

# Body Size and Age Structure of a Breeding Population of the Japanese Common Toad, *Bufo japonicus formosus* (Amphibia: Bufonidae)

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**Abstract:** The body size and age structure of a breeding population of the Japanese common toad, *Bufo japonicus formosus*, was studied at Yamakita-machi in Kanagawa Prefecture for three breeding seasons from 1992 to 1994. Yearly variation in snout-vent length (SVL) was not significant, but the difference in SVL between the sexes was highly significant; the mean SVL of males and females was 125.8 and 134.2 mm, respectively. The breeding adults were aged successfully by skeletochronology using phalanges. The breeding adults were 1–8 yr old. The mean and median ages were 4.5 and 4 yr in males, and 5.2 and 5 yr in females, respectively. The males were estimated to begin to breed when one to four yr old, with a mean of 2.4 yr, while the females were estimated to breed when two to four yr old, with a mean of 2.7 yr. Age at first reproduction was significantly younger for males than females. We discuss the sexual size dimorphism of *B. j. formosus* in relation to the difference in age structure of the breeding population between the sexes clarified by skeletochronology.

Key words: *Bufo japonicus formosus*; Body size; Skeletochronology; Age structure; Age at first reproduction

## INTRODUCTION

Body size and age are two of the most important attributes of life history in population studies of long-lived species such as amphibians. Although body size can be measured easily, it is difficult to determine the age of individual animals. Due to this difficulty, sufficient information has not yet been gathered on the population age structure of

amphibian species. However, this situation may soon change. The recent development of skeletochronology has contributed greatly to gathering information on the age structure. Skeletochronology using phalanges has proven to be an excellent tool for investigating such problems without sacrificing specimens (Castanet et al., 1993; Smirina, 1994). In Japan, this technique has been applied successfully to a number of amphibian species (e.g., Kusano et al., 1995a, 1995b; Marunouchi et al., 2002).

The Japanese common toad, *Bufo japonicus formosus*, is a large and robust species that

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reaches sizes in excess of 100 mm snout-vent length (SVL). It is very common in eastern Japan, from southern Hokkaido to the Kinki and San'in districts of Honshu. It lives in a variety of habitats, from sea level to high mountains, and breeds explosively in early spring in still waters such as roadside ditches, lakes, marshes, and small ponds (Maeda and Matsui, 1989). Due to the fact that local populations of *B. j. formosus* are exposed to a variety of environmental conditions, especially climatological ones, geographic variation of body size is substantial (see Matsui, 1984). Their body size varies greatly from population to population, depending to latitude or altitude (Matsui, 1984; Kadowaki, 1996). However, information on population age structure remains very limited. To the best of our knowledge, the skeletochronological study for *B. japonicus* has not been reported previously. In the present paper, we report on a breeding population of *B. j. formosus* in the southwestern Kanto, with respect to body size, the age structure, age at first reproduction, and growth rates as determined by skeletochronology.

## MATERIALS AND METHODS

### *Study site*

The breeding population of *B. j. formosus* was studied at Hannya-in temple (35°21'N, 139°06'E) in Yamakita-machi, Kanagawa Prefecture, during early to late March in 1992–1994. The temple is located at the foot of a hilly region 100 m above sea level. The study site and its surrounding area were described in detail in our previous reports (Kusano et al., 1995c, 1999). Two small ponds in the temple garden are used by *B. j. formosus* for breeding. There is no other breeding pond near the temple, at least within a radius of a few hundred meters. Both ponds are 7–8 m long and 2–3 m wide; the water is 0.2–0.4 m deep. The linear distance between the two ponds is 30 m. Three species of frog, *Rana ornativentris*, *R. rugosa* and *Rhacophorus schlegelii*, also breed in the study ponds.

### *Body size and determination of age*

In March of 1992–1994, daily visits were made to the study site to survey the breeding population. Toads were captured by hand or a dip net, and sexed using secondary sexual characteristics, such as thumbpad development. They were measured for SVL to the nearest 0.5 mm with a slide caliper and weighed to the nearest 5 g with a spring balance. In 1992, toads were individually marked by toe clipping, while the toads that were first captured in 1993–1994 were marked to denote the year of first capture by clipping a portion of the hind leg webs.

Toes that were clipped for individual identification in 1992 were stored in 10% buffered formalin for skeletochronological analysis. The method that was used to determine the age of each adult was essentially the same as that used by Kusano et al. (1995a, b). The phalanges were decalcified in 5% nitric acid for 2–3 h, cross sectioned (20  $\mu$ m thick) using a freezing microtome, and stained with hematoxylin (Mayer's acid hemalum) for 30 min. Sections with a smaller medullary cavity were selected for observation from the central region of the diaphysis (because the lines of arrested growth were absent or incomplete in the epiphysis) and were examined under a light microscope using an ocular micrometer. Stained sections showed a series of narrow, concentric, hematoxylinophilic rings separated by wider zones of paler background material. These dark stained rings were interpreted as the result of arrested growth during winter; hence, they were regarded as the lines of arrested growth (LAG; see Castanet et al., 1993). The age of each individual was determined by counting the number of LAGs present in the periosteal bone. Due to the fact that specimens were collected during the breeding season before any growth had occurred in the current year, the outer margin of the bone was regarded as an additional LAG (Smirina, 1972). Because these LAGs were nearly circular or elliptical, the longest and shortest axes of each LAG were measured using an ocular micrometer, and the geometric

mean was taken as the diameter of the LAG.

Resorption of the periosteal bone occurs from the inner surface of the bone and is marked by a resorption line: a narrow irregular hematoxylinophilic line (see Hemelaar and van Gelder, 1980). The diameter of the resorption line was also measured.

In the adult toads, the process of resorption may have destroyed the older LAGs, especially at one yr of age (e.g., Hemelaar and Gelder, 1980). In fact, the innermost LAGs of many phalanges were partly destroyed. This partial destruction may present a serious problem for age determination; hence, the degree of resorption should be evaluated by using the diameter of LAGs in individuals of known age, such as one-yr-old juveniles (see Kusano et al., 1995a, b). Unfortunately, due to the fact that we used toes that were clipped for individual identification as materials for skeletochronological analysis, the phalanges that were examined were not necessarily taken from the same toes. Consequently, we could not compare directly the diameters of LAGs between different individuals.

Marunouchi et al. (2000) demonstrated that an individual's body size can be back-calculated accurately from the LAG diameter and the current body size using mark-recapture study. The best valid back-calculation formula (BCF) is the Dahl-Lea method:  $L_i = L_c D_i / D_c$  ( $L$ : SVL in mm;  $D$ : bone diameter in  $\mu\text{m}$ ;  $c$ : at capture;  $i$ : at  $i$ -th winter). We used this formula to evaluate the degree of resorption.

In general, animals allocate a large amount of energy to reproduction. Therefore, their growth slows considerably after reaching sexual maturity (e.g., Kusano, 1982; Ryser, 1989). A smaller interval between two LAGs indicates slow growth and the first of these smaller intervals may indicate the age at sexual maturity (but see Augert and Joly, 1993). That being so, age at first reproduction was estimated for both sexes

#### *Statistical analysis*

We used the software R 2.9.2 (R Development Core Team, 2009) for statistical analyses. The

body mass of breeding adults was compared among sexes and years using a two-way analysis of variance (ANOVA). To test differences in body size distributions among sexes and years, pairwise comparisons were conducted using Welch's test, because of the heterogeneity of variances (Zar, 1996). The differences in age distributions were also tested between both sexes. For this, the Mann-Whitney test was used because of non-normality.

The relationships between age and SVL of breeding adults were analyzed for both sexes using simple regression equations. These regressions were compared between the sexes by an analysis of covariance (ANCOVA).

The significance level used in all tests was  $P < 0.05$ . Acceptance levels for simultaneous statistical tests were adjusted using the sequential Bonferroni procedure (Rice, 1989).

## RESULTS

### *Snout-vent length and body mass*

A total of 195 males and 84 females were captured during the study period. From these breeding adults, SVL and body mass were measured a total of 284 times for males and 104 times for females during three breeding seasons. Their SVLs at the initial capture ranged from 94–168 mm for males and 105–154 mm for females. The mean SVL of males and females was 125.8 mm (SD=13.8) and 134.2 mm (SD=9.5), respectively, and the sexual difference in SVL was highly significant (Welch's test;  $t=5.875$ ,  $df=223.446$ ,  $P < 0.001$ ). Pairwise comparisons of SVL among sexes and years were conducted to test yearly variation of body size distributions of the breeding population. The results showed that the yearly variation was not significant, but that sexual differences were significant except for in 1994 (Fig. 1).

Among 84 females examined, 19 toads had already spawned their eggs when they were weighed. We analyzed the body mass of breeding females excluding these spent females. Their body mass at the initial capture ranged from 45–363 g for males and 135–415 g for females. The mean body mass of males and

females was 202 g (SD=63) and 278 g (SD=62), respectively, and the sexual difference in SVL was highly significant ( $t=8.459$ ,  $df=258$ ,  $P<<0.001$ ). To test the yearly variation of body mass, the two-way ANOVA was conducted using body mass and year as main effects. The result showed that main effects of sex ( $F_{1,360}=79.256$ ,  $P<<0.001$ ) and year ( $F_{2,360}=15.866$ ,  $P<<0.001$ ) were highly significant, although the interaction term was not significant ( $F_{2,360}=1.039$ ,  $P=0.355$ ). The mean body mass of breeding adults varied significantly from year to year, and females were significantly heavier than males during the three years: for males, the mean body mass was 237 g in 1992 (SD=54,  $n=94$ ), 194 g in 1993 (SD=60,  $n=77$ ), and 209 g in 1994 (SD=62,  $n=113$ ), whereas for females it was 320 g in 1992 (SD=61,  $n=19$ ), 271 g in 1993 (SD=57,  $n=28$ ), and 268 g in 1994 (SD=67,  $n=35$ ).

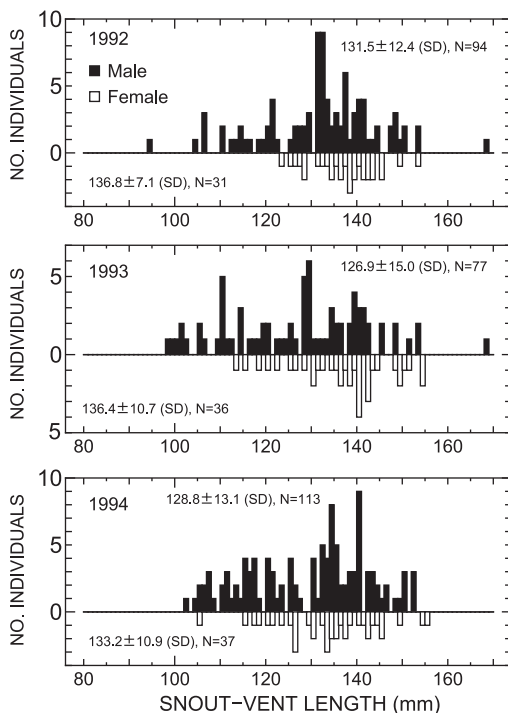


FIG. 1. Snout-vent lengths of breeding adults during the period from 1992 to 1994. Solid and empty histograms indicate males and females, respectively

#### Age structure and age at first reproduction

A total of 79 males and 26 females were aged successfully by skeletochronology. In the phalangeal cross sections, stained LAGs were

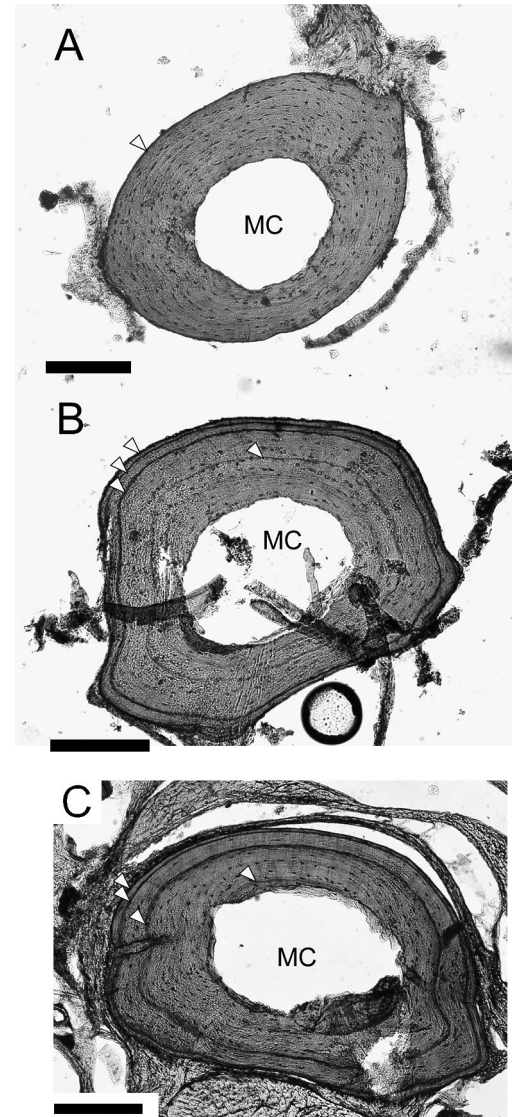


FIG. 2. Hematoxylin-stained cross-sections of the phalangeal bones. White arrows indicate the lines of arrested growth (LAG). A black bar in the lower left corner of each photograph indicates a scale of 0.25 mm; MC=medullary cavity. (A) Adult from the one-yr age class; (B) Adult from the four-yr age class; (C) Adult from the four-yr age class, the innermost LAG was destroyed almost completely.

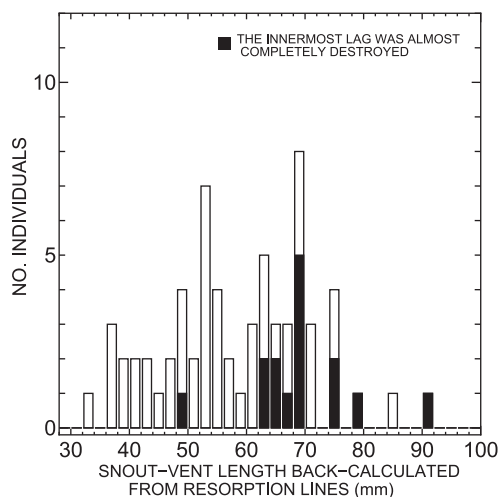


FIG. 3. Frequency distribution of the size of resorption lines. Snout-vent lengths were back-calculated from the diameters of the resorption lines as a relative size so that specimens could be compared. See the text for details.

distinct and easy to count (Fig. 2). The cross sections had 1–8 LAGs. Thus, the donors' ages were estimated to be 1–8 yr.

We back-calculated SVLs from the diameters of resorption lines, and then compared the SVLs between specimens with intact innermost LAGs and those with innermost LAGs that had been destroyed almost completely (Fig. 2C). The back-calculated SVL ranged from 32.6 to 90.2 mm (Fig. 3). Fifteen specimens examined had innermost LAGs that had been destroyed almost completely. The SVLs of these specimens, back-calculated from the resorption lines, were all larger than 62 mm except for one specimen (Fig. 3). We assumed that the LAG at one yr of age had been completely resorbed when the resorption line was >62 mm in the back-calculated SVL, and we added one to the number of LAGs counted for a total of 15 toads.

In other populations of *B. japonicus*, mark-recapture studies showed that SVLs of newly metamorphosed toadlets were only 7–10 mm, but increased to, on the average, about 60 mm by the late autumn of the year (Hisai, 1975, 1981; Okuno, 1984). This fact suggests that it

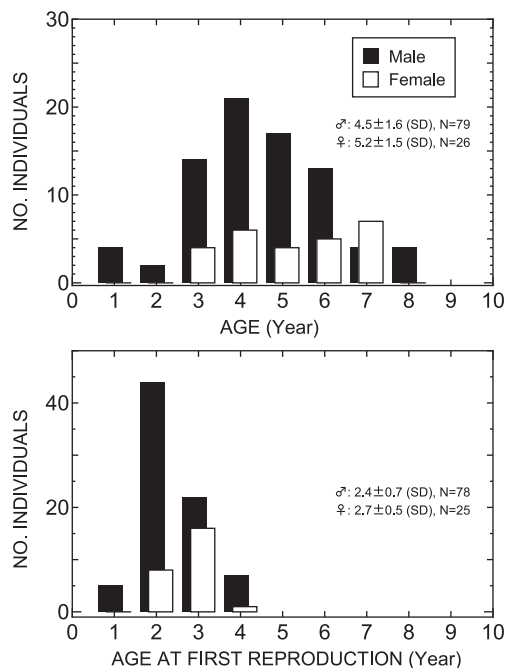


FIG. 4. Age structure of breeding populations (upper) and frequency distribution of age at first reproduction (lower) estimated from the age at which the growth of individuals began to slow considerably. Solid and empty histograms indicate males and females, respectively.

was reasonable for us to make the assumption noted in the preceding paragraph.

Breeding adults were estimated to be 1–8 yr old. The mean and median ages were 4.5 (SD=1.6, n=79) and 4 yr in males, and 5.2 (SD=1.5, n=26) and 5 yr in females, respectively (Fig. 4). We could detect no significant difference in age distribution between the sexes (Mann-Whitney test,  $P=0.071$ ). The youngest males and females were one and three yr old, respectively. This fact may suggest that a difference in the age of sexual maturity in males and females, such that males require only one yr to begin breeding activities, whereas females require three yr. In a stable population, the age distribution should show a fairly steady decline from a maximum in the first year of breeding until the oldest age class. The increase in numbers between the two- and three-yr age classes of males suggests

that the age at first reproduction varied among individuals and that the reproductive success of this population fluctuated annually (Fig. 4).

From the age at which the growth of individuals slowed considerably, males were estimated to begin to breed when one to four yr old, with a mean of 2.4 (SD=0.7, n=78) yr, while females were estimated to breed when two to four yr old, with a mean of 2.7 (SD=0.5, n=25) yr (Fig. 4). The median age was two and three yr, respectively. The modal ages at first reproduction were the same as the medians for both sexes. Age at first reproduction was significantly younger for males than females (Mann-Whitney test,  $P=0.020$ ).

#### Relationship between body size and age

In *B. j. formosus*, we found a significant correlation existed between body size and age in males ( $r=0.511$ ,  $n=79$ ,  $P<0.001$ ), but the correlation was not significant in females ( $r=0.251$ ,  $n=26$ ,  $P=0.215$ ; Fig. 5), perhaps because of the small sample size. We conducted an analysis of covariance (ANCOVA) to compare the regression of SVL on age between

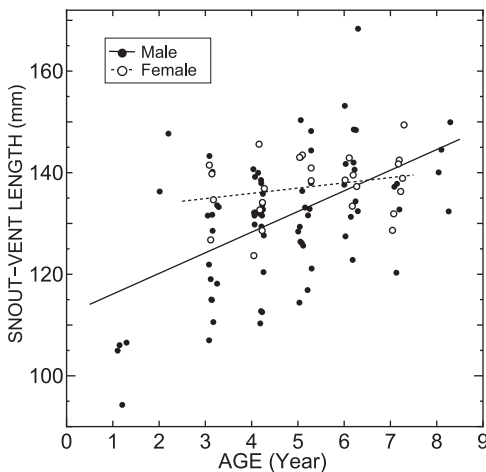


FIG. 5. The relationship between age (X) and body size (Y: snout-vent length). Each symbol indicates one individual. Solid and open circles indicate males and females, respectively. Regression lines calculated from the data for both sexes are shown (male:  $Y=112.6+3.94 X$ ,  $n=79$ ,  $R^2=0.26$ ; female:  $Y=131.5+1.10 X$ ,  $n=26$ ,  $R^2=0.07$ ).

both sexes. We could not detect any significant differences between the sexes in either the slope ( $F_{1,101}=3.702$ ,  $P=0.057$ ) or the intercept ( $F_{1,102}=2.853$ ,  $P=0.094$ ). This result shows that no significant difference in SVL could be detected between the sexes when adjustments were made for age. From the pooled data of both sexes, the common regression line was calculated:  $Y=115.1+3.61 X$ ,  $n=105$ ,  $R^2=0.241$ ,  $F_{1,103}=32.71$ ,  $P<0.001$ ). The estimated slope of the regression shows that the SVL increased by 3–4 mm every year after sexual maturity.

## DISCUSSION

#### Interpopulational variations of body size and age

Matsui (1984) demonstrated that the geographical variation of body size of *B. japonicus* is substantial, such that the population mean of SVL varies from 70.9 to 143.3 mm for males, and from 68.9 to 160.1 mm for females, and the averages of all populations are 116.3 and 126.7 mm, respectively. In the southwestern Kanto, the mean SVL of *B. j. formosus* at Minato-ku in Tokyo is smaller than the average value of all populations: 108.0 mm for males and 118.4 mm for females (Hisai, 1982). However, Serizawa and Kanai (1970) reported a rather larger mean body size of *B. j. formosus* at Hachioji in Tokyo; mean SVLs were 135 and 138 mm, respectively, even though the Hachioji population was not so distant from the Minato-ku population (about 37 km). The mean SVL at Yamakita-machi is near to that at Hachioji: 125.8 and 134.2 mm. High population density and anthropogenic disturbances may affect relatively small body size of the population at Minato-ku, because it inhabits a small isolated forested area surrounded by walls in urban districts (see Matsui, 1984).

The results of the study reported herein show that the age of breeding adults in the studied population was 1–8 yr old and the age at first reproduction was estimated to be 1–4 yr for males and 2–4 yr for females (Fig. 4); the youngest males were only one yr old. Although skeletochronological studies have not previously been reported for *B. japonicus*,

some long-term mark-recapture studies have determined successfully the age of individual toads and reported the age at first reproduction (Hisai, 1981; Okuno, 1984). The minimum age at first reproduction was reported to be 2 yr for males and 2–3 yr for females at Minato-ku in Tokyo and at Kanazawa in Ishikawa Prefecture (Hisai, 1981; Okuno, 1984). At Yamakita-machi, newly metamorphosed toadlets usually emerge from the breeding ponds in May (personal observation) and body size at metamorphosis is not larger than 10 mm in SVL (see Hisai, 1975, 1981; Okuno, 1984). The mean SVL of breeding males at one yr of age was 102.6 mm (SD = 5.8,  $n=4$ ; Fig. 5). It is thus evident that some of the newly metamorphosed toadlets had a strong potential for rapid growth at Yamakita-machi. Their growth in SVL was estimated to attain 90 mm or more in only half a year. However, such males were very few; only 6% (5/78) of all breeding males examined (Fig. 4). The majority of males grew to more than 100 mm in SVL and began to breed when they were two or more years old.

We cannot find any reports showing such early sexual maturity in *B. japonicus* males. Although we cannot exclude the possibility that estimating age yielded an underestimation by the process of resorption of older LAGs, we consider that the likelihood that this happened is low, because the diameters of resorption lines were relatively small for males whose ages were estimated to be one yr old (Fig. 2A).

#### *Sexual size dimorphism*

Sexual size dimorphism has been found in many anuran species. Although females are generally larger than conspecific males, the magnitude of sexual size dimorphism differs markedly among species or populations. In Japanese species, the size ratio (mean female SVL/mean male SVL) varies from nearly 1.0 to 1.45 (see Fukuyama and Kusano, 1989). Matsui (1984) reported geographical variation in the magnitude of size dimorphism between the sexes in *B. j. formosus*. Matsui found that the size ratio of the sex of each population

varied from approximately 0.9 to 1.2, and observed a significant correlation between the size ratio of the sexes and latitude, in that the magnitude of the size dimorphism between the sexes increased in more northern populations (Matsui, 1984). At Minato-ku and Hachioji in Tokyo, the size ratio of the sexes was 1.10 and 1.02, respectively (Serizawa and Kanai, 1970; Hisai, 1982), and the present study showed that it was 1.07 at Yamakita-machi. In southwestern Kanto, the magnitude of sexual dimorphism seems to be moderate among all populations examined (Matsui, 1984).

Several hypotheses have been proposed to explain the direction and magnitude of size dimorphism between the sexes in anurans as a result of sexual selection. Monnet and Cherry (2002) collected morphological and demographic data for anurans from the literature, and showed that most of the variation in size dimorphism in anurans can be explained in terms of differences in the age structure between the sexes in breeding populations. They concluded that if sexual selection has an effect on size dimorphism in anurans, it is likely to be only a secondary one. For *Bufo* species, they collected data from 15 populations of eight species, and showed a clear relationship between the size ratio of the sexes (from 0.88 to 1.27) and age difference (from  $-0.98$  to 1.56 yr; Monnet and Cherry, 2002). In our study, the values of the size ratio of the sexes and age difference of the study population were 1.07 and 0.7, respectively. This result does not contradict the relationship between the size ratio of the sexes and age difference for *Bufo* species presented in Monnet and Cherry (2002). Difference in the age structure of breeding populations is certainly one of the most important factors that determine the direction and magnitude of sexual size dimorphism in anurans.

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