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ORIGINAL PAPER

Morphological differentiation among populations of *Rhinella marina* (Amphibia: Anura) in western Mexico

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Abstract Conspecific populations inhabiting different environments may exhibit morphological differences, potentially reflecting differential local adaptation. In anuran amphibians, morphology of the pelvis and hindlimbs may often experience strong selection due to effects on locomotion. In this study, we used the cane toad *Rhinella marina* to test the hypothesis that populations experiencing a higher abundance of predators should suffer higher mortality rates and exhibit morphological traits associated with enhanced locomotor performance (narrower pelvis and head, longer pelvis and hindlimbs, shorter presacral vertebral column). We investigated inter-population variation in survival rate, abundance of predators, and body shape across five populations in rivers in western Mexico. We conducted (1) mark-recapture experiments to calculate survival rates, (2) linear transects with point counts to estimate abundance of predatory spiders, snakes, and birds, and (3) geometric morphometric analyses to investigate body shape variation. We found significant differences among populations in survival rates, abundance of predators, and body shape. However, these three variables were not necessarily inter-related. Increased predator abundance did not result in decreased survival rates, suggesting other causes of mortality affect these populations. While some morphological differences supported our predictions (trend for longer pelvis, shorter presacral vertebral column, and narrower head in sites with increased abundance of spiders and snakes), other aspects of morphology did not. We discuss alternative explanations for the lack of clear associations between predation, survival, and morphology.

Keywords Geometric morphometrics · Mark-recapture · Survival · Predation · Body shape

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Introduction

Predation represents an important driver of phenotypic variation among populations and species (e.g., Vamosi 2003; Langerhans et al. 2004; Langerhans 2006; Eklöv and Svanbäck 2006). Predators can alter selection on prey populations through lethal and non-lethal interactions, driving evolutionary change, phenotypic plasticity, or both (Walsh and Reznick 2009; Hossie et al. 2010; Scoville and Pfrender 2010). Predation can affect life-history traits such as the timing and size of maturation, the number and size of offspring, and the amount of energy invested in reproduction (Johnson and Belk 2001; Johnson and Zúñiga-Vega 2009; Reznick et al. 2012). Predators can also influence the evolution of behavior, coloration, physiology, and morphology of their prey (Putman and Wratten 1984; Lind and Cresswell 2005; Stoks et al. 2005; Eklöv and Svanbäck 2006). Many prey taxa exhibit morphological responses to predator presence that enhances their survivorship (e.g., DeWitt and Schneider 2004; Lind and Cresswell 2005; Steiner 2007). This phenomenon may often cause phenotypic differentiation among populations of the same species. For example, in populations with higher predation risk certain phenotypes are favored increasing their probability of surviving and reproducing in such high-mortality environments (DeWitt and Langerhans 2003; Teplitsky et al. 2005). On the contrary, in populations with fewer predators and consequently low mortality rates, selection may weaken on these phenotypes, or reverse in direction. The overall result is that individuals from different populations will differ on their average morphology (Dayton et al. 2005; Langerhans and Gifford 2009; Mobley et al. 2011).

For anurans on land, jumping represents a primary means of escaping predators (Zug 1972; Emerson 1978). Jumping ability is affected by the morphology of the hip and hindlimbs, the amount of extensor muscles, and the energy available for jumping (Emerson 1978). A long pelvis, long hindlimbs, and a shortened presacral vertebral column are the main phenotypic attributes associated with jumping, and appear to experience a selective pressure as a result of the intensity of mortality caused by predators (Emerson 1985; Chadwell et al. 2002). Individuals that are exposed to predators during the larval period develop longer forelimbs and hindlimbs and narrower heads and bodies compared to individuals that inhabit sites that are free of predators (Relyea 2001; Capellán and Nicieza 2007).

Skeletal structure of hindlimbs determines jump performance (Choi et al. 2003), with longer hindlimbs resulting in a greater distance of movement during the acceleration phase of take-off, increasing the peak speed (Choi et al. 2000). The ilium is an important attachment site of muscles of the abdominal wall and of the thigh (Duellman and Trueb 1996). It influences the mechanical factors for take-off posture (Choi et al. 2003). We then expect to find morphologies associated with narrow pelvis and head, long urostyle, short presacral vertebral column, and long hindlimbs (femur and tibiofibula) in environments with a higher abundance of predators. The putative cause of this expected association between morphology and predator abundance is an increased mortality rate in environments where predators are abundant, resulting in strong selection on morphological traits that enhance escape performance (Langerhans 2010).

Here we examined morphological variation among populations of the cane toad (*Rhinella marina*, Bufonidae) in western Mexico, and investigated associations between morphology, predator abundance, and mortality rate. We focused on two hypotheses. (1) Populations experiencing a higher abundance of predators should exhibit morphological traits presumably associated with enhanced locomotor performance (narrower pelvis and head, short presacral vertebral column, and longer urostyle and hindlimbs) compared to populations inhabiting environments with lower abundance of predators. (2) High predator abundance should correlate negatively with survival rates. By testing these two hypotheses we aim to provide evidence of an association between predation and morphological divergence while at the same time uncovering a putative ecological mechanism (predator-driven mortality) behind this association.

Materials and methods

Study system

Cane toads (*R. marina,* Linnaeus 1758) are large anurans native to Central and South America, but introduced worldwide (Lever 2001). In Mexico, this species is distributed in the coasts of the Pacific and the Gulf of Mexico (Zug and Zug 1979; López et al. 2009; Solís et al. 2009). This species has an aquatic stage (larva) and a terrestrial stage (post-metamorphic and adults; Zug and Zug 1979). Post-metamorphic individuals exhibit diurnal activity and are found on the edges of rivers mainly on sandy soils (Menin et al. 2008). Egg laying, hatching, larvae development, and metamorphosis occur from March to June (Zug and Zug 1979; López et al. 2009). Post-metamorphic individuals are abundant during these months before the rainy season (in western Mexico the rainy season begins in late June). In this study we focused exclusively on post-metamorphic individuals because adults disperse and move away from water bodies and are less abundant (Freeland and Kerin 1991; Child et al. 2008).

At Gosner stage 46 (when the tail is reabsorbed and metamorphosis is completed; Gosner 1960) newly metamorphosed bufonids form aggregations surrounding the ponds after emerging from water, and are highly susceptible to predation at this time (Arnold and Wassersug 1978). In fact, high mortality rates in terrestrial anurans occur when they develop in land after metamorphosis (Cohen and Alford 1993). In individuals of *R. marina* that just metamorphosed, the main causes of mortality are desiccation, abnormalities during development, starvation, and predation (Cohen and Alford 1993). Individuals during this stage are more active during the day exposing them to diurnal predators such as ants, spiders, reptiles, birds, mammals, and other amphibians including adult individuals of *R. marina* (Zug and Zug 1979; Freeland and Kerin 1991; Toledo 2005; Pizzatto and Shine 2008). As newly metamorphosed bufonids are very susceptible to predation, we therefore expected that this life stage would experience strong selection for traits associated with predator avoidance and escape.

Rhinella marina represents an appropriate taxon to investigate a link between predation, survival, and morphology during their post-metamorphic stage because jumping is likely the main escape strategy to avoid some of their main predators at this stage like spiders and snakes. We recognize that post-metamorphic toads might use additional anti-predator strategies such as crypsis. However, during our behavioral observations of these toads, we confirmed that approaches by spiders and snakes resulted in jumping responses. In addition, toxins are not involved in predator avoidance during the post-metamorphic stage of *R. marina* because toxins are lost during metamorphosis and regained again when they reach the juvenile stage (Zug and Zug 1979). Although the relative importance of the various anti-predator strategies employed by *R. marina* is unknown, we observed high frequencies of jumping behavior all throughout the day and night in most individuals. Hence, we expected strong selection on the morphology associated with jumping performance resulting from predation intensity.

We identified five localities in which newly metamorphosed cane toads were abundant in the states of Colima and Jalisco, Mexico. We worked in five rivers: (1) San Nicolás, (2) Cuixmala, (3) Marabasco, (4) Armería, (5) Coahuayana (Fig. 1). These sampling rivers represent different populations with no inland connection among them. To estimate survival rates, we conducted mark-recapture experiments during May and June of 2011. We sampled populations once a week over a five-week period. At each study site we delimited one 100 m-long portion of the river. In this study plot we captured as many toads as possible on each sampling occasion. Cane toads were toe-clipped following Martof (1953). Weekly sampling allowed us to obtain individualized encounter histories for each marked cane toad. We captured, measured [snout-vent length (SVL)], marked, and followed a total of 3,921 individuals of *R. marina* (Table 1). At each study site we observed several reproductive males and females. Therefore, we assumed that the post-metamorphic individuals that we studied at each site came from clutches of multiple females.

Survival rates

Our first analysis aimed to test for differences in survival rates among sites. We calculated survival rates by means of maximum-likelihood procedures implemented in the program MARK (White and Burnham 1999). We recognize that, in open populations, it is not possible to distinguish between actual mortality and permanent emigration. Hence, MARK estimates “apparent” survival instead of actual survival (Lebreton et al. 1992). However, the time intervals between sampling occasions (1 week) and the total duration of our study (5 weeks) were short enough to minimize the possibility of permanent emigration. In fact, previous studies have demonstrated that post-metamorphic individuals of *R. marina* remain closely associated to a single water body (e.g., a single pond) during the remaining of the dry season after they have undergone metamorphosis (Freeland and Kerin 1991; Child et al. 2008). Therefore, our estimates of survival were unlikely biased strongly by emigration.

We tested different hypotheses about variation in apparent survival (ϕ) and recapture (p) probabilities (Lebreton et al. 1992). First, we tested for the effect of site on survival by contrasting the encounter histories of toads from distinct populations. Second, we tested for the effect of body size on survival by including SVL as a continuous covariate (Lebreton et al. 1992). Including the covariate was equivalent to conducting an analysis of covariance, in which the resulting mean survival rates of each population are adjusted for the effect of the covariate. When including toad size as a covariate in the mark-recapture analysis, MARK is forced to estimate a linear trend (i.e., a single slope) for the relationship between size and survival. The model is specified by the following equation:

$$\phi = \beta_0 + \beta_1 \, \text{size}$$

where size is a continuous covariate represented by the SVL of the individuals and the β terms (one intercept and one slope) are estimated by MARK through maximum-likelihood routines based on our data. Third, we tested for a non-linear relationship between toad size and survival, such as that expected when individuals of intermediate sizes experience either lower or higher survival in comparison with smaller and larger individuals. The model specified has the following equation:

$$\phi = \beta_0 + \beta_1 \, \text{size} + \beta_2 \, \text{size}^2$$

where size is a continuous covariate represented by the SVL of the individuals and the β terms (in this case one intercept and two slopes) are also estimated by MARK through

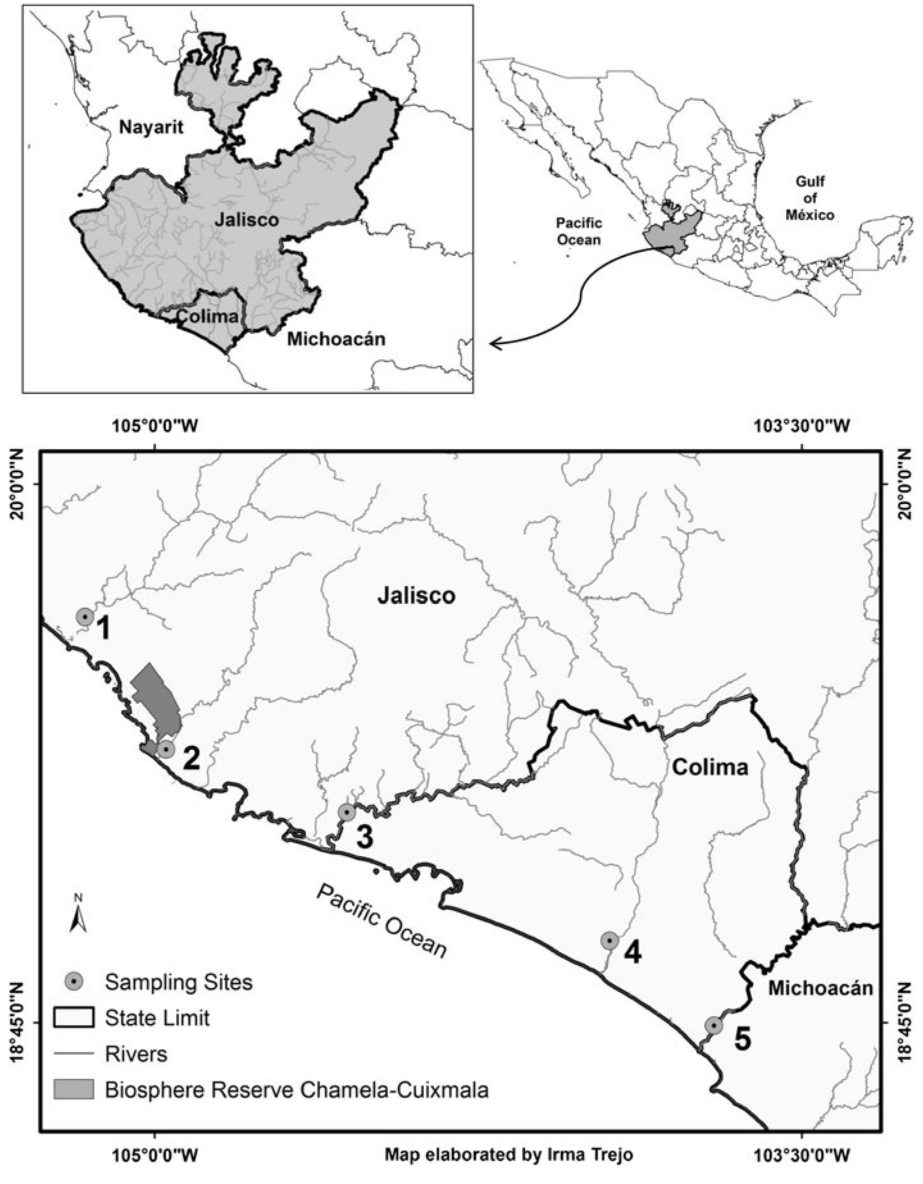

Fig. 1 Location of the five populations of *R. marina* that we studied in the states of Colima and Jalisco, Mexico. *Numbers* represent study sites: (*1*) San Nicolás River, (*2*) Cuixmala River, (*3*) Marabasco River, (*4*) Armería River, (*5*) Coahuayana River

maximum-likelihood routines. We called this type of quadratic model "size^2" to differentiate it from a "size" model, which corresponds to a simple linear relationship between survival and SVL.

Fourth, we implemented models with interactive effects of size and site as well as models with interactive effects of size^2 and site on toad survival. Fifth, we also tested models with constant survival ("null" models: no effects of size, size^2 or site on survival).

Table 1 Sample sizes of *R. marina* and abundance of potential predators in five study sites

Site	Number of marked individuals	Number of individuals recaptured at least once	Average snout-vent length in mm (min–max)	Number of spiders/ Gates index	Number of snakes/ Gates index	Number of birds/ average
1	656	63	12.7 (10.1–17.5)	385/9.04	2/0.58	140/32.1
2	737	159	12.99 (10–17.1)	147/10.7	0	98/21.2
3	870	90	15.43 (10.6–19.4)	110/9.04	0	86/19.8
4	836	147	12.33 (10.2–16.3)	104/7.4	4/0.25	251/54.3
5	822	122	13.17 (10–19.6)	217/16.7	0	72/16

The total observed number of spiders, snakes, and birds is shown per study site. The Gates index calculated for spiders and snakes provides an estimate of the density of these potential predators. For birds, we show the average number of individuals per site and per sampling occasion across observers

Regarding recapture probability (p) we implemented models with differences among sites and models with constant p (“null” models: no differences among sites).

The different combinations of these sources of variation for ϕ and p resulted in a total of 12 models that we fitted to our mark-recapture data. Model fit was evaluated using the Akaike information criterion (AIC), with the lowest AIC score indicating the best-fitting model (Akaike 1973; Burnham and Anderson 2002). A difference between models in AIC (ΔAIC) larger than 2 would indicate considerable support for a real difference in the fit of two models. We computed model-specific Akaike weights (w), which measure the relative support or weight of evidence in the data for each fitted model (Burnham and Anderson 2002). To account for the uncertainty in the process of model selection, we used these w values to calculate for each population model-weighted averages of weekly survival rates as per Burnham and Anderson (2002). These weighted averages of survival rates are calculated by considering the relative support for each competing model. These model-weighted average estimates are more robust than those derived from any single model alone (Johnson and Omland 2004).

Predator abundance

We measured the abundance of potential predators by calculating density of spiders, snakes, and birds at each study site. We only quantified spiders, snakes, and birds as predators of *R. marina* based on previous observations of predation events at the study sites. During our sampling occasions, we observed spiders from the family Lycosidae and birds, mainly of the species *Quiscalus mexicanus*, eating toads at least once at each site. We observed a snake (*Leptodeira maculata*) eating a toad once. We cannot discard the presence of other predators of *R. marina*, such as mammals, lizards, or fish. However, we did not observe any other organism prey on these toads even though we spent an entire 5-week period observing the sites during day and night. Thus, we assumed that the main source of predation for these post-metamorphic toads came from spiders, snakes, and birds.

To estimate density of potential predators, we conducted distance-sampling methods for spiders and snakes and point counts for birds. Spiders and snakes were counted at night along 10-m linear transects, and spotted with a headlamp. The distance at which spiders and snakes were seen was recorded, and we calculated predator densities using the Gates index:

$$\widehat{D} = \lfloor (2n - 1)/2L\bar{r} \rceil$$

where n is the number of individuals observed, L is the transect length, and $\bar{r}$ is the average of the distances to the observed individuals (Southwood and Henderson 2000). We conducted point counts for birds during the day, with four observers recording all bird species seen during a 15-min period. We estimated abundance of birds that eat amphibians based on del Hoyo et al. (1992, 1996, 2001). For each potential predatory bird species we calculated the average number of birds observed per site and per sampling occasion across observers. We then summed these values across all species, yielding an estimate of the total number of potentially predatory birds per site and per sampling occasion. Using abundance estimates for all three types of predators (spiders, snakes, and birds), we conducted a PCA to reduce dimensionality, and then an ANOVA on each principal component to test for differences in overall predation risk among sites.

Morphological variation

To analyze variation among populations in toad morphology we used geometric morphometric techniques (Zelditch et al. 2004). At the end of the mark-recapture experiments, we collected a sample of 40 toads from each population for morphological analyses. We focused on the morphology of the pelvis and head as well as on the length of the hindlimbs (femur and tibiofibula). Choi et al. (2000, 2003) found a functional relationship between the width of the hip (inter-ilial width), the length of the femur, tibiofibula, and foot bone with jumping performance and take-off speed of several anuran species. However, this functional relationship between locomotor performance and morphology has not been tested in *R. marina*. In this study, we assumed that these morphological traits also determine jumping performance and take-off speed in *R. marina* because this functional relationship has been confirmed in a close relative (*Bufo typhonius*; Choi et al. 2000, 2003). However, instead of analyzing the width of the hip by means of the inter-ilial distance, we analyzed the full shape of the pelvis. Similarly to Choi et al. (2000, 2003) we analyzed the length of the femur and tibiofibula. However, we did not measure the length of the foot bone because in small post-metamorphic individuals of *R. marina* the bones of the feet are poorly ossified, and therefore, their length could not be accurately measured. In addition, we analyzed the shape of the head because narrower heads have been found in high-predation environments (Relyea 2001; Capellán and Nicieza 2007).

A digital X-ray radiograph was taken in the dorsal perspective of each cane toad collected. Radiographs were taken using a Hamamatsu L6731-01 microfocus X-ray source with a PaxScan 2520E digital X-ray detector. We digitized the following 16 landmarks on each image using the tpsDig software (Rohlf 2006): (1) posterior tip of the ischium, (2) posterior left tip of the ilium, (3) posterior right tip of the ilium, (4) anterior tip of the pubis, (5) posterior left tip of the sacrum, (6) posterior right tip of the sacrum, (7) anterior tip of the urostyle, (8) anterior center tip of vertebra eight, (9) anterior center tip of vertebra six, (10) anterior center tip of vertebra four, (11) anterior center tip of vertebra two, (12) posterior left junction of the frontoparietal and prootic, (13) posterior right junction of the frontoparietal and prootic, (14) anterior left junction of the frontoparietal and prootic, (15) anterior right junction of the frontoparietal and prootic, and (16) most anterior tip of the nasal (Fig. 2).

We used the tpsRelw software (Rohlf 2007) to perform generalized Procustes analysis (i.e., align landmark coordinates by rotating, translating and scaling coordinates to remove positioning effects and isometric size effects; Bookstein 1991) and obtain geometric shape

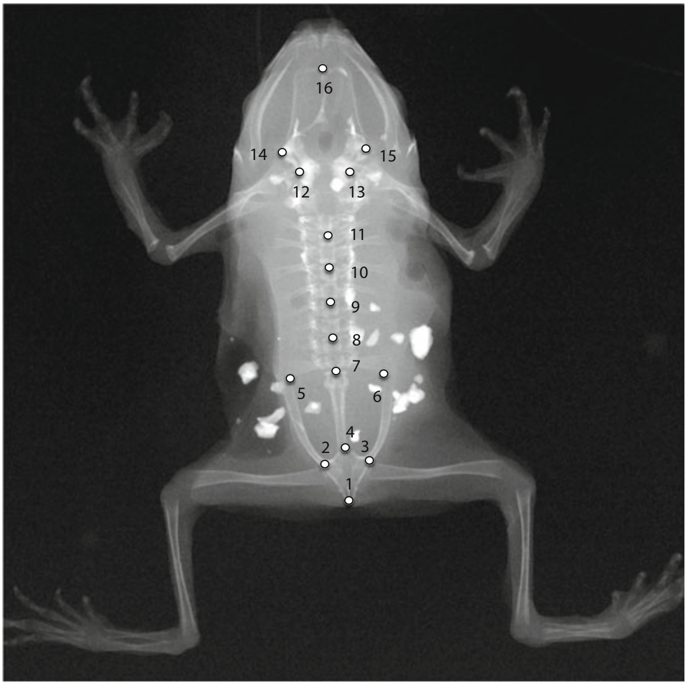

Fig. 2 Landmarks used for morphometric analysis

variables for statistical analysis (relative warps). We examined body shape variation among sites using multivariate analysis of covariance (MANCOVA), where geometric shape variables (relative warps) served as dependent variables, centroid size served as the covariate (to control for multivariate allometry), and site served as the main factor. We also included the interaction between centroid size and site. To assess the relative importance of model terms, we used Wilks's partial η^2 (measure of partial variance explained by a particular term; multivariate approximation of $SS_{effect}/[SS_{effect} + SS_{error}]$; Langerhans and DeWitt 2004). We calculated Wilks's partial η^2 using the full shape space (all 28 relative warps).

We performed canonical analysis of the site term from the MANCOVA following Langerhans (2009) to determine the nature of morphological differences among sites. We performed a principal components analysis (PCA) of the sums of squares and cross-products matrix of the site term to derive eigenvectors of divergence. These divergence vectors describe linear combinations of dependent variables exhibiting the greatest differences between sites, controlling for other factors in the model, in Euclidean space. Divergence vectors were calculated using the full shape space. The vectors with greatest relevance ($\mathbf{d}_1$, $\mathbf{d}_2$) were then visualized using the thin-plate spline approach (Bookstein 1991; Zelditch et al. 2004).

To analyze variation among populations in the hindlimbs (femur and tibiofibula) we conducted a traditional morphometric analysis by measuring these structures directly in the X-ray radiographs. Length of the femur and tibiofibula were measured as distances between the corresponding landmarks. A scale factor was introduced in the X-ray machine for this purpose. We measured the linear distance of these traits, rather than including them in the geometric morphometric analysis, because these articulating structures would have introduced considerable non-shape variation into the geometric morphometric analysis (i.e., position of the preserved limbs do not reflect shape, but rather only reflect the arbitrary spatial position of the articulating structures). We conducted a MANCOVA to determine the nature of morphological differences among sites. The response variables were the lengths of the femur and tibiofibula (log-transformed), the main factor was the source population, and SVL (log-transformed) was used as a covariate.

Relationship among survival rates, abundance of predators, and morphology

Using site means, we performed stepwise regressions to test for the effects of survival rate and predation risk on body size (average centroid size), body shape (divergence vector $\mathbf{d}_1$), and hindlimbs (femur and tibiofibula). The threshold for main effects to be included into the final models was $p = 0.25$. We only used $\mathbf{d}_1$ because it captured the major aspects of shape variation among sites. Because centroid size varied among sites, and was highly correlated with body shape across sites, we obtained residuals of $\mathbf{d}_1$ regressed on centroid size (at the individual level) so that we could more directly address body shape variation independent of allometric effects.

Results

The best fitting-model indicated a quadratic relationship between size and the survival parameter (ϕ; Table 2). This best model also indicated that the shape of this quadratic relationship between size and ϕ differed among populations (i.e., an interaction between site and size^2 affecting ϕ). In this model, p was set as constant across sites. However, another model resulted in ΔAIC = 0.51 with respect to the best-fitting model, indicating some model uncertainty. This second best-fitting model also indicated an interaction between site and size^2 affecting ϕ, but the parameter p was allowed to vary among populations (Table 2). Both models coincided in individuals of intermediate sizes experiencing higher survival probabilities compared to smaller and larger individuals, with differences among populations in the shape of this quadratic relationship between SVL and ϕ (Fig. 3).

Most importantly, both models indicated clear differences in survival probability among sites (Table 2). Model-weighted average estimates of weekly survival for sites 2 and 4 were the highest and similar to each other (mean ± SE: 0.66 ± 0.05; Fig. 4). The lowest survival estimate was observed in site 3 (0.25 ± 0.05). Survival estimates for sites 1 and 5 were 0.39 ± 0.09 and 0.41 ± 0.04, respectively (Fig. 4).

In our PCA of predation risk (i.e., abundance of spiders, snakes, and birds), two principal components explained more than 95 % of the total variance in predator abundance. The first principal component ($\mathrm{PC1}_{\mathrm{pred}}$) explained 60.8 % of the variance and accounted for spiders and snakes (loadings: 0.96 and 0.88, respectively). The second principal component ($\mathrm{PC2}_{\mathrm{pred}}$) explained 34.4 % of the variance and accounted for birds (loading: 0.93). All observed spiders belonged to the family Lycosidae. The observed snake species were *L. maculata* and *Hypsiglena torquata.* The bird species that were more frequently observed were *Q. mexicanus* and *Egretta thula*. We found significant differences among sites in the abundance of predators as indicated by the ANOVAs conducted on the two PCs ($\mathrm{PC1}_{\mathrm{pred}}$: $F_{4,10} = 5.056$, $p = 0.017$; $\mathrm{PC2}_{\mathrm{pred}}$: $F_{4,10} = 4.045$, $p = 0.033$). A Tukey test on $\mathrm{PC1}_{\mathrm{pred}}$ revealed that site 1 had a significantly higher abundance of spiders and snakes

Table 2 Model selection results for the mark-recapture data of the cane toad *R. marina*

Model	AIC	ΔAIC	*w*	Number of parameters
ϕ (s × size^2) p(.)	4005.81	0	0.56	20
ϕ (s × size^2) p(s)	4006.32	0.51	0.44	24
ϕ (size^2) p(s)	4036.22	30.41	0	9
ϕ (s × size) p(s)	4043.29	37.48	0	18
ϕ (s × size) p(.)	4046.63	40.82	0	14
ϕ (size) p(s)	4079.52	73.71	0	8
ϕ (size^2) p(.)	4226.4	220.58	0	5
ϕ (.) p(s)	4237.7	231.89	0	8
ϕ (s) p(.)	4239.25	233.44	0	8
ϕ (s) p(s)	4241.22	235.41	0	12
ϕ (size) p(.)	4280.54	274.73	0	4
ϕ (.) p(.)ϕ	4355.71	349.89	0	4

The 12 models are shown, with each model representing a distinct hypothesis about variation in survival (ϕ) and recapture (p) probabilities. The survival parameter was allowed to be constant (.), different among sites (s), a linear function of toad size (size) or a quadratic function of toad size (size^2). We also tested for interactive (×) effects of size and site on survival. The recapture parameter was allowed to be constant (.) or different among sites (s). AIC represents the Akaike information criterion. The lowest value indicates the model that best fits the data. We show the difference between the corresponding model and the best fitting model in AIC scores (ΔAIC), the relative support (weight) for each model in the data (w), and the number of parameters in each model

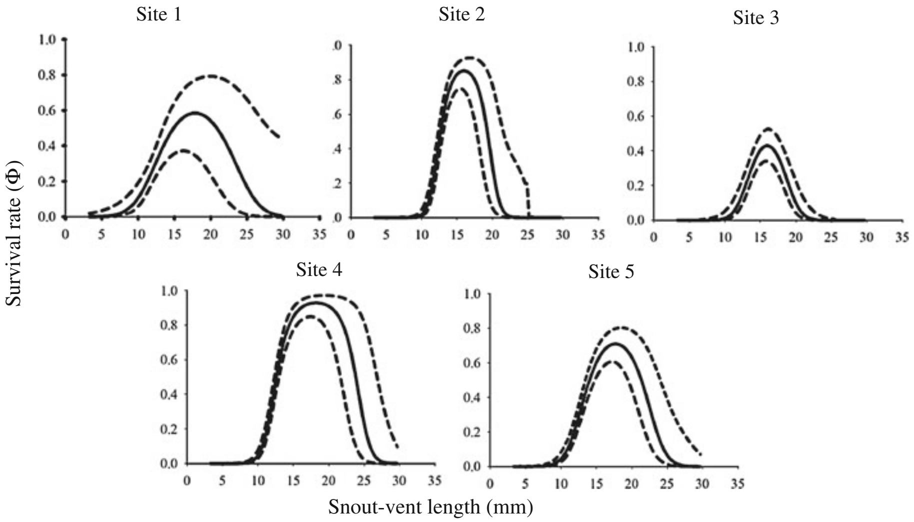

Fig. 3 Estimated relationship between size (snout-vent length) and weekly survival rate for post-metamorphic individuals of *R. marina* in five different populations. *Dashed lines* represent 95 % confidence intervals

compared to the four other sites. A Tukey test on $PC2_{pred}$ revealed that site 4 had a significantly higher abundance of birds. We did not find associations between site-specific predator abundance (the average scores of the populations on the two PCs) and site-specific

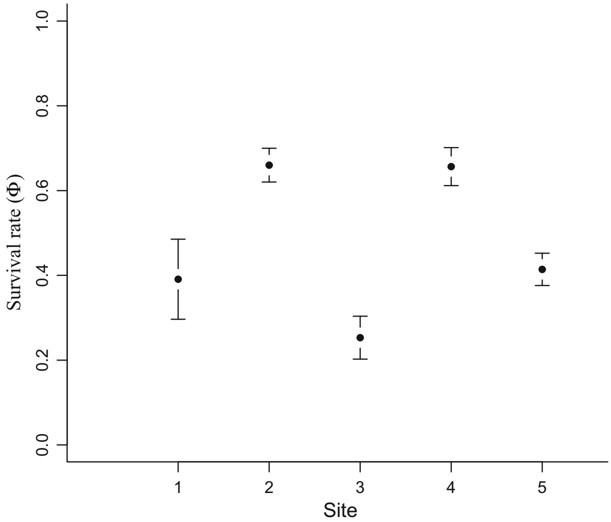

Fig. 4 Weekly size-adjusted survival rates (ϕ) of newly metamorphosed individuals of *R. marina*. *Numbers* represent study sites as per Fig. 1. *Error bars* represent 95 % confidence intervals

Table 3 Results of MANCOVA model that examined morphological variation among five populations of *R. marina*

Factor	F	df	p	Partial η^2	Relative partial η^2
Centroid size	8.072	28, 190	<0.0001	0.581	1
Site	2.826	112, 190	<0.0001	0.326	0.560
Site × centroid size	1.524	112, 190	0.001	0.207	0.356

F-ratios were approximated using Wilks's Λ values for the centroid size, site, and the interaction term. Partial variance explained by each term was estimated using Wilks's partial η^2. Relative variance represents partial variance for a given term divided by the maximum partial variance value in the model

model-weighted survival rates (Pearson correlation coefficients $PC1_{pred}$: $r = -0.39$, $p = 0.52$; $PC2_{pred}$: $r_S = 0.65$, $p = 0.23$).

Populations of *R. marina* showed remarkable differences in morphology. MANCOVA revealed significant effects of centroid size, site, and the interaction term on the relative warps (Table 3). Based on Wilks's partial η^2, centroid size (indicating allometry) was the most important source of variation for body shape. Site had an effect 56 % as strong as the effect of centroid size (Table 3).

Interpretation of the nature of morphological differences using thin-plate spline transformation grids to visualize shape variation along the divergence vectors ($\mathbf{d}_1$ and $\mathbf{d}_2$), revealed significant differences among populations in the shape of the pelvis and the head (Table 3; Fig. 5). From the two divergence vectors retained, $\mathbf{d}_1$ captured major aspects of shape variation among most sites, while $\mathbf{d}_2$ appeared less biologically important. Examination of average population values along $\mathbf{d}_1$ revealed clear inter-population variation in the pelvis and head. In contrast, $\mathbf{d}_2$ indicated a slight rotation of the vertebral column that might reflect an artifact of including images of bent individuals (Fig. 5). According to $\mathbf{d}_1$ toads at site 2 exhibited a narrower and shorter pelvis, wider heads, and a longer presacral

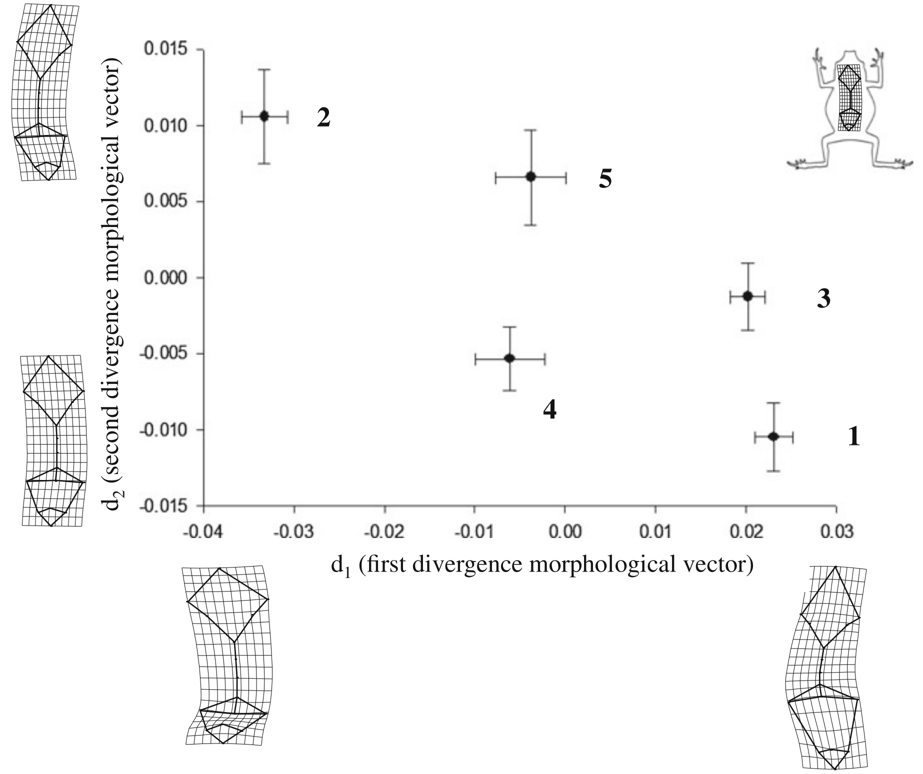

Fig. 5 Morphological divergence among populations of *R. marina*. Body shape variation is described by the divergence vector derived from the site term of the MANCOVA, and illustrated using thin-plate-spline transformations girds relative to mean landmark positions (magnified ×2 to better demonstrate differences). *Solid lines* connecting outer landmarks are drawn to aid interpretation. *Numbers* represent study sites as per Fig. 1

vertebral column. In contrast, toads at sites 1 and 3 exhibited a wider and longer pelvis, narrower heads, and a shorter presacral vertebral column. Toads at sites 4 and 5 exhibited intermediate morphologies between these two extremes (Fig. 5).

We also found differences among sites in the size of the hindlimbs ($\Lambda = 0.149$, $p < 0.001$). Toads at site 2 exhibited significantly shorter femurs compared to those at all other sites (Fig. 6a). Toads at sites 1, 2, and 3 had shorter tibiobifulas than those at sites 4 and 5 (Fig. 6b).

The stepwise regression analysis examining the effects of survival rate and predation risk on size revealed a negative relationship between centroid size and survival, and a negative relationship between centroid size and $PC1_{pred}$ (sites with smaller toads exhibited higher survival and had higher densities of spiders and snakes). We also found a positive relationship between centroid size and $PC2_{pred}$ (sites with larger toads exhibited higher densities of birds; Table 4).

The stepwise regression analysis examining the effects of survival rate and predation risk on shape revealed a suggestive, but marginally non-significant effect of $PC1_{pred}$. This suggested a trend of toads with a longer and wider pelvis, narrower head, and shorter presacral vertebral column in sites with a greater abundance of spiders and snakes. The

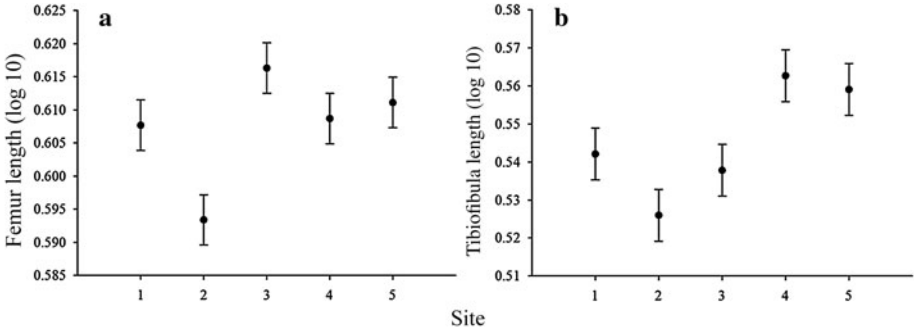

Fig. 6 Size-adjusted average length (log-transformed) of femur (**a**) and tibiofibula (**b**) per study site. Numbers represent study sites as per Fig. 1

Table 4 Results of the stepwise regression analyses that tested the effects of survival and predation (as measured by a principal components analysis conducted on the abundance of spiders, snakes, and birds) on centroid size, morphology (as measured by the first divergence vector), and the size of the hindlimbs (femur and tibiofibula) of post-metamorphic individuals of *R. marina*

Source of variation	F	Regression coefficient	df	*p*
Centroid size = Phi + $PC1_{pred}$ + $PC2_{pred}$				
Survival rate	2,889.722	−699.453	1, 1	0.012
$PC1_{pred}$	328.298	−26.943	1, 1	0.035
$PC2_{pred}$	782.151	69.672	1, 1	0.023
Divergence vector 1 = Phi + $PC1_{pred}$ + $PC2_{pred}$				
$PC1_{pred}$	9.117	0.009	1, 3	0.057
Femur = Phi + $PC1_{pred}$ + $PC2_{pred}$				
Survival rate	3.931	−0.036	1, 3	0.142
Tibiofibula = Phi + $PC1_{pred}$ + $PC2_{pred}$				
–	–	–	–	–

$PC1_{pred}$ stands for the first principal component that represents the abundance of spiders and snakes. $PC2_{pred}$ stands for the second principal component that represents the abundance of birds. The threshold for main effects to be included into the final models was $p = 0.25$. The model for tibiofibula did not include any explanatory variable with a p value < 0.25

stepwise regression analysis examining the effects of survival rate and predation risk on hindlimbs did not reveal any significant relationship (Table 4).

Discussion

Morphological variation among populations of *Rhinella marina*

Toads living in different sites exhibited different morphologies, even after accounting for the effect of body size. Our first divergence vector ($\mathbf{d}_1$) captured morphological changes in the pelvis, head, and presacral vertebral column. Did these morphological differences reflect local adaptation to divergent predation regimes? Previous work has found anurans co-occurring with predators to exhibit a narrower head and pelvis compared to individuals inhabiting predator-free environments (Relyea 2001; Tejedo et al. 2010). Moreover, a long pelvis, long hindlimbs, and a short presacral vertebral column have been shown to enhance jumping performance (Emerson 1985; Choi et al. 2000; Chadwell et al. 2002). Thus, we predicted that *R. marina* populations that experience greater predation pressure should exhibit a narrower head and pelvis, longer hindlimbs and pelvis, and a short presacral vertebral column. Our findings partially supported these predictions, as we found that sites with a higher abundance of spiders and snakes tended to have individuals with a long pelvis, narrow head, and short presacral vertebral column—all matching expectations, albeit marginally non-significant using a two-tailed test. However, in these individuals the pelvis was wide rather than narrow, and we found no relationship between the size of the hindlimbs and predator abundance even though populations differed markedly in relative hindlimb length. Furthermore, inter-population differences in the abundance of birds were not related to morphological differences among sites. In summary, our results yielded inconsistent and weak support for our hypothesis that predation risk should lead to particular morphological differences across populations of *R. marina*.

These findings suggest that while predation from spiders and snakes might have led to some predictable changes in cane toad morphology—consistent with the hypothesis that greater predation pressure should favor enhanced jumping performance—these effects were relatively weak, and some differences were opposite to our predictions. This might partially reflect a lack of detailed knowledge regarding the biomechanics of jumping performance in this particular species (including many-to-one mapping of morphology to jumping performance, sensu Wainwright et al. 2005), or perhaps the importance of other selective agents causing selection on these same traits across these sites, such as other predators, resource competition, and abiotic factors (Toledo et al. 2007; Tejedo et al. 2000; Ortiz-Santaliestra et al. 2012). Future work is required to unravel the causes underlying the observed differences in body morphology among populations.

According to our shape analysis, centroid size explained most of the variation in morphology. This indicates that morphology changes as individuals grow (i.e., allometry). Given this observed effect of body size on the morphology of these toads, one could wonder whether the observed differences among populations might partially reflect inter-population differences in mean age or in their rates of body growth. Because the sizes of toads were broadly similar among populations (e.g., broadly overlapping distributions, see Table 1), this seems unlikely, assuming body size similarly reflects age across sites (Höglund and Säterberg 1989). Therefore, differences that we observed in body shape are unlikely related to age effects. To avoid potential bias due to body growth, we decided to conduct our study during a short period of time (5 weeks). At the end of our study period we confirmed that these post-metamorphic individuals increased in body size 4.8 mm SVL on average across all populations. This average increase was minimal when compared to the size that *R. marina* attain when they become adults ($\sim$240 mm SVL; Child et al. 2008). In addition, we also compared statistically the growth rates among populations and found no significant difference (ANCOVA with growth rates as a response variable, site as a main effect, and toad SVL as covariate; site term: $F_{4,562} = 0.361$, $p = 0.836$). In other words, even though they grew slightly during our study period, all of them grew at a similar rate. Thus, differences in growth rate across sites cannot explain inter-population variation in morphology.

Predator abundance and survival

We did not find a statistical association between predator abundance and survival. Therefore, mortality was likely strongly influenced by other predators such as fish (Toledo et al. 2007) or by other factors besides predation, such as limited food availability, high desiccation rates, or critical temperatures (Alvarez and Nicieza 2002; Relyea and Hoverman 2003; Ficetola and De Bernardi 2006; Johansson et al. 2010). Regarding other potential predators, we did not observe lizards, fishes, or mammals preying on these toads. However, we recognize that differences among sites in the abundance of large-sized and carnivorous lizards such as those from the genus *Ameiva*, fishes from the family Cichlidae, and mammals such as raccoons (*Procyon lotor*) and white-nosed coatis (*Nasua narica*), all of which are present at the study sites (García and Ceballos 1994; Ceballos and Oliva 2005; Miller et al. 2005), might have affected the observed differences among sites in survival rates. In addition, differences among sites in diurnal predators other than birds such as diurnal snakes (e.g., *Conophis vitattus*, *Drymobyus margaritiferusi*; García and Ceballos 1994) or other arthropods (e.g., water bugs from the family Belostomatidae; Rodríguez-Palafox and Corona 2002; Toledo 2003) also might have promoted inter-population differences in survival rates. However, we note that even though we observed these toads during night and day during a 5-week period, we did not observe predation by other arthropods, snakes, lizards, mammals or fishes. Hence, we assumed that predation by these latter potential predators represented only a minor proportion of toad deaths.

Environmental factors other than predator abundance might have caused the observed differences in survival rates. The relative availability of refuges could have been different among populations resulting in toads being more vulnerable at some sites (Ficetola and De Bernardi 2006; Tejedo et al. 2000). Differences among sites in food availability also could have resulted in differences in survival rates. In amphibians, the lack of food during the post-metamorphic stage results in increased mortality (Dahl et al. 2012). Sites with warmer temperatures promote higher desiccation and dehydration rates (Altwegg and Reyer 2003; Tingley et al. 2012). In addition, differences among populations in post-metamorphic mortality could also have been caused by developmental abnormalities associated with differences among rivers in the concentration of toxic compounds in the water (Rouse et al. 1999). Finally, density-dependent factors such as competition or cannibalism may have strong effects on survival rates. Mortality increases in populations with high density of individuals as a result of intense intraspecific competition (Relyea and Hoverman 2003; Ortiz-Santaliestra et al. 2012). In anuran amphibians the rate of cannibalism of juveniles by adult individuals varies among populations (Pizzatto and Shine 2008; Hawley 2009). In summary, several abiotic and biotic factors (including predation) should interact to determine the variation in survival probabilities that we documented here both among and within populations of *R. marina*. These complex interactions should explain why we did not detect an association between site-specific predator abundance and survival.

Effects of size on survival rates

At the inter-population level, sites with larger toads (centroid size) experienced lower overall survival probabilities. Importantly, these site-specific survival probabilities control for within-population effects of body size on survival, and thus reflect the average survival rate of an average-sized toad within each population—this means that this pattern does not conflate within- and between-population processes. We found that populations with relatively larger post-metamorphic toads additionally experienced lower abundances of spiders and snakes and a higher abundance of birds. Size-selectivity of these predators could have caused these patterns, but the lack of an association between predator abundance and survival rates does not support this contention. Sites with larger toads having lower overall survival rates is an unexpected result because previous studies in different taxa have documented that populations consisting of larger individuals have better overall survival probabilities compared to other populations of the same species with relatively smaller individuals (Kingsolver and Pfennig 2004; Uller and Olsson 2010; Cabrera-Guzmán et al. 2013; Harrison et al. 2013). Larger amphibians usually escape more efficiently from predators than smaller individuals, dehydrate less, and are better competitors (Newman and Dunham 1994; Beck and Congdon 2000; Gray and Smith 2005). However, small sizes might provide better survival probabilities when crypsis and immobility are the main strategies to avoid predation (Toledo and Haddad 2009; Touchon et al. 2013). Why sites with smaller post-metamorphic toads resulted in higher overall survival rates deserves further research.

In contrast, at the intra-population level, survival probability of *R. marina* varied as a quadratic (non-linear) function of size. In all five populations, post-metamorphic individuals of intermediate sizes experienced higher survival probabilities compared to smaller and larger individuals. This result represents compelling evidence for stabilizing selection on the size of these toads during their post-metamorphic stage. In this stage of their life cycle, when these toads have small sizes, fitness advantages of fast growth and a large size could be expected (Goater 1994; Arendt 2003). However, our demographic data supports a different scenario. Some hypotheses related to size-specific mortality within populations might explain the observed stabilizing selection on post-metamorphic size. Smaller individuals might be an easier prey for spiders (quite abundant in all five sites) and might suffer higher desiccation rates (Cohen and Alford 1993; Ward-Fear et al. 2010; Tracy et al. 2013). In contrast, birds and snakes may prey selectively on larger post-metamorphic toads because they represent a higher energetic content per capture effort or because they are more conspicuous to these predators (Vincent et al. 2006; Flores et al. 2013; Nakazawa et al. 2013). These hypothetical scenarios may occur only during this post-metamorphic stage when these toads are highly vulnerable to predation by snakes, spiders, birds, and other predators because they are aggregated close to water bodies during the late dry season. Only after the rain begins and these juvenile toads begin dispersing, would the mortality patterns change so that larger individuals might experience higher survival.

Conclusions

The relationships among body shape, predator abundance, and survival were weak to nonexistent. This result was unexpected because several studies have demonstrated that different abundances of predators promote different body shapes presumably through their effects on the differential survival of particular phenotypes (Brönmark and Miner 1992; McCollum and Van Buskirk 1996). It is possible that other predators or environmental factors might have caused the inter-population differences that we observed in body shape and survival of *R. marina*. In addition, we cannot be completely sure that the morphological traits that we studied (the shape of pelvis and head and the length of the hindlimbs) strongly determine jumping performance and take-off speed in *R. marina*. Considerable work has been done on the functional morphology of jumping in anurans (Choi et al. 2000, 2003), but no study has yet directly addressed this subject in this particular species (Chadwell et al. 2002). Therefore, we may have examined the “wrong” morphological traits with respect to locomotor performance, explaining why we could not find a strong and clear association between predation and morphology.

Our results suggest that body size of *R. marina* may play a critical role on survivorship, as body size was associated with survival rates both within and among populations. Moreover, we found clear evidence for body shape differentiation among populations. However this inter-population divergence was only weakly and inconsistently associated with variation in predator abundance, and not associated with survival rates. Further examination of post-metamorphic and adult individuals will provide important insight into the causes of the observed phenotypic divergence.

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