

# Offspring size and survival in the frog *Rana latastei*: from among-population to within-clutch variation

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Egg size is considered to be a major maternal effect for offspring in oviparous organisms. It has profound consequences on fitness, and differences in egg size are viewed as plastic responses to environmental variability. However, it is difficult to identify the effect of egg size *per se* because egg size can covary with genetic features of the mother and with other nongenetic factors. We analysed the relationship between offspring starting size (i.e. a proxy of egg size) and larval survival in the frog *Rana latastei*. We analysed this relationship: (1) among five populations at different altitudes; (2) among clutches laid from different females; and (3) among siblings within clutches, to evaluate the effect of starting size. We observed differences among populations for offspring size, but starting size was not related to altitude or genetic diversity. Mortality was higher in populations and families with small average starting size; however, among siblings, the relationship between starting size and mortality was not verified. The relationship observed among clutches may therefore be caused by covariation between egg size and other effects. This suggests that the covariation between egg size and other effects can result in apparent relationships between egg size and fitness-related traits. Proximate and ultimate factors can cause the phenotypic variation of hatchlings in the wild, and key traits can be related to this variation, but the underlying causes require further investigation. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, 97, 845–853.

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## INTRODUCTION

Nongenetic maternal influence is known to have important effects on key traits of offspring. In oviparous species, egg size-mediated maternal effects can affect offspring size at hatching, survival, growth and development rate, stress tolerance, and other important features in many taxa, including birds, reptiles, amphibians, insects, and molluscs (Mousseau & Fox, 1998; Moran & Emler, 2001). Egg size can influence many life-history traits; therefore, differences between populations in egg size have been frequently interpreted as adaptations to environmental differences among sites (Morrison & Hero, 2003; Olsen & Vollested, 2003; Räsänen, Laurila & Merilä, 2003a,

2005). Moreover, life-history theory predicts a trade-off between offspring number and quality (Krist & Remes, 2004). Females can invest more resources per egg (such as higher yolk content) at the cost of a reduced clutch size, and differences in investment among females can be interpreted as an optimization in function of the different selecting forces that are active (Smith & Fretwell, 1974; Roff, 2002). For these reasons, great attention is given to the study of egg size effects on offspring in evolutionary ecology.

However, it is not easy to evaluate the effect of egg size *per se*. The egg-size-mediated maternal effects can be the result of both the direct effects of egg size, and of the covariation between genetic and environmental effects (Krist & Remes, 2004). For example, egg size can be related to morphological traits of the mother, and morphological traits are known to be highly heritable in many animals. Moreover, there is a possible covariation between egg size and the

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environment that the hatchlings encounter. For example, females living in a more favourable environment can lay larger eggs because of the greater resource availability, and the offspring living in a favourable environment can grow faster. Therefore, it is not easy to disentangle the effect of egg size from that of other maternal or environmental influences (Krist & Remes, 2004).

Krist & Remes (2004) proposed two methods for studying the effect of egg size on offspring performance independently from their covariation with the genetic influence of the mother. First, it is possible to analyse the relationship between egg size and performance among siblings. Given that autosomes segregate at random during meiosis, the covariation between egg size and genetic effects is assumed to be zero among siblings. Second, it is possible to experimentally manipulate the egg size, and thus to evaluate the effect of egg size independently from genetic or other maternal effects. In both methods, the covariation between egg size and post-hatchling environmental effects can be removed by cross-fostering or by rearing hatchlings under a common environment.

In amphibians, egg size correlates with many fitness-related traits, including tadpole survival, morphology, locomotory performance, development rate, and resistance to acidic conditions: larger eggs can enable the offspring to survive better or to metamorphose earlier (Räsänen *et al.*, 2003a, 2005; Dziminski & Alford, 2005; Kaplan & Phillips, 2006; Dziminski, Roberts & Simmons, 2008). Different females lay eggs of different sizes and provide different amounts of resources to the offspring. Differences in maternal investment are expected to affect fitness, and females may modulate their investment as a function of the environment that the offspring will encounter (Kaplan, 1992). Moreover, populations can differ with respect to their average egg size because of local adaptations to the environment (Räsänen *et al.*, 2003a, 2005). However, to date, most studies on the effects of egg size on tadpole performance have used the average egg size of each female as a measure of maternal investment, probably because of the technical difficulties associated with the individual manipulation of amphibian eggs (but see Dziminski & Alford, 2005; Räsänen *et al.*, 2005; Dziminski *et al.*, 2008; for manipulation of jelly capsules, see also Räsänen, Laurila & Merilä, 2003b; Marquis *et al.*, 2006). Using the average egg size of families does not allow studies to control for the covariation between egg size and other effects, either genetic or environmental (Krist & Remes, 2004). Approaches allowing the analysis of egg size independently from other confounding factors are needed to better understand the importance and the evolution of maternal effects in amphibians.

The present study aimed to evaluate the relationship between offspring size at hatching (used as a surrogate of egg size: for justification, see Material and methods) and larval survival in the Italian agile frog, *Rana latastei*. We performed our analyses at three levels. First, for comparison among populations, we evaluated whether populations show differences in starting size, and whether they are related to larval survival. Furthermore, previous studies have suggested that other factors (e.g. genetic and environmental) can have effects on egg size and survival. Egg size can be positively related to genetic diversity (Lesbarrères *et al.*, 2007), high altitude populations often lay larger eggs (Morrison & Hero, 2003), and larval survival of amphibians can be related to genetic diversity/heterozygosity (Beebe, 2005). Therefore, in the comparison among populations, we also tested these hypotheses.

Second, for comparison among families, we evaluated whether, among families, differences in average starting size cause differences in survival. If differences in starting size among populations or among families affect survival, we would expect a lower mortality in populations and families with a larger than average starting body size (SBS) (prediction 1). Comparisons among populations and among families do not allow for the evaluation of the effect of egg size independently from their covariation with genetic effects, or with other unmeasured maternal effects.

Finally, for within-family analysis, we evaluated whether differences in mortality between siblings are related to within-family differences in starting size. If within-family differences in starting size affect mortality, we could expect that, in families where some of the tadpoles died, dead tadpoles had smaller starting sizes (prediction 2), and that, in families where all the tadpoles died, the tadpoles having smaller starting sizes died first (prediction 3). These latter two analyses allow us to detect the effect of egg size independently from their covariation with genetic effects. The ultimate aim of the present study was to evaluate the relative importance of egg size *per se* on tadpole survival. If predictions 2 or 3 are confirmed, this would suggest that egg size has an effect on survival independently from their covariation with other effects (such as genetics). If prediction 1 is confirmed, but predictions 2 or 3 are not confirmed, then egg size does not have an effect independently from their covariation with other effects.

## MATERIAL AND METHODS

### STUDY SPECIES AND POPULATION SAMPLING

*Rana latastei* is a small brown frog endemic to floodplains of Northern Italy and adjacent countries. Each

**Table 1.** Descriptive statistics of starting size and mortality in tadpoles from the five localities

Population	Number of clutches (families)	Altitude (m)	Body length (mean $\pm$ SE) (mm)	Body width (mean $\pm$ SE) (mm)	Average mortality per family (mean $\pm$ SE)
AL	24	320	4.02 $\pm$ 0.07	2.45 $\pm$ 0.04	0.50 $\pm$ 0.16
CU	20	300	4.12 $\pm$ 0.07	2.52 $\pm$ 0.05	0.05 $\pm$ 0.05
MZ	36	175	4.28 $\pm$ 0.05	2.67 $\pm$ 0.03	0.14 $\pm$ 0.06
TC	29	69	4.21 $\pm$ 0.07	2.62 $\pm$ 0.05	0.17 $\pm$ 0.09
ZB	26	75	3.92 $\pm$ 0.06	2.49 $\pm$ 0.05	0.38 $\pm$ 0.13

Mortality was calculated as the average number of tadpoles per family that died before metamorphosis (range = 0–2).

amplectant pair lays a single egg mass (approximately 1300 eggs surrounded by gelatinous capsules) (Barbieri & Mazzotti, 2006). Multiple paternity in a clutch has not been reported for this species; therefore, each clutch is assumed to be a full-sib family. Some studies observed multiple paternity in related species (*Rana dalmatina* and *Rana temporaria*; Lodé & Lesbarrères, 2004; Vieites *et al.*, 2004); however, the offspring of polyandrous mating comprises a small proportion. For example, in *R. dalmatina*, up to 18% of clutches are sired by two males, and the secondary male sires only 24% of eggs (i.e. 4% of all eggs are sired by a secondary male) (Lodé & Lesbarrères, 2004). Similarly, in *R. temporaria*, up to 5% of all eggs are sired by a secondary male (Vieites *et al.*, 2004). These figures represent the highest known values of multiple paternity in related frogs. Even if multiple paternity affected some of our egg masses, no more than one or two families per population would include the offspring of multiple males. Furthermore, even in the case of multiple paternity, the eggs sired by each male would be in close proximity. Our sampling (see below) collected nearby eggs, further decreasing the possibility of sampling multiple paternity. The results obtained are robust to the exclusion of a few individuals from any population. Therefore, we consider that our results are robust with respect to multiple paternity.

Populations show adaptations in larval intrinsic development and growth rate to local climatic conditions (Ficetola & De Bernardi, 2005b; Ficetola & De Bernardi, 2006). We sampled five populations in Northern Italy. Three populations are on the foothills (AL, CU, MZ); two are in a lowland environment (TC, ZB) (Table 1); the among-sites distance is < 60 km, but significant genetic differences among populations are present (Ficetola, Garner & De Bernardi, 2007). For a map of localities, see Ficetola & De Bernardi (2005b). In mid-March 2004, we removed a small portion from each of the 20–36 recently-laid clutches per populations (Table 1). None of the sampled clutches showed signs of predation. The eggs were transferred into 200-mL plastic containers and

returned to the laboratory the next day. This sampling does not appear to influence the survival of collected eggs (Ficetola & De Bernardi, 2005a).

Population-level measures of genetic diversity are available for these five populations. As a measure of genetic diversity, we used the first factor extracted from a principal component analysis (PCA) of allelic richness, percentage of polymorphic loci, and observed heterozygosity, as measured at six microsatellite loci in 28–35 individuals per population (Ficetola *et al.*, 2007). Previous studies showed that this measure of genetic diversity is correlated with multiple measures of fitness in *R. latastei* (Pearman & Garner, 2005; Ficetola *et al.*, 2007). Genetic data have been obtained from samples collected in 2003–2004, and most likely reflect the situation experienced by the individuals that were used in the present study.

#### LABORATORY PROCEDURES

All clutch samples were reared under standard laboratory conditions (constant 20 °C with 12 : 12 h light/dark cycles). In the egg masses included in the present study, embryo development was normal and we did not detect mass mortality as a result of fungal infections. After hatching, on reaching stage 25 (free swimming tadpole: Gosner, 1960), we randomly chose two apparently healthy tadpoles per clutch. Up to stage 25, larvae do not feed; therefore, assuming the maintenance of equivalent environmental conditions, body size at stage 25 (i.e. SBS) is determined by the amount of yolk in the egg. Under constant environmental conditions, previous studies demonstrated a strong correlation between egg size and SBS in frogs belonging to the genus *Rana* (Laugen, Laurila & Merilä, 2003: Pearson's  $r = 0.91$ ) and, thus, starting size is frequently used as a proxy of egg size (Zeisset & Beebe, 2003; Räsänen *et al.*, 2005). In the present study, we used starting size as a surrogate of egg size because eggs are strictly clumped in masses in *R. latastei* (as in most anurans), and attempts to isolate the eggs individually results in the risk of damaging

embryos. Because the present study also aimed to observe the within-clutch differences, we used a method that enabled us to individually measure larvae. Therefore, we assume that differences in SBS indicate differences in egg size.

Tadpoles at stage 25 were photographed under a stereomicroscope to measure SBS, and individually reared in plastic containers filled with 200 mL of aged tap water. All 270 containers (corresponding to two tadpoles from each of 135 full-sib families) were randomly sorted over the same bench of the laboratory. The tadpoles were fed *ad libitum* with rabbit chow and lettuce. The water was changed weekly and tadpoles were monitored daily to record the occurrence of death. Sixty days after hatching, when the first tadpoles approached metamorphosis (Gosner's stage 39), the experiment was stopped. All the tadpoles were released into their wetland of origin.

#### STATISTICAL ANALYSIS

For each tadpole, we measured body length and body width on the basis of photographs (Altig, 2007). All measurements were made by the same individual. Body length and body width were strongly related ( $r = 0.840$ ,  $N = 270$ ,  $P < 0.0001$ ); thus, we used a PCA to replace them with a single factor. The extracted factor (eigenvalue = 1.840) was strongly related to both body length and body width (both  $r = 0.959$ ); thereafter, we refer to this factor as the SBS. As a measure of survival, for each family, we used the number of dead tadpoles at the end of the experiment.

For among-clutches analysis, we used the average SBS of siblings as a measure of family starting size. We used analysis of variance (ANOVA) to determine whether SBS differed among populations. We then used mixed models, considering population of origin as a random factor, to evaluate whether the among-populations differences were related to differences in altitude or in genetic diversity. We used multinomial log-linear models, followed by post-hoc logistic regression, to test whether the number of dead tadpoles (0, 1 or 2) per family was related to SBS and population identity (prediction 1). Significance of multinomial and logistic regressions was tested using likelihood-ratio tests (Venables & Ripley, 2002).

For the within-clutch analysis, we used mixed models to determine whether the dead tadpoles were smaller relative to their surviving siblings (prediction 2), or whether the smallest tadpoles died before their siblings (prediction 3). Family and population identity were included in models as random factors. Within-clutch analysis was possible only for families in which at least one tadpole died. Residual plots were inspected to detect deviations from the model assumptions (normality, homoscedasticity or indepen-

dence of residuals); we did not find strong violations of assumptions. A visual inspection of plots demonstrated that our models were not influenced by outliers. The three predictions were tested using datasets including different sample sizes. Differences in results among analyses might be caused by differences in power; therefore, we also compared effect size of different analyses (Steidl, Hayes & Schaubert, 1997). We performed all the statistical analyses using SPSS, version 13.0 (SPSS Inc.) and R, version 2.2 (<http://www.r-project.org>).

## RESULTS

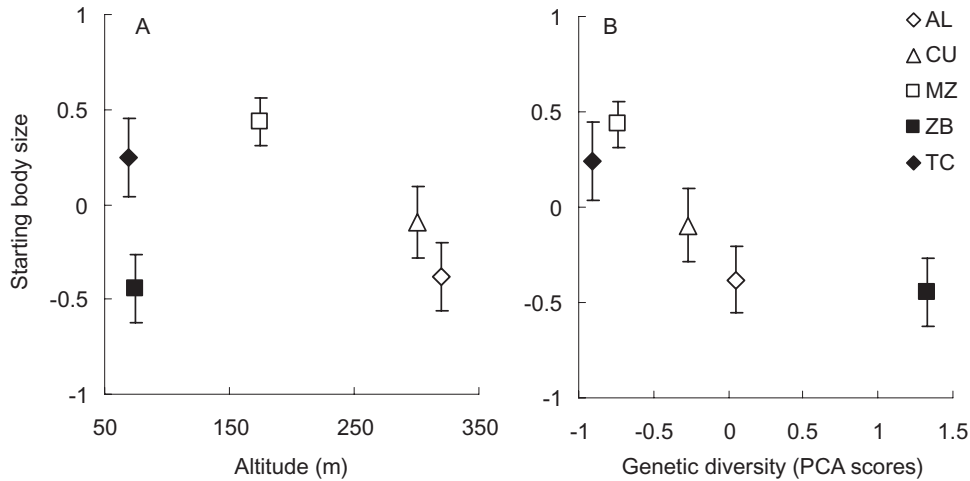
#### ANALYSIS AMONG POPULATIONS

SBS was significantly different among populations (ANOVA:  $F_{4,130} = 5.319$ ,  $P = 0.0005$ ; Fig. 1, Table 1). Differences in SBS among sites were not significantly related to altitude (mixed model:  $F_{1,3} = 0.188$ ,  $P = 0.693$ ; effect of population identity as random factor:  $F_{3,130} = 6.711$ ,  $P = 0.0003$ ) and high elevation populations did not have the largest SBS. For example, the population at the highest elevation (AL) was among the populations with the smallest SBS (Fig. 1A). Contrary to the expectations, populations with the highest genetic diversity tended to have the smallest SBS (Fig. 1B), although the trend was not significant ( $F_{1,3} = 8.075$ ,  $P = 0.07$ ; effect of population identity:  $F_{3,130} = 1.893$ ,  $P = 0.134$ ).

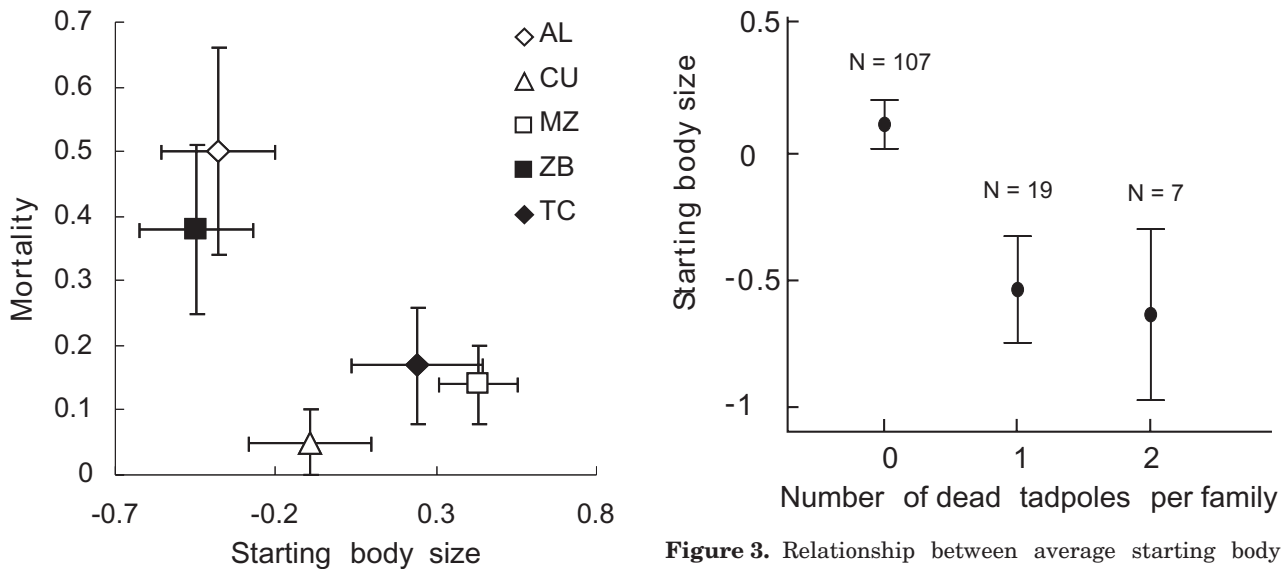
Out of 270 tadpoles, 33 (12.2%) died before the end of the experiment. In 19 families, one tadpole died; in seven families, both tadpoles died. Larval mortality tended to be different across populations (multinomial regression:  $\chi^2 = 15.122$ , d.f. = 8,  $P = 0.056$ ). The populations with higher mortality were AL and ZB, those having tadpoles with smaller starting size (Fig. 2, Table 1). We found no relationship between genetic diversity and averaged population level larval mortality (mixed model:  $F_{1,3} = 1.962$ ,  $P = 0.260$ ; effect of population identity:  $F_{3,130} = 2.463$ ,  $P = 0.065$ ).

#### ANALYSIS AMONG FAMILIES

Within populations, families suffering higher mortality were those having smaller SBS (multinomial regression:  $\chi^2 = 6.506$ , d.f. = 2,  $P = 0.038$ ). After taking into account the effect of SBS, among-population differences were nonsignificant ( $\chi^2 = 11.994$ , d.f. = 8,  $P = 0.151$ ). Mortality increased as average SBS decreased among families (Fig. 3). Tadpoles from families in which at least one tadpole died were significantly smaller than those from families where no tadpole died (logistic regression:  $\chi^2 = 9.582$ , d.f. = 1,  $P = 0.002$ ), but tadpoles from families in which two tadpoles died were not significantly smaller than those from families in which a single tadpole died



**Figure 1.** Relationship between starting body size (first factor extracted using a principal component analysis) and altitude (A) and genetic diversity (B) in five populations of *Rana latastei*. Filled symbols, lowland populations; empty symbols, foothill populations. Error bars represent the SEM. PCA, principle component analysis.



**Figure 2.** Starting body size and larval mortality in *Rana latastei* tadpoles from five populations. Filled symbols, lowland populations; empty symbols, foothill populations. Error bars represent the SEM.

( $\chi^2 = 0.065$ , d.f. = 1,  $P = 0.798$ ). The effect size of SBS in the among-clutches analysis was 2.947.

#### WITHIN-CLUTCH ANALYSIS

We observed within-clutch variability in SBS. Siblings differed for both body length (range of between siblings differences = 0.06–23.7%; mean = 4.16%) and body width (range = 0.09–26.0%; mean = 5.50%). Differences in size between siblings were in the same order of magnitude as the differences among popula-

**Figure 3.** Relationship between average starting body size and larval mortality in *Rana latastei* tadpoles from 135 clutches. Error bars represent the SEM; sample size is different among the three groups (indicated above the error bars).

tions (Table 1). However, we did not observe significant relationships between survival and within-clutch variation in starting size. In the families where only one tadpole survived, the surviving tadpole was not significantly larger than its dead sib ( $F_{1,18} = 0.021$ ,  $P = 0.887$ ; effect of population identity:  $F_{4,14} = 4.016$ ,  $P = 0.023$ ; family identity:  $F_{14,18} = 2.760$ ,  $P = 0.022$ ). Out of 19 families where only one tadpole survived, in ten cases, it was the larger tadpole that was the only the survivor.

Moreover, in families where both tadpoles died, the tadpoles that died first were not significantly

smaller ( $F_{1,5} = 0.034$ ,  $P = 0.861$ ; population identity:  $F_{2,3} = 1.570$ ,  $P = 0.341$ ; family identity:  $F_{3,5} = 20.866$ ,  $P = 0.003$ ). Out of seven families where both tadpoles died, in two cases, the larger tadpole died first, in four cases, the smaller tadpole died first and, in one case, both tadpoles died the same day.

For both the within-clutch analyses, the effect size of the relationship between SBS and survival was very low (families where one tadpole survived: effect size = 0.145; families where both tadpoles died: effect size = 0.185).

## DISCUSSION

The results obtained in the present study are in agreement with the idea that egg size (i.e. as measured by a surrogate, SBS) can affect the survival of tadpoles. Populations demonstrate significant differences in egg size, and survival tended to be higher in populations having larger eggs. Similarly, survival was higher in families having larger average egg sizes. However, in the present study, the lack of within-clutch effects suggests that the influence of egg size on survival cannot be considered independently from other effects. The covariation between egg size and other effects (either genetic or maternal nongenetic ones) most likely explains the relationship that we observed between egg size and survival.

A possible limitation of our study is the smaller sample size of the within-clutch analysis compared to the between-clutches analysis. However, the lack of significance of the within-clutch analysis cannot be explained by a reduced statistical power alone. First, the effect size of the analysis among families was >15-fold greater than that of the within-clutch analysis. Moreover, in the within-clutch analysis, differences among populations and among families remained significant, whereas we did not observe any tendency for tadpoles hatching from smaller eggs to die more frequently or prior to their siblings. For example, in the pairs where only one tadpole died, the proportion of cases in which the larger tadpole died (47.4%) was almost identical to the proportion of cases in which the smaller one died (52.6%). This suggests that the lack of significance of the within-clutch analysis is more likely caused by the lack of a biological effect rather than by a lack of power.

The effect of starting size on family-wide survival was similar to that of average starting size on population-specific survival (Figs 2, 3): small SBS was related to reduced survival. However, none of the within-clutch analyses showed a significant effect, suggesting that the apparent role of egg size on mortality could be explained by their covariation with other fitness-related traits, either genetic or environmental. First, it is possible that SBS covaries with

genetic features of the mother and, in turn, these genetic effects may affect survival (Krist & Remes, 2004). Furthermore, starting size might be affected by environmental factors that are experienced early in development by embryos, such as the presence of fungal infections (Warkentin, Currie & Rehner, 2001). We collected eggs soon after laying to minimize this possibility; nevertheless, unwanted environmental effects are always possible when working with wild populations. Finally, egg size is only one component of the possible nongenetic maternal influences on offspring. Studies investigating the role of maternal environmental effects not related to egg size are scarce in amphibians (Kaplan, 1998; Laugen, Laurila & Merilä, 2002). In *Rana arvalis*, among-population differences exist in the structure of jelly capsules of eggs, and these differences result in differential survival under acidic conditions (Räsänen *et al.*, 2003a). In *R. temporaria*, egg size explains only a small portion of variability in larval traits, whereas maternal identity is important to larval performance, suggesting that maternal effects can be largely independent of egg size (Pakkasmaa, Merilä & O'Hara, 2003).

Studies on other oviparous organisms showed that females can provide compounds such as hormones and antioxidants to offspring, influencing important fitness-related traits. They can also modulate the hormone and antioxidant provisioning in function of extrinsic factors. However, these studies have been mainly limited to birds and, to a lesser extent, reptiles (Gil *et al.*, 1999; Janzen *et al.*, 2002; Lovern & Wade, 2003; Saino *et al.*, 2004; Groothuis *et al.*, 2005).

Despite existing evidence that maternal effects other than egg size can be important in amphibians (Pakkasmaa *et al.*, 2003; Räsänen *et al.*, 2003b; present study), to date, the mechanisms mediating maternal influence are not well understood. The present study hints towards the possibility that there might be covariation between egg size and other maternal effects, allowing the formulation of hypotheses on the underlying mechanisms. In amphibians, egg size is frequently related to female body condition or body size, and body size is usually related to age (Kaplan, 1998; Morrison & Hero, 2003; Weddeling *et al.*, 2005). Therefore, it is possible that females that are older, or are in good condition, lay eggs that have a better quality in terms of yolk content or other compounds important for the offspring. This mechanism is only one example of the many possible pathways explaining the higher survival of larger eggs caused by the covariation between egg size and other maternal effects.

Maternal effects have the potential to generate rapid phenotypic changes, thus enhancing the possibility of rapid adaptation and evolution (Räsänen &

Kruuk, 2007). We therefore stress the importance of studies on a wider range of organisms, for a more complete understanding of these mechanisms and of their evolutionary consequences.

We observed significant differences in SBS among populations divided by small geographical distances and small differences in altitude. In amphibians, populations living at higher altitudes frequently lay larger eggs at the cost of a smaller number of eggs per clutch. The relationship between altitude and egg size is believed to be adaptive because large egg size can increase survival in more stressful, cold environments (Morrison & Hero, 2003). However, our data do not align to this prediction. For example, average SBS was significantly larger in population MZ than in populations living at higher altitudes (Fig. 1A, Table 1). Despite the small absolute differences in altitude, our sites encompass most of the altitudinal range of *R. latastei* (Barbieri & Mazzotti, 2006). The lack of relationship is puzzling because differences in climatic conditions are strong enough to cause variation among populations for intrinsic development rate (Ficetola & De Bernardi, 2005b). Indeed, despite the pattern of increasing egg size with altitude being well documented, there are several cases where differences have not been observed, suggesting that other factors can play a role (Morrison & Hero, 2003). A second expectation, not verified by this dataset, is the association of large starting size with high genetic diversity populations (Lesbarrères *et al.*, 2007). Indeed, the observed (nonsignificant) trend was contrary to the expectation because SBS tended to be smaller in populations with high genetic diversity (Fig. 1B). This may occur, for example, if females with high genetic diversity allocate resources toward clutches with more eggs (McAlpine, 1993).

The lack of a simple pattern suggests that more complex hypotheses are needed to understand the differences among sites in starting size. For example, differences in egg size are frequently related to differences in the size or body conditions of females (Kaplan, 1998; Laugen *et al.*, 2002). Morphometric data on mothers are not available, but considerable variation in the adult body size is known for *R. latastei*. Previous studies showed that the average size of breeding females is significantly larger in population MZ compared to other populations (Ficetola, Scali & De Bernardi, 2006) and this partially corresponds to the pattern of egg size (Fig. 1). Even though morphometric data were not collected in the same years as eggs, the average body size in populations can reflect variation of genetic features, age or climatic conditions among populations (Rosso, Castellano & Giacoma, 2004; Laugen *et al.*, 2005). If the factor(s) responsible for the morphometric variation are still active and lead to differences in female size,

they might at least partially explain the variation in SBS among populations. Differences in egg size could also be interpreted as adaptations to differences among sites not investigated in the present study, such as pH and the risk of predation (Räsänen *et al.*, 2003a, 2005; Van Buskirk & Arioli, 2005), but none of the study populations breed in acidic ponds (G. F. Ficetola, unpublished data). Furthermore, the limited number of populations investigated reduces the possibility of observing significant relationships if they are not clear-cut.

Most importantly, the effects of egg size and starting size on later performance are frequently context dependent (Parichy & Kaplan, 1992; Pakkasmaa *et al.*, 2003; Räsänen *et al.*, 2005; Kaplan & Phillips, 2006). When trying to maximize fitness, females can face a trade-off between egg size and clutch size, and the best combination of egg size and egg number may vary with environmental conditions (Roff, 2002). Therefore, egg size could depend on complex interactions among resource availability, environmental conditions, and optimal allocation.

In summary, the among-population differences in hatchling size observed in the present study were related to differences in survival. However, the strong interpopulation differences cannot be explained by a simple gradient of altitude or genetic diversity. Moreover, our data suggest that the relationship between egg size and survival, as observed among clutches, may indeed be caused by covariation between egg size and other effects. These two results do not fit the two patterns generally hypothesized for amphibians (i.e. the 'altitude-egg size correlation' and the 'egg size as a main maternal effect'), thus offering opportunities for future studies.

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#### REFERENCES

- Altig R.** 2007. A primer for the morphology of anuran tadpoles. *Herpetological Conservation And Biology* **2**: 71–74.
- Barbieri F, Mazzotti S.** 2006. *Rana latastei* Boulenger, 1879. In: Sindaco R, Doria G, Razzetti E, Bernini F, eds. *Atlas of Italian Amphibians and reptiles*. Firenze: Polistampa, 362–367.
- Beebee TJC.** 2005. Conservation genetics of amphibians. *Heredity* **95**: 423–427.

- Dziminski MA, Alford RA. 2005.** Patterns and fitness consequences of intraclutch variation in egg provisioning in tropical Australian frogs. *Oecologia* **146**: 98–109.
- Dziminski MA, Roberts JD, Simmons LW. 2008.** Fitness consequences of parental compatibility in the frog *Crinia georgiana*. *Evolution* **62**: 879–886.
- Ficetola GF, De Bernardi F. 2005a.** Interspecific social interactions and breeding success of the frog *Rana latastei*: a field study. *Ethology* **111**: 764–774.
- Ficetola GF, De Bernardi F. 2005b.** Supplementation or *in situ* conservation? Evidence of local adaptation in the Italian agile frog *Rana latastei* and consequences for the management of populations. *Animal Conservation* **8**: 33–40.
- Ficetola GF, De Bernardi F. 2006.** Trade-off between larval development rate and post-metamorphic traits in the frog *Rana latastei*. *Evolutionary Ecology* **20**: 143–158.
- Ficetola GF, Garner TWJ, De Bernardi F. 2007.** Genetic diversity, but not hatching success, is jointly affected by post glacial colonization and isolation in the threatened frog, *Rana latastei*. *Molecular Ecology* **16**: 1787–1797.
- Ficetola GF, Scali S, De Bernardi F. 2006.** The effects of isolation on fitness and morphometry: *Rana latastei* within the Monza Park. In: Zuffi MAL, ed. *Atti del Vconvegno nazionale Societas Herpetologica Italica*. Firenze: Firenze University Press, 105–112.
- Gil D, Graves J, Hazon N, Wells A. 1999.** Male attractiveness and differential testosterone investment in zebra finch eggs. *Science* **286**: 126–128.
- Gosner KL. 1960.** A simplified table for staging Anuran embryos and larvae with notes on identification. *Herpetologica* **16**: 183–190.
- Groothuis TGG, Muller W, von Engelhardt N, Carere C, Eising C. 2005.** Maternal hormones as a tool to adjust offspring phenotype in avian species. *Neuroscience and Biobehavioral Reviews* **29**: 329–352.
- Janzen FJ, Wilson ME, Tucker JK, Ford SP. 2002.** Experimental manipulation of steroid concentrations in circulation and in egg yolks of turtles. *Journal of Experimental Zoology* **293**: 58–66.
- Kaplan RH. 1992.** Greater maternal investment can decrease offspring survival in the frog *Bombina orientalis*. *Ecology* **73**: 280–288.
- Kaplan RH. 1998.** Maternal effects, developmental plasticity, and life history evolution. An amphibian model. In: Mousseau TA, Fox CW, eds. *Maternal effects as adaptations*. New York, NY: Oxford University Press, 244–260.
- Kaplan RH, Phillips PC. 2006.** Ecological and developmental context of natural selection: maternal effects and thermally induced plasticity in the frog *Bombina orientalis*. *Evolution* **60**: 142–156.
- Krist M, Remes V. 2004.** Maternal effects and offspring performance: in search of the best method. *Oikos* **106**: 422–426.
- Laugen AT, Laurila A, Merilä J. 2003.** Latitudinal and temperature-dependant variation in embryonic development and growth in *Rana temporaria*. *Oecologia* **135**: 548–554.
- Laugen AT, Laurila A, Jonsson KI, Soderman F, Merilä J. 2005.** Do common frogs (*Rana temporaria*) follow Bergmann's rule? *Evolutionary Ecology Research* **7**: 717–731.
- Laugen AT, Laurila A, Merilä J. 2002.** Maternal and genetic contribution to geographical variation in *Rana temporaria* larval life-history traits. *Biological Journal of the Linnean Society* **76**: 61–70.
- Lesbarrères D, Schmeller DS, Primmer CR, Merilä J. 2007.** Genetic variability predicts common frog (*Rana temporaria*) size at metamorphosis in the wild. *Heredity* **99**: 41–46.
- Lodé T, Lesbarrères D. 2004.** Multiple paternity in *Rana dalmatina*, a monogamous territorial breeding anuran. *Naturwissenschaften* **91**: 44–47.
- Lovern MB, Wade J. 2003.** Sex steroids in green anoles (*Anolis carolinensis*): uncoupled maternal plasma and yolk follicle concentrations, potential embryonic steroidogenesis, and evolutionary implications. *General and Comparative Endocrinology* **134**: 109–115.
- McAlpine S. 1993.** Genetic heterozygosity and reproductive success in the green treefrog, *Hyla cinerea*. *Heredity* **70**: 553–558.
- Marquis O, Millery A, Guittonneau S, Miaud C. 2006.** Toxicity of PAHs and jelly protection of eggs in the common frog *Rana temporaria*. *Amphibia-Reptilia* **27**: 472–475.
- Moran AL, Emlet RB. 2001.** Offspring size and performance in variable environments: field studies on a marine snail. *Ecology* **82**: 1597–1612.
- Morrison C, Hero JM. 2003.** Geographic variation in life-history characteristics of amphibians: a review. *Journal of Animal Ecology* **72**: 270–279.
- Mousseau TA, Fox CW. 1998.** *Maternal effects as adaptations*. New York, NY: Oxford University Press.
- Olsen EM, Vollested LA. 2003.** Microgeographical variation in brown trout reproductive traits: possible effects of biotic interactions. *Oikos* **100**: 483–492.
- Pakkasmaa S, Merilä J, O'Hara RB. 2003.** Genetic and maternal effect influences on viability of common frog tadpoles under different environment conditions. *Heredity* **91**: 117–124.
- Parichy DM, Kaplan RH. 1992.** Maternal effects on offspring growth and development depend on environmental quality in the frog *Bombina orientalis*. *Oecologia* **91**: 579–586.
- Pearman PB, Garner TWJ. 2005.** Susceptibility of Italian Agile Frog populations to an emerging *Ranavirus* parallels population genetic diversity. *Ecology Letters* **8**: 401–408.
- Räsänen K, Kruuk LEB. 2007.** Maternal effects and evolution at ecological time-scales. *Functional Ecology* **21**: 408–421.
- Räsänen K, Laurila A, Merilä J. 2003a.** Geographic variation in acid stress tolerance of the moor frog, *Rana arvalis*, I. Local adaptation. *Evolution* **57**: 352–362.
- Räsänen K, Laurila A, Merilä J. 2003b.** Geographic variation in acid stress tolerance of the moor frog, *Rana arvalis*, II. Adaptive maternal effects. *Evolution* **57**: 363–371.
- Räsänen K, Laurila A, Merilä J. 2005.** Maternal investment in egg size: environment- and population-specific effects on offspring performance. *Oecologia* **142**: 546–553.



- Roff DA. 2002.** *Life history evolution*. Sunderland, MA: Sinauer Associates.
- Rosso A, Castellano S, Giacoma C. 2004.** Ecogeographic analysis of morphological and life-history variation in the Italian treefrog. *Evolutionary Ecology* **18**: 303–321.
- Saino N, Romano M, Ambrosini R, Ferrari RP, Moller AP. 2004.** Timing of reproduction and egg quality covary with temperature in the insectivorous Barn Swallow, *Hirundo rustica*. *Functional Ecology* **18**: 50–57.
- Smith CC, Fretwell SD. 1974.** The optimal balance between size and number of offspring. *The American Naturalist* **108**: 499–506.
- Steidl RJ, Hayes JP, Schaubert E. 1997.** Statistical power analysis in wildlife research. *Journal of Wildlife Management* **61**: 270–279.
- Van Buskirk JV, Arioli M. 2005.** Habitat specialization and adaptive phenotypic divergence of anuran populations. *Journal of Evolutionary Biology* **18**: 596–608.
- Venables WN, Ripley BD. 2002.** *Modern applied statistics with S*. New York, NY: Springer.
- Vieites DR, Nieto-Roman S, Barluenga M, Palanca A, Vences M, Meyer A. 2004.** Post-mating clutch piracy in an amphibian. *Nature* **431**: 305–308.
- Warkentin KM, Currie CR, Rehner SA. 2001.** Egg-killing fungus induces early hatching of red-eyed treefrog eggs. *Ecology* **82**: 2860–2869.
- Weddeling K, Bosbach G, Hatchel M, Sander U, Schmidt P, Tarkhnishvili D. 2005.** Egg size versus clutch size: variation and trade-offs in reproductive output of *Rana dalmatina* and *R. temporaria* in a pond near Bonn (Germany). In: Ananjeva N, Tsinenko O, eds. *Herpetologia Petropolitana. Proc. of the 12th Ordinary General Meeting of the Societas Europaea Herpetologica, August 12–16, 2003*. St. Petersburg: Societas Europaea Herpetologica, 238–240.
- Zeisset I, Beebee TJC. 2003.** Population genetics of a successful invader: the marsh frog *Rana ridibunda* in Britain. *Molecular Ecology* **12**: 639–646.