Ann. Zool. Fennici 50: 237–246 ISSN 0003-455X (print), ISSN 1797-2450 (online)
Helsinki 30 August 2013

Understanding the information value of repeated exposure to chemical alarm cues: what can growth patterns tell us?

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Received 16 Nov. 2012, final version received 8 Mar. 2013, accepted 19 Mar. 2013

Jefferson, D. M., Hobson, K. A. & Chivers, D. P. 2013: Understanding the information value of repeated exposure to chemical alarm cues: what can growth patterns tell us? — *Ann. Zool. Fennici* 50: 237–246.

Chemical cues released into the environment from injured prey animals provide a rich source of information about ambient risk. However, these cues could also provide information not associated with predation risk. Here we exposed wood-frog tadpoles (*Lithobates sylvaticus*) to a control diet and one that was soaked in chemical cues released from injured conspecifics, and documented growth and development of the tadpoles. If animals perceive repeated exposure to injured conspecific cues as indicating a high risk environment, then we predict that tadpoles would reduce foraging, prolonging time to metamorphosis, reducing growth rate and initiating adaptive changes in tail morphology. Conversely, if tadpoles interpret repeated exposure to these chemical cues as an indicator of competitor density, they should increase growth rate and body size to become better competitors. We found that tadpoles exposed to chemical cues exhibited significantly larger body width and body length relative to the control. These patterns are inconsistent with a response to risk but correspond with observed responses of wood-frog tadpoles to increased competition.

Introduction

The survival of organisms is dependent on their abilities to detect and respond appropriately to various sources of information (Relyea 2004, Pohnert *et al*. 2007, Moir & Weissburg 2009, Schoeppner & Relyea 2009). Any given species may improve its survivorship by investing in adaptations to evade or deter predation (Dawkins & Krebs 1979), to limit exposure to pathogens (Kiesecker *et al*. 1999), to survive inclement environmental conditions (Wellborn *et al*. 1996, Griffiths 1997, Richter-Boix *et al*. 2007), and to improve competitive success (Relyea 2002, Relyea & Auld 2004, Michel 2012). Various sensory cues are used to track and assess ecological conditions (Petranka & Hayes 1998). Indeed, many species utilize chemical sources of information to identify the origin and degree of potential predation threats (reviewed by Chivers & Smith 1998, Ferrari *et al*. 2010) and the level of competition (Relyea 2002, Michel 2012).

When animals are captured by predators, they often release chemical cues into the environment.

These cues often induce avoidance behaviour in conspecifics (i.e. reduced activity, shelter seeking, and schooling) and hence are often referred to as alarm cues (Hagman & Shine 2008, Moir & Weissburg 2009, Schoeppner & Relyea 2009). Alarm cues that are ingested by predators may also induce the expression of phenotypically plastic morphological traits (i.e. larger bodies, modified locomotory structures) (Van Buskirk & McCollum 2000, Kishida & Nishimura 2004, Relyea 2004, Richter-Boix *et al*. 2007, Chivers *et al*. 2008). Indeed, the responses can be fine-tuned to respond to the nature and intensity of the threat, and the resource availability of the habitat (Relyea 2004). The ability to produce context dependent responses to specific threats can dramatically improve survivorship, while minimizing the incurred cost of the response (Van Buskirk & McCollum 2000, Richter-Boix *et al*. 2007). Therefore, an evolutionary pressure may exist towards the expression of rapid and efficient responses to highly variable conditions (Dawkins & Krebs 1979, Van Buskirk & McCollum 2000, Relyea & Auld 2004, Richter-Boix *et al*. 2007).

An issue not typically appreciated by researchers studying alarm cues is that such cues can in fact provide prey with additional pieces of ecologically relevant information that are not linked to risk. For example, conspecifics may release a suite of informative cues when injured and an individual could interpret information on the frequency of attacks on conspecifics to determine the level of potential competition (Relyea 2002). All else being equal, increasing cue concentrations in the vicinity may identify a higher density of competitors (Relyea 2002).

Cannibalism represents a highly profitable foraging strategy because conspecific tissues contain all essential nutrients, presumably in the appropriate proportions for growth and development (Greenstone 1979, Meffe & Crump 1987, Wildy *et al*. 1998). Such a diet can improve growth, development, and/or survivorship of individuals experiencing dietary stress resulting from reductions in quality and/or quantity of dietary resources (Fox 1975, Meffe & Crump 1987, Babbitt & Meshaka 2000). Incidental exposure to alarm cues released from victims could however dissuade cannibalism by invoking avoidance behaviour in the attackers. Conversely, Crump (1986) observed that initiation of cannibalism among Cuban treefrog tadpoles (*Osteopilus septentrionalis*) appeared to attract additional tadpoles to attack, and this behaviour may have been stimulated by chemical cues. Similarly, Crossland and Shine (2011) identified chemical cues released from late stage embryonic cane toad tadpoles (*Bufo marinus*) that stimulate older, free-feeding conspecifics to cannibalize embryos. Heinen and Abdella (2005) observed that older tadpoles of American toads (*Anaxyrus americanus*) were quicker to consume conspecific tissues than younger individuals. Exposure to conspecific cues may therefore dissuade or promote cannibalism, depending on the ecological context.

The objective of our current work was to study growth patterns of tadpoles to assess how tadpoles interpret repeated information indicating that conspecifics in the vicinity were captured by predators. Theory developed from a risk perspective predicts that tadpoles exposed to alarm cues would show immediate anti-predator behaviour (e.g. Chivers & Smith 1998, Ferrari *et al*. 2008, Moir & Weissburg 2009). Over the long term this would lead to a reduction in foraging with the consequence of increasing time to metamorphosis (Audo *et al*. 1995), lowering growth rate (Crespi & Denver 2004, Schoeppner & Relyea 2005, Fraker *et al*. 2009, Schoeppner & Relyea 2009) and may initiate adaptive changes in tail morphology (Schoeppner & Relyea 2005). In contrast, if tadpoles interpret repeated exposure to alarm cues as an indicator of competitor density, they should have a different pattern of growth (Relyea 2002, Relyea 2004). Specifically they should increase growth rate and body size (width and length) to become better competitors (Relyea 2002, Michel 2012).

Material and methods

Specimen collection

We collected wood-frog egg masses on 3 May 2011 from multiple populations in disjunct wetlands in the Nisbet Provincial Forest area north of Prince Albert, SK, Canada (53°17.188´N, 105°39.226´W). All wetlands were primarily open canopy; however each differed in its proximity to forest cover. All wetlands contained semi-aquatic or biphasic species of predatory invertebrates (e.g. odonates and dytiscids), while only the more permanent wetlands contained species incapable of evading drying periods (i.e. leeches). Plains garter snakes (*Thamnophis radix*) are common and ubiquitous throughout the area and may pose a threat to tadpoles in all collection sites, as do many predatory birds and mammals (e.g. striped skunks, *Mephitis mephitis*; North American raccoons, *Procyon lotor*; red foxes, *Vulpes vulpes*; coyotes, *Canis latrans*; etc). Adult barred tiger salamanders (*Ambystoma mavortium*) are also commonly observed in the area. Egg masses were transported to the University of Saskatchewan, and maintained in a 38-l glass aquarium filled with room temperature de-chlorinated water and pond water.

Experimental procedures

A total of 90 tadpoles at Gosner (1960) stage 25 were individually housed in 980 ml (136.7 × 136.7 × 69.9 mm) plastic containers and randomly assigned to 1 of 2 treatments (45 tadpoles per treatment) in a randomized block arrangement. Tadpoles received water changes 3 days a week (M, W, F) and were fed immediately after all water changes were completed. Water quality (i.e. pH, NH_3, NO_2^-, NO_3^-, GH, KH, Cl) of experimental containers was assessed a minimum of once a week within 24 hours of a feeding using commercially available aquarium test strips. Experiments were performed under a controlled 14:10 h (light:dark) photoperiod. Mean (± SE) water temperatures for experimental containers were 19.7 ± 0.1 °C through the course of the experiment.

Tadpoles were fed a diet of frozen brine shrimp (72.5% dry protein) and generic rodent pellet food (18% dry protein) consistently mixed to a 4:1 ratio by mass. This combined diet was prepared to provide a relatively high protein diet (Schiesari *et al*. 2009) while providing high resource dispersal to encourage constant foraging, and minimize water fouling. Tadpole food was subsequently placed in a solution of conspecific cues in de-chlorinated water (exposure group) or de-chlorinated water alone (control group), homogenized, and allowed to soak in solution for a minimum of 30 min prior to feeding. Conspecific cues were produced by macerating 0.5 g of tadpoles in 150 ml of de-chlorinated water using an ultrasonic homogenizer (to ensure specimens were rapidly killed) (Hagman & Shine 2008). This solution was subsequently filtered using a coarse porosity (25 μm particle retention) crepe fluted filter paper to remove all tadpole tissues thereby minimizing any potential nutritional enhancement from the addition of the conspecific cue solution to the tadpoles' diet. Control solutions consisted of 150 ml of de-chlorinated water that had similarly been homogenized and filtered for consistency. The entire 150 ml of each solution was added to the separate diet preparations intended for each of the treatment groups. The conspecific cue solution was provided to each tadpole with their food to ensure a strong association between the food and the chemical cues. Tadpoles were fed to satiation throughout the study and dietary rations for individual tadpoles were increased uniformly across treatments through experimental progression in response to increased tadpole feeding.

According to Relyea (2004) the magnitude of tadpole growth response should depend on food availability. Consequently, here we used a high quality diet and the effects of competition were negated to promote growth and development. Such conditions were used to identify whether the inclusion of the chemical cues would deter tadpoles from foraging on a profitable diet.

Tadpoles were removed from the study when they achieved Gosner (1960) stage 41 of development (initiation of metamorphosis) and were subsequently euthanized using an overdose of a buffered TMS (tricaine methanesulfonate) solution. Final measurements of mass, body length (BL), body width (BW), maximum tail fin depth (TFD), maximum tail muscle width (TMW), and time to metamorphosis (TTM) were recorded. Measurements of each trait were produced as mean values of 3 replicate measurements per tadpole. Morphometrics were measured using digital calipers, and mass was measured using a Sartorius digital balance, having observed measurement errors of ± 0.02 mm and ± 0.002 g, respectively.

Stable isotope analyses

All tadpole specimens were eviscerated, frozen, and subsequently freeze dried in a Labconco Corp. Freezone® freeze drier for approximately 24 hr in the Department of Biology, University of Saskatchewan, Saskatoon, SK, Canada. Freeze-dried whole-body tadpoles were pulverized to a fine powder, weighed and packaged at National Hydrology Research Center of Environment (NHRC), Saskatoon, SK, Canada. Homogenized whole-body samples were used to compensate for differences in fractionation between animal tissues (Biasatti 2004). Dry powder samples were weighed to approximately 0.100 mg, and packaged in Elemental Microanalysis Ltd. 5 × 3.5 mm tin capsules. Samples were subsequently submitted for C and N stable isotope mass spectrometry analysis to the Stable Isotope Hydrology and Ecology Research Laboratory at NHRC, and the Stable Isotope Laboratory of the Department of Soil Science, U of S. Previous lipid proportions of wild wood frog tadpoles were consistently below 5%. Therefore, no lipid processing of samples submitted for isotope analysis was warranted (Post *et al.* 2007). Values of stable isotopes (δ^{13}C or δ^{15}N) were found as the deviance (δ) of the ratio of heavy to light isotopes (i.e., ^{13}C/^{12}C or ^{15}N/^{14}N) within a sample, to that of an international standard (VPDB, AIR), and expressed in parts per thousand (‰) (Biasatti 2004). Stable isotope values were quantified and reported as described in Jardine *et al.* (2003). Measurement error based on replicate measurements of in-house organic standards (egg albumen, whale baleen) was estimated to be ±0.1‰ and ±0.2‰ for δ^{13}C and δ^{15}N, measurements, respectively.

Statistical analyses

A multivariate analysis of variance (MANOVA) was used to compare time to metamorphosis, mass, and morphometric values of tadpoles between treatments.

Subsequent *post-hoc* tests were conducted to explore potential causes of results from initial analysis. A conservative testing procedure was adopted by adjusting significance levels using the Bonferroni method ($\alpha = 0.01$) to reduce the risk of committing a type I error (Sokal & Rohlf 1995). An analysis of covariance (ANCOVA) was used to compare tadpole body width adjusted for differences in snout–vent length. Additionally, patterns in body growth of tadpoles were tested by comparing the slopes of regression lines against the expected slope for isometric growth (slope of 1) using a one-sample *t*-test of regression lines for body width on body length.

To test for differences in foraging, a total of 23 tadpoles (12 control and 11 exposed) were randomly selected and analyzed for δ^{13}C and δ^{15}N values. Stable isotopic ratios of tadpoles were compared between treatments with MANOVA. Obtained values were subsequently compared to the expected δ^{13}C and δ^{15}N values based on the ratio of diet provided (approximately δ^{13}C = –25.6‰ and δ^{15}N = 5.9‰, based on a 4:1 ratio of brine shrimp to rodent pellet). Proportions of assimilated diets were subsequently assessed using Stable Isotope Sourcing using Sampling (SISUS) implemented in the SISUS package (Erhardt 2007) for R, ver. 2.13.2 (R Development Core Team 2010).

Mortalities were excluded from all analyses. Linearity was assessed among morphometric traits, data was log-transformed where it violated parametric assumptions, and statistical outliers were removed. All analyses, other than SISUS were performed in Systat (Wilkinson 1998).

Results

A total of 26 mortalities occurred throughout the experiment; 12 occurred in the control group and 14 in the exposure group. No aversion to foraging was observed among tadpoles at any point in the study.

Tadpoles exhibited significant difference in response to experimental treatments (Hotelling-Lawley trace: $F_{6,56} = 3.09$, $p = 0.01$). A univariate F-test indicated tadpoles exposed to injured conspecifics cues were significantly larger in body length ($F_{1,61} = 5.96$, $p = 0.01$) with a mean (± SE) of 17.54 ± 0.19 mm *vs.* 16.90 ± 0.17 mm of control tadpoles. Exposed tadpoles also had significantly larger body width ($F_{1,61} = 4.03$,

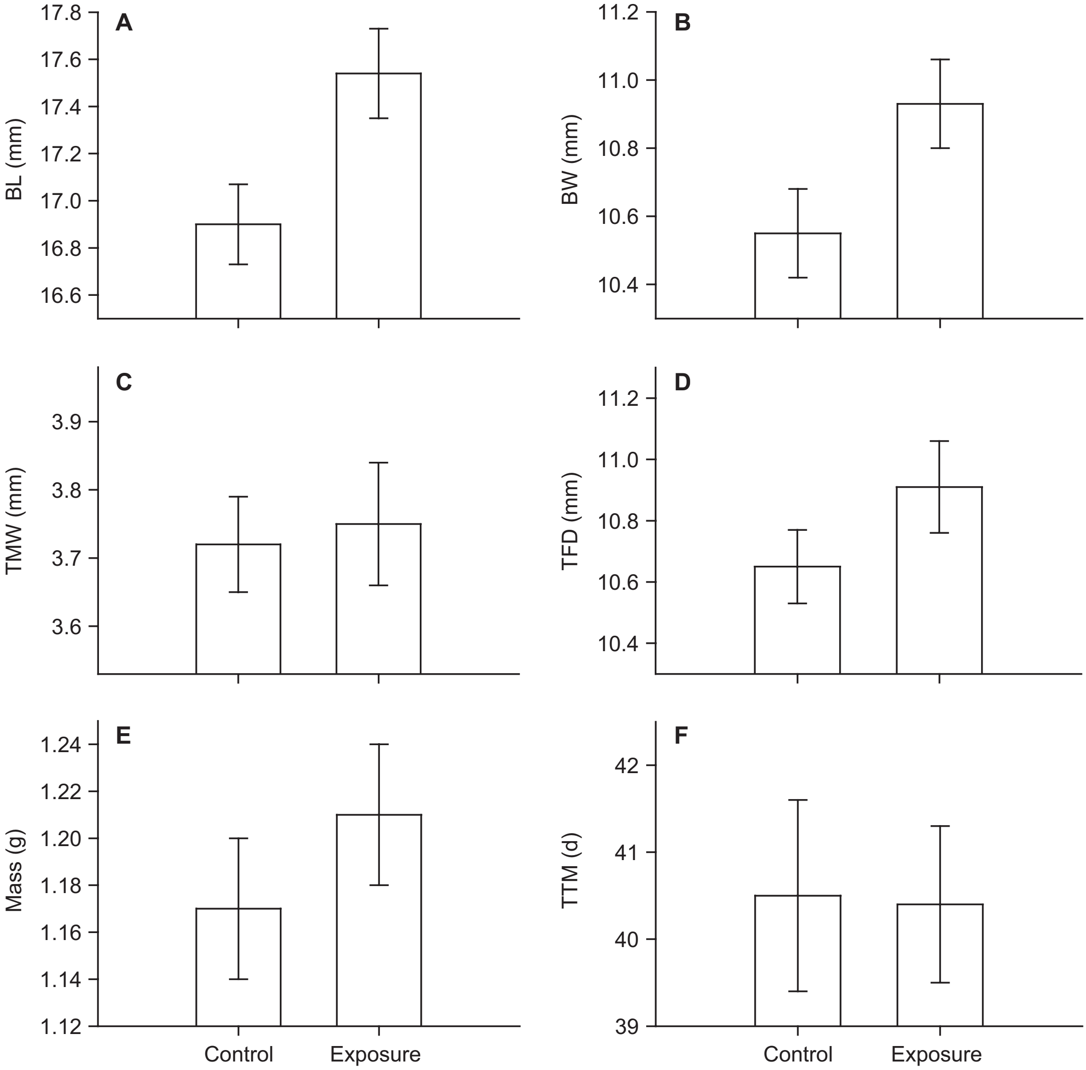

Fig. 1. Tadpole morphometrics and time to metamorphosis between treatments. Comparison of mean tadpole (**A**) body length (BL), (**B**) body width (BW), (**C**) tail muscle width (TMW), (**D**) tail fin depth (TFD), (**E**) mass, and (**F**) time to metamorphosis (TTM) between control and exposure to injured conspecific cues treatments. Error bars represent ± 1SE.

$p = 0.04$) having a mean (± SE) value of 10.93 ± 0.13 mm relative to 10.55 ± 0.13 mm of control tadpoles (Fig. 1). However, no significant differences were found between experimental treatments in mass (mean ± SE; 1.21 ± 0.03 g *vs*. 1.17 ± 0.03 g) or tail morphology (mean ± SE; TFD: 10.91 ± 0.15 mm *vs*. 10.65 ± 0.12 mm; TMW: 3.75 ± 0.09 mm *vs*. 3.72 ± 0.07 mm) (all $p > 0.05$; Fig. 1). Additionally, time to metamorphosis was not significantly different for tadpoles between treatments (40.5 ± 1.1 d *vs*. 40.4 ± 0.9 d, mean ± SE, $p > 0.05$; Fig. 1) (Table 1).

Further comparison of tadpoles between treatments identified no difference in the ratio of body width to body length between groups (equivalent slopes) and no difference in tadpole body width when adjusted for differences in body length (ANCOVA: $F_{1,60} = 0.95$, $p = 0.33$) (Fig. 2). Regression lines of body width against body length exhibited slopes significantly less than 1 for both treatment groups (control: $t_{31} = -89.8$; exposed: $t_{30} = -91.8$; all $p < 0.001$), describing a negative allometric growth pattern. This suggests differences between treatments

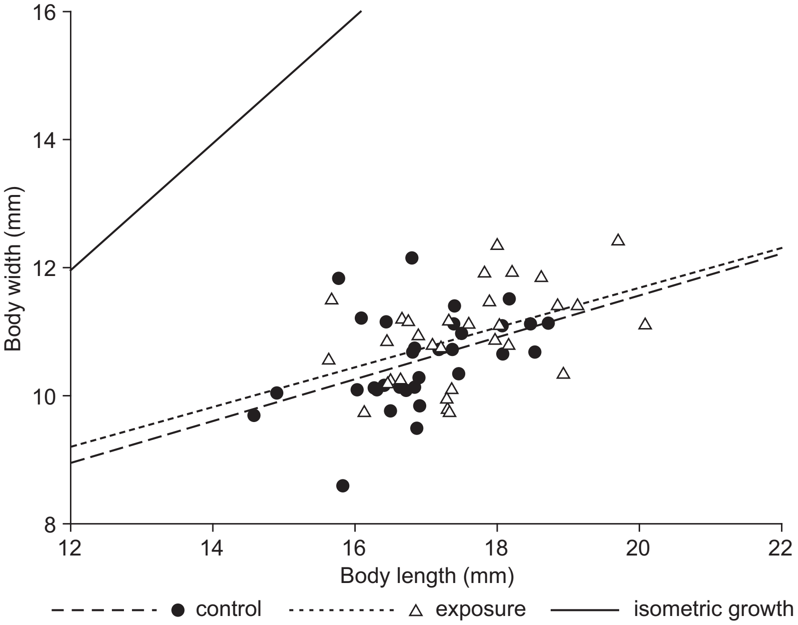

Fig. 2. Body growth of tadpoles. Comparison of negative allometric growth of body width (BW) and body length (BL) observed in tadpoles of the exposed, and control treatments, relative to the regression line of typical isometric growth. Equations of regression lines of BL (x) adjusted BW (y) of exposed tadpoles: $y = 0.31x + 5.49$, $r^2 = 0.21$; control tadpoles: $y = 0.33x + 5.03$, $r^2 = 0.18$; and of the isometric growth line $y = 1\,x\,0$, $r^2 = 1$.

are due to greater overall body size in exposed tadpoles, and growth of body width was less than that of body length at this point in development.

Analyses of δ^{13}C and δ^{15}N values indicated that tadpoles in the two treatments did not differ in foraging or assimilation of dietary resources (Hotelling-Lawley trace: $F_{1,19} = 1.14$, $p = 0.34$). Control and exposed tadpoles exhibited a mean (± SE) δ^{13}C values of –25.8‰ ± 0.3‰ and –26.1‰ ± 0.2‰), respectively, and mean (± SE) δ^{15}N values of 6.4‰ ± 0.4‰ and 5.9‰ ± 0.2‰), respectively. The majority dietary component, frozen brine shrimp ($n = 6$) had mean (± SE) δ^{13}C and δ^{15}N values of –25.0‰ ± 0.0 and 7.2‰ ± 0.0‰. The generic rodent pellet food ($n = 6$) exhibited mean (± SE) δ^{13}C and δ^{15}N values of –27.8‰ ± 0.3‰) and 1.4‰ ± 0.2‰. Based on stable isotopic values, assimilation of the mixed dietary resources was not significantly different from the provided ratio after Bonferroni correction (Control δ^{13}C: $t_{10} = –0.30$, $p = 0.77$; δ^{15}N: $t_{10} = 1.53$, $p = 0.16$; Exposed δ^{13}C: $t_{10} = –2.54$, $p = 0.03$; δ^{15}N: $t_{10} = –0.50$, $p = 0.03$). SISUS (Erhardt 2007) models identified a range of ratios (4.3:1 to 4.7:1) that were similar to the 4:1 ratio of dietary resources provided. These results indicate that tadpoles foraged indiscriminately upon the provided diet, and did not preferentially consume or assimilate resources (Fig. 3).

Table 1. Univariate *F*-test. Comparisons between control and exposure treatment groups of time to metamorphosis (TTM), mass, and morphometrics: body length (BL), body width (BW), tail fin depth (TFD), and tail muscle width (TMW).

Source	SS	df	MS	*F*	*p*
TTM	0.00	1	0.00	0.00	0.99
Error	1.20	61	0.02		
Mass	0.01	1	0.01	0.67	0.42
Error	1.21	61	0.02		
BL	0.02	1	0.02	5.96	0.01
Error	0.22	61	0.00		
BW	0.02	1	0.02	4.03	0.04
Error	0.29	61	0.00		
TFD	0.01	1	0.01	1.79	0.19
Error	0.30	61	0.00		
TMW	0.00	1	0.00	0.04	0.85
Error	0.89	61	0.02		

Discussion

For a variety of prey animals chemical cues of injured conspecifics elicit avoidance behaviour and are associated with reduced activity and foraging (Petranka 1989, Schoeppner & Relyea 2005, Ferrari & Chivers 2008, Fraker *et al.* 2009, Schoeppner & Relyea 2009, Ferrari *et al*. 2010). Such a response improves survivorship by reducing exposure to potential threats and facilitating improved escape (Van Buskirk & McCullum 2000, Relyea 2004). In many tadpoles, alarm cues alone are known to induce dramatic behavioural responses, but long-term exposure may not induce morphological defences (Relyea 2004, Hagman & Shine 2008). However, alarm cues are known to induce adaptive changes in morphology in other taxa (Stabell & Lwin 1997, Chivers *et al*. 2008), and the ability of prey to induce such changes is dependent on the frequency and intensity of risk. Hagman *et al*. (2009) observed that exposing cane toad tadpoles to alarm cues of crushed conspecifics reduced the size of metamorphs, and increased the size of the bufotoxin containing paratoid glands and the amount of the toxin bufalin produced by these toads. Conversely, the results of this study indicate exposed tadpoles exhibited an apparent increase in overall body size with no difference in time to metamorphosis, nor observed tail morphology relative to the control treatment.

Our results are not consistent with the expected results from a predation risk perspective (e.g. Relyea 2004). Tadpoles exposed to conspecific cues in this study also do not conform to the induced "bulgy" morphology, which describes tadpoles with relatively widened and deepened bodies relative to body length (Kishida & Nishimura 2004). Tadpoles generally exhibit isometric growth (Strauss & Altig 1992), and positive allometry is expected in induced morphological responses, such as "bulgy" tadpoles described by Kishida and Nishimura (2004). The negative allometric growth observed in tadpoles of both groups may reflect decreasing body width of tadpoles as they initiated dramatic metamorphic alterations occurring in tadpoles at this stage of development (Gosner 1960). Stable isotopic ratios indicate that exposed tadpoles did not exhibit the preferential foraging of dietary resources observed by Richter-Boix *et al*. (2007). Differences in body size may, therefore have resulted from stimulation of increased foraging or improved nutrient assimilation of exposed tadpoles (Relyea 2004, Relyea & Auld 2004). A previous study by Ferrari *et al*. (2008) identified rapid degradation (within 2 h) of larval wood frog alarm cues under natural conditions. Tadpoles would therefore have experienced repeated acute exposure through the duration of the study. Neuroendocrine responses to stress, leading to avoidance behaviour, may have been initially invoked (Crespi & Denver 2004, Schoeppner & Relyea 2005, Fraker *et al*. 2009, Schoeppner & Relyea 2009); however the exposure period may have been insufficient to sustain suppressed foraging. Conversely, this neuroendocrine response often stimulates appetite subsequent to expression of suppressed feeding and avoidance behaviour (Crespi & Denver 2004). This response acts to increase foraging after a stress or risk has been removed to replenish energy stores (Crespi & Denver 2004). This may have compensated for any initial suppression of feeding imposed upon tadpoles by acute exposure to these chemical cues. However, this does not account for the observed increased body size, or the absence of a reduction in developmental rate.

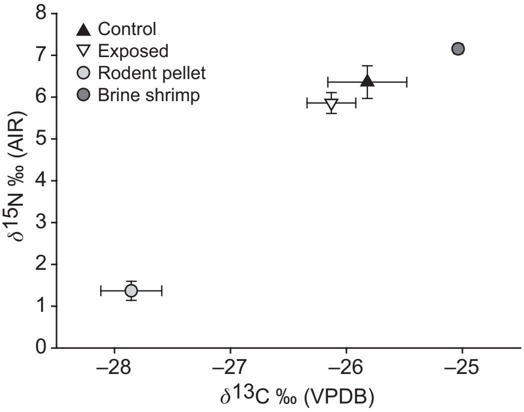

Fig. 3. Tadpole stable isotopic values. Mean δ^{13}C and δ^{15}N values of tadpoles in control (no conspecific cues added) and exposed (conspecific cues added) treatments, and the two components of their mixed diet; brine shrimp and generic rodent pellet. Error bars represent ± 1SE.

The discrepancy between the observed and expected results from a risk perspective may be explained by the interpretation of the cues tadpoles were exposed to. Relyea (2004) observed that tadpoles specifically adjust their behavioral and phenotypic responses to efficiently adapt to the overall ecological risks (i.e. predation, competition) and available resources (i.e. quality and quantity of food) to improve their survival.

The specificity and/or duration of the tadpoles' response may also relate to the degree of the perceived risk, whereby animals may exhibit a much greater or prolonged response in situations of high-risk, and a lesser and/or shorter response under low risk situations (Lima & Bednekoff 1999, Mirza *et al*. 2006, Ferrari *et al*. 2009). The observed morphological response of tadpoles does not correspond with typical responses of exposure to alarm cues; however it does resemble findings from studies of increased intraspecific competition (Relyea 2002, Michel 2012). Multiple studies have found increased body size in wood frog tadpoles in response to high competition (Relyea 2000, Relyea 2002, Relyea 2004, Relyea & Auld 2004, Michel 2012). Relyea (2002) observed that tadpoles exhibit similar responses regardless of whether tadpole density or food density was manipulated and therefore asserts that tadpoles assess levels of competition based on chemical cues. Tadpoles may identify increasing concentrations of innate chemicals (e.g. urine or feces) within the habitat as a sign of increased competition (Relyea 2002). Relyea and Auld (2004) identified increased intestinal length associated with increased competition. The authors further suggest that this increased intestinal length may account for the larger body sizes of tadpoles under increased competition (e.g. Relyea 2002, Relyea & Auld 2004). It is possible that in our current study these chemical cues were present in the solution provided to tadpoles, and may have induced the observed responses.

However, results are not consistent among all studies of wood frog tadpoles. For example, results of this study and those of Relyea (2002) identified increased tadpole body width in response to high competition, while Michel (2012) found no such increase in body width in the same species. Similarly, Relyea and Auld (2004) recorded increased gut length in response to increased competition, and Michel (2012) found no such difference. Relyea (2002) and Michel (2012) both observed decreases in tail morphometrics, which were not observed in this study. Relyea (2002) also observed reduced growth rate (g d^{-1}) as competition increased, while the results of this study suggest no such difference between exposed and control treatments. These differences may simply reflect the plasticity of wood frog response observed by Relyea (2004). He suggests that tadpoles respond in accordance to ecological context (Relyea 2004). In this study it is possible that tadpoles identified a relatively low-risk situation, and focused on a perceived risk of intraspecific competition. The observed differences in body size between the exposed and control groups may have been the result of increased intestinal length in exposed tadpoles as suggested by Relyea and Auld (2004). In the absence of competition or any difference in diet, the cost of the increased intestinal length may have off-set any benefit this adaptation provided to the exposed tadpoles, therefore negating any expected increase in mass.

Our results suggest tadpoles may shift their perception of or focal response to specific risks. We suggest that early in the experiment tadpoles may have responded to chemical cues as an indication of risk until these cues degraded (i.e. Ferrari *et al*. 2008). The remaining chemical cues, combined with metabolic waste released from experimental tadpoles, would then persist within the experimental container and possibly be interpreted as cues of increasing competition (i.e. Relyea 2002). After prolonged acute exposures to these chemical cues in the absence of a threat, tadpoles ceased responding to the risk cues and began to respond to the putative cues of increasing competition. The ability of tadpoles to correctly assume risk level is paramount to their immediate survival; to shift focus from risk of predation and towards increasing competition suggest that tadpoles identify this as a looming threat to survival or fitness. The results of this study indicate tadpoles are capable of assessing multiple, possibly conflicting ecological conditions and subsequently respond accordingly to efficiently mitigate risks. Although further investigation is necessary, these observations provide further insight into the risk assessment of tadpoles and may highlight the evolutionary pressures that act towards development of response plasticity.

Acknowledgments

DMJ was supported by a Dean's Scholarship from the University of Saskatchewan. Research funding was provided by a Natural Sciences and Engineering Research Council Discovery Grant to DPC, and Environment Canada operating funds to KAH. Shelly Fisher assisted in field collections, and Chantel Showers assisted in sample preparation. Jill Johnstone and Maud Ferrari provided statistical advice. All work was conducted in accordance with the guidelines of the Canadian Council on Animal Care and was approved by the University of Saskatchewan Animal Ethics Committee (protocol #: 20100115). Field collections followed the code of practice outlined by the Declining Amphibian Task Force (DAPTF), and were conducted under a special permit for scientific research granted by the Saskatchewan Ministry of Environment (permit no. 11FW093).

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