ECOPHYSIOLOGY

Food supply modifies the trade-off between past and future reproduction in a sexual parasite–host system (*Rana esculenta, Rana lessonae*)

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Abstract Life history theory is concerned with the costs of survival, growth and reproduction under different ecological conditions and the allocation of resources to meet these costs. Typical approaches used to address these topics include manipulation of food resources, followed by measures of subsequent reproductive traits, and measures of the relationship between current and future reproductive investment. Rarely, however, do studies test for the interaction of past investment, present resource availability and future investment simultaneously. Here, we investigate this interaction in females of a sexual parasite–host system consisting of the hybridogenetic frog *Rana esculenta* (E) and one of its parental species *Rana lessonae* (L). We kept females from each of two groups (with or without previous reproduction) under two food treatments (low or high) and regularly recorded their growth as well as their body condition and hormone titres as measures of future reproductive condition. After keeping them in hibernation until the following spring, we exposed the females to males, recorded whether they spawned or not and related this response to their condition in the previous autumn. Past reproduction negatively affected growth during summer and condition during autumn which, in turn, reduced the following year's reproductive output. These costs of previous reproduction were less pronounced under the high than under the low food treatment and lower in *R. lessonae* than in *R. esculenta*. Increasing food supply improved reproductive condition more in L than in E females. These species differences in reproductive costs and food requirements provide a mechanistic explanation for why E females skip annual reproduction almost twice as often as L females. Since *R. esculenta* is a sexual parasite that depends on *R. lessonae* for successful reproduction, these species-specific life history patterns not only affect individual fitness but also the spatial structure and temporal dynamics of mixed LE populations.

Keywords Life history theory · Costs of reproduction · Resource availability · Metabolic rate · Population dynamics

Introduction

Life history studies address the question of how organisms allocate resources to fitness-relevant parameters, such as present reproduction, survival, growth and future reproduction (Lessells 1991; Stearns 1992; Roff 2002). Theory predicts trade-offs between allocation to these individual fitness parameters and that the optimal compromise will vary with reproductive potential (e.g. fecundity) and reproductive costs (e.g. mortality). Empirical studies, however, often do not find the predicted trade-offs (e.g. Huber and During 2000; Lardner and Loman 2003; Castellano et al. 2004), because differences in age, body size, individual quality, resource availability and other covariates are not controlled for (Van Noordwijk and de Jong 1986; Roff 2002). When the effects of such confounding

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covariates are statistically or experimentally removed, the underlying trade-offs are usually unmasked (e.g. Brown 2003; Uller and Olsson 2005).

One commonly performed type of experiment is manipulation of current reproductive effort, followed by measures of subsequent growth, survival and/or reproductive traits, such as number and quality of offspring or number of broods per season (e.g. Koivula et al. 2003; Bize et al. 2004; Parejo and Danchin 2006). Most of these experiments, however, ignore variation in food resources. Another common type of experiment manipulates the quantity or quality of food resources and then measures the treatment effects on future performance (e.g. Svensson 1995; Nager et al. 1997; Covas et al. 2004; Gignac and Gregory 2005), but these studies usually do not simultaneously control for previous reproductive investment. However, depending on whether and how much an individual has invested in the past, the initial level of energy resources to which the provided food is added may differ substantially. This is true for both ''capital breeders'', in which reproduction depends on long-term energy stores, and ''income breeders'', in which reproduction is fuelled by recently acquired resources (Drent and Daan 1980). As a result, even experimental manipulations of either current reproductive effort or food availability may fail to detect trade-offs between past and future reproduction, simply because they did not control for available resources and previous reproductive investment, respectively.

Here, we test the past investment × resource interaction on performance in females of a sexual parasite–host system (*Rana esculenta*, *Rana lessonae*). In these water frogs, resource allocation to reproduction not only affects individual fitness but also the structure and dynamics of their mixed-species populations. *Rana esculenta* (genotype LR) is originally a hybrid between *R. lessonae* (LL) and *Rana ridibunda* (RR) (Berger 1977; Günther 1990). It reproduces through hybridogenesis (Schultz 1969), i.e. it eliminates one of its parental genomes prior to meiosis and produces haploid gametes containing only the unrecombined genome of the other parental species. Because hybrid × hybrid matings usually do not result in viable offspring, due probably to an accumulation of deleterious mutations on the unrecombined genome (Vorburger 2001; Guex et al. 2002), hybrid populations can persist only in sympatry with the parental species whose genome they exclude. Typically, this is *R. lessonae* (LE system; Uzzell and Berger 1975). Fertilization of R gametes produced by *R. esculenta* with L gametes from *R. lessonae* restores somatic hybridity (LR) in the next generation (Graf and Polls Pelaz 1989).

Species composition in such mixed LE populations is highly variable among ponds, ranging from 5 to 95% *R. lessonae* (Berger 1977; Blankenhorn 1977). Within ponds, however, L/E ratios remain fairly stable over time even when population size fluctuates (Holenweg Peter et al. 2002). This stability arises from complicated interactions between the outcome of various mating combinations, overt and cryptic female choice, male–male competition, female fertility, male fertilization success and survival of adults and larvae (Hellriegel and Reyer 2000; Som et al. 2000; Lengagne et al. 2006). Some of these factors favour numerical superiority of the hybrid, whereas others enhance the success of the parental species. For a summary and references to the relevant literature see the Introduction and Discussion in Reyer et al. (2004).

One of the factors favouring the parental species is more regular breeding. In a field study, Reyer et al. (2004) found that the proportion of annually reproducing females was, on average, 1.90 times higher for *R. lessonae* than for *R. esculenta*. In that study, the causes for the species' difference in skipping reproduction remained unclear, but the authors hypothesized that they may lie in body mass-related species' differences in metabolic rates. Although the mass-specific rate scales with body mass at a power of <1 (and therefore decreases with increasing size), the absolute metabolic rate, and hence the energy requirement for maintenance and reproduction, is substantially higher for the larger E- than the smaller L-females (see Eq. 1).

Given that egg development involves complicated physiological processes (summarized in Reyer and Bättig 2004) and gonads with ripe eggs weigh up to 20% of the total body mass (Redshaw 1972), reproductive costs are to be expected and have, indeed, been found in several amphibian species (e.g. Jørgensen 1986; Elmberg 1991; Ritke and Lessman 1994; Lardner and Loman 2003; Camargo et al. 2005). In water frogs, reproducing females grow less and tend to have lower survival than non-reproducing ones (Reyer et al. 2004), and the higher the mass-specific number of eggs released in spring, the lower the body condition and hormone titres in the subsequent autumn, and the smaller the clutch size the following year (Reyer et al. 1999). In contrast to these results from water frog females kept under standardized laboratory conditions, spawning and non-spawning females in natural ponds only differed in growth rate, but not in survival, hormone titres and subsequent reproduction (Reyer et al. 2004; see also Jørgensen 1986). The contrast may partly be due to differences between frogs in captivity and the field in previous reproductive effort (e.g. clutch size) and availability of food resources. With energy requirements being higher in the larger *R. esculenta* than in the smaller *R. lessonae* we expect that different combinations of past reproductive investment and present food availability will affect future reproduction in the two species differently, including the probability of skipping reproduction.

In this paper, we report results of an experiment that was designed to test this hypothesis. For females of both species, we quantified reproductive costs by measuring their

growth rates, body conditions, hormone titres and future reproduction in relation to two categories of past reproductive effort (spawning vs. no spawning) and two levels of food supply (high vs. low).

Materials and methods

Animals

The frogs used in our experiment were caught during 5 nights between 14 May and 10 June 2000 in two ponds, one at Hellberg near Hinwil (47°18′04″N, 8°50′10″E) and the other at Pfaffensee near Andelfingen (47°35′45″N, 8°40′42″E). Both ponds contain mixed water frog populations, with a *R. lessonae/R. esculenta* ratio in favour of *R. lessonae* at Hellberg and a ratio in favour of *R. esculenta* at Pfaffensee. The animals were transported to the laboratory, where the same night we identified their sex through the presence (males) or absence (females) of thumb pads and vocal sacs and their species through the size and shape of the inner metatarsal tubercle (*callus internus*) (Berger 1977) and took blood from all females (see below). The following day, snout–vent lengths (SVL) of the animals were measured to the nearest 0.5 mm and they were weighed to the nearest 0.1 g using an electronic balance (Sartorius PT600; Sartorius, Göttingen, Germany). From these two measures, we calculated a body condition index (BCI) according to the equation $BCI = 10^4 \times weight/SVL^3$. This index has also been used to quantify body condition in other vertebrates, including amphibians (Jakob et al. 1996; Lüddecke 1997; Green 2001).

Animals were individually marked by inserting a 0.1g passive integrated transponder (Trovan ID 100; Euro ID, Weilerwist, Germany) subcutaneously into the lateral lymph sac (Sinsch 1992). Since species identification from the callus internus and other morphometric measures has its limits (Pagano and Joly 1999), even when there are no triploids as in our study populations, lymph was taken from a cut into the base of a hind foot web and later subjected to enzyme electrophoresis to unambiguously determine the frog species from albumin patterns (Uzzell and Berger 1975).

Experimental design

Before beginning the experiment described here, we subjected the females to two pre-treatments, one producing a category of 33 females with high reproductive output (category 1) and the other yielding 27 females that did not reproduce (category 2).

Female category 1 (with reproduction)

In this treatment, females were injected with 100 μl fish hormone/10 g body weight (LHRH-Salmon, H-7525; Bachem Feinchemikalien, Switzerland) to induce ovulation through elevated gonadotropin hormones (Van der Kraak et al. 1983). Twenty-three females were then put together with two *R. lessonae* males each and allowed to spawn naturally during amplexus. The other ten females were stripped of their eggs using a pair of tweezers (Berger et al. 1994). Both these methods usually lead to the oviposition of all ripe eggs a female has and, hence, guarantee a high reproductive investment. The reason for obtaining eggs in different ways was that the same 33 females were used to collect data for two other studies, one on mating behaviour (n = 23) and the other for performing an artificial fertilization experiment (n = 10).

Female category 2 (no reproduction)

Females in this category had previously been kept in artificial ponds, where they did not spawn. This does not necessarily mean that they did not carry any eggs; but they did not release them and, hence, were able to reduce their possible initial reproductive effort by resorbing them (Reyer et al. 1999).

After the pre-treatment, all 60 females were measured and weighed and their blood was sampled. Thereafter, each category was further split into two sub-categories by randomly assigning individuals to two food level treatments. For each of the resulting spawning (yes/no) × food (low/high) treatment combination, we created four replicates. In three of them, females were held in groups of four (two L and two E); in the fourth replicate they were held in variable group sizes and species ratios, due to the unequal number of spawners and non-spawners and of L- and E-females.

Initially (i.e. from 8 June on), each group was kept in a green polyethylene/polypropylene tub of 80 l volume and 0.29 m² surface area. In each tub, one-third of the bottom was covered with turf and soil; the remaining area was filled with approximately 10 l water. A cover made of fibreglass window screen prevented the animals from escaping and protected them from predation and intense sunshine. The tubs were arranged in rows in an open field near the University of Zürich–Irchel. However, when some animals died during a period of extremely high temperatures, all tubs were moved indoors for a short period (14 June). Half of the female groups remained indoors in the tubs, the other half was transferred to fenced artificial ponds on 18 June, again with females belonging to the same spawning × food combination entering the same pond. Each pond (2.5 × 1.5 × 0.4 m deep), contained 12 plants in flowerpots, was surrounded by a narrow band of grass and covered with a net to keep birds, cats and other potential predators away. The distribution of females over two locations and keeping them

in different densities (0.13 m²/frog in tubs, 1.93 m²/frog in ponds) was necessary because we had neither sufficient indoor space nor enough outdoor ponds to keep all animals in the same place and in equal densities.

All females were fed crickets 2–4 times a week but in different quantities. Food requirements were calculated by dividing resting metabolic rate (RMR) of frogs at 20°C through the average cricket energy content (CEC). RMR was calculated from frog body mass (g) according to the following equation:

$$RMR \ (\text{cal/week}) = \left(0.102 \times \text{body mass}^{0.82}\right) \\ \times 4.8 \times 24 \times 7 \quad (1)$$

Here, the expression in parentheses (from Table 12.2 in Gatten et al. 1992) calculates O_2 (ml/h) which is converted into heat production (cal/h) by the factor 4.8 (Schmidt-Nielsen 1983) and extrapolated to cal/week through multiplying the value by 24 h and 7 days. Cricket energy content was calculated as:

$$CEC \ (\text{cal/g dry weight}) = 0.3 \times (1 - 0.71) \times 5,360 \quad (2)$$

Here 0.3 is the average body mass of the crickets we used, 0.71 is the water content and 5,360 the energy content (cal) per gram cricket dry weight (values for Saltatoria, taken from Appendix 1 in Brodmann and Reyer 1999). The calculations resulted in weekly food requirements of 1.9 crickets per average *R. lessonae* (18.1 g at the beginning of the experiment) and 3.8 per average *R. esculenta* (42.2 g). Frogs in the high food treatment received roughly 400% of this calculated number, those in the low food treatment only about 80%. Since RMR is only the level for minimal activity, and metabolic rates for amphibians engaging in natural behaviour are, on average, about 3 times higher (see Table 14.4 in Pough et al. 1992), the high food treatment must have been plentiful, whereas the low food treatment must have been inadequate. We set the dosage that low to avoid, under our experimental conditions, the provision of enough energy from the low food treatment to replenish resources lost in previous reproduction and the build up of energy stores for future reproductive investment. As the frogs gained weight during the season (see below) and/or when group size decreased because of mortality, the absolute number of crickets added to a tub or pond was adjusted accordingly. Since females were kept in groups, it was not possible to monitor food intake of individuals which might have been influenced by interference competition from others in the same container. Individually kept water frogs, however, are stressed and do not behave normally; hence, there was no alternative to holding them in groups. Potential influences of this methodological constraint will be addressed in the ''Discussion''.

Data collection

Growth and body condition

The females were kept in the environments described above from the first half of June until late September, the time when in nature frogs leave their ponds for hibernation (Holenweg and Reyer 2000). Every other week the females were caught and immersed in a box with pond water for at least an hour to annul potential evaporative water loss which can amount to up to 10% of the body weight (Sinsch 1983). Thereafter, the frogs were dried with a towel, measured and then weighed on an electronic balance. The condition index was calculated and the subsequent amount of food adjusted to the new body mass.

Hormone titres

Presence or absence of mature follicles was deduced from testosterone titres which in water frogs provide a fairly reliable measure of gravidity when ethical reasons or re-use of frogs in subsequent studies prohibit checking for eggs the invasive way. Reyer and Bättig (2004) found that titres ≥ 20 ng/ml correctly predicted the presence of eggs in about 90% of the females whereas less than 40% of the females with titres ≤ 10 ng/ml were found to carry eggs. Once a month the blood of females was sampled by making a small incision between two toes of a hind foot. Blood was taken immediately after catching the female to insure that androgen levels did not drop due to the stress of captivity (Licht et al. 1983; Paolucci et al. 1990). Subsequent treatment of blood samples and testosterone analysis followed the procedure described in Reyer and Bättig (2004), with the exception that we applied a different antibody (AK 8/4 E instead of AK 8/3). It had specific binding between 42.0 and 53.8%; the unspecific binding varied between 2.1 and 3.3%. The lowest testosterone concentration that could be interpreted with confidence ranged from 0.50 to 5.70 ng/ml.

Data analysis

We used analysis of covariance (ANCOVA) to relate growth and reproductive condition, respectively, to species (LL or LR), two treatment effects with two levels each (spawning, yes or no; food, high or low) and their two- and three-way interactions plus two covariates (date, SVL at the beginning of the experiment). We also included location (indoor tubs versus outdoor ponds) in the analysis. Prior to the analysis we tested the treatment × covariate interactions for analyses (growth) and (reproductive condition) and all covariates shown in Table 1. With all $P \geq 0.150$, homogeneity of slopes, a necessary condition for ANCOVA, could be assumed. Since females were kept

in groups to reduce stress, we used tub and pond means, respectively, for each species, rather than values from individuals for the analyses. Reproductive condition was expressed by the scores from the first (and only) principal component of a principal components analysis (PCA) based on BCI and testosterone titres. Prior to these analyses, the continuous variables (BCI and testosterone titers) were ln-transformed to increase additivity of effects and equality of variance and percent growth was arcsin(square root) transformed (Snedecor and Cochran 1980). The relationship between spawning in the following spring (yes, no) and species, last year's growth and reproductive condition scores in the previous autumn was analysed by means of stepwise logistic regression analysis with $P = 0.10$ as the inclusion/exclusion criterion.

Results

Seasonal pattern of growth and reproductive condition

During the experiment, SVL, body condition and testosterone titres all changed from low to high values. Growth over the 4 months was 3 times higher in the non-reproducing than in the reproducing females (7.2 vs. 2.4% increase in SVL). Condition indices increased from very low values in early June to high indices in late July/early August that were then maintained until the end of September (Fig. 1). This development was similar in females that had reproduced prior to the start of the experiment and in those that had not, but reproducing females had lower condition indices at any one point in time. Testosterone titres were initially slightly higher in the non-reproducing females than in those that had spawned, but then dropped and remained low in both female groups until the second half of July. Thereafter, they rose only slightly in females that had reproduced but sharply in those that had not.

In late September, hormone titres and condition indices of individuals were significantly correlated ($r = 0.713$, $n = 36$, $P < 0.001$) and, thus, did not represent independent measures of reproductive condition. For the analyses below, we therefore replaced the two original variables by the factor scores obtained from a PCA. In the PCA, testosterone and condition had high positive loadings (0.925) on the same factor which accounted for 86% of the total variance. Hence, the scores of this factor provide an adequate measure of reproductive condition.

Determinants of growth and reproductive condition in autumn

Growth from early June to late September and reproductive condition in late September were clearly related

Table 1 Results from two analyses of covariance (ANCOVA) testing for the effects of species, spawning, food treatment and their two- and three-way interactions on growth [=percent increase in snout–vent length (SVL)] and reproductive condition in autumn, measured by the factor scores from a principal components analysis performed on testosterone titres and body condition index. Also included in the analysis were the location of the experiment (outdoor ponds vs. indoor tubs) and two covariates: SVL at the beginning of the season and time

Source	SS	df	F	P
Growth ($R^2 = 0.744$)				
Species	0.0	1	0.0	0.975
Spawning	13.1	1	1.7	0.217
Food	23.0	1	23.0	0.108
Location	3.4	1	0.4	0.517
Species × spawning	3.4	1	0.4	0.518
Species × food	9.0	1	1.2	0.301
Spawning × food	42.2	1	5.4	0.036
Species × food × spawning	16.4	1	2.1	0.170
SVL	10.7	1	1.4	0.260
Time[a]	3.0	1	0.4	0.547
Error	100.8	13		
Reproductive condition ($R^2 = 0.934$)				
Species	0.8	1	4.9	0.054
Spawning	0.2	1	1.5	0.250
Food	7.3	1	46.9	<0.001
Location	0.2	1	1.3	0.287
Species × spawning	0.9	1	5.8	0.039
Species × food	0.7	1	4.7	0.059
Spawning × food	0.9	1	5.8	0.040
Species × food × spawning	0.1	1	0.9	0.368
SVL	0.9	1	5.9	0.038
Time[b]	0.1	1	0.4	0.562
Error	1.4	9		

[a] Number of days between the first and the last SVL measurement

[b] Day of the season when the sample for testosterone analysis was taken and the condition index was measured

to experimental treatments (Table 1). The most obvious and consistent effect is the significant interaction between spawning and food provisioning (Fig. 2a, b). Overall, females that had spawned grew less and attained a poorer reproductive condition than females that had not; but these costs of reproduction were modified by the amount of food they received ($P = 0.036$ and $P = 0.040$, respectively). Reproductive condition in spawners was much lower in the low than in the high food treatment, whereas in non-spawners the difference between food levels was not very pronounced (Fig. 2b). Conversely, food treatment had no effect on growth of spawners, but a large one on non-spawners: those receiving little food grew more than those receiving lots of food (Fig. 2a). This antagonistic effect of food on growth and reproductive
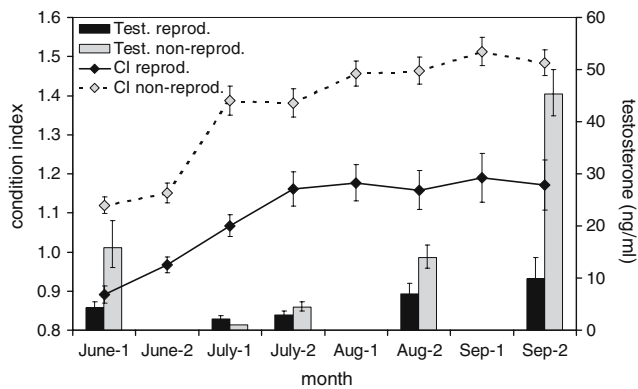
Fig. 1 Seasonal development of body condition index (*lines*) and testosterone titres (*bars*) in females that had (*black*) or had not reproduced (*grey*). Values are least square means and SEs from the analyses in Table 1. Data are shown separately for the first and second half of each month [e.g. first half of June (*June-1*), second half of June (*June-2*), etc.]. Absence of bars indicates that testosterone was not measured during the time interval. *CI* Confidence interval, *Aug* August, *Sep* September

condition is clearly visible in Fig. 2c, where values for spawners and non-spawners have been pooled.

There were also differences between the two species for reproductive condition, but not for growth. Overall, *R. lessonae* reached higher reproductive scores in late September than *R. esculenta* (Fig. 2d, e), but the species difference was modified by previous reproduction and food supply, as indicated by the respective two-way interactions in Table 1. The species difference tended to increase with increasing food supply (Fig. 2d) and was significantly larger in spawners than in non-spawners (Fig. 2e). The latter result was due to the fact that previous reproduction negatively affected future reproductive condition in *R. escuelenta*, but not in *R. lessonae*.

Finally, there was a significant effect of initial body size on subsequent reproductive condition ($P < 0.038$). The smaller a female was at the beginning of the experiment, the higher was the reproductive condition in autumn (Fig. 2f). The time between the start and the end of the experiment varied from 88 to 111 days, but this had no effect on either growth ($P = 0.547$) or reproductive condition ($P = 0.562$). Also, it made no difference for growth and reproduction whether females were kept in outdoor ponds or indoor tubs ($P = 0.517$ and $P = 0.287$, respectively, for location).

Following year's reproduction

Of the 24 females (12 of each species) that hibernated in captivity, three *R. lessonae* died during the winter. Among the 21 survivors there were highly significant positive correlations between SVLs before (late September 2000)

and after hibernation (April 2001) ($r = 0.929$, $P < 0.001$) as well as between reproductive conditions before and after hibernation ($r = 0.784$, $P < 0.001$). When put together with *R. lessonae* males in spring 2001, seven *R. lessonae* and eight *R. esculenta* mated and spawned while two *R. lessonae* and four *R. esculenta* did not. A stepwise logistic regression analysis revealed no effect of species and growth between June and late September of the previous year on reproduction. But spawning and non-spawning females differed significantly in their reproductive scores of the previous autumn ($P = 0.028$). Females that did mate and lay eggs in spring 2001 had an average autumn score of 0.31 (± 0.18), while those that did not had one of −1.41 (± 0.61).

Discussion

The results from our experiment confirm those from previous studies (Reyer et al. 1999, 2004) that reproduction in water frog females is costly. Past reproduction negatively affected growth during summer and reproductive condition during autumn (Figs. 1, 2a, b) which, in turn, reduced the following year's reproductive success. In addition, our experiment reveals that these costs are modified by the available food resources (spawning × food interaction; Fig. 2b) and that the response to past reproductive effort and available food resources differs between *R. lessonae* and *R. esculenta* females. Overall, future reproduction is less impaired by past reproduction and more enhanced by benign food conditions in the parental species than in the hybrid (Fig. 2d, e). This explains our previous finding that hybrid females skip reproduction almost twice as often as females of the parental species (Reyer et al. 2004).

The negative effects of past reproductive effort on subsequent growth and reproductive condition are most pronounced for females raised under the low food treatment. Evidence for low fecundity of undernourished frogs also comes from a study on *Rana cyanophlyctis* (Girish and Saidapur 2000). In our study, reproductive costs were less obvious or even absent in females with high amounts of food (Fig. 2a, b). Hence, energy and resource depletion during present reproduction can, but do not necessarily impair future reproduction. If sufficient food is available, females reproducing in one year can recover and grow and invest as much into the following year's reproduction as females that have not paid the costs of previous reproduction (cf. Clutton-Brock et al. 1983). Surprisingly, females that had not reproduced grew less under the high than under the low food treatment (Fig. 2a). This puzzling result can be explained by the antagonistic effect of food on growth and reproductive condition shown in Fig. 2c. The trade-off suggests that females primarily invest in growth

Fig. 2 Growth from early June to late September and reproductive condition scores in late September in relation to previous reproduction (with, without), amount of food received (low, high) and body size (snout–vent length; SVL) at the beginning of the experiment. Shown are least square means and SEs for significant interactions from the analyses in Table 1. **a** and **c** (*filled circles*) Growth, plotted as percentage increase in SVL on the left *y*-axis. **b**, **d**, **e** and **c** (*open circles*) Reproductive condition indices, plotted as factor scores on the right *y*-axis. **a–c** Values are pooled over both species. **d**, **e** Values are presented separately for *Rana lessonae* and *Rana esculenta* since the species × spawning interaction (**e**) was significant and the species × food interaction (**d**) was almost significant. Values for reproductive condition in **f** are estimates after statistically removing the effects of the other variables in the analysis of covariance from Table 1
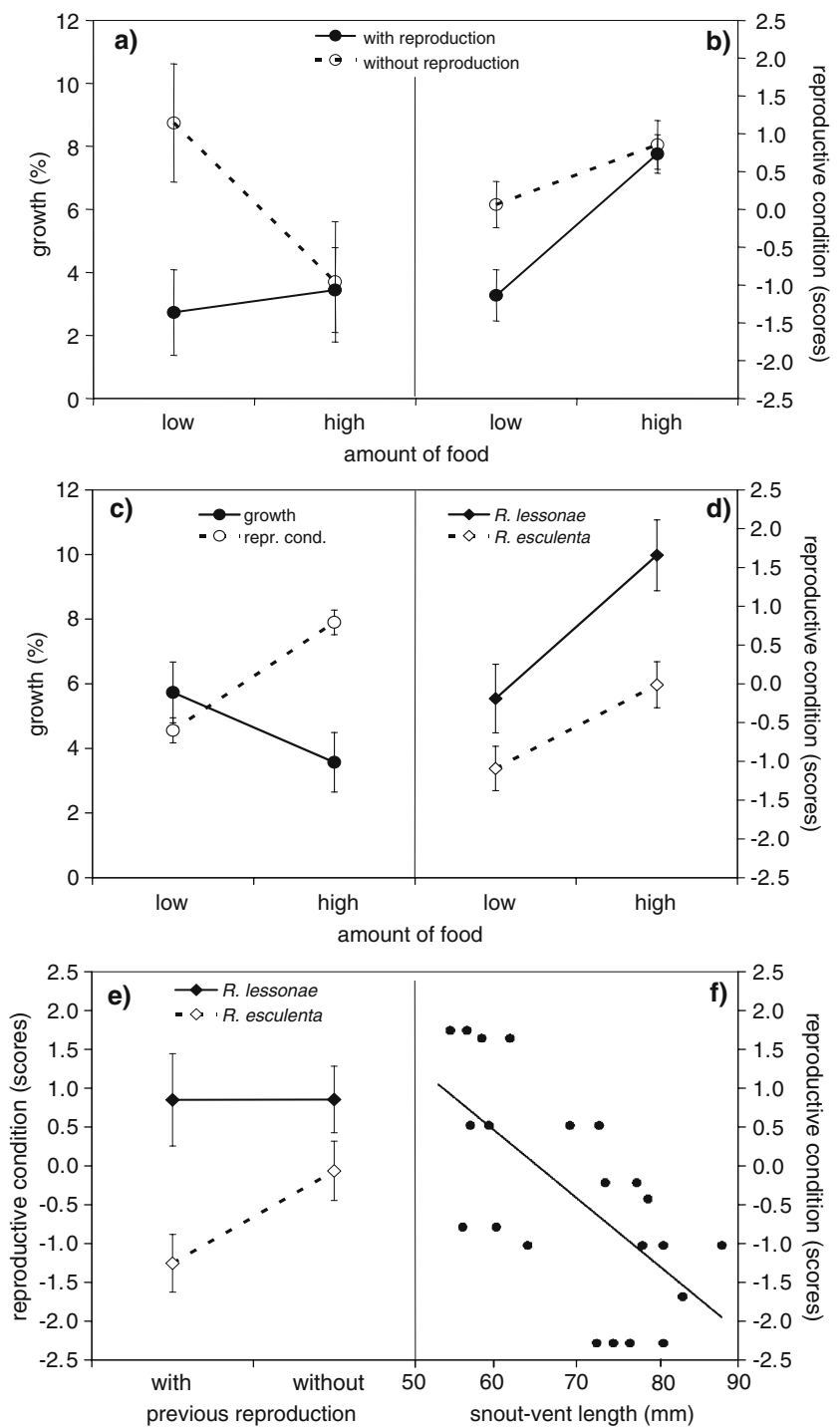
when food conditions are poor, but in future reproduction when they are good. To our knowledge, this shift in allocation rules with food availability has never been reported before for anurans, although the observed negative relationship between reproductive and somatic investment is not exceptional (e.g. Elmberg 1991; Camargo et al. 2005). It is, however, not universal either. Some studies on anurans have found fixed, rather than flexible, allocation patterns, with positive correlations between reproductive and

somatic investment (Lampo and Medialdea 1996; Lardner and Loman 2003). To determine whether such diverse relationships really represent different allocation strategies or merely reflect different resource availabilities as suggested by the Van Noordwijk and de Jong (1986) model, we will have to study food intake and resource allocation at the level of individuals. In the present study this was not possible, due to logistic problems and the need to keep frogs in groups to reduce stress.

Reproductive condition in autumn also differed between species. Overall, *R. lessonae* reached higher reproductive scores than *R. esculenta*, but the extent of the species difference varied with the treatment as indicated by the significant species × spawning and the nearly significant species × food interactions (Table 1). The species × spawning interaction (Fig. 2e) indicates that reproductive effort is higher in hybrid than in parental females. Based on investment in eggs, this was not to be expected. Egg number per gram body mass is 16% (Juszczyk 1974 cited in Günther 1990) to 36% (Berger and Uzzell 1980) higher for an average *R. esculenta* than for an average *R. lessonae* female (mean = 26%). Conversely, average volume of the individual egg is 25% higher in L- than in E-females [calculated from equations in Berger and Uzzell (1980) with the average SVL values for L- (58.0 mm) and E-females (75.4 mm) from our study]. With 26% higher mass-specific egg number in the hybrid versus a 25% higher egg volume in the parental species, the energetic investment alone cannot explain why reproductive costs are higher in *R. esculenta* than in *R. lessonae*. Maybe, this calculation is too simple to capture the real costs. Also, we have to consider that E/L egg ratios and egg sizes vary widely among populations, female size classes and individuals (Berger and Uzzell 1980; Abt and Reyer 2004), as well as with the mating combination (Reyer et al. 1999).

The almost significant species × food interaction on reproductive condition (Fig. 2d) indicates that L- and E-females differ in their response to resources. Although food supply had been adjusted to body mass (see "Materials and methods") increasing supply tended to improve reproductive condition more in *R. lessonae* than in *R. esculenta* ($P = 0.059$); and averaged over both food treatments there was a tendency for better condition in the parental species ($P = 0.054$). The most plausible interpretation is that—relative to the species-specific energy requirements—even the low food treatment was already better for the smaller L- than for the larger E-females; this advantage increased under the high food treatment. The same argument can explain why—independent of species—there was a significant negative relationship between body size and reproductive condition (Fig. 2f). Since, with increasing food levels, the (lower) energy requirements of small females are met earlier than the (higher) requirements of large females, the small individuals are more likely to reach reproductive condition.

As females were kept in mixed LE groups, our results and conclusions are potentially affected by the extent of interference competition between the two species. With no competition and equal chances to encounter a cricket, which was likely under the low female densities in ponds, the smaller L-females might obtain more food relative to their body size than the larger E-females. With competitive

superiority of the larger E-females the reverse would be true, in particular under the high female densities in tubs. Since we included body size as a co-variable in the analysis and also found no significant effect of location on either growth or reproductive condition (Table 1) in spite of the about 15-fold density difference between tubs and ponds (see "Materials and methods"), we feel that the observed genotype differences are not spurious results of body size-related competitive success and food intake. Moreover, even if they were, this would not change our explanation why in nature *R. esculenta* females skip reproduction almost twice as often as *R. lessonae* females (Reyer et al. 2004). In natural ponds they also occur in mixed LE groups and hybrids are, on average, larger than the parental species. Hence, our experimental setup mirrors the natural situation much better than individual rearing would have done.

Although growth can be considered an investment for future reproduction, because fecundity increases with body size (Berger 1977), the fairly small difference in average growth (5.8 vs. 1.6 mm SVL increment) will give non-spawning females only about a 15–20% advantage in the following year's fecundity over spawning females [calculated from equations in Berger and Uzzell (1980) and the average SVL values for L and E females from our study]. This gain is far too small to compensate for the 100% loss incurred by skipping the present reproductive season and the more than 35% average probability of not surviving to the next (Holenweg Peter 2001; Anholt et al. 2003). Since average age structures and survival probabilities do not seem to differ markedly and consistently between *R. lessonae* and *R. esculenta* populations (Neveu 1991; Holenweg Peter 2001; Anholt et al. 2003; Reyer et al. 2004), the species differences in the frequency of breeding will not be counterbalanced by differences in lifespan either. Hence, the 1.9-times higher frequency of skipping reproduction in E- than in L-females found earlier (Reyer et al. 2004) must be viewed as an energetic constraint on *R. esculenta*, rather than a reproductive strategy that is different from that of *R. lessonae*.

This energetic constraint will shift the numerical balance towards the parental species. However, as shown in this paper, reproduction of *R. esculenta* and *R. lessonae* females varies with the amount of available food (Fig. 2d), due to size-related differences in metabolic rates. As a result, we may expect that, where resource availability varies in space and/or time, the proportions of L and E females ready to spawn will differ among ponds and/or between years. Combined with the outcome of the four possible mating combinations—with L × L yielding L, L × E and E × L yielding E and E × E producing no viable offspring—this may contribute to the wide range of L/E ratios that have been found in nature (Berger 1977; Blankenhorn 1977;

Holenweg Peter et al. 2002). Thus, the LE system clearly illustrates that trade-offs within generations will translate into effects across generations and, hence, can have great significance for population structure and dynamics. This is not a new idea (see e.g. Beckerman et al. 2002; Benton et al. 2005 and literature therein), but the direct causal link between the individuals' life histories and subsequent events on the population and community level are likely to become more visible in systems like ours, where differences in individual performance have qualitative consequences, namely the production of either L or E offspring, rather than weak quantitative consequences (e.g. Erelli and Elkinton 2000; Banks and Powell 2004).

References

Abt G, Reyer H-U (2004) Larval development and recruitment of juveniles in a natural population of *Rana lessonae* and *Rana esculenta*. Copeia 2004:638–646

Anholt BR, Hotz H, Guex G-D, Semlitsch RD (2003) Overwinter survival of *Rana lessonae* and its hemiclonal associate *Rana esculenta*. Ecology 84:391–397

Banks PB, Powell F (2004) Does maternal condition or predation risk influence small mammal population dynamics? Oikos 106:176–184

Beckerman A, Benton TG, Ranta E, Kaitala V, Lundberg P (2002) Population dynamic consequences of delayed life-history effects. Trends Ecol Evol 17:263–269

Benton TG, Plaistow SJ, Beckerman AP, Lapsley CT, Littlejohns S (2005) Changes in maternal investment in eggs can affect population dynamics. Proc R Soc Lond B 272:1351–1356

Berger L (1977) Systematics and hybridization in the *Rana esculenta* complex. In: Taylor DH, Guttmann SI (eds) The reproductive biology of amphibians. Plenum, New York, pp 367–388

Berger L, Uzzell T (1980) The eggs of European water frogs (*Rana esculenta* complex) and their hybrids. Folia Biol (Kraków) 28:3-25

Berger L, Rybacki M, Hotz H (1994) Artificial fertilization of water frogs. Amphib Reptil 15:408-413

Bize P, Roulin A, Tella JL, Bersier LF, Richner H (2004) Additive effects of ectoparasites over reproductive attempts in the long-lived alpine swift. J Anim Ecol 73:1080–1088

Blankenhorn HJ (1977) Reproduction and mating behavior in *Rana lessonae–Rana esculenta* mixed populations. In: Taylor DH, Guttmann SI (eds) The reproductive biology of amphibians. Plenum, New York, pp 389–410

Brodmann PA, Reyer H-U (1999) Nestling provisioning in water pipits (*Anthus spinoletta*): do parents go for specific nutrients or profitable prey? Oecologia 120:506–514

Brown CA (2003) Offspring size-number trade-offs in scorpions: an empirical test of the Van Noordwijk and de Jong model. Evolution 57:2184–2190

Camargo A, Naya DE, Canavero A, da Rosa I, Maneyro R (2005) Seasonal activity and the body size-fecundity relationship in a population of *Physalaemus gracilis* (Boulenger, 1883) (Anura, Leptodactylidae) from Uruguay. Ann Zool Fenn 42:513–521

Castellano S, Cucco M, Giacoma C (2004) Reproductive investment of female green toads (*Bufo viridis*). Copeia 2004:659–664

Clutton-Brock T, Guiness FE, Albon SD (1983) The costs of reproduction to red deer hinds. J Anim Ecol 52:367–383

Covas R, Doutrleant C, du Plessis MA (2004) Experimental evidence of a link between breeding conditions and the decision to breed or help in a colonial cooperative bird. Proc R Soc Lond B 271:827–832

Drent RH, Daan S (1980) The prudent parent: energetic adjustments in avian breeding. Ardea 80:225–252

Elmberg J (1991) Ovarian cyclicity and fecundity in boreal common frogs *Rana temporaria* L. along a climatic gradient. Funct Ecol 5:340–350

Erelli MC, Elkinton JS (2000) Maternal effects on gypsy moth (Lepidoptera: Lymantriidae) population dynamics: a field experiment. Environ Entomol 29:476–488

Gatten RE Jr, Miller K, Full RJ (1992) Energetics at rest and locomotion. In: Feder ME, Burggren WW (eds) Environmental physiology of amphibians. The University of Chicago Press, Chicago, Ill., pp 314–377

Gignac A, Gregory PT (2005) The effects of body size, age, and food intake during pregnancy on reproductive traits of a viviparous snake, *Thamnophis ordinoides*. Ecoscience 12:236–243

Girish S, Saidapur SK (2000) Interrelationship between food availability, fat body, and ovarian cycles in the frog, *Rana tigrina*, with a discussion on the role of fat body in anuran reproduction. J Exp Zool 286:487–493

Graf J-D, Polls Pelaz (1989) Evolutionary genetics of the *Rana esculenta* complex. In: Dawley RM, Bogart, JP (eds) Evolution and ecology of unisexual vertebrates. New York State Mus Bull 466:289–302

Green AJ (2001) Mass/length residuals: measures of body condition or generators of spurious results? Ecology 82:1473-1483

Günther R (1990) Die Wasserfrösche Europas. Ziemsen, Wittenberg

Guex G-D, Hotz H, Semlitsch RD (2002) Deleterious alleles and differential viability in progeny of natural hemiclonal frogs. Evolution 56:1036–1044

Hellriegel B, Reyer H-U (2000) Factors influencing the composition of mixed populations of a hemiclonal hybrid and its sexual host. J Evol Biol 13:906–918

Holenweg Peter A-K (2001) Survival in adults of the water frog *Rana lessonae* and its hybridogenetic associate *Rana esculenta*. Can J Zool 79:652–661

Holenweg A-K, Reyer H-U (2000) Hibernating behavior of *Rana lessonae* and *R. esculenta* in theirnatural habitat. Oecologia 123:41–47

Holenweg Peter A-K, Reyer H-U, Abt Tietje G (2002) Ecological factors affecting genotype and sex ratios in mixed populations of *Rana lessonae*, *R. ridibunda* and their hybridogenetic associate *R. esculenta*. Ecoscience 9:1–11

Huber H, During HJ (2000) No long-term costs of meristem allocation to flowering in stolonferous *Trifolium* species. Evol Ecol 14:731–748

Jakob EM, Marshall SD, Uetz GW (1996) Estimating fitness: a comparison of body condition indices. Oikos 77:61-67

Jørgensen CB (1986) Effect of fat body excision in female *Bufo bufo* on ipsilateral ovary, with a discussion of fat body–gonad relationships. Acta Zool 67:5-10

Koivula M, Koskela E, Mappes T, Oksanen TA (2003) Cost of reproduction in the wild: manipulation of reproductive effort in the bank vole. Ecology 84:398–405

Lampo M, Medialdea V (1996) Energy allocation patterns in *Bufo marinus* from two habitats in Venezuela. J Trop Ecol 12:321–331

Lardner B, Loman J (2003) Growth or reproduction? Resource allocation by female frogs *Rana temporaria*. Oecologia 137:541–546

Lengagne T, Grolet O, Joly P (2006) Male mating speed promote hybridization in the *Rana lessonae–Rana esculenta* waterfrog system. Behav Ecol Sociobiol 60:123–130

Lessells CM (1991) The evolution of life histories. In: Krebs JR, Davies NB (eds) Behavioural ecology, 3rd edn. Blackwell, Oxford, pp 32–68

Licht P, Mc Creery BR, Barnes R, Pang R (1983) Seasonal and stress related changes in plasma gonadotropins, sex steroids, and corticosterone in the bullfrog, *Rana catesbeiana*. Gen Comp Endocrinol 50:124-145

Lüddecke H (1997) Field reproductive potential of tropical high mountain *Hyla labialis* females: direct and indirect evidence from mark–recapture data. Amphib Reptil 18:357–368

Nager RG, Ruegger C, Van Noordwijk AJ (1997) Nutrient or energy limitation on egg formation: a feeding experiment in great tits. J Anim Ecol 66:495–507

Neveu A (1991) Structures démographiques de populations adultes de grenouilles vertes du complexe *R. esculenta*. Bull Fr Peche Oiscicult 321:55–71

Pagano A, Joly P (1999) Limits of the morphometric method for field identification of water frogs. Alytes 16:130–138

Paolucci M, Esposito V, Difiore MM, Botte V (1990) Effects of short postcapture confinement on plasma reproductive hormone and corticosterone profiles in *Rana esculenta* during the sexual cycle. Boll Zool 57:253-259

Parejo D, Danchin E (2006) Brood size manipulation affects frequency of second clutches in the blue tit. Behav Ecol Sociobiol 60:184–194

Pough FH, Magnussen WE, Ryan MJ Wells KD, Taigen TL (1992) Behavioral energetics. In: Feder ME, Burggren WW (eds) Environmental physiology of amphibians. The University of Chicago Press, Chicago, Ill., pp 395–436

Redshaw MR (1972) The hormonal control of the amphibian ovary. Am Zool 12:289-306

Reyer H-U, Bättig B (2004) Identification of reproductive status in female frogs—a quantitative comparison of nine methods. Herpetologica 60:349–357

Reyer H-U, Frei G, Som C (1999) Cryptic female choice: frogs reduce clutch size when amplexed by undesired males. Proc R Soc Lond B 266:2101-2107

Reyer H-U, Wälti M-O, Bättig I, Altwegg R, Hellriegel B (2004) Low proportions of reproducing hemiclonal females increase the stability of a sexual parasite–host system (*Rana esculenta, R. lessonae*). J Anim Ecol 73:1089–1101

Ritke ME, Lessman CA (1994) Longitudinal-study of ovarian dynamics in female gray treefrogs (*Hyla chrysoscelis*). Copeia 1994:1014–1022

Roff DA (2002) Life history evolution. Sinauer, Sunderland, Mass.

Schmidt-Nielsen K (1983) Animal physiology: adaptation and environment, 3rd edn. Cambridge University Press, Cambridge

Schultz RJ (1969) Hybridization, unisexuality, and polyploidy in the teleost *Poeciliopsis* (Poeciliidae) and other vertebrates. Am Nat 103:605-619

Sinsch U (1983) Wasserhaushalt und tagesperiodisches Verhalten von drei europäischen Rana-Arten (Amphibia: Anura). PhD thesis, University of Köln, Germany

Sinsch U (1992) Two new tagging methods for individual identification of amphibians in long-term field studies: first experiences with natterjacks. Salamandra 28:116-128

Snedecor GW, Cochran WG (1980) Statistical methods, 15th edn. Iowa State University Press, Ames, Iowa

Som C, Anholt BR, Reyer H-U (2000) The effect of assortative mating on the coexistence of a hybridogenetic waterfrog and its sexual host. Am Nat 156:34-46

Stearns SC (1992) The evolution of life histories. Oxford University Press, Oxford

Svensson E (1995) Avian reproductive timing—when should parents be prudent? Anim Behav 49:2569–1575

Uller T, Olsson M (2005) Trade-offs between offspring size and number in the lizard *Lacerta vivipara*: a comparison between field and laboratory conditions. J Zool 265:295–299

Uzzell T, Berger L (1975) Electrophoretic phenotypes of *Rana ridibunda, Rana lessonae* and their hybridogenetic associate, *Rana esculenta*. Proc Acad Nat Sci Philadelphia 127:13-24

Van der Kraak G, Lin HR, Donaldson EM, Dye HM, Hunter GA (1983) Effects of LH-RH and des-Gly[10](D-Ala[6])LH-RH-ethylamide on plasma gonadotropin levels and oocyte maturation in adult female coho salmon (*Oncorhynchus kisutch*). Gen Comp Endocrinol 40:470–476

Van Noordwijk AJ, de Jong G (1986) Acquisition and allocation of resources—their influence on variation in life-history tactics. Am Nat 128:137–142

Vorburger C (2001) Fixation of deleterious mutations in clonal lineages: evidence from hybridogenetic frogs. Evolution 55:2319–2332