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THE USE OF PHALANGES FOR AGE DETERMINATION IN THE SMOOTH NEWT, *TRITURUS VULGARIS* L.

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Since the early work of Senning (1940), skeletochronology has been used to gain an insight into the population biology of a wide range of amphibians (e.g. Smirina & Rocek, 1976; Gittins, Steed & Williams, 1982; Gibbons & McCarthy, 1983; Caetano, Castanet & Francillon, 1985; Verrell & Francillon, 1986). This technique has been shown to be the most reliable method of ageing amphibians when mark-recapture data are unavailable (Halliday & Verrell, 1988). A pattern apparent in the long bones, of rapid summer growth bands and winter lines of arrested growth (LAGs), analogous to tree rings, has been well documented and proven to be annual by comparison with data from marked animals caught over a number of years (Gibbons & McCarthy, 1983) and also by the injection of fluorescent dyes into captive animals (Caetano & Castanet, 1987).

For the larger amphibians, particularly the anurans, the phalanges are normally examined (e.g. Gittins *et al.* 1982; Gibbons & McCarthy, 1983; Reading, 1991; Flageole & Leclair, 1992; Guarino, Angelini & Cammarota, 1995), precluding the need to sacrifice any animals and thus making the technique compatible with mark-recapture or other ecological investigations. However, the understanding of the population biology of small-bodied amphibians, such as the smooth newt (*Triturus vulgaris*), has been hampered by the need to sacrifice large numbers of animals to extract the humerus or femur. Miaud (1991) investigated the age structure of three species of *Triturus* (*T. cristatus*, *T. alpestris* and *T. helveticus*) using skeletochronology. For the two larger species he was able to examine phalanges, but for *T. helveticus* he found it necessary to utilise the humerus. Guyetant *et al.* (1991), however, did manage to use the phalanges of *T. helveticus* for skeletochronology. Francillon-Vieillot, Arntzen & Geraudie (1990) have used the phlanages to determine age in *Triturus cristatus* and *T. marmoratus*. Halliday & Verrell (1988) suggested that the phalanges of the smooth newt are unsuitable for skeletochronology, being too cartilaginous and subject to remodelling. The immediate problem, however, is the tiny size of the toe bones (0.5 - 1mm in length). This note outlines how both the difficulties of bone structure and bone size have been overcome, allowing the reliable use of the phalanges in the determination of age in the smooth newt.

As part of a three year field study, 167 smooth newts were caught at a small pond (elevation 60 m) and marked by removing the 4th toe of the hind right leg. Animals ranged in size from 71 - 95 mm total length. The removed digits were preserved in 70% alcohol and stored in labelled tubes. In the laboratory the proximal phalanx was dissected from the digit, decalcified for 1 hour in Rapid Decal© and thoroughly rinsed. Because of the size of the bones, it was not possible to rinse them under running water; instead, they were placed in sealed tubes of water and agitated vigorously. The bones were then subjected to a standard wax-embedding procedure (Gabe, 1976), but dehydration and wax-embedding of the bones was carried out by hand, again because of their small size, as automated manipulation by Histokinette© (American Optical, Type E7326) was found to be unreliable, with considerable bone losses occurring during the process. Before wax-impregnation it was found useful to stain the phalanges briefly in Eosin. This made the bones more visible for the final embedding when the tissue needed to be aligned perpendicularly in the wax mould, and also allowed the bones to be seen through the wax when trimming the wax blocks for sectioning.

Serial sectioning of the phalanges showed that the mid-diaphyseal region of the shaft contained the greatest breadth of periosteal bone (in which the growth bands are laid down), and the smallest marrow cavity diameter. Endosteal growth, which can sometimes obscure the earliest growth rings, was also found to be at a minimum in this region. The phalanges were sectioned transversely at a thickness of 10 - 12 μm, heat-mounted onto microscope slides and left to dry for 24 hours. The wax was then removed using xylene and the sections were gradually rehydrated over a period of 4 hours. Harris' Haematoxylin was used to stain the tissue and immersion for as little as five minutes proved sufficient to show up the LAGs. Differentiation with acid alcohol was found to be unnecessary.

While the LAGs in the humerus of *Triturus vulgaris* are known to be laid down annually (Hagström, 1977; Verrell & Francillon, 1986), it was necessary to show that the pattern of haematoxylinophilic lines visible in the phalanges corresponded to the LAGs in the humerus. To this end 10 animals, including two juveniles caught terrestrially, were sacrificed (under licence) and a comparison made between the number of LAGs visible in the humerus and in the phalanges. Table 1 shows that a close approximation was obtained between the two bones.

Endosteal resorption was found to be no more severe in the phalanx than the humerus. In animals older than 2 years of age the first LAG was found to be resorbed to some extent, but normally this remodelling was asymmetrical and some part of the first LAG was always visible, even in the oldest animals. In some phalanges false lines and partial double lines confused the reading, as also reported by Verrell & Francillon (1986) for

TABLE 1. A comparison of the numbers of LAGs recorded from humerus and phalanx bones in 10 newts.

Newt No.	Humerus	Phalanx
1	5	5
2	3/4	4
3	5/6	5/6
4	1	1
5	3	3
6	3	3
7	2	2
8	3	3
9	3	3
10	4	3/4

the humerus of this species. While it was usually possible to come to a conclusion as to the number of true LAGs in these bones by examining a series of sections, 25 (15%) were not sufficiently clear to allow an age estimate - a proportion comparable to the skeletochronological study of the phalanges of *T. cristatus* by Miaud, Joly & Castanet (1993). Of the remaining 142 phalanges, it was possible to give a precise age estimate in all but eight animals (5.6%). For these eight animals an error margin of 1 LAG was employed (e.g. 4/5 LAG; 5/6 LAG).

The youngest animal encountered in the study pond showed three LAGs, while the oldest animal had seven. (A fuller analysis of the demography of this study population is being prepared.) It is suggested that the method commonly used when referring to fish ages i.e. 3+, 4+ etc. should also be used in skeletochronological investigations of amphibians, as it is normally the number of winters an animal has survived that is counted rather than the true age of the animal.

In conclusion, by modifying standard skeletochronological techniques it has proved possible to determine the age of smooth newts by using the phalanges. In 80% of cases a precise age estimate was reached, in a further 5% an error margin of 1 LAG was necessary; and for 15% of the sample it was not possible to make an estimate. It is envisaged that this adapted technique could be applied to all small-bodied amphibians as it is compatible with mark-recapture and other ecological investigations and it removes the need for large scale sacrifices as required in humerus-based age determination.

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