EFFECTS OF WESTERN MOSQUITOFISH (*GAMBUSIA AFFINIS*) ON TADPOLE PRODUCTION OF GRAY TREEFROGS (*HYLA VERSICOLOR*)

GEOFFREY R. SMITH[1,3] AND LOGAN E. SMITH[2]

[1]*Department of Biology, Denison University, Granville, Ohio 43023 USA*
[2]*Granville High School, 248 New Burg St., Granville, Ohio 43023 USA, e-mail: 19smithlogan@gmail.com*
[3]*Corresponding author, e-mail: smithg@denison.edu*

Abstract.—The introduction of fish into fishless habitats is a concern for the conservation of native amphibians. Invasion of freshwater habitats by mosquitofish (*Gambusia holbrooki* and *G. affinis*) often results in declines in native amphibian populations. Predation on eggs, embryos, and/or tadpoles appears to be the primary mechanism by which mosquitofish cause declines. Here, we examine the effects of Western Mosquitofish (*G. affinis*) on eggs and hatchlings of Gray Treefrogs (*Hyla versicolor*). In particular we determine which stage (i.e., egg or hatchling) is most vulnerable to consumption and compare the impact of male and female mosquitofish on eggs and hatchlings. The proportion of eggs successfully producing hatchlings did not differ between treatments containing no mosquitofish, a male mosquitofish, or a female mosquitofish. However, the proportion of eggs producing tadpoles at the end of the experiment was significantly lower in treatments with male mosquitofish than in control treatments, but did not differ from the control in treatments with female mosquitofish. Our results suggest *G. affinis* has the potential to limit the ability of Gray Treefrogs to successfully colonize ponds through predation on hatchlings and/or early tadpoles, rather than predation on eggs.

Key Words.—Eggs; fish; frogs; hatchlings; predation; test

INTRODUCTION

Mosquitofish (*Gambusia holbrooki* and *Gambusia affinis*) have been introduced into freshwater ecosystems around the world, often resulting in declines in native fish, invertebrate, and amphibian populations (reviewed in Pyke 2008). These declines may be driven by either competition or predation but in the case of amphibians, predation on eggs, embryos, and/or tadpoles appears to be the primary mechanism by which mosquitofish cause declines (reviewed in Pyke 2008). Indeed, experiments have demonstrated negative effects of mosquitofish on the survival of tadpoles (e.g., Karraker et al. 2010; Smith and Dibble 2012; Smith et al. 2013), as well as high rates of consumption of tadpoles in the laboratory (e.g., > 70–75% consumption, Zeiber et al. 2008; Hunter et al. 2011; Kerby et al. 2012). In particular, hatchlings and tadpoles (especially smaller tadpoles) of several species frequently appear to be more susceptible to mosquitofish predation than are eggs (e.g., Komak and Crossland 2000; Zeiber et al. 2008; Reynolds 2009); however, some species suffer predation during all aquatic stages (e.g., Grubb 1972; Komak and Crossland 2000; Pyke and White 2000). Additionally, injury rates (particularly of tail or limbs) in anuran tadpoles are higher in the presence of mosquitofish (Lane and Mahony 2002; Gregoire and Gunzburger 2008; Shulse and Semlitsch 2014), which are known to show only partial consumption of tadpole prey (Walls et al. 2002). However, not all anuran species appear to be susceptible to mosquitofish predation or, alternatively, mosquitofish are not efficient predators of tadpoles of certain species (e.g., Lawler et al. 1999; Gunzburger and Travis 2004; Zeiber et al. 2008). Such differences in susceptibility to mosquitofish predation likely parallel patterns of differential susceptibility observed among species and populations of other amphibians to fish predators, which reflect a variety of underlying causes, including the distinct evolutionary histories of populations (see Gunzburger and Travis 2005).

Gray Treefrog (*Hyla versicolor*) tadpoles are palatable to fish (Adams et al. 2011), and the abundance of Gray Treefrogs is often lower in the presence of mosquitofish (Shulse et al. 2013). Gray Treefrogs can also use cues to avoid oviposition in ponds with fish (Takahashi 2007). In addition, mosquitofish are able to colonize temporary ponds in wet years and/or they can be introduced to help control mosquitoes (e.g., Alemadi and Jenkins 2008; Caillouët et al. 2008). Indeed, we have observed Western Mosquitofish (*G. affinis*) occasionally present in a local temporary pond where Gray Treefrogs typically breed that is normally fishless (Geoffrey Smith, pers. obs.). Here, we examine the effects of non-native *G. affinis* on Gray Treefrog eggs and hatchlings. In addition, we determine which stage (i.e., egg or hatchling) is more vulnerable to consumption and compare the effects of male and female *G. affinis* on the eggs and hatchlings of Gray Treefrogs. We compared the potential effects of male and female *G. affinis* because they show dramatic sexual size dimorphism
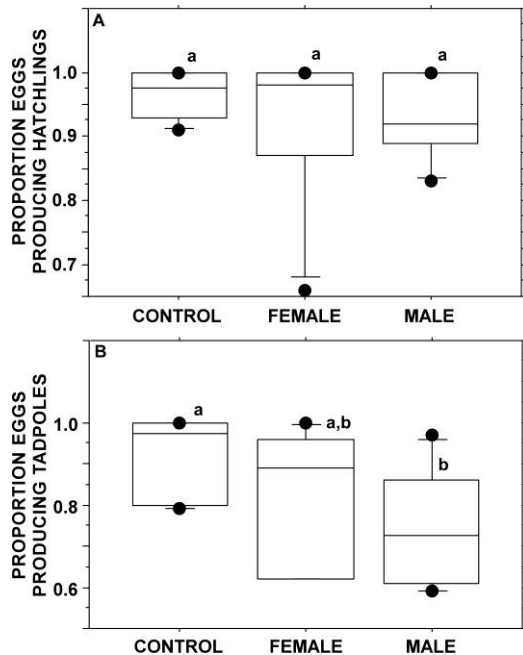
FIGURE 1. Box plots of the proportion of (A) Gray Treefrog eggs producing hatchlings and (B) Gray Treefrog eggs producing tadpoles after exposure to nothing (control), a female, or a male Western Mosquitofish (*Gambusia affinis*). Median is given by the line within the box, the box represents the 2nd and 3rd quartiles, vertical lines are 10th and 90th percentiles, and dots represent values below the 10th percentile and above the 90th percentile. Treatments sharing a letter are not significantly different based on Wilcoxon pairwise post-hoc tests ($P \geq 0.05$).

with females being much larger than males (Pyke 2005). Thus, we might predict differences in their impact on Gray Treefrogs with females having a greater effect due to their larger size.

MATERIALS AND METHODS

On 18 June 2012, we collected six Gray Treefrog egg clusters from three experimental ponds (1135 L Stock Tanks, Rubbermaid Commercial Products, Winchester, Virginia, USA) on the Denison University Biological Reserve, Granville, Licking County, Ohio, USA (40°05'07.32"N, 82°30'33.92"W; datum: WGS84). We collected egg clusters within 12 h of deposition. Because we collected egg clusters from three different experimental ponds separated by several meters, it is likely that egg clusters collected from different ponds were laid by different females, and thus the egg clusters likely represented ≥ 3 females. We dipnetted male and female *G. affinis* from a local pond that does not appear to have Gray Treefrogs, thus they probably did not have previous experience with this species. We housed fish in the laboratory without feeding for 24 h prior to introduction into the experiment. Total length of male

G. affinis was 25–27 mm and total length of female *G. affinis* was 45–50 mm.

For the experiment, we randomly divided each of six individual egg clusters into three subunits of between 58 and 80 eggs (mean ± 1 S.E. = 66.9 ± 1.32 eggs). We then assigned one of the three subunits from each egg cluster to one of three mosquitofish treatments: control (no mosquitofish), female mosquitofish (a single adult female mosquitofish), and male mosquitofish (a single adult male mosquitofish). Therefore, eggs from each cluster were exposed to each treatment with six replicates per treatment. We established experimental treatments in 5.7-L plastic containers (32 cm L × 19 cm W × 11 cm H) filled with aged well-water. In both mosquitofish treatments, mosquitofish had access to the entire container. We monitored the number of eggs, hatchlings (Gosner Stages 19–24; Gosner 1960), and tadpoles (Gosner Stage ≥ 25) daily for 5 d. During the experiment we did not feed mosquitofish any other food. We performed the experiment in a climate-controlled field station building at a temperature of 20–21 °C and with a natural light:dark regime.

Hatching took place between the daily checks on 20 June and 21 June (i.e., between days 2 and 3 of the experiment). We therefore analyzed the effects of mosquitofish on eggs remaining at the 20 June check to determine the consumption of eggs prior to the beginning of hatching. We also analyzed the effects of mosquitofish on the production of tadpoles at the end of the experiment on 23 June.

Because data were not normally distributed, even after an arcsine-square root transformation (Shapiro-Wilk test, $P < 0.05$), we used a nonparametric Kruskal-Wallis Test to analyze the effects of male and female *G. affinis* on the survival of eggs to hatching (i.e., the proportion of eggs that produced hatchlings) and on the proportion of eggs that produced tadpoles at the end of the experiment (i.e., number of tadpoles at the end of the experiment divided by the number of eggs at the start of the experiment). We used the number of eggs at the start of the experiment in the calculation of the proportion of eggs producing tadpoles at the end of the experiment because we were interested in the effect of *G. affinis* on the recruitment of tadpoles from the eggs initially produced by a female Gray Treefrog. We included egg cluster of origin as a blocking factor to account for any variation among egg clusters in viability. Significant overall treatment effects were followed by nonparametric comparisons for each treatment pair using the Wilcoxon method. We used JMP 10 (SAS Institute, Cary, North Carolina, USA) to analyze the data. We set statistical significance at α = 0.05.

RESULTS

The proportion of eggs remaining on 20 June (i.e., just prior to hatching) did not differ among mosquitofish treatments (Fig. 1A; $H = 1.17$, df $= 2$, $P = 0.56$). The proportion of eggs producing tadpoles at the end of the experiment was significantly different among mosquitofish treatments (Fig. 1B; $H = 7.17$, df $= 2$, $P = 0.028$). Post-hoc comparisons showed that tadpole production was significantly lower in containers with male mosquitofish (median 72.5%) compared to control containers (median 97.5%; $W = -2.48$. $P = 0.013$). In contrast, tadpole production was not significantly different between female containers (median 89%) and control containers ($W = -1.28$, $P = 0.20$) nor was it different between male and female containers ($W = -1.36$, $P = 0.17$).

DISCUSSION

Our experiment demonstrates that the presence of *G. affinis* has a negative effect on the ability of Gray Treefrogs to successfully produce tadpoles from the eggs they lay. Previous studies have also shown mosquitofish can prey upon early life stages of amphibians (e.g., Komak and Crossland 2000; Zeiber et al. 2008; Reynolds 2009), and therefore our results are not unexpected. However, in addition to demonstrating the negative effects of *G. affinis* on Gray Treefrog tadpole production, our results indicate the negative impact of *G. affinis* on Gray Treefrog tadpole production is due to the consumption of hatchlings and early tadpoles rather than the consumption of eggs. We note, however, that our experiment only addressed the impact on the egg to tadpole transition, and not the potential for mosquitofish to impact tadpole survival to metamorphosis or size at metamorphosis.

Our conclusion that the negative impact of *G. affinis* was due to the consumption of hatchlings rather than eggs is based on the lack of differences among treatments in the proportion of eggs producing hatchlings, the relatively high proportion of eggs producing hatchlings in all treatments (> 90% in all treatments), and significant differences among treatments in the proportion of eggs producing tadpoles. Our results are consistent with several other studies that found eggs of several species were consumed by mosquitofish less, or not at all, relative to hatchlings or tadpoles (e.g., Komak and Crossland 2000; Zeiber et al. 2008; Reynolds 2009). Some anuran eggs appear to be protected by their jelly coats, as eggs protected by jelly were consumed less than eggs that have had their jelly coat removed (e.g., Werschkul and Christensen 1977). In addition Grubb (1972) found anuran species with larger eggs, including *Hyla chrysoscelis*, a close relative of the Grey Treefrog, experienced lower egg predation rates by *G. affinis*, than those species with smaller eggs. Eggs and hatchlings also appear to be less palatable than tadpoles across most groups of amphibians (Gunzburger and Travis 2005). It also appears that the movement of hatchlings and tadpoles may elicit attacks from mosquitofish, whereas eggs do not move as much and are therefore not attacked (Braid et al. 1994; Drake et al. 2014).

Our results also suggest that male *G. affinis* may have a greater impact on Grey Treefrog tadpole production than female *G. affinis*. Although tadpole production was not significantly different between male and female mosquitofish treatments, it was significantly lower in the male treatments compared to control treatments (females were not different than controls). Because of their larger gape size (see Henkanaththegedara and Stockwell 2013), we expected female mosquitofish to consume more eggs or hatchlings than males. Indeed, Henkanaththegedara and Stockwell (2013) found that female *G. affinis* preyed upon larval Mohave Tui Chub (*Siphateles bicolor mohavensis*) at much higher rates than males. In our experiment, hatchling Gray Treefrogs may be small enough to pose no impediment to consumption by males. In addition, because mosquitofish are known to prey upon amphibian larvae by nipping at bodies and fins, rather than necessarily consuming the whole tadpole or larva (Walls et al. 2002; Gregoire and Gunzburger 2008; Shulse and Semlitsch 2014), gape size may not limit the ability of mosquitofish to consume or kill hatchlings or tadpoles. The potentially greater effect of males may therefore come from the fact that male mosquitofish are generally more aggressive to conspecifics and heterospecifics than females, especially in the lab (Martin 1975; Carmona-Catot et al. 2013). However, other studies have shown that female mosquitofish tend to have higher attack rates on food items than males (e.g., Shankuntala 1977; Blanco et al. 2004; Arrington et al. 2009). Further experiments that observe the behavior and foraging of male and female mosquitofish on eggs, hatchlings, and tadpoles are needed to fully understand the potential for each sex to affect the successful production of anuran tadpoles.

Although our experiment took place in a simple laboratory environment, our results demonstrate that *G. affinis* have the potential to affect the ability of Gray Treefrogs to successfully colonize ponds through predation on hatchlings and/or tadpoles. This is in addition to the potential for mosquitofish to cause Gray Treefrogs to avoid oviposition in ponds with fish (Takahashi 2007), including *G. affinis* (Geoffrey Smith and Johanna Harmon, unpubl. data). Therefore, the introduction of mosquitofish into previously fishless ponds could have negative impacts on the distribution and abundance of Gray Treefrogs and likely other species of anurans.

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GEOFFREY R. SMITH received his B.A. in Biology from Earlham College and his Ph.D. in Biological Sciences from the University of Nebraska-Lincoln. He is a Professor of Biology at Denison University in Granville, Ohio. Geoff's research focuses on how human modifications of the environment affect amphibian and reptile populations and communities. (Photographed by Jamie Hale).

LOGAN E. SMITH is currently a student at Granville High School. In addition to the experiment reported in this manuscript, Logan has participated in research examining the ecology of tadpoles, frog metamorphs, turtles, and fish. (Photographed by Geoff Smith).