

Reproductive Investment of Female Green Toads (*Bufo viridis*)

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Females of iteroparous species may compromise between contrasting reproductive strategies. They should balance the amount of energy devoted to reproduction against the energy saved for growth and survival, and they should compromise between the quantity versus the quality of offspring. In the present paper, we analyze these trade-offs in European Green Toads (*Bufo viridis*). We define reproductive investment in terms of clutch dry mass, number of eggs, and mean ovum dry mass, whereas we describe the amount of resources saved for growth and survival (somatic investment) with a fat index derived from the total body electrical conductivity (TO-BEC) of postspawning females. Our results indicate that larger individuals produce larger clutches, both in the number and size of eggs. Females with higher reproductive effort, however, also show a larger somatic investment regardless of size. We show that females of similar size invest similar proportions of resources in reproduction, so that those in good condition not only show larger reproductive effort than lower-quality females but also store larger amounts of resources for growth, survival, and future reproduction.

AN understanding of life-history evolution requires analysis of the balance of energy devoted to growth, development, and reproduction and how this balance can be adjusted to maximize lifetime reproductive success (Stearns, 1992; Roff, 2002). From a life-history perspective, reproduction requires animals to compromise between contrasting alternatives. For instance, there should be a trade-off with respect to the time and energy allotted to reproduction versus the time and energy directed toward growth, survival, and future reproduction. Furthermore, for females there is often a trade-off between the quantity versus the quality of their offspring.

For female anurans without parental care, reproductive effort depends largely on the total amount of energy allocated to oocytes (Grafe et al., 1992). These resources are known to be directly affected by female condition, in particular by the amount of energy stored in fat bodies (Jørgensen, 1992). However, several allocation strategies might result in a positive relationship between body-condition and clutch-size. Figure 1 models three alternative hypotheses in which females can adjust reproductive effort to their amount of available energy: (A) Females show a high, condition-dependent reproductive investment and a low, condition-independent somatic investment; (B) Females short of allocable energy invest less in reproduction (both in absolute and relative terms) than females with more resources, so that, after spawning, females in poor condition will have more amounts of energy for growth and future reproduction; or (C) Females with abundant resources show

greater reproductive effort than females with scarce resources, but, at the same time, they save more energy for somatic growth and, after reproduction, they still maintain higher amounts of available energy.

Female reproductive success depends not only on the total amount of resources allocated to reproduction but also on the way these resources are distributed among offspring (i.e., on the number and quality of zygotes). Female control over offspring fitness, therefore, can be significantly influenced by allocation of energy reserves (Kaplan, 1998) and potentially by genetic quality through mate choice (Andersson, 1994; Welch et al., 1999). For example, larger eggs tend to produce larger tadpoles, which grow and develop faster, resulting in an increased probability of reaching metamorphosis than tadpoles hatching from smaller eggs (Laugen et al., 2002; Loman, 2002; but for environmental effects on the egg-size versus larval survival relationships, see Kaplan, 1992).

Body size and body condition are known to play an important role in influencing energetic allocation to offspring in female anurans. For example, there is typically a positive association between fecundity and snout-vent length (SVL; review in Duellman and Trueb, 1986), which may be an important determinant of sexual size dimorphism (Howard, 1988). The relationship between ovum-size to body size is not as clear as the relationship between clutch-size and body-size, because of the negative correlation between ovum size and clutch size (Duellman and Trueb, 1986; Jørgensen, 1992) and the high within-individual variation of ovum size (review in Jørgensen 1992; Kaplan and King, 1997).

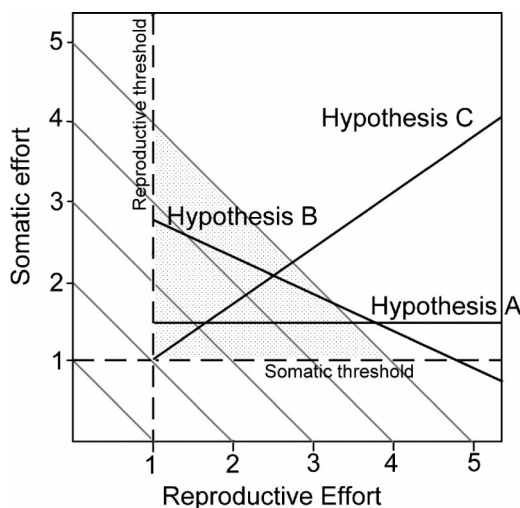


Fig. 1. Models of condition-dependent reproductive allocation strategies. Females with different amounts of available resources move along different diagonal lines. The horizontal dash line represents the minimum somatic investment compatible with survival, whereas the vertical dash line represents the minimum amount of resources necessary for reproduction. The stippled area represent potential reproductive production. Three strategies are presented, all resulting in a positive correlation between reproductive effort and female condition. According to the Hypothesis A, females invest most of their available resources in reproduction, and similar amounts of resources in growth and survival. According to Hypothesis B, females in poor condition save larger amounts of resources for growth and survival than those in better condition. According to Hypothesis C, females invest similar proportions of available energy to reproduction, those having invested more in reproduction saved also larger amounts of energy for growth.

We studied female reproductive investment in a population of the European Green Toad (*Bufo viridis*). The aim of this research was twofold. First, we analyzed the relationship between reproductive and somatic investment to determine which of the patterns presented in Figure 1 best explained the allocation strategy of female green toads. Second, we investigated the effects, if any, of body size and postspawning body condition on the trade-off between number and size of eggs. We described the reproductive effort in terms of clutch dry mass and distinguished between the number of eggs (female fecundity) and mean ovum size (offspring quality). We quantified the amount of resources saved for growth and survival (somatic investment) by means of a fat index, derived from the total body electrical conductivity (TOBEC) of postspawning females.

MATERIALS AND METHODS

In April and May 2002, amplexant male and female Green Toads were caught in a pond near Zucchea (NW Italy, 44°49'N, 7°26'E), kept in a moist sack at 5 C to prevent egg deposition, and carried to the laboratory. There, pairs were settled into separate tanks (60 × 40 cm, 30 cm tall) with shallow water (whose temperature varied between 12 and 16 C), and left there overnight. Spawning usually started a few hours later and egg deposition was completed by the following morning. Pairs were released in the same place where they were captured the day before.

Clutch weight and number of eggs.—Egg strings were divided into 4–6 portions of similar length. Each segment was coiled in a glass dish, dried with blotting paper, weighed to the nearest 0.1 mg, and photographed with a digital camera. Half the clutch portions were dehydrated in a fan convection oven at 60 C for about 24 h to obtain dry-mass. To minimize our impact on the Green Toad population, the remaining clutch portions were placed into 80-liter aquaria and allowed to hatch and tadpoles develop to stage 24 of Gosner (1960), before their release into the parental pond. To infer the dry mass of these clutch portions, we multiplied their wet weight by the dry mass to wet mass ratio of the dehydrated portions.

To quantify individual female reproductive investment, we measured three parameters: (1) clutch dry-mass; (2) number of eggs per clutch; and (3) mean dry-mass of a single egg. We counted the total number of eggs per clutch on the digital pictures of coiled egg string portions using the software Image Tool 3.0 (University of Texas Health Science Center in San Antonio). This program automatically counted the eggs it identified and showed their outlines on the original picture. When necessary, we adjusted the automatic result by manually counting those eggs that the program failed to identify. The mean dry mass of a single egg was obtained by averaging the mean individual egg mass values from each dehydrated portion.

Female size and condition.—To characterize size and condition of females, we employed three parameters: (1) snout-vent length (SVL), measured with a digital calliper to the nearest 0.01 mm on anaesthetized individuals (see below); (2) body mass, measured with a digital balance to the nearest 0.1 g, both before and after egg deposition; and (3) an after-spawning Fat Index, calculated as the residuals of a regression between total body mass and an indirect estimate

TABLE 1. DESCRIPTIVE STATISTICS OF THE VARIABLES EMPLOYED TO DESCRIBE THE REPRODUCTIVE INVESTMENT AND THE PHENOTYPE OF FEMALES.

	<i>n</i>	Minimum	Maximum	Mean	SD	CV (%)
Body size and condition						
SVL (mm)	28	59.59	77.14	70.04	4.35	6.2
After-spawning Fat Index	28	-3.47	2.65	-0.02	1.69	
Before-spawning body mass (g)	28	21.6	52.6	38.7	7.9	20.3
After-spawning body mass (g)	27	15.3	37.8	26.5	5.4	20.6
Reproductive investment						
Egg dry mass (mg)	27	0.44	0.72	0.57	0.08	13.5
Number of eggs	27	5687	17602	8913	2550	28.6
Clutch dry mass (mg)	27	2718.3	8203.3	5089.0	1316.0	25.8

of the lean body mass. The composition of the body was evaluated nondestructively by electroconductivity with the TOBEC (Total Body Electrical Conductivity) system. This device gives an indication of the lean body mass, since the contribution of lipid tissue to conductivity is negligible (Walsberg, 1988; Castro et al., 1990; Angilletta, 1999).

To measure the electrical conductivity index, each individual was placed in the detection chamber (44 mm diameter) of a SA-3000 Small Animal Body Composition Analyzer (EM-SCAN, Inc., Springfield, Illinois) for 5–6 sec. TOBEC values were derived from the formula: TOBEC index = $(S-E) / R$, where S = measurement with the specimen, E = empty measurement, and R = reference number (detailed instructions in the EM-SCAN operator's manual). For each individual the measurement was repeated three times and the average value was employed in successive analyses. As body electrical conductivity is sensitive to temperature, hydration and position of the subject in the measurement chamber, toads were measured at a constant

room temperature of about 20 C. In addition, TOBEC measures of each toad were taken within 3 to 4 min subsequent to being held in water for 5–10 min to control for hydration. Following the protocol developed by Fisher et al. (1996) for aquatic species, we sedated toads in a 0.2% solution of MS222 (Sandoz) to avoid movement, and each individual was centered head first on the carrier device of the apparatus. We checked machine calibration following readings taken on every third toad by using a calibration tube supplied with the device.

RESULTS

Table 1 shows descriptive statistics of the variables employed to describe the phenotype and the reproductive investment of females. Clutch mass shows high individual variation (CV = 25.9%), which is mostly caused by variation of eggs per clutch (CV = 28.6%) rather than by the mass variation of individual eggs (CV = 13.6%) within the clutch. Because of egg deposition, females lose on average 12.2 g, which is about a quarter (23%) of their mass. However, a large portion of this loss is water, because the mean dry mass of clutches (5.1 g) represents only 41.8% of the total loss.

Factors affecting variation of clutch mass.—A multiple regression analysis between clutch mass (dependent variable) and SVL and Fat Index (independent variables) was highly significant ($R^2 = 0.864$; $F_{2,23} = 73.071$; $P < 0.001$): larger clutches tended to be laid by larger females (Fig. 2; partial regression coefficient [b_{SVL}] = 283.05; $P < 0.001$) with higher values of the postspawning Fat Index (partial regression coefficient [$b_{\text{Fat Index}}$] = 170.785; $P = 0.013$). Thus, independent of size, females with higher reproductive effort retained more resources for

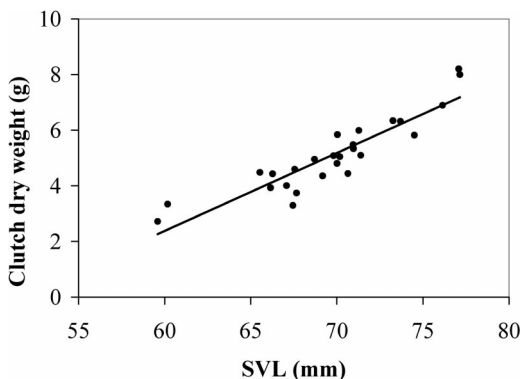


Fig. 2. Relationship between female SVL and clutch size ($r = 0.748$; $F = 30.464$; $df = 1, 24$; $P < 0.001$).

growth and future reproduction, as predicted by Hypothesis C (Fig. 1).

Clutches may differ in dry mass because they contain a different number of eggs and/or because their eggs differ in size. Simple regression analyses show the former of the two hypotheses as the most important to explain variation in female reproductive investment. As expected, the number of eggs correlated significantly to the clutch dry mass ($R^2 = 0.745$; $b = 0.445$; $F_{1,25} = 72.9$; $P < 0.001$), whereas the mean mass of single eggs had no significant effect ($R^2 = 0.056$; $b = 4002.5$; $F_{1,25} = 1.5$; $P > 0.1$). Although ovum-mass had minimal effect on clutch-size variation, it was significantly different among females (Kruskal-Wallis: $\chi^2 = 66.3$; $df = 27$; $P < 0.001$), suggesting that females differed not only in the number of eggs they produced but also in the amount of resources they invested per single eggs.

Factors affecting egg mass and number.—The multiple regression analysis between the number of eggs per clutch (dependent variable) and SVL and Fat Index (independent variables) was highly significant ($R^2 = 0.604$; $F_{2,23} = 17.565$; $P < 0.001$). However, only SVL appeared to affect the dependent variable (partial regression coefficient $b_{SVL} = 451.4$; $P < 0.001$); the Fat Index did not show any significant effect (partial regression coefficient $b_{Fat\ Index} = 337.54$; $P > 0.1$).

To analyse the causal factors responsible for egg-mass variation, we used as predictors of the multiple regression model SVL, Fat Index, and the total number of eggs per clutch. The analysis showed highly significant results ($R^2 = 0.715$; $F_{3,22} = 18.4$; $P < 0.001$), with significant effects of all three independent variables: SVL ($b_{SVL} = 0.023$; $P < 0.001$) and the Fat Index ($b_{Fat\ Index} = 0.015$; $P = 0.016$) weighed positively on the mean egg mass, whereas the number of eggs per clutch weighed negatively ($b_{egg-number} = -0.001$; $P < 0.001$).

DISCUSSION

Our study showed that (1) large females invested more in reproduction than small females; (2) independent of body size; females in good condition laid larger clutches and stored larger amounts of energy for growth and survival than females in poorer condition; (3) independent of body size and condition, females that produced a larger number of eggs invested less energy per single egg. Figure 3 summarizes the pattern of associations between body size and reproductive and somatic effort of females.

In Green Toads, as in many other anuran spe-

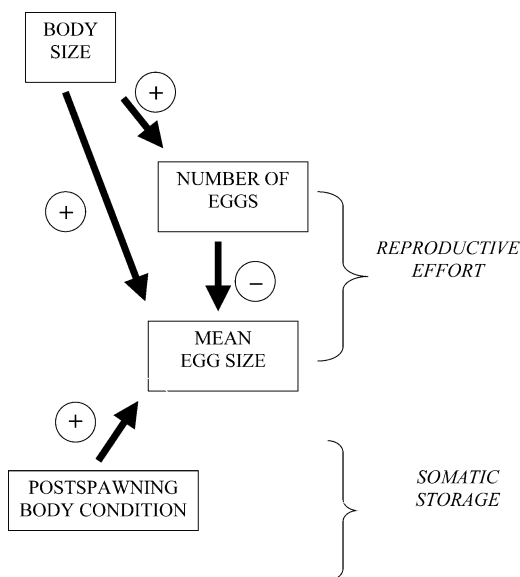


Fig. 3. Pattern of association among the variables employed to describe female state (SVL), somatic effort (postspawning fat index), and reproductive effort (number and mean size of eggs).

cies (review by Duellman and Trueb, 1986), body size is the most important phenotypic trait affecting variation in female reproductive effort. In other anurans (review in Halliday and Verrel, 1988) and in Green Toads as well (Castellano et al., 1999), body size is often found to correlate positively (though weakly) to age; thus, the body-size to clutch-size positive association may reveal an increase of reproductive effort with age. Large, old females, due based on their reduced expected future life, should invest proportionally more in reproduction and less in growth and survival than their younger counterparts. Our results, however, by failing to find any negative association between reproductive and somatic storage, provide no evidence to support this hypothesis and suggest that the strong positive correlation between body size and clutch size may result from a developmental constraint caused by the allometric relationships between gonad and overall body size. Better conditioned females, as more efficient consumers of environmental resources, can grow faster than females of poor quality, and attain a larger size, therefore affording them greater reproductive investment.

The large among-female variation in reproductive effort was more likely caused by variation in the number rather than by the size (mass) of eggs. In our study, the largest clutch had three times more eggs than the smallest clutch, whereas the highest ovum dry mass was

less than twice as heavy as the lightest egg. Large clutch size variation has also been observed within other conspecific populations. Jørgensen (1984), in comparing the reproductive investment of Danish and Israeli female green toads, found the mean number of full-grown vitellogenic oocytes per clutch amounting to about 11,000 in the Danish and 9000 in the Israeli populations. Furthermore, in the Israeli toad population a negative correlation was found between the number and size of eggs in a clutch. A similar negative relationship was observed also in *Rana temporaria* (Gibbons and McCarthy, 1986) and *Rana sylvatica* (Berven, 1988), where variation was correlated with age: clutch egg number decreasing and egg mass increasing with age.

Differing from the observed Israeli population data, we did not find a negative association between the number and size of eggs, unless we took into account the effect of both body size and postspawning body condition. Indeed, large females with large amounts of allocable energy not only recruited more oocytes to vitellogenic growth but also produced larger eggs than smaller females. Moreover, we found that resources were equally partitioned to both reproductive and somatic investments, which supports the condition-dependent reproductive hypothesis (Fig. 1, Hypothesis C). This strategy allows females to retain a proportion of their available resources for future reproductive contributions while contributing to the current reproductive cohort (van Noordwijk and de Jong, 1986).

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