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Research Note

Observations on the Histomorphogical Structure of Some Long Bones of the Water Frog (*Rana bedriagae*) from the İzmir Area

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Abstract: Histomorphological structures of the femur and the second phalange of the second outer toe of the left frog leg were investigated using the decalcifying method in *Rana bedriagae*. Skeleton Growth Marks (SGMs) were clearly observed in *Rana bedriagae* and it was shown that the skeletochronological technique is convenient and applicable for the age determination of this species.

Key Words: Bone, Skeletochronology, Age determination, *Rana bedriagae*.

İzmir Civarindaki Su Kurbağası (*Rana bedriagae*)'nin Bazı Uzun Kemiklerinin Histomorfolojisi Üzerinde Gözlemler

Özet: *Rana bedriagae*'nın femur ve parmak kemiklerinin histomorfolojik yapısı dekalsifikasyon yöntemi kullanılarak incelenmiştir. *Rana bedriagae*'da yaşa bağlı büyüme halkaları bariz şekilde gözlenmiş ve söz konusu tekniğin bu türe uygun ve uygulanabilir olduğu anlaşılmıştır.

Anahtar Sözcükler: Kemik, İskeletkronolojisi, Yaş tayini, *Rana bedriagae*.

Introduction

The age of amphibians can be determined from histological structures in their skeleton. Many studies concerning population dynamics and taxonomical studies depending on morphometrical measurement require the age determination of individual animals.

Several methods have been proposed to assess the age determinaton of amphibians and reptiles. To date, these are eyes lens weight, testis lobation, size–frequency data, mark-release recapture and skeletochronology (1-5). The best method would be to follow each individual starting the from birth by the mark-release recapture technique but such a practice is labor intensive, requiring a very long time to yield results. Moreover, a such technique can not be used with fossil material. The skeletochronology method for individual aging uses biological marks recorded in the hard tissuses. This method is fundamentally different from techniques that use morphometric data (1 -5).

Bone, showing obvious variations and differences during the course of an animal's life, is dynamic tissue that carries their biological records. Seitz (6), who was the first to observe the histomorphology of bone, noted the layered structure of compact bone in connection with growth periodicity. Later on, the works of Wallis (7) on turtles and of Emelianov (8) on snakes connected the number of layers in bone with the age of animals.

Both flat and long bones of the urodeles and anurans can be used to the determine the age of individuals (2-5,10-13). However, it is diffucult to find all the skeleton growth marks (SGMs) in the flat bones; therefore other bones are more suitable for determining the age of specimens. Some long bones such as phalanges in some cases are not useful for age determination because of their immature structure (9-11).

Studies using SGMs for the age determination of some species of urodela and anurans reported that seasonal climate changes have a dramatic effect on the physiological activity of amphibians. These changes particularly affect bone growth, resulting in an active growth period during the warm season, followed by reduced or arrested bone growth during the cold season (4,5,9-12). On the stained cross sections of the long bones, SGMs appear as a concentric zones of periosteal

zone separated by narrow dark rest lines. In the temperate zone, rest lines are formed annually during hibernation and they mark the bone at that particular age (4,5,9-15).

In our work, the histolomorphological structure of the femur and the second phalange of the second outer toe of the left frog leg of specimens of *Rana bedriagae* species were investigated as to whether they are suitable for age determination or not, and to observe the existence of SGMs in these bones.

Materials and Methods

Specimens of *Rana bedriagae* (N:10) were collected from their natural locations in İzmir. The average water temperature is 8-12°C between March and April.

For this study, two different long bones were used: the femur and the second phalange of the second outer toe of the left frog leg. After being clipped and preserved in 70% solution of alcohol, these bones were decalcified in a 5% solution of nitric acid for 4 hours and then common histogical procedures were used (16).

Sections from the central region of the diaphysis of the second phalange and the femur, which were obtained transversely at a thickness of 10-12 µm, were selected for observations and were examined under a light microscope using X40 and X100 magnification for degree of vascularizatons, cellular distributions, and the presence of SGMs.

Results and Discussion

Skeletochronology and bone histology have been demonstrated to be perfect tools for appraising the physiological activity induced in amphibians by seasonal changes. Such changes lead to the formation of skeleton growth marks (SGMs), such as zones of thicker layers of bone laid down during periods of fast osteogenesis, and lines of arrested growth (LAGs) or annuli formed in period when osteogenesis is very slow. A zone followed by a LAG corresponds to a single annual cyle of activity in cold or temperate regions. SGMs have been considered to result from a genetically controlled cycle of growth, strongly synchronized with and reinforced by seasonality. The presence of SGMs, zones alternating with annuli, were expected in the frog populations studied at high altitudes, where climatic conditions impose a cessation of activity during part of the year. The weak expression of SGMs would be expected in frogs from areas with a less seasonal climate (2,9-15,17,19).

In observed specimens, the histological structure of the midshaft diaphysis of both the femur and the second phalange of *Rana bedriagae* was relatively uniform. Stained sections of these bones are nearly circular. The bone matrix is essentially parallel–fibered.

Stained sections of the second phalange were characterized by alternating regions, called growth zones and annuli or lags, which resemble the growth rings of a tree (9,18-19). Annuli the narrower stucturs, refer to LAGs, which formed during the hibernation period of *R. bedriagae* individuals. The other zones, well wider than LAGs, refer to growth zones, which formed during the estivation period of *R. bedriagae* (Figure 1).

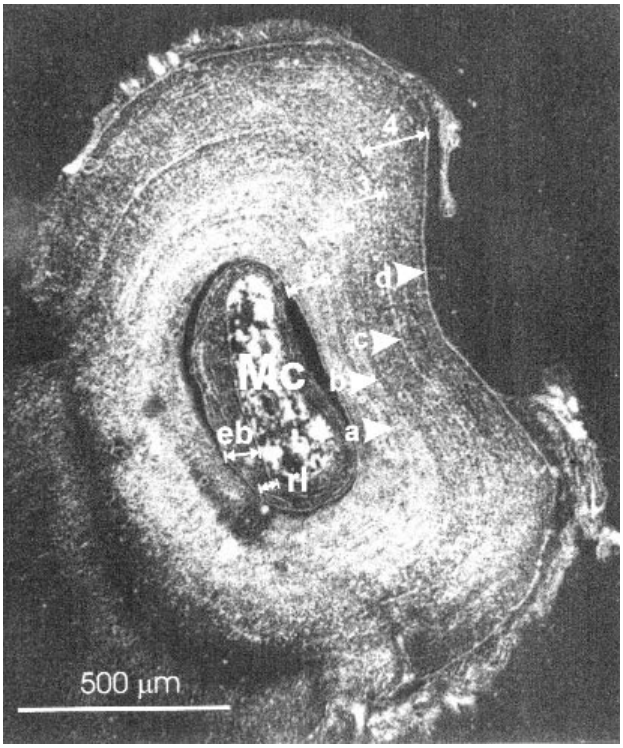

Figure 1. Cross section taken at the middle of the second phalange diaphysis of the second outer toe of the left frog leg of *Rana bedriagae*. Four Lines of Arrested Growth (LAGs) are visible (a-d); Growth zone: 1-4; Mc: Medullar cavity; rl: resorption line.

Since one growth zone and annulus or LAGs are deposited during the course of a single year, the age of *Rana bedriagae* individuals can be determined by counting the growth rings.

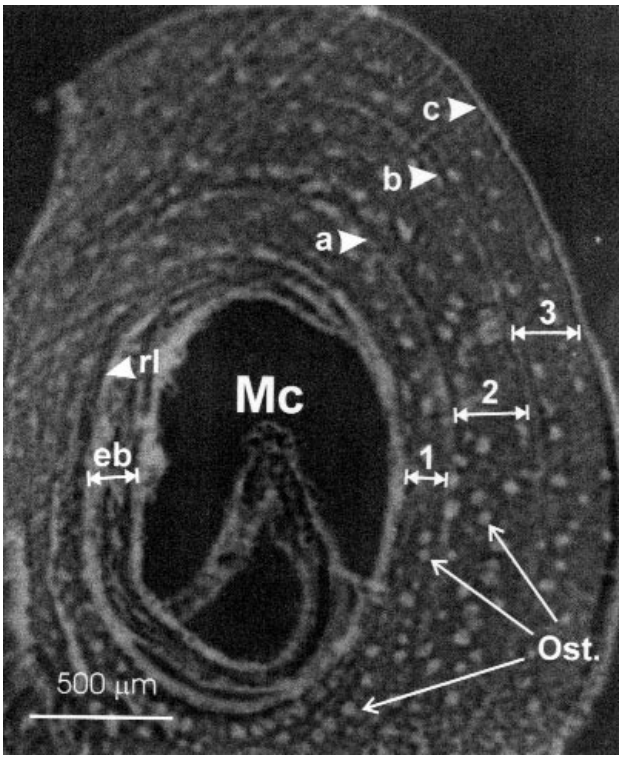

Figure 2. Cross section taken at femur diaphysis of left frog leg of *Rana bedriagae*. Four Lines of Arrested Growth (LAGs) are visible (a-c); Growth zone: 1-3; Mc: Medullar cavity; rl : resorption line, Ost: primary osteon

The histomorphological structure of the femur of *R. bedriagae* is similar to that of its second phalange when compared. SGMs of both bones are mainly formed by the parallel-fibered bone periphery of the bone. Osteocytes are large and abundant and randomly distributed. The structure of long bones of *R. bedriagae* is observed as primary vascular canal, longitudinal type, run in directions essentially parallel to the long axis of the bone. The basic stucture of LAGs is relatively translucent cement and avascularized (Figure 2).

Newly collected data on declining amphibian populations in many parts of the world have caused increased concerns about the negative effects of global enviromental changes on biodiversity (11). Amphibians are thought to be good indicators of environmental stress.

Both the histomorphology of bones in anura species and their individual ages can be determined by skeletochronological investigations. However, flat bones in small species are thin and cause some errors in the determination of SGMs in tranversal sections of bone. The long bones of the limbs are the most suitable part of the skeleton for skeletochronological investigations (9-15,18-20). When compared with previous papers (9-23) the bone stucture of the middle of the diapsis of t he phalange and femur in our samples did not differ from those of the other species of the genus *Rana*.

Therefore, we found that histomorphological structures of the phalange and femur of *R. bedriagae* are suitable for skeletochronological analysis and, of these, the former bone could be used for age determination without sacrificing them.

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