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# ON THE POSSIBILITY OF USING ANNUAL BONE LAYERS OF ALPINE NEWTS, TRITURUS ALPESTRIS (AMPHIBIA: URODELA), FOR THEIR AGE DETERMINATION

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Abstract: In the paper there is an account of the age analysis of the population sample of the Alpine newts (*Triturus alpestris* Laurenti, 1768), from the locality of Mladé Buky (NE Bohemia) by means of the method of stating the annual bone layers number. The results of this analysis are completed by ecological and bionomical characteristics of the population and they are also discussed from this point of view. The authors have come to the conclusion that it is possible to determine the age by bone layers in the Alpine newts with the accuracy of ± 1 year.

## INTRODUCTION

The first attempt at the age determination of amphibians according to the bone layers was made by Senning (1940) who also found out the layers in the clarified bones of the *Necturus maculosus* skull. Although the layers were visible in many bones the observing was restricted to parasphenoid as to the most low-laying bone. Senning has disclosed the fact that in young animals the bone layers are spread out in long distances while in adult individuals the distances between layers become much shorter on and after the eight and the layers on and after the 15th—16th were very closely adjacent to one another so that it was no more possible to count them.

It is perceptible after the size curve given by Senning that the growth in the *Necturus maculosus* goes considerably fast up to the attainment of sexual maturity which comes at the age of the seventh to the eighth year of their life. Then the growth becomes considerably slow. The number of layers in parasphenoids of five specimens of the mentioned species with known age was in accordance with the number of years lived through.

On the basis of the results obtained by studying Senning has come to the conclusion that it is possible to determine the ages of *Necturus maculosus* specimens with the exception of the oldest in which the layers corresponding to the last years of their life are so narrow and closely adjacent to one another that counting them is simply not possible.

Schroeder and Baskett (1968), studying the possibility of age determination of the Bullfrog (*Rana catesbeiana*), observed flat bones which were clarified by the light running through and found out annual layers in many of them. The most outstanding picture was shown in the back wing of the os pterygoideum. During the checking on the frogs marked with indi-

vidual marks both authors have stated that it is possible to determine the age after the number of these layers. In spite of it both Senning and Schroeder & Baskett consider as impossible to carry out determining the age by using this method in the oldest animals because in these specimens the latter annual layers are narrow and very closely adjacent to one another and counting them is considerably difficult.

The investigations carried out on Common frogs (*Rana temporaria*) and Common toads (*Bufo bufo*) have shown that it is more advisable to count annual layers in the bones on the transversal sections in the middle of the diaphysis of the tubular bones (including the toes phalangs) stained with Ehrlich's haematoxylin (Klejnenberg and Smirina, 1969; Smirina, 1972, 1974b). In this case layers can be seen much more clearly and counting them, including the oldest animals, is considerably simplified. The attempts using vital stainings of bones — alizarin red S and oxytetracyclin which both leave a lasting picture in the bones — and also the investigations of sections from toes phalangs of animals marked with individual marks have confirmed that in the bones of amphibians collected in the region of continental climate every year one layer really arises. This layer consists of a broad zone of the usual bone tissue which is formed during the period of activity of the animal and of a narrow compact layer which is formed in the period of winter restriction of growth (Smirina, 1972). It is necessary to take into consideration, however, that along with accruing of new bone tissue layers from the periostal side, the formerly arisen bone tissue from the side of endoost which beds the marrow cavity of the tubular bones is resorbed. When determining the age after bone layers it is necessary to determine and take into consideration the rate of this process of resorption in every species. It usually gets slow or stops completely after the attainment of sexual maturity.

The aim of this paper is to clear up the problem of the existence of layers in the bones of the Alpine newts (*Triturus alpestris*) which could be held after the analogy with other amphibians as annual ones and to determine the age after them.

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## MATERIAL AND METHODS

For our investigations we had 40 specimens of adult Alpine newts (20 males, 20 females). The body length from the fore end of the head to the cloacal aperture was 43—58 mm in the females and 40—51 mm in the males. We suppose that these specimens were sexually matured (Roček, 1974). There was a shortage of specimens from the first growth groups.

The material was collected on May 5, 1971, in the pond about 1 km NE from the village Mladé Buky (5 km N from the town of Trutnov, NE Bohemia). This locality is situated on the southern foot of the eastern part of the Krkonoše Mts. (Rýchory). The whole region is orographically included into the geographical unit of West Sudeten (Zoubek, Kunský & coll., 1968). The locality itself is an overgrowing pond about 20 by 30 m large and represents by its nature a typical locality of the Alpine newt in this region. It is situated in the elevation of 490 m.

The climatological, precipitation, snow and phenological characteristics were obtained from long-termed averages (mostly from the period 1901—1950) of measurements of the meteorological station of Trutnov. This station is situated in the elevation of 427 m (the geographical position 50°34′, 15°55′) about 5 km SE from the mentioned locality. Some characteristics were completed by the data of the meteorological station of Žacléř which is situated on the eastern foot of the Rýchory Mts. Its elevation is 604 m, the geographical position 50°40′, 15°56′.

For the study of layers the transversal sections from the middle part of diaphysis of femur were made and then they were stained with Ehrlich's haematoxylin. The bones were preliminarily

Tab. 1. Climatological, precipitation, snow and phenological characteristics obtained by measurements of meteorological stations of Trutnov and Žacléř in the period 1901—1950

| Station | Climatol. character | Average air temperature annual | Average air temperature IV—IX | Average relat. air humidity annual | Average relat. air humidity IV—IX | Mean of annual minimums of relat. air humidity |
|---|---|---|---|---|---|---|
| Trutnov | 6.8 °C | 13 °C | | 82% | | 28% |
| Žacléř | 6.1 °C | 12.2 °C | | — | | — |

| Station | Precipit. and snow character. | Average total of precipitations annual | Average total of precipitations IV—IX | Average total of precipitations X—III | Average number of days with snow-storm | Average number of days with snow cover |
|---|---|---|---|---|---|---|
| Trutnov | | 778 mm | 418 mm | 384 mm | 53.1 | 85.4 |
| Žacléř | | 857 mm | 473 mm | 384 mm | — | — |

| Station | Phenolog. character. | Beginning of apple-trees blossom |
|---|---|---|
| Trutnov | | May 10 |

decalcified in the 5% solution of nitric acid. The sections were obtained on a freezing microtome. The smallest number of visible layers on the sections was 2—3, the greatest was 9—10 (see Table 2).

Considering that among basic lines of adhesion which correspond to the hibernations it is possible to see more or less clearly the supplementary lines of adhesion (Fig. 1a) or owing to the decomposition of the lines of adhesion it is difficult to count the layers. Therefore we think it is right and more suitable in such cases not to try to count accurately the number of layers, because there could be mistakes, but only to estimate approximately their number: e.g. instead of 3 layers only 3—4 etc. It is possible to state in general that the layers in the bones of Alpine newts are well visible and counting them does not make any particular troubles (Fig. 1).

## ECOLOGICAL AND BIONOMICAL CHARACTERISTICS

The locality in which the newts were collected belongs to the mildly cold region with the average number of "summer days" (max. temperature more than 25 °C) 0—10 a year and with the average number of "cold days" (max. temperature less than —0.1 °C) 60—70 a year. The average date of the first "frosty day" (min. temperature —0.1 °C or lower) is Apr. 21 to May 11, the average date of the first day with snow cover is Oct. 21 — Nov. 1, the average date of the last day with snow cover is Apr. 21 — May 1. Further most important characteristics are given in Table 1. The average period of the snow cover duration is a bit longer than it is mentioned in the table because the locality is situated closely to a mountain massif and to large wood complexes. Also other characteristics are influenced in a similar way. It is possible to state that the region considered is situated roughly in the transition between the oceanic climate and the continental one.

The climatological characteristics mentioned also determine the biology of the Alpine newt in this region. The beginning of hibernation comes in

approximately towards the end of October (it is difficult to determine it more accurately because the most of Alpine newts act on the land during that period); the hibernation ends approximately in the middle of April. The beginning and the end of the hibernation is strongly influenced by the course of the weather during the year but it can considerably change from year to year. It is necessary, however, to state in general that the period of hibernation takes roughly about 6 months but it can be also shorter. The period of hibernation can be broken by the periods of activity only at the beginning when short time periods of warm weather can come. These short-time changes, however, cannot influence essentially the formation of the bone tissue.

The period of reproduction comes in practically immediately after coming into water with those specimens which hibernated on the land. Some of the newts, however, also hibernate in water which can be seen from the fact that they act in water already when there is still ice and snow on the shores. The nature of the locality is thus very important. The reproduction itself takes no more than 14—21 days. The newts, however, stay further in water and leave it, earlier or later after circumstances uncleared so far. Therefore the length of the stay in water is considerably varied. Sometimes we can even observe, in the region of Czechoslovakia, that the Alpine newts stay during the whole summer period in water and they do not come on the land. It is still necessary to note that under normal circumstances when newts come on the land males leave water earlier than females.

The period of larval development takes about 4 months (June—September). But if the coming of the winter period is accelerated the metamorphosis can come as late as in the spring of the next year. These cases happen especially in the localities of all regions of Czechoslovakia of higher elevation but they are not abundant.

## RESULTS AND DISCUSSION

Unfortunately, as it was mentioned above we had at the disposal no specimens from the first age-groups so that we cannot state assuredly the rate of resorption of the bone tissue from the side of endoost, but judging by some indirect indications this rate is not great. So on the sections is some specimens it is possible to see around the marrow cavity one line of adhesion which is more or less resorbed. We cannot state if it is the rest of the resorbing line of adhesion which corresponds to the first hibernation or if it belongs to the second one. But we have never observed two lines of adhesion afflicted by the process of resorption in the newts that we have observed, e.g., in Common frogs or in Common toads. So it is possible that even the first line of adhesion in Alpine newts cannot be resorbed completely by the sexual maturity. This presupposition is also confirmed by the fact that the first from the visible lines of adhesion (which is closely situated to the marrow cavity) is regularly inexpressive, incontrast and it has no clear outlines. This is characteristic of the lines of adhesion to the first hibernation as it was already stated in the case of mammals (Klevezal and Klejnenberg, 1967) and as we also observed in other amphibians and reptiles. It is possible (probably in mammals, too) to clear up this fact so that in the first year (but in some species with the slow growth rate — "in first years") either the seasonal variations of the growth rate of animal as a whole or also the opposition of the bone tissue are less sharp than in the following years. In

Table 2. Number of layers in the middle of diaphysis of femur in the Alpine newts (*Triturus alpestris*)

| Number of specimen | Body length in mm | Number of bone layers | Number of specimen | Body length in mm | Number of bone layers |
|---|---|---|---|---|---|
| 1 | 51 | 9—10 | 21 | 50 | 3 |
| 2 | 56 | 7—8 | 22 | 47 | 3 |
| 3 | 52 | 4 | 23 | 42 | 3 |
| 4 | 55 | 5 | 24 | 47 | 3 |
| 5 | 58 | 9—10 | 25 | 47 | 3 |
| 6 | 50 | 5 | 26 | 47 | 5 |
| 7 | 50 | 9—10 | 27 | 44 | 3—4 |
| 8 | 48 | 5 | 28 | 51 | 5 |
| 9 | 51 | 8 | 29 | 47 | 3 |
| 10 | 54 | 6 | 30 | 47 | 6—7 |
| 11 | 49 | 5 | 31 | 44 | 3 |
| 12 | 52 | 5 | 32 | 47 | 4 |
| 13 | 50 | 5—6 | 33 | 47 | 5 |
| 14 | 47 | 8 | 34 | 50 | 9 |
| 15 | 57 | 5 | 35 | 46 | 4 |
| 16 | 43 | 3 | 36 | 43 | 3—4 |
| 17 | 54 | 6 | 37 | 47 | 3—4 |
| 18 | 50 | 8 | 38 | 46 | 4 |
| 19 | 45 | 5 | 39 | 42 | 2—3 |
| 20 | 45 | 6 | 40 | 40 | 3 |

this case owing to the partial retarding of the growth in winter the line of adhesion which corresponds to this hibernation is formed less expressive and in the case of discontinued hibernation double, therefold etc. lines of adhesion are formed. The sharper and longer is the suppressing of the growth, the more expressive is the corresponding line of adhesion.

G. A. Klevezal (1973) distinguishes three basic categories of annual additional layers according to the stage of their expressiveness on the stained sections:

(1) Expressive layers. They are in contrast, easily separated from the supplementary lines of adhesion; counting them is without troubles.

(2) Inexpressive layers. With regard to the little contrast of the lines of adhesion or to the numerous supplementary lines it is almost impossible to state the limit of annual layers and count them.

(3) Layers of the medium expression. This is the transient category where it is possible to separate and count the layers only with difficulty.

From the 40 specimens of the Alpine newt investigated 27 ones had expressive layers (67.5%) and 13 ones had the layers of the medium expression (32.5%). We had no specimens with inexpressive layers which would be impossible to count.

It is perceptible from Fig. 2 that even when there is no sharp dependence of the increasing number of bone layers on the increasing length of the body, there is a certain tendency in that regard. The fact that the biggest specimens are not always the oldest as well need not be surprising. This phenomenon was already observed in reptiles (Smirina, 1974a) and it was also stated and cleared up in fishes (Ricker, 1969).

SUMMARY

Although we had no dated material we can state according to the analogy with other species of amphibians and the nature of the distribution of bone layers that these are annual ones and that it is possible to determine the age after them with the accuracy of ± 1 year.. This accuracy could be higher after the determination of the resorption rate.

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The plate (Figs. 1, 2) will be found at the end of this issue.

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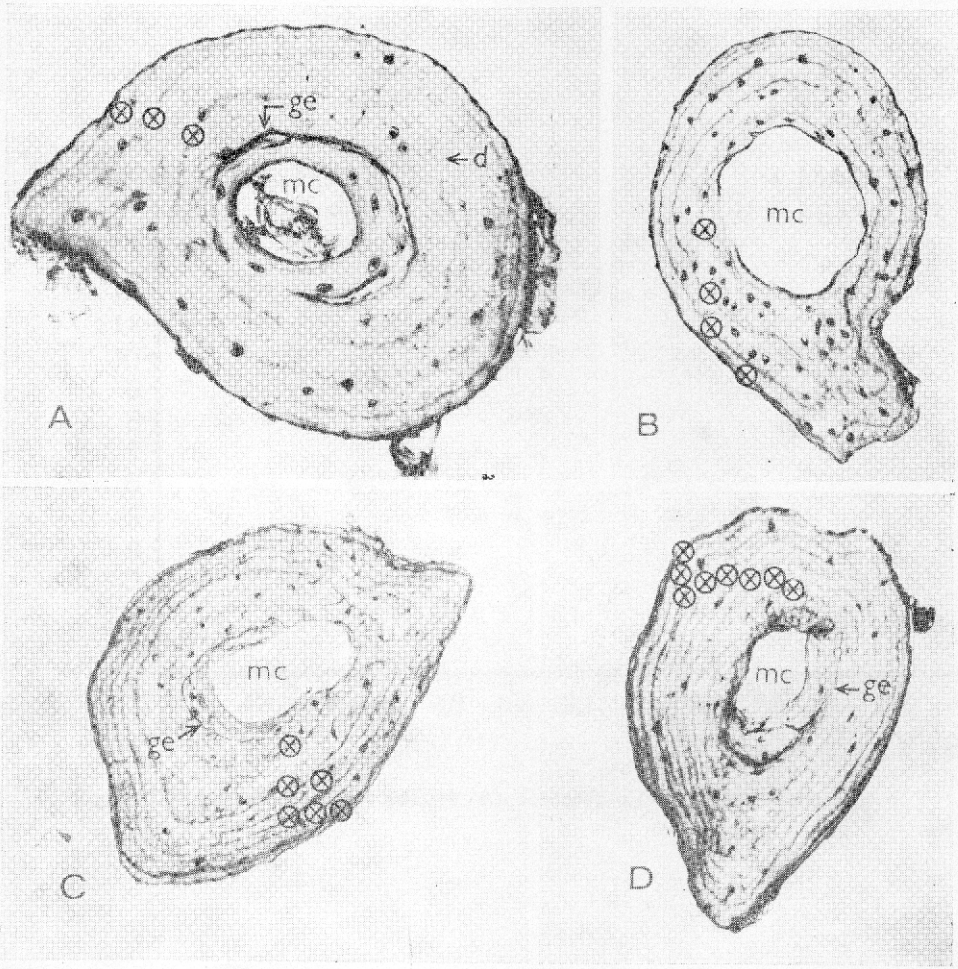


Fig. 1. The transversal sections in the femur of the Alpine newt. A — 3 layers; B — 4 layers: C — 6 layers; E — 8 layers. Abbreviations: ge — the limits of endoostal bone zone; d — supplementary lines; mc — marrow cavity. The layers are marked by crosses.

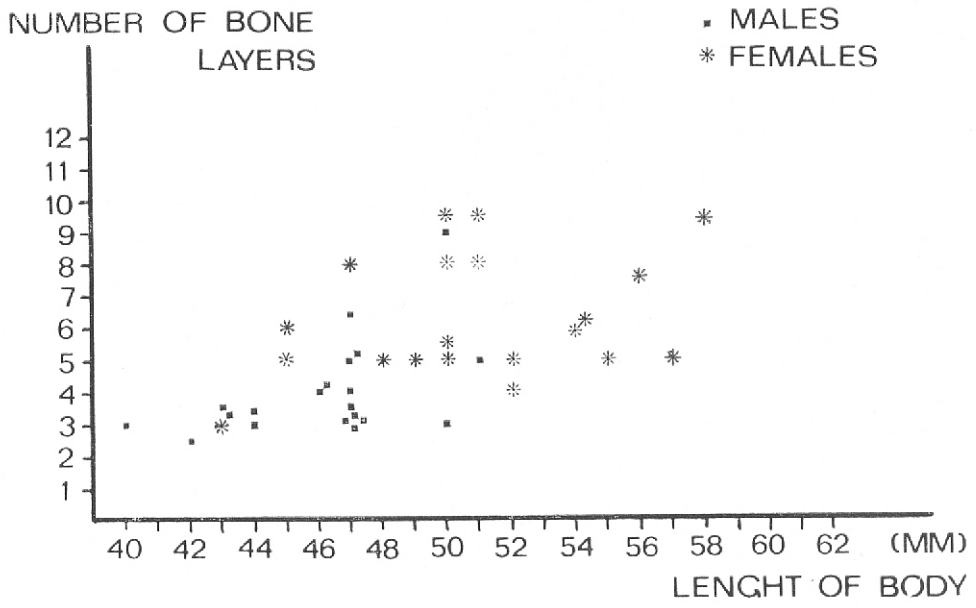


Fig. 2. The dependence of the number of bone layers on the length of the body.