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

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Running title:

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Abstract

Egg size is considered a major maternal effect for the 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*, since egg size can covary with genetic features of the mother and with other non genetic factors. We analyzed the relationship between offspring starting size (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 *per se*. 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 need further investigation.

Key words: altitudinal gradient; amphibian; egg size; intraspecific variation; maternal effects; microsatellites.
INTRODUCTION

Non-genetic 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 & Emlet, 2001). Egg size can influence many life history traits, therefore differences between populations in egg size has been frequently interpreted as adaptations to environmental differences among sites (Morrison & Hero, 2003; Olsen & Vollested, 2003; Räsänen, Laurila & Merilä, 2003a; Räsänen, Laurila & Merilä, 2005). Moreover, life history theory predicts a trade-off between offspring number and quality (Krist & Remes, 2004). That is, 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 optimisation in function of different selecting forces 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 instance, egg size can be related to morphological traits of the mother, and morphological traits are known to be highly heritable in many animals. Moreover, a covariation is possible between the egg size and the environment 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 and Remes (2004) proposed two methods to study 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; Dziminski & Alford, 2005; Räsänen et al., 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 in function of the environment the offspring will encounter (Kaplan, 1992). Moreover, populations can differ in their average egg size because of local adaptations to the environment (Räsänen et al., 2003a; Räsänen et al., 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 to the individual manipulation of amphibian eggs (but see Dziminski & Alford, 2005; Räsänen et al., 2005; Dziminski et al., 2008; see also Räsänen, Laurila & Merilä, 2003b; Marquis et al., 2006 for manipulation of the jelly capsules). Using the average egg size of families does not allow 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 aim of this study was to evaluate the relationship between offspring size at hatching (used as a surrogate of egg size: see methods for justification) and larval survival in the Italian agile
frog, *Rana latastei*. We performed our analyses at three levels. First (*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 (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 negatively related to genetic diversity/heterozygosity (Beebee, 2005). Therefore, in the comparison among populations, we also tested these hypotheses.

Second (*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 larger average starting body size (prediction 1). The comparisons among populations and among families does 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 (*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, within families when some of the tadpoles died, dead tadpoles had smaller starting sizes (prediction 2), and that, in families in which all 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 this 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 none of predictions 2 or 3 are confirmed, than egg size does not have an effect independently from their covariation with other effects.
**MATERIALS AND METHODS**

**STUDY SPECIES AND POPULATION SAMPLING**

*Rana latastei* is a small brown frog endemic to floodplains of Northern Italy and adjacent countries. Each amplexant pair lays a single egg mass (about 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 (*R. dalmatina* and *R. 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 values of multiple paternity known in related frogs. Even if multiple paternity affected some of our egg masses, no more than 1-2 families per population would include the offspring of multiple males. Furthermore, even in 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. Our results are robust to the exclusion of a few individuals from any population. Therefore, we believe that our results would be robust 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); among sites distance is < 60km, but significant genetic differences among populations are present (Ficetola, Garner & De Bernardi, 2007). See Ficetola and De Bernardi (2005b) for a map of localities. In mid March 2004, we removed a small portion from
each of 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 seem 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 of allelic richness, percentage of polymorphic loci and observed heterozygosity, 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 therefore probably reflect the situation of individuals used by the present study.

LABORATORY PROCEDURES

All clutch samples were reared under standard laboratory conditions (constant 20°C with 12-h dark-light cycles). In the egg masses included in this study, embryo development was normal and we did not detect mass mortality caused by fungal infections. After hatching, on reaching stage 25 (free swimming tadpole: (Gosner, 1960), we haphazardly chose two apparently healthy tadpoles per clutch. Up to stage 25 larvae do not feed, therefore, keeping equal environmental conditions, body size at stage 25 (thereafter “starting body size”) is mainly determined by the amount of yolk in the egg. Under constant environmental conditions, previous studies showed a strong correlation between egg size and starting body size in frogs belonging to the genus Rana (e.g., Laugen et al., 2003: Pearson’s $r = 0.91$), and thus starting size is frequently used as a proxy of egg size (Zeisset & Beebee, 2003; Räsänen et al., 2005). In our study, we used starting size as a surrogate of egg size since in R. latastei (as in most anurans) eggs are strictly clumped in masses, and attempts to isolate
individually the eggs result in the risk of damaging embryos. Since the aim of this study was to observe also the within-clutch differences, we used a method that enabled us to individually measure larvae. Therefore, throughout the text, we assume that differences in starting body size indicate differences in egg size.

Tadpoles at stage 25 were photographed under a stereomicroscope for measurement of starting body size, and individually reared in plastic containers filled with 200ml 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 in their wetland of origin.

**DATA ANALYSIS**

For each tadpole, we measured body length and body width on the basis of pictures (Altig, 2007). All measurements were made by the same person. Body length and body width were strongly related (*r = 0.840, N = 270, P < 0.0001*), thus we used a Principal Component Analysis (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 call this factor starting body size (SBS). As a measure of survival, for each family we used the number of dead tadpoles at the end of the experiment.

In the among clutches analyses, we used the average SBS of siblings as a measure of family starting size. We used analysis of variance (ANOVA) to evaluate if 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).

In the within-clutch analysis, we used mixed models to evaluate 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 independence of residuals); we did not find strong violations of assumptions. Visual inspection of plots showed 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 & Schauber, 1997). We performed statistical analyses using SPSS 13.0 and R 2.2 (www.r-project.org).

RESULTS

ANALYSIS AMONG POPULATIONS

Starting body size was significantly different among populations (ANOVA: $F_{4,130} = 5.319$, $P = 0.0005$; Table 1; Fig. 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), but 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 7 families both tadpoles died. Larval mortality tended to be different across populations (multinomial regression: $\chi^2_8 = 15.122$, $P = 0.056$). The populations with higher mortality were AL and ZB, those having tadpoles with smaller starting size (Table 1, Fig. 2). 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 starting body size (multinomial regression: $\chi^2_2 = 6.506$, $P = 0.038$). After taking into account the effect of SBS, among population differences were non significant ($\chi^2_8 = 11.994$, $P = 0.151$). Mortality increased as average starting body size 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: $\chi^2_1 = 9.582$, $P = 0.002$), but tadpoles from families in which two tadpoles died were not significantly smaller than those from families in which a single tadpole died ($\chi^2_1 = 0.065$, $P = 0.798$). The effect size of SBS in the among-clutches analysis was 2.947.

**WITHIN-CLUTCH ANALYSIS**

We observed within-clutch variability in starting body size. Siblings differed for both body length (range of between siblings differences: 0.06% - 23.7%; average: 4.16%) and body width (range: 0.09% - 26.0%; average: 5.50%). Differences in size between siblings were in the same order of magnitude as the differences among populations (see 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 10 cases it was the larger tadpole that was the only 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 7 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**

Our results align to the idea that egg size (here measured by a surrogate, starting body size) can affect the survival of tadpoles. Populations show 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 our study case, the lack of within-clutch effects suggest that the influence of egg size on survival can not be considered independently from other effects. The covariation between egg size and other effects (either genetic or maternal non genetic ones) is a likely explanation for the relationship we observed between egg size and survival.

A possible limitation of our study is the smaller sample size of the within-clutch analysis, if compared to the between clutches analysis. However, the lack of significance of the within-clutch analysis cannot be explained only by reduced statistical power. First, the effect size of the analysis
among families was $> 15$ times larger than that of the within-clutch analysis. Moreover, in the within-clutch analysis, differences among populations and among families remained significant, while we did not observe any tendency of tadpoles hatched 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 lack of biological effect rather than by lack of power.

The effect of starting size on family-wide survival was similar to that of average starting size on population-specific survival (Fig. 2, Fig. 3): small starting body size 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 starting body size 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 experienced early by embryos, such as the presence of fungal infections (Warkentin, Currie & Rehner, 2001). We collected eggs soon after laying to minimize this possibility; nevertheless, environmental unwanted effects are always possible when working with wild populations. Finally, egg size is only one component of the possible non-genetic 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 while maternal identity is important to larval performance, suggesting that maternal effects can be largely independent from 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. Yet, these studies have been mainly limited to birds and, to a lesser extent, to 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; this study), to date the mechanisms mediating maternal influence are not well understood. Our work hints towards the possibility that there might be covariation between egg size and other maternal effects, allowing to formulating 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, Bosbach, Hatchel, Sander, Schmidt & Tarkhnishvili, 2005). Therefore, it is possible that females that are older or are in good condition lay eggs which 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, since 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 (Table 1, Fig. 1A). 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, as differences in climatic conditions are
strong enough to cause variation among populations for intrinsic development rate (Ficetola & De
Bernardi, 2005b). Indeed, despite that the pattern of increasing egg size with altitude is 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 that expectation, as SBS
tended to be smaller in populations with high genetic diversity (Fig. 1B). This may occur, for
instance, 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 than in the other populations (Ficetola, Scali & De
Bernardi, 2006): 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) causing the
morphometric variation are still active and cause 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 by our study, such as pH and
risk of predation (Räsänen *et al.*, 2003a; Räsänen *et al.*, 2005; Van Buskirk & Arioli, 2005), but
none of the study populations breed in acidic ponds (G.F.F. unpublished data). Furthermore, the limited number of populations investigated reduces the possibility to observe significant relationships if they are not clear-cut.

Most importantly, the effects of egg size and starting size on later performance is frequently context dependent (Parichy & Kaplan, 1992; Pakkasmaa et al., 2003; Räsänen et al., 2005; Kaplan & Phillips, 2006). When trying to maximise 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 resources availability, environmental conditions and optimal allocation.

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

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REFERENCES


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

Mortality was calculated as the average number of tadpoles per family which died before metamorphosis (range: 0-2).

<table>
<thead>
<tr>
<th>Population</th>
<th>N of clutches (families)</th>
<th>Altitude (m)</th>
<th>Body length ± SE (mm)</th>
<th>Body width ± SE (mm)</th>
<th>Average mortality per family ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL</td>
<td>24</td>
<td>320</td>
<td>4.02 ± 0.07</td>
<td>2.45 ± 0.04</td>
<td>0.50 ± 0.16</td>
</tr>
<tr>
<td>CU</td>
<td>20</td>
<td>300</td>
<td>4.12 ± 0.07</td>
<td>2.52 ± 0.05</td>
<td>0.05 ± 0.05</td>
</tr>
<tr>
<td>MZ</td>
<td>36</td>
<td>175</td>
<td>4.28 ± 0.05</td>
<td>2.67 ± 0.03</td>
<td>0.14 ± 0.06</td>
</tr>
<tr>
<td>TC</td>
<td>29</td>
<td>69</td>
<td>4.21 ± 0.07</td>
<td>2.62 ± 0.05</td>
<td>0.17 ± 0.09</td>
</tr>
<tr>
<td>ZB</td>
<td>26</td>
<td>75</td>
<td>3.92 ± 0.06</td>
<td>2.49 ± 0.05</td>
<td>0.38 ± 0.13</td>
</tr>
</tbody>
</table>
Figure 1. Relationship between starting body size (first factor extracted using a Principal Component Analysis) and (A) altitude and (B) genetic diversity in five populations of *Rana latastei*. Filled symbols: lowland populations; empty symbols: foothill populations. Error bars represent standard errors of the mean.
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 standard errors of the mean.
Figure 3. Relationship between average starting body size and larval mortality in *R. latastei* tadpoles from 135 clutches. Error bars represent standard errors of the mean; sample size is different among the three groups (indicated above the error bars).