

Consequences of an amphibian malformity for development and fitness in complex environments

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SUMMARY

1. Environmental stressors have both lethal and sublethal effects, such as altered developmental rates and the induction of malformations. Ecological interactions, including predation and competition, often amplify such effects, for instance by inducing behavioural changes that increase susceptibility to the stress.
2. Using experimental mesocosms, we asked whether the density of conspecific competitors and predation risk from larval water beetles (*Dytiscus* spp.) affect the development of malformations in tadpoles of the wood frog (*Rana sylvatica*). We also examined whether such malformities increase the susceptibility of tadpoles to predation.
3. The risk of predation decreased the frequency of malformities in both low- and high-density treatments, although this effect was greater at low density. Behavioural observations suggested that reductions in activity by amphibian larvae induced by predators mediated these responses by decreasing cumulative exposure to ultraviolet-B radiation, the putative stressor causing the observed malformity. These results suggest that predators can reduce negative impacts of stressors by inducing behavioural changes in prey organisms.
4. Malformed individuals were twice as vulnerable to predators as non-malformed individuals, suggesting that sublethal effects can ultimately cause increased mortality.

Keywords: antipredator behaviour, environmental stressor, scoliosis, swim speed, ultraviolet-B radiation

Introduction

Many species are negatively affected by a variety of abiotic and biotic stressors acting alone or synergistically (Cech, Wilson & Crosby, 1998; Bancroft, Baker & Blaustein, 2008). Ecological interactions, such as competition and predation, often amplify the negative impacts of these stressors by adding further stress to the individual (Relyea & Mills, 2001; Relyea, 2003) or inducing phenotypic changes (i.e. changes in physiology, morphology or behaviour) that render the individual more susceptible to the effects of the initial stressor (Thiemann & Wassersug, 2000; Decaestecker,

De Meester & Ebert, 2002; Van Der Veen, 2005; Johnson *et al.*, 2006). For example, prey may take refuge from predators in habitats that increase the risk of parasites and disease (e.g. Decaestecker *et al.*, 2002). However, recent research has suggested that ecological interactions may also reduce the harmful impacts of environmental stressors, through similar induced changes in habitat selection or activity (e.g. Laws *et al.*, 2009). Because of these conflicting outcomes, it is important to examine further the impacts of stressors within complex ecological settings and to determine the mechanisms by which individuals become more or less susceptible to stressors. Based on these previous studies, it is evident that one important mechanism may be the plastic phenotypic responses of organisms to various environmental conditions.

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The effects of stressors on individuals can range from directly lethal to various sublethal effects such as malformities or reduced fecundity. In contrast to direct mortality, the fitness consequences of sublethal effects are less clear (Von Westernhagen, 1988) and often depend on environmental context. For example, individuals that develop a malformity upon exposure to a stressor may still grow and develop normally under laboratory conditions (e.g. Browne, Pomeroy & Hamer, 2003), but may not be able to survive in more realistic conditions when ecological pressures, such as competition or predation, may reduce the performance and fitness of malformed individuals. Thus, such sublethal effects may become lethal when considered in natural habitats, although relatively few studies have examined the potential fitness consequences of sublethal effects (but see Romansic *et al.*, 2009).

Amphibians are vulnerable to various stressors including parasites (e.g. Thiemann & Wassersug, 2000; Johnson *et al.*, 2006), pathogens (reviewed by Daszak *et al.*, 1999), pollutants (e.g. Relyea & Mills, 2001; Relyea, 2003) and ultraviolet-B radiation (reviewed by Bancroft *et al.*, 2008; Croteau *et al.*, 2008a). Exposure to such stressors can increase mortality (Blaustein & Kiesecker, 2002; Collins & Storer, 2003), alter the hatching success of eggs (e.g. Bancroft *et al.*, 2008) and cause various malformities (Ankley *et al.*, 2004). Consequently, these stressors are thought to be a major factor contributing to the current global amphibian decline (reviewed by Blaustein & Kiesecker, 2002; Collins & Storer, 2003; Lannoo, 2005). While many studies have examined the effects of these stressors on amphibians in isolation from other environmental variables, our knowledge of the impacts of stressors and in more ecologically realistic habitats is comparatively slight (but see Johnson *et al.*, 2006; Alton, Wilson & Franklin, 2010).

In previous research, we noticed that a small proportion of wood frog (*Rana sylvatica* LeConte) tadpoles raised in outdoor mesocosms had developed scoliosis, defined here as a lateral curvature of the tail muscle (Fig. 1; Hisaoka & List, 1957). Scoliosis affects a wide range of amphibians, and its expression is linked to various stressors, including oestrogenic chemicals (Croteau *et al.*, 2008b), heavy metals (Hopkins, Congdon & Ray, 2000; Chen, Gross & Karasov, 2006), insecticides (Alvarez, Honrubia & Herráez,

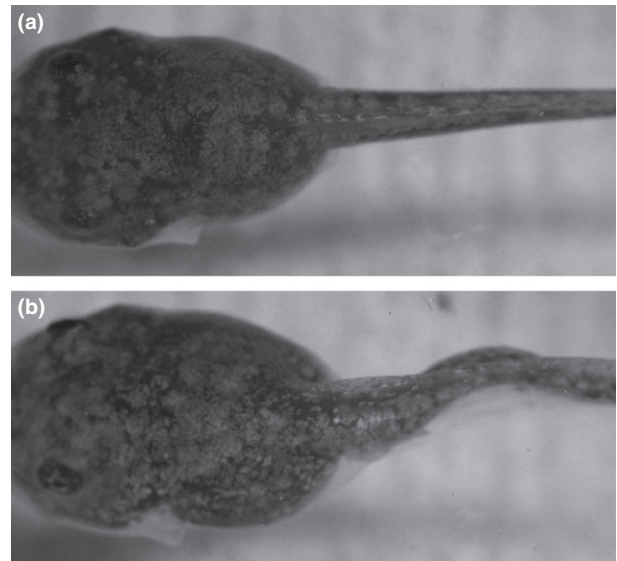


Fig. 1 (a) Non-scoliotic and (b) scoliotic tadpoles.

1995; Ankley *et al.*, 1998), malnourishment (Martínez *et al.*, 1992) and overexposure to ultraviolet-B radiation (UVB; Worrest & Kimeldorf, 1976; Pakkala, Laurila & Merilä, 2001; Weyrauch & Grubb, 2006; Van Uitregt, Wilson & Franklin, 2007; Croteau *et al.*, 2008a,b). However, few studies have examined the development and subsequent fitness consequences of scoliosis in natural environments. As *R. sylvatica* tadpoles exhibit behavioural plasticity in response to changes in several environmental conditions, including predation risk (Van Buskirk, 2002) and density of conspecifics (Relyea, 2002), such plasticity may mediate their susceptibility to stressors causing scoliosis.

In this study, we examine how three environmental variables – predation risk, tadpole density and physical habitat complexity – affect the development of scoliosis in *R. sylvatica* tadpoles. Additionally, we quantified tadpole activity to assess whether the effects of predators and tadpole densities on scoliosis development were mediated by tadpole behaviour in experimentally manipulated environments. To determine the potential performance consequences of scoliosis, we tested the burst swimming speeds of scoliotic and non-scoliotic tadpoles. Additionally, to determine the fitness consequences of scoliosis in a realistic environment, we examined the survival of scoliotic and non-scoliotic tadpoles exposed to the risk of predation. We predicted that scoliotic tadpoles

would have reduced burst swimming speeds and, consequently, suffer greater mortality from predation.

Methods

Experiment I: Effects of environmental variables on the frequency of scoliosis

In May 2006, we collected *R. sylvatica* tadpoles of approximately Gosner developmental stage 25 (Gosner, 1960) from three temporary, open-canopy ponds within the University of Notre Dame Environmental Research Center (Gogebic Co., MI) and placed them in 1-m-diameter wading pools kept outside. After approximately 1 week, we randomly selected healthy tadpoles and placed them into various 60.5-L experimental tubs in which one level of each of the following factors was applied: tadpole density (seven or 21 total *R. sylvatica* tadpoles), predation risk (no predator or one caged predator) and vertical structural complexity (0, 28 or 84 strands of green polypropylene rope, simulating aquatic vegetation). One large (>4 cm) diving beetle larva (*Dytiscus* spp., instar IV or V) was used as the predator and fed 500 mg of *R. sylvatica* tadpoles every other day. *Dytiscus* larvae are common predators of *R. sylvatica* tadpoles and were collected from the same three temporary ponds. Rope strands were tied to a black plastic hardware net anchored to the bottom of the tub with four flat rocks. We fully crossed each factor ($2 \times 2 \times 3$) and replicated each treatment five times for a total of 60 experimental tubs. We filled all tubs to a depth of 0.25 m with well water originating from the same source, to diminish the possibility that variation in water quality produced differences in the frequency of scoliosis. Two weeks before the experiment, we inoculated each tub with 1 g crushed rabbit food and 500 mL of pond water to stimulate the growth of algae for the tadpoles to eat. We kept all tubs outside where they experienced ambient sunlight and temperature and covered each tub with fibreglass screening to prevent colonisation by aquatic insects. During the experiment, we quantified tadpole activity (the number of active tadpoles divided by the number of visible tadpoles) using scan sampling. These observations were performed once (morning or afternoon) on five randomly selected days throughout the duration of the experiment. After 22 days, we removed all

surviving tadpoles from the tubs and preserved them in 10% formalin. We later examined all individuals for scoliosis and calculated a ratio of scoliotic : non-scoliotic tadpoles for each tub.

We used a binomial modelling approach with tadpole density, predation risk, structural complexity and their interactions as categorical independent variables and scoliotic : non-scoliotic phenotype ratio as the dependent variable (Crawley, 2005). We started with the full model and began removing non-significant interactions and then main effects. Because there was evidence of overdispersion when all factors were included, we employed quasibinomial errors (Crawley, 2005). At each step, we tested whether the removal of the term improved the fit of the model by comparing estimates of deviance with the previous model using an analysis of deviance (ANODEV). We saved the most parsimonious model and tested for the significance of included effects using an ANODEV. Significance testing of models was conducted using R 2.7.0 (R Core Development Team, 2008).

To test the effect of tadpole activity on scoliosis development, we used a logistic regression with scoliotic : non-scoliotic phenotype ratios of each tub as the dependent variable and mean overall activity of each tub (after an arcsine, square root transformation) as the independent variable. Because an analysis of the residuals demonstrated violations of the assumptions of parametric statistics, we used a Monte Carlo approach to test the hypothesis that activity has no effect on scoliosis development. Scoliotic : non-scoliotic phenotype ratios were randomly selected, with replacement, and paired with measures of activity. Using a generalised linear model, we obtained an estimate of the slope and repeated this process 5000 times. A one-tailed *P*-value for the slope estimate was obtained by dividing the number of randomly generated slope estimates that were greater than the slope estimate of the actual data by the 5000 simulations. Monte Carlo simulations were performed with a code written in R 2.7.0 (R Core Development Team, 2008).

Experiment II: Effects of scoliosis on survival in the presence of predators

In late April 2008, we collected portions of two to three *R. sylvatica* egg masses from each of five ponds and placed them in five outdoor wading pools filled with well water, so that each pool contained egg

masses from one pond. In early May, after eggs hatched, we randomly selected 50 tadpoles from each of the five pools and placed them into two 1-m-diameter wading pools (water depth: 17.8 cm) kept outdoors. We covered each pool with a lid composed of fibreglass window screening to discourage colonisation by aquatic organisms. When tadpoles were approximately at Gosner developmental stage 31, we haphazardly selected 50 scoliotic and 50 non-scoliotic tadpoles from the two pools to be used in swimming and survival trials.

To test tadpole burst swimming speed, we constructed a Plexiglas chamber (35 cm × 7.5 cm) filled with approximately 3 cm of well water at ambient temperature; no flow was present inside the chamber. At the bottom of the chamber, we delineated a 1 cm × 1 cm grid and then placed a Sony digital camcorder (29.97 frames s⁻¹) 0.4 m above the water surface to obtain a top-down view. For each tadpole (50 scoliotic and 50 non-scoliotic), we first obtained a mass (mean ± 1 SE: scoliotic 0.338 g ± 0.011; normal 0.306 g ± 0.012) and then placed the individual in the chamber. After a one-minute acclimation time, we simulated a predator attack by flicking the tadpole at the base of the tail muscle with a dissecting probe (Dayton *et al.*, 2005). Three successive simulated attacks were conducted for each tadpole, with a 30-s rest period in between each attack. We alternated swimming trials between scoliotic and non-scoliotic tadpoles. During digital playback of the video, we calculated burst speeds (mm s⁻¹) within the first 0.3–0.6 s following a simulated attack by measuring the distance travelled by the tadpole and the elapsed time. Of the three burst speed measurements, we took the fastest for statistical analyses.

After the swimming speed test, each tadpole was placed into one of two 60.5-L tubs, according to phenotype, for a 24-h recovery period. We then randomly selected five scoliotic and five non-scoliotic individuals and placed them together into one 60.5-L tub containing 24 strands of green polypropylene rope to provide basic habitat structure. We allowed 24-h acclimation and then introduced one late instar *Dytiscus* larva (mean mass ± 1 SE: 0.964 g ± 0.068) to each tub. We observed tubs at 9, 15 and 18 h after introduction of the predator to ensure that the predator did not consume all tadpoles. When four to six tadpoles remained in a tub, we collected the

predator and sorted the survivors by phenotype. All mortality was assumed to be because of predation, since no tadpole carcasses were found. Ten such predator selection trials were conducted simultaneously.

To analyse the effects of scoliosis on burst swimming speed, we log-transformed speed and body mass and then conducted an analysis of covariance (ANCOVA) with phenotype as the factor and body mass as the covariate. The interaction between phenotype and body size was not significant ($P = 0.993$), indicating slope homogeneity in estimates of burst swimming speed. For the predation trials, we used a paired *t*-test to test the null hypothesis that the mean difference between the number of non-scoliotic and scoliotic individuals that survived predation did not differ from zero. Each predation trial ($n = 10$) was used as a replicate. Both of these analyses were conducted using SYSTAT 10.0 (Systat Software, Chicago, IL, USA).

Results

Experiment I

Tadpole survival was high (96.2%) and did not differ between treatments. Overall, 8.7% of tadpoles exhibited some degree of scoliosis. The most parsimonious model for explaining scoliotic : non-scoliotic phenotype ratios included terms for tadpole density, predation risk and their interaction (see Appendix S1 for model-building steps). The results from the ANODEV indicated that predation risk ($F_1 = 4.78$, $P = 0.033$) and its interaction with tadpole density ($F_1 = 5.25$, $P = 0.026$) affected the proportion of scoliotic tadpoles, although density as a main factor did not ($F_1 = 2.25$, $P = 0.123$). Predation risk induced a lower frequency of scoliosis across all treatments, but the effect was stronger at low tadpole density (Fig. 2). Structural complexity had no significant effect on the frequency of scoliosis, although the variance in the frequency of scoliosis was high. With five replicates, the power for the statistical test of structure was estimated as 0.366; 15 replicates would have been required to obtain a power of 0.80 (Appendix S2). Tadpole activity was positively correlated with the proportion of scoliotic tadpoles (slope ± 1 SE; $2.50 ± 1.97$; Fig. 3). This slope value ranked 330th of 5000 Monte Carlo simulations, yielding a *P*-value of 0.066.

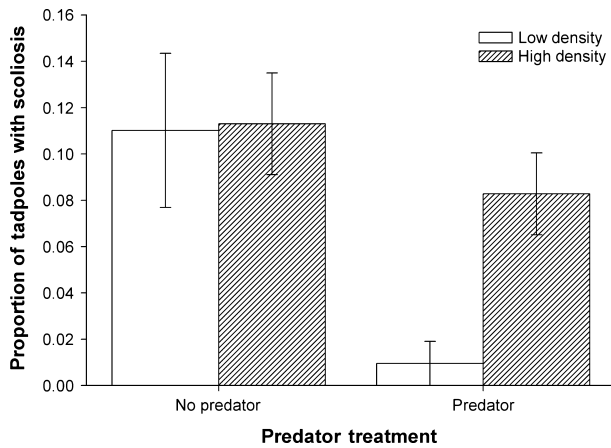


Fig. 2 Mean (\pm SE) proportion of tadpoles with scoliosis from tadpole density and predation risk treatment tubs. Open bars indicate low-density treatment, and hatched bars indicate high-density treatment.

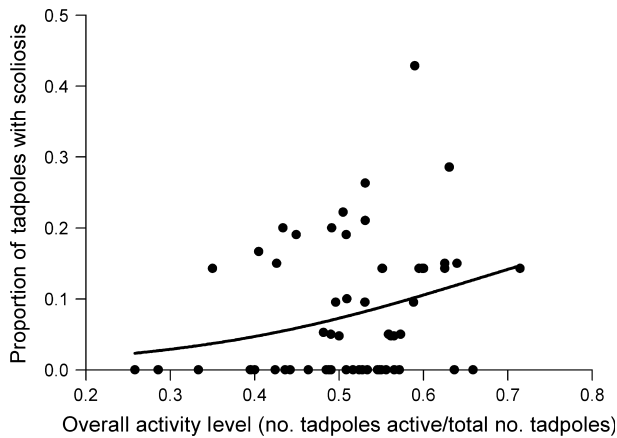


Fig. 3 Relationship between the proportion of tadpoles with scoliosis and the overall activity levels of tadpoles, with best-fitting logistic trendline. Each point represents one of the 60 experimental tubs.

Experiment II

Observations of tadpole swimming suggest that scoliosis restricts tail movement to the portion of the tail distal to the tail muscle curvature. Mean burst swimming speed of normal tadpoles was approximately 14% greater than that of tadpoles afflicted with scoliosis ($F_{1,97} = 14.59$, $P < 0.001$; Fig. 4) after controlling for variation in body size. More non-scoliotic tadpoles survived predation than scoliotic tadpoles ($t = 4.32$, d.f. = 9, $P = 0.002$). On average, 66% of the normal phenotypes survived compared to 30% of the scoliosis phenotype.

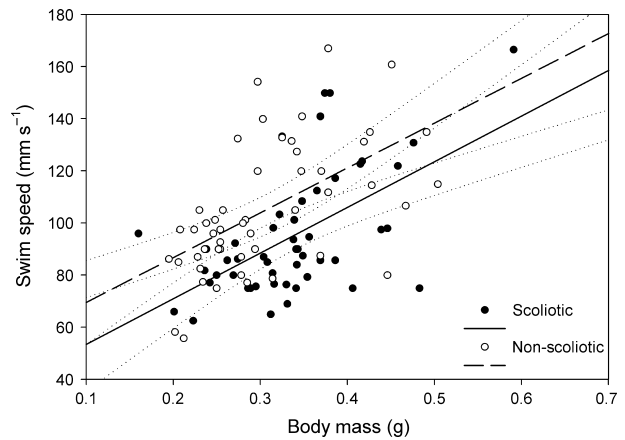


Fig. 4 Burst swimming speeds of scoliotic (filled circles, solid line) and non-scoliotic tadpoles (open circles, dashed line) as a function of body mass. Dotted lines are 95% confidence interval bands.

Discussion

The effects of environmental stressors on organisms can be modified by ecological interactions and, specifically, the effects of these ecological interactions on particular organismal traits. Our results indicate that the development of tadpole scoliosis depended on the environment in which the tadpole was raised. The presence of predators reduced the frequency of scoliosis in both low and high tadpole density environments. Higher tadpole densities did not alter scoliosis rate in the absence of predation risk, but did cause a slight increase in the presence of predators. Additionally, the frequency of scoliosis was related to activity, in that tubs with more active tadpoles produced a higher proportion of scoliotic tadpoles. Because the effects of predation risk and competition on the development of tadpole scoliosis were similar to their effects on tadpole activity, it is possible that these behavioural changes modified the development of scoliosis.

To determine how predation risk and competition intensity altered patterns of scoliosis development in *R. sylvatica* tadpoles, it is important to establish a likely cause for the observed scoliosis. Pollutants or pesticides are probably not the cause, as all tubs were filled using the same water, and tests confirmed the absence of heavy metals and harmful chemicals. Malnutrition is a possible cause (Martínez *et al.*, 1992), especially considering that competitor density and predation risk are known to affect tadpole growth

(Relyea, 2002; Steiner & Van Buskirk, 2008) in a manner similar to the effect of these treatments on the frequency of scoliosis. However, scoliotic tadpoles can grow as large as non-scoliotic tadpoles (see Fig. 1; Methods: Experiment II) and the growth rate of tadpoles in Experiment I was not correlated with scoliosis frequency (Appendix S3). Further, previous studies have found that diet had no effect on scoliosis development (Martínez *et al.*, 1996; Browne, Pomeroy & Hamer, 2003). Thus, malnutrition is unlikely to have caused scoliosis in this experiment.

Tadpoles were, however, raised outdoors and exposed to full sunlight under a screening that transmits UVB. Many studies have documented that larvae of several amphibian species, including *R. sylvatica*, develop scoliosis after exposure to ambient or increased UVB (Worrest & Kimeldorf, 1976; Pahkala *et al.*, 2001; Weyrauch & Grubb, 2006; Van Uitregt *et al.*, 2007; Croteau *et al.*, 2008a,b). In addition, we found a lower frequency of scoliosis in tadpoles raised under a thick shade cloth (60% shade) than in those under a window screening that transmits more sunlight (M. Michel, unpubl. data). Therefore, of all the known causes of scoliosis, exposure to UVB radiation seems the most probable, although future studies are needed.

If UVB was the cause of scoliosis, then the behavioural responses of tadpoles to predation risk and tadpole density can explain how these environmental factors affected scoliosis development. Chemical cues from predators induce reductions in larval amphibian activity (e.g. Lawler, 1989; Van Buskirk, 2002; reviewed in Anholt & Werner, 1999; Benard, 2004), spatial avoidance of predators (e.g. Van Buskirk & Schmidt, 2000) and increased use of benthic leaf litter as refugia (e.g. Van Buskirk, 2001), whereas the increased competitor density induces an increase in overall activity (Relyea, 2002). UVB attenuates with depth, and the degree of this attenuation depends on several factors. For example, in various wetland ponds of northern Minnesota and Wisconsin, 99% of UVB was attenuated within depths of 0.05–0.2 m (Peterson *et al.*, 2002). Therefore, predation may decrease overall exposure to UVB by promoting sedentary, cryptic behaviour. Similarly, by promoting greater activity, increased conspecific densities may increase overall exposure to UVB. These hypotheses are supported by the positive correlation between overall tadpole activity and proportion of scoliosis

(Fig. 3). However, because the strength of this relationship was weak, other changes to tadpole behaviour, such as the proportion of time spent at the bottom of the tubs, may be as important. Interestingly, a recent study (Alton *et al.*, 2010) reported that predation risk increased mortality because of UVB in tadpoles of the striped marsh frog (*Limnodynastes peronii* Duméril and Bibron). However, because the experimental containers had only 2 cm water, there was little opportunity for a predator-induced habitat shift of tadpoles to deeper water, where UVB is reduced.

Scoliotic tadpoles were approximately 50% more susceptible to predators, possibly as a result of reduced swimming ability. Burst swimming speed is important for determining prey escape from a predator attack (Lima & Dill, 1990), especially for tadpoles (Dayton *et al.*, 2005). Similarly, Hopkins *et al.* (2000) found that scoliotic bullfrog (*Rana catesbeiana* Shaw) tadpoles swam approximately 34% slower than normal tadpoles; however, no predation trials were conducted. In contrast, Romansic *et al.* (2009) found that tail deformities did not increase the susceptibility of cascades frog (*Rana cascadae* Slater) tadpoles to predation from newts (*Taricha granulosa* Skilton). This discrepancy may reflect differences between the foraging abilities of *Dytiscus* and the faster swimming *Taricha*.

Our results have interesting implications for the prevalence of tadpole scoliosis in natural ponds. Ponds range from small, ephemeral water bodies containing few predators to larger, more permanent ponds containing a higher diversity and density of predators (Wellborn, Skelly & Werner, 1996). Tadpole activity often reflects this gradient, as tadpoles in smaller ponds are usually more active and have faster growth and development, while tadpoles in larger ponds are less active, presumably reducing predation (Wellborn *et al.*, 1996; Richardson, 2001; Van Buskirk, 2002). If activity and habitat selection are important mechanisms underlying the development of scoliosis, then it is expected that the latter would be more prevalent in the small, ephemeral ponds than the larger ponds. In addition, the predators in these larger ponds would preferentially consume scoliotic tadpoles.

This study demonstrates that scoliosis affects the performance and fitness of tadpoles and that its development depends on environmental conditions.

In addition, this study suggests that behavioural plasticity of tadpoles in response to these environments may affect their susceptibility to developing scoliosis. Because behaviourally plastic responses are widespread among many aquatic species (Tollrian & Harvell, 1999), they may be an important mechanism in determining the vulnerability of species to environmental stressors in many freshwater habitats.

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References

- Alton L.A., Wilson R.S. & Franklin C.E. (2010) Risk of predation enhances the lethal effects of UV-B in amphibians. *Global Change Biology*, **16**, 538–545.
- Alvarez R., Honrubia M.P. & Herráez M.P. (1995) Skeletal malformations induced by the insecticides ZZ-Aphox® and Folidol® during larval development of *Rana perezi*. *Archives of Environmental Contamination and Toxicology*, **28**, 349–356.
- Anholt B.R. & Werner E.E. (1999) Density-dependent consequences of induced behavior. In: *The Ecology and Evolution of Inducible Defenses* (Eds R. Tollrian & D. Harvell), pp. 218–230. Princeton University Press, Princeton, NJ.
- Ankley G.T., Tietge J.E., DeFoe D.L., Jensen K.M., Holcombe G.W., Durhan E.J. *et al.* (1998) Effects of ultraviolet light and methoprene on survival and development of *Rana pipiens*. *Environmental Toxicology and Chemistry*, **17**, 2530–2542.
- Ankley G.T., Degitz S.J., Diamond S.A. & Tietge J.E. (2004) Assessment of environmental stressors potentially responsible for malformation in North American anuran amphibians. *Ecotoxicology and Environmental Safety*, **58**, 7–16.
- Bancroft B.A., Baker N.J. & Blaustein A.R. (2008) A meta-analysis of the effects of ultraviolet B radiation and its synergistic interactions with pH, contaminants, and disease on amphibian survival. *Conservation Biology*, **22**, 987–996.
- Benard M.F. (2004) Predator-induced phenotypic plasticity in organisms with complex life histories. *Annual Review of Ecology, Evolution, and Systematics*, **35**, 651–673.
- Blaustein A.R. & Kiesecker J.M. (2002) Complexity in conservation: lessons from the global decline of amphibian populations. *Ecology Letters*, **5**, 597–608.
- Browne R.K., Pomeroy M. & Hamer A.J. (2003) High density effects on the growth, development and survival of *Litoria aurea* tadpoles. *Aquaculture*, **215**, 109–121.
- Cech J.J. Jr, Wilson B.W. & Crosby D.G. (1998) *Multiple Stresses in Ecosystems*. Lewis Publishers, Boca Raton.
- Chen T.-H., Gross J.A. & Karasov W.H. (2006) Sublethal effects of lead on northern leopard frog (*Rana pipiens*) tadpoles. *Environmental Toxicology and Chemistry*, **25**, 1383–1389.
- Collins J.P. & Storfer A. (2003) Global amphibian declines: sorting the hypotheses. *Diversity and Distributions*, **9**, 89–98.
- Crawley M.J. (2005) *Statistics: An Introduction Using R*. John Wiley and Sons, Hoboken.
- Croteau M.C., Davidson M.A., Lean D.R.S. & Trudeau V.L. (2008a) Global increases in ultraviolet B radiation: potential impacts on amphibian development and metamorphosis. *Physiological and Biochemical Zoology*, **81**, 743–761.
- Croteau M.C., Martyniuk C.J., Trudeau V.L. & Lean D.R.S. (2008b) Chronic exposure of *Rana pipiens* tadpoles to UVB radiation and the estrogenic chemical 4-tert-octylphenol. *Journal of Toxicology and Environmental Health – Part A*, **71**, 134–144.
- Daszak P., Berger L., Cunningham A.A., Hyatt A.D., Green D.E. & Speare R. (1999) Emerging infectious diseases and amphibian population declines. *Emerging Infectious Diseases*, **5**, 735–748.
- Dayton G.H., Saenz D., Baum K.A., Langerhans R.B. & DeWitt T.J. (2005) Body shape, burst speed and escape behavior of larval anurans. *Oikos*, **111**, 582–591.
- Decaestecker E., De Meester L. & Ebert D. (2002) In deep trouble: habitat selection constrained by multiple enemies in zooplankton. *Proceedings of the National Academy of Sciences*, **99**, 5481–5485.
- Gosner K.L. (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, **16**, 183–190.
- Hisaoka K.K. & List J.C. (1957) The spontaneous occurrence of scoliosis in larvae of *Rana sylvatica*. *Transactions of the American Microscopical Society*, **76**, 381–387.
- Hopkins W.A., Congdon J. & Ray J.K. (2000) Incidence and impact of axial malformations in larval bullfrogs (*Rana catesbeiana*) developing in sites polluted by a

- coal-burning power plant. *Environmental Toxicology and Chemistry*, **19**, 862–868.
- Johnson P.T.J., Preu E.R., Sutherland D.R., Romansic J.M., Han B. & Blaustein A.R. (2006) Adding infection to injury: synergistic effects of predation and parasitism on amphibian malformations. *Ecology*, **87**, 2227–2235.
- Lannoo M.J. (2005) *Amphibian Declines: The Conservation Status of U.S. Amphibians*. University of California Press, Berkeley.
- Lawler S.P. (1989) Behavioural responses to predators and predation risk in four species of larval anurans. *Animal Behaviour*, **38**, 1039–1047.
- Laws A.N., Frauendorf T.C., Gómez J.E. & Algaze I.M. (2009) Predators mediate the effects of a fungal pathogen on prey: an experiment with grasshoppers, wolf spiders and fungal pathogens. *Ecological Entomology*, **34**, 702–708.
- Lima S.L. & Dill L.M. (1990) Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, **68**, 619–640.
- Martínez I.P., Álvarez R., Herráez I. & Herráez M.P. (1992) Skeletal malformations in hatchery reared *Rana perezi* tadpoles. *Anatomical Record*, **233**, 314–320.
- Martínez I.P., Álvarez R., Herráez I. & Herráez M.P. (1996) Growth and metamorphosis of *Rana perezi* larvae in culture: effects of larval density. *Aquaculture*, **142**, 163–170.
- Pahkala M., Laurila A. & Merilä J. (2001) Carry-over effects of ultraviolet-B radiation on larval fitness in *Rana temporaria*. *Proceedings of the Royal Society B – Biological Sciences*, **268**, 1699–1706.
- Peterson G.S., Johnson L.B., Axler R.P. & Diamond S.A. (2002) Assessment of the risk of solar ultraviolet radiation to amphibians. II. *In situ* characterization of exposure in amphibian habitats. *Environmental Science and Technology*, **36**, 2859–2865.
- R Core Development Team (2008). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.
- Relyea R.A. (2002) Competitor-induced plasticity in tadpoles: consequences, cues, and connections to predator-induced plasticity. *Ecological Monographs*, **72**, 523–540.
- Relyea R.A. (2003) Predator cues and pesticides: a double dose of danger for amphibians. *Ecological Applications*, **13**, 1515–1521.
- Relyea R.A. & Mills N. (2001) Predator-induced stress makes the pesticide carbaryl more deadly to gray treefrog tadpoles (*Hyla versicolor*). *Proceedings of the National Academy of Sciences*, **98**, 2491–2496.
- Richardson J.M.L. (2001) A comparative study of activity levels in larval anurans and response to the presence of different predators. *Behavioral Ecology*, **12**, 51–58.
- Romansic J.M., Waggener A.A., Bancroft B.A. & Blaustein A.R. (2009) Influence of ultraviolet-B radiation on growth, prevalence of deformities, and susceptibility to predation in Cascades frog (*Rana cascadae*) larvae. *Hydrobiologia*, **624**, 219–233.
- Steiner U.K. & Van Buskirk J. (2008) Environmental stress and the costs of whole-organism phenotypic plasticity in tadpoles. *Journal of Evolutionary Biology*, **21**, 97–103.
- Thiemann G.W. & Wassersug R.J. (2000) Patterns and consequences of behavioural responses to predators and parasite in *Rana* tadpoles. *Biological Journal of the Linnean Society*, **71**, 513–528.
- Tollrian R. & Harvell C.D. (1999) *The Ecology and Evolution of Inducible Defenses*. Princeton University Press, Princeton, NJ.
- Van Buskirk J. (2001) Specific induced responses to different predator species in anuran larvae. *Journal of Evolutionary Biology*, **14**, 482–489.
- Van Buskirk J. (2002) A comparative test of the adaptive plasticity hypothesis: relationships between habitat and phenotype in anuran larvae. *American Naturalist*, **160**, 87–102.
- Van Buskirk J. & Schmidt B.R. (2000) Predator-induced phenotypic plasticity in larval newts: trade-offs, selection, and variation in nature. *Ecology*, **81**, 3009–3028.
- Van Der Veen I.T. (2005) Costly carotenoids: a trade-off between predation and infection risk? *Journal of Evolutionary Biology*, **18**, 992–999.
- Van Uitregt V.O., Wilson R.S. & Franklin C.E. (2007) Cooler temperatures increase sensitivity to ultraviolet B radiation in embryos and larvae of the frog *Limnodynastes peronii*. *Global Change Biology*, **13**, 1114–1121.
- Von Westernhagen H. (1988) Sublethal effects of pollutants on fish eggs and larvae. In: *Fish Physiology, Volume XI: The Physiology of Developing Fish, Part A: Eggs and Larvae* (Eds W.S. Hoar & D.J. Randall), pp. 253–346. Academic Press, New York.
- Wellborn G.A., Skelly D.K. & Werner E.E. (1996) Mechanisms creating community structure across a freshwater habitat gradient. *Annual Review of Ecology and Systematics*, **27**, 337–363.
- Weyrauch S.L. & Grubb T.C. Jr (2006) Effects of the interaction between genetic diversity and UV-B radiation on wood frog fitness. *Conservation Biology*, **20**, 802–810.
- Worrest R.C. & Kimeldorf D.J. (1976) Distortions in amphibian development induced by ultraviolet-B enhancement (290–315 nm) of a simulated solar spectrum. *Photochemistry and Photobiology*, **24**, 377–382.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Full statistical results for ANODEV.

Appendix S2. Power tests for ANODEV.

Appendix S3. Relationship between growth rates and scoliosis frequency.

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