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Sexual size dimorphism in the Tyrrhenian Treefrog, *Hyla sarda*: a life-history perspective

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Abstract

Sexual size dimorphism (SSD) is often explained as the differential equilibrium between stabilizing survival selection and directional sexual/fecundity selection on the body size of males and females. Provided that survival selection is similar in both sexes, female-biased SSD is thought to occur when fecundity selection on female body size is stronger than sexual selection on male body size. However, in animals with indeterminate growth, body size depends on several life-history traits, thus, to understand why SSD has evolved one should understand how it arises. We investigate SSD in the Tyrrhenian Treefrog, *Hyla sarda*, by describing sexual dimorphism in age and growth and by assessing how body-size affects their reproductive success. Females are 16% larger than males because they mature one year later, live one year longer, and reach a larger asymptotic body size. Furthermore, body size correlates positively with female fecundity, but not with male mating success. These results suggest that SSD arises from differential optimal tradeoffs between the expected number of reproductive episodes (which decreases with prolonging growth) and the expected success in each reproductive episode (which increases with prolonging growth).

Introduction

Sexual size dimorphism (SSD) is a pattern observed in most animal groups (Andersson, 1994; Fairbairn, Blanckenhorn & Székely, 2010). In species where males and females have highly overlapping ecological niches and, thus, experience similar regimes of survival selection, SSD evolves as the indirect consequence of the different reproductive role of males and females (Darwin, 1871; Andersson, 1994). Strictly speaking, SSD is not an adaptation. SSD is a population property emerging from sexual differences in life-history (Halliday & Verrell, 1986; Monnet & Cherry, 2002). Life-history strategies are adaptations, because they evolve to maximize individual lifetime reproductive success. For this reason, to understand the processes responsible for the pattern of SSD, a life-history perspective is required. Such an approach poses not only the question of why one sex is larger in size than the other, but it also asks how these differences in size are attained.

The advantages of this approach are particularly evident in those species that show a strong plasticity in body size, due to biphasic growth with asymptotic growth after maturity (Stamps, 1993). In these animals, in fact, adult body size depends on the amount of energy that individuals invest in growth both before and after the attainment of sexual maturity (Jörgensen, 1992). For this reason, sex-differences in life history traits (i.e. pre- and post-maturation growth rate, age at first reproduction and longevity) may represent different allocation strategies responsible for both the direction and the extent of SSD.

Anurans are a well studied example of species that show indeterminate growth pattern. In frogs and toads, somatic growth continues after maturation, though at rates decreasing with age (Gibbons & McCarthy, 1984; Ryser, 1988; Gramapurohit, Shanbhag & Saidapur, 2004), and SSD is widespread, being females larger than males in the majority of species (Shine, 1979).

The pattern of SSD has been usually explained by the “differential-equilibrium model”, often employed to explain the SSD in amphibian species as well. This model suggests that SSD arises when conflicting selective pressures on body size (i.e. sexual and survival selection, in males, and fecundity and survival selection, in females) equilibrate differently in the two sexes (Blanckenhorn *et al.*, 2007). When survival selection is similar in both sexes, a female-biased SSD results from sexual differences in reproductive selection gradients (Lande & Arnold, 1983; Arnold & Wade, 1984): fecundity selection on female body size (large females can accommodate more eggs in their body than smaller females) is stronger than sexual selection on male body size (Arak, 1988). The predictions of the differential-equilibrium model have been supported by several studies on anurans, which show that the correlation between reproductive success and body size is stronger in females than in males (Howard & Kluge, 1985; Vargas-Salinas, 2006).

However, in animals with indeterminate growth, selection does not act on adult body size *per se*, but on the different life-history traits that affect body size. Taking into account the entire individual lifetime, optimal body size depends on the reproductive benefits of *being* large and the costs of *becoming* large (review in Blanckenhorn, 2000). Indeed, it takes time to grow large and the longer the time devoted to growth the lower the probability to survive to first reproduction and the lower the number of expected reproductive episodes (Shine, 1988). When costs and benefits differ between sexes, selection may favour the evolution of different life history strategies in males and females. To analyse the effects of between-sex life-history variation on SSD, Monnet and Cherry (2002) conducted a comparative study of SSD in anurans and found that most of the among-species variation in SSD was explained by sex differences in age structure. Similar results have been observed at the intra-specific scale (Liao & Lu, 2010; Lyapkov, Cherdantsev & Cherdantseva, 2010), suggesting that longevity and age at first reproduction are the main determinants of SSD in anurans.

In the present study, we examine the SSD pattern in a population of Tyrrhenian Treefrog, *Hyla sarda*, an endemic species of the Tyrrhenian islands (Corsica, Sardinia and the Tuscan Archipelago). *H. sarda* belongs to the *H. arborea* group (Stöck, Dubey & Klütsch, 2008) and, as all the species of this group, it shows a *lek* mating pattern (Friedl & Klump, 2005; Castellano *et al.*, 2009a, b). During the breeding season, males aggregate in choruses at the breeding site; females are attracted by male advertisement calls (Castellano *et al.*, 2002; Rosso, Castellano & Giacoma, 2004a) and actively choose their mates on the basis of several acoustic properties of males' call (Castellano & Rosso, 2006, 2007).

By means of skeletochronology techniques, we describe the age structure and the growth curves of males and females in our tree-frog population. Skeletochronological technique is considered the best method for age determination in anurans and has been already used in other tree-frog species (Friedl & Klump, 1997; Rosso, Castellano & Giacoma, 2004b). Furthermore, for both sexes we describe the relationship between body size and 'single-season' reproductive success. By combining these two types of information, we test the hypothesis that the observed SSD pattern arises as the effect of between-sex differences in optimal life-history strategies.

Materials and Methods

Study Site

The field study was carried out in Caprera (NW Sardinia, Italy, 41°19'N, 09°45'E), a small island inside "Parco Nazionale dell'Arcipelago della Maddalena". The study population of Tyrrhenian treefrogs (*H. sarda*) reproduced in a pond originated from a dismissed clay pit. This pond was the largest (110 m of perimeter) and the deepest (maximum profundity of 1.2 m) of a group of temporary pools that formed after spring rainfalls, a few hundred meters from the south-eastern coastline. Unlike most of these pools, which dried up at the end of May, the breeding pond maintained the water level constant for the entire breeding season, in both years of study. Treefrogs were the only amphibians breeding in the pond.

Sample Collection and Marking Technique

We carried out this study in the 2007 and 2008 breeding seasons, during the peak of reproductive activity (from 3 April to 12 May in 2007 and from 1 April to 29 April in 2008). Every night, from 21:00 to 0:30, we captured male and female treefrogs found in breeding activity, by moving along the shoreline. Marked individuals were identified and immediately released in the pond, whereas pairs and unmarked individuals were carried to the laboratory, where pairs were let to spawn overnight in separate plastic boxes (25x20x10 cm) filled up with tap water.

The following morning, we photographed the clutches of eggs with a digital camera (Canon Power Shot -A75, CANON) to allow successive counting of the number of eggs and measured all the unmarked individuals. Treefrogs were anaesthetized in a 0.2% solution of MS-222 Sandoz, weighed (± 0.1 g) with a digital scale (Multifunction Pocket Scale MF-250, MTI Weight Systems, Inc., Kingstown, RI, USA), their Snout-Vent Length (SVL) was measured (± 0.01 mm) with a digital calliper (MITUTOYO CD-15C, Mitutoyo Inc., Mississauga, ON, Canada), and they were individually marked by implanting a fluorescent alphanumeric tag (VI Alpha Tags, size 1.0x2.5 mm, 0.1 mm thick, Northwest Marine Technology Inc., Shaw Island, Washington, U.S.A) beneath the skin of the ventral side of the right hindlimb thigh (Castellano *et al.*, 2009a, b). Furthermore, in 2008, treefrogs were toe-clipped by cutting out the last two phalanges of the fourth toe of their right hind limb. Phalanges were preserved in a 70% ethanol solution and successively used in skeletochronological analyses.

Age determination

We determined individual age by means of skeletochronological techniques (Smirina, 1972; Francillon & Castanet, 1985). Preserved phalanges were cleaned of surrounding tissues, decalcified in 5% nitric acid for about 30 min, and soaked in tap water overnight. Phalanx cross-sections were obtained with a freezing microtome at 16 µm, stained with hematoxylin for 20 min and washed in water for 10 min. We selected the mid-diaphyseal sections with the narrowest medullar cavity and mounted them on microscope slides using Aquamount. For each section, two observers counted independently the number of Lines of Arrested Growth (LAGs) under a light microscope and later compared results. Dubious cases were discounted. The most peripheral edge of the cross-sections was counted as an additional LAG because the specimens were collected during the breeding seasons after emergence from hibernation (Rogers & Harvey, 1994).

Set of variables and statistical analyses

We described sexual size dimorphism (SSD) in terms of both body-length (SVL) and body-weight (BW) differences. To investigate how SSD was attained, that is, whether it was due to sexual differences in either the age structure or the pattern of growth or both, we analyzed the age structure and the age-size relationship in both sexes separately. As predicted by the theory of biphasic somatic growth (Quince *et al.*, 2008), we assumed that, in adult treefrogs, body size did not increase linearly with age, but asymptotically, as described by the von Bertalanffy (vB) growth function (Day & Taylor, 1997; Lester, Shuter & Abrams, 2004):

$$SVL_t = SVL_{MAX} - (SVL_{MAX} - SVL_{MIN})e^{-k(t-t_{MIN})}$$

SVL_t, the body size at age t, was calculated using two parameters: SVL_{MIN}, the mean body length at age of first reproduction (one year for males, two years for females), and SVL_{MAX}, the asymptotic body length, defined as the highest SVL observed (38.20 mm in males, 44.53 mm in females). The growth coefficient k of the vB function (the rate at which SVL_{MAX} is approached) was inferred by means of nonlinear regression techniques using PASW vs. 18 (IBM corporation IBM, Somers, NY). By comparing k-values between sexes, we tested the null-hypothesis that males and females, once attained sexual maturity, showed statistically similar growth.

To investigate the ultimate causes of SSD, we tested the hypothesis that SSD was the consequence of different selective pressures acting on female and male body size. To analyse the effect of body size on female reproductive success (expressed as the total number of eggs laid), we used a General Linear Model (GLM), in which the year was a random factor and the female SVL and the ratio between male and female SVLs were the covariates. The male-female SVL ratio was entered in the model under the hypothesis that females adjusted the number of eggs on the basis of the relative size of their mates.

In males, reproductive success was described either as the number of matings obtained by a male during the entire breeding season (mating success) or as the number of eggs laid by a male's mate (fecundity success). Mating success was used in a GLM with Poisson errors and log-link function, in which year was the random factor and SVL, BW, and chorus tenure were the covariates. Chorus tenure was a measure of mating effort and was defined as the number of days between the last and the first capture of a male. Fecundity success was used in a general linear model with Gaussian errors and with year as a random factor and male SVL as a covariate.

Results

Sexual differences in body size and age

Table 1 shows the descriptive statistics of body size (SVL) and body weight (BW) of male and female treefrogs in both the 2007 and 2008 samples. Mixed two-way ANOVAs, with sex as a fixed factor and year as a random factor, showed between-sex highly significant differences in both SVL ($F_{1,373} = 251.71$, $P < 0.001$) and BW ($F_{1,334} = 189.38$, $P < 0.001$), with females being larger and heavier than males; whereas these morphometric characters showed weak (SVL: $F_{1,373} = 3.603$, $P = 0.058$) or no differences (BW: $F_{1,334} = 0.213$, $P = 0.645$) between years.

Skeletochronological analysis was carried out successfully on 115 adult individuals (58% of the 2008 sample): 70 males and 45 females (Table 1). Age distribution differed significantly between sexes ($\chi^2 = 25.17$, df = 4, $P < 0.001$): adult-male age ranged from one to four years, with most of males (80%) being two or three years old; adult females were about one-year older than males, their age ranging from two to five years, with most of females (71%) falling into the three- and four-year age classes (Fig. 1).

Figure 2 shows the relationship between age and SVL in male and female Sardinian treefrogs. While, in males, the average per-year increase of SVL does not vary markedly with age (2.3 mm, 2.6 mm and 1.8 mm, respectively, between 2 and 1, 3 and 2, and 4 and 3 years), in females it varies much more abruptly: the mean difference in SVL between three- and two-year old females (4.7 mm) is about five times larger than that between four- and three-year old females (0.9 mm), which, in turn, is about five times larger than the difference in size between five- and four-year old females (0.2 mm). Although the vB growth coefficient (k) was smaller in males ($k = 0.360$, 95% Confidence Interval ($CI_{95\%}$): 0.30 - 0.42) than in females ($k = 0.442$, $CI_{95\%}$: 0.33 - 0.55), this difference was not statistically significant, suggesting that the male and female growth curves differed in the asymptote, but not in the rate at which the asymptote was reached.

Body size and reproductive success in females

During the 2007 and 2008 breeding seasons, we captured 75 females, 45 were paired and for 37 of them we measured fecundity (the total number of eggs produced). Fecundity was positively affected by the female SVL ($F_{1,31} = 6.78$, $P = 0.014$), but not by the mated male-female SVL ratio ($F_{1,31} = 0.02$, $P = 0.881$). The effect of body size on fecundity (Fig. 3) differed significantly between the years ($F_{1,31} = 5.3$, $P = 0.028$). In the 2008 breeding season, females produced clutches that were, on average, less than half the size of those produced in the previous year (Table 1). Such a decrease in fecundity was size dependent, because, in 2008, large females laid a proportionally smaller number of eggs than smaller females (Fig. 3).

Body size and male mating success in males

Fig. 4 shows the frequency distribution of male mating success in both the 2007 and 2008 breeding seasons. In 2008, the mean male mating success (Table 1) was higher than that observed in the previous season (coefficient = 0.819, SE = 0.347, P = 0.018) and this difference can be explained by the higher sex ratio found in 2007: 7.9 male/female *versus* 2.6 in 2008. Independent of the year, however, the longer the time spent at the breeding site the higher the mating success (b = 0.054, SE = 0.017, P = 0.001). In contrast, neither SVL (b = -0.002, SE = 0.148, P = 0.986) nor BW (b = 0.669, SE = 0.611, P = 0.273) showed a statistically significant relationship with male mating success.

To further investigate the effect of body size on male reproductive success, we analyzed the relationships between the SVL of males and either the SVL or the number of eggs of their mates. We found no evidence for a large male advantage in this population: independent of the breeding season, the SVL of males was not positively associated either with the SVL of paired females (b = 0.023, $F_{1,39}$ = 0.025, P = 0.875) or with the number of eggs that could have been possibly sired (b = 3.870, $F_{1,33}$ = 0.152, P = 0.700).

Discussion

In this study, we have adopted a life-history approach to understand the evolution of SSD in Tyrrhenian treefrogs. Our study provides three main results. First, it shows that body size differs between sexes, females being 16% larger than males. Second, it shows that this pattern depends on differences in age at first reproduction and in asymptotic body size. Third, it shows that the effect of body size on within-season reproductive success is stronger in females than in males. This latter result seems consistent with the differential-equilibrium model of the evolution of SSD (Blanckenhorn, 2000), because it suggests that selection on body size be stronger on females than on males.

In females, reproductive success mainly depends on the number of eggs they produce and large females can accommodate more eggs than smaller females. In both years, we found a positive correlation between female body size and the number of eggs. Quite unexpectedly, we also observed that such relationship differed markedly between years: the mean number of eggs in 2007 being twice as large as that in 2008. We do not know what causes these differences in fecundity, but we observe that differences increase with female body size, suggesting that, under favourable conditions, large females can increase fecundity more than smaller females do. Several studies have shown a positive association between body size and the number of eggs, both at the intra-specific (Lardner & Loman, 2003; Castellano, Cucco & Giacoma, 2004; Vargas-Salinas, 2006) and at the inter-specific level (Kuramoto, 1978; Kaplan & Salthe, 1979). Furthermore, in some species, female body size has been observed to correlate positively with egg size, which, in turn, correlates positively with hatchling size (Kaplan, 1980; Crump, 1984) and tadpole growth rate (Travis, 1984) and survival (Travis, 1983). Overall, these results provide strong evidence that body size affects female fecundity and that the expected benefits of increasing body size by delaying reproduction might be high.

In males, reproductive success mainly depends on their number of matings, which, in turn, depends on mating patterns and operational sex ratios (Wells, 1977; Sullivan, Ryan & Verrell, 1995). In lekking anurans, sexual selection on males is usually strong and operates through two distinct mechanisms: endurance rivalry and female choice (Castellano *et al.*, 2009a). Sexual selection on male body size is expected if large males spend more time at leks or if they are more effective in attracting females than smaller males. In the study population, mating success was found to correlate positively with the number of days spent at the breeding site, but not with male body size. Similar results were found in other two tree-frog species, phylogenetically close to the Tyrrhenian Treefrog, *H. intermedia* (Castellano *et al.*, 2009a) and *H. arborea* (Friedl & Klump, 2005). In both these species, male mating success was strongly correlated with male attendance, and in *H.*

intermedia also with some acoustic properties of male advertisement calls. In no cases, however, male mating success was found to correlate with body size. These results provide evidence that, in males, the expected benefits of increasing size by postponing reproduction are much lower than in females.

According to life-history theory, the optimal age at first reproduction is that at which the benefits and costs of maturation at different ages balance at a stable equilibrium (Stearns, 1992). In species with indeterminate growth, the main benefit of delayed maturation is the large body size that can be attained and its effects on reproductive performance, whereas the main cost is the low probability of surviving to first reproduction (Arak, 1988; Andersson, 1994; Blanckenhorn, 2000). If the benefits of delayed maturation differ between males and females more than the costs do, then life-history theory predicts between- sex differences in age at first reproduction (Kozlowski & Wiegert, 1987; Kozlowski, 1992). In many species with indeterminate growth, females tend to mature later and at a larger size than males and this sexual bimaturism has been explained in terms of increased fecundity of large and old females (Stamps & Krishnan, 1997). In anurans, in particular, delayed maturation of females has been observed in several species with female biased-SSD (review in Monnet & Cherry, 2002). Tyrrhenian treefrogs conform to this general pattern and provide further evidence that SSD arises because females benefit from delaying reproduction more than males do.

Shine (1990) suggested that if individual body size differs between sexes at sexual maturity, then the largest sex maintains this advantage over the other one also during the adult phase. In several amphibian species with female-biased SSD, however, females not only reach sexual maturity at an older age, but they also show a higher post-maturation growth rate (Ma & Lu, 2009; Hasumi, 2010; Guarino *et al.*, 2003), which further increases SSD in the adult population. Our results provide no clear evidence for sexual differences in adult growth rate. From the one hand, we observe that male body size increases slowly but at an almost constant rate, whereas female body size increases from the two- to the three-year age class, but then growth markedly decreases. On the other hand, however, we find no statistically significant differences between the vB growth constants, *k*. Furthermore, the growth curves in our sample should be considered cautiously, because of the within-sex variation in age at maturity. In fact, since not all males reach sexual maturity at one year of age and not all females at two, the observed body-size differences between one- and two-year old males and between two- and three-year old females are the average growth rates of both early- and late-maturing individuals and they are thus expected to overestimate the true individual growth rate. Because of these limitations, our results cannot exclude that some sexual differences in adult growth rate exist, but they provide strong evidence that the main cause of SSD in Sardinian treefrogs lies in the between-sex differences in age at first reproduction.

In conclusion, this study shows that SSD in the Tyrrhenian Treefrog arises from differences in the life-history strategies of the two sexes, that is, from the different way males and females use their time to guarantee a future to their genes. In this species, females grow larger than males because they invest more time in pre-adult growth and this occurs because of the different optimal compromise between body size and developmental time: females benefit from attaining large size by prolonging developmental time much more than males do.

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References

Andersson, M. (1994). *Sexual Selection*. Princeton, New Jersey. Princeton Univ. Press.

Arak, A. (1988). Sexual dimorphism in body size: a model and a test. *Evolution* **42**: 820-25.

Arnold, S.J. & Wade, M.J. (1984). On the measurement of natural and sexual selection: theory. *Evolution* **38**: 709-719.

Blanckenhorn, W.U. (2000). The evolution of body size: what keeps organisms small? *Q. Rev. Biol*. **75**: 385-407.

Blanckenhorn, W.U, Dixon, A.F.G., Fairbairn, D.J., Foellmer, M.W., Gibert, P., van Der Linde, K., Meier, R., Sören, N., Pitnick, S., Schoff, C., Signorelli, M., Teder, T., Wiklund, C. (2007). Proximate causes of Rensch's rule: does sexual size dimorphism in arthropods result from sex differences in development time? *Am. Nat.* **169**: 245-257.

Castellano, S., Cuatto, B., Rinella, R., Rosso, A. & Giacoma, C. (2002). The advertisement call of the European treefrogs (*Hyla arborea*): a multilevel study of variation. *Ethology* **108**: 75-89.

Castellano, S., Cucco, M. & Giacoma, C. (2004). Reproductive investment of female green toads (*Bufo viridis*). *Copeia* **2004**: 659-664.

Castellano, S. & Rosso, A. (2006). Variation in call temporal properties and female preferences in *Hyla intermedia. Behaviour* **143**: 405-424.

Castellano, S. & Rosso, A. (2007). Female preferences for multiple attributes in the acoustic signals of the Italian treefrog, *Hyla intermedia*. *Behav. Ecol. Sociobiol*. **61**: 1293-1302.

Castellano, S., Zanollo, V., Marconi, V. & Berto, G. (2009a). The mechanisms of sexual selection in a lek-breeding anuran, *Hyla intermedia*. *Anim. Behav.* **77**: 213-224.

Castellano, S., Marconi, V., Zanollo, V. & Berto, G. (2009b). Alternative mating tactics in the Italian treefrog, *Hyla intermedia*. *Behav. Ecol. Sociobiol.* **63**: 1109-1118.

Crump, M.L. (1984). Intraclutch egg size variability in *Hyla crucifer* (Anura: Hylidae). *Copei*a **1984**: 302-308.

Darwin, C. (1871). *The descent of man and selection in relation to sex*. London, J. Murray.

Day, T. & Taylor, P.D. (1997). Von Bertalanffy's growth equation should not be used to model age and size at maturity. *Am. Nat.* **149**: 381-393.

Fairbairn, D.J., Blanckenhorn, W.U. & Székely, T. (2010). *Sex, size and gender roles: evolutionary studies of sexual size dimorphism*. New York, Oxford Univ. Press.

Francillon, H. & Castanet, J. (1985). Mise en evidence expérimentale du caractère annuel des lignes d'arrète de croissance squelettique chez *Rana esculenta* (Amphibia: Anura). *C. R. Acad. Sci. Paris* **300**: 327-332.

Friedl, T.W.P. & Klump, G.M. (1997). Some aspects of population biology in the European Treefrog, *Hyla arborea*. *Herpetologica* **53**: 321-330.

Friedl, T.W.P. & Klump, G.M. (2005). Sexual selection in the lek-breeding European treefrog: body size, chorus attendance, random mating and good genes. *Anim. Behav*. **70**: 1141-1154.

Gibbons, M.M. & McCarthy, T.K. (1984). Growth, maturation and survival of frogs *Rana temporaria* L. *Holarc. Ecol.* **7**: 419-427.

Gramapurohit, N.P., Shanbhag, B.A. & Saidapur; S.K. (2004). Growth, sexual maturation and body Size dimorphism in the Indian Bullfrog, *Hoplobatrachus tigerinus* (Daud.). *Herpetologica* **4**: 414-419.

Guarino, F.M., Lunardi, S., Carlomagno, M. & Mazzotti, S. (2003). A skeletochronological study of growth, longevity, and age at sexual maturity in a population of *Rana latastei* (Amphibia, Anura). *J. Biosci.* **28**: 775-782.

Halliday, T.R. & Verrell, P.A. (1986). Review: sexual selection and body size in amphibians. *Herpetol. J.* **1**: 86-92.

Hasumi, M. (2010). Age, body size, and sexual dimorphism in size and shape in *Salamandrella keyserlingii* (Caudata: Hynobiidae). *Evol. Biol.* **37**: 38-48.

Howard, R. & Kluge, A.G. (1985). Proximate mechanisms of sexual selection in wood frogs. *Evolution* **39**: 260-277.

Jörgensen, C.B. (1992). Growth and reproduction. In: *Environmental physiology of the amphibians*: 439-466. Feder, M.E. & Burggren, W.W. (Eds). Chicago, Chicago Univ. Press.

Kaplan, R.B. & Salthe, S.N. (1979). The Allometry of reproduction: an empirical view in salamanders. *Am. Nat.* **113**: 671-689.

Kaplan, R.H. (1980). The implications of ovum size variability for offspring fitness and clutch size within several populations of salamanders (*Ambystoma*). *Evolution* **34**: 51-64.

Kozlowski, J. & Wiegert, R.G. (1987). Optimal age and size at maturity in annuals and perennials with determinate growth. *Evol. Ecol.* **1**: 231-244.

Kozlowski, J. (1992). Optimal allocation of resources to growth and reproduction: implications for age and size at maturity. *Trends Ecol. Evol.* **7**: 15-19.

Kuramoto, M. (1978). Correlations of quantitative parameters of fecundity in amphibians. *Evolution* **32**: 287-296.

Lande, R. & Arnold, S.J. (1983). The measurement of selection on correlated characters. *Evolution* **37**: 1210-1226.

Lardner, B. & Loman, J. (2003). Growth or reproduction? Resource allocation by female frogs *Rana temporaria*. *Oecologia* **137**: 541-546.

Lester, N.P., Shuter, B.J. & Abrams, P.A. (2004). Interpreting the von Bertalanffy model of somatic growth in fishes: the cost of reproduction. *Proc. R. Soc. B* **271**: 1625-1631.

Liao, W.B. & Lu, X. (2010). Age structure and body size of the Chuanxi Treefrog *Hyla annectans chuanxiensis* from two different elevations in Sichuan (China). *Zool. Anz*. **248**: 255-263.

Lyapkov, S.M., Cherdantsev, V.G. & Cherdantseva, E.M. (2010). Geographic variation of sexual dimorphism in the moor frog (*Rana arvalis*) as a result of differences in reproductive strategies. *Zhurnal. Obshchei Biol*. **71**: 337-358.

Ma, X. & Lu, X. (2009). Sexual size dimorphism in relation to age and growth based on skeletochronological analysis in a Tibetan frog. *Amphibia-Reptilia* **30**: 351-359.

Monnet, J.M. & Cherry, M.I. (2002). Sexual size dimorphism in anurans. *Proc. R. Soc. B.* **269**: 2301-2307.

Quince, C., Abrams, P.A., Shuter, B.J. & Lester, N.P. (2008). Biphasic growth in fish I: theoretical foundations. *J. Theor. Biol.* **254**: 197–206.

Rogers, K.L. & Harvey, L. (1994**).** A skeletochronological assessment of fossil and recent *Bufo cognatus* from south-central Colorado. *J. Herpetol.* **28**: 133-140.

Rosso, A., Castellano, S. & Giacoma, C. (2004a). The advertisement call of *Hyla intermedia* and *H. sarda*. *Ital. J. Zool.* **S2**: 169-173.

Rosso, A., Castellano, S. & Giacoma, C. (2004b). Ecogeographic analysis of morphological and life-history variation in the Italian treefrog. *Evol. Ecol***.** **18**: 303-321.

Ryser, J. (1988). Determination of growth and maturation in the common frog, *Rana temporaria,* by scheletochronology. *J. Zool.(Lond.)* **216**: 673-685.

Shine, R. (1979). Sexual selection and sexual dimorphism in the Amphibia. *Copeia* **1979**: 297-306.

Shine, R. (1988). The evolution of large body size in females: a critique of Darwin's "fecundity advantage" model. *Am. Nat.* **131**: 124-131.

Shine, R. (1990). Proximate determinants of sexual differences in adult body size. *Am. Nat.* **135**: 278-283.

Smirina, E.M. (1972). Annual layers in bones of *Rana temporaria*. *Zool. Zhurnal.* **51**: 1529-1534.

Stamps, J.A. (1993). Sexual size dimorphism in species with asymptotic growth after maturity. *Biol. J. Linn. Soc*. **50**: 123-145.

Stamps, J.A. & Krishnan, V. (1997). Sexual bimaturation and sexual size dimorphism in animals with asymptotic growth after maturity. *Evol. Ecol*. **11**: 21-39.

Stearns, S.C. (1992). *The evolution of life histories*. Oxford, Oxford Univ. Press.

Stöck, M., Dubey, S. & Klütsch, C. (2008**).** Mitochondrial and nuclear phylogeny of circum-Mediterranean tree frogs from the *Hyla arborea* group. *Mol. Phylogenet. Evol.* **49**: 1019-1024.

Sullivan, B.K., Ryan, M.J. & Verrell, P. (1995). Sexual selection and mating systems in amphibians. In: *Amphibian biology*, vol. 2 Social behavior: 469-517. Sullivan, B.K. & Heatwole, H. (Eds). New York, Surrey Beatty & Sons.

Travis, J. (1983). Variation in growth and survival of *Hyla gratiosa* larvae in experimental enclosures. *Copeia* **1983**: 232-237.

Travis, J. (1984). Anuran size at metamorphosis: experimental test of a model based on intraspecific competition. *Ecology* **65**: 1155-1160.

Vargas-Salinas, F. (2006). Sexual size dimorphism in the Cuban treefrog *Osteopilus septentrionalis. Amphibia-Reptilia* **27**: 419-426

Wells, K.D. (1977). The social behaviour of anurans amphibians. *Anim. Behav*. **25**: 666-693.

FIGURE CAPTIONS

Figure 1. Age-frequency distribution of male (a) and female (b) breeding tree-frogs. Dashed lines show the normal curves of the two distributions.

Figure 2. Relationship between age and body size (SVL) of males (open circles) and females (solid dots). The vB growth functions show that females (solid line) reach a larger asymptotic body size than that reached by males (dashed line).

Figure 3.

The number of eggs laid by females as a function of their SVL. Females laid more eggs in 2007 (solid dots) than in 2008 (open dots). Although, in both years, the number of eggs increases with female SVL, the regression coefficient was significantly higher in 2007 (solid line) than in 2008 (dashed line).

Figure 4. Frequency distribution of the number of mating obtained by males in 2007 and 2008.

Table 1. Descriptive statistics of body size (SVL and weight), age and reproductive success in males and females in the two breeding seasons .

		MALES			FEMALES		
Trait		N	Mean	*SD*	N	Mean	*SD*
SVL (mm)	2007	158	32.51	1.9	20	36.60	2.84
	2008	143	32.78	2.38	55	38.17	3.24
Weight (gr)	2007	158	2.52	0.49	13	3.76	0.87
	2008	144	2.47	0.53	22	3.97	0.99
Age (years)	2008	70	2.54	0.81	45	3.44	0.92
Mating success (n. of matings)	2007	158	0.08	0.28	13	1	0
	2008	136	0.22	0.47	32	1	0
Reproductive success (n. of eggs)	2007	158	60	211	13	727	239
	2008	132	63	147	24	344	80

FIGURE 1

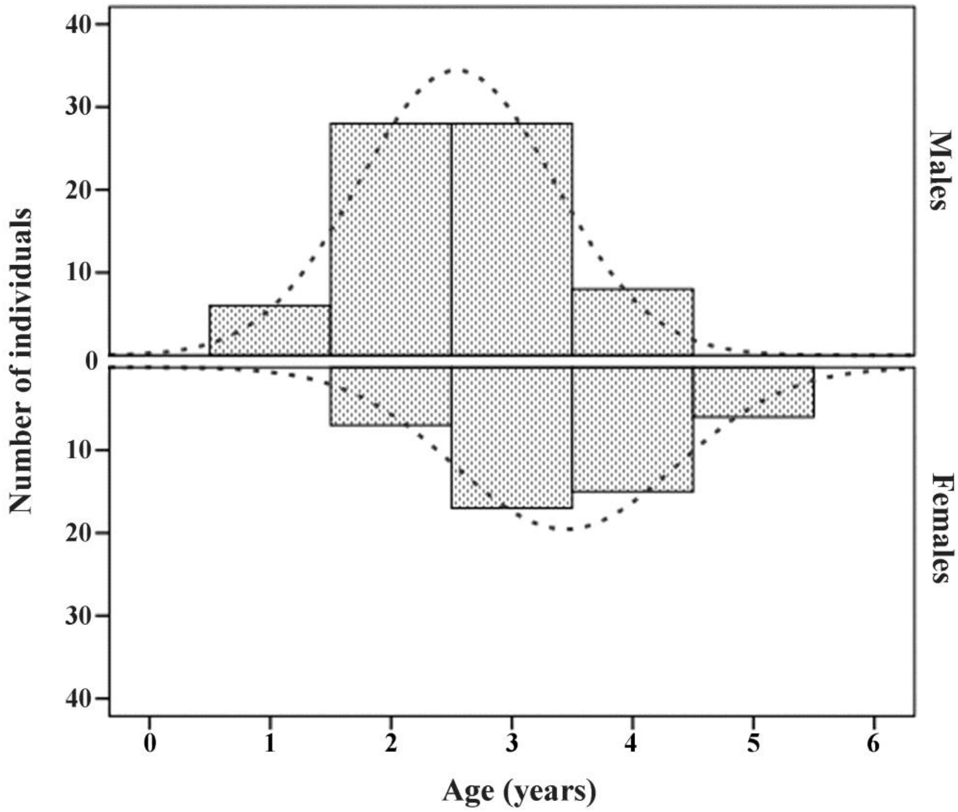
40
30
20
10
0
10
20
30
40
Number of individuals
Males
Females
0
1
2
3
4
5
6
Age (years)

FIGURE 2

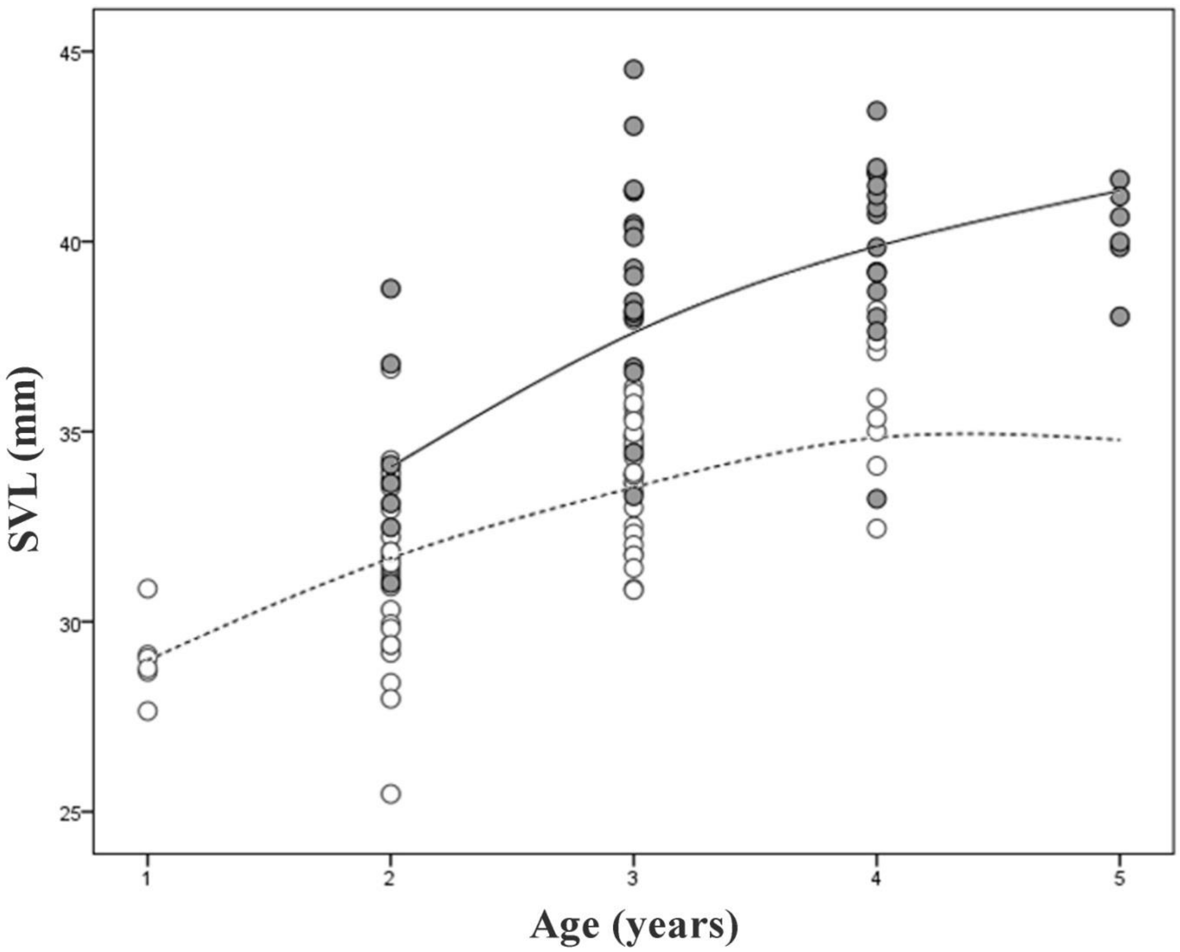

FIGURE 3

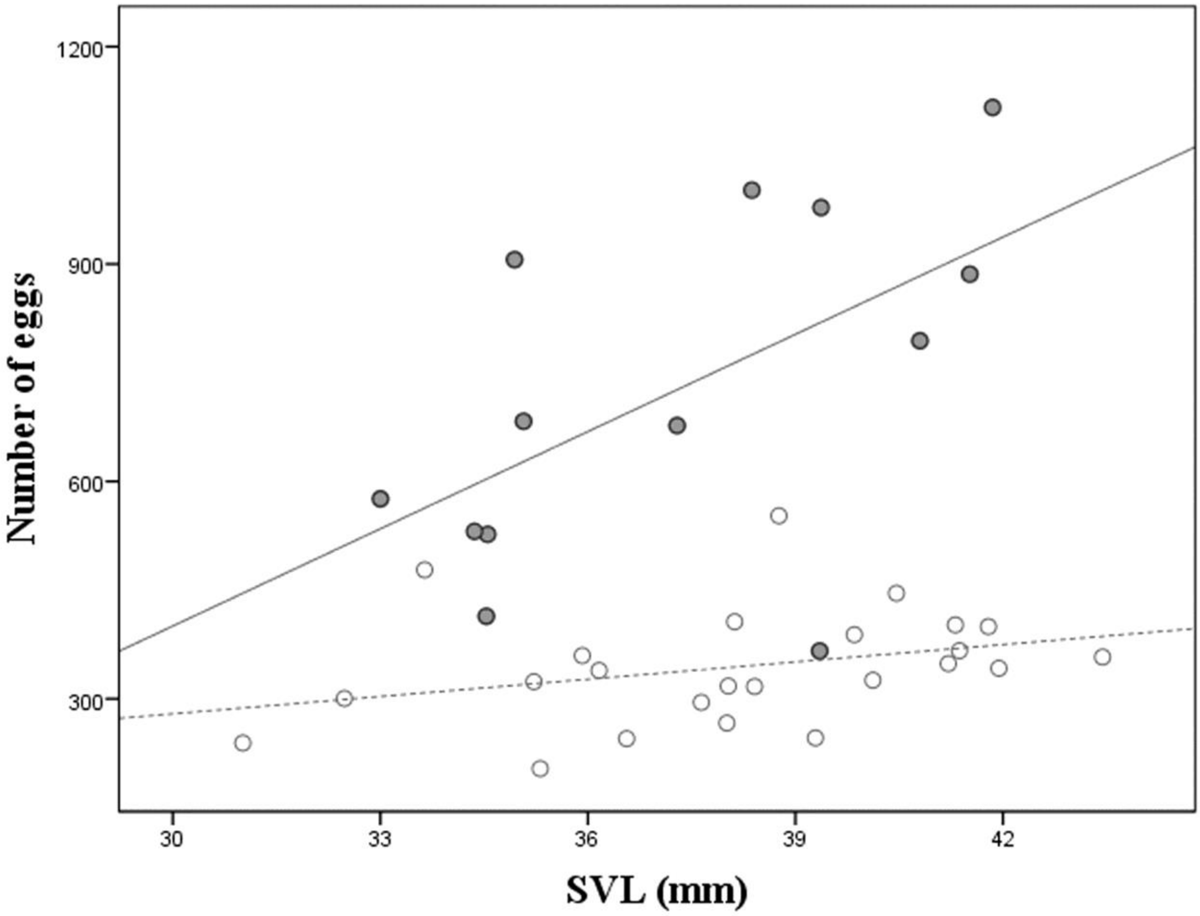

FIGURE 4