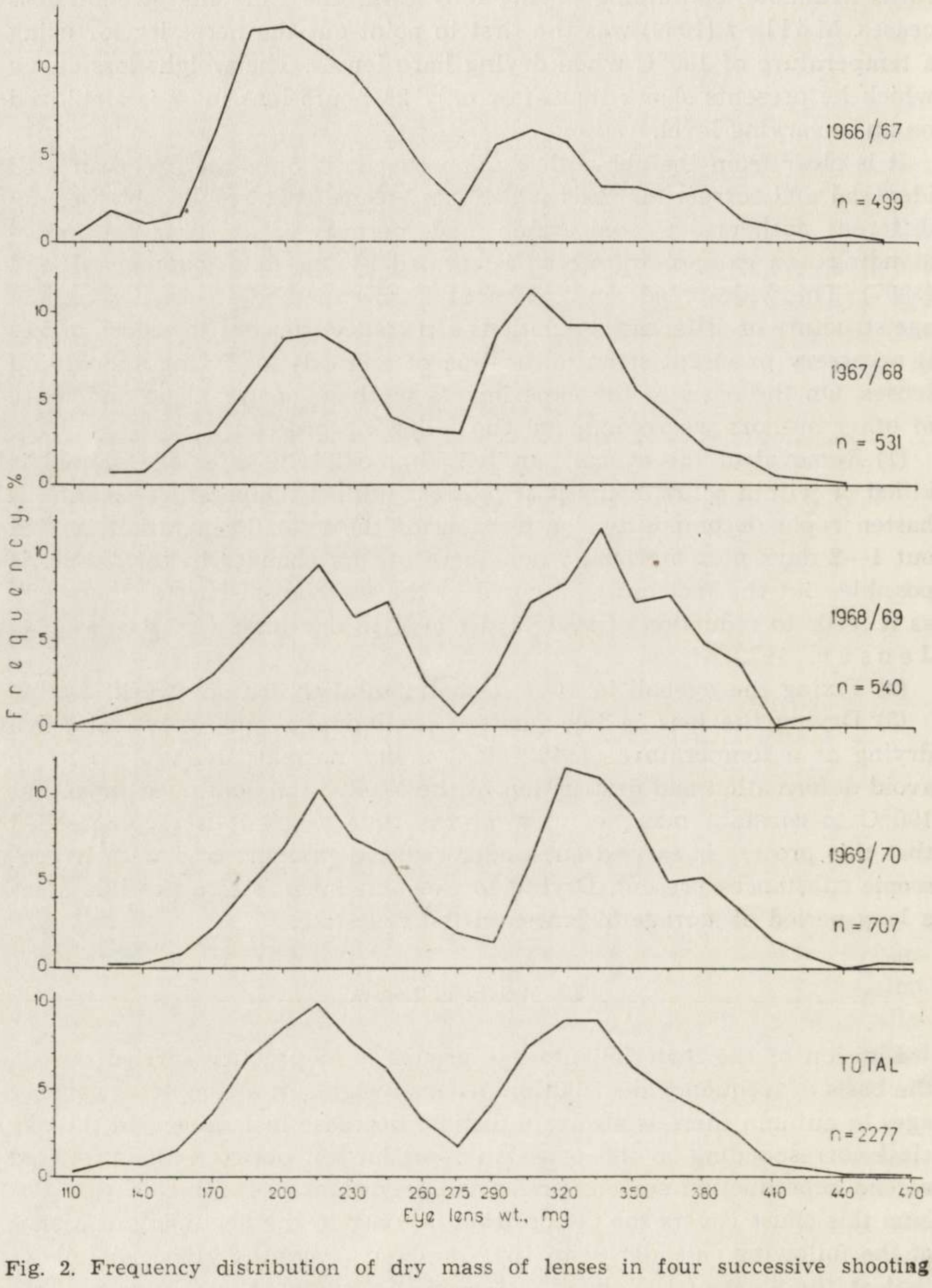

Fig. 2. Frequency distribution of dry mass of lenses in four successive shooting seasons.

during the shooting season the majority of the hares from the »young« group are still in the period of physical development and uncompleted growth. Growth processes of the skeleton defined on the basis of closure of the epiphyses of the long bones end at the age of 8—9 months (A n d e r s e n, 1958), while processes of sexual maturation of young hares are not manifested until January (R a c z y ń s k i, 1964). If in addition the fact is taken into consideration that the participation of the oldest group in realized increase should, according to R a c z y ń s k i' s assumptions (1964), be negligible, then a collection of this kind fulfils the conditions guaranteeing correct division into the two basic age groups.

Distribution of frequency of lens weight in the studied population in four consecutive shooting seasons is illustrated in Fig. 2. The considerable constancy of the extreme value of lens weight for three seasons and in the collective material in the 275 mg class (268—282 mg) is remarkable. It was only in the 1969/1970 season that the extreme zone shifted to the 290 mg class (283—297 mg). This is accompanied by a shift in the minimum values for young hares, which suggests, in our opinion, that in this case we have to do with the result of a shift in the reproduction season. R a c z y ń s k i (1964) encountered *e.g.* the phenomenon of an early start to reproduction in material from the Poznań voivodship for the 1959/1960 breeding season.

In material from West Europe the extreme zone of lens weight for young hares lies in the region of 270 mg (R i e c k, 1962 — 275 mg; W a l h o v d, 1966 — 250—270 mg; W a n d e l e r & H u b e r, 1967 — 260—270 mg: M ö l l e r, 1969 — class 270 mg). M ö l l e r' s (1969) results, based on a large amount of material (n = 8368), are evidence of great precision of division — only 0.58% of the whole sample comes within the dividing zone (270 mg).

Analysis of our material shows that it is of no use to accept a constant extreme value for lens weight, despite the fact that it is regularly observed to occur. Suitable exactitude ensures that this limit is defined in each homogeneous sample (in a given year) on the basis of the frequency distribution of lens weight. The only possible procedure for division is arbitrary allocation of individuals from each side of the dividing line to separate groups. It must be underlined that shifts in the narrow zone of contact are possible as the result of the phenomenon of individual variation in lens weight.

3.3. Epiphyseal Protrusion

The disappearance of the protrusion and epiphyseal cartilage in young hares was analysed using a large amount of material (n = 1165), making comparisons with increase in lens weight (Fig. 3). As increase in lens mass in young individuals is closely related to age (cf. curve of lens weight: Lord, 1959; Rongstad, 1966) the increasing classes of lens weight represent successive age groups. The materials presented prove that there is considerable age variation in the ossification process. In an extreme case cartilage disappearance began as early as in the 155 mg class (range 147—162 mg). This process becomes distinct as from the 200 mg class, while in the 230 mg class disappearance of the epiphyseal cartilage was found in more than half the young hares. In the dividing class of lens weight — 275 mg — only 2% of the hares have a perceptible cartilage, but examination by palpation does not permit of distinguishing this group. No cartilage was found in the next classes of lens

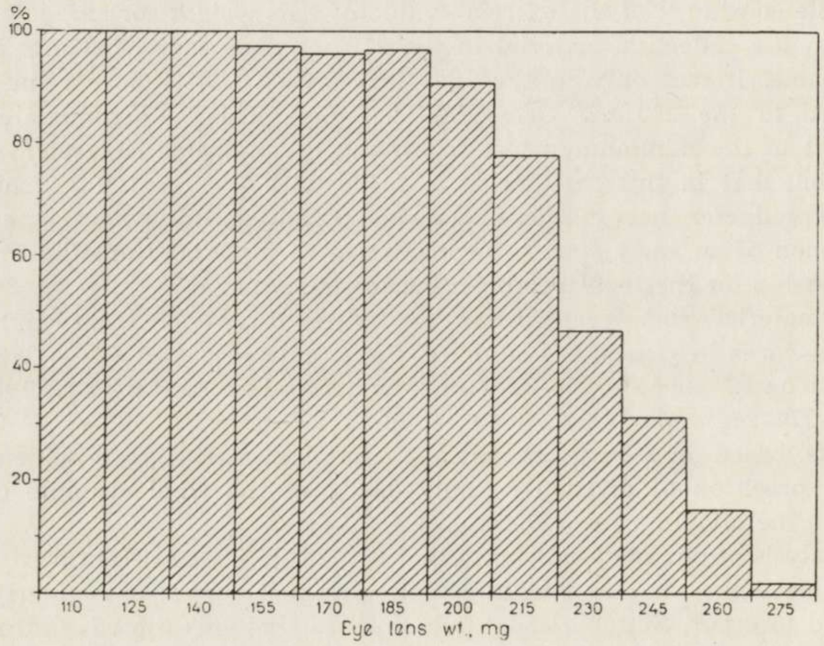

Fig. 3. Percentage of hares with epiphyseal protrusion or epiphyseal cartilage present in lens weight classes.

weight. It would appear that the phenomenon described may be the expression not only of individual variation in the ossification process and closure of the epiphyses, but also of a different rate of growth in different generations (autumn and spring litters). It is unfortunately impossible to present the phenomena described on an absolute age scale owing to the lack of pattern data for the study population.

The error in definitions of age caused by disappearance of the protrusion increases in successive months of the shooting season as the absolute age of young hares increases. In the study material in the second half of the shooting season about 40% of the individuals in the group of young hares (distinguished on the basis of lens weight) were allocated to the »adult« class on the grounds of absence of the epiphyseal protrusion (Fig. 4). An even greater error in distinguishing young hares, attaining 60% in January, was found by Möller (1969). The average error calculated for four consecutive shooting seasons was similar, being about 30%. This can be explained by the very similar arrangement in time of sampling periods in all the study years.

The usefulness of Stroh's method for defining the age of hares has already been critically evaluated by Bujalska *et al.* (1965) and Walhovd (1966). Attention has been drawn by Rieck (1967, cited

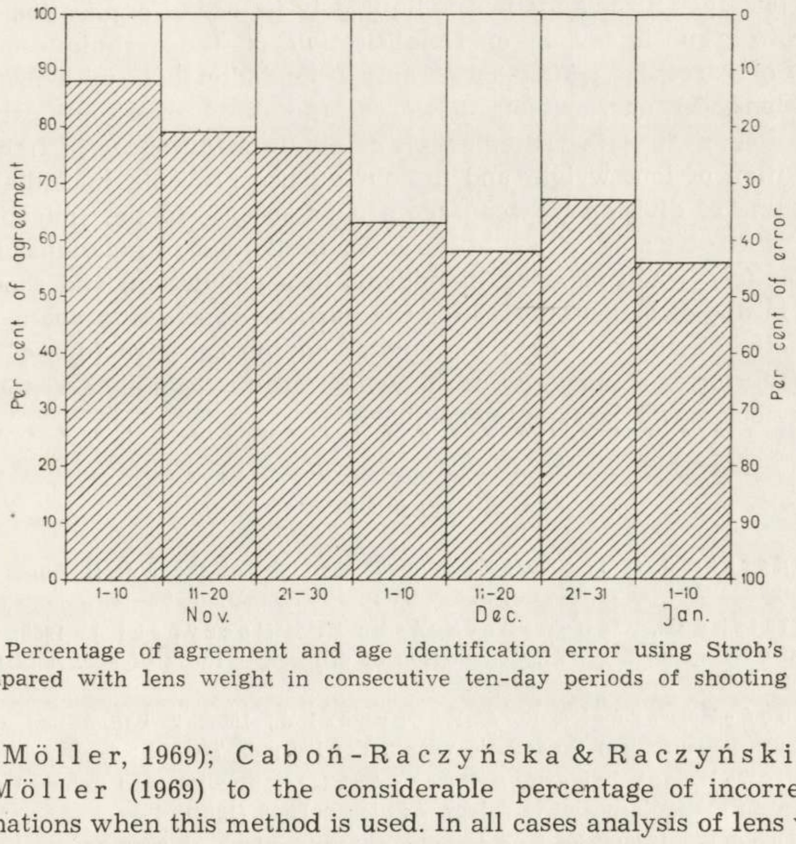

Fig. 4. Percentage of agreement and age identification error using Stroh's method as compared with lens weight in consecutive ten-day periods of shooting season.

after Möller, 1969); Caboń-Raczyńska & Raczyński (1968) and Möller (1969) to the considerable percentage of incorrect determinations when this method is used. In all cases analysis of lens weight has been accepted as a basis for evaluation of Stroh's method.

Under the conditions in Polish hunting-grounds definitions of age made by Stroh's method can be taken as reliable in October, but in November estimation of population growth made by this method may be underestimated by 10 to 20% of the head of young hares (»young«), which are allocated to the »adult« group. Since these divergences depend in each year and each collection on the age structure of young hares, the introduction of any constant corrections of materials segregated by Stroh's method in order to use them for consideration of the extent of increase in young is unjustified.

It should be emphasised that the study material does not in any case throw doubt on the essence of Stroh's method, based on the process of skeletal growth changes. Not a single individual with a protrusion or trace of epiphyseal cartilage was encountered which would be allocated to the »adult« group on the basis of lens weight. At the same time measurement of breadth of the protrusion in young hares was as a rule in reverse proportion to lens mass.

The fact that Stroh's method is limited in its useful application would appear to argue in favour of simplification of the technique of mass studies of hares, by taking lenses only from individuals with negligible protrusions. In our opinion, however, this kind of simplification is undesirable, as it makes it impossible to obtain a full curve of frequency distribution of lens weight and in consequence prevents final definition of the line of division between age groups specific to the collection.

Use of Stroh's method would therefore be limited to individual identifications (*e.g.* when marking individuals in a population) and should be aimed in one direction only — for the purpose of selecting young hares. The method cannot be used for studies on the age structure of populations during the shooting season, which require complete segregation into age classes of the sample examined.

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METODYKA OZNACZANIA WIEKU ZAJĄCA SZARAKA

Streszczenie

Porównywano oznaczenia wieku (podział na 2 grupy: tegoroczne — „juv" i starsze niż rok — „ad") zajęcy odstrzelonych w jednym łowisku w czterech kolejnych sezonach polowań (1966/67 — 1969/70) na podstawie (1) występowania zgrubienia nasadowego lub śladów chrząstki nasadowej na kości łokciowej (cecha Stroh'a); (2) suchej masy soczewki oka.

Stwierdzono, że poprawne wyniki oznaczeń na podstawie ciężaru soczewki oka zależą od techniki postępowania z materiałem. W celu zapewnienia porównywalności oznaczeń zaproponowano następującą metodykę: konserwowanie oka lub soczewki w 10% formalinie (rozcieńczenie stężonego formaldehydu 1 : 3) przez okres 1 tygodnia, suszenie w temperaturze 60—80°C przez 4—5 dni i dosuszanie do stałej masy w temperaturze 100°C, pod zmniejszonym ciśnieniem i przy absorpcji wody (Ryc. 1). Graniczną wartość ciężaru należy określić dla każdego jednorodnego zbioru indywidualnie na podstawie rozkładu frekwencji (Ryc. 2). Wyniki uzyskane tą drogą przedstawiają najdokładniejszy aktualnie do osiągnięcia obraz struktury wiekowej populacji zająca.

Metoda Stroh'a daje zmienny błąd jednokierunkowy (zaniżanie udziału młodych) spowodowany procesem zanikania chrząstki nasadowej u ca 10% młodych zajęcy w listopadzie i 40% w styczniu (Ryc. 4). Dlatego nie może być stosowana w bada-

niach populacyjnych. Kostnienie dystalnej nasady kości łokciowej wykazuje znaczną zmienność: rozpoczyna się przy ciężarze soczewki 155—200 mg, zaś w pobliżu granicznej wartości 275 mg obejmuje 98% młodych (Ryc. 3). Wszystkie zające z zachowaną chrząstką nasadową miały ciężar soczewki charakteryzujący grupę młodych.