

When Males Are Larger than Females in Ectotherms: Reproductive Investment in the Eastern Spadefoot Toad *Pelobates syriacus*

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The way animals allocate resources to growth and reproduction is of major importance in understanding life-history trade-offs. Because the investment in reproduction of the two sexes differs, comparing the costs and the reproductive strategies adopted offers an insight into the evolutionary forces that shaped them. In the Eastern Spadefoot Toad, *Pelobates syriacus* the males are larger than females in body length, but not in body mass. We studied the reproductive effort and output in a population of *P. syriacus* from the Danube Delta Biosphere Reserve (Romania), during two consecutive years (2010 and 2011). Body mass loss during mating and egg deposition in females averaged 27%, while in males it was 3.9%. Body mass loss in females was proportional with size, with larger females losing more weight than smaller ones. Initial body mass and body mass loss during reproduction were significantly correlated with clutch size, but egg size was not correlated to female size or body mass loss. Year had a significant effect on body size in both sexes, suggesting high plasticity and the effect of local environmental factors on growth and reproduction. Age of adults ranged between 2–12 years and did not differ significantly between sexes. A nonrandom, but not completely assortative, mating was observed, with the age and body size of paired animals slightly positively correlated, indicating an explosive breeder reproductive strategy.

Life-history theory is concerned with the way individuals allocate resources to growth and reproduction (Roff, 1992). When resources are limited there are physiological constraints on growth and reproduction, especially in indeterminate growers like amphibians (Kozlowski and Uchmanski, 1987; Heino and Kaitala, 1999; Lardner and Loman, 2003). Animals have thus the choice of allocating limited resources to growth, reproduction, or a combination of the two (Kozlowski, 1996). Because the clutch size increases with body mass in most animal species, allocating to growth is an investment in future reproduction. Maximizing reproductive success, while minimizing physiological costs, is a major selection pressure in amphibian reproductive biology (McCauley et al., 2000). Because amphibian growth and adult body size are highly plastic and indeterminate (Jørgensen, 1992), this implies that environmental factors affect growth patterns (Sebens, 1987). The disparity in parental investment between sexes means that the potential reproductive rate of males usually exceeds that of females. Thus females become a limiting resource and the object of competition among males (Wells, 2007). Traits that enhance the ability of males to locate, attract, capture, or retain possession of females will be favored by sexual selection (Arnold and Duval, 1994). It is expected that the two sexes will be under different selective pressures and differ in a variety of parameters, including size, weight, and age.

The spadefoot toads (*Pelobates* spp.) are highly specialized and have a narrow ecological niche: they are obligatory burrowing species, and landscape features have a strong influence on population spread and abundance (e.g., Nöllert, 1990). Typical terrestrial habitats include sandy areas, heath lands, and deciduous woodlands with loamy

soils. The aquatic habitats used for reproduction include a variety of permanent and semi-permanent ponds. The larval stage of spadefoot toads is of a relatively long duration (2 to 4 months), the larvae being much larger than other European anuran species. Traditionally the four species of the genus *Pelobates* have been considered explosive breeders, with a mating system that can be described as scramble competition (Hels, 2002; Wells, 2007). Other authors disagree with this opinion, with two species, *P. fuscus* and *P. cultripes*, described as prolonged breeders (Salvidio and Quero, 1987; Nöllert, 1990; Salvador and Carrascal, 1990; Lizana et al., 1994; Eggert and Guyétant, 2003), with mating seasons lasting roughly a month. For *P. syriacus* there are scarce data on the mating strategies, but we consider *P. syriacus* as an explosive breeder, with mating season lasting only a few days (Székely, 2010). The Eastern Spadefoot Toad *P. syriacus* is the largest of the four species of the genus *Pelobates*. The males of this species are also larger than females; explanations for this type of sexual dimorphism include male–male interactions (Shine, 1979), female choice for larger males (Halliday and Verrell, 1986), or differences in age structure between sexes that lead to differences in body size (Monnet and Cherry, 2002).

We studied the reproductive ecology in a population of *P. syriacus* from the Danube Delta Biosphere Reserve (Romania), at the northern limit of its range. We compared body size measures in breeding pairs coupled with reproductive output, and quantified the reproductive effort of both sexes. Because reproductive investment is usually size dependent, we checked for assortative mating (i.e., pairs formed between animals of similar size), based on the hypothesis that larger males will preferentially breed with larger females and thus maximize their reproductive success.

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MATERIALS AND METHODS

Study area.—The study site is located in the southern part of the Danube Delta Biosphere Reserve, on a natural levee (Grindul Lupilor) separating two lakes, Zmeica and Sinoe ($44^{\circ}37'N$, $28^{\circ}48'E$). It is a flat sand dune of Holocene origin, ranging in altitude between 0.5–1.5 m. The area is covered by steppe vegetation, partly on salinized land, bordered by permanent and temporary wetlands covered with reed (*Phragmites australis*) and rushes (*Typha* sp.) that grow in shallow water. The area has a very low annual precipitation (350 mm/year), with 65% of the rainfall in summer, the maximum in June, and very little snow in the winter. About 30–35% of the rainfall is in quantities around 1 mm/day. The air humidity is high due to the vicinity of the sea and the lagoons (Bogdan, 2008).

Data collection.—Reproduction of the population of *P. syriacus* on Grindul Lupilor was studied in spring during two consecutive years (2010–2011). The spadefoot toads are strictly nocturnal, so all field activities were carried out at night. Toads in amplexus were present in the open, shallow parts of a temporarily flooded area bordering a sand dune. The paired animals were collected separately in buckets, briefly dried on filter paper, weighed with a precision of 0.01 g on an electronic precision balance (Kern PCB), and their snout–vent length (SVL) measured with dial calipers to 0.1 mm precision. We collected 22 pairs in 2010 and 16 pairs in 2011. The pairs were taken to the laboratory and kept individually in 10 l capacity plastic buckets, with 4 l water and a plastic decorative plant for wrapping the egg clutch. Most pairs deposited during the first night. The pairs were kept for several additional days to ensure all eggs were laid. After deposition the adults were weighed again and a toe was removed and stored in alcohol for age assessment using skeletochronology. All animals and the unhatched egg clutches were released at the site of capture, after less than a week in the lab.

The skeletochronological method was a slightly modified version of that of Castanet and Smirina (1990). We decalcified the second phalange for 3 hrs with 5% HNO₃, followed by inclusion in TissueTek, freezing, and sectioning with a Tehsys CR 3000 cryotome at 16 µm. The cross sections were stained with Ehrlich's haematoxylin for 3 hrs. In addition to the 76 adults we also collected toes from 12 juveniles used for calibration. Age was estimated from the Lines of Arrested Growth (LAGs) using an Olympus CX 31 microscope and Quick Photo Micro 2.3 software. In four animals (one female and three males) age could not be assessed because LAGs were not clearly distinguishable, due to multiple lines.

The egg-cords were divided in portions and all the eggs were counted. The egg clutch was then photographed in a large Petri dish over plotting paper and egg diameter, as a measure of offspring quality, was measured in a sample of 30–50 eggs per clutch using UTHSCSA ImageTool vers. 3 program (University of Texas Health Science Center at San Antonio, Texas and available via anonymous FTP from maxrad6.uthscsa.edu).

Data analysis.—Prior to analysis, body mass and SVL data sets were tested for normality using Shapiro-Wilk test. We estimated reproductive effort from the difference in body mass before and after egg deposition for females and before and after mating for males. To compare reproductive effort

we used repeated measure ANOVA with body mass before and after egg deposition for females and before and after mating for males, as within subjects factor variables, year as factor and SVL and age as covariates. To test for sexual dimorphism in body mass we used ANOVA with sex as fixed factor, year as random factor and SVL and age as covariates. Multivariate regression analysis was used to examine the relation between egg clutch parameters and different measures of body size. All analyses were done using SPSS version 17 (SPSS Inc., Chicago, IL).

RESULTS

Snout–vent length of the male was greater than that of the female in 25 pairs out of 38 (66%; Fig. 1A). Males were heavier than females in 15 pairs (39%; Fig. 1B). Body mass and SVL of paired males and females were positively correlated (body mass: R² = 0.18, P < 0.05; SVL: R² = 0.35, P < 0.05; n = 38). Because males lost less weight than females during reproduction, the proportion of heavier males than females after egg deposition was 66% (Fig. 1C). Snout–vent length and body mass were significantly correlated in males and females, both before and after reproduction (females before R² = 0.81, n = 30, P < 0.001; after R² = 0.83, n = 38, P < 0.001; males before R² = 0.91, n = 38, P < 0.001; after R² = 0.90, n = 38, P < 0.001; Fig. 2). Body mass loss during reproduction was higher in females than in males (Mann-Whitney U-test, z = -7.365, n = 75, P < 0.001), with an average of 27% loss in females (range 11.9–40.9%) and 3.9% loss in males (range 0.1–10.1%). Body mass loss during reproduction in females was positively correlated with initial body mass (R² = 0.86, n = 38, P < 0.001). The average clutch size was 2817 ± 1551 eggs (mean ± standard deviation; range 865–5812). In females, initial body mass and body mass loss during reproduction were positively correlated with clutch size (R² = 0.94, n = 35, P < 0.001; and R² = 0.86, n = 35, P < 0.001, respectively; Fig. 3). Average egg size (1.82 ± 0.18 , range 1.47–2.34) was not correlated to clutch size or female initial mass (R² = 0.24, n = 36, P < 0.14; and R² = 0.29, n = 36, P = 0.08, respectively).

The average age of females was 7.3 years (range 3–12), and of males of 7.5 (range 4–11). Age did not differ significantly between sexes (t-test, t = 0.46, df = 70, P = 0.64). Sexual maturity was reached at an average age of 3.2 years (range 2–4) in females and at 3.5 years (range 2–6) in males. There was no correlation between the ages of paired animals (R² = 0.005, n = 31, P = 0.69). Age was correlated with initial body mass in females (R² = 0.16, n = 37, P < 0.01), but not in males (R² = 0.02, n = 35, P < 0.001). Year had a significant effect on SVL in both females ($F_{1,37} = 6.122$, P = 0.019) and males ($F_{1,37} = 11.053$, P = 0.002), and on body mass before ($F_{1,37} = 9.226$, P = 0.005) and after ($F_{1,37} = 10.427$, P = 0.005) reproduction in males (Table 1).

Repeated measures ANOVA showed a significant main effect of egg laying and mating on females' body mass and a significant interaction between egg laying and SVL, whereas in males no significant effect of reproduction (i.e., mating) was found (Table 2). Body mass loss during reproduction was higher in females (Table 3). Multivariate regression revealed that clutch size was correlated with female body mass measured before reproduction (Table 4).

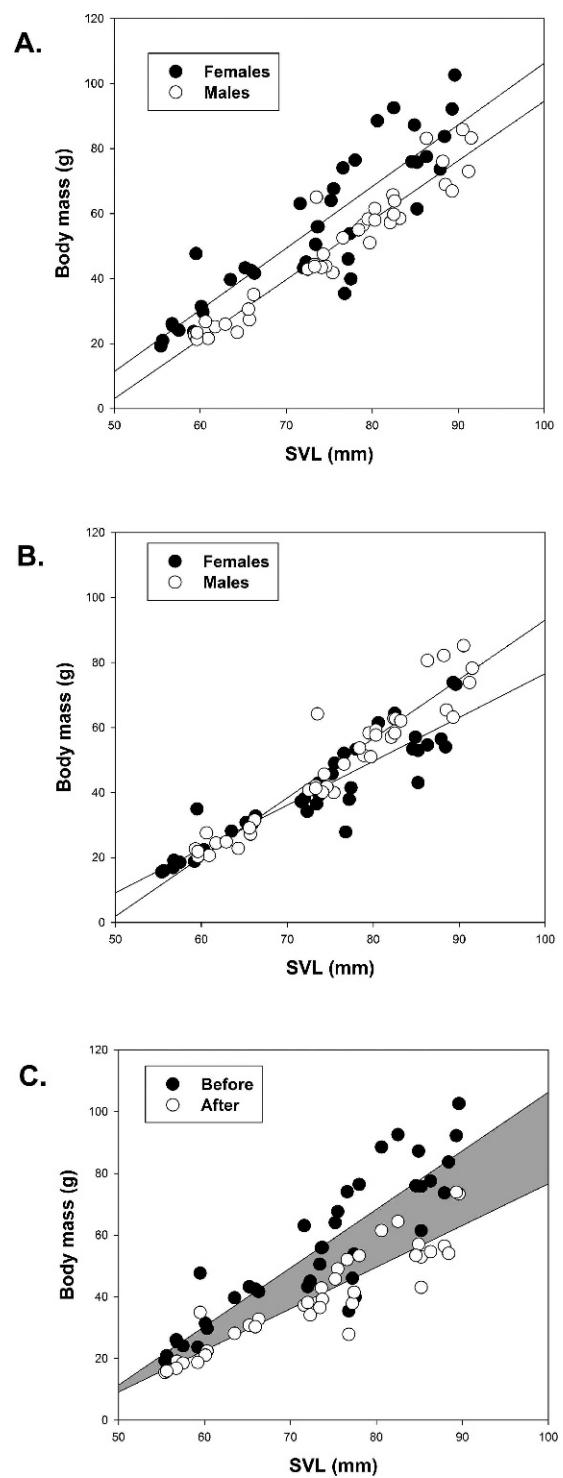
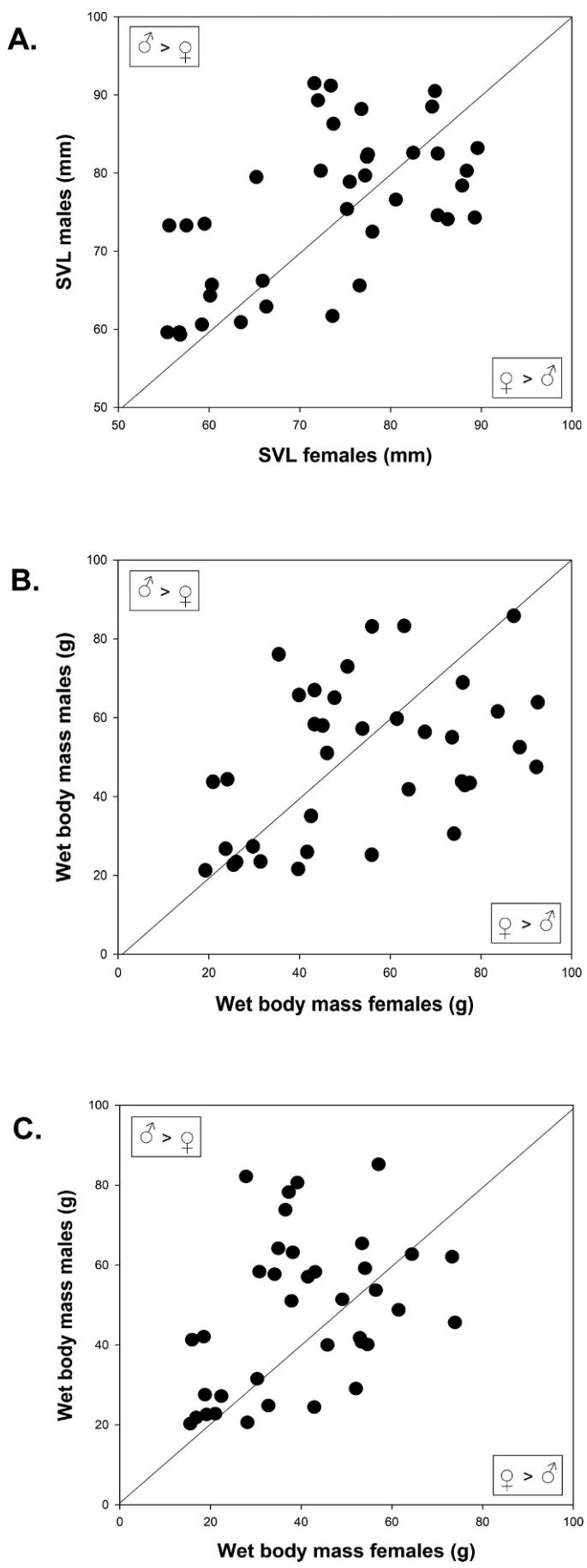


Fig. 2. SVL–body mass relationships in males and females before (A) and after (B) reproduction. The decrease in body mass during reproduction in females increases with size and is represented by the shaded area (C).

Fig. 1. Body size dimensions of pairs of *P. syriacus* in amplexus (A) and (B) are before reproduction, while (C) is after. The points positioned on the line indicate pairs in which both sexes had the same body dimension. Points situated above the line correspond to pairs in which males are larger/heavier than females, while points situated below indicate pairs with larger females.

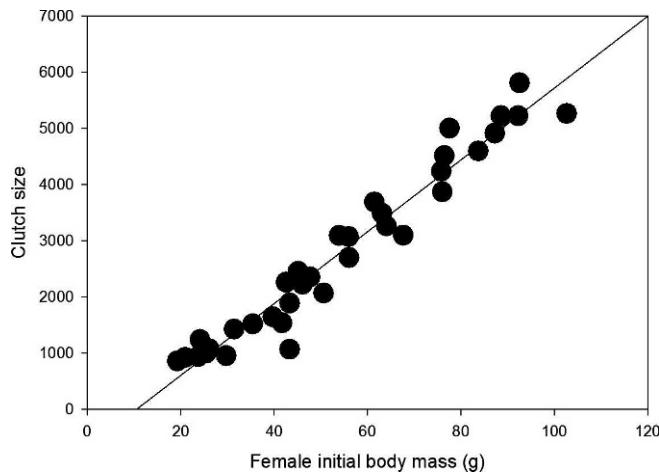


Fig. 3. Correlation between the initial body mass of females and clutch size ($n = 35$, $R^2 = 0.94$).

DISCUSSION

Our study has revealed nonrandom, but not completely assortative, mating in *P. syriacus*, despite the significant correlation between female body size and egg clutch size that would suggest a benefit to male–male competition for larger females. We did not test for other aspects of sexual selection, such as large-male advantage in mating success. We frequently have observed larger males attached to an already formed pair and attempting to displace the breeding male. Therefore it can be assumed that larger males achieve greater reproductive success. Sexual size dimorphism in anurans has been extensively debated (Shine, 1979; Woolbright, 1983; Halliday and Verrell, 1986; Monnet and Cherry, 2002), but no clear pattern is apparent. Shine (1979) reviewed for the first time the literature and observed that in 90% of anurans, females grow larger than males. He concluded that males are larger in species in which there is male competition for females. Halliday and Verrell (1986) refuted his conclusion and suggested that because mating systems in anurans are too complex, no generalization is possible. Males of *P. syriacus* are larger than females in body length, but not in body mass before reproduction. This pattern has been reported in populations throughout the species' range (Rot-Nikčević et al., 2001; Ugurtaş et al., 2002). Larger animals were older and larger females deposited more eggs. The fact that females allocate significantly more resources than males for reproduction means that males can allocate more marginal resources (surplus energy) to growth, and indeed males had larger SVLs than females in our study.

In a later study Monnet and Cherry (2002) concluded that sexual size dimorphism can be explained by the differences in age structure between the sexes (i.e., larger males being older). The males and females in our study did not differ in age structure, rebutting the hypothesis of Monnet and Cherry (2002). This is consistent with previous studies on desert anurans that indicate rapid growth to maturity, but short life span as adults in response to the highly variable, harsh environment (Sullivan and Fernandez, 1999). It is also consistent with a study of a Lower Danube floodplain amphibian community where two newt species and a small-bodied anuran (*Bombina bombina*) had shorter longevity and smaller body size than reported for other populations (Cogălniceanu and Miaud, 2003).

Table 1. Mean Body Mass of Male and Female *P. syriacus* Measured before Reproduction. Mean value \pm SD, minimum and maximum values in parentheses.

	2010 (n = 22)	2011 (n = 16)
Males	42.59 ± 18.2 (21.28–83.28)	59.64 ± 15.99 (26.74–85.84)
Females	50.57 ± 21.59 (19.24–92.55)	61.58 ± 24.53 (20.91–102.62)

The studied population of *P. syriacus* inhabits a highly unpredictable environment. The extent and persistence of water bodies are extremely variable in the study area, being influenced not only by local conditions, but mostly by the hydrology of the Danube River which is 2,860 km long and has a drainage basin of 801,500 km². The average water flow of the river upstream of the delta is 6,300 m³/s, ranging between extreme values of 1,500 and 19,000 m³/s (Cogălniceanu, 2012). The high variability of the environment is related to the observed differences in size among seasons. In a population of European tree frog (*Hyla arborea*), the size differences among seasons were related to differences in the age structure (Fiedl and Klump, 2005). Tadpoles of *P. syriacus* are well adapted to the arid environment of the study site: in a controlled experiment, tadpoles from that population showed phenotypic plasticity and were able to respond to pond drying by speeding up their development, thus increasing their survival rate (Székely et al., 2010). Strong variation in larval survival selects for decreased annual reproductive effort and higher adult survival (Seger and Brockmann, 1987). An alternative to prolonged adult life span may be to have offspring attain maturity in different years (Wilbur and Rudolf, 2006). This is consistent with our results of sexual maturity ranging between 2–4 years in females and 2–6 years in males.

An experiment using *Rana dalmatina* has shown that mate acquisition is a trial-and-error process, not supporting the hypothesis that males discriminate among females of different size (Hettyey et al., 2005). Males in explosive mating aggregations often have to struggle for females, and unpaired males try to displace those already in amplexus. Multiple males clasping has been observed in the studied population. Usually large males displace smaller males and are thus more successful in acquiring mates (Wells, 2007), but in other species like the European tree frog (*Hyla arborea*), pairs in amplex are never disturbed or attacked by conspecifics (Marquez and Tejedo, 1990; Fiedl and Klump, 2005). Halliday and Verrell (1988) argued that female choice for larger males in anurans favors not older males, but males that have shown rapid juvenile growth. Because the mating period in the studied population lasted 4–6 days, this resulted in scramble competition and multiple males clasping a single female.

It has been hypothesized that there is a negative trade-off between current reproductive effort and future reproductive output (Reznick, 1985; Nilsson and Svensson, 1996). This was not sustained in our studied population, characterized by few reproductive bouts, during the relatively short life span. The highly unpredictable environment makes reproductive success (i.e., metamorphosis of tadpoles) also unpredictable. Thus there is no predictable advantage of

Table 2. Results of Repeated Measure ANOVA Testing the Effect of Body Mass of Females and Males on the Reproductive Effort (Egg Laying and Mating) in *P. syriacus*. df = degrees of freedom; SS = sum of squares; F = F statistic; P-value = probability. Significant results are marked with bold.

Source	SS	df	MS	F	P-value
Females					
Reproductive effort	84.308	1	84.308	6.517	0.022
Reproductive effort*SVL	266.359	1	266.359	20.589	0.000
Reproductive effort*age	14.371	1	7.185	0.555	0.585
Reproductive effort*year	7.724	1	7.724	0.597	0.452
Error (egg laying)	194.051	37	12.937		
Males					
Mating	1.877	1	1.877	0.793	0.381
Mating*age	2.328	1	2.328	0.983	0.33
Mating*SVL	1.27	1	1.27	0.536	0.47
Mating*year	6.037	1	6.037	2.549	0.122
Error (mating)	66.313	37	2.368		

saving resources during reproduction in one year for the next year. In our study females allocated to reproduction a predictable amount related to their body mass, suggesting a constant allocation of resources to reproduction. This is consistent with a study on resource allocation by female in *Rana temporaria*, another explosive breeding frog, which showed that different feeding rates do not influence the relative allocation of resources to growth versus reproduction (Lardner and Loman, 2003). In a population of European treefrog (*Hyla arborea*) there was no size- or age-assortative mating. Male mating success was significantly correlated with the number of nights spent calling at the breeding site (Friedl and Klump, 2005).

The cost of reproduction is a trade-off between different life-history variables (e.g., current and future reproductive effort or parental survival), but is difficult to measure. In the present study we estimated the reproductive effort of females as the difference between the body mass before and after egg deposition. While animal body mass can be greatly influenced by variation in water content, the short period between measurements (ranging between 12–36 hours) and the fact that the animals stayed only in

water, limited this bias. It was a measure not only of clutch size mass, but also of mating expenditure. The reproductive investment of females in our study was high, consistent with other temperate species of similar body size (Kuramoto, 1978; Berger and Uzzell, 1980; Jørgensen, 1984; Ryser, 1989; Castellano et al., 2004). Several measures of reproductive effort were used in similar studies, but many involved sacrificing the animals, an alternative avoided in our study. Overall, the published data on reproductive effort are scarce and the measures used vary and are often not comparable. An analysis of the available data that used comparable measures reveals a slight positive correlation between initial female body mass and reproductive effort (Fig. 4). This indicates that females of larger species produce more spawn.

As in many species we found a positive correlation between female body size and clutch size (Duellman and Trueb, 1986) that would suggest a benefit to male-male competition for larger females. The relative short life span and limited potential reproductive events should also favor it. The observed nonrandom, weakly assortative mating in *P. syriacus* was probably due to the short period of

Table 3. Comparisons between Sexes and Years of Body Mass (SVL and Age Adjusted) Measured Before and After Reproductive Effort (Egg Laying and Mating). df = degrees of freedom; SS = sum of squares; F = F statistic; P-value = probability. Significant results are marked with bold.

Source	SS	df	MS	F	P-value
Before					
Intercept	9034.458	1	9034.458	137.69	0.000
Sex	1864.624	1	1864.624	28.418	0.000
Year	239.998	1	239.998	3.658	0.06
Sex*year	72.648	1	72.648	1.107	0.297
Error	4133.719	75	65.615		
After					
Intercept	5128.301	1	5128.301	144.668	0.000
Sex	338.666	1	338.666	9.554	0.003
Year	50.871	1	50.871	1.435	0.236
Sex*year	436.33	1	436.33	12.309	0.001
Error	2197.828	75	35.449		

Table 4. Multiple Regression Analyses of Number of Eggs and Mean Egg Size as a Function of SVL and Body Mass Before and After Reproduction, and Age in Females of *P. syriacus*. Significant results are marked with bold.

Variable	B	SE	t	P-value
Number of eggs				
(Constant)	1250.997	1721.38	0.727	0.476
Female SVL	19.205	19.742	0.973	0.343
Female before body mass	82.52	16.247	5.079	0.000
Female after body mass	-33.851	25.121	-1.348	0.194
Female age	-42.96	51.236	-0.838	0.412
Mean egg size				
(Constant)	2.067	0.729	2.834	0.011
Female SVL	0.009	0.008	1.051	0.307
Female before body mass	0.007	0.007	1.087	0.29
Female after body mass	-0.011	0.011	-0.998	0.331
Female age	0.012	0.022	0.573	0.573

reproduction and the low intensity call of males. Contrary to other species where male calls are loud, spadefoot toads call underwater and thus provide few clues regarding size and/or fitness to incoming females. The short reproductive period is also limiting mate search for both males and females.

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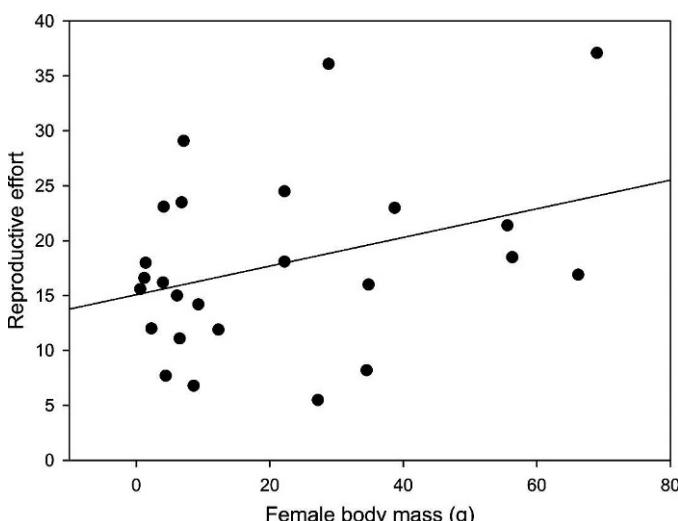


Fig. 4. The relationship between female body mass and the reproductive effort (estimated as percentage of egg mass) in 25 anuran species from six families ($R^2 = 0.12$, $n = 25$, $P = 0.09$). See Appendix 1 for details.

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Appendix 1. Reproductive Effort in Different Species of Anura Reported in the Literature.

Family	Species	Body mass (g)	Reproductive effort	Measure	Source
Bufoidae	<i>Bufo viridis</i>	38.7	23.0	Absolute reproductive output (mean mass of ovaries as percentage of body mass)	Jørgensen, 1984; Castellano et al., 2004
Hylidae	<i>Hyla japonica</i>	4.1	23.1	Clutch ratio (clutch weight/female standard weight)	Kuramoto, 1978
Hylidae	<i>Hyla nana</i>	0.6	15.6	Percentage of ovary mass in total body mass	Prado and Haddad, 2005
Hylidae	<i>Hyla raniceps</i>	12.3	11.9	Percentage of ovary mass in total body mass	Prado and Haddad, 2005
Hylidae	<i>Phrynohyas venulosa</i>	34.5	8.2	Percentage of ovary mass in total body mass	Prado and Haddad, 2005
Hylidae	<i>Phyllomedusa hypochondrialis</i>	4	16.2	Percentage of ovary mass in total body mass	Prado and Haddad, 2005
Hylidae	<i>Pseudis paradoxa</i>	27.2	5.5	Percentage of ovary mass in total body mass	Prado and Haddad, 2005
Hylidae	<i>Scinax acuminatus</i>	6.5	11.1	Percentage of ovary mass in total body mass	Prado and Haddad, 2005
Leiuperidae	<i>Physalaemus gracilis</i>	2.25	12.0	Percentage of ovary mass in total body mass	Camargo et al., 2008
Leiuperidae	<i>Physalaemus biligonigerus</i>	4.41	7.7	Percentage of ovary mass in total body mass	Camargo et al., 2008
Leptodactylidae	<i>Leptodactylus chaquensis</i>	34.8	16	Percentage of ovary mass in total body mass	Prado and Haddad, 2005
Leptodactylidae	<i>Leptodactylus fuscus</i>	8.6	6.8	Percentage of ovary mass in total body mass	Prado and Haddad, 2005
Leptodactylidae	<i>Leptodactylus podicipinus</i>	6.1	15	Percentage of ovary mass in total body mass	Prado and Haddad, 2005
Leptodactylidae	<i>Physalaemus albonotatus</i>	1.2	16.6	Percentage of ovary mass in total body mass	Prado and Haddad, 2005
Microhylidae	<i>Elachistocleis bicolor</i>	1.4	18	Percentage of ovary mass in total body mass	Prado and Haddad, 2005
Ranidae	<i>Rana temporaria</i>	69	37.1	Weight loss during reproduction (long period)	Ryser, 1989
Ranidae	<i>Pelophylax lessonae</i>	22.2	18.1	Percentage of ovary mass in total body mass	Berger and Uzzell, 1980
Ranidae	<i>Pelophylax esculentus</i>	56.32	18.5	Percentage of ovary mass in total body mass	Berger and Uzzell, 1980
Ranidae	<i>Pelophylax ridibundus</i>	66.2	16.9	Percentage of ovary mass in total body mass	Berger and Uzzell, 1980
Ranidae	<i>Rana japonica</i>	28.8	36.1	Clutch ratio (clutch weight/female standard weight)	Kuramoto, 1978
Ranidae	<i>Rana tsushimensis</i>	7.1	29.1	Clutch ratio (clutch weight/female standard weight)	Kuramoto, 1978
Ranidae	<i>Rana tagoi</i>	6.8	23.5	Clutch ratio (clutch weight/female standard weight)	Kuramoto, 1978
Ranidae	<i>Rana nigromaculata</i>	55.6	21.4	Clutch ratio (clutch weight/female standard weight)	Kuramoto, 1978
Ranidae	<i>Rana brevipoda</i>	22.2	24.5	Clutch ratio (clutch weight/female standard weight)	Kuramoto, 1978
Ranidae	<i>Rana limnocharis</i>	9.3	14.2	Clutch ratio (clutch weight/female standard weight)	Kuramoto, 1978