



BRILL

No evidence of selection by predators on tadpole boldness

Author(s): Bradley E. Carlson and Tracy Langkilde

Source: *Behaviour*, Vol. 151, No. 1 (2014), pp. 23-45

Published by: Brill

Stable URL: <https://www.jstor.org/stable/24526054>

Accessed: 12-08-2021 22:03 UTC

REFERENCES

Linked references are available on JSTOR for this article:

https://www.jstor.org/stable/24526054?seq=1&cid=pdf-reference#references_tab_contents

You may need to log in to JSTOR to access the linked references.

JSTOR is a not-for-profit service that helps scholars, researchers, and students discover, use, and build upon a wide range of content in a trusted digital archive. We use information technology and tools to increase productivity and facilitate new forms of scholarship. For more information about JSTOR, please contact support@jstor.org.

Your use of the JSTOR archive indicates your acceptance of the Terms & Conditions of Use, available at <https://about.jstor.org/terms>



JSTOR

Brill is collaborating with JSTOR to digitize, preserve and extend access to *Behaviour*



No evidence of selection by predators on tadpole boldness

Bradley E. Carlson^{a,b,*} and Tracy Langkilde^{a,b}

^a Department of Biology, The Pennsylvania State University, University Park, PA, USA

^b Intercollege Graduate Degree Program in Ecology, The Pennsylvania State University, University Park, PA, USA

* Corresponding author's e-mail address: bec169@psu.edu

Accepted 6 August 2013; published online 2 October 2013

Abstract

Animals typically exhibit adaptive behaviors that reduce their risk of predation. The term 'boldness' describes individual variation in the propensity to exhibit risk-reducing behavior and is the subject of much research attention. Predators should select against boldness, and this has been supported by empirical studies and behavioral ecology theory. We tested whether a standardized assay of three boldness-associated behaviors in wood frog (*Lithobates sylvaticus*) tadpoles predicted survival when faced with a predator. Tadpole behavior was assayed in an open field and then tadpoles were placed, in pairs, in an enclosure with a predator (newt or larval dragonfly). Survival did not depend on differences in measured boldness, and this result held when we accounted for interactions between different boldness behaviors and between behavior and size or predator identity. The absence of selection by predators against bolder tadpoles is counterintuitive and inconsistent with our understanding of the behavioral ecology of these animals. Two possible explanations are offered for this result. First, selection against boldness may be minimized by other phenotypic traits, such as escape ability. Alternatively, the potential lack of consistency between standardized boldness assays and natural encounters with predators may limit our capacity to study the evolution of boldness, cautioning against this approach. These results highlight the complexities of the relationships between behavioral traits and fitness and the challenges associated with their study.

Keywords

anti-predator behavior, boldness, *Lithobates sylvaticus*, natural selection, predation, *Rana sylvatica*, tadpoles.

1. Introduction

Predation is a common source of selection on behavioral traits (Smith & Blumstein, 2007; Calsbeek & Cox, 2010). Individuals, populations and

species experiencing predation pressure typically perform behaviors that reduce the probability of being detected, captured, and/or consumed by predators (Lima & Dill, 1990). These behavioral mechanisms for avoiding predation can be fixed (e.g., nocturnality) or plastically respond to temporal or spatial variation in predation risk (e.g., refraining from foraging when predators are present; Lima & Dill, 1990). A general paradigm in behavioral ecology is that greater activity rates are positively associated with both energy intake (i.e., foraging) and predation mortality (due to increased encounters with and detection by predators; Werner & Anholt, 1993). Hence, there is typically a trade-off between growth and mortality, mediated by activity (Werner & Anholt, 1993). Many species thus plastically alter activity in response to cues that indicate the probable presence of a predator, reducing foraging or other activities only until cues of the threat are diminished (Ball & Baker, 1996). Animals can also behaviorally respond to cues of predator presence by avoiding areas where predation risk is higher ('spatial avoidance'; Lima & Dill, 1990; Sergio et al., 2003). This can, in part, provide a solution to the growth/mortality trade-off by enabling the prey to continue foraging by selecting low risk habitat patches (Sih, 1982). Activity reductions and directional movement away from predators are ubiquitous and their survival value under most circumstances is readily apparent.

Within a single species, individuals often substantially and consistently vary in their responsiveness to threats; for example, some individuals may reduce activity levels to a lesser degree or exhibit lower spatial avoidance of predators than conspecifics (Wilson et al., 1993). This variability among individuals in risk-taking propensity (whether in a predation context or otherwise) is commonly labeled the 'boldness–shyness' axis of behavioral variation and is a focus of research on behavioral differences among conspecifics and species (Wilson et al., 1994; Gosling, 2001; Sih et al., 2004; Réale et al., 2007). Shyer animals, as opposed to bolder ones, tend to respond more strongly to predators by engaging in behaviors that generally decrease the risk of predation, and we will henceforth use 'boldness' to refer to reduced performance of any behaviors that decrease risk (e.g., activity level in the presence of predators or spatial avoidance; Réale et al., 2007). Such variation provides the substrate upon which phenotypic selection can act (Houle, 1992). We expect predators to shape behavioral phenotypes within populations, reducing boldness through plastic changes in potentially risky behaviors and/or through selection against bolder phenotypes. Where predation

risk is greater, survival selection ought to favor reduced boldness, though ultimately the fitness consequences of a trait will depend on the balance with other simultaneously-acting selective agents (e.g., resource acquisition or mate competition; Schluter et al., 1991). Comparisons of the behavior of wild populations exposed to divergent predation regimes provide support for the evolutionary prediction of reduced boldness where predation risk is high (e.g., Åbjörnsson et al., 2004; Bell, 2005; Van Buskirk & Arioli, 2005; Dalesman et al., 2007; Brydges et al., 2008; Smee & Weissburg, 2008) though the opposite pattern also can occur (e.g., Brown et al., 2005; Urban, 2007; Riesch et al., 2009), or there can be no relationship between predation regime and boldness (e.g., Laurila, 2000; Messler et al., 2007). Empirical studies directly comparing predation of species or individuals typically confirm that bolder animals are indeed more susceptible to predators (Lawler, 1989; Downes, 2002; Smith & Blumstein, 2007; Carter et al., 2010), but others have demonstrated no relationship (Bell & Sih, 2007) or a positive relationship (Godin & Davis, 1995; Réale & Festa-Bianchet, 2003; Smith & Blumstein, 2010) between boldness and survival. These discrepancies may be due, in part, to the fact that selection on boldness may depend on the behavior of individual predators (Altwegg, 2003; Smith & Blumstein, 2010; Pruitt et al., 2012). Further study of selection on boldness with many species and under different conditions will better enable us to construct a general framework for understanding the variable nature of predator-mediated selection on boldness.

However, there are at least two reasons that selection against boldness may not occur. First, a number of behaviors and other phenotypic traits will jointly influence susceptibility to predation. Though a particular isolated behavior may increase risk, this consequence may be compensated for by other traits (Lind & Cresswell, 2005). For instance, higher activity rates may increase detection by predators, but those individuals that are more capable of deterring or escaping predators may be more active due to their reduced vulnerability (Smith & Blumstein, 2010). Second, a particular measured boldness-associated behavior may not be under selection in a given situation. ‘Boldness’ is an underlying trait manifested in multiple behaviors that will vary depending on the species and context, and is often quantified by measuring selected behaviors under artificially staged conditions in boldness assays (Bell et al., 2009). These assays may be implemented in the field (e.g., docility of bighorn sheep during handling by humans; Reale & Festa-Bianchet, 2003) or in the laboratory (e.g., emergence from shelters in guppies; Harris

et al., 2010) and often produce individually-consistent boldness scores (Bell et al., 2009) that have, in some cases, been correlated with survival (Smith & Blumstein, 2007). The assumption that behaviors measured in contrived assays are consistent with those expressed under natural predation risk may be untenable in some cases, especially under laboratory conditions (Brown et al., 2005). If behavior in assays and natural conditions is sufficiently decoupled, boldness assays may have little ecological relevance and therefore fail to predict survival rates.

The larvae of frogs and toads (tadpoles) are an excellent model system of behavioral interactions between predators and prey, and of predator-induced shifts in the behavior of the tadpole prey. Tadpoles are vulnerable to predatory insects, fish and aquatic salamanders, and typically respond to cues of predation risk by reducing foraging and swimming activity and spatially avoiding predators (e.g., Lawler, 1989; Skelly & Werner, 1990; Petranka & Hayes, 1998; Relyea & Werner, 1999; Eklov & Werner, 2000). These responses often vary among predator species, resulting in defenses that appear to match predator-specific risk (Relyea, 2001). Previous research has provided some evidence that activity in the presence of predators increases predation risk in tadpoles, both within (Skelly, 1994; Anholt & Werner, 1998; Van Buskirk & McCollum, 2000; Altwegg, 2003) and between species (Lawler, 1989). However, most of these studies manipulated behavior rather than testing innate behavioral differences (but see Van Buskirk & McCollum, 2000), and we know of no studies that examined selection on spatial avoidance of predators by tadpoles. Furthermore, there is a rapidly growing body of research on individual variation in boldness and other personality traits in tadpoles (Beaty, 2012; Brodin et al., 2012; Koprivnikar et al., 2012; Wilson & Krause, 2012; Carlson & Langkilde, 2013b). These studies measured boldness under laboratory conditions using different methods that likely vary in ecological realism. For example, Carlson & Langkilde (2013b) quantified boldness as a reduced preference for the periphery of an open field, Wilson & Krause (2012) used shorter latencies to exit a simulated refuge as indicative of greater boldness, and Beaty (2012) measured activity changes in response to chemical, visual, and water-disturbance cues of predation risk. These studies did not evaluate whether the measured behaviors are associated with predation risk, which is important for understanding their ecological relevance.

We tested whether individual differences in boldness of wood frog tadpoles (*Lithobates sylvaticus* (= *Rana sylvatica*)), as measured in standardized assays, were associated with susceptibility to predation. We hypothesized that there would be greater predation on tadpoles that were more active when exposed to predator cues and showed less avoidance of areas of greater risk in laboratory boldness assays. Alternatively, behaviors measured in the assays may not predict survival during predator encounters, either because the typical behaviors measured may inadequately characterize the many factors that determine predation risk or due to a lack of consistency in behavior between the two environments.

2. Material and methods

2.1. Subjects

Wood frogs (*Lithobates sylvaticus*) are abundant, predominantly terrestrial inhabitants of forests across Canada and the northeastern United States (Redmer & Trauth, 2005). They breed primarily in ephemeral ponds early in the spring, where the tadpoles develop over two to three months (Redmer & Trauth, 2005). The most common predators of wood frog tadpoles across their range and within the study area include salamanders (particularly newts (*Notophthalmus* spp.) and larval *Ambystoma* spp.) and aquatic insects, such as predaceous diving beetles (Coleoptera: Dytiscidae), backswimmers (Heteroptera: Notonectidae), and damer dragonfly nymphs (Odonata: Aeshnidae) (Redmer & Trauth, 2005; Michel, 2011; B.E.C., pers. obs.). Behavioral responses of *L. sylvaticus* to chemical cues of these and other predators have been extensively studied; they exhibit reduced activity and spatial avoidance of cues, as is typical of anuran larvae (Petranka & Hayes, 1998; Relyea, 2001).

We collected 120 partial *L. sylvaticus* egg masses (approximately 50–200 eggs each) from 15 ponds (8 masses/pond) in Pennsylvania during March 2012. We hatched the eggs outdoors in plastic containers with three liters of water and randomly removed a subset of the tadpoles for use in other experiments after they began feeding (stage 25; Gosner, 1960); all clutches reached this stage within a 13-day period. The remainder were transferred to two 1100-liter round cattle tanks filled with well water, to which we had previously added leaf litter, rabbit chow, and an inoculate of pond water to

simulate natural pond conditions and food resources (Relyea, 2002). Tadpoles from all clutches were mixed together in these cattle tanks, so we were not able to account for clutch or population of origin in the analysis. When most tadpoles were between stages 25 and 30 (early in hindlimb development; Gosner, 1960), we began haphazardly selecting tadpoles each day from these tanks for use in behavioral assays and selection experiments. We subsequently housed these tadpoles in smaller groups (approx. 30 tadpoles) for several days in tubs (L × W × D 40 × 26 × 18 cm) with 9 liters of cattle tank water and excess food and leaf litter. This allowed them to forage ad libitum prior to trials, as the initial density of tadpoles in the cattle tanks appeared to result in high competition for food.

The day before performing a set of trials, we removed tadpoles from these tubs and randomly-selected half for marking with methylene blue, a common biological stain that has been used effectively to mark tadpoles. Because marking with methylene blue was recently reported to affect the behavioral responses of tadpoles to predator cues and increase predation risk (Carlson & Langkilde, 2013a), we controlled for these marking effects statistically (below). It was not within the purview of this study, however, to determine whether behavioral differences among tadpoles were intrinsic or the result of experience, state (e.g., hunger), or marking effects. We marked tadpoles by keeping them overnight in tubs with six liters of a 0.00025% methylene blue solution at a density of 20–30 tadpoles per tub (Waldman, 1982); unmarked tadpoles were kept under identical conditions except for the absence of the stain. The marking procedure produced a faint blue hue in tadpole skin; the intestines and fecal matter (examined after euthanizing) were more distinctly colored. Densities during marking and in the housing tubs prior to marking varied among days, but all tadpoles assayed and exposed to predators together had been kept under the same conditions and randomly allocated to marking treatments. Excess amounts of algae and rabbit chow were provided to both groups. The behavior of individual tadpoles was assayed the day following marking.

2.2. Behavioral assays

To measure behaviors associated with boldness, we used a modified form of the open-field test, a common method of measuring behavioral traits such as activity, boldness, and exploration (Walsh & Cummins, 1976; Burns, 2008). In open-field tests, an individual animal is placed in an open and typically

novel space and its behavior observed and quantified as metrics of underlying behavioral tendencies (Walsh & Cummins, 1976). We specifically measured (1) the activity level when predator cues were present (hereafter ‘activity’), (2) the proportional change in activity level from baseline conditions (when predator cues were absent) after adding the predator cue (‘responsiveness’) and (3) avoidance of the location where predator cue was added (‘avoidance’). Our basic testing procedure consisted of placing one tadpole in an open-field, allowing it to acclimate to the testing environment, measuring its baseline activity level, adding the scent of a predator to one end, and then observing its behavior again. We assayed 20 tadpoles simultaneously (in different open-fields), which would later be paired in 10 predator selection trials (below). For each set of trials, ten marked and ten unmarked tadpoles were haphazardly selected from the tubs in which they had been kept overnight and were transported with a pipette to the center of separate open-field tubs. As open-fields we used $46 \times 14 \times 12$ cm (L \times W \times D) plastic tubs filled with 4.5 liters of water from a third cattle tank similar to those used for rearing the tadpoles (to minimize the olfactory novelty associated with the assay environment; Figure 1a). A plastic divider with a 5×7 cm hole covered in fiberglass mesh was used to section off 5 cm of both ends of the tub, leaving a central area of 36×14 cm in which the tadpoles were free to swim (Figure 1a). The dividers slowed the dissipation of predator cues injected into one of the ends (see below), thus maintaining a chemical gradient. Three lines

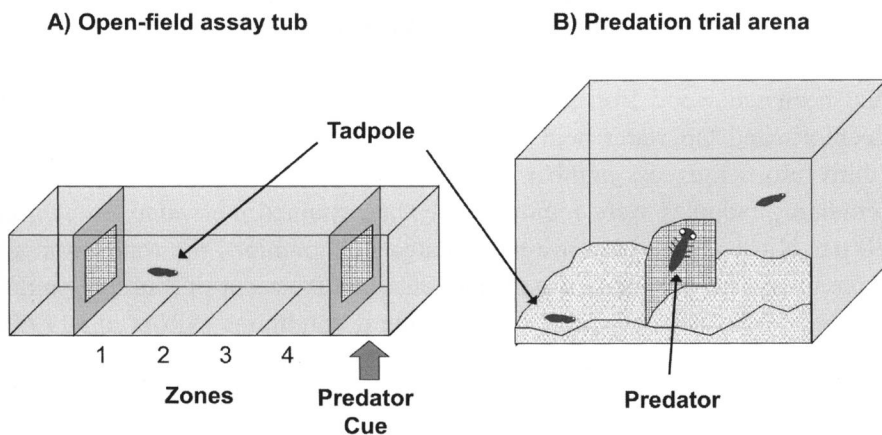


Figure 1. Diagram of experimental setups for (a) assaying behavior in an open-field and (b) predator selection trials. Not to scale.

drawn widthwise on the bottom of the tub marked four equal-sized zones (1–4), and we considered the zone nearest the end to which the predator cue would later be added to be zone 4 (Figure 1a).

We allowed the tadpoles to acclimate for 30 min after transferring them to the open-field tubs. The response to a novel environment or situation is characteristic of the ‘exploration-avoidance’ axis of behavioral variation. Since we were interested in boldness, it was necessary to allow the tadpoles to become acclimated and resume normal behavioral patterns. Initial pilot tests indicated that behavior remained consistent for at least two hours after this 30-min acclimation period, suggesting they were likely no longer in an exploratory phase. This acclimation period is also consistent with other studies in this species (Koprivnikar et al., 2012). After acclimation, we noted whether each tadpole was still or moving once per minute for 30 min and calculated the proportion of time spent active. The observer was seated next to the open-field tubs, with minimal movement needed to view the tadpoles. The tadpoles did not exhibit stereotypical startle responses when observations were made, suggesting that the observer was not disturbing them. We cannot rule out, however, that the tadpoles were aware of the presence of the observer and would have been more active if he had been absent.

We then added 15 ml of predator cue behind the mesh divider near zone 4. Tadpoles exhibit strong behavioral, morphological, and physiological responses to olfactory cues of conspecifics being consumed by predators (Fraker et al., 2009). We prepared a predation cue solution by collecting water in which larval dragonflies (all *Anax junius*, except for a single *A. longipes*) and adult eastern red-spotted newts (*Notophthalmus v. viridescens*) had been fed wood frog tadpoles ad libitum. Three tubs with 3 liters of dechlorinated tap water held either 17 dragonflies (2 tubs), or five newts (third tub). An excess number of tadpoles were added and, after 20 h, the remaining tadpoles were removed. We then strained the water through an 80 μm Nitex mesh to remove particulates and combined the water from all three tubs. This produced water that contained the scent of multiple predators, as would occur typically under natural conditions (Sih et al., 1997), though it was likely dominated by the predators with the highest rate of consumption in this experiment (*A. junius*; B.E.C., pers. obs.). After thorough mixing, the water was stored frozen in 200-ml portions and thawed immediately prior to use, preventing natural degradation of the cue (Ferrari et al., 2007). We acknowledge that this procedure cannot be used to repeatedly

produce chemical cues of the same concentration and composition; however, the single well-mixed batch we used for all behavioral assays ensured that all tadpoles in this experiment were responding to similar cues.

After injecting the predator cue into one end of the open field tub, we waited 5 min to allow the cue to begin to disperse and establish a gradient. We then continued observations of the tadpoles once per minute for 30 min, noting whether they were moving and which zone they were occupying. In addition to the proportion of time spent moving, we calculated a spatial avoidance score for each tadpole by averaging the zone location scores over the 30 trials. A spatial avoidance score of 1 thus indicates complete avoidance of the predator cue, and a score of 4 indicates a strong preference for the cue. We thoroughly cleaned the open-field tubs with pressurized water before reusing in further trials.

2.3. Predator selection experiments

Immediately after completing behavioral assays of 20 tadpoles, we paired tadpoles (one marked, one unmarked) for use in the predator selection experiments. Pairs were chosen to minimize size differences between tadpoles and to maximize the contrast in behavior (Watkins, 1996). This was done by visual comparison of size and a cursory examination of the recorded data from the open-field assays. Given the limited number of tadpoles in each set of assays, we were not always able to achieve this goal, resulting in some pairings of tadpoles of different sizes and/or with similar open-field behavior. We prioritized establishing pairs with dissimilar behavior, as size differences were minor. We noted those cases in which one tadpole was visibly larger for inclusion as a covariate in analyses.

We transferred pairs of tadpoles to each of ten $53 \times 37 \times 33$ cm (L \times W \times D) tubs with an uneven substrate layer of approx. 2–3 cm of mixed sand and pea gravel and approximately 10 cm of water (Figure 1b). A U-shaped 11×30 cm plastic mesh with large (2.5 cm) holes was placed upright in the gravel to provide structure to the environment and a perching site for predators (Figure 1b). The water originated from the same cattle tank that was used for the behavioral assays, but the tubs had housed either an adult male newt (five tubs) or a late-instar *A. junius* dragonfly nymph (remaining five tubs) for at least 48 h prior to the trials. The same individual predators were held in the same tubs and were reused over the course of the experiment. We fed the predators one wood frog tadpole daily (usually the tadpole eaten during the

selection experiments) and, therefore, the tubs contained the olfactory cues of the predators themselves as well as consumed and digested conspecific tadpoles. We included both predator species in our selection experiment because different foraging behaviors among and within predator species may affect the fitness consequences of prey behavior (Altwegg, 2003). Some behaviors may make prey less susceptible to one predator while having no effect on, or even increasing, risk of predation by another species or individual (Altwegg, 2003; Pruitt et al., 2012). Dragonfly nymphs are typically characterized as sit-and-wait, ambush predators that depend primarily on visual cues for predator detection while newts tend to actively move around their environment and may use both olfactory and visual cues to locate prey. Our design allows us to test whether selection on boldness differs between these two disparate predators.

To commence the predator selection trials, we temporarily removed the predators from the predator tubs, introduced the paired tadpoles, and added 10 ml of predator cue from a second batch prepared in a similar manner to that used in the behavioral assays. The cue was added to a standardized location and served to ensure the presence of 'fresh' predator cues (as opposed to the cues from the most recent feeding of the predator about 24 h prior), as cues under natural conditions may degrade within a few hours (Ferrari et al., 2007). We allowed the tadpoles to acclimate undisturbed for 30 min, after which the predators (newt or dragonfly nymph) were returned to the tubs at the same location as we added the predator cue.

After adding the predators, we inspected each tub at least once every three minutes until one tadpole had been eaten or 90 min had passed, whichever occurred first. This was done by visually scanning the tubs, taking care not to disturb either predators or prey. We did not notice any changes in behavior in newts, dragonflies, or tadpoles when these observations were made. When only one tadpole could be found, or when a tadpole was seen to have been captured by the predator, the survivor was removed and held separately to later identify whether or not they were marked (enabling us to match identities of surviving and captured tadpoles to our behavioral data). We recorded the time until one tadpole was eaten, rounding up to the nearest three-minute mark. Trials in which neither tadpole was captured or both were completely consumed between observations were not used in analysis, resulting in a final sample size of 36 newt and 40 dragonfly nymph trials.

2.4. Statistical analysis

We used multiple logistic regression to assess the effects of the relative values of activity, responsiveness, and avoidance on the identity of the surviving tadpole, with a binomial response variable indicating whether the unmarked tadpole survived. For the behavioral measures, we used the difference in scores between the two tadpoles in each trial, subtracting the marked tadpole's score from the unmarked tadpole's score. For example, a positive difference in activity indicates that the unmarked tadpole was more active, which we would expect to increase the probability of it being consumed (a '0' in the logistic regression). Temperature differed among open-field tubs during the behavioral assays, both between days and between tubs tested the same day. To determine if we needed to account for the effect of water temperature, we regressed each variable against temperature during the assays. Temperature had a significant positive effect on activity ($F_{1,150} = 7.80$, $p = 0.006$) and a nearly significant negative effect on avoidance ($F_{1,150} = 3.20$, $p = 0.08$). We could not include temperature as a covariate in the regression because each tadpole had a unique temperature value, so we instead used the residuals from the above regressions as behavioral variables in later analyses. Temperature did not affect responsiveness ($F_{1,150} = 1.91$, $p = 0.66$) and we therefore used the raw values for this behavior.

We fit several logistic regression models in which different covariates and interactions between terms were included, as selection on a given trait may depend on other trait values (e.g., correlational selection; Brodie, 1992) or the predator species. In such cases, the fitness effects of a trait may be obscured if these interactions are not accounted for. Many such models could have been analyzed, but we restricted our investigation to five models that were chosen a priori. The 'reduced' model included the three behavioral scores as independent variables (Model 1; Table 1). In the second model, we also allowed two-way interactions between each of the three behaviors (Model 2; Table 1), which would permit us to test whether selection on one boldness behavior (e.g., activity) depended on another (e.g., avoidance). In Model 3, we included two-way interaction terms between each behavior and predator identity, allowing selection on behavioral traits to differ between the two predator types (Table 1). We also tested whether the size difference between paired tadpoles influenced survival by including a variable to denote whether the unmarked (1), marked (-1), or neither tadpole (0) was the larger of the pair, as well as two-way interactions between size

Table 1.

Summary of logistic regression models predicting survival of the unmarked tadpoles.

Model	Terms	Significance	Compared to Model 1
1 (Reduced)	Behavior	$p = 0.71$ ($\chi^2_3 = 1.39$)	–
2	Behavior \times behavior	$p = 0.51$ ($\chi^2_6 = 5.30$)	$p = 0.27$ ($\chi^2_3 = 3.91$)
3	Behavior \times predator	$p = 0.71$ ($\chi^2_7 = 4.61$)	$p = 0.52$ ($\chi^2_4 = 3.22$)
4	Behavior \times size	$p = 0.25$ ($\chi^2_7 = 9.03$)	$p = 0.11$ ($\chi^2_4 = 7.64$)
5	All two-way interactions	$p = 0.27$ ($\chi^2_{15} = 17.86$)	$p = 0.17$ ($\chi^2_{12} = 16.48$)

Behavior includes all three measures of boldness (activity, responsiveness and avoidance). All statistical tests are likelihood ratio tests (LRT) comparing fitted models to either a null model (to evaluate overall significance of all parameters) or Model 1 (to test marginal improvement in fit over the reduced model).

and each behavior (Model 4; Table 1). Finally, we fit a model including all main effects and two-way interactions as in Models 1–4, as well as between predator species and size (Model 5; Table 1). We tested the overall significance of each model using likelihood ratio tests (LRT) comparing it to the null (intercept-only) model, simultaneously assessing the significance of all independent variables. We also used LRT to compare Models 2–5 with the ‘reduced’ Model 1 to evaluate the contribution of interactions, predator identity, and size to model fits. Individual parameters in Model 1 were tested using the Wald statistic, and we evaluated the fit of models by performing a Hosmer–Lemeshow goodness-of-fit test. Examination of variance inflation factors (VIF) revealed that multicollinearity between independent variables is unlikely to have significantly impacted our conclusions (all VIF < 5.9, and <1.1 in Model 1). Because we always modeled the survival of the unmarked tadpole, the intercept of the logistic regression provided an estimate of the difference in survival probability of unmarked tadpoles (relative to the marked tadpole). We reported marking effects elsewhere (Carlson & Langkilde, 2013a) and do not consider them further here.

We chose to analyze the effects of relative boldness on survival because whether an individual or species is considered ‘bold’ is largely dependent on a frame of reference (i.e., the behavior of conspecifics or heterospecifics, respectively). However, we performed the same analyses as above (Models 1–5) using absolute values of boldness behaviors rather than differences between paired tadpoles (relative boldness). We used either all the marked or all the unmarked tadpoles in the analyses, to avoid issues of non-independence

of tadpoles in the same predator trial. These analyses yielded qualitatively similar results to those using the relative scores, and we therefore do not consider them further in this paper.

We conducted similar analyses using Cox proportional hazards survival analysis to evaluate whether the time until death was affected by behavior, because another similar study found significant effects of behavior on survival using this approach (Van Buskirk & McCollum, 2000). As this analysis yielded qualitatively similar results, we do not describe it further here.

Selection on prey behavior may depend on phenotypes of individual predators (Pruitt et al., 2012). Predators were reused in multiple trials (5–9 useable trials for each individual predator), allowing us to test whether the risk associated with bold behavior differed among individual predators. We did this with a generalized linear mixed model (GLMM) in which we allowed the effect of behavioral differences on survival to vary among individual predators (i.e., a random slopes model; Bolker et al., 2009); the fixed effects remained identical to Model 1. We tested the significance of each random slope with LRT, dividing p -values by 2 to prevent overly conservative tests caused by boundary effects (Bolker et al., 2009).

All analyses were conducted using program R v 2.15.0 (R Core Development Team, 2012) and two-tailed tests with $\alpha = 0.05$ significance level. The package ‘lme4’ was used to fit GLMMs (Bates et al., 2001).

3. Results

Behavioral assays revealed substantial variation in tadpole behavior (Figure 2), suggesting that there was ample opportunity for selection to act. In predation trials, predators usually consumed one tadpole within 30 min (Figure 3). Newts tended to pursue the tadpoles and required multiple attempts to capture prey, while dragonfly larvae were more successful and usually stationary, striking when approached (pers. obs.).

We found no evidence that relative boldness of two tadpoles influenced their probability of being preyed upon. The ‘reduced’ Model 1, in which we only included the three boldness behaviors as predictors of survival, did not fit significantly better than a null model ($\chi^2_3 = 1.41$, $p = 0.70$; Table 1). Accordingly, differences in activity ($\chi^2_1 = 1.16$, $p = 0.28$, odds ratio 95% confidence interval (CI) = [0.90, 1.03]; Figure 4a), responsiveness ($\chi^2_1 = 0.16$, $p = 0.69$, 95% CI = [0.55, 1.49]; Figure 4b), and avoidance ($\chi^2_1 =$

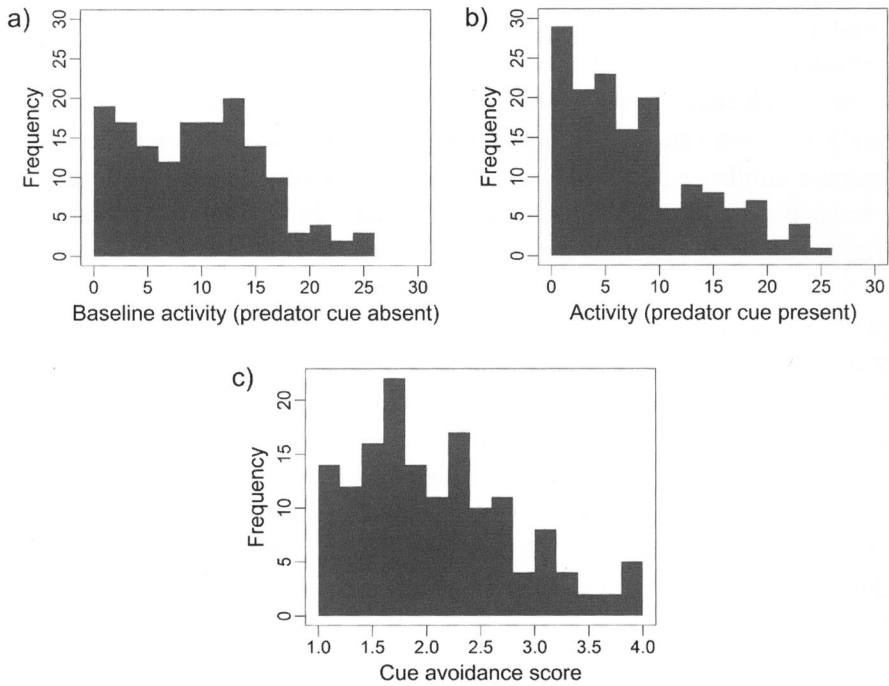


Figure 2. Distributions of behavioral scores for activity level in the (a) absence and (b) presence of predator cues, and (c) avoidance of predator cues. Activity levels represent the number of observations (out of 30) in which tadpoles were observed moving, while low numbers indicate avoidance of the predator cues.

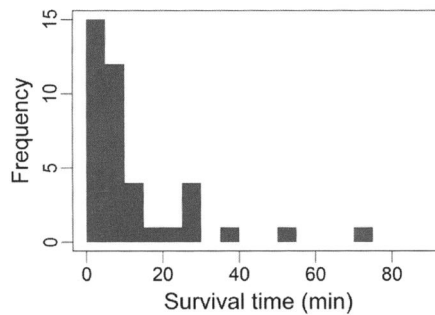
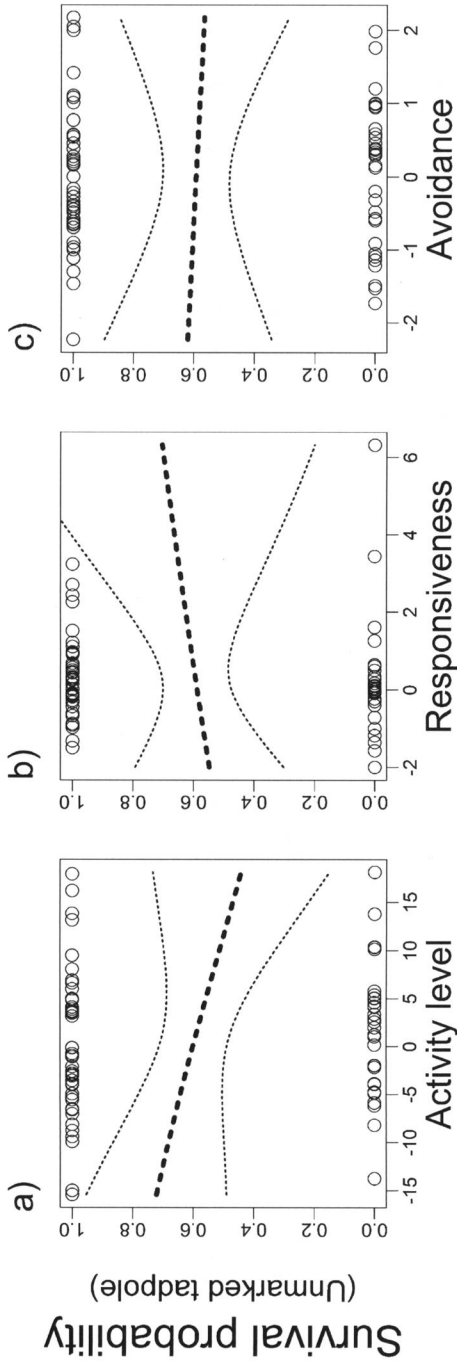


Figure 3. Distribution of durations of predator selection trials used in the analyses. Trials were terminated when one tadpole was captured or eaten.



Relative behavioral scores (Unmarked - marked)

Figure 4. Relationships between survival of focal tadpoles in predation trials and relative differences in boldness-related behavior between the focal tadpole and the conspecific with which it was paired during a trial. Behaviors are (a) activity level in the presence of predator cues, (b) responsiveness (change in activity after adding predator cues) and (c) spatial avoidance of predator cues. Lines indicate 95% confidence intervals of predicted survival probability using logistic regressions with a single independent variable.

0.03, $p = 0.87$, 95% CI = [0.70, 1.52]; Figure 4c) in the open-field assay did not affect survival in the predator selection experiment. Models 2–5 did not fit significantly better than Model 1 or the null model (Table 1), indicating that interactions between the behaviors and each other, predator species identity, and size were also non-significant.

The GLMM with random slopes revealed that there was no variation among individual predators in selection on differences in activity level ($\chi_4^2 = 0.55$, $p = 0.48$), responsiveness ($\chi_4^2 = 0.77$, $p = 0.47$) and avoidance ($\chi_4^2 = 1.72$, $p = 0.39$). This suggests that the effects of variation in boldness were consistent across individual predators and the lack of a clear relationship between behavior and predation risk was not due to interactions between predator and prey behavioral types.

4. Discussion

Contrary to predictions, tadpoles that were bolder (more active, less responsive to predator cues, or exhibited less avoidance of predator cues) during open-field behavioral assays were not less likely to survive in predation trials. This finding contradicts the theoretical expectation of a trade-off between activity and predation mortality (Werner & Anholt, 1993), as well as abundant empirical evidence of a positive relationship between predation risk and boldness in a number of species (Smith & Blumstein, 2007), including tadpoles (Skelly, 1994; Van Buskirk & McCollum, 2000). The lack of detectable selection on individual behaviors could not be attributed to correlational selection on trait combinations, as there were no significant interactions between behaviors or between behavior and size, or differential selection by the two predator species used. There was also no indication that selection on boldness differences varied among individual predators, as has been found in some cases (Smith & Blumstein, 2010; Pruitt et al., 2012). The 95% confidence intervals (CI) for the effects of responsiveness and avoidance of predator cues were large and centered on an odds ratio of 1, indicating it is unlikely that a reasonably increased sample size would alter the conclusion for these traits. Though the CI was narrower for activity (95% CI = [0.90, 1.03]), the estimated odds ratio was 0.96 and it is therefore unlikely to have anything but a small effect on survival, even with a larger sample size.

While there are few studies of selection on behavior in natural populations (Kingsolver et al., 2001), bolder individual animals often have reduced survival (reviewed in Smith & Blumstein, 2007), an effect likely attributable to

increased predation rates. These short-term evolutionary studies are corroborated by the products of many generations of selection: local adaptation to predator regimes often involves reduced activity levels or boldness (e.g., Van Buskirk & Arioli, 2005), and the innate behavioral response to cues of predation in many organisms (including tadpoles) is a reduction in activity and spatial avoidance (Lima & Dill, 1990). A large amount of direct evidence for predator-mediated selection against activity has been derived from laboratory studies or similarly controlled experiments on tadpoles and other prey organisms (e.g., Skelly, 1994; Anholt & Werner, 1998; Brodin & Johansson, 2008). There is thus voluminous support, both empirical and theoretical, for survival costs of boldness behaviors in general and in anuran larvae. In this study, however, an assay inspired by standard methods for measuring boldness in tadpoles was not predictive of the ability to survive predation.

Selection on boldness in this system may be fairly weak. The strongest evidence for selection by predators against higher activity in tadpoles has come from studies that associated interspecific differences in predation and activity (Lawler, 1989) or that manipulated activity of tadpoles by administering anesthetics (Skelly, 1994), controlling tadpole hunger levels (Anholt & Werner, 1998; Altwegg, 2003), or inducing activity reduction by increasing predator cues (Anholt & Werner, 1998). Fewer studies have exploited ‘natural’ intraspecific variation in behavior (e.g., Van Buskirk & McCollum, 2000), presumably largely due to the logistical constraint of performing many behavioral assays. Notably, Van Buskirk et al. (1997) found that predation rates within groups of wood frog tadpoles were not related to the level of activity in the groups. They suggested that this could be the consequence of stronger selection for traits that affect escape ability, such as tail muscle width and tail fin depth, than those that affect prey detection and encounter rates (i.e., movement; Van Buskirk et al., 1997). Similarly, selection on boldness in this study may have been limited by greater selection on other traits (Lind & Cresswell, 2005). Factors such as swim speed, that affect escape ability, may be especially important, as individuals with greater capacity to escape predators may act more boldly with little negative consequence (Smith & Blumstein, 2010). Together, these findings from tadpole systems suggest that selection by predators generally favors large reductions in activity, but that the among-individual behavioral variation that occurs naturally in many populations may be low enough that the response to selection on activity is often weak relative to other traits. A long history of selection may

have eliminated extreme individual variation in behavior, with the remaining variation producing small fitness differences that are overwhelmed by other more variable traits under selection.

Detecting selection on behavior is also complicated by the difficulty of accurately measuring the relevant behavioral traits. If behavior is measured in an environment different from that in which selection occurs — all attempts at ecological realism notwithstanding — it may poorly represent the behavior exhibited by the animal when it is actually exposed to the predator (Brown et al., 2005; but see, e.g., Smith & Blumstein, 2007). This contrasts with morphological traits, for instance, which remain essentially constant over short time periods. We would expect a stronger correlation between survival and boldness when tested in identical settings. Behavior in the open-field and selection arena environments may have been sufficiently decoupled as to make it unlikely for the assayed behavior to predict predation mortality. Nonetheless, the two settings we used were likely more similar than most standardized behavioral assays are to the environments in which the fitness consequences of the behavior are realized (Smith & Blumstein, 2007). As a noteworthy exception, Van Buskirk & McCollum (2000) conducted a similar study but found that more active tadpoles were more susceptible to predators, and indeed the tadpoles were assayed in the same environment to which the predators were introduced. The consistent setting likely produced more consistent behavior than would occur when assay and selection environments differ as in this and many other experiments.

Our results point to a complex and nuanced relationship between boldness and mortality, and suggest that greater boldness may not invariably lead to greater predation risk. Instead, the fitness consequences of boldness may depend strongly on context and, under some conditions, differences in boldness may effectively be selectively neutral with regard to predation. In particular, the among-individual variation in boldness that occurs naturally in populations might sometimes be small enough so as to be evolutionarily inconsequential. This could occur if selection sets limits to boldness such that no individuals exhibit extreme behavioral phenotypes that would cause a selective disadvantage. In effect, all individuals could be considered ‘bold’, for example, with respect to the range of possible phenotypic values, but with minor differences between individuals occupying the same general region of this behavioral spectrum. The emphasis on providing adaptive explanations for variation in boldness is usually predicated on enhanced predation risk in

bolder animals. Our work suggests that differences in boldness may sometimes be negligible for survival. This should motivate increased examination of alternative adaptive and non-adaptive paradigms for the evolution of variation in boldness.

Behavioral diversity has recently been increasingly recognized as an important factor in ecology (Sih et al., 2012; Wolf & Weissing, 2012) and boldness is a prominent axis of behavioral variation in animals (Réale et al., 2007), mediating interactions with predators and thus influencing ecological processes such as population dynamics and behaviorally-mediated trophic cascades (Schmitz et al., 1997). Selection by predators should favor risk-avoidance behaviors, a prediction borne out by examining plastic responses to predators (e.g., Relyea, 2001), comparing populations that differ in predation regime (e.g., Bell, 2005), and measuring predator attack rates on alternative behavioral phenotypes (Carter et al., 2010). Nonetheless, the role of predator-mediated selection in shaping animal boldness remains unclear, as demonstrated in this study. Future work should investigate limits to detecting selection on boldness by examining other traits that may compensate for the risks of high boldness and evaluating the consistency and realism of different assay methods. Moreover, increased attention should be given to cases in which selection by predators is not detected, as these apparent exceptions may ultimately prove to be more instructive than the findings typically reported.

Acknowledgements

We thank J. Newman, D. Knapp and G. Carlson for assistance throughout the experiment, and B. Chitterlings for helpful comments on the manuscript. Two anonymous reviewers also provided constructive feedback. Funding in support of this project was provided by an Animal Behavior Society research award and a Sigma Xi Grant-in-Aid of Research (both to B.E.C.). All animals were collected under scientific collecting permit No. 488 from the Pennsylvania Fish and Boat Commission and access to sites was granted by the Pennsylvania Game Commission, Bureau of Forestry, and Bureau of State Parks. The procedures used in this study were approved by the Penn State University Institutional Animal Care and Use Committee (permit No. 33469).

References

- Åbjörnsson, K., Hansson, L.-A. & Brönmark, C. (2004). Responses of prey from habitats with different predator regimes: local adaptation and heritability. — *Ecology* 85: 1859-1866.
- Altwegg, R. (2003). Hungry predators render predator-avoidance behavior in tadpoles ineffective. — *Oikos* 100: 311-316.
- Anholt, B.R. & Werner, E.E. (1998). Predictable changes in predation mortality as a consequence of changes in food availability and predation risk. — *Evol. Ecol.* 12: 729-738.
- Ball, S.L. & Baker, R.L. (1996). Predator-induced life history changes: antipredator behavior costs or facultative life history shifts? — *Ecology* 77: 1116.
- Bates, D., Maechler, M. & Bolker, B. (2011). lme4: linear mixed-effects models using Eigen and Eigenfaces. R Package Version 0.999375-42. — Available online at: <http://www.R-project.org> (accessed 3 August 2012).
- Beaty, L. (2012). The effect of body size and personality on anuran fitness. — Masters thesis, Texas Tech University, Lubbock, TX.
- Bell, A.M. (2005). Behavioural differences between individuals and two populations of stickleback (*Gasterosteus aculeatus*). — *J. Evol. Biol.* 18: 464-473.
- Bell, A.M. & Sih, A. (2007). Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). — *Ecol. Lett.* 15: 278-289.
- Bell, A.M., Hankison, S.J. & Laskowski, K.L. (2009). The repeatability of behaviour: a meta-analysis. — *Anim. Behav.* 77: 771-783.
- Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H. & White, J.-S.S. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. — *Trends Ecol. Evol.* 24: 127-135.
- Brodie III, E.D. (1992). Correlational selection for color pattern and antipredator behavior in the garter snake *Thamnophis ordinoides*. — *Evolution* 46: 1284-1298.
- Brodin, T. & Johansson, F. (2008). Conflicting selection pressures on the growth/predation-risk trade-off in a damselfly. — *Ecology* 85: 2927-2932.
- Brodin, T., Lind, M.I., Wiberg, M.K. & Johansson, F. (2012). Personality trait differences between mainland and island populations in the common frog (*Rana temporaria*). — *Behav. Ecol. Sociobiol.* 67: 135-143.
- Brown, C., Jones, F. & Braithwaite, V. (2005). In situ examination of boldness–shyness traits in the tropical poeciliid, *Brachyraphis episcopi*. — *Anim. Behav.* 70: 1003-1009.
- Brydges, N.M., Colegrave, N., Heathcote, R.J.P. & Braithwaite, V.A. (2008). Habitat stability and predation pressure affect temperament behaviours in populations of three-spined sticklebacks. — *J. Anim. Ecol.* 77: 229-235.
- Burns, J.G. (2008). The validity of three tests of temperament in guppies (*Poecilia reticulata*). — *J. Comp. Psychol.* 122: 344-356.
- Calsbeek, R. & Cox, R.M. (2010). Experimentally assessing the relative importance of predation and competition as agents of selection. — *Nature* 465: 613-616.
- Carlson, B.E. & Langkilde, T. (2013a). A common marking technique affects tadpole behavior and risk of predation. — *Ethology* 119: 167-177.

- Carlson, B.E. & Langkilde, T. (2013b). Personality traits are expressed in Bullfrog tadpoles during open-field trials. — *J. Herpetol.* 47: 378-383.
- Carter, A.J., Goldizen, A.W. & Tromp, S.A. (2010). Agamas exhibit behavioral syndromes: bolder males bask and feed more but may suffer higher predation. — *Behav. Ecol.* 21: 655-661.
- Dalesman, S., Rundle, S.D. & Cotton, P.A. (2007). Predator regime influences innate anti-predator behaviour in the freshwater gastropod *Lymnaea stagnalis*. — *Freshwater Biol.* 52: 2134-2140.
- Downes, S. (2002). Does responsiveness to predator scents affect lizard survivorship? — *Behav. Ecol. Sociobiol.* 52: 38-42.
- Eklov, P. & Werner, E.E. (2000). Multiple predator effects on size-dependent behavior and mortality of two species of anuran larvae. — *Oikos* 88: 250-258.
- Ferrari, M.C.O., Messier, F. & Chivers, D.P. (2007). Degradation of chemical alarm cues under natural conditions: risk assessment by larval woodfrogs. — *Chemoecology* 17: 263-266.
- Fraker, M.E., Hu, F., Cuddapah, V., McCollum, S.A., Relyea, R.A., Hempel, J. & Denver, R.J. (2009). Characterization of an alarm pheromone secreted by amphibian tadpoles that induces behavioral inhibition and suppression of the neuroendocrine stress axis. — *Horm. Behav.* 55: 520-529.
- Godin, J.-G.J. & Davis, S.A. (1995). Who dares, benefits: predator approach behaviour in the guppy (*Poecilia reticulata*) deters predator pursuit. — *Proc. Roy. Soc. Lond. B: Biol. Sci.* 259: 193-200.
- Gosling, S.D. (2001). From mice to men: what can we learn about personality from animal research? — *Psychol. Bull.* 127: 45-86.
- Gosner, K.L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. — *Herpetologica* 16: 183-190.
- Harris, S., Ramnarine, I.W., Smith, H.G. & Pettersson, L.B. (2010). Picking personalities apart: estimating the influence of predation, sex and body size on boldness in the guppy *Poecilia reticulata*. — *Oikos* 119: 1711-1718.
- Houle, D. (1992). Comparing evolvability and variability of quantitative traits. — *Genetics* 130: 195-204.
- Kingsolver, J.G., Hoekstra, H.E., Hoekstra, J.M., Berrigan, D., Vignieri, S.N., Hill, C.E., Hoang, A., Gibert, P. & Beerli, P. (2001). The strength of phenotypic selection in natural populations. — *Am. Nat.* 157: 245-261.
- Koprivnikar, J., Gibson, C.H. & Redfern, J.C. (2012). Infectious personalities: behavioural syndromes and disease risk in larval amphibians. — *Proc. Roy. Soc. Lond. B: Biol. Sci.* 279: 1544-1550.
- Laurila, A. (2000). Behavioural responses to predator chemical cues and local variation in antipredator performance in *Rana temporaria* tadpoles. — *Oikos* 88: 159-168.
- Lawler, S.P. (1989). Behavioural responses to predators and predation risk in four species of larval anurans. — *Anim. Behav.* 38: 1039-1047.
- Lima, S.L. & Dill, L.M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. — *Can. J. Zool.* 68: 619-640.

- Lind, J. & Cresswell, W. (2005). Determining the fitness consequences of anti-predation behaviour. — *Behav. Ecol.* 16: 945-956.
- Messler, A., Wund, M.A., Baker, J.A. & Foster, S.A. (2007). The effects of relaxed and reversed selection by predators on the antipredator behavior of the threespine stickleback, *Gasterosteus aculeatus*. — *Ethology* 113: 953-963.
- Michel, M.J. (2011). Spatial dependence of phenotype-environment associations for tadpoles in natural ponds. — *Evol. Ecol.* 25: 915-932.
- Petranka, J. & Hayes, L. (1998). Chemically mediated avoidance of a predatory odonate (*Anax junius*) by American toad (*Bufo americanus*) and wood frog (*Rana sylvatica*) tadpoles. — *Behav. Ecol. Sociobiol.* 42: 263-271.
- Pruitt, J.N., Stachowicz, J.J. & Sih, A. (2012). Behavioral types of predator and prey jointly determine prey survival: potential implications for the maintenance of within-species behavioral variation. — *Am. Nat.* 179: 217-227.
- R Development Core Team (2012). R: a language and environment for statistical computing, 2.15.0 edn. — R Foundation for Statistical Computing, Vienna, available online at: <http://www.R-project.org> (accessed 3 August 2012).
- Réale, D. & Festa-Bianchet, M. (2003). Predator-induced natural selection on temperament in bighorn ewes. — *Anim. Behav.* 65: 463-470.
- Réale, D., Reader, S.M., Sol, D., McDougall, P.T. & Dingemanse, N.J. (2007). Integrating animal temperament within ecology and evolution. — *Biol. Rev.* 82: 291-318.
- Redmer, M. & Trauth, S. (2005). *Rana sylvatica* LeConte, 1825: wood frog. — In: *Amphibian declines: the conservation status of United States species* (Lannoo, M., ed.). University of California Press, Berkeley, CA, p. 590-593.
- Relyea, R.A. (2001). Morphological and behavioral plasticity of larval anurans in response to different predators. — *Ecology* 82: 523-540.
- Relyea, R.A. (2002). Competitor-induced plasticity in tadpoles: consequences, cues, and connections to predator-induced plasticity. — *Ecol. Monogr.* 72: 523-540.
- Relyea, R.A. & Werner, E.E. (1999). Quantifying the relation between predator-induced behavior and growth performance in larval anurans. — *Ecology* 80: 2117-2124.
- Riesch, R., Duwe, V., Herrmann, N., Padur, L., Ramm, A., Scharnweber, K., Schulte, M., Schulz-Mirbach, T., Ziege, M. & Plath, M. (2009). Variation along the shy–bold continuum in extremophile fishes (*Poecilia mexicana*, *Poecilia sulphuraria*). — *Behav. Ecol. Sociobiol.* 63: 1515-1526.
- Schluter, D., Price, T.D. & Rowe, L. (1991). Conflicting selection pressures and life history trade-offs. — *Proc. Roy. Soc. Lond. B: Biol. Sci.* 246: 11-17.
- Schmitz, O.J., Beckerman, A.P. & O'Brien, K.M. (1997). Behaviorally mediated trophic cascades: effects of predation risk on food web interactions. — *Ecology* 78: 1388-1399.
- Sergio, F., Marchesi, L. & Pedrini, P. (2003). Spatial refugia and the coexistence of a diurnal raptor with its intraguild owl predator. — *J. Anim. Ecol.* 72: 232-245.
- Sih, A. (1982). Foraging strategies and the avoidance of predation by an aquatic insect, *Notonecta hoffmanni*. — *Ecology* 63: 786-796.
- Sih, A., Bell, A. & Johnson, J.C. (2004). Behavioral syndromes: an ecological and evolutionary overview. — *Trends Ecol. Evol.* 19: 372-378.

- Sih, A., Cote, J., Evans, M., Fogarty, S. & Pruitt, J. (2012). Ecological implications of behavioural syndromes. — *Ecol. Lett.* 19: 372-378.
- Sih, A., Englund, G. & Wooster, D. (1997). Emergent impacts of multiple predators on prey. — *Trends Ecol. Evol.* 13: 350-355.
- Skelly, D.K. (1994). Activity level and the susceptibility of anuran larvae to predation. — *Anim. Behav.* 47: 465-468.
- Skelly, D.K. & Werner, E.E. (1990). Behavioral and life-historical responses of larval American toads to an odonate predator. — *Ecology* 71: 2313-2322.
- Smee, D.L. & Weissburg, M.J. (2008). Heightened prey responses in risky habitats: predation pressure increases prey sensitivity to predation risk. — *Mar. Ecol. Prog. Ser.* 363: 39-50.
- Smith, B.R. & Blumstein, D.T. (2007). Fitness consequences of personality: a meta-analysis. — *Behav. Ecol.* 19: 448-455.
- Smith, B.R. & Blumstein, D.T. (2010). Behavioral types as predictors of survival in Trinidadian guppies (*Poecilia reticulata*). — *Behav. Ecol.* 21: 919-926.
- Urban, M.C. (2007). Risky prey behavior evolves in risky habitats. — *Proc. Natl. Acad. Sci. USA* 104: 14377-14382.
- Van Buskirk, J. & Arioli, M. (2005). Habitat specialization and adaptive phenotypic divergence of anuran populations. — *J. Evol. Biol.* 18: 596-608.
- Van Buskirk, J. & McCollum, S.A. (2000). Functional mechanisms of an inducible defence in tadpoles: morphology and behaviour influence mortality risk from predation. — *J. Evol. Biol.* 13: 336-347.
- Van Buskirk, J., McCollum, S.A. & Werner, E.E. (1997). Natural selection for environmentally induced phenotypes in tadpoles. — *Evolution* 51: 1983-1992.
- Waldman, B. (1982). Sibling association among schooling toad tadpoles: field evidence and implications. — *Anim. Behav.* 30: 700-713.
- Walsh, R. & Cummins, R. (1976). The open-field test: a critical review. — *Psychol. Bull.* 83: 482-504.
- Watkins, T.B. (1996). Predator-mediated selection on burst swimming performance in tadpoles of the Pacific tree frog *Pseudacris regilla*. — *Physiol. Zool.* 69: 154-167.
- Werner, E.E. & Anholt, B.R. (1993). Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. — *Am. Nat.* 142: 242-272.
- Wilson, A.D.M. & Krause, J. (2012). Personality and metamorphosis: is behavioral variation consistent across ontogenetic niche shifts? — *Behav. Ecol.* 23: 1316-1323.
- Wilson, D.S., Clark, A.B., Coleman, K. & Dearstyne, T. (1994). Shyness and boldness in humans and other animals. — *Trends Ecol. Evol.* 9: 442-446.
- Wilson, D.S., Coleman, K., Clark, A.B. & Biederman, L. (1993). Shy-bold continuum in pumpkinseed sunfish (*Lepomis gibbosus*): an ecological study of a psychological trait. — *J. Comp. Psychol.* 107: 250-260.
- Wolf, M. & Weissing, F.J. (2012). Animal personalities: consequences for ecology and evolution. — *Trends Ecol. Evol.* 27: 452-461.