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PREDATION OF EGGS AND RECENTLY HATCHED LARVAE OF ENDEMIC RINGED SALAMANDERS (*AMBYSTOMA ANNULATUM*) BY NATIVE AND INTRODUCED AQUATIC PREDATORS

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ABSTRACT: Predation is a key determinant of pond community structure, yet not all predators are equally effective and not all life stages of potential prey are similarly susceptible. Understanding the effects of native and introduced species is essential to informing management strategies, especially for endemic and species of conservation concern. We examined the effects of five common predators (three native: Central Newts [*Notophthalmus viridescens louisianensis*], aeshnid dragonfly naiads [Aeshnidae], and Southern Leopard Frog tadpoles [*Lithobates sphenoccephalus*]; and two introduced: Fathead Minnows [*Pimephales promelas*] and Mosquitofish [*Gambusia affinis*]) on survival of eggs and recently hatched larvae of Ringed Salamanders (*Ambystoma annulatum*). We also examined the effect of supplemental food or cover availability on survival at each stage. Predators primarily showed a binary response to eggs, consuming all or none of them. Supplemental food did not influence whether eggs or larvae were consumed. Larvae were consumed by all predator species although the effect varied. The presence of cover did not reduce the impacts of the other predators on larval survival. Overall, the two introduced fish species had a greater impact on survival of the early stages of Ringed Salamanders than did the native predators. Further inquiries into the susceptibility of different life stages and survival will improve conservation strategies for rare and endemic species such as Ringed Salamanders.

Key words: Amphibians; Community structure; Food choice; Hatchlings; Management; Palatability; Refuge; Survival

PREDATION plays a key role in structuring amphibian communities in ponds (Morin, 1981, 1983; Wellborn et al., 1996; Wilbur, 1997) and includes both direct (consumptive) and indirect (nonconsumptive or trait-mediated) effects. Direct predation effects cause immediate mortality of the prey, with no subsequent chance for survival. Indirect effects of predators alter many aspects of the ecology of the early life stages of amphibians, including time to hatching (Sih and Moore, 1993; Vonesh, 2005) or limiting foraging behavior and reducing growth rates of larvae and, thus, overall size at metamorphosis (Holomuzki, 1986; Morin, 1986; Semlitsch, 1987; Kats et al., 1988). Indirect effects of predation also include injury (Semlitsch and Reichling, 1989; Shulse and Semlitsch, 2014) and compensatory changes in larval morphology (Van Buskirk and Schmidt, 2000).

Several factors influence the impact of predation on the early life stages of amphib-

ians. Predator density and composition can vary according to the time of year and hydroperiod of aquatic habitats, determining which predators and prey temporally overlap within ponds (Babbitt et al., 2003; Baber et al., 2004). Availability of alternative food sources can redirect predation attempts to other organisms, limiting their direct effects on amphibians (Hunter et al., 2011; but see Goodsell and Kats, 1999). Foraging mode (i.e., sit-and-wait versus actively searching predators) can alter behavioral patterns of larval amphibians, reduce foraging rates, and influence microhabitat selection within ponds (Preisser et al., 2007). The presence of complex habitat and prey refuge often interacts with different hunting strategies, where sit-and-wait predators utilize cover to ambush prey while active predators might have difficulty navigating dense vegetation, limiting their effectiveness (Warfe and Barmuta, 2004). Gape limitations and other oral morphology of the predator also effect prey susceptibility (Urban, 2007; Anderson et al.,

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2013). Relative size differences between prey, such as larval amphibians, and their predators matter in gape-limited predators such as fish and newts, with predators with larger mouths being able to consume larger prey in its entirety. Gape limitations do not exclude some predators from eating larger prey. Mosquitofish (*Gambusia affinis*), for example, have been reported to inflict damage on prey items larger than themselves by nibbling (Shulse and Semlitsch, 2014). Differences in feeding mechanisms (i.e., whether a predator is a gulper or chewer) also play a role in prey susceptibility. For example, dragonfly naiads (Aeshnidae), with chewing mouthparts, are able to consume large tadpoles and larvae (Urban, 2007).

Many amphibian eggs and larvae have physical, chemical, or inducible defenses (and any combination) that reduce or eliminate the likelihood of being consumed by predators (reviewed in Gunzberger and Travis, 2005). Aquatic eggs of amphibians are often covered in a gelatinous matrix that varies in thickness, density, and palatability by species and exposure to predators. For example, Spotted Salamanders (*Ambystoma maculatum*) have egg masses with a thick, gelatinous matrix surrounding the eggs, which excludes many predators (Ward and Sexton, 1981; Semlitsch, 1988), whereas eggs of other species of *Ambystoma*, including Ringed Salamanders (*Ambystoma annulatum*), have only a thin, gelatinous covering over the eggs that is penetrable by several predators (Walters, 1975; Anderson et al., 2013). Chemical defenses resulting in unpalatability are common in some amphibians, allowing them to persist with many types of predators, including fish, but are generally absent among egg and larval stages of ambystomatids (Gunzberger and Travis, 2005). Overall, the interplay between the defense strategies amphibians use to increase their survival in the presence of multiple predators, and the factors predators encounter that affect their effectiveness, vary widely by species but have important consequences for community structure and population dynamics of both groups.

While the effects of predation on the aquatic life stages have been reported for several species of *Ambystoma* (e.g., Walters,

1975; Semlitsch, 1987; Sih and Moore, 1993; Urban, 2007; Anderson et al., 2013), a paucity of natural history information regarding predation exists for Ringed Salamanders, *Ambystoma annulatum*. This species is endemic to the Ozark Plateau of Missouri, Arkansas, and Oklahoma and utilizes semipermanent to permanent ponds for breeding in the autumn (Petranka, 1998; Semlitsch et al., 2014). Larvae of Ringed Salamanders overwinter in ponds and metamorphose between April and June (Semlitsch et al., 2014). Some studies have documented the indirect effects of predators on Ringed Salamanders (e.g., Mathis et al., 2003), but experimental studies examining the direct effects of native predators on early life stages of this species are limited (e.g., Wilson, 1993), and none have examined the effects of introduced predators. Given its conservation status over most of its range (e.g., a species of conservation concern in Missouri), comparing the effects of native and introduced predators is important to answer both basic ecological questions and inform conservation decisions.

We investigated the direct effects of predation on the eggs and recently hatched larvae of Ringed Salamanders by six native and introduced, sympatric, aquatic predators. We tested whether these predators would consume eggs or larvae (or both) of Ringed Salamanders and whether they would do so under two experimental conditions: (1) the presence of an alternate food source; and (2) the presence of cover objects.

MATERIALS AND METHODS

We conducted all experiments in 38-L glass aquaria in a controlled laboratory at the University of Missouri, USA, during November 2012 and 2013. Each aquarium was surrounded with white paper blinds to limit external stimuli that could influence predator and prey behavior. Lighting in the room was set to a 11:13-h light:dark schedule reflective of the natural schedule at the time of the experiments. All aquaria were filled with 26.5 L of charcoal-filtered UV-sterilized water at least 2 d before observations began, and all aquaria were cleaned between experiments. We collected all aquatic predators and late-stage egg masses of Ringed Salamanders in

November 2012 and 2013 from wildlife ponds on the military post Fort Leonard Wood, Pulaski County, Missouri.

Food Experiments: Eggs and Recently Hatched Larvae

Late-stage egg masses of Ringed Salamanders and their potential aquatic predators were collected at least 4 d prior to the commencement of experimental observations. The predators used in these experiments were commonly found in ponds where Ringed Salamanders were known to oviposit. They included Central Newts (*Notophthalmus viridescens louisianensis*), overwintering tadpoles of Southern Leopard Frogs (*Lithobates sphenoccephalus*), and two introduced fishes commonly used by managers, Mosquitofish (*Gambusia affinis*) and Fathead Minnows (*Pimephales promelas*). The newts were first-year back to the pond, returning after having left the ponds during their postmetamorphic terrestrial eft stage (mean [± 1 SE] snout-vent length [SVL] = 3.9 ± 0.04 cm), and the tadpoles were first-year tadpoles at developmental stages 34–36 (Gosner, 1960; mean total length [TL] = 7.2 ± 0.12 cm). There were two size classes of Mosquitofish, small (mean TL = 2.0 ± 0.02 cm) and large (mean TL = 3.9 ± 0.04 cm). Because the small Mosquitofish are gape limited with regard to the relative size of the developing salamander embryos, and because there would be a notable biomass difference between a small Mosquitofish and the other predators in this study, treatments using the small Mosquitofish as predators included five individuals, rather than one, per aquarium. Because of the limited number of eggs available for the study, only one size class of Fathead Minnows was used (mean TL = 4.7 ± 0.07 cm).

All predators were housed in the laboratory for 3 d prior to the study and were offered alternative food options to verify their willingness to consume them. Newts and fish were offered a combination of zooplankton, mosquito larvae, and commercial fish flakes, and tadpoles were offered commercially available algae discs and flakes. Individuals that did not consume the provided food options were omitted from assignment to treatments. During the experiments, predators in the food

treatment were fed a combination of these food options (minus the fish flakes for the newts) at least once per day and more frequently if none of the added food was seen during scheduled observations.

We randomly assigned treatments to aquaria, with a total of 12 treatments (five predator treatments and a control, all crossed with the presence-absence of alternative food sources), with five replicates per treatment. Predators were added to the tanks on Day 1 of the experiment. Whole egg masses (mean number of eggs/mass = 20 ± 3.2) were introduced to the aquaria on Day 2 after the number of eggs per mass was counted and recorded. Observations were made of each aquarium at 3-h intervals, beginning 30 min after the introduction of the egg masses to the aquaria to allow time for the predators to settle from the introduction disturbance. We continued observations at each aquarium until all eggs or larvae were consumed or up to 204 h when the study ended (approximately 8 d). The number of eggs or larvae remaining in each aquarium, and additional behavioral observations, were noted at each observation period. A headlamp with a red filter was used for nocturnal observations.

Cover Experiments: Recently Hatched Larvae

We collected predators and eggs of Ringed Salamanders from the same location as those used in the Food Experiments during the second week of November 2012. The predators used in these experiments included newts, overwintering 4th or 5th instar dragonfly naiads (Aeshnidae), and Mosquitofish. The newts were in a size class similar to those used in the Food Experiments, and the Mosquitofish were equivalent to the large size class designated in the Food Experiments. All predators were stored in a cold room (10°C) until the initiation of the experiment.

There were eight treatment combinations using three predators and a control, as well as the presence-absence of cover with 10 replicates of each treatment. The experiments were conducted in two temporal blocks, with 40 aquaria in each block ($n = 5$ of each treatment within each block) that each were conducted for 5 d. We added 20 larvae of *A. annulatum* to each aquarium and allowed 30

min before adding either *N. viridescens* ($n = 1$), *G. affinis* ($n = 3$), or aeshnid naiad ($n = 1$) as predators. Three Mosquitofish were used because this species is normally a schooling top-minnow, and solitary individuals might not behave similarly to natural conditions (we acknowledge that predator density is confounded by our design). Cover objects consisted of 10 strips of gray window screen (20 × 5 cm; 1.6 mm pore size) that were pinched and held together in the center in a bowtie shape with a small drop of glue to create an irregular surface. In the Cover treatments, one half of the bottom of each aquarium was without cover, and mesh strips were haphazardly distributed on the other half of the bottom; the screen allowed for visual determination of larval location, even while under the cover. Predators used in the first temporal block were added 2 d after collection and were not fed while maintained in the aquaria. Predators used in the second temporal block were fed two larval Ringed Salamanders approximately 3 d prior to the start of their experiments (total duration prior to use = 7 d). Aquaria were observed three times per day between 0800 and 2300 h ($n = 18$ total observations) over 5 d within each block, and the number of remaining larvae was recorded at each observation. A headlamp with a red filter was used for nocturnal observations.

Analyses

We assessed survival from all experiments using generalized linear models with a quasi-binomial error distribution to account for overdispersion in R (R Core Development Team, 2013). There were two response variables in the Food Experiments: egg survival (the number of eggs surviving until the first larva hatched) and overall survival posthatching. Egg masses did not fully hatch in five aquaria because of slow development (large Mosquitofish, $n = 3$ and small Mosquitofish, $n = 2$) and were excluded from the posthatching analysis. We tested for the main effects manipulated in each experiment (Food: alternative food, predator identity, and their interaction; Cover: cover, predator identity, and their interaction). We also examined the mean number of hours before predators began consuming eggs. Because

only two predators were primarily responsible for consuming eggs, we did not statistically analyze these temporal data. For the Cover Experiments, we initially included the temporal block in the larval analysis, but it was not significant and was subsequently removed.

RESULTS

Food Experiments: Eggs and Recently Hatched Larvae

Developing embryos were consumed by all but one of the predators (tadpoles), and only the actual embryo was eaten, not the egg material (i.e., vitelline membrane, gelatinous exostructure). Egg (embryo) predation was affected by predator identity ($\chi^2 = 69.49$, $df = 4$, $P < 0.001$; Fig. 1A). The addition of food and the interaction of food and predator did not affect predation in the treatments. Survival of eggs to hatching was low with Fathead Minnows (0%) and Central Newts (44%) but high with large Mosquitofish (95%), small Mosquitofish (100%), tadpoles (100%), and in the controls (100%). Egg survival with newts averaged 44%, but their consumption was nearly always 100% or 0%, regardless of food treatment. The mean number of hours to the first recorded egg that was consumed varied by predator species but did not differ based on food manipulation. On average, newts first began eating eggs 21.2 h into the experiment whereas Fathead Minnows took an average 54.3 h. Mosquitofish consumed eggs in only one aquarium each, and it was 81 h to initial consumption in those instances. Individual Fathead Minnows ate a maximum of 19 embryos/3 h.

Larvae were consumed by all predator species immediately posthatching, except Fathead Minnows, as no eggs survived to hatching with this predator. As with eggs, survival of larvae was affected by predator species ($\chi^2 = 49.769$, $df = 3$, $P < 0.001$), and food manipulation and the interaction of predator and food manipulation were not significant (Fig. 1B). Survival was highest in tanks with tadpoles (95%), whereas survival was low with large and small Mosquitofish and newts (20%, 18%, and 19%, respectively; we did not calculate survival with Fathead Minnows as all eggs were consumed prior to

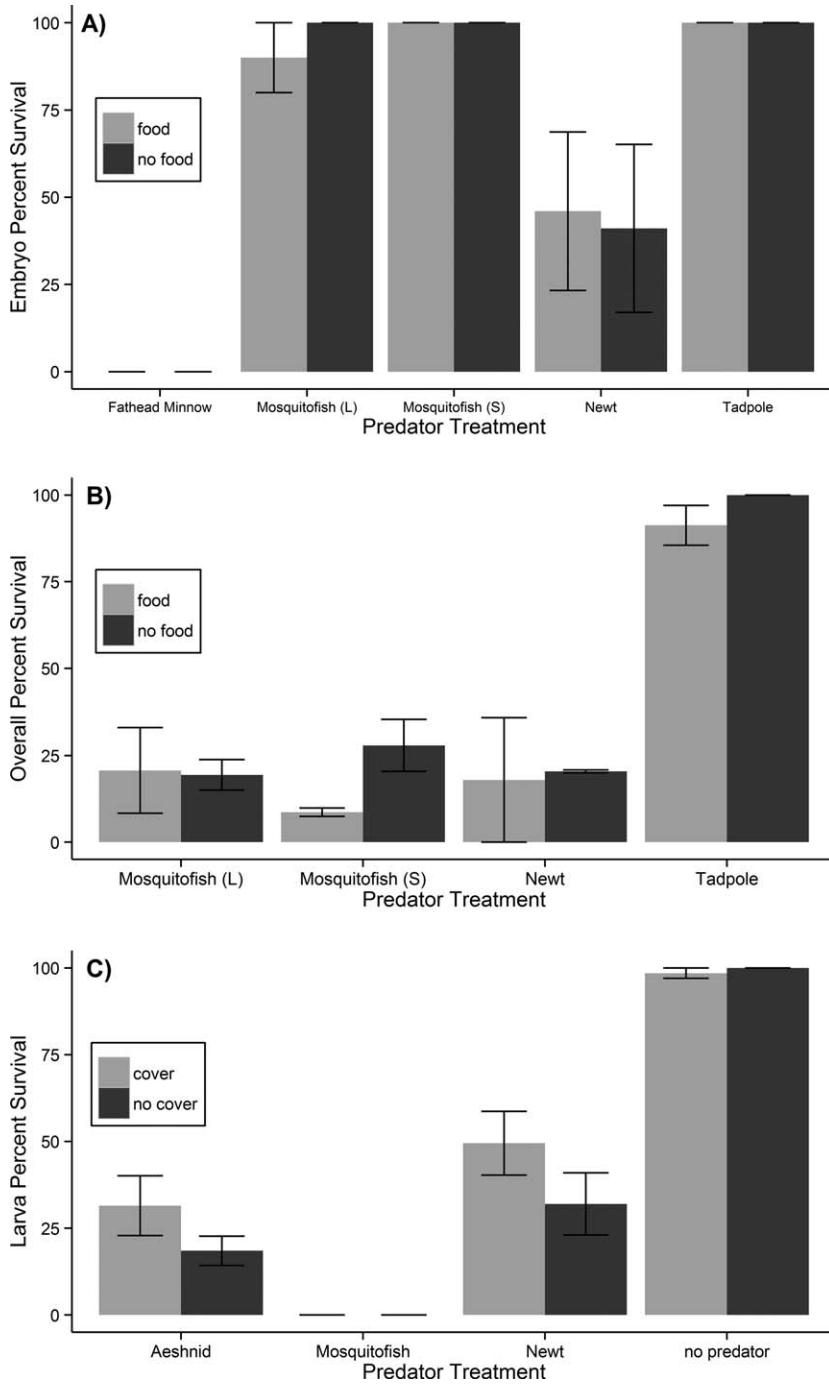


FIG. 1.—Mean (± 1 SE) survival of different life-history stages of Ringed Salamanders (*Ambystoma annulatum*). (A) eggs; (B) eggs plus recently hatched larvae with the presence–absence of alternative food (overall survival during aquatic stages); and (C) survival of recently hatched larvae with the presence–absence of cover. Sample sizes of the panels (A) and (B) are $n = 5$ aquaria per species, total $n = 50$ aquaria. Sample size of panel (C) is $n = 10$ aquaria per treatment, total $n = 80$ aquaria. The S and L indicate small and large Mosquitofish, respectively.

hatching). The mean number of hours to first larva eaten was similar among predators (newts = 148.5 ± 1.4 h; small Mosquitofish = 156.3 ± 11.3 h, large Mosquitofish = 153.6 ± 17.4 h; tadpoles = 150 h). Recently hatched larvae in all but the tadpole treatments were consumed immediately upon hatching. Fish in the large and small Mosquitofish treatments consumed a maximum of 15 and 13 larvae within 3 h, respectively.

Cover Experiments: Recently Hatched Larvae

Survival of larvae varied as a function of predator species ($\chi^2 = 164.3$, $df = 3$, $P < 0.001$) but cover had only marginal effects ($P = 0.1$), and their interaction was not significant (Fig. 1C). Hatchling survival averaged 99% in controls and was 0% with Mosquitofish, regardless of cover. All larvae were consumed by Mosquitofish within the first 2 d for each temporal block. Survival of larvae with Central Newts was greater in cover treatments than in treatments with no cover ($50 \pm 1.8\%$ and $32 \pm 1.8\%$, respectively). Larval survival was $31 \pm 1.7\%$ with cover, and $19 \pm 0.8\%$ without cover, in aquaria with aeshnid naiads.

DISCUSSION

We found that all of the predator species in this experiment consumed early life stages of Ringed Salamanders, that the consumption of eggs and recently hatched larvae of Ringed Salamanders varied by life stage and predator, and that our two independent variables (presence of cover and alternate food) had no effects on egg or larval survival. These results indicate that predation by both native and introduced predators can have strong effects on survival of the early life stages of Ringed Salamanders. All predatory species in our study overwinter in ponds at the site from which we collected specimens, in some combination, at Ringed Salamander breeding ponds (Peterman et al., 2014). Thus, a greater understanding of the relative effects of different predators and predator combinations (both native and introduced) will provide insight into which predators have the greatest effects on survival and distributional patterns across the landscape. Furthermore, because Ringed Salamanders breed in the autumn and require ponds with hydroperiods of at least 6–

8.5 mo (the time necessary to complete metamorphosis; Hutcherson et al., 1989; Semlitsch et al., 2014), ponds where they breed might be more likely to be colonized by fish, serve as locations for stocking bait such as minnows, or both.

There are several factors that influence predator–prey interactions including the type and limitations of the predator and the accessibility and behavior of the potential prey. In our experiments, we used primarily visual predators (Martin et al., 1974; Attar and Maly, 1980) with different gape limitations (gape largest to smallest: Central Newts, Fathead Minnows, and Mosquitofish), but also one that uses olfaction as its primary means of food detection (tadpoles; Taylor et al., 1995). Accessibility of the eggs and developing embryos were restricted by the gelatinous matrix and egg membranes surrounding the embryos. Behavior of the embryos changed over time, with activity levels increasing as they approached hatching but decreasing immediately after hatching, when they sat quiescent on the egg mass or aquarium bottom. Behavior of both size classes of Mosquitofish also changed as activity levels increased within the egg masses, with Mosquitofish increasing proximity and orientation toward the egg masses as they neared, and during, hatching. Predation rates on recently hatched larvae were high and occurred quickly with most predators. The limited swimming faculties of recently hatched larvae likely contributed to their increased vulnerability to multiple predators (Anderson et al., 1971). Dragonfly naiads likely have longer handling times than do fish and newts because of their use of labial claws when chewing captured prey.

Food manipulation likely did not affect outcomes in the experiments because all predatory species used were obligate feeders. The predators in our study demonstrated an ability to consume large quantities of eggs, larvae, or both in short periods of time, regardless of having consumed all of the other food available in the food treatments. Our results are similar to those of Goodsell and Kats (1999), where they found no difference in survival of tadpoles in the treatments with just Mosquitofish or with Mosquitofish and

mosquito larvae. They also reported that consumption rates of the Mosquitofish were the same whether Mosquitofish were added to high or low densities of mosquito larvae (i.e., the fish ate more when more food was available, as was apparent in our experiments).

We observed no effects of cover on predation rates of recently hatched larvae. Similar to our study, mortality induced by Mosquitofish was very high for several anuran tadpoles and salamander larvae and was not influenced by prey refuge (Tarr and Babbitt, 2002; Baber and Babbitt, 2004; Segev et al., 2009). Baber and Babbitt (2004) suggested that Mosquitofish can navigate dense cover that otherwise excludes other fish predators. Cover can increase predation rates by aeshnid dragonflies, as they are sit-and-wait predators that utilize the habitat complexity to ambush prey (Babbitt and Tanner, 1998; Tarr and Babbitt, 2002), but cover can also limit their effectiveness (Hossie and Murray, 2010). Predation rates in our experiments indicate that the handling times or attack rates might vary between the different predators on salamander larvae. Fish consumed all of the free-swimming larvae relatively quickly whereas the newts and dragonflies showed similar patterns of predation, although our design is confounded by predator density (three fish versus one newt or dragonfly).

Newts are widely documented natural predators of a variety of amphibian eggs and larvae, including the eggs of Spotted Salamanders, which are surrounded by a thick gelatinous matrix (a barrier to many predators; Walters, 1975; Petranka, 1998; Gunzburger and Travis, 2005). The eggs of Ringed Salamanders have a much-thinner external gelatinous matrix than those of Spotted Salamanders, more like the encapsulated and naked egg stages used by Ward and Sexton (1981). Newts have been reported to penetrate the egg masses of Spotted Salamanders, yet were unsuccessful at accessing embryos of Jefferson's Salamanders (*Ambystoma jeffersonianum*) unless the gelatinous matrix had been damaged (Walters, 1975). Wilson (1993) found newts to be capable predators on eggs and larvae of Ringed Salamanders. We observed a strong binary response in the newts to the eggs, where either all or none of

the developing embryos were consumed, suggesting individual variability in the propensity to consume eggs.

There are many reports of oophagy and predation of amphibian larvae by tadpoles, including those of leopard frogs (Ward and Sexton, 1981; Gunzburger and Travis, 2005; Drake, 2010). Tadpoles of Southern Leopard Frogs and Wood Frogs (*Lithobates sylvaticus*) were also reported to eat eggs of Spotted Salamanders (Ward and Sexton, 1981; Burley et al., 2006). In Missouri, leopard frogs exhibit bimodal breeding, with occasional summer breeding (Drake and Ousterhout, 2011), and larvae are present in many of the ponds throughout the oviposition and larval development stages of the Ringed Salamanders. Although a few larvae of Ringed Salamanders were consumed during our study, the *L. sphenoccephalus* tadpoles seemed to have little interest in consuming the eggs, embryos, or larvae, despite tadpoles being observed adjacent to and on top of the egg masses and larvae during the experiments. It is unlikely that oral disc morphology would explain the lack of egg consumption by these tadpoles as they, like most North American ranid species, have keratinized jaw sheaths for rasping and have been capable of penetrating gelatinous matrices and egg membranes of other amphibian species (Altig and McDiarmid, 1999). Eggs and embryos are energy rich and contain calcium and phosphorus, both necessary for skeletal development in metamorphosing tadpoles (Crump, 1983). A likely explanation for the high survival of eggs of Ringed Salamanders is that the eggs or their gelatinous matrix coat were unpalatable to the tadpoles.

Our results have important conservation implications because both fish species used in this study are often introduced in bodies of water used by amphibians. Quantifying their impacts relative to native species is necessary to understand the threats amphibians face from these different predators. Mosquitofish are introduced throughout the world as mosquito control agents, but often have strong negative effects on nontarget species, and can limit or eliminate recruitment out of pond habitats under both experimental and natural conditions (Segev et al., 2009; Shulse et al., 2013). Alternatively, predation by Red-spotted

Newts (*Notophthalmus viridescens viridescens*) on eggs of Tiger Salamanders (*Ambystoma tigrinum*) was enough to exclude the latter species from a pond (Morin, 1983), indicating that newts also have the potential to be detrimental predators. Concurrent research suggests that survival of Ringed Salamanders can be high with low densities of Mosquitofish under experimental conditions (T. L. Anderson, personal observation), contrary to that for most published accounts of amphibians with Mosquitofish (e.g., Baber and Babbitt, 2004; Segev et al., 2009). Mosquitofish fecundity often results in extremely high densities, however, suggesting their deleterious effects may be density-dependent for Ringed Salamanders (Shulze et al., 2013).

Fathead Minnows are widely used as bait and are introduced as a feeder fish when establishing stocked game-fish ponds, yet little information is available in the literature on their natural diet, especially as relates to amphibians. We have confirmed that Fathead Minnows consume eggs of Ringed Salamanders, and that they did so more effectively than Mosquitofish, and we suggest that the minnows would have consumed larvae had any of the developing embryos not been consumed prior to hatching. Further research should determine which amphibian species are limited by Fathead Minnow presence. One study found that Fathead Minnows consumed larvae of Small-mouthed Salamanders, *Ambystoma texanum* (Petranka, 1983). Bluntnose Minnows (*Pimephales notatus*) have been reported to consume eggs of Spotted Salamanders (Ward and Sexton, 1981). The difference in egg consumption between Mosquitofish and Fathead Minnows could be explained by differences in mouth orientation and gape size between these two species of small fish. The Mosquitofish is a surface feeder, with an upturned mouth that is smaller than that of the Fathead Minnow, while the Fathead is a mid-column feeder with a larger terminal mouth (Page and Burr, 2011). Thus, consideration of the oral morphology and feeding ecology of introduced predators may determine which life stages of amphibians are most at risk.

Our results indicate that native and introduced predators can have varying effects on survival of different life history stages of Ringed Salamanders. Knowledge of the effects of native predators can provide a baseline against which nonnative predators can be gauged. Future studies should investigate other impacts of invasive predators relative to native threats including their density-dependent effects, food requirements, and preferences. Such research should also identify the long-term consequences of predation on different life stages including pond community structure, survival to metamorphosis, and success and persistence of populations. Examinations of functional response curves of predators under different experimental conditions to estimate handling time and attack rates would further quantify interspecific differences between predators and prey stages. These rates are important, for both native and introduced predators, to understand the ability of a predator to consume or exclude native amphibians as well as the conditions under which predatory effectiveness might be altered.

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