Larval long-toed salamanders incur nonconsumptive effects in the presence of nonnative trout

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Abstract. Predators can influence prey directly through consumption or indirectly through nonconsumptive effects (NCEs) by altering prey behavior, morphology, and life history. We investigated whether predator-avoidance behaviors by larval long-toed salamanders (Ambystoma macrodactylum) in lakes with nonnative trout result in NCEs on morphology and development. Field studies in lakes with and without trout were corroborated by experimental enclosures, where prey were exposed only to visual and chemical cues of predators. We found that salamanders in lakes with trout were consistently smaller than in lakes without trout: 38% lower weight, 24% shorter body length, and 29% shorter tail length. Similarly, salamanders in protective enclosures grew 2.9 times slower when exposed to visual and olfactory trout cues than when no trout cues were present. Salamanders in trout-free lakes and enclosures were 22.7 times and 1.48 times, respectively, more likely to metamorphose during the summer season than those exposed to trout in lakes and/or their cues. Observed changes in larval growth rate and development likely resulted from a facultative response to predator-avoidance behavior and demonstrate NCEs occurred even when predation risk was only perceived. Reduced body size and growth, as well as delayed metamorphosis, could have ecological consequences for salamander populations existing with fish if those effects carry-over into lower recruitment, survival, and fecundity.

Key words: Ambystoma macrodactylum; amphibian larvae; avoidance behavior; body morphology; energy acquisition; field experiment; metamorphosis; mountain lakes; NCEs; phenotypic plasticity; predator cues; predator–prey dynamics.

INTRODUCTION

To avoid predation, prey use tactile, visual, or chemical cues to detect predators and respond with appropriate defense tactics (Kats et al. 1988, Stauffer and Semlitsch 1993, Lima 1998). Some defenses are always present, such as unpalatable toxins in skin of newts and toads (Daly 1995). Other defenses are facultative and induced by the presence of predators, resulting in changes in behavior, morphology, and life history of prey (Lima 1998, Benard 2004). Although such defensive strategies may reduce the probability of predation, they often come with a cost (Lima 1998).

Nonconsumptive effects (NCEs) of predators can alter energy acquisition, activity level, and diet of prey species (Werner et al. 1983, Davenport et al. 2014). Individuals that reduce activity are less likely to be detected and captured by predators, but...
also acquire fewer resources (Werner et al. 1983). Furthermore, foraging activity that does occur often is restricted to low-risk, resource-poor areas to reduce predator encounter rates, ultimately resulting in reduced size and growth (Werner et al. 1983). The eventual consequences of reduced energy intake are poorly known; however, decreases in body size are associated with reduced survival, increased susceptibility to disease, and reduced reproductive success (Werner et al. 1983, Lima 1998, Peckarsky et al. 2002). Moreover, NCEs incurred by prey may be equally or even more influential on population dynamics than predator consumption (Werner and McPeek 1994, Peaco and Werner 2001). Therefore, prey must appropriately balance the advantages of predator defenses with the costs of NCEs (Lima 1998).

Dynamics between predators and prey become more complex when predators become established in novel environments (Salo et al. 2007, Sih et al. 2010). Unlike prey that have co-occurred, and perhaps co-evolved, with native predators for long periods of time, prey naïve to non-native predators may lack chemical or morphological defenses and instead must rely on changes in behavior or life history traits (Kiesecker and Blaustein 1997, Salo et al. 2007). Trout, salmon, and char (family Salmonidae) are predatory fish that have been introduced to most continents and many water bodies outside of their historic distribution, often resulting in considerable ecological effects on native species and ecosystems (Crawford and Muir 2008, Cucherousset and Olden 2011). The negative effects of nonnative trout on amphibian populations are particularly well documented for high-elevation lakes, most of which were historically fishless prior to stocking (see review by Pilliod et al. 2012).

In western North America, the long-toed salamander (Ambystoma macrodactylum) is particularly sensitive to fish predation, exhibiting lower abundance or even local extirpation in the presence of fish predators (Tyler et al. 1998a, Pilliod and Peterson 2001, Bull and Marx 2002, Welsh et al. 2006, Pilliod et al. 2010). Long-toed salamanders are palatable to trout and susceptible to predation during all developmental stages from egg to adult (Tyler et al. 1998b, Pearson and Goater 2009). Furthermore, coexistence of long-toed salamanders and trout is rare, but has been observed in the Rocky Mountains where complex structure provides suitable refugia from predation (Pilliod et al. 2013).

Previous research has focused primarily on the direct, consumptive effects of introduced trout on long-toed salamanders (Tyler et al. 1998a). Less is known about potential NCEs of trout on salamander larvae (Pilliod and Peterson 2001, Pilliod et al. 2010). Salamander larvae can detect chemical and visual cues of fish predators and increase refuge use to avoid predation within laboratory settings; this defensive strategy is associated with reduced foraging activity, energy intake, growth, and size during the larval stage (Tyler et al. 1998b, Pearson and Goater 2009). Furthermore, amphibians can induce early metamorphosis facultatively under high levels of perceived risk to leave a dangerous environment, but often do so at smaller sizes (Werner 1986, Kiesecker et al. 2002). Although extirpations of salamander populations and the rarity of coexistence between these species are thought to be a result of direct predation, NCEs may play an important role in population dynamics.

We sampled long-toed salamander larvae in lakes with and without trout to test for evidence of NCEs of trout on body morphology and metamorphosis of larval salamanders. In addition, we conducted a field experiment using enclosures to test if NCEs on growth and probability of metamorphosis can be induced via exposure to chemical and visual cues of fish predators alone. Quantifying NCEs in both the lake-wide sampling and field experiments will provide corroborating evidence that salamanders can: (1) detect fish predators; and (2) display morphological and life history plasticity in response to predation risk, regardless of consumptive effects. We hypothesized that salamanders exposed to trout or their cues would be smaller in size and would have shorter larval periods.

**Methods**

**Study lakes**

We sampled permanent lakes in the South Fork Flathead River and Swan River drainages in northwestern Montana during the summers of 2012 and 2013. We did not randomly select lakes. Instead, we selected potential study lakes based on previous surveys that confirmed presence of salamanders and trout (B. Maxell and M. Boyer, personal communication). We then
located nearby lakes of similar sizes that contained salamanders but lacked fish; these sites served as trout-free reference locations in our sampling design. In 2012, we sampled 14 lakes occupied by salamanders, seven lakes with trout and seven without trout. In 2013, we sampled six of the original 14 lakes, three with trout and three without. We identified trout in the study lakes as rainbow trout (*Oncorhynchus mykiss*), cutthroat trout (*O. clarki*), or hybrids of the two species based on field observations, angler interviews, and Montana stocking reports (Montana Fish, Wildlife & Parks 2004, 2005, 2007, 2010, 2011). To account for conditions in study lakes, we measured dissolved oxygen, pH, and water temperature using a YSI Pro 1030 during each sampling session in 2012 and measured lake perimeter and recorded elevation of each lake.

**Lake-wide sampling**

We captured long-toed salamander larvae with minnow traps, which are especially useful to trap small, cryptic larvae, are associated with little mortality or injury, and capture individuals passively without bait (Adams et al. 1997). We set all traps in the littoral zone around the perimeter of each lake at depths ≤ 1m, with the number of traps dependent upon lake size. In 2012, the total number of traps in each lake ranged from 6 to 16. To increase trapping effort in 2013, we placed one trap every 20 m; the total number of traps in each lake ranged from 7 to 48. We deployed traps for multiple 4-d capture sessions from July through August, with a total of four sampling sessions in 2012 and three in 2013, and examined the number of individuals captured per unit effort as our measure of abundance.

**Field experiment**

In conjunction with lake-wide sampling during the summer of 2013, we conducted a field experiment in five of the six study lakes, two lakes with trout and three without trout. We constructed experimental field enclosures with PVC pipe and fiberglass window screening (2 × 1 × 1 m, LWH, Sredl and Collins 1991, Kiesecker and Blaustein 1998). We placed eight enclosures in lakes with trout and four enclosures in lakes without trout, added ~10 L of lake water to enclosures at each visit to provide additional food resources (Stenhouse 1985).

**Data collection**

During each sampling session and enclosure visit, we collected salamanders from traps or enclosures, anesthetized individuals with MS-222, measured weight, total length, and tail length with an electronic scale (to 0.001 g) and calipers (to 0.1 mm) and marked individuals with visual implant elastomer in the middle of the tail. Elastomer is a common marking technique for larval salamanders, which is long-lasting and does not affect survival or metamorphosis (Grant 2008). We marked individuals captured during lake-wide sampling with a batch mark specific to the sampling session number, to identify them as recaptures. In field enclosures, we marked each salamander uniquely. We also noted whether each salamander had initiated metamorphosis, defined as any evidence of gill absorption (Dodd and Dodd 1976, Duellman and Trueb 1986). We anesthetized, marked, and handled all captured individuals in accordance with Montana State University Institutional Animal Care and Use Committee protocols 2012-28 and 2013-04. We released all individuals at their original capture location or in their assigned enclosure after data collection.

**Statistical analyses**

We compared measurements of body morphology (weight, total length, and tail length, depths and distances from each other (Sredl and Collins 1991, Kenison 2014). This design allowed us to expose salamanders to chemical and visual signals of trout, while still excluding fish and the risk of direct predation.

We captured salamanders with minnow traps and randomly assigned 20 salamanders that were comparable in size to each enclosure; all salamanders remained in the lake where they were captured. We selected this density of salamanders as it was comparable to previous studies that did not observe cannibalism and where adequate amounts of food persisted throughout study periods (e.g., Semlitsch 1987, Tyler et al. 1998b, Pearson and Goater 2009). We visited each enclosure four times during July and August 2013. Although we expected zooplankton and other small crustaceans to be able to pass through enclosure screen, we added ~10 L of lake water to enclosures at each visit to provide additional food resources (Stenhouse 1985).
each as a separate response variable) for larval salamanders collected from lake-wide sampling and field enclosures in lakes with and without trout. We made separate comparisons of salamanders that metamorphosed and those that did not. We also compared the probability of salamander metamorphosis for lakes with and without trout; these estimates were based on the number of individuals exhibiting signs of metamorphosis relative to the total number of individuals trapped in lakes or present in enclosures.

We used a generalized linear mixed model approach for all analyses, selecting the appropriate distribution and link function for each response variable. We included the presence or absence of trout or their cues as the main effect in our analytical models. We treated lakes as subjects and included a three-tiered, nested data structure—(1) multiple sampling sessions nested within lakes nested within years or (2) multiple salamanders nested within enclosures nested within lakes—as random effects in all analyses to account for repeated measurements and variation among lakes (Zuur et al. 2009). We also included year and number of days since the first sampling visit to account for changes over time and to test for interactive effects (trout × day). We removed interactions that did not explain sufficient variation (P > 0.1), but retained all simple explanatory variables (trout, year, and day) in models for inference. We also included all lake-level covariates (perimeter, elevation, and repeated measurements of dissolved oxygen, pH, and temperature) as random effects within models to account for variation between lakes with and without trout. We log-transformed response variables when appropriate to meet assumptions. We ran all statistical analyses in program R version 3.0.2 and used the “nlme” and “MASS” packages (R Development Core Team 2013). We report mean values, mean and percent differences, probability of metamorphosis, and 95% confidence intervals, along with t statistics and P values in text; we back-transformed these values when appropriate.

RESULTS

Lake-wide sampling

Some lake-level covariates differed between study sites; lakes with trout had higher levels of DO (1.87 ppm difference, t12 = 2.79, P = 0.02), higher pH (0.54, t12 = 2.65, P = 0.02), and larger perimeters (354.3 m, t12 = 2.68, P = 0.02), but elevation (294.6 m, t12 = 1.83, P = 0.09) and temperature (0.26°C, t12 = 0.17, P = 0.87) were similar between lakes with and without trout, after accounting for year. Despite some differences in physical attributes of lakes, we considered them to be comparable given that densities of salamanders were similar on the basis of catch per unit effort (difference of 6 salamanders/100 m, 95% CI = −22 to 34 salamanders/100 m, t12 = −0.45, P = 0.66) in lakes with and without trout.

Salamander larvae were markedly smaller in lakes with trout compared to lakes without trout (Fig. 1). In lakes with trout, salamander larvae weighed 38% less (95% CI = 3–72%, t12 = −2.13, P = 0.05), were 24% shorter in total length (6–43%, t12 = −2.60, P = 0.02), and had 29% shorter tails (7–50%, t12 = −3.08, P = 0.01, Fig. 2A). These differences were present at the beginning of the summer, did not differ between years, and the magnitude of difference did not change over the summer seasons (all P values associated with time effects > 0.1).

By the end of the summer, larval salamanders in lakes without trout were 22.7 times (95% CI=1.83–62.5 times) more likely to metamorphose and they began metamorphosing earlier in the summer (t1553 = −2.25, P = 0.03, Fig. 3) compared to larvae in lakes with trout. Moreover, in lakes with trout, the salamanders that did metamorphose were considerably smaller, averaging 8.8% shorter in total length (3–14.9%, t6 = −2.98, P = 0.03) and 16.9% shorter in tail length (7–26%, t6 = −3.59, P = 0.01, Fig. 2B). However, we did not detect differences in weight of metamorphosing salamanders between lakes with and without trout (t6 = −0.59, P = 0.58).

Field experiment

Measurements of salamander body morphology increased over time in all enclosures, but larvae reared in the absence of trout cues grew faster (trout × day). On average, salamanders in enclosures without trout cues increased their weight 2.9 times (95% CI = 2.8–3.1 times, t1272 = 25.24, P < 0.001), total length 2.8 times (2.7–3.0 times, t1272 = 22.25, P < 0.001), and tail length 2.8 times faster (2.6–3.1 times, t1272 = 15.38, 43%,
$P < 0.001$) compared to salamanders in enclosures with trout cues (Fig. 4). Moreover, salamanders in enclosures without trout cues were 1.48 times (1.00–5.66 times) more likely to have metamorphosed by the end of the summer ($t_{1349} = \text{-}5.84$, $P < 0.001$, Fig. 5). Metamorphosing salamanders in enclosures were similar in weight ($\text{-}0.19$ g difference, 95% CI = $\text{-}1.10$ to 0.73 g, $t_3 = \text{-}0.65$, $P = 0.57$), total length ($\text{-}3.47$ cm, $\text{-}17.75$ to 10.80 cm, $t_3 = \text{-}0.78$, $P = 0.49$), and tail length ($\text{-}2.29$ cm, $\text{-}10.31$ to 5.72 cm, $t_3 = \text{-}0.91$, $P = 0.44$), with and without trout cues.

**Discussion**

We observed distinct differences in body size and probability of metamorphosis of larval salamanders in natural lake settings with trout and in experimental field enclosures with trout cues. Our results provide clear evidence of NCEs given that we observed such distinct differences in morphology and life history when larvae were reared in the presence of predator cues alone. These NCEs are considerably higher than others reported in the literature (Davenport et al. 2014). Long-toed salamander larvae were capable of detecting predatory trout or their cues, and our results showed compromised growth and development of larvae when they co-existed with these predators. The magnitude of the differences in body morphology and probability of metamorphosis may have significant effects on fitness of adult salamanders; thus, long-term population viability in the presence of a nonnative predator warrants further investigation (Arntzen 1994).

Amphibians are highly plastic in their response to predation risk (McCollum and Leimberger 1997, Van Buskirk and Schmidt 2000). Facultative changes in morphology have been observed in salamander larvae (e.g., *Ambystoma laterale*, *A. maculatum*, and *A. tigrinum*, Yurewicz 2004), newts (e.g., *Triturus alpestris* and *T. helveticus*, Van Buskirk and Schmidt 2000), and tadpoles.
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(e.g., *Hyla chrysoscelis*, McCollum and Leimberger 1997) that co-occur with native predators. For example, larvae that naturally co-occur with native aquatic predators exhibit longer tails and tail muscles and deeper tail fins, which likely result in increased swimming speed and maneuverability, reducing predation risk (McCollum and Leimberger 1997, Van Buskirk and Schmidt 2000). However, we observed the opposite response in our system when native long-toed salamanders co-occurred with nonnative trout.

In the presence of predators, amphibian larvae often increase refuge use, which can result in as much as a twofold decrease in feeding (Semlitsch 1987, Orizaola and Brana 2003). An increase in refuge use restricts the time that an animal is exposed to predators, but also reduces opportunities for foraging, and therefore, reduces energy intake, resource acquisition, and growth (Semlitsch 1987, Skelly and Werner 1990, Tyler et al. 1998b). In addition, persisting in environments that are perceived as high-risk can increase stress

Fig. 3. The probability of metamorphosis in salamanders in lakes without trout (*n* = 15 of 177 salamanders in seven lakes) and with trout (*n* = 52 of 1372 salamanders in seven lakes) over time, northwestern Montana, summer 2012 and 2013. “Day” represents the number of days since first sampling session. Estimates are based on the number of metamorphosing individuals relative to the number of individuals trapped over time.

Fig. 2. (A) Body morphology of nonmetamorphosing salamanders (means and 95% CIs) in lakes without trout (*n* = 177 salamanders in seven lakes) and lakes with trout (*n* = 1372 salamanders in seven lakes) and (B) body morphology of metamorphosing salamanders (means and 95% CIs) in lakes without trout (*n* = 15 salamanders in seven lakes) and lakes with trout (*n* = 52 salamanders in seven lakes), northwestern Montana, summer 2012 and 2013.
levels, which can further inhibit food intake and suppress appetite (Crespi and Denver 2005). Therefore, increased refuge use, reduced foraging, and increased stress, in combination, may have resulted in reduced size and growth over the larval period within our study system.

Several studies have demonstrated that chemical cues from fish can elicit responses in larval amphibians that are similar, if not stronger, to those induced by the physical presence of a fish predator (Petranka et al. 1987, Kats et al. 1988, Stauffer and Semlitsch 1993). Similar to the differences we observed between salamanders in lakes with and without trout, tadpoles of California red-legged frogs (Rana aurora draytonii) weigh 34% less in the presence of mosquito-fish (Gambusia affinis, Lawler et al. 1999) and body sizes of larval mole salamanders (Ambystoma talpoideum) are reduced by 18% in the presence of bluegill sunfish (Lepomis macrochirus, Semlitsch 1987). In our lake-wide sampling, the small body sizes of larval salamanders we observed were likely not a direct effect of consumption. If fish were limited by gape size there would have been differential predation of smaller, more susceptible individuals, not larger ones (Caldwell et al. 1980, Pagnucco and Paszkowski 2011). In addition, we can confidently rule out size-selective predation in our study, because trout were able to induce NCEs even without the physical risk of predation during the field experiment; chemical, visual, or both types of cues in enclosures were sufficient for salamanders to detect predators and elicited changes in morphology and life history.

Small body sizes during the larval stage may have directly influenced timing and probability of salamander metamorphosis, as metamorphosis is initiated after a minimum body size is attained (Wilbur and Collins 1973, Duellman and Trueb 1986). Salamanders commonly spend multiple years in the larval stage, so they can acquire

Fig. 4. Changes in body morphology over time of salamanders in enclosures in lakes without trout (n = 240 salamanders in 12 enclosures) and lakes with trout (n = 320 salamanders in 16 enclosures), northwestern Montana, summer 2013.

Fig. 5. The probability of metamorphosis in salamanders in enclosures in lakes without trout (n = 43 of 143 salamanders in 12 enclosures) and lakes with trout (n = 83 of 320 salamanders in 16 enclosures) over time, day represents the number of days since first enclosure visit, northwestern Montana, summer 2012 and 2013. Estimates are based on the number of metamorphosing individuals relative to the number of individuals captured over time.
adequate resources to reach the necessary size for metamorphosis (Howard and Wallace 1985). Alternatively, in the presence of a predator, we predicted long-toed salamanders would shorten their larval periods, similar to western toads that reduce their time to metamorphosis by ~10 d to escape risky environments (Chiavers et al. 1999). Salamanders did not metamorphose earlier; instead, they remained in lakes with trout for longer periods of time. Predator-avoidance behaviors that reduce foraging and decrease energy acquisition will subsequently require extended larval periods to compensate for reduced growth rates (Wilbur and Collins 1973, Nicieza 2000). For example, newts (Triturus alpestris and T. helveticus) exposed to caged predators weigh 22% less and extend larval periods by 19% or 16 d (Van Buskirk and Schmidt 2000). However, increasing the duration of the larval period can increase exposure and susceptibility to trout predation, especially during winter months when larvae must move from shallow, vegetated areas into open, deep sections of the lake that do not freeze (Wilbur and Collins 1973). We did not recapture any individuals marked in 2012 during sampling in 2013, which may provide evidence of increased predation on individuals that have an extended larval stage. Other researchers have observed few second-year long-toed salamander larvae in lakes with fish (Pilliod and Peterson 2001, Bull and Marx 2002).

In addition to delayed metamorphosis, we also observed reduced size at metamorphosis, which may reduce fecundity and fitness in females (Berven 1988). Smaller body sizes at metamorphosis may decrease reproductive potential, as the number and size of eggs are positively correlated with female size in amphibians (Kaplan and Salthe 1979, Berven 1988, Prado and Haddad 2005, Green 2015). A decrease of 3 mm in body length of wood frogs (Lithobates sylvaticus) results in a decrease of 90 eggs, which represents a 15% decrease in mean clutch size (Berven 1988). Tail length also is positively related to clutch size of female golden-striped salamanders (Chioglossa lusitanica), as tails serve as food storage and may provide resources for reproduction (Maiorana 1977, Arntzen 1994).

We argue observed differences in morphology and life history are evidence of NCEs of trout on salamander larvae; however, variables such as pathogen infection, genetics, and other environmental factors also can influence body size and life history. For example, long-toed salamanders infected with Ambystoma tigrinum virus are significantly smaller (snout-vent length) at metamorphosis and western chorus frogs (Pseudacris triseriata) infected with chytrid fungus (Batrachochytrium dendrobatidis) gain 15–42% less weight compared to uninfected individuals (Forson and Storfer 2006, Retallick and Miera 2007). Although fish are capable of passing infection to amphibians, we did not observe common symptoms of disease such as hemorrhaging in the extremities, sloughing of the skin, edema, or thick mucus from the cloaca in any of our salamanders (Forson and Storfer 2006, Brenes et al. 2014). In addition, salamanders co-existing with predatory trout may evolve different phenotypes and plasticity of those phenotypes compared to salamanders naïve to trout and their risk (i.e., those living in trout-free water, Relyea 2002). Variations in body morphology and life history could be a function of genetic variation, however, long-toed salamander populations in Idaho and western Montana have high levels of genetic similarity and low divergence among ponds (Tallmon et al. 2000). Furthermore, random mating events and between pond migrations are sufficient enough to make populations in multiple ponds within a single basin genetically indistinguishable (Tallmon et al. 2000). Finally, trout and larval salamander larvae have overlapping diets and fish introductions have been associated with reduced populations of macroinvertebrates, zooplankton, and subsequently, amphibians (Knapp et al. 2001). Changes in food availability in lakes with trout may have influenced salamander growth and size, however, we argue that observed differences are still a function of trout given that resource competition is inherently related to nonconsumptive effects.

Reduced body sizes and delayed metamorphosis of long-toed salamanders in lakes with trout provide clear expressions of the morphological and metamorphic costs associated with coexistence with a nonnative predator (Tyler et al. 1998, Pagnucco and Paszkowski 2011). Our results suggest that the combination of consumptive and nonconsumptive effects of nonnative trout influence amphibian extirpations across western North America. Researchers can build
on these ideas by focusing on whether costs incurred by larvae through the period of metamorphosis carry-over to the adult stage, and specifically, how NCEs of trout may influence long-toed salamander population dynamics and persistence over time.

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**Supporting Information**

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.1258/supinfo