



Carryover effects of phenotypic plasticity: Embryonic environment and larval response to predation risk in wood (*Lithobates sylvaticus*) and Northern leopard (*Lithobates pipiens*) frogs

Journal:	<i>Canadian Journal of Zoology</i>
Manuscript ID	cjz-2015-0129.R1
Manuscript Type:	Article
Date Submitted by the Author:	27-Aug-2015
Complete List of Authors:	Bennett, Amanda; Trent University, Environmental and Life Sciences Murray, D.L.; Trent University, Biology
Keyword:	Trade-off, Plasticity-history limitation, Predation risk, Tadpole, ECOLOGY < Discipline, LIFE HISTORY < Discipline, ANURA < Taxon



1 **Carryover effects of phenotypic plasticity: Embryonic environment and larval response to**
2 **predation risk in wood (*Lithobates sylvaticus*) and Northern leopard (*Lithobates pipiens*)**
3 **frogs**

4

5 **A. M. Bennett**¹

6 Environmental & Life Sciences, Environmental Science Building, Suite A211, Trent University,
7 1600 West Bank Drive, Peterborough, Ontario, K9J 7B8 (email: amandabennett2@trentu.ca)

8 **D. L. Murray**

9 Department of Biology, Life & Health Sciences Building, Block D, Trent University, 2140 East
10 Bank Drive, Peterborough, Ontario, K9J 7B8 (email: dennismurray@trentu.ca)

11

12

13 ¹Correspondence: A.M. Bennett, Environmental & Life Sciences, Environmental Science
14 Building, Suite A211, Trent University, 1600 West Bank Drive, Peterborough, Ontario, K9J 7B8
15 email: amandabennett2@trentu.ca

16 phone: (705) 760-5722

17 fax: (705) 748-1139

18 Carryover effects of phenotypic plasticity: Embryonic environment and larval response to
19 predation risk in wood (*Lithobates sylvaticus*) and Northern leopard (*Lithobates pipiens*) frogs

20 A. M. Bennett and D. L. Murray

21

22 **Abstract**

23 Limitations of phenotypic plasticity affect the success of individuals and populations in changing
24 environments. We assessed the plasticity-history limitation on predator-induced defenses in
25 anurans (wood frogs: *Lithobates sylvaticus* (LeConte, 1825); Northern leopard frogs: *L. pipiens*
26 (Schreber, 1782)), predicting that plastic responses to predation risk by dragonfly larvae (*Aeshna*
27 spp.) in the embryonic environment would limit the defensive response to predators in the larval
28 environment. Predator-conditioned wood frog embryos increased relative tail depth in response
29 to those same cues as larvae, whereas predator-naïve tadpoles did not. However, no carryover
30 effect was noted in the behavioural response of wood frog tadpoles to predation risk. Predator-
31 naïve Northern leopard frog tadpoles increased relative tail depth in response to predation risk in
32 the larval environment. Predator-conditioned leopard frog embryos hatched with, and
33 maintained, a marginal increase in tail depth as larvae in the absence of predation risk. Predator-
34 conditioned leopard frog embryos exposed to predation risk as larvae showed no morphological
35 response. While we find no strong support for the plasticity-history limitation *per se*, carryover
36 effects across embryonic and larval life-history stages were noted in both wood and leopard
37 frogs, suggesting that predation risk early in ontogeny can influence the outcome of future
38 interactions with predators.

39

40 **Key words:** Trade-off, wood frog, Northern leopard frog, *Lithobates pipiens*, *L. sylvaticus*

41 Introduction

42 Plasticity, the capacity of a phenotypic trait to be modified in response to environmental
43 cues within the lifespan of an individual, is adaptive, and the ability to be plastic is itself a
44 heritable trait favoured by selection when environmental conditions are variable but constrained
45 to a predictable gradient (Scheiner 1993; Via et al. 1995). Plasticity in behaviour, morphology,
46 and life history can have direct influences on population-level processes under different
47 environmental conditions (Kishida et al. 2010; Reed et al. 2010). More specifically, plasticity in
48 response to predation risk can alter food web dynamics (Beckermann et al. 1997; Van Buskirk
49 and Schmidt 2000), and influence antagonistic and mutualistic interactions between species
50 (Agrawal 2001), thereby driving evolutionary divergence in novel habitats (Prokopy et al. 1982;
51 Pigliucci et al. 2006). Understanding the limitations acting on plasticity is therefore fundamental
52 both to our knowledge of the current ecology and evolutionary history of species, and to our
53 ability to predict the capacity of those species to respond to novel environmental changes.

54 Plastic responses early in ontogeny may negatively affect an organism's ability to respond
55 to those same environmental cues later in ontogeny; this is called a plasticity-history limitation
56 (Weinig and Delph 2001; Van Kleunen and Fischer 2005; Auld et al. 2010). Plasticity-history
57 limits are expressed when the magnitude of plastic response by an organism to particular
58 environmental cues is constrained by a previous (i.e., earlier in ontogeny) trait change (Auld et
59 al. 2010). For example, Weinig and Delph (2001) describe how morphological responses (stem
60 elongation) to a reduction in the ratio of red:far red wavelengths of light caused seedlings of
61 *Abutilon theophrasti* Medic. to become less responsive to a second reduction in the red:far red
62 ratio later in development. In this case, the competitive advantage of elongated stems in the
63 environment was constrained by limitations on the structural integrity of the plant (Weinig and

64 Delph 2001). However, this plasticity-history limit could be compensated for in a high density
65 environment where neighbouring plants can increase stability for long-stemmed individuals,
66 suggesting plasticity-history limitations can reflect both developmental and environmental
67 constraints (Weinig and Delph 2001).

68 A similar plasticity-history limitation could be manifested in organisms displaying
69 inducible defenses in response to predation risk. For example, plasticity-history limits on
70 defensive traits expressed early in development, such as a decrease in foraging activity, should
71 be costly to manifest repeatedly over time, as a reduction in resource acquisition would constrain
72 growth and development. To compensate for such a limitation, we would predict high
73 reversibility of the behavioural response and a decrease in responsiveness, as the energetic
74 demands for other processes grow. Both of these compensatory mechanisms have been
75 documented, to some extent, in the behavioural defenses of amphibian larvae (Relyea 2003;
76 Laurila et al. 2004, respectively). Under natural conditions, the direct effects of predators (i.e.,
77 decreased conspecific density due to removal of individuals from the population) can act as
78 environmental compensation by lowering competition for resources in later life-history stages
79 (Vonesh 2005). There is also the potential for developmental compensation if other defensive
80 traits (such as morphological plasticity) are traded-off with behavioural responses during
81 ontogeny (Relyea 2003). The manifestation of any of these compensatory mechanisms would
82 provide empirical evidence of limitations acting on the inducible defenses of prey.

83 An explicit test of the plasticity-history limitation on inducible defenses would examine
84 how plasticity in response to predation risk during an early life-history stage affects the
85 magnitude and/or type of response to predation risk later in development. Studies that examined
86 carryover effects of predation risk across life-history stages have been largely focused on

87 transitions that are accompanied by dramatic changes in environment, notably an air-water
88 transition (Van Buskirk and Saxer 2001; Vonesh 2005; Niecieza et al. 2006; Capellàn and Niecieza
89 2007; Gomez-Mestre et al. 2010). In these studies, both abiotic and biotic conditions are vastly
90 different between early and later life-history stages, however, the plasticity-history limitation is
91 predicated on the limitation imposed by sequential responses to the same environmental cues.
92 Therefore, in our study we examine limitations imposed by plastic responses to predation risk
93 cues in an aquatic environment alone.

94 Many amphibian species are known to detect and respond to predation risk at both the
95 embryonic and larval stages (Chivers et al. 2001; Laurila et al. 2002; Ireland et al. 2007;
96 Mandrillon and Saglio 2007). Embryos can learn to associate risk with novel predators when
97 exposed to chemical cues from both the predator and injured conspecifics (Ferrari and Chivers
98 2009, 2011). Embryos may delay hatching in response to larval predators, so that hatchlings are
99 larger and more developmentally advanced (Sih and Moore 1993; Ireland et al. 2007). Tadpoles
100 exposed to odonate predation risk typically reduce their activity and develop deeper tails relative
101 to body size (Relyea and Werner 2000; Relyea 2001). Lower activity levels decrease encounter
102 rates with ambush predators and therefore increase survival (Anholt and Werner 1995; Van
103 Buskirk and McCollum 2000). Similarly, deeper tails can increase survivorship by acting as lures
104 to draw dragonfly strikes away from the vulnerable head and body (Van Buskirk et al. 2003).
105 Tadpoles lacking these inducible defenses suffer greater mortality rates in high predation risk
106 environments (Van Buskirk and McCollum 2000; Van Buskirk et al. 2003). If larval plasticity is
107 limited by plasticity in early ontogeny, an embryonic response to predation risk should constrain
108 the magnitude of potential responses during larval development. Therefore, a plasticity-history
109 limitation would be manifest if tadpoles hatched from predator-exposed eggs did not, in turn,

110 demonstrate an induced defense in response to larval predation risk. Alternatively, in the absence
111 of a plasticity-history limitation, a carryover effect would be evident if the induced defense is
112 maintained in the larval stage in the absence of larval predators.

113 The purpose of our experiment was to explicitly test for a plasticity-history limitation on
114 phenotypic plasticity in a vertebrate system. We explored plasticity-history limitations in the
115 wood frog (*Lithobates sylvaticus* (LeConte, 1825)) and Northern leopard frog (*Lithobates pipiens*
116 (Schreber, 1782)) by examining risk-related trade-offs and carryover effects between the
117 embryonic and larval life-history stages. Both frog species are early spring breeders and can
118 overlap in reproductive habitat (Werner 1992; Werner and Glennemeier 1999); they have similar
119 larval responses to predation risk from odonate predators, including reducing activity and
120 increasing tail fin or tail muscle depth (Relyea and Werner 2000; Relyea 2001). However, wood
121 frog spring breeding habitats are typically fish-free vernal ponds or small pools (Petranka and
122 Thomas 1995; Baldwin et al. 2006). In contrast, Northern leopard frogs choose a wider variety of
123 breeding habitats, including the floodplains of permanent bodies of water (Gilbert et al. 1994).
124 Therefore, variability in plasticity is also expected between species, given the variability in
125 selection pressures such as hydroperiod, interspecific competition, and predation risk among
126 populations. We hypothesize that if plasticity-history limitation exists, it will be manifest
127 between embryonic and larval responses to predation risk as a trade-off in which eggs exposed to
128 larval predation risk will alter hatching phenology and/or embryonic morphology, and that those
129 previously exposed embryos will develop into tadpoles showing reduced predator-induced
130 phenotypes in the presence of larval predator cues.

131

132 **Materials and Methods**

133 *Egg collection and rearing*

134 The experiment was conducted using frog eggs collected near Peterborough, ON
135 (44°20'N 78°11'W) over two summers: six wood frog (WF) egg masses were collected in May
136 2011, and six Northern leopard frog (LF) egg masses were collected from captive-bred wild
137 frogs in May 2013. Predators (late-instar dragonfly nymphs, Aeshnidae) were collected from
138 nearby ponds in both years. Six wood frog clutches were split into strands of 10-15 eggs each,
139 and then mixed into groups of approximately 100 eggs, which were placed in plastic tanks filled
140 with 10 L of aged tap water. Each tank contained a plastic floating breeding cage with a short
141 piece of rubber tubing to act as a dragonfly perch, and half of the cages contained a late-instar
142 dragonfly nymph (predator treatment, $n = 6$ replicates) while the other half remained empty
143 (control treatment, $n = 6$ replicates). Hatching was defined as the timing of embryo emergence
144 from their jelly coats, and tanks were checked every two (during the day) to six (overnight) hours
145 for hatching success. Within 24 hours of complete hatching, five embryos were haphazardly
146 chosen from each tank and photographed laterally against a scale bar. Embryo total length (mm)
147 and tail depth (mm) were then measured from photographs using the program ImageJ (Rasband
148 2012). After all eggs had hatched, discarded jelly coats were removed and half of the tadpoles (n
149 ≈ 45) were placed in a new plastic tank filled with 10 L aged tap water, where they were exposed
150 to presence/absence of the caged dragonfly nymph predator ($n = 6$ replicates per treatment). The
151 other half of the tadpoles remained in the same tank (after a full water change) with the same
152 treatment as their embryonic exposure. Our experiment was thus a 2x2 factorial design: i) no
153 predator in either environment (control) ii) predator present during embryonic development only;
154 iii) predator present during larval development only; and iv) predator present during both
155 embryonic and larval development. Tadpoles were fed ground algae discs (Wardley Algae

156 Discs™ or Omega One Veggie Rounds™) *ad libitum*, and half water changes were done twice a
157 week. Tanks were placed in an outdoor enclosure and thereby subject to natural light cycles and
158 temperatures. Dragonfly nymphs were maintained on three conspecific tadpoles, fed twice
159 weekly. The above experiment was duplicated in 2013 using Northern leopard frogs from five
160 clutches (the sixth clutch collected was not viable).

161 Once tadpoles had reached Gosner stage 25 (Gosner 1960), we conducted behavioural
162 assays four days per week, between 0800 and 1000 hrs, by counting the number of tadpoles that
163 were active (showed any movement) within 30 seconds. Tadpole morphology was recorded
164 every week for six weeks by haphazardly removing and photographing five tadpoles from each
165 tank using a Nikon D70 digital SLR camera equipped with a Tamron 90 mm macro lens against
166 a scale bar. Tadpoles were blotted dry, weighed on a microbalance, then staged (according to
167 Gosner 1960) by viewing under a dissecting microscope, before being returned to the treatment
168 tank. Experiments ended on 5-July-2011 for wood frogs and 2-July-2013 for leopard frogs, at
169 which point tadpoles were euthanized with an overdose of tricaine methanesulfonate (MS-222).
170 All experiments were carried out according to the guidelines of the Canadian Council on Animal
171 Care, under Trent University Animal Use Protocols 12022 and 13002, and complied with
172 Canadian law.

173 *Statistical Analyses*

174 *Hatching:*

175 Hatching timing was analyzed for each species using time to 50% hatching (hrs) and total
176 hatching time (from onset to 100% hatched; hrs) and hatchling relative tail depth was calculated
177 as the residuals of an ordinary least-squares regression of tail depth (mm) on total length (mm)
178 for each species (WF: $R^2 = 0.66$, $P < 0.001$; LF: $R^2 = 0.30$, $P < 0.001$). Tanks were arranged in

179 rows (three rows in 2011 and two rows in 2013) against a concrete wall in such a way that rows
180 farther from the wall were exposed to sunlight for longer periods of time, effectively increasing
181 the temperature in these tanks on sunny days. We therefore analyzed dependent variables using a
182 nested design with treatment as a fixed factor nested within row. Tank temperatures were
183 measured daily as part of our water quality monitoring and we noted that the temperature
184 difference among rows was lost as vegetation leafed out over the fenced roof of the enclosure
185 after hatching, effectively shading all tanks. To account for potential carryover effects of
186 embryonic temperature on larval traits, we originally included row as an additional factor in all
187 larval analyses. However, we found no significant main or interactive effects of row on larval
188 traits for either species, therefore, we excluded row as a factor in the larval trait analyses and
189 results reported below.

190 Behaviour:

191 We used a repeated-measures ANOVA, with embryonic and larval environment as fixed
192 factors, to assess differences in the proportion (arcsin squareroot transformed) of active tadpoles
193 per tank, over 14 days from 30-May to 28-Jun for *L. sylvaticus* (2011) and *L. pipiens* (2013).

194 Morphology:

195 A variety of methods are used to describe size-independent shape changes in response to
196 predation risk in tadpoles, ranging from geometric morphometrics (Dayton et al. 2005; Johnson et
197 al. 2008) to linear measurements (Van Buskirk and McCollum 2000; Relyea 2001; Van Buskirk
198 and Arioli 2002); however, morphological responses are consistently related to changes in the
199 tail fin depth relative to body size. As such, we decided to use mass-corrected tail depth as our
200 response variable of morphological plasticity. To calculate mass-independent tail depth, we first
201 conducted ANCOVAs of raw tail depth (mm) by treatment (categorical factor with four levels:

202 predator always present, predators never present, predators in the embryonic environment,
203 predators in the larval environment) with tadpole mass (g) as the covariate for each week. We
204 used data from weeks 3 to 6 only, as tadpole mass interacted significantly with treatment during
205 weeks 1 and 2, thus violating the requirement that regression lines be parallel between treatments
206 in our size correction (Schoeppner and Relyea 2009). Mass-independent estimates of tail depth
207 were then calculated for every tadpole by adding the residuals from the ANCOVAs to the
208 estimated marginal means of each treatment (McCoy et al. 2006; Schoeppner and Relyea 2008,
209 2009). We then used the tank means of mass-corrected tail depths in a repeated-measures
210 ANOVA, with larval and embryonic environment as fixed factors and week as the repeated
211 measure.

212 Growth and Development:

213 To analyze the effect of predation risk in the larval and embryonic environments on
214 growth and development, we used a repeated-measures ANOVA with tank means of mass (g) or
215 developmental stage from weeks three through six as the response variables and embryonic and
216 larval environments as fixed factors. As developmental stage was the tank mean, it was treated as
217 a continuous variable (Laurila et al. 2002; Ireland et al. 2007).

218 All statistical analyses were performed (and graphs were created) using Statistica 10
219 (StatSoft 2011). Assumptions of statistical analyses were tested and met, following
220 transformations as noted above. Significance was set at $\alpha < 0.05$. Carryover effects are predicted
221 to be manifest through significant interactions between embryonic and larval environment on
222 dependent variables.

223

224 **Results**

225 *Wood frogs (L. sylvaticus)*

226 Hatching:

227 We found no effect of predation risk on hatching timing (time to 50% hatch: $F_{[3,6]} = 0.51$,
228 $P = 0.69$; total hatching time: $F_{[3,6]} = 0.56$, $P = 0.66$), however, we do note a non-significant
229 trend towards an increase in relative tail depth in wood frog hatchlings exposed to predation risk
230 ($F_{[3,6]} = 3.77$, $P = 0.08$; Fig. 1A). Row did not affect hatching timing (time to 50% hatch: $F_{[3,6]} =$
231 0.72 , $P = 0.53$; total hatching time: $F_{[3,6]} = 0.79$, $P = 0.50$), nor did row significantly affect
232 hatchling morphology in wood frog tadpoles ($F_{[3,6]} = 2.62$, $P = 0.15$).

233 Behaviour:

234 Wood frog tadpoles showed a significant decline in activity when exposed to predation
235 risk in the larval environment alone ($F_{[1,20]} = 7.67$, $P = 0.01$; Fig. 2A). Exposure to predation risk
236 in the embryonic environment had no effect on tadpole behaviour ($F_{[1,20]} = 0.60$, $P = 0.45$), nor
237 was there an interaction between embryonic and larval treatment on tadpole activity ($F_{[1,20]} =$
238 1.18 , $P = 0.29$). Activity varied by day ($F_{[13,260]} = 25.54$, $P < 0.001$) and we also noted a
239 significant two-way interaction between day and larval environment ($F_{[13,260]} = 1.80$, $P = 0.04$).
240 There was no interaction, however, between day and embryonic environment ($F_{[13,260]} = 0.93$, P
241 $= 0.53$), nor between day, larval, and embryonic environment ($F_{[13,260]} = 1.73$, $P = 0.06$) on wood
242 frog tadpole activity. It is difficult to infer any particular biological meaning from the statistical
243 interaction between day and larval treatment, though it likely reflects the naturally high variation
244 in activity level between days coupled with variability in the concentration of predation risk cues
245 on any given day (Fig. 2C).

246 Morphology:

247 Wood frog tadpoles exposed to predation risk in both the embryonic and larval

248 environments had significantly deeper tails than tadpoles exposed in either environment alone, as
249 well as those tadpoles that had never been exposed to predator cues (embryonic*larval: $F_{[1,20]} =$
250 9.51, $P = 0.01$; Fig. 3A). Exposure to predator cues in either environment alone did not affect
251 relative tail depth in wood frog tadpoles (embryonic: $F_{[1,20]} = 1.65$, $P = 0.21$; larval: $F_{[1,20]} =$
252 0.57, $P = 0.48$).

253 Relative tail depth increased significantly over four weeks ($F_{[3,60]} = 79.98$, $P < 0.001$).
254 Tadpoles exposed to predation risk in the embryonic environment had relatively deeper tails than
255 controls during weeks three and four, however, this difference was lost in weeks five and six
256 ($F_{[3,60]} = 3.84$, $P = 0.01$; Fig. 3B). There was no interaction between larval exposure and week on
257 relative tail depth ($F_{[3,60]} = 0.09$, $P = 0.97$), nor was there any three-way interaction effect on tail
258 depth (embryonic*larval*week: $F_{[3,60]} = 0.47$, $P = 0.71$).

259 Growth and Development:

260 As expected, wood frog tadpoles increased in mass ($F_{[3,60]} = 53.56$, $P < 0.001$) and
261 developmental stage ($F_{[3,60]} = 96.83$, $P < 0.001$) over four weeks. However, there was no effect of
262 predation risk on either mass (embryonic: $F_{[1,20]} = 0.70$, $P = 0.41$; larval: $F_{[1,20]} = 0.10$, $P = 0.75$;
263 embryonic*larval: $F_{[1,20]} = 0.78$, $P = 0.39$) or developmental stage (embryonic: $F_{[1,20]} = 0.26$, $P =$
264 0.62; larval: $F_{[1,20]} = 1.50$, $P = 0.23$; embryonic*larval: $F_{[1,20]} = 0.00$, $P = 0.97$). We do note a
265 significant three-way interaction between week, embryonic, and larval environment on tadpole
266 development ($F_{[3,60]} = 3.46$, $P = 0.02$). Tadpole development was slower for those individuals
267 exposed to predators in the larval environment only, but only during weeks four and five (Fig. 4).
268 By week six, there are no differences in developmental stage between treatment groups (Fig. 4).
269 There were no other significant interactions between week and environmental exposure for either
270 mass or stage ($P > 0.05$, in all cases).

271

272 *Northern leopard frogs (L. pipiens)*

273 Hatching:

274 Northern leopard frog eggs in the sunniest row hatched more quickly than in the shaded
275 row (time to 50% hatch: $F_{[1,8]} = 8.64$, $P = 0.02$; total hatching time: $F_{[1,8]} = 8.63$, $P = 0.02$),
276 however, we found no effect of predation risk on hatching timing (time to 50% hatch: $F_{[2,8]} =$
277 0.96 $P = 0.42$; total hatching time: $F_{[2,8]} = 0.02$, $P = 0.98$). Row did not affect hatching
278 morphology in leopard frog tadpoles ($F_{[1,8]} = 0.04$, $P = 0.85$), though, like the wood frogs,
279 hatchlings exposed to predation risk as eggs showed a non-significant trend towards relatively
280 deeper tails ($F_{[2,8]} = 3.71$, $P = 0.07$; Fig. 1B).

281 Behaviour:

282 Northern leopard frog tadpoles did not respond behaviourally to predation risk in either
283 the larval ($F_{[1,20]} = 3.00$, $P = 0.10$) or embryonic environment ($F_{[1,20]} = 0.71$, $P = 0.41$; Fig. 2B),
284 nor was there an interaction between the embryonic and larval environments on activity ($F_{[1,20]} =$
285 0.25 , $P = 0.70$). Activity varied significantly by day ($F_{[13,260]} = 18.42$, $P < 0.001$), though there
286 were no significant interactions between day and either embryonic or larval environment on
287 activity ($P > 0.10$, in all cases).

288 Morphology:

289 There was a significant interaction between embryonic and larval exposure on relative tail
290 depth (embryonic*larval: $F_{[1,20]} = 39.74$, $P < 0.001$). Tadpoles exposed to larval predator cues in
291 the embryonic environment tended to have relatively deeper tails than predator-naive tadpoles
292 when there was no risk of predation in the larval environment (Fig. 5A). However, this increased
293 tail depth in predator-conditioned tadpoles was not observed if tadpoles were also exposed

294 predation risk during larval development (Fig. 5A). Predator-naive tadpoles exposed to predation
295 risk cues as larvae responded by increasing relative tail depth ($F_{[1,20]} = 9.35, P < 0.001$) and there
296 was no effect of embryonic exposure alone on relative tail depth ($F_{[1,20]} = 0.41, P = 0.53$).

297 Relative tail depth increased over four weeks ($F_{[3,60]} = 144.81, P < 0.001$), and we noted a
298 significant three-way interaction between week, embryonic, and larval exposure to predation risk
299 ($F_{[3,60]} = 3.79, P = 0.02$) in which differences in relative tail depth between treatment groups
300 diminished by the fifth and sixth week of exposure (Fig. 5B). There were no other significant
301 interactions between week and embryonic or larval treatment ($P > 0.05$, in both cases).

302 **Growth and Development:**

303 Northern leopard frog tadpoles increased significantly in mass and developmental stage
304 over four weeks (mass: $F_{[3,60]} = 75.50, P < 0.001$; stage: $F_{[3,60]} = 100.04, P < 0.001$). There was a
305 significant three-way interaction between week, embryonic exposure, and larval exposure to
306 predation risk on tadpole mass in that tadpoles exposed to risk in either environment grew more
307 quickly in week six than those never exposed to predation risk, and those exposed as both
308 embryos and larvae ($F_{[3,60]} = 2.96, P = 0.04$; Fig. 6). There were no other significant effects of
309 predation risk nor interactions on either tadpole mass or developmental stage ($P > 0.05$, in all
310 cases).

311

312 **Discussion**

313 *Wood frogs (L. sylvaticus)*

314 Wood frog tadpoles demonstrated a carryover effect of embryonic exposure to predation
315 risk in morphological plasticity during larval development. Embryonic exposure to larval
316 predator cues resulted in hatchlings with a marginally significant increase in relative tail depth;

317 continued exposure to larval predator cues during larval development resulted in tadpoles with
318 significantly deeper tails in comparison to controls. A similar morphological response was not
319 found when tadpoles were predator-naive (i.e., not exposed to larval predator cues in the
320 embryonic environment). This lack of morphological response in predator-naive tadpoles is
321 surprising, given the volume of literature describing an increased relative tail depth in studies of
322 larval wood frogs (typically starting at Gosner stage 25) exposed to dragonfly predator cues (Van
323 Buskirk and Relyea 1998; Relyea 2002; Middlemis Maher et al. 2013). However, the timing of
324 exposure to predation-risk cues has been found to influence the induction of defensive
325 morphologies in Northern leopard frogs (Hossie and Murray 2012) and behavioural responses in
326 grey treefrogs (Relyea 2003). Tadpoles are more responsive (morphologically or behaviourally,
327 respectively) to exposure to predation risk cues early in larval development (Relyea 2003; Hossie
328 and Murray 2012). Additionally, wood frog embryos are able to associate predation risk with
329 chemical cues from novel predators when those predator cues are paired with the alarm cues of
330 injured, larval conspecifics (Mathis et al. 2008). It is possible that our population of wood frogs
331 are developing associations between predation risk and predator cues early in ontogeny, and that
332 the lack of opportunity to create these associations may limit the response to predators later in
333 development for predator-naive tadpoles. Indeed, the marginally significant effect of embryonic
334 exposure on relative tail depth was notable for up to three and four weeks post-hatching,
335 regardless of the continued presence of larval predators, suggesting defensive morphology in
336 wood frog tadpoles is influenced by embryonic exposure to cues of larval predation risk.

337 Unlike morphological responses, behavioural responses to predation risk cues among our
338 wood frog tadpoles do not appear to be the result of a learned association, as tadpoles decreased
339 activity in response to cues from conspecific-fed dragonfly larvae regardless of embryonic

340 experience. However, this lack of evidence of a learned association is not a general characteristic
341 of behavioural responses in wood frog tadpoles. Mathis et al. (2008) found that wood frog
342 tadpoles could learn as embryos to associate predation risk with unfamiliar predators through
343 conditioning with novel predators and injured conspecific cues, as evidenced by behavioural
344 changes when exposed to those same predator cues as tadpoles. Furthermore, learned
345 behavioural responses are threat-sensitive, with tadpoles increasing their subsequent antipredator
346 behavioural response to predator cues when, as embryos, they were exposed to higher
347 concentrations of injured tadpole cues coupled those same novel predator cues (Ferrari and
348 Chivers 2010). Thus, behavioural responses in wood frogs to predation risk can result from
349 embryonic conditioning to predator cues.

350 In our study, behavioural response to predation risk in wood frog tadpoles appears to be
351 innate, as it was dependent on the current presence or absence of larval predators and was not
352 affected by prior exposure to larval predator cues in the embryonic environment. In a common-
353 garden experiment on wood frog tadpoles from the E.S. George Reserve in Michigan, USA,
354 Relyea (2002) found tadpoles from eight different populations all responded to dragonfly
355 predator cues by decreasing activity. While the magnitude of the behavioural response to
356 predation risk varied by population, none of the tadpoles had prior embryonic exposure to either
357 dragonfly cues or cues from older, injured conspecifics (Relyea 2002). Thus, the behavioural
358 responses to predation risk by dragonfly larvae among these and our populations appear to
359 represent a genetically-based, naturally selected response to a specific predator.

360 *Northern leopard frogs (L. pipiens)*

361 Northern leopard frog tadpoles showed evidence of a carryover effect in our experiment,
362 however, the nature of this carryover effect was a trade-off between exposure in the embryonic

363 and exposure in the larval environment to larval predator cues. Predator-naive leopard frog
364 tadpoles responded to dragonfly predator cues by increasing relative tail depth. Predator-
365 conditioned tadpoles showed no such morphological response, though embryos exposed to
366 dragonfly predator cues hatched with marginally significantly deeper tails that were maintained
367 throughout larval development in the absence of predation risk. Furthermore, tadpoles exposed in
368 either the embryonic or larval environment showed increased growth rate at six weeks post-
369 hatching, while tadpoles exposed continuously throughout development had comparable growth
370 rates to controls. Interestingly, Northern leopard frog tadpoles did not show any significant
371 behavioural response to predation risk in any environment, suggesting that, as in wood frog
372 tadpoles, morphological and behavioural responses to predation risk are functionally
373 independent.

374 Tadpoles with relatively deeper tails have higher survivorship in the presence of
375 predatory dragonfly larvae than tadpoles with shallower tail fins (Van Buskirk and Relyea 1998).
376 In our study, Northern leopard frog embryos exposed to larval predator cues hatched with, and
377 retained, marginally deeper tails than predator-naive tadpoles. Predator-naive tadpoles, however,
378 increased their relative tail depth significantly in response to predation risk over three to four
379 weeks of exposure. Interestingly, tail depth effects were lost over time as all tadpoles, regardless
380 of predation risk exposure, had similarly deep tail fins after six weeks of development. Hossie
381 and Murray (2012) observed a similar phenomenon in *L. pipiens* tadpoles after six weeks of
382 development, and suggest that either the increased tail depth affords greater thrust to a swimming
383 tadpole to offset the drag caused by the developing limb buds later in development (energy
384 efficiency hypothesis), or that tadpoles become increasingly vulnerable to predators as they near
385 metamorphosis (innate predator defense hypothesis). While our experiments cannot distinguish

386 between the two hypotheses, we do provide evidence that this phenomenon is both repeatable
387 and measureable in multiple species. Furthermore, Northern leopard frog tadpoles in our
388 experiment showed a curious effect in which growth was accelerated at week six in those same
389 treatment groups that had relatively deeper tails at weeks three and four. A size refugium can
390 alter the growth-predation risk trade-off by increasing survivorship once prey reach a body size
391 larger than a predators gape (Babbitt and Tanner 1998, Chase 1999, Bell et al. 2011). It is
392 possible that tadpoles are switching anti-predator tactics at this time to increase body size as a
393 response to changes in relative vulnerability as they near metamorphosis, though further research
394 is required to test this hypothesis.

395 *General Conclusion*

396 The plasticity-history limitation hypothesis postulates that plastic responses are
397 constrained by previous responses earlier in ontogeny (Auld et al. 2010). While we note
398 significant carryover effects of exposure to larval predator cues for both wood and Northern
399 leopard frog tadpoles, the nature of these effects does not support the predictions of the
400 plasticity-history limitation in wood frogs and only provides weak support in Northern leopard
401 frogs. Wood frog tadpoles increased the magnitude of morphological response later in ontogeny
402 when conditioned to predation risk cues as embryos. Instead of a limitation, early exposure to
403 predator cues appears to have created or strengthened the association tadpoles developed
404 between dragonfly larvae and predation risk in wood frogs. In contrast, Northern leopard frogs
405 did exhibit limited morphological plasticity in response to predation risk exposure during both
406 embryonic and larval development, however, we did not find a significant morphological
407 response to predation risk in the embryonic environment alone. Therefore, while we do not show
408 evidence of the plasticity-history limitation *per se*, Northern leopard frog tadpoles appear to be

409 either constrained morphologically, or potentially desensitized, by the presence of larval predator
410 cues in both the embryonic and larval environment. Taken together, our findings suggest that
411 environmental cues experienced early in ontogeny, even in the embryonic environment, can have
412 fitness consequences on later life-history stages.

413

414 **Acknowledgments**

415 Thanks to K. Landolt, N. Hughes, M. Keevil, T. Isherwood and E. Kusari for help in the lab and
416 field. Trent University provided logistical support. Thanks as well to anonymous reviewers for
417 helpful comments and suggestions on a previous draft of this manuscript. This project was
418 funded by the Natural Sciences and Engineering Research Council of Canada and the Canada
419 Research Chairs program. The authors declare that they have no conflict of interest.

420 **Literature Cited**

- 421 Agrawal, A.A. 2001. Phenotypic plasticity in the interactions and evolution of species. *Science*,
422 **294**(5541): 321-326. doi: 10.1126/science.1060701.
- 423 Anholt, B.R., and Werner, E.E. 1995. Interaction between food availability and predation
424 mortality mediated by adaptive behavior. *Ecology*, **76**: 2230-2234. Available from
425 <http://www.jstor.org/stable/1941696> [accessed 20 January 2015].
- 426 Auld, J., Agrawal, A., and Relyea, R. 2010. Re-evaluating the costs and limits of adaptive
427 phenotypic plasticity. *Proc. R. Soc. Lond. B Biol. Sci.* **277**(1681): 503-511. doi:
428 10.1098/rspb.2009.1355.
- 429 Babbitt, K.J., and Tanner, G.W. 1998. Effects of cover and predator size on survival and
430 development of *Rana utricularia* tadpoles. *Oecologia*, **114**(2): 258-262. doi:
431 10.1007/s004420050444.
- 432 Baldwin, R.F., Calhoun, A.J.K., and deMaynadier, P.G. 2006. Conservation planning for
433 amphibian species with complex habitat requirements: a case study using movements and habitat
434 selection of the wood frog *Rana sylvatica*. *J. Herpetol.* **40**(4): 442-453. doi: 10.1670/0022-
435 1511(2006)40[442:CPFASW]2.0.CO;2.
- 436 Beckermann, A.P., Uriarte, M., and Schmitz, O.J. 1997. Experimental evidence for a behavior-
437 mediated trophic cascade in a terrestrial food chain. *Proc. Natl. Acad. Sci. U.S.A.* **94**(20): 10735-
438 10738. doi: 10.1073/pnas.94.20.10735.
- 439 Bell, A.M., Dingemanse, N.J., Hankison, S.J., Langenhof, M.B.W., and Rollins, K. 2011. Early
440 exposure to nonlethal predation risk by size-selective predators increases somatic growth and
441 decreases size at adulthood in three-spined sticklebacks. *J. Evol. Biol.* **24**(5): 943-53. doi:

- 442 10.1111/j.1420-9101.2011.02247.x.
- 443 Capellàn, E. and Nicieza, A.G. 2007. Trade-offs across life stages: does predator-induced
444 hatching plasticity reduce anuran post-metamorphic performance? *Evol. Ecol.* **21**(4): 445-458.
445 doi: 10.1007/s.10682-006-9133-9.
- 446 Chase, J.M. 1999. Food web effects prey size refugia: variable interactions and alternative stable
447 equilibria. *Am. Nat.* **154**(5): 559-570. doi: 10.1086/303260.
- 448 Chivers, D.P., Kiesecker, J.M., Marco, A., De Vito, J., Anderson, M.T., and Blaustein, A.R.
449 2001. Predator-induced life history changes in amphibians: egg predation induces hatching.
450 *Oikos*, **92**(1): 135-142. doi: 10.1034/j.1600-0706.2001.920116.x.
- 451 Dayton, G.H., Saenz, D., Baum, K.A., Langerhans, R.B., and DeWitt, T.J. 2005. Body shape,
452 burst speed and escape behaviour of larval anurans. *Oikos*, **111**(3): 582-591. doi: 10.1111/j.1600-
453 0706.2005.14340.x.
- 454 Ferrari, M.C.O., and Chivers, D.P. 2009. Latent inhibition of predator recognition by embryonic
455 amphibians. *Biol. Lett.* **5**(2): 160-162. doi: 10.1098/rsbl.2008.0641.
- 456 Ferrari, M.C.O., and Chivers, D.P. 2010. The ghost of predation future: Threat-sensitive and
457 temporal assessment of risk by embryonic wood frogs. *Behav. Ecol. Sociobiol.* **64**(4): 549-555.
458 doi: 10.1007/s00265-009-0870-y.
- 459 Ferrari, M.C.O., and Chivers, D.P. 2011. Learning about non-predators and safe places: the
460 forgotten elements of risk assessment. *Anim. Cogn.* **14**(3): 309-316. doi: 10.1007/s10071-010-
461 0363-4.
- 462 Gilbert, M., Leclair Jr., R., and Fortin, R. 1994. Reproduction of the Northern leopard frog (*Rana*

- 463 *pipiens*) in floodplain habitat in the Richelieu River, P. Quebec, Canada. *J. Herpetol.* **28**(4): 465-
464 470. Available from <http://www.jstor.org/stable/1564959> [accessed 26 January 2015].
- 465 Gomez-Mestre, I., Saccoccio, V.L., Iijima, T., Collins, E.M., Rosenthal, G.G., and Warkentin,
466 K.M. 2010. The shape of things to come: linking developmental plasticity to post-metamorphic
467 morphology in anurans. *J. Evol. Biol.* **23**(7): 1364-1373. doi: 10.1111/j.1420-9101.2010.02016.x.
- 468 Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on
469 identification. *Herpetologica*, **16**(3): 183-190. Available from
470 <http://www.jstor.org/stable/3890061> [accessed 20 January 2015].
- 471 Hossie, T.J., and Murray, D.L. 2012. Assessing behavioural and morphological responses of
472 frog tadpoles to temporal variability in predation risk. *J. Zool.* **288**(4): 275-282. doi:
473 10.1111/j.1469-7998.2012.00955.x.
- 474 Ireland D.H., Wirsing, A.J., and Murray, D.L. 2007. Phenotypically plastic responses of green
475 frog embryos to conflicting predation risk. *Oecologia*, **152**(1): 162-168. doi: 10.1007/s00442-
476 006-0637-3.
- 477 Johnson, J.B., Burt, D.B., and DeWitt, T.J. 2008. Form, function, and fitness: pathways to
478 survival. *Evolution*, **62**(5): 1243-1251. doi: 10.1111/j.1558.5646.2008.00343.x.
- 479 Kishida, O., Trussell, G.C., Mougi, A., and Nishimura, K. 2010. Evolutionary ecology of
480 inducible morphological plasticity in predator-prey interactions: toward the practical links with
481 population ecology. *Popul. Ecol.* **52** (1): 37-46. doi: 10.1007/s10144-009-0182-0.
- 482 Laurila, A., Pakkasmaa, S., Crochet, P.-A., and Merilä, J. 2002. Predator-induced plasticity in
483 early life history and morphology in two anuran amphibians. *Oecologia*, **132**(4): 524-530. doi:

- 484 10.1007/s00442-002-0984-7.
- 485 Laurila, A., Järvi-Laturi, M., Pakkasmaa, S., and Merilä, J. 2004. Temporal variation in
486 predation risk: stage-dependency, graded responses and fitness costs in tadpole antipredator
487 defences. *Oikos*, **107**(1): 90-99. doi: 10.1111/j.0030-1299.2004.13126.x.
- 488 Mandrillon, A.-L., and Saglio, P. 2007. Effects of embryonic exposure to conspecific chemical
489 cues on hatching and larval traits in the common frog (*Rana temporaria*). *Chemoecology*, **17**(3):
490 169-175. doi: 10.1007/s00049-007-0376-x.
- 491 Mathis, A., Ferrari, M.C.O., Windel, N., Messier, F., and Chivers, D.P. 2008. Learning by
492 embryos and the ghost of predation future. *Proc. R. Soc. Lond. B Biol. Sci.* **275**: 2603-2607. doi:
493 10.1098/rspb.2008.0754.
- 494 McCoy, M., Bolker, B.M., Osenberg, C.W., Miner, B.G., and Vonesh, J.R. 2006. Size
495 correction: Comparing morphological traits among populations and environments. *Oecologia*,
496 **148**(4): 547-554. doi: 10.1007/s00442-006-0403-6.
- 497 Middlemis Maher, J., Werner, E.E., and Denver, R.J. 2013. Stress hormones mediate predator-
498 induced phenotypic plasticity in amphibian tadpoles. *Proc. R. Soc. Lond. B Biol. Sci.* **280**:
499 20123075. doi: 10.1098/rspb.2012.3075.
- 500 Niecieza A.G., Alvarez, D., and Atienza, E.M.S. 2006. Delayed effects of larval predation risk
501 and food quality on anuran juvenile performance. *J. Evol. Biol.* **19**(4): 1092-1103. doi:
502 10.1111/j.1420-9101.2006.01100.x.
- 503 Petranka, J.W., and Thomas, D.A.G. 1995. Explosive breeding reduces egg and tadpole
504 cannibalism in the wood frog, *Rana sylvatica*. *Anim. Behav.* **50**(3): 731-739. doi: 10.1016/0003-

- 505 3472(95)80133-2.
- 506 Pigliucci, M., Murren, C.J., and Schlichting, C.D. 2006. Phenotypic plasticity and evolution by
507 genetic assimilation. *J. Exp. Biol.* **209**: 2362-2367. doi: 10.1242/jeb.02070.
- 508 Prokopy, R.J., Averill, A.L., Cooley, S.S., and Roitberg, C.A. 1982. Associative learning in
509 egg-laying site selection by apple maggot flies. *Science*, **218**(4567): 76-77. doi:
510 10.1126/science.218.4567.76.
- 511 Rasband, W.S. 2012. ImageJ. U.S. National Institutes of Health, Bethesda, Maryland, USA.
512 Available from <http://imagej.nih.gov/ij/> [Accessed 20 January 2015].
- 513 Reed, T.E., Waples, R.S., Schindler, D.E., Hard, J.J., and Kinnison, T. 2010. Phenotypic
514 plasticity and population viability: the importance of environmental predictability. *Proc. R. Soc.
515 Lond. B Biol. Sci.* **277**: 3391-3400. doi: 10.1098/rspb.2010.0771.
- 516 Relyea, R.A. 2001. Morphological and behavioral plasticity of larval anurans in response to
517 different predators. *Ecology*, **82**(2): 523-540. doi: 10.1890/0012-
518 9658(2001)082[0523:MABPOL]2.0.CO;2.
- 519 Relyea, R.A. 2002. Local population differences in phenotypic plasticity: Predator-induced
520 changes in wood frog tadpoles. *Ecol. Monogr.* **72**(1): 77-93. Available from
521 <http://www.jstor.org/stable/310086> [Accessed 19 June 2015].
- 522 Relyea, R.A. 2003. Predators come and predators go: the reversibility of predator-induced traits.
523 *Ecology*, **84**(7): 1840-1848. doi: 10.1890/0012-9658(2003)084[1840:PCAPGT]2.0.CO;2.
- 524 Relyea, R.A., and Werner, E.E. 2000. Morphological plasticity in four larval anurans distributed
525 along an environmental gradient. *Copeia*, 2000(1): 178-190. doi: 10.1643/0045-

- 526 8511(2000)2000[0178:MPIFLA]2.0.CO;2.
- 527 Scheiner, S.M. 1993. Genetics and evolution of phenotypic plasticity. *Annu. Rev. Ecol. Syst.* **24**:
528 35-68. Available from <http://www.jstor.org/stable/2097172> [accessed 20 January 2015].
- 529 Schoeppner, N.M., and Relyea, R.A. 2009. Interpreting the smells of predation: how alarm cues
530 and kairomones induce different prey defenses. *Funct. Ecol.* **23**(6): 1114-1121. doi:
531 10.1111/j.1365.2435.2009.01578.x.
- 532 Sih, A., and Moore, R.D. 1993. Delayed hatching of salamander eggs in response to enhanced
533 larval predation risk. *Am. Nat.* **142**(6): 947-960. Available from
534 <http://www.jstor.org/stable/2462693> [accessed 20 January 2015].
- 535 Van Buskirk, J., and Relyea, R.A. 1998. Selection for phenotypic plasticity in *Rana sylvatica*
536 tadpoles. *Biol. J. Linn. Soc.* **65**: 301-328. 10.1006/bijl.1998.2049.
- 537 Van Buskirk, J., and Arioli, M. 2002. Dosage response of an induced defense: How sensitive are
538 tadpoles to predation risk? *Ecology*, **83**(6): 1580-1585. doi: 10.1890/0012-
539 09658(2002)083[1580:DROAID]2.0.CO;2.
- 540 Van Buskirk, J., and McCollum, S.A. 2000. Functional mechanisms of an inducible defence in
541 tadpoles: morphology and behaviour influence mortality risk from predation. *J. Evol. Biol.* **13**(2):
542 336-347. doi: 10.1046/j.1420-9101.2000.00173.x.
- 543 Van Buskirk, J., and Saxer, G. 2001. Delayed costs of an induced defense in tadpoles?
544 Morphology, hopping, and development rate at metamorphosis. *Evolution*, **55**(4): 821-829. doi:
545 10.1111/j.0014-3820.2001.tb00817.x.
- 546 Van Buskirk, J., and Schmidt, B.R. 2000. Predator-induced phenotypic plasticity in larval newts:

- 547 trade-offs, selection, and variation in nature. *Ecology*, **81**(11): 3009-3028. doi: 10.1890/0012-
548 9658(2000)081[3009:PIPPIL]2.0.CO;2.
- 549 Van Buskirk, J., Anderwald, P., Lüpold, S., Reinhardt, L., and Schuler, H. 2003. The lure effect,
550 tadpole tail shape, and the target of dragonfly strikes. *J. Herpetol.* **37**(2): 420-424. doi:
551 10.1670/0022-1511(2003)037[0420:TLETTTS]2.0.CO;2.
- 552 Van Kleunen, M., and Fischer, M. 2005. Constraints on the evolution of adaptive phenotypic
553 plasticity in plants. *New Phytol.* **166**(1): 49-60. doi: 10.1111/j.1469-8137.2004.01296.x.
- 554 Via, S., Gomulkiewicz, R., De Jon, G., Scheiner, S.M., Schlichting, C.D., and VanTienderen,
555 P.H. 1995. Adaptive phenotypic plasticity: consensus and controversy. *Trends Ecol. Evol.* **10**(5):
556 212-217. doi: 10.1016/S0169-5347(00)89061-8.
- 557 Vonesh, J.R. 2005. Sequential predator effects across three life stages of the African tree frog,
558 *Hyperolius spinigularis*. *Oecologia*, **143**(2): 280-290. doi: 10.1007/s00442-004-1806-x.
- 559 Weinig, C., and Delph, L.F. 2001. Phenotypic plasticity early in life constrains developmental
560 responses later. *Evolution*, **55**(5): 930-936. doi: 10.1111/j.0014-3820.2001.tb00610.x.
- 561 Werner, E.E. 1992. Competitive interactions between wood frog and Northern leopard frog
562 larvae: the influence of size and activity. *Copeia*, 1992(1): 26-35. Available from
563 <http://www.jstor.org/stable/1446532> [accessed 20 January 2015].
- 564 Werner, E.E., and Glennemeier, K.S. 1999. Influence of forest canopy cover on the breeding
565 pond distributions of several amphibian species. *Copeia*, 1999(1): 1-12. Available from
566 <http://www.jstor.org/stable/1447379> [accessed 20 January 2015].

567 **Figure 1:** Boxplot (mean \pm standard error (box) \pm standard deviation (whisker)) of relative tail
568 depth (residuals of tail depth (mm) regressed against total length (mm): see text for detail) of
569 wood frog (*Lithobates sylvaticus* (LeConte, 1825): A) and Northern leopard frog (*L. pipiens*
570 (Schreber, 1782): B) hatchlings after exposure to predation risk (caged Aeshnidae dragonfly
571 nymph) or an empty cage (control) during embryonic development.

572

573 **Figure 2:** Mean (\pm standard error) proportion of active wood frog (*Lithobates sylvaticus*
574 (LeConte, 1825): A) and Northern leopard frog (*L. pipiens* (Schreber, 1782): B) tadpoles over six
575 weeks, and mean (\pm standard error) proportion by day of active wood frog tadpoles (C) per tank
576 in response to predation risk (caged Aeshnidae dragonfly nymph) or control (empty cage) in the
577 larval environment.

578

579 **Figure 3:** Mean (\pm standard error) relative tail depth (residuals of tail depth (mm) regressed
580 against body size (PC1: see text for details on how body size was calculated)) of wood frog
581 tadpoles (*Lithobates sylvaticus* (LeConte, 1825)) exposed to caged dragonfly nymphs
582 (Aeshnidae) in the embryonic and/or larval environment averaged over six weeks (A), and in the
583 embryonic environment alone averaged by week from weeks three to six (B).

584

585 **Figure 4:** Mean (\pm standard error) developmental stage (Gosner 1960) averaged by week for
586 weeks three through six post-hatching of wood frog (*Lithobates sylvaticus* (LeConte, 1825))
587 tadpoles exposed to caged dragonfly nymphs (Aeshnidae) in the embryonic and/or larval
588 environment.

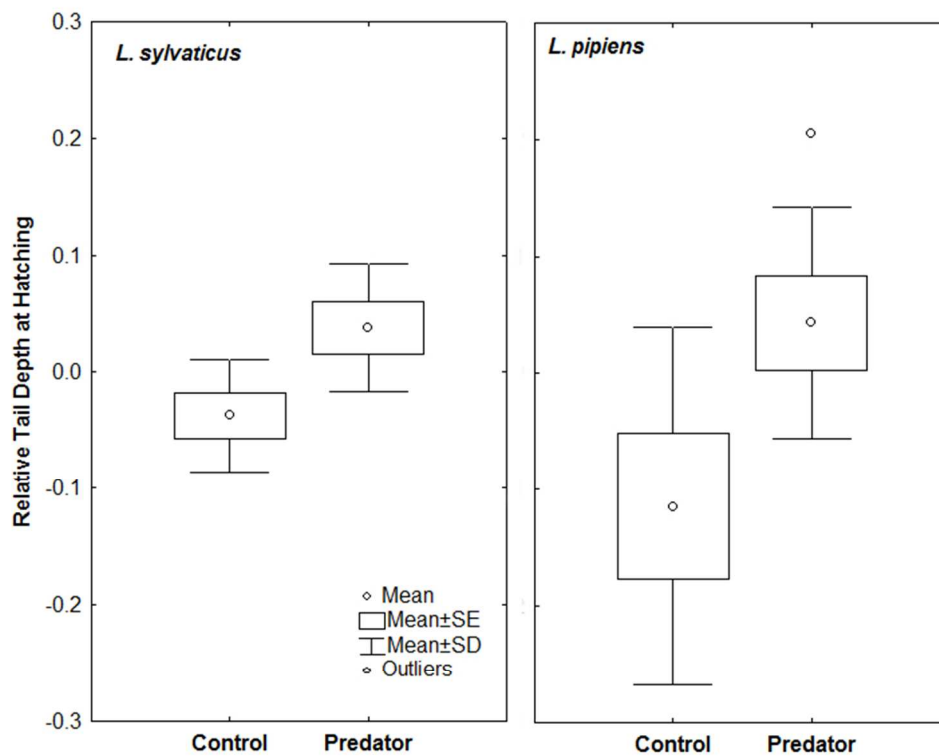
589

590 **Figure 5:** Mean (\pm standard error) relative tail depth (residuals of tail depth (mm) regressed
591 against body size (PC1: see text for details on how body size was calculated)) of wood frog
592 tadpoles (*Lithobates pipiens* (Schreber, 1782)) exposed to caged dragonfly nymphs (Aeshnidae)
593 in the embryonic and/or larval environment averaged over six weeks (A), and averaged by week
594 from weeks three to six (B).

595

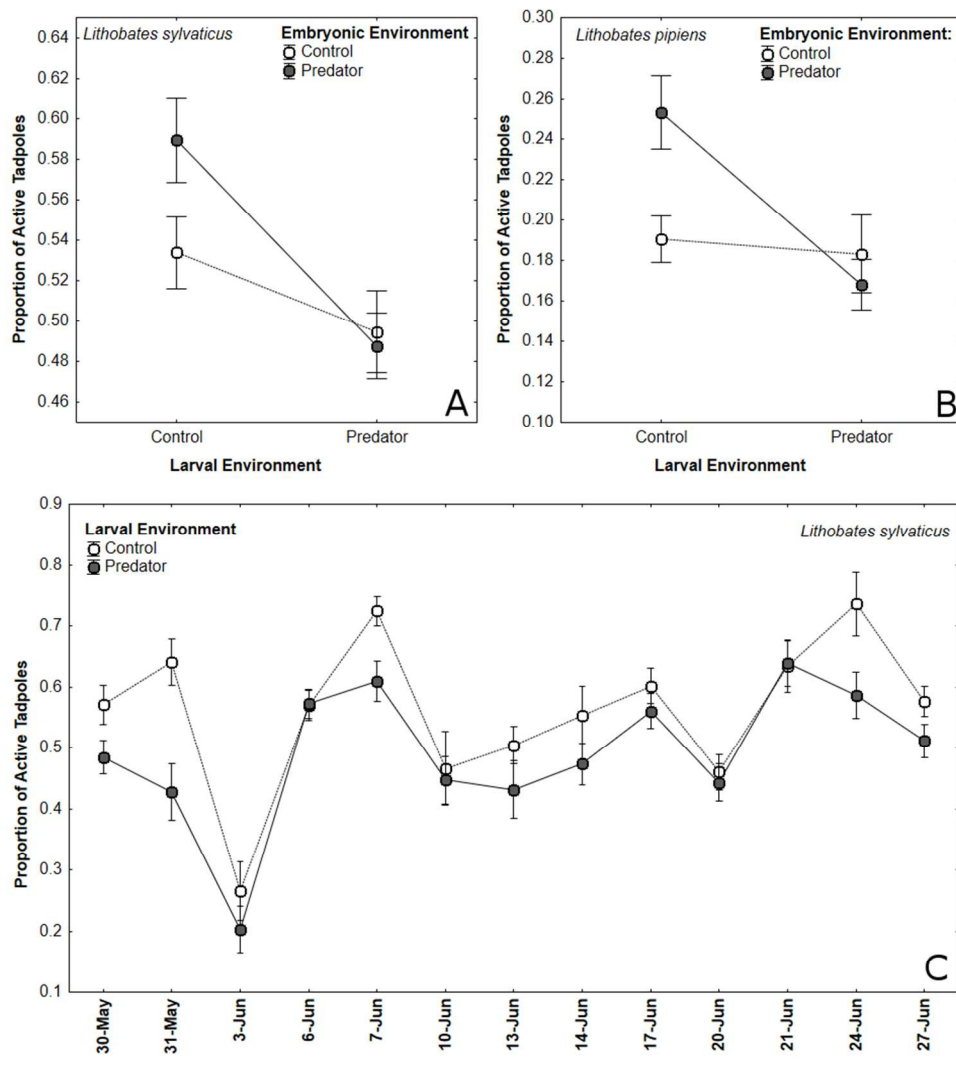
596 **Figure 6:** Mean (\pm standard error) mass (g) averaged by week for weeks three through six post-
597 hatching of Northern leopard frog (*Lithobates pipiens* (Schreber, 1782)) tadpoles exposed to
598 caged dragonfly nymphs (Aeshnidae) in the embryonic and/or larval environment.

Draft

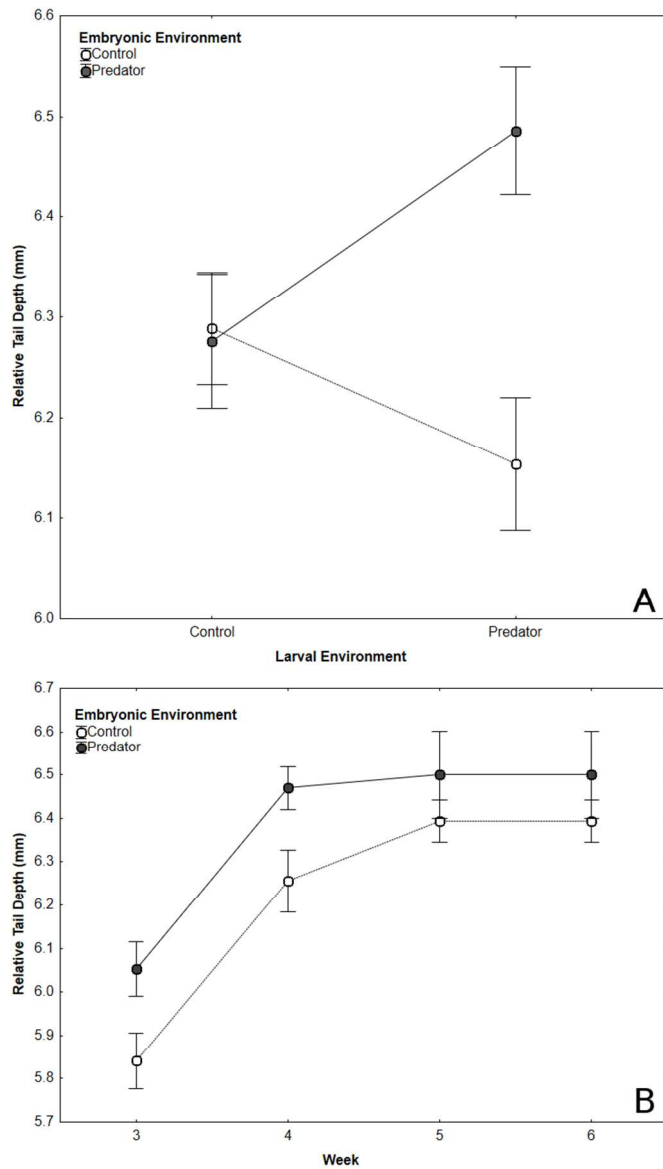


Boxplot (mean \pm standard error (box) \pm standard deviation (whisker)) of relative tail depth (residuals of tail depth (mm) regressed against total length (mm): see text for detail) of wood frog (*Lithobates sylvaticus* (LeConte, 1825): A) and Northern leopard frog (*L. pipiens* (Schreber, 1782): B) hatchlings after exposure to predation risk (caged Aeshnidae dragonfly nymph) or an empty cage (control) during embryonic development.

63x48mm (300 x 300 DPI)

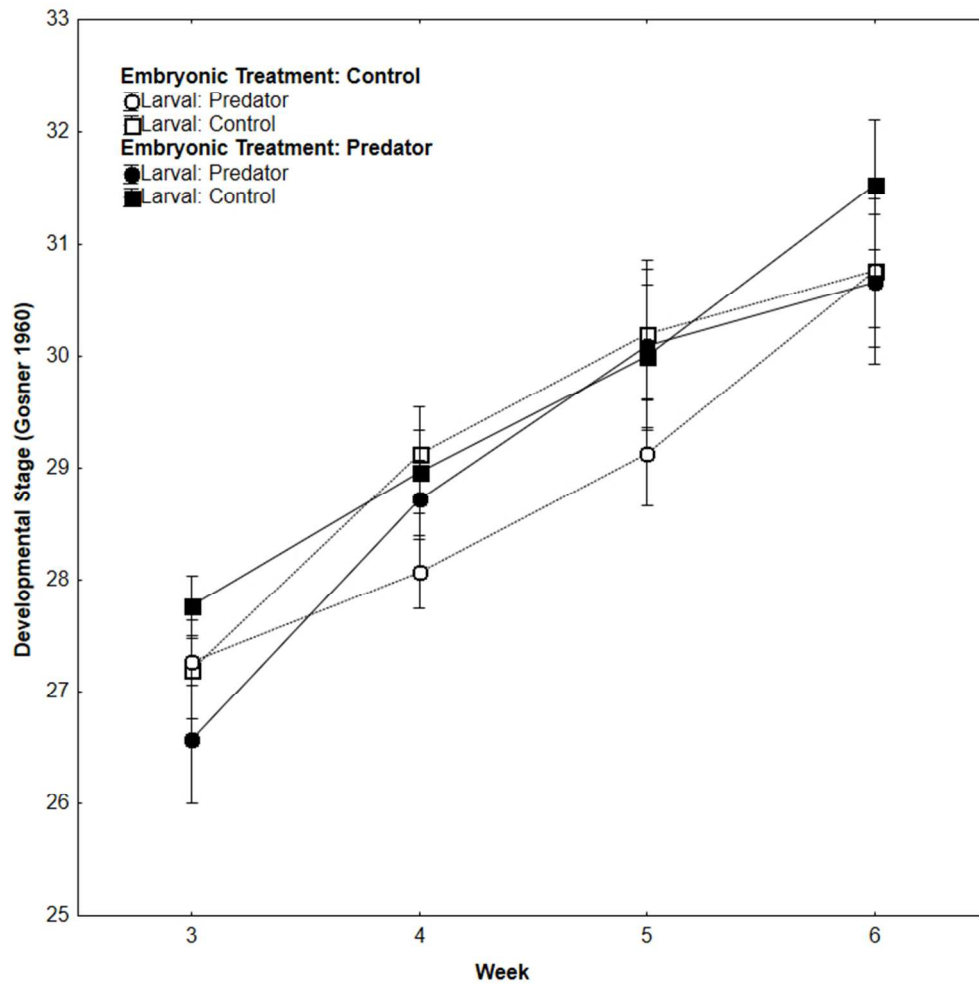


Mean (\pm standard error) proportion of active wood frog (*Lithobates sylvaticus* (LeConte, 1825): A) and Northern leopard frog (*L. pipiens* (Schreber, 1782): B) tadpoles over six weeks, and mean (\pm standard error) proportion by day of active wood frog tadpoles (C) per tank in response to predation risk (caged Aeshnidae dragonfly nymph) or control (empty cage) in the larval environment. 83x89mm (300 x 300 DPI)

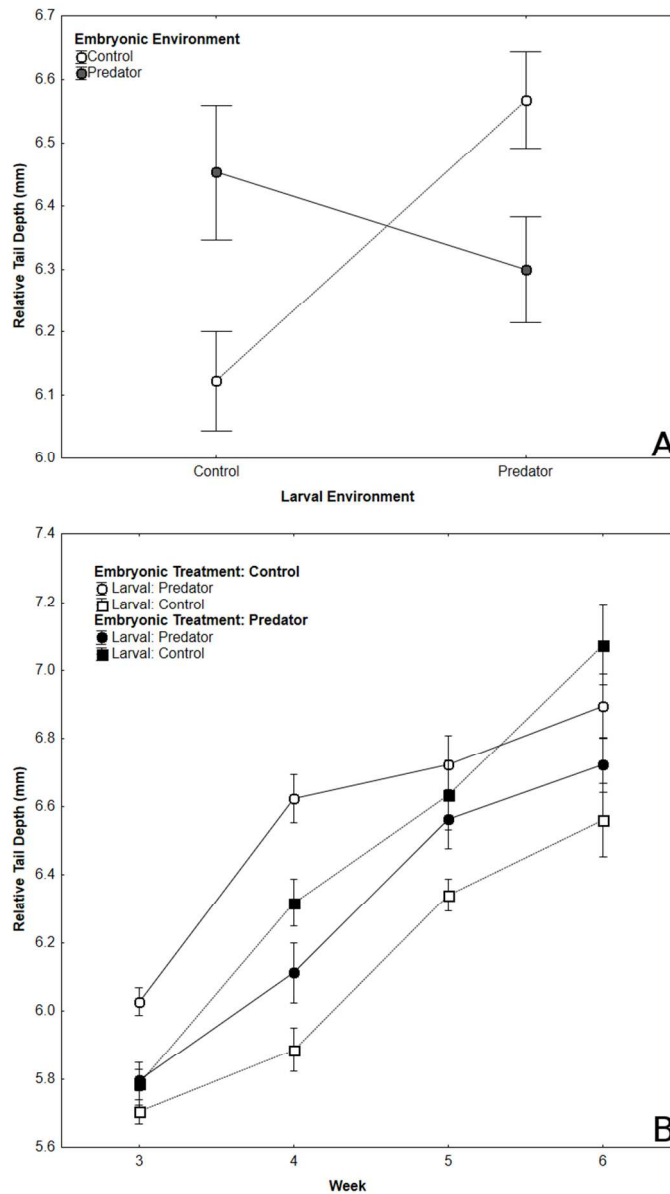


Mean (\pm standard error) relative tail depth (residuals of tail depth (mm) regressed against body size (PC1: see text for details on how body size was calculated)) of wood frog tadpoles (*Lithobates sylvaticus* (LeConte, 1825)) exposed to caged dragonfly nymphs (Aeshnidae) in the embryonic and/or larval environment averaged over six weeks (A), and in the embryonic environment alone averaged by week from weeks three to six (B).

66x116mm (300 x 300 DPI)

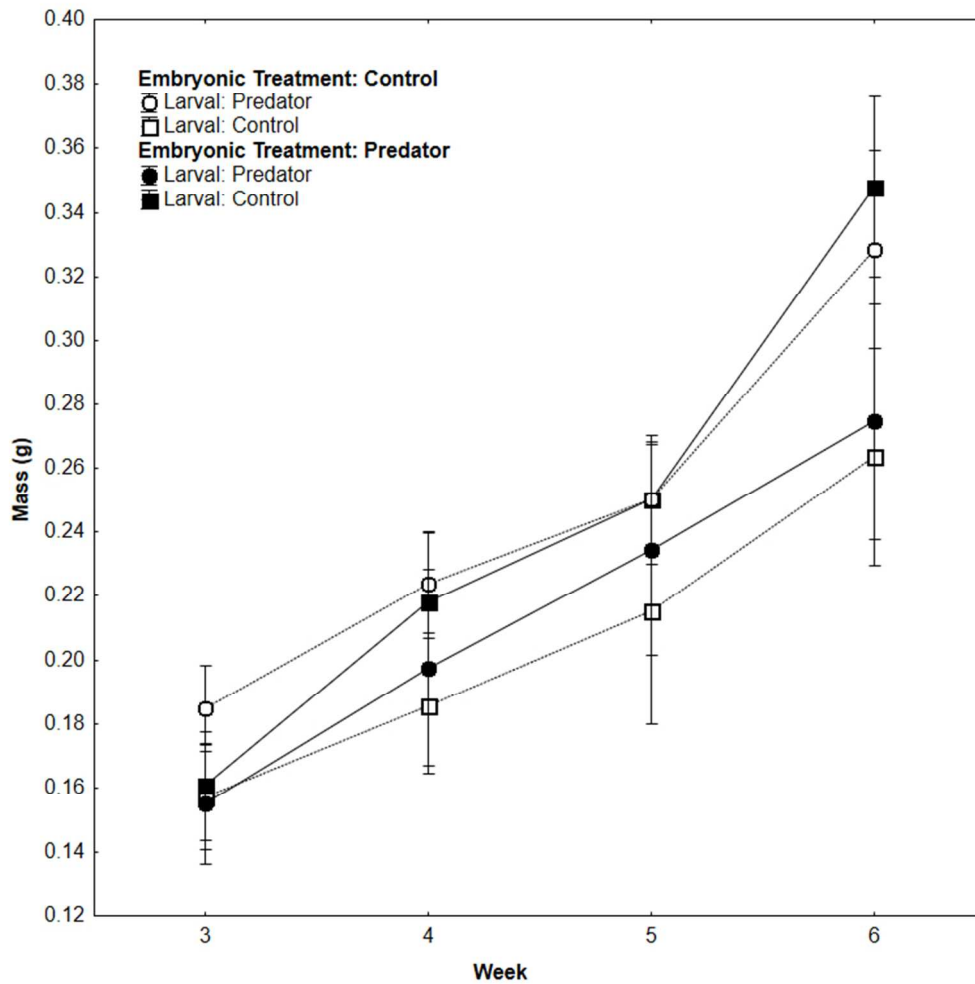


Mean (\pm standard error) developmental stage (Gosner 1960) averaged by week for weeks three through six post-hatching of wood frog (*Lithobates sylvaticus* (LeConte, 1825)) tadpoles exposed to caged dragonfly nymphs (Aeshnidae) in the embryonic and/or larval environment.
203x203mm (96 x 96 DPI)



Mean (\pm standard error) relative tail depth (residuals of tail depth (mm) regressed against body size (PC1: see text for details on how body size was calculated)) of wood frog tadpoles (*Lithobates pipiens* (Schreber, 1782)) exposed to caged dragonfly nymphs (Aeshnidae) in the embryonic and/or larval environment averaged over six weeks (A), and averaged by week from weeks three to six (B).

67x113mm (300 x 300 DPI)



Mean (\pm standard error) mass (g) averaged by week for weeks three through six post-hatching of Northern leopard frog (*Lithobates pipiens* (Schreber, 1782)) tadpoles exposed to caged dragonfly nymphs (Aeshnidae) in the embryonic and/or larval environment.
203x203mm (96 x 96 DPI)