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

perspective

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1 Abstract

Sexual size dimorphism (SSD) is often explained as the differential equilibrium between stabilizing 2 survival selection and directional sexual/fecundity selection on the body size of males and females. 3 Provided that survival selection is similar in both sexes, female-biased SSD is thought to occur 4 5 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, 6 thus, to understand why SSD has evolved one should understand how it arises. We investigate SSD 7 8 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 9 because they mature one year later, live one year longer, and reach a larger asymptotic body size. 10 Furthermore, body size correlates positively with female fecundity, but not with male mating 11 success. These results suggest that SSD arises from differential optimal tradeoffs between the 12 expected number of reproductive episodes (which decreases with prolonging growth) and the 13 expected success in each reproductive episode (which increases with prolonging growth). 14

15

17 Introduction

Sexual size dimorphism (SSD) is a pattern observed in most animal groups (Andersson, 1994; 18 Fairbairn, Blanckenhorn & Székely, 2010). In species where males and females have highly 19 overlapping ecological niches and, thus, experience similar regimes of survival selection, SSD 20 evolves as the indirect consequence of the different reproductive role of males and females 21 (Darwin, 1871; Andersson, 1994). Strictly speaking, SSD is not an adaptation. SSD is a population 22 property emerging from sexual differences in life-history (Halliday & Verrell, 1986; Monnet & 23 24 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 25 pattern of SSD, a life-history perspective is required. Such an approach poses not only the question 26 of why one sex is larger in size than the other, but it also asks how these differences in size are 27 attained. 28 29 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 40 employed to explain the SSD in amphibian species as well. This model suggests that SSD arises 41 when conflicting selective pressures on body size (i.e. sexual and survival selection, in males, and 42 fecundity and survival selection, in females) equilibrate differently in the two sexes (Blanckenhorn 43 et al., 2007). When survival selection is similar in both sexes, a female-biased SSD results from 44 sexual differences in reproductive selection gradients (Lande & Arnold, 1983; Arnold & Wade, 45 1984): fecundity selection on female body size (large females can accommodate more eggs in their 46 body than smaller females) is stronger than sexual selection on male body size (Arak, 1988). The 47 predictions of the differential-equilibrium model have been supported by several studies on anurans, 48 49 which show that the correlation between reproductive success and body size is stronger in females than in males (Howard & Kluge, 1985; Vargas-Salinas, 2006). 50

However, in animals with indeterminate growth, selection does not act on adult body size *per se*, 51 52 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 53 becoming large (review in Blanckenhorn, 2000). Indeed, it takes time to grow large and the longer 54 the time devoted to growth the lower the probability to survive to first reproduction and the lower 55 the number of expected reproductive episodes (Shine, 1988). When costs and benefits differ 56 between sexes, selection may favour the evolution of different life history strategies in males and 57 females. To analyse the effects of between-sex life-history variation on SSD, Monnet and Cherry 58 (2002) conducted a comparative study of SSD in anurans and found that most of the among-species 59 variation in SSD was explained by sex differences in age structure. Similar results have been 60 observed at the intra-specific scale (Liao & Lu, 2010; Lyapkov, Cherdantsev & Cherdantseva, 61 2010), suggesting that longevity and age at first reproduction are the main determinants of SSD in 62 63 anurans.

64	In the present study, we examine the SSD pattern in a population of Tyrrhenian Treefrog, Hyla
65	sarda, an endemic species of the Tyrrhenian islands (Corsica, Sardinia and the Tuscan
66	Archipelago). H. sarda belongs to the H. arborea group (Stöck, Dubey & Klütsch, 2008) and, as all
67	the species of this group, it shows a lek mating pattern (Friedl & Klump, 2005; Castellano et al.,
68	2009a, b). During the breeding season, males aggregate in choruses at the breeding site; females are
69	attracted by male advertisement calls (Castellano et al., 2002; Rosso, Castellano & Giacoma,
70	2004a) and actively choose their mates on the basis of several acoustic properties of males' call
71	(Castellano & Rosso, 2006, 2007).
72	By means of skeletochronology techniques, we describe the age structure and the growth curves of
73	males and females in our tree-frog population. Skeletochronological technique is considered the
74	best method for age determination in anurans and has been already used in other tree-frog species
75	(Friedl & Klump, 1997; Rosso, Castellano & Giacoma, 2004b). Furthermore, for both sexes we
76	describe the relationship between body size and 'single-season' reproductive success. By combining
77	these two types of information, we test the hypothesis that the observed SSD pattern arises as the
78	effect of between-sex differences in optimal life-history strategies.
79	

80 Materials and Methods

81 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.

90 Sample Collection and Marking Technique

91 We carried out this study in the 2007 and 2008 breeding seasons, during the peak of reproductive

92 activity (from 3 April to 12 May in 2007 and from 1 April to 29 April in 2008). Every night, from

93 21:00 to 0:30, we captured male and female treefrogs found in breeding activity, by moving along

94 the shoreline. Marked individuals were identified and immediately released in the pond, whereas

95 pairs and unmarked individuals were carried to the laboratory, where pairs were let to spawn

96 overnight in separate plastic boxes (25x20x10 cm) filled up with tap water.

97 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

99 unmarked individuals. Treefrogs were anaesthetized in a 0.2% solution of MS-222 Sandoz, weighed

100 $(\pm 0.1 \text{ g})$ with a digital scale (Multifunction Pocket Scale MF-250, MTI Weight Systems, Inc.,

101 Kingstown, RI, USA), their Snout-Vent Length (SVL) was measured (± 0.01 mm) with a digital

102 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

104 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

106 2008, treefrogs were toe-clipped by cutting out the last two phalanges of the fourth toe of their right

- 107 hind limb. Phalanges were preserved in a 70% ethanol solution and successively used in
- 108 skeletochronological analyses.

109 Age determination

110 We determined individual age by means of skeletochronological techniques (Smirina, 1972;

111 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 112 113 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 114 mounted them on microscope slides using Aquamount. For each section, two observers counted 115 independently the number of Lines of Arrested Growth (LAGs) under a light microscope and later 116 compared results. Dubious cases were discounted. The most peripheral edge of the cross-sections 117 was counted as an additional LAG because the specimens were collected during the breeding 118 seasons after emergence from hibernation (Rogers & Harvey, 1994). 119

120 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):

128
$$SVL_{t} = SVL_{MAX} - (SVL_{MAX} - SVL_{MIN})e^{-k(t-t_{MIN})}$$

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.

150

151 **Results**

152 Sexual differences in body size and age

153 Table 1 shows the descriptive statistics of body size (SVL) and body weight (BW) of male and

154 female treefrogs in both the 2007 and 2008 samples. Mixed two-way ANOVAs, with sex as a fixed

155 factor and year as a random factor, showed between-sex highly significant differences in both SVL

156 $(F_{1,373} = 251.71, P < 0.001)$ 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 =
- 158 0.058) or no differences (BW: $F_{1,334} = 0.213$, P = 0.645) between years.
- 159 Skeletochronological analysis was carried out successfully on 115 adult individuals (58% of the
- 160 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.

166 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

169 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%

172 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

174 differed in the asymptote, but not in the rate at which the asymptote was reached.

175

167

176 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 177 them we measured fecundity (the total number of eggs produced). Fecundity was positively affected 178 by the female SVL ($F_{1,31} = 6.78$, P = 0.014), but not by the mated male-female SVL ratio ($F_{1,31} =$ 179 0.02, P = 0.881). The effect of body size on fecundity (Fig. 3) differed significantly between the 180 years ($F_{1,31} = 5.3$, P = 0.028). In the 2008 breeding season, females produced clutches that were, on 181 average, less than half the size of those produced in the previous year (Table 1). Such a decrease in 182 fecundity was size dependent, because, in 2008, large females laid a proportionally smaller number 183 of eggs than smaller females (Fig. 3). 184

186 **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 187 seasons. In 2008, the mean male mating success (Table 1) was higher than that observed in the 188 previous season (coefficient = 0.819, SE = 0.347, P = 0.018) and this difference can be explained by 189 the higher sex ratio found in 2007: 7.9 male/female versus 2.6 in 2008. Independent of the year, 190 however, the longer the time spent at the breeding site the higher the mating success (b = 0.054, SE 191 = 0.017, P = 0.001). In contrast, neither SVL (b = -0.002, SE = 0.148, P = 0.986) nor BW (b = -0.017, P = 0.001). 192 0.669, SE = 0.611, P = 0.273) showed a statistically significant relationship with male mating 193 194 success. To further investigate the effect of body size on male reproductive success, we analyzed the 195 relationships between the SVL of males and either the SVL or the number of eggs of their mates. 196 We found no evidence for a large male advantage in this population: independent of the breeding 197 198 season, the SVL of males was not positively associated either with the SVL of paired females (b =

199 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).

201

202 **Discussion**

203 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 204 between sexes, females being 16% larger than males. Second, it shows that this pattern depends on 205 differences in age at first reproduction and in asymptotic body size. Third, it shows that the effect of 206 body size on within-season reproductive success is stronger in females than in males. This latter 207 208 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 209 on males. 210

In females, reproductive success mainly depends on the number of eggs they produce and large 211 212 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 213 observed that such relationship differed markedly between years: the mean number of eggs in 2007 214 being twice as large as that in 2008. We do not know what causes these differences in fecundity, but 215 we observe that differences increase with female body size, suggesting that, under favourable 216 217 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 218 (Lardner & Loman, 2003; Castellano, Cucco & Giacoma, 2004; Vargas-Salinas, 2006) and at the 219 220 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 221 positively with hatchling size (Kaplan, 1980; Crump, 1984) and tadpole growth rate (Travis, 1984) 222 223 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 224 225 might be high.

226 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 227 228 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 229 male body size is expected if large males spend more time at leks or if they are more effective in 230 231 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. 232 Similar results were found in other two tree-frog species, phylogenetically close to the Tyrrhenian 233 Treefrog, H. intermedia (Castellano et al., 2009a) and H. arborea (Friedl & Klump, 2005). In both 234 these species, male mating success was strongly correlated with male attendance, and in H. 235

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 240 and costs of maturation at different ages balance at a stable equilibrium (Stearns, 1992). In species 241 242 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 243 surviving to first reproduction (Arak, 1988; Andersson, 1994; Blanckenhorn, 2000). If the benefits 244 245 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; 246 Kozlowski, 1992). In many species with indeterminate growth, females tend to mature later and at a 247 248 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 249 250 maturation of females has been observed in several species with female biased-SSD (review in 251 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. 252 253 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 254 amphibian species with female-biased SSD, however, females not only reach sexual maturity at an 255 256 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 257 clear evidence for sexual differences in adult growth rate. From the one hand, we observe that male 258 body size increases slowly but at an almost constant rate, whereas female body size increases from 259 the two- to the three-year age class, but then growth markedly decreases. On the other hand, 260

however, we find no statistically significant differences between the vB growth constants, k. 261 262 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 263 of age and not all females at two, the observed body-size differences between one- and two-year old 264 males and between two- and three-year old females are the average growth rates of both early- and 265 late-maturing individuals and they are thus expected to overestimate the true individual growth rate. 266 267 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 268 treefrogs lies in the between-sex differences in age at first reproduction. 269 In conclusion, this study shows that SSD in the Tyrrhenian Treefrog arises from differences in the 270 life-history strategies of the two sexes, that is, from the different way males and females use their 271 time to guarantee a future to their genes. In this species, females grow larger than males because 272 273 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 274

- by prolonging developmental time much more than males do.
- 276

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282

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410 FIGURE CAPTIONS

411

412	Figure 1. Age-frequency distribution of male (a) and female (b) breeding tree-frogs. Dashed lines
413	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).

417 **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).

422

Figure 4. Frequency distribution of the number of mating obtained by males in 2007 and 2008.
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427
428 Table 1. Descriptive statistics of body size (SVL and weight), age and reproductive success in
429 males and females in the two breeding seasons .

		I	MALES		FEMALES		
Trait		Ν	Mean	SD	Ν	Mean	SD
SVI (mm)	2007	158	32.51	1.9	20	36.60	2.84
	2008	143	32.78	2.38	55	38.17	3.24
Waisht (an)	2007	158	2.52	0.49	13	3.76	0.87
weight (gr)	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	2007	158	60	211	13	727	239
eggs)	2008	132	63	147	24	344	80

436 FIGURE 1





450 FIGURE 2



462 FIGURE 3





- 474 FIGURE 4



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