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Age Structure of a Spadefoot Toad *Pelobates fuscus* (Pelobatidae) Population

CHRISTOPHE EGGERT AND ROBERT GUYETANT

Age structure of a *Pelobates fuscus* population portion, which visits ponds during the 1996 breeding period, was studied using skeletochronological methods. The studied population lives in northeast France, in the western border of the species area. The age distributions were strongly different between the sexes : males were represented mainly by the two_ and three-years-old individuals, when females were older. A lack of three-years-old migrating females was observed and discussed.

THE holartic Pelobatidae species (genus *Scaphiopus*, *Spea* and *Pelobates*), are fossorial and adapted to sandy places (Duellman and Trueb, 1986). Although in North America *Scaphiopus* and *Spea* species inhabit xeric environments (deserts and grasslands ; Newman, 1989; Feder, 1992), the spadefoot toads, *Pelobates fuscus*, live in steppic zones of Europe, which sustain high thermic contrasts with very cold winters (Nöllert, 1997).

Although the populations of *Pelobates fuscus* are declining in most places where they are found (Nöllert, 1997), very little is known about the population biology of this species. In France, it is one of the rarest and most endangered Anurans, and population numbers decreased dramatically during this century (Lescure, 1984). Recent long-term studies point out the very high level of fluctuation (up to 90%) in population sizes (Jehle R. et al., 1995; König and Diemer, 1995; Wiener, 1997), which can decrease to near-extinction. The origins and processes of these fluctuations are unknown, and precise knowledge on demographic traits is needed for the analysis of population dynamics.

The purpose of this study is to determine, using skeletochronological methods, the age structure, the fraction of a population which visits ponds during the breeding period. Skeletochronological analysis is now a reliable increasingly used technique to determine age structure in amphibian populations (see Castanet and Smirina, 1990; Smirina, 1994; Caetano and Leclair, 1996). Applied to phalanges, it is a powerful method for assessing growth (distance between two successive Lines of Arrested Growth, LAG), age of sexual maturity, longevity and periodic or aperiodic annual activity (Castanet et al., 1992). The need for only a finger to observe the LAGs in phalanges cross-sections is important in conservation biology, particularly with small populations, because it avoids having to kill individuals. Moreover, the tissue (skin and muscle)

removed with the bone can be used for genetical analyses (Gonser and Collura, 1996).

MATERIALS AND METHODS.

The breeding site, in northeast France within the Sarre River watershed (49°8'N, 6°4'E), includes four temporary ponds located very close to each other (less than 10 meters between any two), in a sandy area (255m elevation). The surrounding 50 ha of moors were isolated by a highway and an industrial area. The surface area was 20 m² (depth: 40cm), for the smallest pond, and 5000 m² (depth: 150 cm), for the largest, at maximum water level. There were no other known breeding ponds in the region. Spadefoot toads were caught during their nocturnal breeding migration in 1996 using a drift fence with pitfall traps that surrounded the breeding ponds (see Gibbons and Semlitsch, 1982). The traps were inspected every morning from 13 April (after a long period of frosty nights), to 14 July (50 days after final oviposition). PIT tags (Passive Integrated Transponders) were used for individual identification, and the second phalange of the third toe of the right forelimb was removed. Secondary sexual characters were used to identify males and females. Individuals without any secondary sexual characters were recorded as juveniles. Then after registration, animals were released on the opposite site of the fence straightaway.

For the skeletochronological analysis, we used the phalanges of the 118 individuals who stayed for more than one day inside the encircled area (87 males, 29 females, and 2 juveniles). Few adults were mating because only six layings were found in the ponds. Standard techniques (Leclair and Castanet, 1987; Miaud et al., 1993) were used. Preserved in 70% alcohol, the phalanges were cleared of muscles and skin and the bones decalcified in 3% nitric acid for 8 h. After 12 h

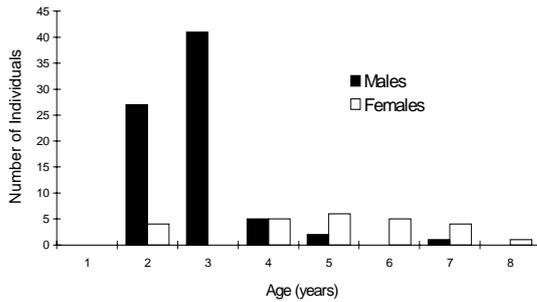


Fig. 1: Age distribution in the *Pelobates fuscus* population.

washing under running tap water, the diaphyseal portion was cross-sectioned at 14 μm with a freezing microtome. The sections were stained with Ehrlich's hematoxylin, mounted between slides and observed under a light microscope. Resorption of the first Lines of Arrested Growth (LAG) linked to endostal cavity growth was estimated using comparisons with the rest lines of recently metamorphosed individuals and with phalanges showing a low level of resorption (metamorphosis line and first rest line still visible).

RESULTS

Age estimation was possible in 86.2% of the females ($n=25$) and in 87.3% of the males ($n=76$) and for the two juveniles. Because spacing between the outermost LAGs in the oldest individuals became smaller and sometimes difficult to distinguish, or because resorption level was sometimes hard to estimate, age was estimated with an error rate of one year in 23 cross sections (22%). We never observed any inflammations in 116 recaptured toe-clipped individuals, over a period of 2 to 119 days after marking. Age of mature adults ranged from two to seven years for the males and two to eight years for the females (Fig.1). Mean estimated age was 2.8 (SD = 0.8) years old for the males and 5.0 (SD = 1.7) years for the females. The mode was 3 for males and five for females. The age distributions were significantly different (Mann-Whitney U -test, $U = 296$, $P < 0.0001$). Both juveniles were two years old. Males were represented mainly by two- and three-year-old individuals, which constituted 89.5 % ($n = 68$) of the total, whereas these age classes represented only 16% ($n = 4$) of the females. Moreover, the sex ratios of the sampled population (3.04:1) differed significantly from 1:1 in favor of the males ($\chi^2 = 25.75$; $df=1$; $P < 0.00001$). This bias was mainly due to two- and three-year-old males, and the sex ratio was biased in favor of the females (0.38:1) for the oldest age group (four to eight years old combined, $\chi^2 = 5.82$; $df = 1$; $P < 0.02$).

DISCUSSION

A long-term study in Vienna (Austria) with recapture data for *P. fuscus* showed that females reached sexual maturity at the earliest age of two and males at one, but the majority of males came to the breeding site at two and females at three years old (Wiener, 1997). In the French population, earliest age at maturity was at two years old in both sexes. The eight-year-old female was caught again in 1997, so reached the age of nine. Population size was strongly influenced by two- and three-year-old males, that is, recruitment occurring 2-3 years before our sampling. In Austria, adult population size was mainly dependent on the number of metamorphs two years before (Wiener, 1997). In the same way Artzen and Teunis (1993) with *Triturus cristatus* and Semlitsch et al. (1996) with *Ambystoma opacum*, *A. tigrinum*, *Eurycea quadridigitata*, *Pseudoacris nigrata* and *P. ornata* observed that adult population size depended on the number of metamorphs one and two years earlier. In *Bufo calamita*, Banks et al. (1993) found that breeding female population size depended on success of metamorphs three years before. Similar dominance of a cohort was observed by Gibbons and McCarthy (1984) in an Irish population of *Rana temporaria*. Life history traits, such as longevity, adult survival rate, and fecundity, and migrations to and from other breeding sites can reduce the level of fluctuation in breeding population size (Miaud et al., 1993; Berven, 1995; Semlitsch et al., 1996). Because of these large variations, adult survival rates cannot be estimated from an age table, but only from mark-release-recapture methods (Gibbons and McCarthy, 1984).

A predominance of males is common in anura breeding-part populations (Duellman and Trueb, 1986) and has already been observed in *Pelobates* (Nöllert, 1990; Lizana et al., 1994). In this study, the sex ratio was skewed because of the two- and three-year-old males and more specifically because of the lack of three-year-old females. A delayed maturation in females, in comparison to males, was proposed to explain the deficit of two- and three-year-old individuals in a *Rana temporaria* population (Gibbons and McCarthy, 1984), and *Rana sylvatica* (Berven, 1990). This could probably explain the low number of two-year-old females but probably not the absence of three-year-old females. The hypothesis of a four-year maturation period is not in agreement with the interpretation of skeletochronological patterns that supports a three-year maturation. Moreover, the females caught and aged in 1997 ($n=34$) did not metamorphose in 1993 (unpubl. Data). The lack of three-year-old migrating females in this study could result from their dispersal or a skewed sex ratio at metamorphosis (primary sex ratio).

Distorted primary sex ratio is not well documented in amphibians (Dournon et al., 1990). They occur in some complexes of hybridogenetic frogs (see Schmidt, 1993), and environmental sex determination, particularly temperature-dependant sex determination (TSD), occurs in several species (e.g. *Rana temporaria*, *Pleurodeles waltl* and *P. poireti*). In the laboratory, normal ambient temperatures lead to a balanced sex ratio, whereas extreme temperatures result in a biased ratio (see Dournon et al., 1990). Did TSD occur in *Pelobates fuscus*? In some of our studied ponds we observed very high water temperatures (up to 30 C) on some days, and those high temperatures occurred only when water level was low, in relation to rainfall pattern. This relation between age structure and causes of skewed sex ratio is now under study.

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