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

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1 **Abstract**

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

15

16

17 **Introduction**

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

29 The advantages of this approach are particularly evident in those species that show a strong
30 plasticity in body size, due to biphasic growth with asymptotic growth after maturity (Stamps,
31 1993). In these animals, in fact, adult body size depends on the amount of energy that individuals
32 invest in growth both before and after the attainment of sexual maturity (Jørgensen, 1992). For this
33 reason, sex-differences in life history traits (i.e. pre- and post-maturation growth rate, age at first
34 reproduction and longevity) may represent different allocation strategies responsible for both the
35 direction and the extent of SSD.

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

40 The pattern of SSD has been usually explained by the “differential-equilibrium model”, often
41 employed to explain the SSD in amphibian species as well. This model suggests that SSD arises
42 when conflicting selective pressures on body size (i.e. sexual and survival selection, in males, and
43 fecundity and survival selection, in females) equilibrate differently in the two sexes (Blanckenhorn
44 *et al.*, 2007). When survival selection is similar in both sexes, a female-biased SSD results from
45 sexual differences in reproductive selection gradients (Lande & Arnold, 1983; Arnold & Wade,
46 1984): fecundity selection on female body size (large females can accommodate more eggs in their
47 body than smaller females) is stronger than sexual selection on male body size (Arak, 1988). The
48 predictions of the differential-equilibrium model have been supported by several studies on anurans,
49 which show that the correlation between reproductive success and body size is stronger in females
50 than in males (Howard & Kluge, 1985; Vargas-Salinas, 2006).

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

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

88 maintained the water level constant for the entire breeding season, in both years of study. Treefrogs
89 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
98 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 (± 0.1 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
103 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
105 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

112 in 5% nitric acid for about 30 min, and soaked in tap water overnight. Phalanx cross-sections were
113 obtained with a freezing microtome at 16 μm , stained with hematoxylin for 20 min and washed in
114 water for 10 min. We selected the mid-diaphyseal sections with the narrowest medullar cavity and
115 mounted them on microscope slides using Aquamount. For each section, two observers counted
116 independently the number of Lines of Arrested Growth (LAGs) under a light microscope and later
117 compared results. Dubious cases were discounted. The most peripheral edge of the cross-sections
118 was counted as an additional LAG because the specimens were collected during the breeding
119 seasons after emergence from hibernation (Rogers & Harvey, 1994).

120 **Set of variables and statistical analyses**

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

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

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

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

143 In males, reproductive success was described either as the number of matings obtained by a male
144 during the entire breeding season (mating success) or as the number of eggs laid by a male's mate
145 (fecundity success). Mating success was used in a GLM with Poisson errors and log-link function,
146 in which year was the random factor and SVL, BW, and chorus tenure were the covariates. Chorus
147 tenure was a measure of mating effort and was defined as the number of days between the last and
148 the first capture of a male. Fecundity success was used in a general linear model with Gaussian
149 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
157 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

161 sexes ($\chi^2 = 25.17$, $df = 4$, $P < 0.001$): adult-male age ranged from one to four years, with most of
162 males (80%) being two or three years old; adult females were about one-year older than males, their
163 age ranging from two to five years, with most of females (71%) falling into the three- and four-year
164 age classes (Fig. 1).

165 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,
167 2.6 mm and 1.8 mm, respectively, between 2 and 1, 3 and 2, and 4 and 3 years), in females it varies
168 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,
170 in turn, is about five times larger than the difference in size between five- and four-year old
171 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
173 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

176 **Body size and reproductive success in females**

177 During the 2007 and 2008 breeding seasons, we captured 75 females, 45 were paired and for 37 of
178 them we measured fecundity (the total number of eggs produced). Fecundity was positively affected
179 by the female SVL ($F_{1,31} = 6.78$, $P = 0.014$), but not by the mated male-female SVL ratio ($F_{1,31} =$
180 0.02 , $P = 0.881$). The effect of body size on fecundity (Fig. 3) differed significantly between the
181 years ($F_{1,31} = 5.3$, $P = 0.028$). In the 2008 breeding season, females produced clutches that were, on
182 average, less than half the size of those produced in the previous year (Table 1). Such a decrease in
183 fecundity was size dependent, because, in 2008, large females laid a proportionally smaller number
184 of eggs than smaller females (Fig. 3).

185

186 **Body size and male mating success in males**

187 Fig. 4 shows the frequency distribution of male mating success in both the 2007 and 2008 breeding
188 seasons. In 2008, the mean male mating success (Table 1) was higher than that observed in the
189 previous season (coefficient = 0.819, SE = 0.347, P = 0.018) and this difference can be explained by
190 the higher sex ratio found in 2007: 7.9 male/female *versus* 2.6 in 2008. Independent of the year,
191 however, the longer the time spent at the breeding site the higher the mating success (b = 0.054, SE
192 = 0.017, P = 0.001). In contrast, neither SVL (b = -0.002, SE = 0.148, P = 0.986) nor BW (b =
193 0.669, SE = 0.611, P = 0.273) showed a statistically significant relationship with male mating
194 success.

195 To further investigate the effect of body size on male reproductive success, we analyzed the
196 relationships between the SVL of males and either the SVL or the number of eggs of their mates.
197 We found no evidence for a large male advantage in this population: independent of the breeding
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 =
200 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
204 Tyrrhenian treefrogs. Our study provides three main results. First, it shows that body size differs
205 between sexes, females being 16% larger than males. Second, it shows that this pattern depends on
206 differences in age at first reproduction and in asymptotic body size. Third, it shows that the effect of
207 body size on within-season reproductive success is stronger in females than in males. This latter
208 result seems consistent with the differential-equilibrium model of the evolution of SSD
209 (Blanckenhorn, 2000), because it suggests that selection on body size be stronger on females than
210 on males.

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

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

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

240 According to life-history theory, the optimal age at first reproduction is that at which the benefits
241 and costs of maturation at different ages balance at a stable equilibrium (Stearns, 1992). In species
242 with indeterminate growth, the main benefit of delayed maturation is the large body size that can be
243 attained and its effects on reproductive performance, whereas the main cost is the low probability of
244 surviving to first reproduction (Arak, 1988; Andersson, 1994; Blanckenhorn, 2000). If the benefits
245 of delayed maturation differ between males and females more than the costs do, then life-history
246 theory predicts between- sex differences in age at first reproduction (Kozłowski & Wiegert, 1987;
247 Kozłowski, 1992). In many species with indeterminate growth, females tend to mature later and at a
248 larger size than males and this sexual bimaturism has been explained in terms of increased
249 fecundity of large and old females (Stamps & Krishnan, 1997). In anurans, in particular, delayed
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
252 evidence that SSD arises because females benefit from delaying reproduction more than males do.
253 Shine (1990) suggested that if individual body size differs between sexes at sexual maturity, then
254 the largest sex maintains this advantage over the other one also during the adult phase. In several
255 amphibian species with female-biased SSD, however, females not only reach sexual maturity at an
256 older age, but they also show a higher post-maturation growth rate (Ma & Lu, 2009; Hasumi, 2010;
257 Guarino *et al.*, 2003), which further increases SSD in the adult population. Our results provide no
258 clear evidence for sexual differences in adult growth rate. From the one hand, we observe that male
259 body size increases slowly but at an almost constant rate, whereas female body size increases from
260 the two- to the three-year age class, but then growth markedly decreases. On the other hand,

261 however, we find no statistically significant differences between the vB growth constants, k .
262 Furthermore, the growth curves in our sample should be considered cautiously, because of the
263 within-sex variation in age at maturity. In fact, since not all males reach sexual maturity at one year
264 of age and not all females at two, the observed body-size differences between one- and two-year old
265 males and between two- and three-year old females are the average growth rates of both early- and
266 late-maturing individuals and they are thus expected to overestimate the true individual growth rate.
267 Because of these limitations, our results cannot exclude that some sexual differences in adult
268 growth rate exist, but they provide strong evidence that the main cause of SSD in Sardinian
269 treefrogs lies in the between-sex differences in age at first reproduction.
270 In conclusion, this study shows that SSD in the Tyrrhenian Treefrog arises from differences in the
271 life-history strategies of the two sexes, that is, from the different way males and females use their
272 time to guarantee a future to their genes. In this species, females grow larger than males because
273 they invest more time in pre-adult growth and this occurs because of the different optimal
274 compromise between body size and developmental time: females benefit from attaining large size
275 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.

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

417 **Figure 3.**

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

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

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 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 .

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Trait		MALES			FEMALES		
		N	Mean	<i>SD</i>	N	Mean	<i>SD</i>
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

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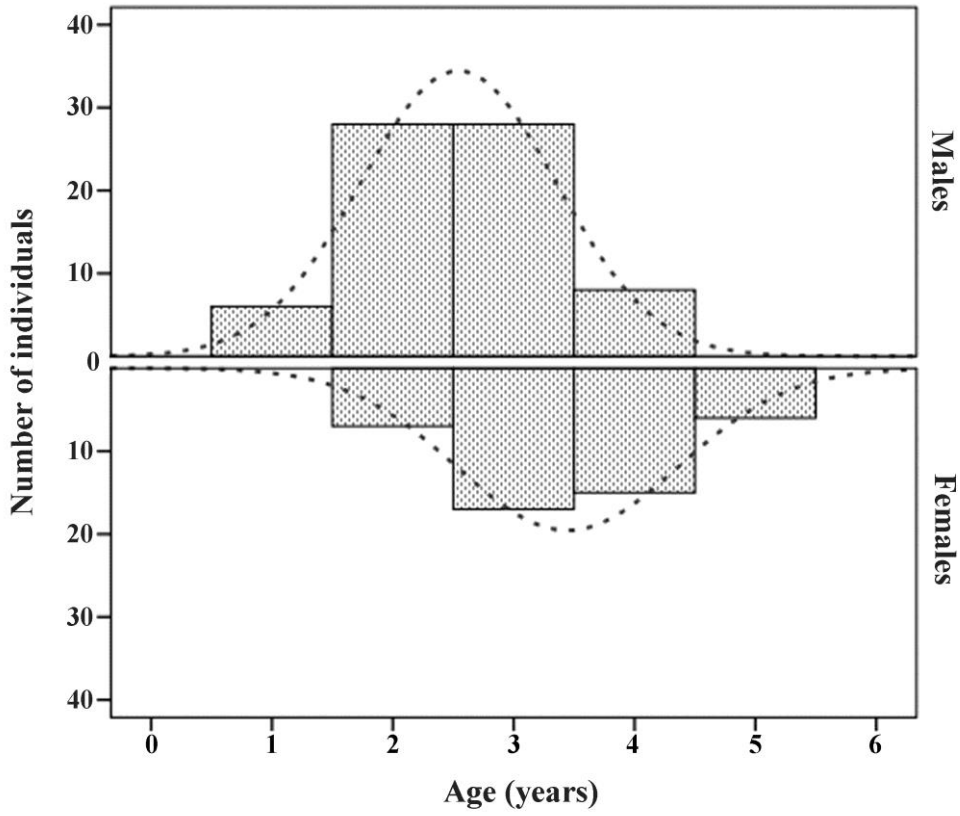
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436 FIGURE 1

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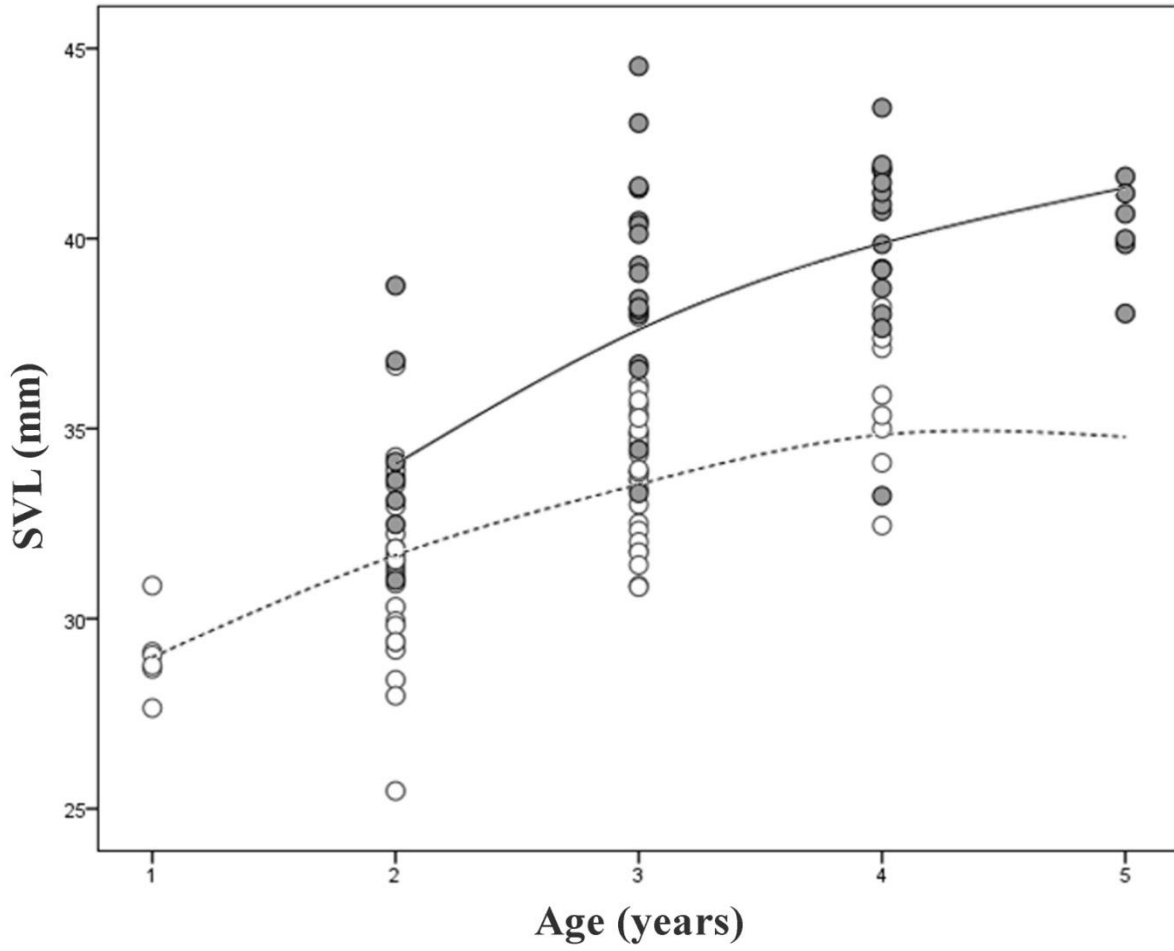
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450 FIGURE 2

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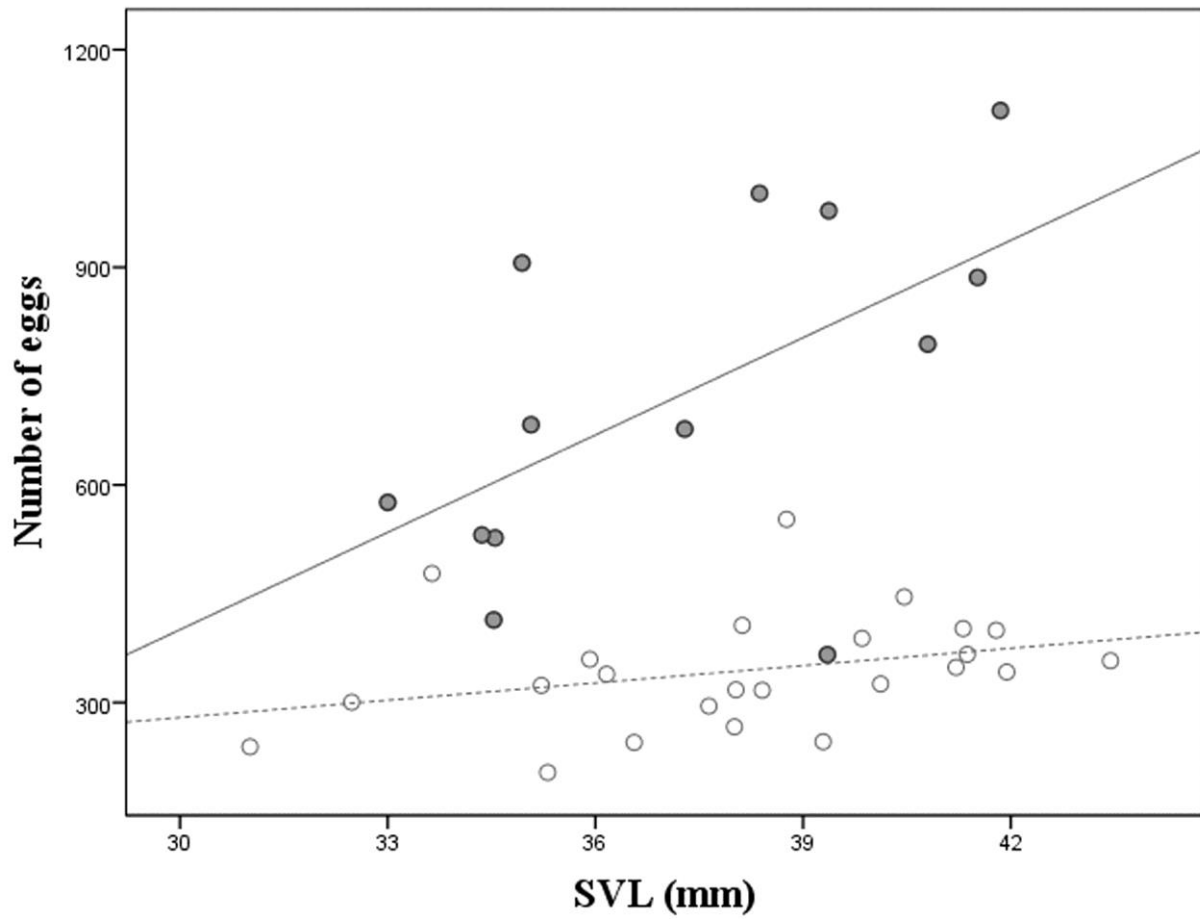
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462 FIGURE 3

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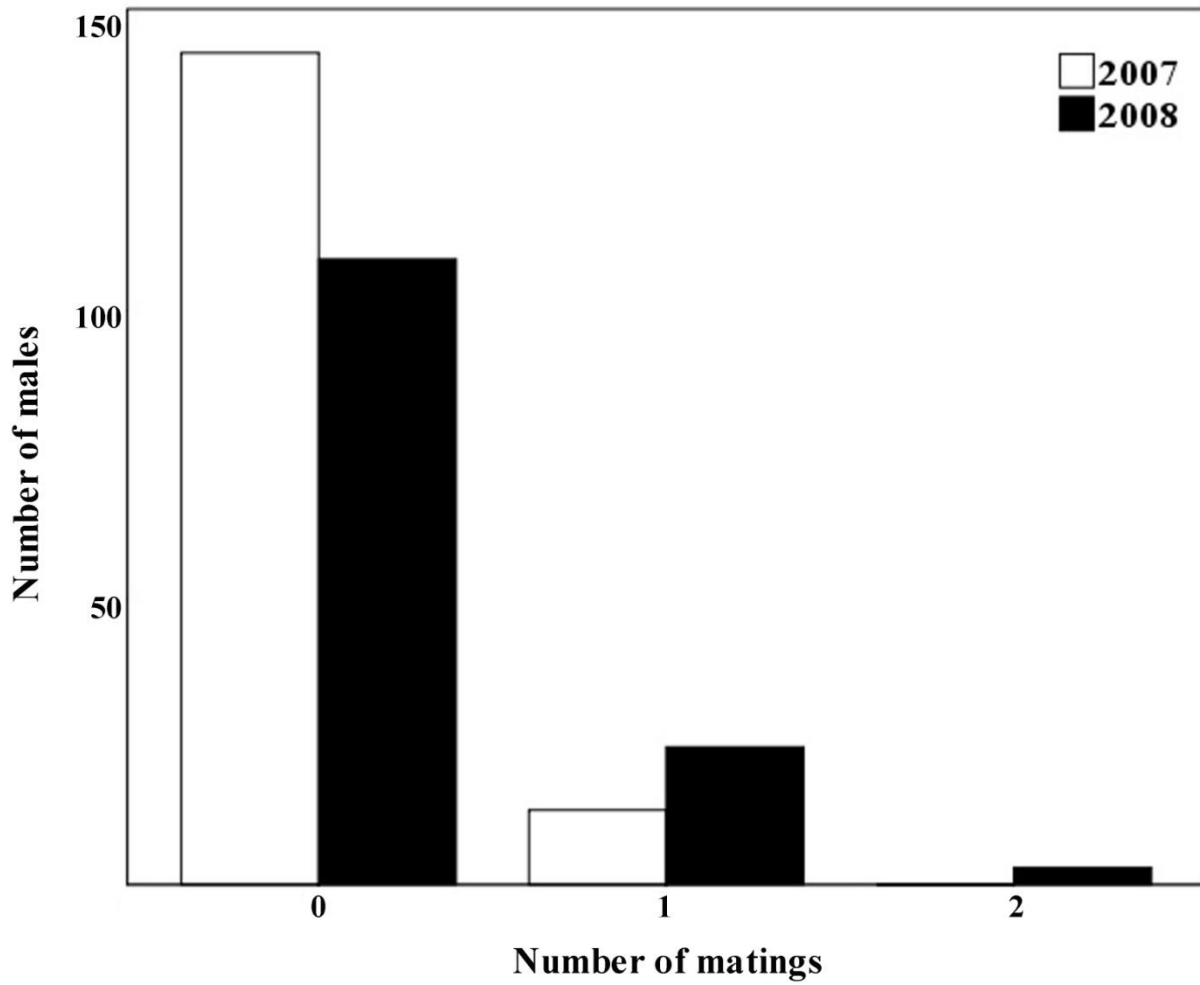
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474 FIGURE 4

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