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Use of Skelettochronology in Age Determination of American Toads (Bufo americanus) from East Central Illinois

Philip M. Acker
Eastern Illinois University

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Use of Skelettochronology in Age Determination of American Toads (Bufo americanus) from East Central, Illinois

(TITLE)

BY

Philip M. Acker

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Abstract

A skelettochronological technique was applied to the phalanges of the American toad, Bufo americanus, from east central, IL. In hematoxylin-stained cross-sections of the phalanges, suspected year rings are formed which can be used to age individuals. This method allows for aging without destructive sampling of toad populations.

A majority of the male toads from two choruses were estimated to be 2 or 3 years old, while most of the females from the same two sites were in the 3 or 4 year age class. Females were larger than males in the 3 and 4 year age classes, and overall, female length was found to be greater than male length. Considerable overlaps were found between the lengths and estimated age classes of both male and female toads. Because of the large variance of size within an age class, it appears that it is not possible to assume that a larger individual is necessarily older.

Acknowledgments

I wish to thank R. Gawlik and Dr. K. Kruse for their help in field collections, Dr. A. Hemelaar for help with the initial technique refinement, and Dr. J. Speer for assistance in photographing my thesis material. To my family and special friends, I am grateful for your continual love and understanding. I am also indebted to my graduate committee, who made this thesis project possible and who have given me valuable insights into the realm of biology.

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Introduction

During the past decade, there has been a renewed interest in sexual selection studies. Anurans appear to be an excellent vehicle for sexual selection research, and bufonid species in particular have received wide attention (Davies and Halliday, 1977, 1979; Fairchild, 1981, 1984; Gatz, 1981; Gittins et al., 1980; Hillis et al., 1984; Kruse, 1981, 1982; Kruse and Mounce, 1982; Licht, 1976; Sullivan, 1982, 1983; Wells, 1977; Wilbur et al., 1978; Woodward, 1982 a, b). Much of this research demonstrated that larger male toads participate in a disproportionate amount of the mating because females prefer the calls of larger males (Fairchild, 1981; Wilbur et al., 1978) or because larger males can displace smaller males in amplexus (Davies and Halliday, 1977, 1979; Gittens et al., 1980; Kruse, 1982; Licht, 1976). In general, it has been assumed that larger individuals are older because toads are indeterminate growers.

In studies of sexual selection and population dynamics, the need for accurate methods of age determination are well recognized. As previously mentioned, toads are indeterminate growers, therefore, aging individuals by visual methods is problematic (eg. small=young, large=old). The use of bone material in which layered structures are formed (see Peabody, 1961)

1968); the long bones of Rana temporaria (Smirina, 1972); and the phalanges of Bufo bufo (Hemelaar and van Gelder, 1980).

The application of skelettochronology is possible only if the amount of resorption of bone due to "remodelling" can also be determined. According to Ham and Leeson (1961), as new layers of bone are added to the outside of the shaft of a long bone during the growing period, there is a natural resorption of bone from the inside that must also occur in order for the bone to increase in diameter. Smirina (1972) found that in the phalanges of Rana temporaria from Russia it is always the first two resting lines that are effected by bone resorption. On the other hand, Hemelaar (1981) found the degree of resorption in Bufo bufo differs between individuals, bone used, and the climatic area in which the specimen was collected. The range of ring resorption varied in B. bufo phalanges from 0 to 2 rings.

Because of the vast amount of sexual selection research being applied to bufonids, especially Bufo americanus, a technique for aging individuals without destructive sampling is needed. Consequently, the purpose of this thesis project was to determine: (1) if the age of American toads, Bufo americanus, could be ascertained using a skelettochronological technique similar to that of Hemelaar and van Gelder (1980), and

(2) the association, if any, between the size of the toad and the age of the toad as found in (1).

Material and Methods

Between April 13 and April 25, 1983, approximately 300 American toads (Bufo americanus) were collected at a spawning site located about 8 km southeast of Charleston, (Coles Co.) IL. All toads were weighed to the nearest 0.1 gm and snout-vent lengths (SVL) were estimated to the nearest 1.0 mm. The fourth phalange of the left hind limb was removed from each toad and separately stored at -20°C in the laboratory. After marking all individuals by toe-clipping (Fig. 1A), the toads were released the same night at the edge of the pond.

The males were divided into five weight classes (<20.0 g, 20.1-24.0 g, 24.1-28.0 g, 28.1-32.0 g, and > 32.0 g) and a random sample of six individuals was selected from each class for age determination. Similarly, the females were divided into five classes according to their SVL (<67 mm, 67-70 mm, 71-74 mm, 75-78 mm, and >78 mm) and six individuals were again randomly selected from each class.

To prepare the toes for sectioning, they were first placed in 1-2% trypsin for 5 hrs (Hemelaar, 1981). The soft tissues were then fleshed by hand and the bone was decalcified in RDO decalcifier (Du Page Kinetic Laboratories, Inc.) for approximately 2 hrs. Hemelaar

and van Gelder (1980) report a histological technique for sectioning the phalanges using a freezing microtome. Not having access to this type of equipment, the tissues were sectioned using a standard rotary microtome (American Optical "820"). After experimenting with the dehydration and staining procedures, the following methods were found to give the most satisfactory results.

The decalcified bone was then left in distilled water for 24 hrs and dehydration of the bone was completed by running it through successive isopropynol stages of 50, 70, 85, 95, and absolute percents for approximately 1.5 hrs in each.

Tissues were infiltrated with successive paraffin changes for 6 hrs in a paraffin oven at 63°C. The tissues were then embedded in small paraffin blocks and allowed to harden overnight. The embedded tissues were attached to wooden blocks for sectioning. Cross-sections through the diaphysis of the proximal phalangeal bone (Fig. 1B) were cut at 15 μm intervals using a microtome knife.

The sections were attached to slides using albumen and left on a warming tray 6-8 hrs. To prepare the slides for staining, they were hydrated through a series of alcohol steps, which included two minutes in each of: two Xylol steps, absolute, 95, 85, 70, 50, and 35 percent isopropynol steps, and water. The slides were

stained in Delafield's hematoxylin for 30-35 minutes. Dehydration was completed by running the slides through the reverse order of the above alcohol steps. Sections were protected using glass coverslips and mounted in Permount.

Sections from the middle of the diaphysis (see Hemelaar and van Gelder, 1980) were examined using a light microscope at 100X to 400X magnification. These sections were also examined by the concensus agreement of three individuals using a projecting microscope (Leitz: Neo-Promar); this proved to be the preferred method of examination. The age was determined by counting the number of resting lines; the outer margin of the bone was also considered a resting line (Smirina, 1972, as cited by Hemelaar, 1981).

On April 26, 1984, seven amplecting pairs of American toads were collected from a semi-permanent pond located 5 km east of Charleston, (Coles Co.) IL. After the females had oviposited in the laboratory (except for one pair), all specimens were weighed and measured, killed in an ether jar, and separately stored at -20° C in the laboratory. Both the right and left pterygoid bones were removed by dissection, boiled in water for 60 sec, fleshed by hand to further clean them, and separately stored. The fourth phalange from the left hind limb was also removed for a comparison age determination study by the method previously noted.

The pterygoid bones were placed in toluene for 20 minutes and then soaked in mineral oil after the toluene had evaporated. Since McAuliffe (1978) found this method helped to illuminate growth annuli in bullfrog pterygoid bones, this method was applied to the pterygoids of Bufo americanus used in this study. Annuli were inspected using transmitted light from a dissecting microscope according to the method of Schroeder and Baskett (1968) for bullfrogs.

Results

The projecting microscope was used for group examination of the sections and the age of all toads was determined to a particular year. Resting lines were not always distinct, especially near the outer bone margins, and some sections may have been underestimated with respect to age. Figure 2A-C shows photographs taken of sections from three representative age classes.

Although resorption of initial resting lines has been a problem (Hemelaar and van Gelder, 1980; Smirina, 1972), it did not cause difficulty in this study. In only 4 of 73 (5.5 %) specimens did it appear that resorption had obscured the first resting line. This estimation was based on the large marrow cavities of those specimens and the diameter of the first resting line from individuals with little or no resorption. In no sections was the second resting line effected by resorption. The appearance of false annuli and double lines did not cause any serious problems (Fig. 2D), and these were treated according to the methods of Hemelaar and van Gelder (1980).

Results from the analysis of the male phalanges used from both collecting sites, indicate that there is a considerable degree of overlap of the lengths (Fig. 3) and the weights (data not shown) between the various age

classes of the males. A one-way analysis of variance suggests that there is no statistical difference ($F=0.86$; $df=2,33$; $P=0.566$) between male length in the three age classes. The majority of these males were estimated to be 2 and 3 yrs old (34/36 = 94.5%), with a mean age of 2.6 yrs for the individuals used in this study. When the age determined for females from both collecting sites was compared to SVL, an overlap between age classes and length was again noted (Fig. 3) and there was also no evidence to suggest a statistical difference ($F=1.00$; $df=3,33$; $P=0.406$) between female length in the four age classes. No age and weight comparison could be made between female toads because some individuals had oviposited during collection procedures. Females were mostly estimated to be in the third and fourth year age classes (26/37 = 78.4%), with a mean age of 3.5 yrs for those individuals used. Percentages of individuals in the various estimated age classes in this study differ from those of Hemelaar (1983), who found that the majority of male Bufo bufo were in the 4 to 6 yr age classes, while females were mostly in the 6 yr age class.

Statistically, there was no difference between the lengths of Bufo americanus males and females in the 2 yr age class ($F=0.57$; $df=1,18$; $P=0.533$). There was, however, a statistical difference between the lengths of males and females in the 3 yr age class ($F=15.68$;

df=1,33; P=0.001) and in the 4 yr age class (F=8.78; df=1,13; P=0.011); the lengths of females being greater in both cases. When the lengths of all males were compared to the lengths of all females, females were statistically larger (F=24.85; df=1,71; P<0.001).

Analysis of the pterygoid bones showed possible growth annuli in only 8 of the 13 paired pterygoids (one pair was unusable)(Table 1). Of these 8 pairs, only two pairs showed the same number of annuli in both pterygoid bones. Age determination by use of the phalanges from these toads did not agree with the results of the pterygoids in any instance, except for the left pterygoid of toad 6 (Table 1).

Discussion

The use of skelettochronology appears to be a valuable tool as an aging technique for Bufo americanus. Although the rings found appear to be annual growth marks, this cannot be substantiated until known age toads are available. Unfortunately, known age toads were not available for this study. I was also unable to recapture any of the marked population during the following breeding season (April, 1984). Thus, it is not known for certain that new growth rings are added each year in B. americanus, but it is suspected that this toad would act similar to that of B. bufo (Hemelaar, 1981) in this respect because of the similarity of the climate (i.e. temperate, with a distinct winter that would cause torpidity and growth cessation).

It is apparent from my results that a considerable degree of overlap exists between what are apparent age classes and length (Fig. 3). This observation was also noted by Hemelaar (1983) in the age classes of Bufo bufo. Although Hemelaar found statistically significant differences between the lengths and age classes of both male and female B. bufo, there was no statistical difference between these components in B. americanus. On the other hand, except in the 2 yr age class, there was a difference between male length and female length

in the 3 and 4 yr age classes, and an overall difference in length between males and females. In this respect, B. americanus was similar to B. bufo (Hemelaar, 1983).

Because of the large overlap between length and suspected age of both males and females, it is not possible to estimate age merely by the length of the toad. Since toads are indeterminate growers, they will tend to increase in length and weight as they get older. The overlap observed in this study could be a result of ecological and/or genetic factors; not all toads of the same age class will grow at the same rate as their conspecifics. As a result, there exists a large variance in size within a single age class with some individuals surpassing those of older age classes in size parameters.

Another possible explanation for the overlap between age classes may be related to the fact that Coles Co., IL is at the northern edge of the intergrade zone of the two subspecies of Bufo americanus. Smith (1961) notes that the range of the prairie form of the American toad (Bufo americanus americanus) extends southerly through Coles Co., while the intergrade zone between this form and the dwarf form (Bufo americanus charlesmithi) extends northward into this area. The subspecies charlesmithi is somewhat smaller in length and less common than that of the subspecies americanus in this area (Smith, 1961). Therefore, it is quite

possible that my sample, used for age determination studies, contained members from both of these subspecies and possibly included intergrades between these two apparently genetically distinct forms. This would result in individuals of the same suspected age class having a wider distribution with respect to length.

Schroeder and Baskett (1968) state that their technique for aging bullfrogs by use of the pterygoid bones might be applied successfully to anurans with shorter life spans than that of the bullfrog. The findings of this study, however, indicate that the use of the pterygoids from Bufo americanus as an alternate or comparison method of age determination does not appear to be useful. Ages determined in this pterygoid study do not approximate the estimated ages found in the analysis of the phalanges of the same individuals (Table 1). It may be noted that an alternate form of illuminating annular marks was attempted (wetting the pterygoids with 30 percent ethanol--Schroeder and Baskett, 1968), but this method gave even less definitive results than the toluene/mineral oil method. It is possible that other alcohol mixtures may result in more definitive readings of the pterygoid bones.

In conclusion, my aging results suggest that larger toads are not necessarily the older individuals in the population. Wilbur et al. (1978) suggest that females may prefer larger toads because they are older and,

therefore, more "nature tested." My results suggest, however, if larger males are in fact preferred by females, it is not age related, but rather resource accrual related.

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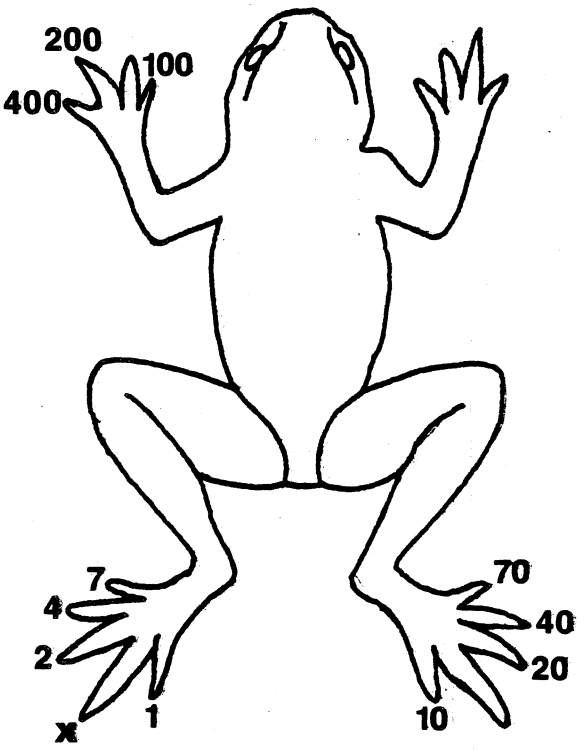
Fig. 1. A. Dorsal view of toad showing toe-clip numbering scheme used for identification (after McAuliffe, 1978). (X) indicates phalange clipped for study. B. Skeletal view of left hind foot. Shaded phalageal bone was that used for age determination.

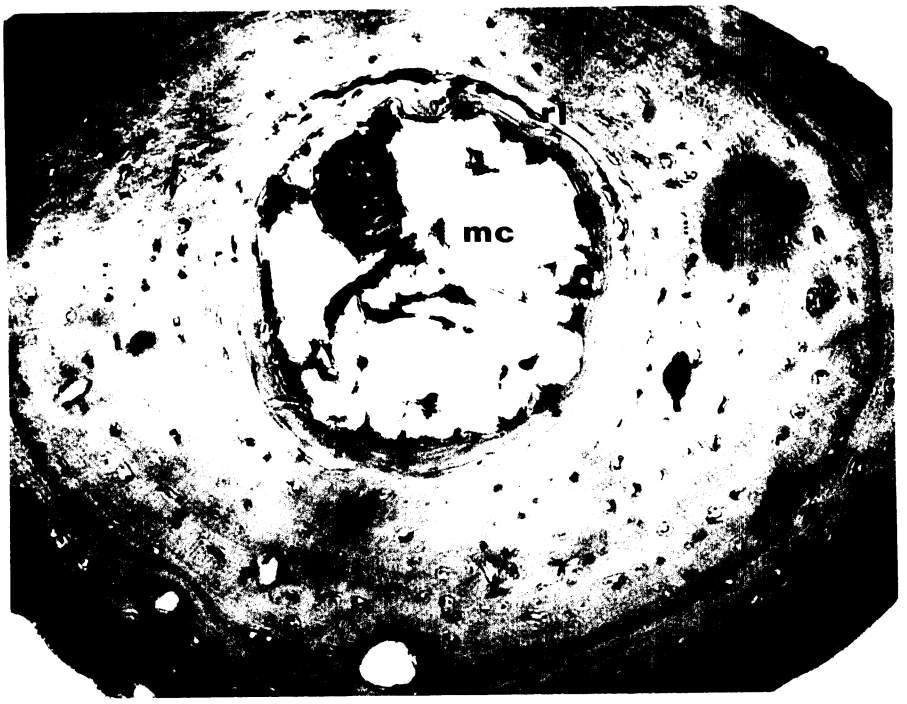


A

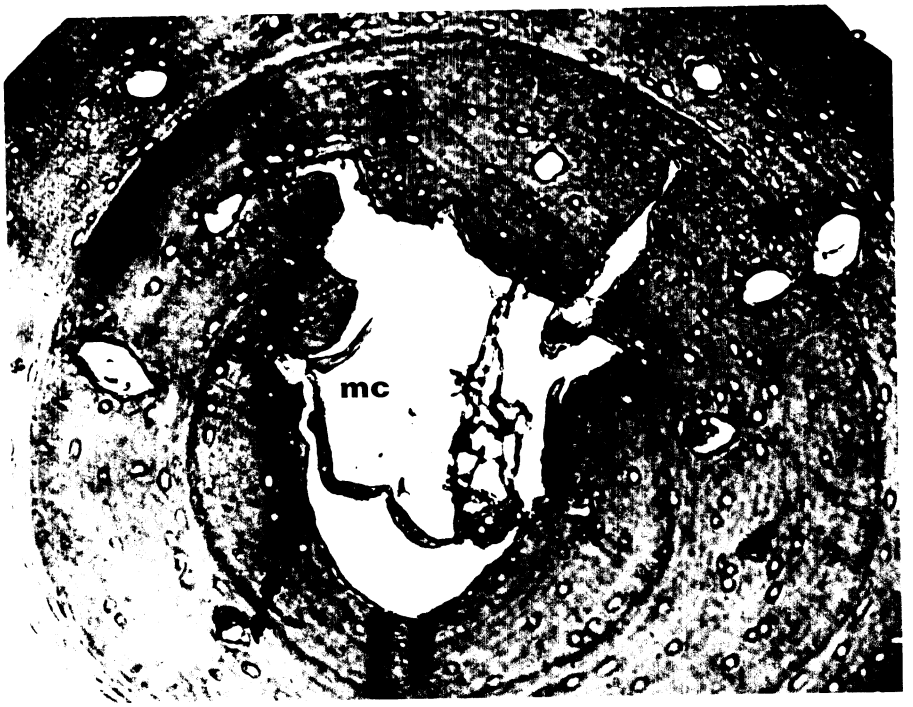
B

Fig. 2. Hematoxylin-stained cross-sections of the proximal phalangeal bone of Bufo americanus. Numbers refer to resting lines; mc = marrow cavity; rl = resorption line. A. Male from 2 yr age class. B. Male from 3 yr age class. C. Female from 4 yr age class. D. Male from 3 yr age class with a double line (dl) seen in the first resting line.

A



B



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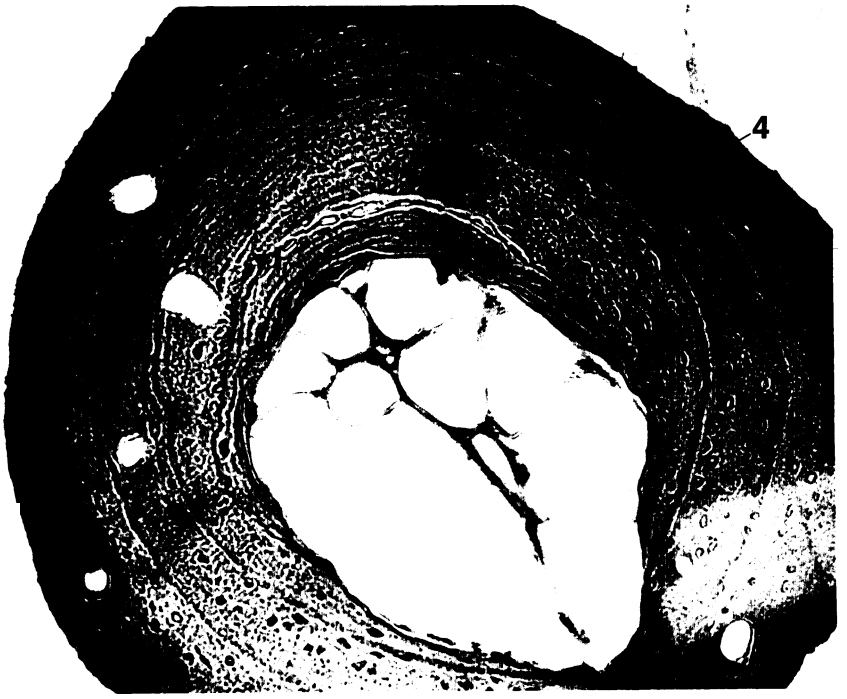

D

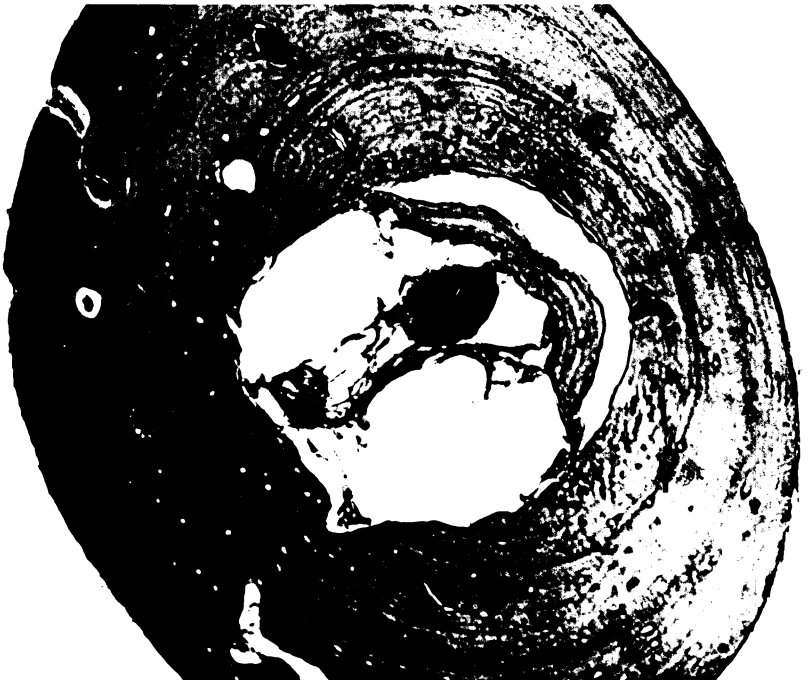

Fig. 3. Mean length (cross), standard deviation (column), range (bars) and frequency (number in parenthesis) of the toads used for age determination from both collecting sites. Males are indicated by open columns, females by shaded columns.

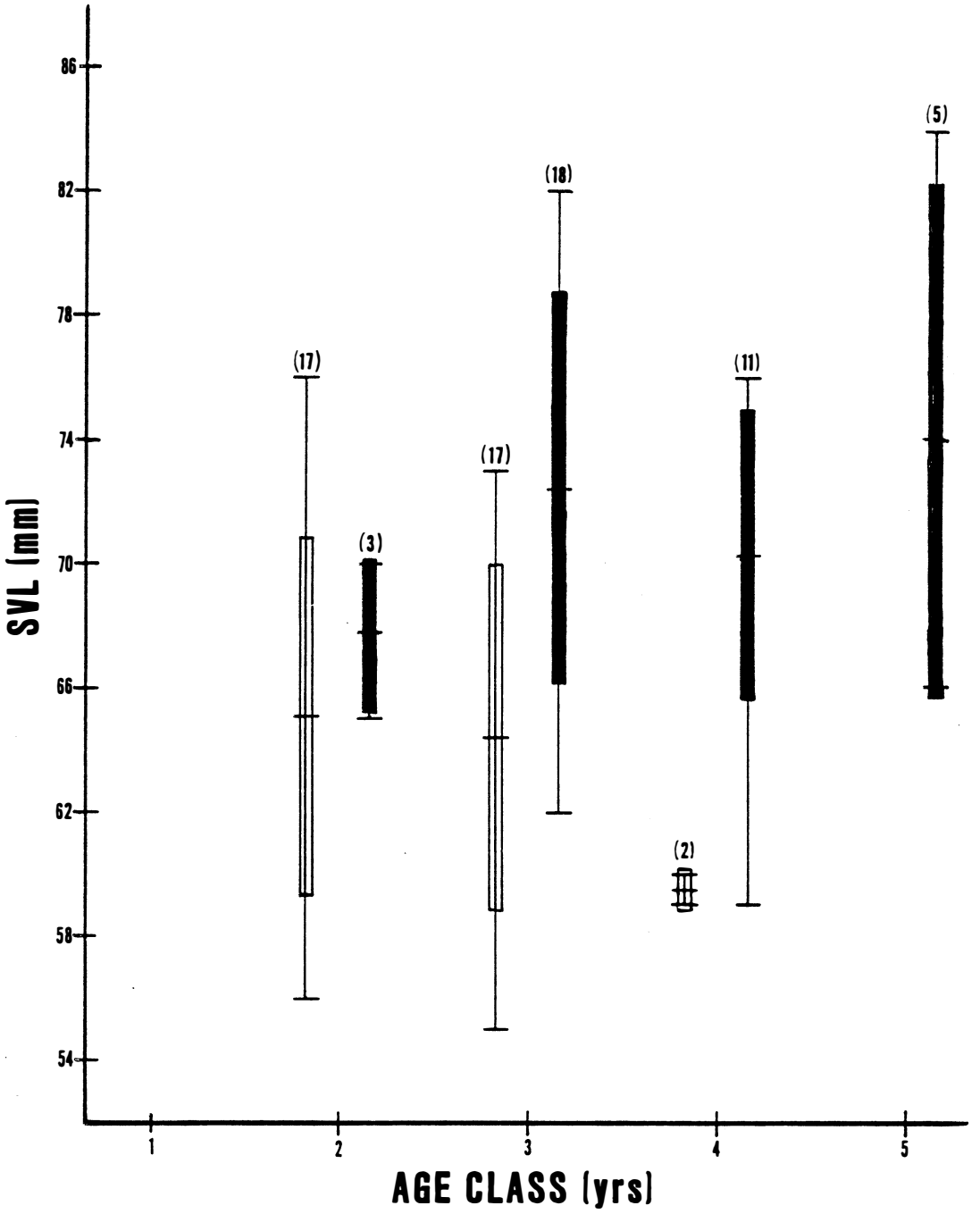
86
82
78
74
70
66
62
58
54
SVL (mm)
(17)
(3)
(17)
(18)
(2)
(11)
(5)
1
2
3
4
5
AGE CLASS (yrs)

Table 1. Comparison of estimated ages by the two skelettochronological techniques used on amplectant pairs of toads from a semi-permanent pond located 5 km east of Charleston, (Coles Co.) IL. L = left pterygoid; R = right pterygoid; * = unusable bone tissue; 0 = no discernable annuli.

Toad No.	Age Determination Technique		
	Pterygoid		Phalange
	L	R	
1	3	3	5
2	1	0	2
3	0	1	4
4	0	1	3
5	2	1	5
6	2	0	2
7	*	*	5
8	0	0	3
9	0	0	4
10	0	0	*
11	0	0	4
12	0	1	4
13	0	0	4
14	1	1	3