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Larval salamanders are as effective at short-term mosquito predation as mosquitofish

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Draft

Abstract: Biological control of mosquitoes can have unintended ecological consequences. One example is the introduction of mosquitofish (*Gambusia* spp.) into ponds and wetlands.

Mosquitofish are invasive in many parts of the world and have the potential to alter ecosystems by changing trophic interactions and extirpating amphibians. We sought to determine whether larval amphibians are capable of consuming larval mosquitoes as effectively as mosquitofish. We tested the predation ability of larval spotted salamanders (*Ambystoma maculatum* Shaw, 1802; $n = 13$), southern leopard frog tadpoles (*Lithobates sphenoccephalus* Cope, 1886; $n = 12$) and mosquitofish (*Gambusia affinis* Baird and Girard, 1853; $n = 13$) across a range of body sizes in laboratory experiments. Our results showed that over a 24-h period, salamander larvae (mean $\pm$ SE; 238 ± 14) and mosquitofish (195 ± 17) consumed a statistically equivalent number of mosquito larvae, while tadpoles consumed a large number (113 ± 14) but significantly less. All species had significant (or marginally significant) positive relationships between body size and rate of mosquito consumption. Further studies into the ability of native larval amphibians to consume mosquito larvae are needed to assess whether amphibians can be successful at mosquito control instead of introducing nonnative species to new areas.

Keywords: *Ambystoma maculatum*; *Gambusia affinis*; *Lithobates sphenoccephalus*; mosquito control; mosquitofish; spotted salamander; southern leopard frog

Introduction

Mosquito-borne human diseases are flourishing worldwide (Tolle 2009). For example, West Nile virus (*Flavivirus*) has spread rapidly through the U.S. via mosquito vectors (Turell et al. 2005), where it has infected more than 40,000 people and caused more than 1,400 fatalities (Centers for Disease Control and Prevention 2014). More recently, the World Health Organization declared the mosquito-transmitted Zika virus as a global public health emergency (Gulland 2016). To stop the spread of mosquito-borne diseases, many countries have implemented various control strategies in mosquito breeding grounds. Some examples are the use of insecticides, draining bodies of water, releasing pathogens or parasites of larval mosquitoes, placing an oil film on the surface of the water, or introducing fish species such as the mosquitofish (*Gambusia affinis* (Baird & Girard, 1853) and *G. holbrooki* (Girard, 1859); Pyke 2008).

Mosquitofish are generalists with high reproductive potential that easily adapt to new environments. Mosquitofish can be voracious predators of mosquito larvae (reviewed in Walton 2007). For example, DuRant and Hopkins (2008) found a 1.3 g fish consumed an average of 992 third instar mosquito larvae in 24 h. However, mosquitofish can disrupt food webs through competition for resources and predation (Pyke 2008; Shulse et al. 2013). Introduction of mosquitofish into water bodies that previously lacked them has been shown to negatively impact amphibian (Shulse and Semlitsch 2014) and fish populations (Blanco et al. 2004). In fact, the International Union for the Conservation of Nature (IUCN) lists mosquitofish among the 100 worst invasive species worldwide (Lowe et al. 2000).

Introductions of mosquitofish also may be unnecessary, as amphibians could potentially reduce mosquitoes on their own. Salamander larvae are obligate predators and anuran larvae can also be predatory (Schiesari et al. 2009). Previous work found that larval mole salamanders

(*Ambystoma talpoideum* Holbrook, 1838) and red-spotted newts (*Notophthalmus viridescens* *viridescens* Rafinesque, 1820) consistently consumed 200–400 third-instar mosquito larvae per day (DuRant and Hopkins 2008). Others found that larval and pupal mosquitoes were the third largest component of larval tiger salamander (*A. tigrinum* Green, 1825) diets (Brodman and Dorton 2006). Wood frog tadpoles (*Rana sylvatica* LeConte, 1825) and green frog tadpoles (*R. clamitans* Latreille, 1801) can also be major predators of macroinvertebrates in ponds (Petranka and Kennedy 1999; Schiesari et al. 2009), but surprisingly few studies exist on mosquito predation by tadpoles. Cuban treefrog (*Osteopilus septentrionalis* Duméril & Bibron, 1841) tadpoles have been documented to consume between 13 (Spielman and Sullivan 1974) and 22 mosquito larvae per tadpole per day (Rodríguez and González 2000). Additionally, some mosquito species avoid ovipositing in pools with tadpoles present (Mokany and Shine 2003a). Anuran larvae may also reduce mosquito survival through competition for resources (Blaustein and Margalit 1994; Mokany and Shine 2003b). Together, these studies suggest that larval amphibians may be an underappreciated source of mosquito control, but more data are needed.

Our aim was to compare the predatory ability of tadpoles, larval salamanders, and mosquitofish across the body size gradients found in natural systems. We hypothesized there would be differences in mosquito consumption across our three test species. Salamander larvae are predators that can have strong effects on lower trophic levels in fishless ponds (Morin 1983; Holomuzki et al. 1994), thus we predicted they would have a similar predation ability as fish. The limited information on tadpoles indicates they could be weak predators of mosquito larvae. Specifically, we predicted mosquitofish and salamander larvae would consume equivalent numbers of mosquito larvae, and tadpoles fewer mosquitoes. Previous work has mainly tested mosquito consumption in size-matched fish and salamanders, but few studies have expanded the

research to include animals across a range of sizes. We also predicted that mosquito consumption would increase linearly with size for all species. These data provide new information on how native amphibians could potentially control mosquito populations.

Materials and Methods

To test the efficacy of tadpoles, larval salamanders and mosquitofish at mosquito predation, we conducted a series of experiments between May–July 2015 in controlled animal care space at the Botany Greenhouse near the University of Missouri campus, Columbia, MO, USA. Prior to the experiments, we set up three 1,000 L cattle tanks as holding ponds at a fenced outdoor research facility adjacent to the greenhouse on 15 March 2015. Cattle tanks are often used to create experimental ponds that mimic the plankton, algal and bacterial communities of natural ponds (Semlitsch and Boone 2009). In early April 2015, we collected southern leopard frog (*Lithobates sphenoccephalus* Cope, 1886) and spotted salamander (*Ambystoma maculatum* Shaw, 1802) egg masses from several ponds at Fort Leonard Wood, Missouri, USA. We collected a minimum of three egg masses for each species to increase representation of population-level genetic diversity. We cut each egg mass into at least four pieces and distributed them between four plastic containers for each species (approximately 2 L volume) placed on a table in a covered outdoor space at the greenhouse. We changed the water every other day until tadpoles had hatched and reached the free-swimming stage (Gosner Stage 25; Gosner 1960); salamander larvae were added to their holding pond five days post-hatching. We stocked one cattle tank with 48 southern leopard frog tadpoles, another with 24 spotted salamander hatchlings, and the third with approximately 100 Western mosquitofish (*Gambusia affinis* Baird and Girard, 1853) of both sexes collected from an established high-density population in a pond at Fort Leonard Wood. By stocking hatchling amphibians, we were able to take advantage of the

size changes across ontogeny. We also attempted to capture natural size variation in mosquitofish by sampling a large number of mosquitofish from a natural population. Mosquito larvae (Diptera: Culicidae) were collected from untreated small containers and puddles around the greenhouse. We required a large number (~5000) of mosquito larvae for each trial, so we used a mix of *Culex* spp., and *Anopheles* spp. larvae in instars 3 and 4.

Experimental Design

To perform the experiments, we filled 10-gallon aquaria with 11 L of dechlorinated tap water. We added Prime (Seachem Laboratories Inc., Madison, GA USA) to remove ammonia from the water. We covered the sides of the aquaria with white paper to limit external stimuli and the top was covered with a netted lid. Lighting in the room was set to a 11:13-h light:dark schedule. Animals were dip-netted out of holding ponds and starved 24-h in advance of trials. We used a range of animal sizes for each species to assess how mosquito consumption varied with size. Before each trial, we dipnetted out at least one visually-assessed small-, medium-, and large-sized individual of each species. Each animal was then photographed, randomly assigned to an aquarium, and allowed to acclimate to the aquarium conditions for 24 h before we added mosquito larvae. Mosquito larvae were counted into groups of 100 the day before each trial, and any mosquito larvae that progressed to the pupal stage were removed and replaced before the experiments. Mosquito larvae were added to the aquaria at midnight (time 0), and immediately after we took photos every four hours for a full 24 h to assess mosquito survival. We used Image J (Rasband 2012) to count surviving mosquitoes and measure predator body size. We used total body length, defined as the distance from snout to tail tip along the central dorsal line, to assess body size because it could be measured from a photograph to minimize stress and potential injury to predators before experiments.

To determine the number of mosquito larvae to stock in experiments, we conducted an experiment on 5 June 2015 to estimate mosquito consumption of each species in a 24-h period. Three individuals of each species were given 500 mosquito larvae (after the acclimation period) and left undisturbed for 24 h. We then set the mosquito larvae densities to be higher than the maximum observed for each species (i.e., salamander larvae, mosquitofish, and tadpoles).

The first trial was performed on 12 June 2015, the second on 19 June 2015, and the third on 9 July 2015. For the first two trials, we had five replicates of each species. By the third trial we struggled to find enough mosquito larvae, and so reduced our replication to three tadpoles and mosquitofish, and four salamander larvae. For the first trial, we added 500 mosquito larvae to the salamander and mosquitofish aquaria and 150 mosquito larvae to the tadpole aquaria. For the second and third trials, we added 300 mosquito larvae to all aquaria. We recognize that having different prey densities in the first trial would alter predator encounter rates, potentially biasing our results. To account for this, we ran all of our analyses with only trials 2 and 3 included and compared the results to our full dataset. The results were very similar¹, so we present data from all three trials.

Analyses

We performed all statistical analyses in R (R Core Team 2017). We performed ANOVAs with post-hoc Tukey HSD tests to compare body sizes and mosquito consumption of the three species (i.e., salamander larvae, mosquitofish, and tadpoles). To test how mosquito consumption varied with body size, we fit generalized linear models using the ‘lme4’ package (Bates et al. 2015). We fit a separate model for each species, with mosquito consumption predicted by body size in a binomial distribution with a logit link function. We assessed model fit by examining residual plots and removed influential outliers identified by ‘outlierTest’ in the ‘car’ package

¹ Supplementary data cjz-2017-0267 suppl a

(Fox and Weisberg 2011). We then used the 'Anova' function in the 'car' package to conduct Type II Wald χ^2 tests to assess fit of regression parameters.

Results

There were significant differences in body size among the three species (i.e., salamander larvae, mosquitofish, and tadpoles; $F_{2,35} = 27.77$, $P < 0.001$; Fig. 1a). Post-hoc Tukey tests indicated mosquitofish were significantly smaller than the other two species (Fig. 1a). Mosquitofish were approximately 70% smaller than the other two species; the mean body size of mosquitofish was 20 ± 1 mm (mean $\pm$ SE), whereas salamander larvae averaged 34 ± 1 mm and tadpoles 35 ± 3 mm. Tadpoles had the largest size range (19–44 mm). There was no size overlap between mosquitofish (14–25 mm) and salamander larvae (30–40 mm).

We found significant differences in 24-h larval mosquito consumption among the species ($F_{2,35} = 17.14$, $P < 0.001$; Fig. 1b). Post-hoc Tukey tests indicated salamander larvae and mosquitofish consumed a statistically equivalent number of mosquito larvae, and tadpoles significantly fewer. Salamander larvae consumed a mean of 238 mosquito larvae $\pm$ (SE) 14, and mosquitofish slightly, but insignificantly fewer (195 ± 17 ; Fig. 1b). Tadpoles consumed approximately half as many mosquito larvae (113 ± 14) as salamanders and fish.

The data suggested a significant positive relationship between body size and mosquito consumption for salamanders ($\chi^2 = 362.15$, $df = 1$, $P < 0.001$, $n = 12$; Fig. 2) and tadpoles ($\chi^2 = 8.31$, $df = 1$, $P = 0.004$, $n = 11$; Fig. 2). Mosquitofish consumption marginally increased with body size ($\chi^2 = 3.67$, $df = 1$, $P = 0.055$, $n = 12$).

Discussion

Scientists continue to search for biological ways to control pest species. Mosquitofish are often introduced to areas where they are non-native as mosquito control, yet naturally occurring

larval amphibians may be just as effective. Our data suggest that, at least under short-term laboratory conditions, amphibians were effective predators of mosquito larvae. Both salamander larvae and mosquitofish consumed ~200 mosquito larvae in 24 h, and tadpoles about half (~100) of the other two species in our study. Salamander larvae and tadpoles had significant positive relationships between body size and rate of mosquito consumption (and mosquitofish marginally significant). Thus, although mosquitofish are good at reducing mosquito densities, under certain scenarios amphibians may be better.

We found salamanders to be effective predators of mosquito larvae, in agreement with previous work (DuRant and Hopkins 2008; Rubbo et al. 2011). Previous experiments show that eastern newts (*Notophthalmus viridescens* Rafinesque, 1820) and larval mole salamanders (*A. talpoideum*) were consistently capable of consuming 200–400 third-instar mosquito larvae per day (DuRant and Hopkins 2008), and we found spotted salamander larvae were in this range. Although our data are limited to lab studies, field surveys have shown low survival of mosquitoes in pools with salamander larvae present and some species of mosquitoes even avoid ovipositing in pools with salamander larvae (Rubbo et al. 2011; Blaustein et al. 2014). Additionally, mosquito larvae also tend to be 10–100 times less abundant in wetlands with *Ambystoma* larvae present compared to those without (Brodman et al. 2003). Our data show larval salamander predation of mosquito larvae is more strongly related to body size than in the other two species, adding to the limited existing data that amystomatid salamander larvae scale mosquito consumption with body size (DuRant and Hopkins 2008). Thus, when salamander and mosquito phenology overlaps (e.g., spring-breeding salamanders and early summer-breeding mosquitoes), salamanders could regulate mosquito population size, especially when salamander

larvae are large. Taken together, these findings suggest that salamander larvae are effective mosquito predators in natural pools and wetlands.

Unsurprisingly, we found mosquitofish were effective predators of mosquito larvae. Despite their smaller size, mosquitofish consumed an equivalent number of mosquitos as salamander larvae. Other experiments with direct comparisons have reported that larger mosquitofish can consume approximately 5x as many mosquito larvae (~1000) as *A. talpoideum* of the same size (DuRant and Hopkins 2008). We found a marginally significant relationship between mosquitofish body size and consumption, suggesting larger mosquitofish are better at mosquito predation. However, we did not distinguish between sexes, and *Gambusia* can have strong sexual dimorphism where females are much larger (Blanco et al. 2004). It may be that the size effect we saw was due to sex differences, and this should be considered in future studies. Nevertheless, our fish were within the range of overlap between males and females (Blanco et al. 2004). Natural populations of *Gambusia* are often dominated by mosquitofish are smaller than larval salamanders. Previous work has reported size ranges for mosquitofish of 20–55 mm, with the majority less than 30 mm (Hughes 1985). In June and July (when our experiments were conducted), most mosquitofish were < 20 mm (Daniels and Felley 1992). Thus, our size ranges reflect natural variation present during summer, i.e., when the mosquitofish population is largely dominated by young-of-year. Although per capita rates of mosquito predation by salamanders and mosquitofish in our experiment were equivalent, mosquitofish have the potential to attain much higher population sizes as the season progresses in permanent water bodies because of rapid reproduction and development (Meffe 1992), which should be considered in future studies.

Although it is increasingly recognized that tadpoles are omnivorous (Schiesari et al. 2009), the predatory role of tadpoles on mosquito larvae has often been overlooked. Tadpoles in

our experiment consumed a large number of mosquitoes, adding to the limited existing information about the predation ability of tadpoles. Tadpoles have been shown to reduce mosquito populations in the field and lab (Spielman and Sullivan 1974; Rodríguez and González 2000), and influence oviposition of mosquitoes (Mokany and Shine 2003a; Rubbo et al. 2011). We found that southern leopard frog tadpoles consumed approximately 5x more mosquitoes than previously documented for Cuban treefrogs (Spielman and Sullivan 1974; Rodríguez and González 2000). Furthermore, as the tadpoles grew they consumed more mosquitoes. Given the high densities of tadpoles frequently observed in ponds and wetlands, the contribution of tadpoles to mosquito control could be substantial.

Tadpoles may be important in controlling mosquito populations, but not just through predation. Tadpoles can strongly affect ecosystem properties such as nutrient cycling, primary production, and plankton dynamics in ponds (Seale 1980), so they likely limit mosquito growth through competition, and this effect may be even more important than direct consumption. In our experiments, the tadpoles grew to larger sizes than the other species, and consumption increased with size. Competitive ability likely scales with size as well. Previous research has found tadpoles can be strong competitors for shared resources (such algae and detritus) with mosquitoes, as well as preyed upon by mosquito larvae at small sizes (Blaustein and Margalit 1994; Mokany and Shine 2003b). Whether tadpoles prey on, are preyed upon, or compete with mosquito larvae depends on the order in which the organisms colonize (Blaustein and Margalit 1996), and the tadpole and mosquito species present.

Although mosquitofish may be a good mosquito control strategy in areas where they are native, introduction of non-indigenous species such as mosquitofish can have negative and unintended impacts on the native biota present in aquatic systems. Introduced populations of

mosquitofish alter food webs (Pyke 2008) and negatively affect amphibians and fishes (Blanco et al. 2004; Zeiber et al. 2008; Segev et al. 2009; Drake et al. 2014; Shulse and Semlitsch 2014). Instead of introducing mosquitofish to areas where they are non-native, ensuring healthy breeding populations of salamanders and anurans could be a good mosquito control measure. This is especially true in temporary pools, which pond-breeding amphibians can colonize because of their complex life cycle (Wilbur 1980). Thus, amphibians could be important for controlling mosquitos in aquatic systems that dry semi-regularly or regularly, which would be unsuitable for mosquitofish.

Our study suggests that at least over the short periods, salamander larvae may be as effective as mosquitofish, and anuran tadpoles less so, at mosquito control in small aquatic ecosystems without the secondary negative effects of introducing non-indigenous organisms such as mosquitofish. Global declines of amphibian species (Stuart et al. 2004) may have a large effect on transmission of mosquito-borne diseases. We suggest restoration of amphibian habitats, rather than introduction of non-native species, may be an effective mosquito control measure.

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Figure captions

Fig. 1. Experimental ranges of (A) total body lengths and (B) 24-h mosquito larvae predation in spotted salamander larvae (*Ambystoma maculatum*), mosquitofish (*Gambusia affinis*) and southern leopard frog tadpoles (*Lithobates sphenoccephalus*). Different letters indicate significant differences in post-hoc Tukey HSD tests.

Fig. 2. Total body length (mm) vs. proportional consumption of mosquito larvae over 24 h for (A) salamander larvae (*Ambystoma maculatum*), (B) mosquitofish (*Gambusia affinis*), and (C) tadpoles (*Lithobates sphenoccephalus*). Models indicated mosquito predation increased significantly with body size in salamander larvae and tadpoles, and marginally for mosquitofish.

Draft

Figure 1.

Fig. 1

224x392mm (300 x 300 DPI)