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Environmentally determined juvenile growth rates dictate the degree of sexual size dimorphism in the Sardinian brook newt

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Abstract Sexual size dimorphism (SSD) is widespread among amphibians, and it is attributed to fecundity selection, invoked for female-biased-SSD species, sexual selection, usually invoked for male-biased-SSD species, or other forms of natural selection. Further, SSD interplays with body size variability at population level. By using a male-biased-SSD newt (*Euproctus platycephalus*) as model species, we investigated body size and SSD variability across the whole species' range, the island of Sardinia, looking for the main evolutionary force behind SSD. We found geographic variation of body size and SSD: newts from northern populations were generally larger than those from southern ones, with a larger degree of male biased dimorphism in the former. The mean age of newts varied among populations, but it did not follow any geographical pattern, and it did not differ between sexes. Southern populations reached sexual maturity earlier than northern ones, and maturity was positively correlated with temperature. Growth curves show that northern populations achieved male-biased SSD before reaching sexual maturity, whilst in the south males become larger than females after 6 years in age, following a non-asymptotic growth curve. The geographical pattern of the adult body size variation is attributable to delayed maturation of the larger newts in more northerly populations, and earlier sexual maturation

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due to warmer temperature in southern populations, which leads to smaller body size. SSD varies consistently with body size: it is evident in the north, where sexual bimaturation caused larger males, but it is weak in the south, where warmer temperature caused an earlier maturation of both sexes.

Keywords *Euproctus platycephalus* · Body size · Intraspecific variability · Sexual maturity · Sexual size dimorphism · Sardinia

Introduction

Sexual size dimorphism (SSD), the body size difference between males and females of the same species or population, is widespread within animals and plants. Despite the large body of evidences for its occurrence in a range of taxonomic groups, the evolutionary processes and the proximate mechanisms that underlie SSD vary across species and habitats, making SSD a field of active research and new findings (e.g., Fairbairn 2005; Blanckenhorn et al. 2007; Cox and Calsbeek 2009; Barrett and Hough 2013). Within vertebrates, it is generally assumed that males of lizards, birds and mammals are larger than females, whilst females of fishes and amphibians are larger than respective males. SSD in amphibians has been studied for decades and is phylogenetically widespread (Shine 1979; Woolbright 1983; Halliday and Verrell 1986; Monnet and Cherry 2002; Kupfer 2007, 2009; Cadeddu et al. 2012). Even if female-biased SSD is the common pattern within amphibians, still some species show male-biased SSD or no SSD.

The evolution of SSD is generally attributed to sexual selection, fecundity selection, or other forms of natural selection due to divergent, sex-specific ecological niches (Fairbairn et al. 2007). Where males are the larger sex, sexual selection is commonly invoked due to the presence of phenotypically variable male traits that confer reproductive advantage, especially during the mating (Shine 1979; Halliday and Tejedo 1995; Howard et al. 1997; Gabor and Halliday 1997; Gabor et al. 2000; Bovero et al. 2003). When large females have better reproductive outputs, female-biased SSD is usually explained as the result of fecundity selection for larger females (Hedrick and Temeles 1989): e.g., larger amphibian females produce more and/or larger eggs (reviewed in Wells 2007).

Sex-specific growth rates appear near-ubiquitous within amphibians (Monnet and Cherry 2002) and affect the adult SSD patterns: growth rates resulting in SSD frequently arise from sex-specific selection on age at first reproduction (Shine 1990), and fecundity selection is frequently invoked as an explanation for accelerated growth of sexually immature females, positive female lifetime growth rates and female-biased SSD (Miaud et al. 2000; Blackwell et al. 2003; Hasumi 2010). For example, male juvenile western spadefoot toads grow faster, reach sexual maturity earlier and are larger at sexual maturity than females, likely due to the advantage large body size confers during male–male competition for access to breeding females (Morey and Reznick 2001). However, females eventually exceed males in size, since females continue to grow after males reached maturity and reduce their growth rates, presumably due to fecundity selection. In caudate amphibians, either sex may end up the larger, highlighting the possible roles of different selection pressures (Bruce 1993; Malmgren and Thollesson 1999; Miaud et al. 2000; Blackwell et al. 2003; Howard 2009; Hasumi 2010).

Irrespective of the selective force that causes SSD, the degree of divergence in sex-specific growth rates is expected to be influenced by environmental variation (Post et al. 1999;

Blanckenhorn et al. 2006) because a given selective pressure will vary in strength in different environments (Fairbairn 1997; Badyaev 2002; Badyaev et al. 2003; Le Galliard et al. 2006). Notwithstanding, when environmental variation influences the degree of SSD exhibited among populations, SSD should still be associated with the predominant evolutionary force that leads to the evolution of sex-specific growth rates (Badyaev et al. 2003). If SSD evolves as the result of sexual or fecundity selection, the expectation is that plasticity should be predominantly expressed in one sex, in traits under sexual selection, or in traits that impart fecundity benefits (Preziosi and Fairbairn 2000; Fairbairn 2005). If SSD arises due to other forms of natural selection it is often affected by phenotypic plasticity, predominantly during early development (Cornwallis and Uller 2010). In this case, both sexes may exhibit plasticity and trait variation should not be restricted to traits under sexual selection (differential plasticity hypothesis). However, it has been also shown that canalization can influence intraspecific patterns of SSD (differential adaptive canalization hypothesis, Fairbairn 2005). Overall, explanations for the evolution of SSD in caudates are based on sexual and fecundity selection rather than other forms of natural selection (but see Shine 1990). However, differential equilibrium among these selective pressures (Blanckenhorn 2005) has to be taken into account, as well as ecologically mediated SSD should not be discounted (Shine 1989). Expression of both male and female traits is often conditional, though, and as a result strong within-population variation can be observed (Haerty et al. 2007; Malmgren and Enghag 2008). Because growth rates of both sexes are strongly influenced by environmental conditions, it is difficult to conclude whether plastic growth rates reflect sexual and/or fecundity selection or environmental constraints on body size that are (or are not) independent of sex (Miaud et al. 2000, but see Howard 2009).

We investigated the body size and SSD variation of the Sardinian brook newt, *Euproctus platycephalus* (Gravenhorst 1829), a caudate amphibian that exhibits male-biased SSD, which is uncommon in amphibians (Kupfer 2007). A single population study showed that males are the larger sex, and have longer limbs and longer and larger heads, but proportionally shorter tails than females (Bovero et al. 2003). Bovero et al. (2003) concluded that differences in morphology among the sexes arose through sexual selection because males use their jaws, limbs and tail to physically grasp females while attempting forcible insemination. This is an unusual mating system for a newt. More typically, male newts use courtship without any physical restraint to encourage females to pick up spermatophores deposited on the substrate, rather than inserting them directly (Halliday and Tejedo 1995). When male physically restrains the female during the mating, male-biased sexual dimorphism due to sexual selection is predicted (Shine 1979), and shown for one caudate amphibian (Jones et al. 2004). Jones et al. (2004) showed that a consistently male-biased strength of sexual selection in Rough-skinned newts supports the conclusion that tail height is an important determinant of male mating success and that size dimorphism of this trait arose through sexual selection.

However, it is not easy to disentangle how multiple evolutionary pressures may be contibuting to SSD and evaluate their relative and absolute importance (Blanckenhorn 2005). Here we attempted to assess the relative contributions of evolutionary and ecological factors on SSD. Our first goal was to determine if SSD in *E. platycephalus* is consistent across the species range or if intraspecific variation in SSD exists for this species, and, in the case of the latter, how the observed variation correlates with body size variation at population level. Intraspecific SSD variation has been described in many and diverse taxa with different explanations for why it occurs (Fairbairn et al. 2007; Lengkeek et al. 2008; Stillwell and Fox 2009; Cox and Calsbeek 2010). Our working hypotheses was that if sexual selection was the predominant force behind newt body size, then SSD should be relatively consistent across the species range. However, if ecological constraints were more important for determining newt body size, and as a result SSD, then significant intraspecific variation in SSD should be detectable due to environmental variation across the species range.

Whatever the evolutionary force dictating SSD, it is usually determined at early ages or through divergent ontogenies, particularly in ectotherms that continue to grow for the whole lifespan (Badyaev 2002; Monnet and Cherry 2002; Blanckenhorn et al. 2007; Zhang and Lu 2013). Because investment in most traits associated with SSD in amphibians should be strongly influenced by investment in growth, where growth is impaired the degree of SSD should be diminished or only evident when slow growth in the early stages is compensated for later in life. For this reason, our second goal was to assess if body size and/or SSD variation could be attributed to age differences among populations and sexes, or to divergent ontogenetic trajectories. Here our working hypothesis was that if sexes did not have different age structure, growth duration (and its timing) and/or growth trajectories would be responsible for SSD.

As a tertiary goal, we also tested if male tail length is sexually selected, as expected for body features males use to restrain females during mating (Shine 1979) and as confirmed in the Rough-skinned newt (Jones et al. 2004). Our hypothesis was that if tail length is directly under sexual selection, then only males should exhibit allometric tail growth with respect to body size.

Materials and methods

Euproctus platycephalus is a relatively long-living, endangered caudate amphibian (Bovero et al. 2008; Romano et al. 2012). Although the species is restricted to a single Mediterranean island, Sardinia, significant latitudinal variation in environmental metrics, influenced by changes in elevation, occurs on the island, even over short, straight-line distances (Delitala et al. 2000). Brook newts are typically found from 400 to 1,200 m elevation, and inhabit northern, central and southern mountain regions of the eastern slope of the island (Lecis 2007): different populations are likely to experience different environmental conditions. Brook newts are notoriously difficult to sample, as they are shy, cryptic and rarely encountered at high densities (Lecis and Norris 2003; pers. obs.). Unlike many European amphibians, they do not breed explosively: eggs are deposited singly under rocks or on aquatic vegetation and are difficult to find, and larvae are rarely encountered in the wild. Because of this, no field data on reproductive rates and clutch size exist, but captive animals can breed for four out of seven years (Funnell and Tapley 2009).

For 5 years (2004–2008) we surveyed locations across the mountainous areas of Sardinia where *E. platycephalus* was reported to occur, or locations we deemed likely to support newt populations. We restricted our searches to streams, pools and small lakes, including cave locations, since *E. platycephalus* is presumed to be active only in water, even though newts can spend time on land (Lecis 2007; pers. obs.). For each site used in this study, we recorded coordinates and elevation, and subsequently obtained the relevant mean annual air temperatures from WorldClim, based on 30-arc-seconds cells (Hijmans et al. 2005). These two environmental metrics have been shown to influence amphibian growth in the temperate zone (e.g., Morrison and Hero 2003; Cvetković et al. 2009). Although we have detected newts at more than 30 locations (Sotgiu et al. 2010), surveys of only a subset of these sites (nine, Table 1; Fig. 1) generated numbers of newts

Table 1 Studied sites of *E. platycephalus*: site name (geographic location in brackets), code used in the text, type of water-body, altitude of the site, mean annual temperature (basing on Hijmans et al. 2005), sampling period ("spr." is for spring, "sum." for summer, "aut." for autumn) and size (sample size refers to SVL, for more detailed information on sample sizes see Supplementary material, Appendix 1)

Site	Code	Type	Altitude (m a.s.l.)	Mean annual temperature (°C)	Sampling period and size
Rio Pisciaroni (Limbara mountains)	L1	Stream	960–1,000	11	2008 (spr., sum.) 21 males, 14 females
Riu Fundu di Monti tributary (Limbara mountains)	L2	Stream	970–990	11	2007 (sum.); 2008 (spr., sum.) 38 males, 20 females
Gorroppu (Supramonte mountains)	SC	Residual pond	625	13.7	2004 (sum.); 2005 (sum., aut.); 2006 (spr.) 78 males, 38 females
Perdasdefogu Gennargentu mountains	PF	Cave brook	486	13.8	2007 (aut.) 10 males, 16 females
Rio Picocca tributary (Sette fratelli mountains)	S1	Stream	150–200	15.9	2007 (spr.) 20 males, 15 females
Rio Picocca tributary (Sette fratelli mountains)	S2	Stream	200–300	14.8	2007 (spr.) 7 males, 21 females
Rio Picocca tributary (Sette fratelli mountains)	S3	Stream	300–400	15.5	2007 (spr.) 12 males, 16 females
Rio Maidopis (Sette fratelli mountains)	S4	Stream	450–550	13.9	2005 (sum.); 2007 (spr.); 2008 (spr.) 20 males, 8 females
Rio Suergiu Mannu tributary (Sette fratelli mountains)	S5	Stream	550–600	14.2	2007 (spr.) 12 males, 9 females

(Supplementary material, Appendix 1) we deemed sufficient for comparing morphology and inferring from this life history variation. These sites were located in all the three major mountain ranges on the island (the northern Limbara Mountains, the central Gennargentu Range and the southern Sette Fratelli mountains) and encompass the entire species range of *E. platycephalus* (Fig. 1). Lecis and Norris (2004) found genetic structure in the species, but it has no geographic basis; further, they considered that gene flow occurred at least in the recent past, so that ongoing speciation among our populations is unlikely. We pooled data across years for sites where we had multiyear samples after confirming that variation among years at a site only accounted for 5 % of the total variance (nested ANOVA, analysis not shown). Individuals were sexed based on cloacal morphology (Bovero et al. 2003) and measured for both total (TL) and snout-to-vent length (SVL) to the nearest millimeter. We calculated tail length (tL) as the difference between TL and SVL.

We determined age of newts using skeletochronology (Castanet and Smirina 1990) of the third toe of one hind foot following the protocol used by Bovero et al. (2003; see details in the Supplementary material, Appendix 2). The bone sections were surveyed at 400× magnification (Nikon Eclipse e200) for lines of arrested growth (LAGs), the sum of which

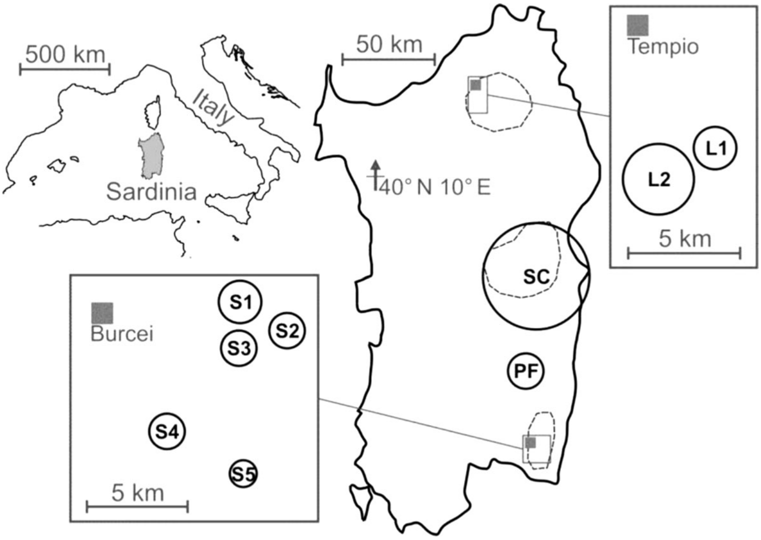

Fig. 1 Map of Sardinia with the location of the study populations; *dotted lines* encircle the *E. platycephalus*' range according to IUCN; *circle* size is proportional to the number of individuals used for the analysis

is the estimate of age (Castanet and Smirna 1990). Occasionally, the first LAG can be eliminated by the resorption of bone tissue (Francillon-Vieillot et al. 1990). To evaluate this, we performed osteometrical analysis on the sections (Guarino et al. 2003) and, when needed, we added 1 year to our estimate of adult age of that individual (Supplementary material, Appendix 2). Our estimation of age at sexual maturity is based on the fact that in amphibians maturity is associated with reduced investment in somatic growth (Hemelaar 1988). As a result, the deposition of LAGs during adulthood occurs more closely together, and the distance between LAGs (annulus) during juvenile growth is significantly greater than when somatic growth is traded off against investment in reproduction (Halliday and Verrell 1988). The result is a pattern of one, or a few, internally located interLAG annuli that are spaced significantly farther apart than annuli located peripheral to the wider annuli. We confirmed this pattern in *E. platycephalus* (Supplementary material, Appendix 2). We therefore considered age at first reproduction for each newt as the year (LAG) at which interLAG spaces shifted in size.

We used ANOVA to first seek evidence of variation in body size (SVL) among populations and the sexes and used ANOVA-based analyses to explore whether the sexes varied in age structure. We incorporated a variable coding for the population in which the data was collected in these models because if environmental variability influenced SSD, trait variation would vary amongst populations with different environmental regimes. To ascertain whether both morphological traits (SVL, tL) varied significantly between sexes and among populations, we used two-way factorial analysis of variance (ANOVA), with population, sex and their interaction as factors. We again used two-way factorial ANOVA to assess the effects population, sex and their interaction may have had on adult age. For post hoc comparisons we used the Tukey HSD test for unequal samples (homogenity: $\alpha = 0.05$). We also measured the effect of sex and population on age at sexual maturity, but used a Kruskal–Wallis ANOVA, because data on age at sexual maturity did not meet the assumptions of normality. Sample sizes are reported in Table 1 and Supplementary material, Appendix 1.

Because sex significantly affected both size and age at sexual maturity in the previous analyses, we used a Pearson correlation test to ascertain if older individuals were, on average, larger than their younger counterparts, as would be expected. Age and body size (SVL) were significantly correlated (see results) so we reanalyzed the effect of population, sex and their interaction had on SVL using two-way analysis of covariance (ANCOVA) with age controlled for as a covariate. We chose SVL for this analysis as age should affect this trait similarly in both sexes, or exhibit female bias if fecundity selection was important. To determine if tL was biased towards males, as would be the case if tL was a trait under male-biased sexual selection (Tomkins et al. 2005), we reanalyzed the effect of population, sex and their interaction on tL using ANCOVA as above, but with SVL controlled for as a covariate and assuming non-homogeneity of slopes (see below).

To investigate how specific environmental factors may have influenced growth rates, we again used Pearson correlations to describe the relationships between each of local air temperature or altitude with average age at sexual maturity or age of newts of both sexes in a population. Population was a significant factor in the initial ANOVA-based analyses (see below) so we examined how geographic variation of body size may link to life history variation by constructing models of annual adult growth based on the Von Bertalanffy (1938) equation. Models were built for each sex, split amongst regions for which we detected significant differences in body size (see results). To do this, we used measurements of all sexually mature individuals we sampled in a region, and applied the equation $\mathrm{SVL_t} = \mathrm{SVL_{max}} - (\mathrm{SVL_{max}} - \mathrm{SVL_{met}})e^{-k(t - \mathrm{tmet})}$, where t is the estimated age in years, $\mathrm{SVL_{max}}$ is the estimated maximum SVL that can be reached, $\mathrm{SVL_{met}}$ is the measured average SVL of metamorphosing individuals and k is the growth coefficient, with t_{met} fixed at zero (see Arntzen 2000, and Miaud and Guillaume 2005). We fitted curves using non-linear estimation (1,000 iterations).

Last, we compared the degree of sexual size dimorphism among populations by calculating a size dimorphism index (SDI) (Lovich and Gibbons 1992) for each population {SDI = [(mean SVL of females/mean SVL of males) − 1]} following convention. We then calculated a corrected SDI by using the deviations of SVL from the regression between age and SVL, i.e. after the age effect on body size had been removed. We used Pearson correlation tests to verify if the corrected SDI was associated with average age of each sex at sexual maturity and to evaluated to what extent the corrected SDI correlated with the mean female–male age difference (MAD, Monnet and Cherry 2002), i.e. by the sex-specific population age structure. We used the software STATISTICA 7.0 (StatSoft Inc. 2004) for all statistical analyses.

Results

Population, sex and their interaction all influenced SVL (Table 2; see Supplementary material, Appendix 1 for data summary). Males were larger than females and body size variation clustered into two distinct geographic regions: newts from the north and central parts of the distribution were larger than newts from the south (Sette Fratelli mountains) (Fig. 2 and Supplementary material, Appendix 3). Post hoc comparisons also showed that sex-specific differences in SVL were less evident among males and females from populations located in the southern part of the species distribution, in the Sette Fratelli mountain range (Fig. 2 and Supplementary material, Appendix 4). Similarly, tail length (tL) was

Table 2 Effects of population, sex and their interaction on snout-vent length (SVL), tail length (tL) and age of *E. platycephalus* (two-way factorial ANOVAs)

Effect	SVL (error $df = 357$)	tL (error $df = 311$)	Age (error $df = 212$)
Population	$F = 108.3$, $P < 0.001$	$F = 101.3$, $P < 0.001$	$F = 11.5$, $P < 0.001$
Sex	$F = 30.6$, $P < 0.001$	$F = 45.9$, $P < 0.001$	$F = 0.5$, $P = 0.47$
Population × sex	$F = 6.2$, $P < 0.001$	$F = 3.8$, $P < 0.001$	$F = 1.3$, $P = 0.23$

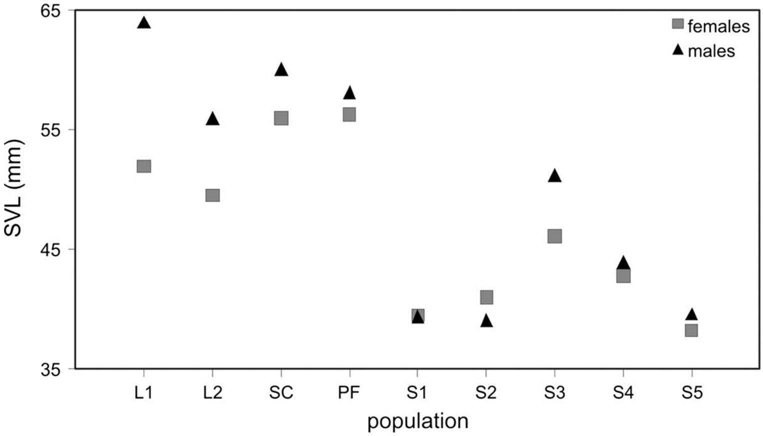

Fig. 2 Mean snout-vent length (SVL) of *E. platycephalus* basing on sex and population

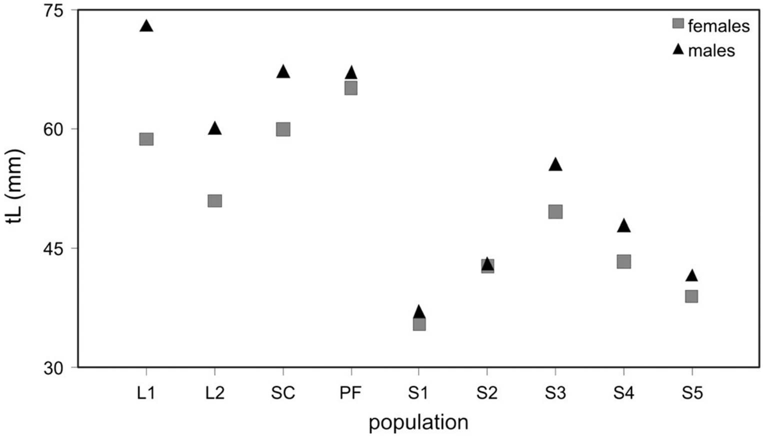

Fig. 3 Mean tail length (tL) of *E. platycephalus* basing on sex and population

significantly affected by population, sex and their interaction (Table 2). Again, males have longer tail, and newts from the south smaller (Fig. 3 and Supplementary material, Appendix 1 and 3), with differences among the sexes muted in southern populations. Mean age was only influenced by population and did not map to the two regions that were

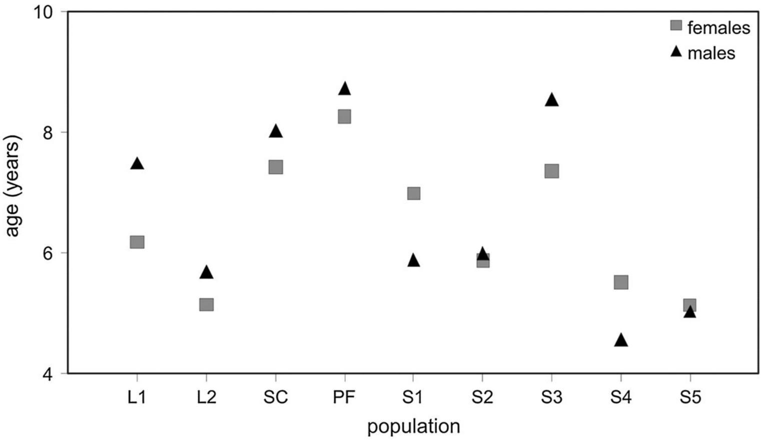

Fig. 4 Mean age of *E. platycephalus* basing on sex and population

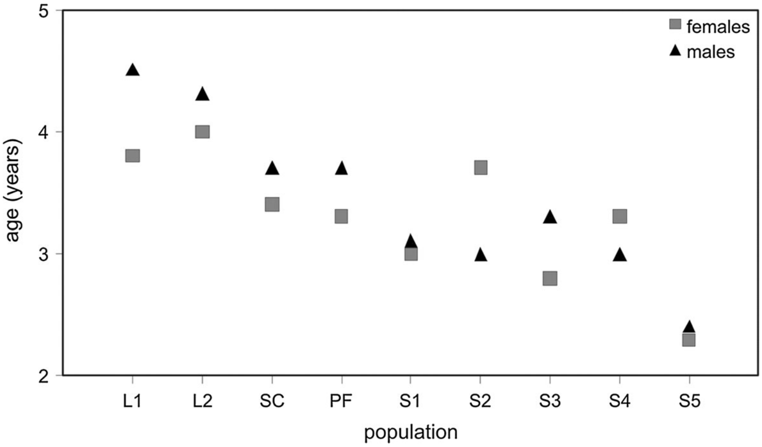

Fig. 5 Mean age at sexual maturity of *E. platycephalus* basing on sex and population

associated with significant differences in body size (Fig. 4 and Supplementary material, Appendix 1 and 3). However, age at which sexual maturity was reached (Fig. 5, Supplementary material, Appendix 1) also varied among the two regions (Kruskal–Wallis ANOVA, $H_{8,168} = 73.7$, $P < 0.001$), with newts in the south reaching sexual maturity on average 0.8 years before those from the north and central regions (Fig. 6c). Overall, females tended to attain sexual maturity at an earlier age (Kruskal–Wallis ANOVA, $H_{1,168} = 3.6$, $P = 0.057$) but patterns among regions were not consistent (see below; Fig. 6c).

As predicted, body size (SVL) was correlated with age ($r_{\mathrm{Pearson}} = 0.58$, $P < 0.001$), and the slopes of the relationship did not differ based on population ($F_{8,194} = 0.744$, $P = 0.65$), sex ($F_{1,194} = 0.1$, $P = 0.76$) or their interaction ($F_{8,194} = 1.1$, $P = 0.34$). When we controlled for the effect of age on SVL, all factors (population, sex and the interaction term) still had a significant effect on body size (ANCOVA; population:

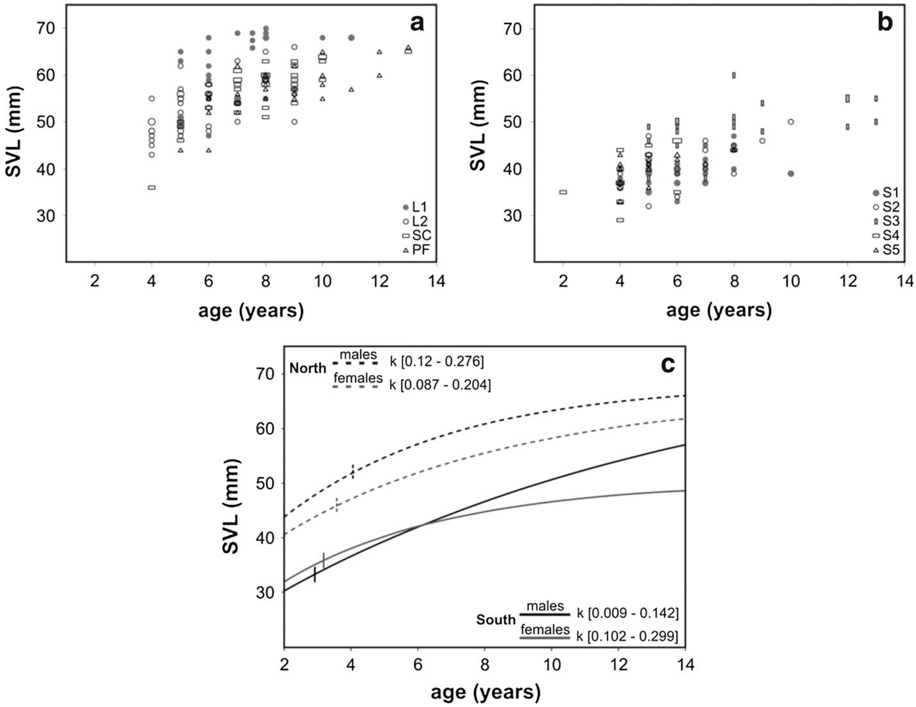

Fig. 6 The relationships between age and size (snout-vent length, SVL) of *E. platycephalus* plotted by population and regions: **a** plot of age versus SVL of newts (sexes combined) sampled from northern and central populations (L1, L2, SC, PF); **b** plot of age versus SVL of newts (sexes combined) sampled from southern populations (S1–S5). *Symbol* size is proportional to the number of individuals (representing from one to three individuals). **c** Von Bertalanffy's growth curves for northern/central versus southern populations of *E. platycephalus*, split by sex; mean age at maturity for each sex in each region is represented as a *vertical bar* on each *line*; 95 % confidence intervals for growth coefficients (k) for each *line* are embedded in the figure

$F_{8,211} = 78.2$, $P < 0.001$, Fig. 6a, b; sex: $F_{1,211} = 21.2$, $P < 0.001$; population × sex: $F_{8,211} = 5.1$, $P < 0.001$) and followed the geographical patterns reported above (larger males in the north, smaller newts in the south, reduced evidence of divergent body sizes among the sexes in the south). Conversely, slopes of relationships of factors to tL were not homogeneous due to the significant difference of the slopes for one interaction term (population × SVL: $F_{8,293} = 8.7$, $P < 0.001$). A separate slopes ANCOVA model revealed that only population had a significant effect on tail length ($F_{8,293} = 10$, $P < 0.001$) and that males did not have longer tails when the effect of body size (SVL) was controlled for ($F_{1,293} = 2.1$, $P = 0.15$). The interaction between sex and population also had no significant effect on tL ($F_{8,293} = 1$, $P = 0.33$).

Across all sites mean annual air temperatures ranged from 11 to 15.9 °C (Table 1). Temperature followed both a latitudinal and altitudinal gradient (respectively: $r_P = 0.92$, $P < 0.001$; $r_P = -0.97$, $P < 0.001$), with temperature increasing from north to south, and from high to low elevation. Sexual maturity was reached earlier by newts at warmer sites (males: $r_P = -0.78$, $P < 0.02$; females: $r_P = -0.66$, $P < 0.05$; sexes combined: $r_P = -0.77$, $P < 0.02$). Increasing altitude correlated positively with age of sexual maturity in male newts ($r_P = 0.72$, $P < 0.03$), but not females ($r_P = 0.49$, $P = 0.18$; sexes combined: $r_P = 0.65$, $P = 0.06$). Neither cofactor correlated with average population age (temperature, males: $r_P = 0.01$, $P = 0.98$; females: $r_P = 0.38$, $P = 0.31$; sexes combined: $r_P = 0.12$, $P = 0.76$; altitude, males: $r_P = 0.03$, $P = 0.95$: females: $r_P = -0.37$, $P = 0.33$; sexes combined: $r_P = -0.09$, $P = 0.81$).

Von Bertalanffy growth curves (Fig. 6c) illustrate the pattern of body size variation among the sexes and the geographic regions that post hoc tests suggested were divergent (northern sites: L1, L2, SC, PF; southern sites: S1, S2, S3, S4, S5). Male newts from northern sites were larger than females and achieve this size difference before the onset of sexual maturity, but a divergent pattern in body size is still not evident at the same age in newts in the south. Males only achieve larger body size in the south after 6 years of age (Fig. 6c). Newts from the north exhibit decreasing growth rates, as do females from the south, but males in the south appear to maintain a high rate of growth throughout life. Patterns for sex-specific age of sexual maturity switch between regions: in the north, males mature later, while in the south, females are the late-maturing sex (Fig. 6c, see also Fig. 5 and Supplementary material, Appendix 1).

Mean SDI was -0.056 ± 0.025 SE; populations S1 and S2 located in the Sette Fratelli Mts. exhibited a female bias (0.002 and 0.062 respectively), and the minimum SDI value was calculated using data from population L1 located in the Limbara Mts (−0.189). Corrected SDI ranged from −0.174 to 0.068 (mean corrected SDI = -0.043 ± 0.027 SE, S1, S2 and S4 from the Sette Fratelli Mts. exhibited positive SDI, 0.026, 0.068 and 0.06 respectively). Male age at sexual maturity negatively correlated with corrected SDI: the younger that males achieved sexual maturity, the less male-biased and the more female-biased was the corrected SDI ($r_P = -0.76$, $P < 0.05$). No such relationship existed between female age at sexual maturity and corrected SDI ($r_P = -0.25$, $P = 0.51$). Corrected SDI was positively correlated with the sex-specific population age structure, i.e. increased with increasing MAD ($r_P = 0.81$, $P < 0.01$).

Discussion

Our results confirm that morphological and life history can vary significantly amongst populations over very short geographic distances, and that even within such a limited range there can exist large amounts of variation in the levels of SSD amongst populations. Here we discuss these patterns and outline some possible mechanisms that may help explain that patterns that we observed. Of all the Mediterranean islands, Sardinia is second only to Sicily in terms of land area. It is still a relatively small land mass (about 24,090 km^2) that measures approximately 270 km at its longest point, and 145 km at its widest. The Sardinian endemic *E. platycephalus* occupies only a subset of the available land mass of the island, as currently it is restricted to sites in the three major mountainous regions. Given the restricted distribution of the study species, it is striking that we detected significant variation in newt life-history traits. Significant differences were detected over a straight line distance of just 35 km and exhibited a spatial pattern. Newts sampled from populations located in the southern mountain range were, on average, significantly smaller and matured earlier than those from central and northern populations (Table 2, Figs. 2, 5, 6). Variation in our two environmental variables mirrored this pattern but it did non affect consistently variation of life history. Sette Fratelli populations were, on average, located at lower elevations, and higher mean annual air temperature experienced by newts in these southern populations was significantly correlated with earlier sexual maturity and smaller body size at a given age. However, lifespan, as inferred from mean population age, did not correlate with either environmental metric. Our findings suggest that warmer temperatures lead to earlier sexual maturation (Miaud and Guyétant 1998; Marunouchi et al. 2000), which implies a shift of resource allocation from body growth to reproduction at a younger age (Hemelaar 1988; Atkinson 1996) by newts in the South, resulting in smaller body size.

Geographic variation of morphology and life history influenced the degree of SSD in *E. platycephalus*. Males were clearly larger than females in the north at the onset of sexual maturity and for all years after that, but newts in the south either exhibited some degree of female-biased SSD or no evidence of SSD at younger ages (Fig. 6c). This pattern was in part associated with sex-specific growth rates, in that females exhibited consistent, asymptotic growth across regions irrespective of age of sexual maturity (Fig. 6c). In comparison, male growth rate was not consistently asymptotic and varied geographically, as well as differing from female rates (Fig. 6c), a pattern significantly associated with variation in male age at sexual maturity. It was plasticity in male, but not female, age at sexual maturity that correlated with our age-adjusted estimate of sexual size dimorphism (SDI) indicating that variation in SSD was predominantly driven by variation in male life history.

Overall, our results indicate that the predominant evolutionary mechanism responsible for variation of SSD in *E. platycephalus* across populations is unlikely to be sexual selection. The link between male age at sexual maturity and temperature and the absence of male biased SSD in southern populations where males mature earlier, argues for an effect of environment on body size that occurs before sexual maturity is reached. Males respond more strongly to temperature variation and only males respond to altitude by altering growth rates, as predicted by the differential plasticity hypothesis (Fairbairn 2005). While environmentally mediated developmental plasticity in amphibians is well-documented for larval amphibians (e.g. Van Buskirk 2002), little empirical evidence is available regarding sex-specific growth rates directly measured before sexual maturity in amphibians. Two studies that tested for sex-specific patterns of pre-metamorphic growth in species with SSD detected none (Vorburger 2001; Howard 2009). Trait divergence during very early development should be unlikely, as SSD may arise due to negative intersexual genetic correlations and larval development is positively correlated among the sexes (Rice and Chippindale 2001). Sex-specific and post-metamorphic growth rates resulting in SSD are predicted to be more likely, as Howard (2009) showed empirically in Tiger salamanders. We conclude that growth before sexual maturity but after the larval period is the main factor responsible for SSD in the northern populations and likely plays a strong role in determining body size variation among populations. However, growth after sexual maturity driven by sexual selection is likely responsible for SSD in southern populations. In the south males reach sexual maturity at approximately the same body size as females (Fig. 6c). At these southern sites males maintain a relatively rapid and positive, not asymptotic rate of growth throughout adulthood that eventually results in larger male body size, although not as large, in absolute terms, as males from northern populations. Because *Euproctus* males have to physically restrain females to achieve insemination, small male body size when sexually mature would disadvantage males attempting to inseminate a resistant female. In this situation sexual selection for rapid, male growth rate after sexual maturity should be strong and agrees with the hypothesis that sexual selection should result in male-biased SSD in species with forcible insemination (Shine 1979).

Males also have longer tails that grew disproportionately when compared to SVL, which on the face of it would support the conclusion that tail length is a sexually selected trait.

However, allometric growth was exhibited by both sexes and longer tails exhibited by males was the result of their larger body size and not the result of direct sexual selection on tail length. Bovero et al. (2003) have shown that other traits are sexually dimorphic in *E. platycephalus* due to sex-based allometric growth. The overall pattern does support the hypothesis that sexual selection selects for larger male body size and allometric investment in growth of some traits measured by Bovero et al. (2003) beyond what would arise through selection on body size. Overall, sexual selection plays a role in determining *Euproctus* SSD, even if its role is diminished by environmental variation and becomes evident only when environment limits the growth and, then, SSD. Size and growth of females appear less variable then of males. This suggests females are developmentally canalized with respect to males (Fairbairn 2005) and female body size is subject to stabilizing selection, possibly due to fecundity selection.

In conclusion, we found that adult newt body size varies geographically, and that larger newts we found in northern Sardinia achieve greater body size through delayed maturation, unlike their counterparts in the South. Different ontogenies and not sexual selection (Shine 1990; Stamps and Krishnan 1997), appears to be the primary cause for SSD in northern newts. However, in the south where sexual bimaturation is nullified by increased temperature, sexual selection may be important too, favouring males with a high growth rate after sexual maturity. More in general, these divergent growth schedules manifest as divergent patterns of SSD that are consistent with Rensch's rule (Rensch 1950; Fairbairn 1997), since increasing body size exacerbates SSD when males are the larger sex. Furthermore, our findings support the role of environmental features in driving the magnitude and pattern of SSD variation by acting on male growth, as postulated by Cox and Calsbeek (2010).

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