

Eastern mosquitofish resists invasion by nonindigenous poeciliids through agonistic behaviors

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Abstract Florida is a hotspot for nonindigenous fishes with over 30 species established, although few of these are small-bodied species. One hypothesis for this pattern is that biotic resistance of native species is reducing the success of small-bodied, introduced fishes. The eastern mosquitofish *Gambusia holbrooki* is common in many freshwater habitats in Florida and although small-bodied (<50 mm), it is a predator and aggressive competitor. We conducted four mesocosm experiments to examine the potential for biotic resistance by eastern mosquitofish to two small-bodied nonindigenous fishes, variable platyfish (*Xiphophorus variatus*) and swordtail (*X. hellerii*). Experiments tested: (1) effect of eastern mosquitofish density on adult survival, (2) effect of eastern mosquitofish on a stage-structured population, (3) role of habitat structural complexity on nonindigenous adult survival, and (4) behavioral effects of eastern mosquitofish presence

and habitat complexity. Eastern mosquitofish attacked and killed non-native poeciliids with especially strong effects on juveniles of both species. Higher eastern mosquitofish density resulted in greater effects. Predation on swordtails increased with increasing habitat complexity. Eastern mosquitofish also actively drove swordtails from cover, which could expose non-native fish to other predators under field conditions. Our results suggest that eastern mosquitofish may limit invasion success.

Keywords Biotic resistance · *Gambusia holbrooki* · *Xiphophorus* · Predation · Aggressive competition · Mesocosm · Nonindigenous species

Introduction

Biotic resistance is one of several hypotheses proposed to explain patterns of invasion success. The concept has a long history, dating back to Elton (1958) who hypothesized that more diverse communities are more resistant to invasion through competitive processes. Predation is another form of biotic resistance (Maron and Vila 2001; DeRivera et al. 2005; Ruesink 2007). Predation may directly eliminate introduced populations, facilitate extirpation by other mechanisms, or reduce the distribution and abundance of established invaders. Reduced invasion success as a function of

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native predators has been demonstrated in marine systems (Harding 2003; Hunt and Yamada 2003; DeRivera et al. 2005) as well as in some freshwater habitats. Baltz and Moyle (1993) hypothesized that native fishes limited success of nonindigenous fishes in California and subsequent experiments suggested that a combination of native and nonindigenous predatory fishes curbed the range of an introduced minnow (Harvey et al. 2004).

Florida (USA) is a hot spot for fish invasions with over 130 species reported or collected from inland waters, and with at least 30 of these species established (Fuller et al. 1999; Nico and Fuller 1999; Shafland et al. 2008). Most established nonindigenous fishes in peninsular Florida have medium to large adult body size (≥ 15 cm total length [TL]) (Nico and Fuller 1999; Nico 2005). Out of 45 small-bodied species introduced (< 15 cm TL), approximately six have reproducing populations in Florida and just one of these, the African jewelfish (*Hemichromis letourneuxi*), is common and widespread (USGS 2007; Shafland et al. 2008).

The low incidence of nonindigenous small-bodied fishes with established populations in Florida is counter to the findings of Ruesink (2005) in which high establishment success was found for fish families characterized by small body size (e.g., Poeciliidae) in a global analysis of introductions. The ornamental fish trade is an important pathway for fish introductions (Duggan et al. 2006) and has been active in Florida since the 1930s. Currently, hundreds of small-bodied fishes are cultured and traded within Florida, many of which are maintained in outdoor ponds (Hill and Yanong 2002). Environmental and ecological gradients influence the abundance and distribution of nonindigenous fishes, especially in habitats with strong hydrological cycles such as in South Florida (Trexler et al. 2000). Nevertheless, Florida's relatively mild, subtropical climate and diverse aquatic habitats favors establishment of many tropical or subtropical species common in the industry (Nico and Fuller 1999).

Given high propagule pressure and a suitable environment, the low incidence of established, small-bodied species may be due to resistance from the biotic community through predation or competition. For example, largemouth bass (*Micropterus salmoides*) prey on small-bodied introduced species

(Hill unpublished data). However, many large-bodied predatory fishes are inefficient predators in shallow or densely-vegetated areas where small-bodied species often take refuge (Savino and Stein 1982; Anderson 1984). A smaller species that may influence introduced fishes in structurally complex habitat is the ubiquitous and abundant eastern mosquitofish (*Gambusia holbrooki*) (Hill et al. 2011). Although small bodied (adults typically 20–40 mm TL; Page and Burr 1991), eastern mosquitofish are aggressive and commonly attack and occasionally prey on other small fishes (Meffe 1985; Schaefer et al. 1994; Rincon et al. 2002). Three lines of evidence suggest that eastern mosquitofish may strongly interact with small-bodied introduced fishes: (1) mosquitofish (*G. holbrooki* and closely related western mosquitofish *G. affinis*), when introduced themselves, have caused declines of native small-bodied fishes (Meffe et al. 1983; Courtenay and Meffe 1989; Galat and Robertson 1992), (2) eastern mosquitofish have large effects on naturally co-occurring cyprinodontiform fishes in Florida (Belk and Lydeard 1994; Schaefer et al. 1994; Taylor et al. 2001), and (3) eastern mosquitofish is a pest of ornamental aquaculture, causing dramatic declines of production in infested ponds (Hill personal observations). These effects may be due to competition (Rincon et al. 2002); however, most studies have shown the strongest factor is mosquitofish predation on heterospecific juveniles (e.g., Meffe 1985; Taylor et al. 2001; Laha and Mattingly 2007). Mosquitofish are also known to be aggressive, biting and harassing other fishes even without consumption (Meffe and Snelson 1989), potentially affecting behavior, growth, and survival of other fishes.

The objective of our study was to test the hypothesis that predation and aggression by native eastern mosquitofish resists invasions of small-bodied fishes in Florida's freshwater habitats. We conducted a series of mesocosm experiments to determine the effect of eastern mosquitofish on two nonindigenous small-bodied freshwater fishes of the genus *Xiphophorus*. Both are poeciliids that are similar in body shape and size, but potentially differ in their anti-predator behaviors. Experiments were designed to evaluate the effects of different eastern mosquitofish densities on non-native fish behavior and survival on both adult and stage-structured populations, as well as different levels of habitat structural complexity.

Methods

Study species

Two nonindigenous poeciliids, variable platyfish (*Xiphophorus variatus*) and a variant of the green swordtail (*X. hellerii*), were tested separately in all mesocosm studies. Both are common in ornamental aquaculture production in Florida (Hill and Yanong 2002) and the aquarium hobby. The variable platyfish is native to drainages along portions of Mexico (Rosen 1979; Page and Burr 1991). The green swordtail has a larger native distribution, ranging from Veracruz, Mexico south into Honduras (Page and Burr 1991; Greenfield and Thomerson 1997). These fishes are generalized omnivores, feeding on a variety of small invertebrates, plant material, and detritus (Mills and Vevers 1989). Maximum total length (TL) for variable platyfish is approximately 70 mm, whereas green swordtails grow to 80 mm TL not including the sword; males reach greater overall total length due to sexual dimorphism of the caudal fin (Page and Burr 1991). Localized and ephemeral populations of both species have been found in Florida (USGS 2007; Shafland et al. 2008; Hill and Cichra unpublished data).

Eastern mosquitofish were obtained from a detention pond of the UF/IFAS Tropical Aquaculture Laboratory (TAL), Ruskin, Florida and a commercial producer in Venus, Florida. The gonopodial structure of >30 male specimens from both sources were examined to verify that specimens were *Gambusia holbrooki* and not *G. affinis*, (Page and Burr 1991). Variable platyfish (hereafter platyfish) were obtained from a commercial producer. Green swordtails (hereafter swordtails) used in experiments were the “velvet wag” variety (red body with black fins) collected from aquaculture ponds at TAL. Swordtails are markedly sexually dimorphic, with males having a “sword” or extended portion of the lower part of the caudal fin. The sex ratio used in the experiments was not manipulated and was representative of the culture ponds with the majority of individuals being females (mean \pm SD = 32 \pm 3.5% male).

For all experiments, adults of the nonindigenous species were size selected to represent fish commonly found in aquaria. Eastern mosquitofish were 28 \pm 5 mm TL and 0.27 \pm 0.16 g, platyfish were 40 \pm 4 mm TL and 1.20 \pm 0.35 g, and swordtails were 50 \pm 3 mm TL (not including sword on males) and

1.73 \pm 0.41 g across all experiments. The two nonindigenous species were significantly longer (two-sample t test: platyfish $t_{0.05, 238} = -20.30$, $P < 0.0001$; swordtails $t_{0.05, 238} = -40.41$, $P < 0.0001$) and heavier (platyfish $t_{0.05, 238} = -25.67$, $P < 0.0001$; swordtails $t_{0.05, 238} = -37.83$, $P < 0.0001$) than eastern mosquitofish in all experiments.

Mesocosm overview

A series of mesocosm experiments was conducted to investigate possible predatory effects of eastern mosquitofish on the study species (details in Table 1). Experimental units were black, oval polyethylene tanks with an area of approximately 1.2 m² at the base and with a water surface area of 1.4 m². Water depth was maintained at 23 cm, similar to small streams and shallow littoral zones commonly inhabited by eastern mosquitofish. Tanks were arranged on a flow-through system, and each tank was aerated by a single air stone and had a single standpipe. Structural complexity was provided in each tank by artificial vegetation consisting of multiple strips of black plastic (2 cm \times 40 cm) glued to a rectangular plastic lighting grate (91 \times 61 cm) covering 49% of tank bottom (cf. Savino and Stein 1982).

Water quality parameters varied little during the experiments. Hardness (485.7 \pm 47.1 ppm), alkalinity (177.8 \pm 16.4 ppm), nitrites (0.13 \pm 0.23 ppm), and unionized ammonia nitrogen (undetectable) were measured using a Hach[®] fish farmer’s water quality kit (Hach Company, Loveland, CO, USA). Temperature (27.0 \pm 0.23 C), pH (8.0 \pm 0), dissolved oxygen (6.68 \pm 0.57 ppm), and salinity (0.4 \pm 0 ppt) were measured using a YSI handheld meter (YSI Inc., Yellow Springs, OH, USA).

Eastern mosquitofish were stocked 4 days prior to the introduction of nonindigenous fish except in Experiment 2. Each tank was inspected at least once daily and collectively the tanks were observed for two or more hours daily on nearly all days. Dead eastern mosquitofish found in tanks during the acclimation period were removed and replaced to maintain target density. Mortalities of nonindigenous fish were removed once or twice daily. No fish were replaced during the trials. Eastern mosquitofish were fed 5% body weight per day of a commercial feed (Purina 33% Tropical Fish Chow, Purina Mills, St. Louis, MO, USA) and feed weights were adjusted upward upon the

Table 1 Overview of four different mesocosm experiments testing the effects of mosquitofish predation on two nonindigenous poeciliids

Expt.	Independent variable	Treatment levels	Replicates	Introduced population	MF added	Variable platyfish trial duration (days)	Swordtail trial duration (days)	Response variables
1	MF density	21,43,86 MF/m ²	5	10 adults	4 days prior	11	5	Adult survival
2	MF density	0,21,86 MF/m ²	5	10 adults, 10 juveniles	Same time	4	4	Adult survival, juvenile survival
3	Stem density	72,216,645 stems/m ²	5	10 adults	4 days prior	11	5	Adult survival
4a	Stem density	72,216,645 stems/m ²	3	10 adults	4 days prior	3	3	Number of attacks, proportion of fish in vegetation
4b	MF presence	0,86 MF/m ² , or 86 zebra danios/m ²	3	10 adults	4 days prior	3	3	Number of attacks, proportion of fish in vegetation

Stem density was standardized at 216 stems/m² in experiments 1, 2, and 4b. Mosquitofish density was standardized at 86 fish/m² in experiments 3 and 4a. Fin damage score (0 = no damage, 1 = moderate damage, and 2 = severe damage) for surviving adults of the introduced species was a response in all experiments as well MF mosquitofish

addition of the nonindigenous species. At the end of each trial, the artificial vegetation was removed and all remaining fish were counted. All surviving adult platyfish and swordtails were inspected for damage to the caudal fin and scored based on amount of caudal fin loss with the values of: 0 for no damage, 1 for moderate damage (less than 50% of fin area), and 2 for severe damage (greater than 50%) (Meffe 1985; Galat and Robertson 1992; Hill et al. 2011).

Experiment 1: Adult introduction

Experiment 1 investigated effects of eastern mosquitofish on introduced adult platyfish and swordtails at three (low, medium and high) eastern mosquitofish densities chosen to be within the range observed in natural systems (Trexler et al. 2005, supplemental material; Hill unpublished data) with a constant, intermediate stem density (Table 1). After eastern mosquitofish were established in the mesocosms for a period of 4 days, 10 adult platyfish or swordtails were introduced into the tanks. This experiment mimicked an introduction event that might occur when marketable-sized ornamental fish are introduced by escape or home aquaria release into an environment already containing eastern mosquitofish. Trials lasted 11 days for platyfish and 5 days for swordtails as a result of differing mortality between the two species.

Experiment 2: Stage-structured population

This experiment was designed to test the effect of eastern mosquitofish densities on survival rates in stage-structured populations of nonindigenous fish. Similar to Experiment 1, three eastern mosquitofish densities were used with the stem density held constant. Unlike the other experiments, 10 adult and 10 juvenile platyfish or swordtails were stocked into mesocosms to represent a mixed reproducing population (Table 1). Eastern mosquitofish and the nonindigenous species were added to the experimental units at the same time to mimic co-existing populations of eastern mosquitofish and the two introduced species, as well as prevent an artificially high predation effect on introduced juveniles when initially added to tanks. A treatment without eastern mosquitofish was used to control for possible cannibalism of the juveniles by conspecific adults. Trials lasted 4 days for both species due to high mortality of the juveniles.

Juveniles used were the smallest individuals available at the time and subsamples ($n = 30$) of each species were used to estimate lengths and weights of the donor population. Juvenile platyfish were 10 ± 2 mm TL and 0.02 ± 0.01 g. Juvenile swordtails were 14 ± 1 mm TL and 0.07 ± 0.01 g.

Appearance of offspring was noted during the experiment and analyzed as a response to eastern mosquitofish treatment. Neonates were noticeably smaller and could be readily distinguished from the stocked juveniles. Female poeciliids are capable of bearing live young nearly year round (Meffe and Snelson 1989) and gravid females were commonly included as part of the 10 introduced adult fish in all experiments. Due to the difficulty in differentiating reproductive stage, no attempt was made to select females of equal reproductive potential (e.g., embryo number and developmental stage).

Experiment 3: Effects of structural complexity

Experiment 3 was designed to test the influence of habitat structural complexity on survival of introduced poeciliids in the presence of eastern mosquitofish. The high density treatment level (86 fish/m²) from previous experiments was used across three vegetation densities (Table 1). Stem densities were chosen to be within the range of native plant densities and other experimental work on habitat complexity and fish predation (e.g., Savino and Stein 1982; Anderson 1984; Hayse and Wissing 1996). The introduced population size and the trial duration was the same as used in Experiment 1.

Experiment 4: Behavioral measurements

Eastern mosquitofish were closely associated with artificial vegetation during Experiments 1, 2 and 3, rarely moving outside areas of stem cover; the introduced species were found primarily outside the artificial cover, potentially excluded due to eastern mosquitofish aggression. Experiment 4 was designed to test behavioral changes of the platyfish and swordtails more quantitatively based on those preliminary observations. Two main hypotheses were tested in this experiment: (1) introduced poeciliids would encounter differential attack rates and utilize habitat in the tank differently across varied stem densities in the presence of eastern mosquitofish; and (2) at a constant stem density, habitat use patterns of

the introduced poeciliids would differ depending on the presence versus absence of eastern mosquitofish. Analysis of this experiment was separated into two groups of treatments.

4a: Analysis of differing stem densities

This portion of Experiment 4 tested the effect of habitat complexity on eastern mosquitofish attack rates and habitat use of the two introduced species. The treatments were the same as used in Experiment 3 with three stem densities and one constant (high) density of eastern mosquitofish established beforehand in the mesocosms (Table 1).

4b: Analysis of eastern mosquitofish presence

This second set of treatments in Experiment 4 tested the effect of eastern mosquitofish presence on attack rate and habitat use across a constant stem density. The treatment groups included the medium stem density treatment (216 stems/m²) with high eastern mosquitofish density, which was also analyzed in Experiment 4a (treatment referred to as MF in the analysis of Experiment 4b), and two control treatments at the same stem density (Table 1). One control had no eastern mosquitofish or any other fish stocked previous to the introduction (hereafter NoMF) and the second control had zebra danios (*Danio rerio*) stocked at high density (hereafter Danios) (Table 1). The Danios control was used to control for any density effects on behavior by the eastern mosquitofish not linked to aggression. The zebra danio is a small cyprinid fish from Asia that is a common ornamental fish and research animal. This species was chosen to serve as a control for the experiment because it is similar in size, appearance, and activity level to eastern mosquitofish (Thompson and Hill personal observations), but is generally not aggressive (Mills and Vevers 1989).

In both experiment 4a and 4b, behavior was measured in two ways. The first was a focal animal sampling protocol (Martin and Bateson 1993) whereby individuals of the introduced species were observed over time to obtain a quantitative estimate of the number of attacks an introduced fish sustains at different eastern mosquitofish or stem density treatments (Table 1). Following Laha and Mattingly (2007) an attack was defined as the sum of all aggressive movements, including chases and nips

(bites to the caudal fin area). Conspecific attacks of the introduced species were also recorded. Observations were made on an arbitrarily chosen individual that was followed for a maximum of 5 min or until lost after which another fish was chosen. The total focal animal sampling period was 30 min for each tank.

The second behavioral measure was achieved with scan sampling (Martin and Bateson 1993). This protocol was used to quantify the habitat use patterns of the introduced species as a response to eastern mosquitofish presence or stem density. In this procedure, the position of all 10 introduced fish was recorded every 5 min for 30 min giving 7 total position observations per sampling period. The recorded positions were either “in the vegetation,” describing fish in or above the simulated vegetation mat, or “outside the vegetation,” to describe those fish outside of the area defined. If a fish was on the border, the position was assigned to the habitat containing >50% of the body length based on visual estimation.

Both types of behavior observations were made twice during this experiment. A focal animal sampling period of 30 min began immediately when the study fish were introduced (Day 1). This was followed up with a scan sampling period approximately 4 h later on the same day. The focal animal sampling and scan sampling periods were repeated on Day 3 to allow for observation after a period of acclimation for the nonindigenous study species, where habitat use patterns may be more indicative of preference and anti-predatory behavior may be more apparent. In all behavioral observations, recording began after allowing 5 min for the fish to acclimate to the observer.

Data analysis

Unless otherwise noted, all statistical analyses were conducted at a Type-I error rate of $\alpha = 0.05$ using SAS version 8.1 (SAS Institute, Cary, NC, USA). To normalize the percent survival of the introduced species in each experiment the raw data were arcsine, square-root transformed (Gotelli and Ellison 2004) and analyzed using one-way analysis of variance (ANOVA). Following a significant ANOVA, Tukey’s post hoc pairwise tests were used to determine which treatments differed. In all of the experiments, the assumptions of ANOVA were checked using Levene’s test for homogeneity as well as the Shapiro–Wilk test for normality on the residuals of the ANOVA model.

For Experiment 2, juvenile and adult percent survival were analyzed separately. Also in Experiment 2, the additional recruit count data were square-root transformed and then analyzed using a one-way ANOVA. Power was estimated post hoc for non-significant ANOVAs.

The Experiment 4 total attacks data were analyzed across stem density (Experiment 4a) or eastern mosquitofish treatment (Experiment 4b). Total attack count data were analyzed using the non-parametric Kruskal–Wallis (K–W) rank-sum test because the data (both raw and square-root transformed) violated the assumptions of normality and homogeneity of variance required in the ANOVA model. Dunn’s non-parametric multiple comparison procedure (Hollander and Wolfe 1973) was used post hoc to determine which treatment groups differed significantly. Because of the conservative nature of Dunn’s procedure, a type-I error rate of $\alpha = 0.15$ was used (Hollander and Wolfe 1973). For the scan sampling data in Experiment 4, the proportion of introduced fish in the artificial vegetation was analyzed after being arcsine, square-root transformed. The least-squares means procedure was used post hoc to determine which treatments differed.

Fin damage scores were analyzed using the non-parametric K–W rank-sum test. Dunn’s non-parametric multiple comparison procedure (Hollander and Wolfe 1973) was used post hoc to determine which treatment groups differed significantly.

Results

Experiment 1: Adult introduction

Eastern mosquitofish harassed and nipped the fins of the adults of both nonindigenous species in all tanks, causing mortalities in all treatments except for platyfish in the low density treatment. There was little mortality in the low and medium density treatments with survival near 100% (Fig. 1). However, survival of nonindigenous species significantly decreased with increasing eastern mosquitofish density (platyfish $F_{2, 12} = 13.96$; $P = 0.0007$; swordtail $F_{2, 12} = 6.77$; $P = 0.018$) (Fig. 1).

Caudal fin damage was noted in a portion of surviving individuals of both platyfish and swordtails. However, no statistical differences were found in the extent of fin damage among the different treatments

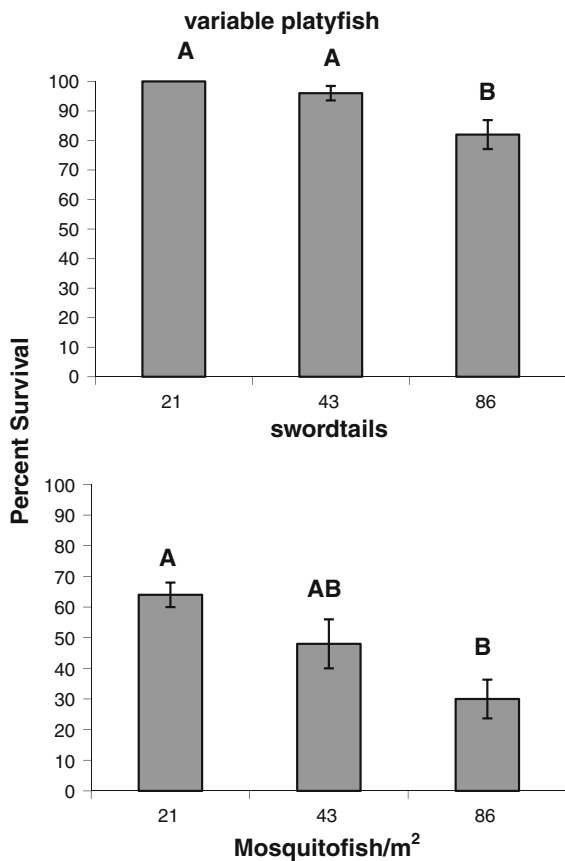


Fig. 1 Results from Experiment 1. Average percent survival (\pm SE) of adult poeciliids introduced into previously-acclimated population of mosquitofish across a range of mosquitofish densities with a constant stem density. Means with different letters are significantly differed based on Tukey's post hoc test. Trials lasted 11 days for platyfish and 5 days for swordtails

for either species (platyfish $\chi^2 = 2.25$, $P = 0.325$; swordtails $\chi^2 = 2.02$, $P = 0.364$). Most surviving platyfish had no damage ($\geq 96\%$) and none were found with severe damage. No damage was found on at least 90% of the surviving swordtails in these treatments, with 100% showing no damage in the high density treatment. The low and medium density treatments had 3% with moderate damage and some (3% for low density and 7% at high density) with severe damage. In contrast, all mortalities of both species exhibited substantial damage to the caudal fin and caudal peduncle. Fin damage of dead fish was severe and would have been scored as 2 (i.e., $>50\%$ of caudal fin missing) if they had survived to the end of the experiment. A similar pattern was observed throughout all subsequent experiments.

Experiment 2: Stage-structured population

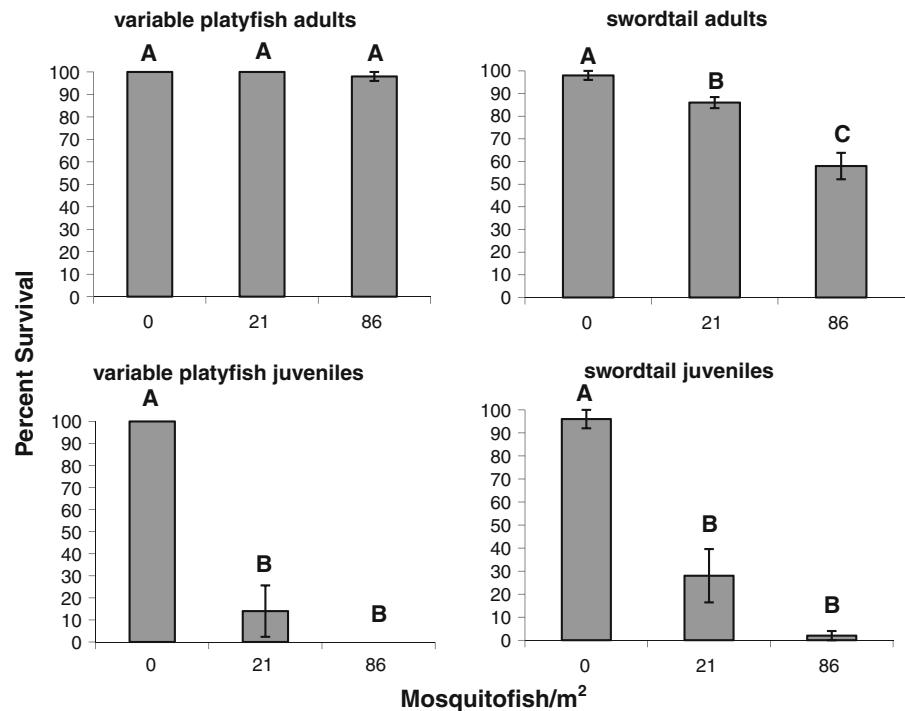
Eastern mosquitofish exerted variable mortality effects on adult platyfish and swordtails, but had strong negative effects on juvenile survival of the two species. Eastern mosquitofish density had little effect on adult survival of platyfish in this shorter experiment ($F_{2, 12} = 1.00$; $P = 0.3966$), with mean survival near 100% across all treatments (Fig. 2). Conversely, eastern mosquitofish density had a significant effect on adult swordtails ($F_{2, 12} = 32.87$; $P < 0.0001$) with survival reduced to $86 \pm 2.4\%$ in the low density and $58 \pm 5.8\%$ in the high density treatments (Fig. 2). In the absence of eastern mosquitofish, no platyfish died and swordtail survival was $98 \pm 2.0\%$.

Eastern mosquitofish density had a significant negative effect on juvenile survival (platyfish $F_{2, 12} = 71.92$; $P < 0.0001$; swordtail $F_{2, 12} = 34.08$; $P < 0.0001$) (Fig. 2). Eastern mosquitofish presence was the critical factor with juvenile survival in the controls being 3–7 times higher than in the low density treatment. Only one juvenile swordtail survived in the high density treatment. Dead juveniles were not observed in the tanks, indicating that the eastern mosquitofish completely consumed them. All juvenile platyfish survived in the control treatment, evidence that adults did not cannibalize their young (Fig. 2). Swordtail adults exhibited a small cannibalistic effect with juvenile survival of $96 \pm 4.0\%$ in the controls.

Platyfish recruitment in this experiment varied significantly with eastern mosquitofish density ($F_{2, 12} = 6.45$; $P = 0.0125$). Additional recruits were noted in four of five control replicates for the platyfish experiment, averaging an additional 6.8 ± 4.2 fish per mesocosm. In contrast, neonate platyfish were not observed in the two eastern mosquitofish treatments. There was no additional swordtail recruitment observed in the control or eastern mosquitofish treatments.

Significant differences were observed in the level of fin damage among adults of both species (platyfish $\chi^2 = 17.64$, $P = 0.001$; swordtails $\chi^2 = 8.51$, $P = 0.014$). Of the platyfish in the high density eastern mosquitofish treatment, 91.4% had a score of 0, 4.2% had a score of 1, and the remaining 4.4% were found with severe damage (score of 2). In contrast, all surviving platyfish from the control and low eastern mosquitofish density treatments had a score of 0. Swordtails from the high density treatment sustained

Fig. 2 Results from Experiment 2. Average percent survival (\pm SE) of adult and juvenile variable platyfish and swordtails when sympatric with three different densities of eastern mosquitofish. Means with different letters are significantly differed based on Tukey's post hoc test. Trials lasted 4 days for each species



significantly more damage than those in the control treatment. All fish recovered from control were scored 0, whereas in the high density 64.3% were scored 0, 21.2% were scored 1, and 14.5% were scored 2. Swordtails from the medium density treatment were not significantly different from either the control or high density with 90.6% given a score of 0 and the remaining 9.4% had a score of 1.

Experiment 3: Effects of structural complexity

Mortalities of both study species were observed across all stem density treatments. Stem density had no significant effect on platyfish survival ($F_{2, 12} = 1.79$; $P = 0.208$) (Fig. 3). Conversely, swordtail survival in the low and medium stem density treatments was about 2 times higher than in the high stem density treatment ($F_{2, 12} = 5.86$; $P = 0.0168$) (Fig. 3).

None of the surviving platyfish exhibited fin damage in this experiment, but some swordtails had caudal fin damage. However, there was no significant difference among the different stem densities in level of fin damage for the adult swordtails in this experiment ($\chi^2 = 0.00$, $P = 1.00$).

Experiment 4a: Analysis of differing stem densities

Day 1

As observed qualitatively in Experiments 1–3, eastern mosquitofish commonly attacked individuals of both introduced species. For platyfish, there were no statistical differences in number of attacks among stem densities on the day of introduction ($\chi^2 = 0.2667$, $P = 0.8725$). Mean numbers of attacks were between 12 and 14 for the 30 min of observation across treatments, but the data were highly variable (ranging from 2 to 35). Similarly, total attack results were not significantly different among stem densities for swordtails ($\chi^2 = 1.681$, $P = 0.4316$). Mean number of attacks were 12 (SE = 9) for the high stem density and 25 for the low (SE = 18) and medium (SE = 20). Number of attacks across the treatments ranged from 2 to 45, again showing high variability.

Although differences were not detected in the mean number of attacks, the proportion of nonindigenous fish in artificial vegetation in the presence of a high density of eastern mosquitofish differed significantly by stem density for both platyfish and swordtails on

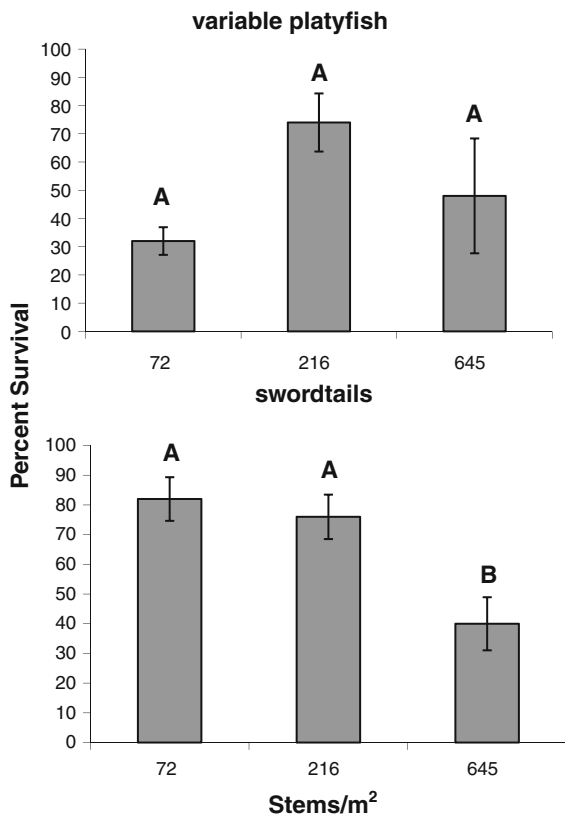


Fig. 3 Results from Experiment 3. Average percent survival (\pm SE) of poeciliids introduced into a previously-established population of mosquitofish across a range of artificial vegetation stem density with constant mosquitofish density. Means with different letters are significantly differed based on Tukey's post hoc test. Trials lasted 11 days for platyfish and 5 days for swordtails

the day of introduction (platyfish $F_{2,6} = 5.74$; $P = 0.0404$; swordtail $F_{2,6} = 5.84$; $P = 0.0391$) (Fig. 4). In general, the highest proportion of fish (about 0.5) in vegetated areas was found at the highest stem density. The numbers of individuals observed in the vegetation was 1.7 and 4.5 times greater than that observed in the low density treatment for platyfish and swordtails respectively (Fig. 4).

Day 3

After 2 days of exposure to eastern mosquitofish, attacks on the study species continued. However, the only mortality was a single dead platyfish found in the

medium stem density treatment. Similar to Day 1, there were no significant differences in attack number across stem densities for either species (platyfish $\chi^2 = 2.529$, $P = 0.2823$; swordtail $\chi^2 = 2.056$, $P = 0.3576$). Mean attack numbers were 17 ± 2 in the low, 11 ± 5 in the medium and 15 ± 4 in the high stem density for platyfish (the range of attacks was 8–19 across all replicates). Mean attacks were 10 ± 5 in the low, 11 ± 3 in the medium and 6 ± 5 in the high treatment for swordtails (across all replicates the range was from 1 to 13 attacks).

Unlike Day 1 results, there was no significant difference in use of vegetation by platyfish across stem densities ($F_{2,6} = 1.04$; $P = 0.4080$) (Fig. 4). In contrast, swordtail results were similar to Day 1, with the proportion of individuals in the vegetation at the high stem density significantly greater than in the low and medium stem densities ($F_{2,6} = 6.75$; $P = 0.0292$) (Fig. 4). There was no significant pattern in fin damage by stem density for either species (platyfish $\chi^2 = 0.640$, $P = 0.726$; swordtails $\chi^2 = 0.48$, $P = 0.783$).

Experiment 4b: Analysis of eastern mosquitofish presence

Day 1

Eastern mosquitofish were again observed attacking both introduced species. However, there were no conspecific attacks observed in either the Danios or NoMF treatments or any attacks observed by the zebra danios on either study species. The only attacks noted were in the MF treatment and the analysis showed that the number of attacks was significantly different among treatments for both species (platyfish $\chi^2 = 7.623$, $P = 0.0221$; swordtail $\chi^2 = 7.624$, $P = 0.0220$). Mean total attacks on platyfish observed in the MF treatment was 11 ± 18 , with 25 ± 20 in the swordtail trials.

The scan sampling data showed that habitat use patterns differed by treatment with platyfish, with more individuals using vegetation in the presence of eastern mosquitofish ($F_{2,6} = 6.98$; $P = 0.0271$). The mean proportion of platyfish in the vegetation in the NoMF and Danios treatments were 2–5 times less than in the MF treatment (Fig. 5). Swordtails showed no significant difference in habitat use among treatments ($F_{2,6} = 1.78$; $p = 0.2474$) (Fig. 5).

Fig. 4 Habitat use results from Experiment 4a. Mean proportion of adult introduced poeciliids observed in the vegetated area of the experimental mesocosm (\pm SE) when introduced into a previously-established population of mosquitofish across a range of artificial vegetation stem density. Means with different letters are significantly differed based on Tukey's post hoc test

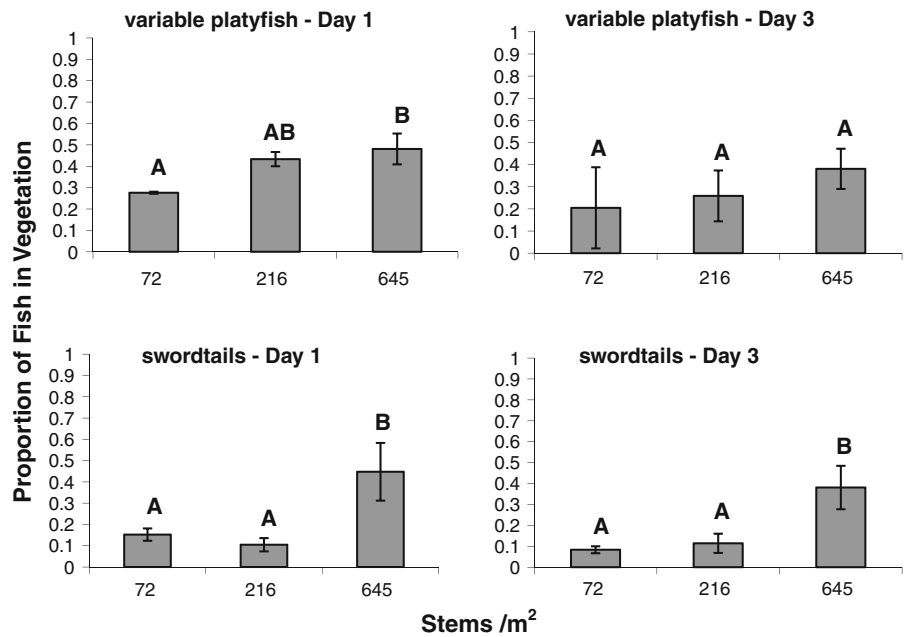
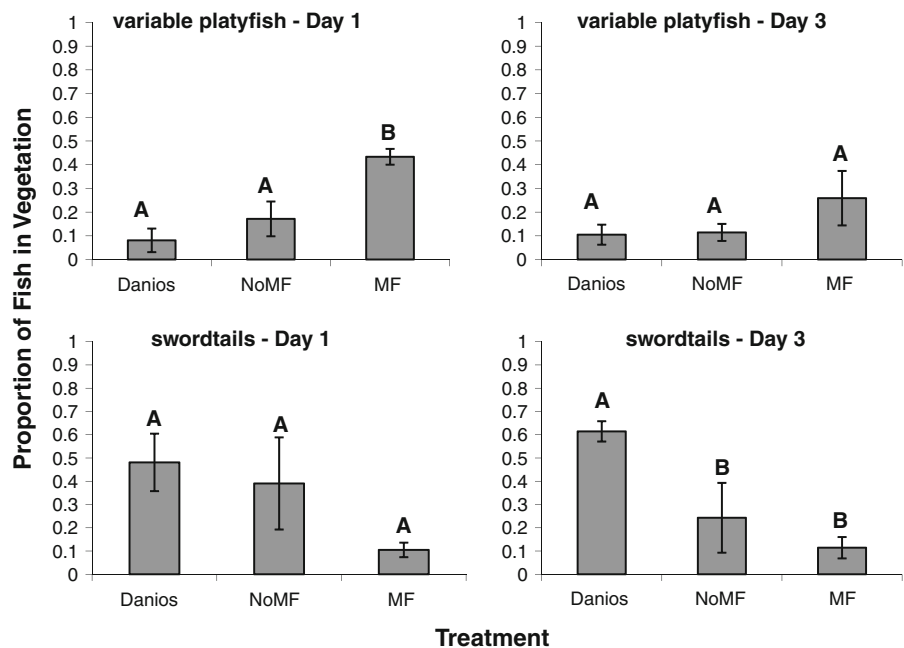


Fig. 5 Habitat use results from Experiment 4b. Mean proportion of adult introduced poeciliids observed in the vegetated area of the experimental mesocosm (\pm SE) when introduced into a previously-established population of mosquitofish across a range of artificial vegetation stem density. Means with different letters are significantly differed based on Tukey's post hoc test



Day 3

Attacks were again noted on Day 3 of observation among these treatments for both species. The single platyfish mortality in the medium density treatment was reported in the results for Experiment 4a (this

treatment was shared by 4a and 4b). There were no other mortalities in the other two treatments for either species. Pattern of attacks by treatment was the same as in Day 1, with all observed attacks occurring in the MF treatment (platyfish; $\chi^2 = 7.714, P = 0.0211$; swordtail $\chi^2 = 7.624, P = 0.0220$). The mean number

of attacks on both platyfish and swordtails was 11 (platyfish SE = 5; swordtail SE = 2) in the MF treatment.

On this day of observation, platyfish did not show a significant difference in habitat use ($F_{2,6} = 1.04$; $P = 0.4080$) whereas the proportion of swordtails in the vegetation was significantly different by treatment ($F_{2,6} = 6.99$; $P = 0.0271$) (Fig. 5). The proportion of swordtails using the vegetated area in the tanks was 3–5 times higher in the Danios treatment compared to the NoMF and MF treatments (Fig. 5).

Both species showed similar patterns of fin damage in this experiment with significantly greater amounts of fin damage in the MF treatment than either the NoMF or Danios treatments (platyfish $\chi^2 = 15.52$, $P = 0.004$; swordtails $\chi^2 = 5.82$, $P = 0.054$). All platyfish recovered from the Danios and NoMF had no observable damage to the caudal fin, but of those from the MF treatment, 76.3% had no damage, 17.0% had moderate damage, and the remaining 6.7% had severe damage. At least 90% of the swordtails recovered showed no damage to the caudal fin and none were given a score of 2 from both the Danios and NoMF treatment. In contrast, 16.7% percent of swordtails recovered from the MF treatment tanks had moderate damage (score of 1). In this treatment no fish were scored a 2.

Discussion

Our laboratory experiments demonstrated that eastern mosquitofish have strong negative effects through aggression and predation on survival of platyfish and swordtails. Eastern mosquitofish killed both adults and juveniles, indicating an influence on various stages of the invasion process (Maron and Vila 2001). Experimental results support the hypothesis that eastern mosquitofish may be limiting the invasions of nonindigenous poeciliids and other small-bodied fishes in Florida's freshwater systems.

Previous authors have noted that mosquitofish prey on the young or larvae of a variety of native and introduced fish (Schoenherr 1981, Meffe 1985). Meffe (1985) and Hill et al. (2011) also noted that mosquitofish were capable of harassing and killing fish of similar size. However, the present study is the first experimental demonstration that mosquitofish are capable of killing fish substantially larger than

themselves. In this experiment, eastern mosquitofish killed fish 1.5–2 times longer and 4–6 times heavier than themselves. The substantial differences in size and weight of eastern mosquitofish and the nonindigenous poeciliids used in this experiment is evidence that mosquitofish may impact a larger size range of fish species than previously shown (cf. Meffe 1985; Taylor et al. 2001; Laha and Mattingly 2007).

Similar to observations by others (Meffe 1985; Baber and Babbitt 2004; Laha and Mattingly 2007; Hill et al. 2011), eastern mosquitofish in this study initiated attacks by first biting the caudal fin of prey fish prior to killing them. All mortalities recovered from experimental units showed damage to the caudal region, often with large portions of the caudal fin missing. Even in instances in which the attacked fish survived, the damage sustained would cause the fish to be more susceptible to further attacks or later death from wound infection (Noga 1996; Wildgoose 2001). Although *Xiphophorus* species are known to exhibit intraspecific aggression (Earley 2006), no intraspecific attacks were observed in treatments lacking eastern mosquitofish and few attacks (≤ 2 in three observation periods) were observed in treatments where eastern mosquitofish were present. Platyfish and swordtails are commonly held at high densities in aquaculture tanks without losses due to intraspecific aggression (Hill personal observations). These results indicate that all or most mortality among swordtails and platyfish was the result of eastern mosquitofish attacks and aggression.

Increasing eastern mosquitofish density was shown to have a negative effect on survival of adults of the two nonindigenous study species. This was expected because predator density increases can increase predation effects overall (Relyea 2003) and mosquitofish predation effects have been demonstrated as density dependent in mesocosm experiments (Belk and Lydeard 1994; Taylor et al. 2001; Mills et al. 2004). There was some variation in the effect eastern mosquitofish density had on the survival of adult platyfish and swordtails between experiments 1 and 2. These experimental findings may be explained by differences in trial duration (see Table 1), with higher survival in shorter experiments. It is also possible that eastern mosquitofish are slower to exhibit aggressive behavior when concurrently introduced into tanks with other fish, as opposed to treatments in which the eastern mosquitofish were placed into tanks a few days

before nonindigenous fish and subsequently became residents as part of an acclimation period (Chellappa et al. 1999; Earley 2006).

Strong effects on platyfish and swordtail juveniles are similar to previous published information describing predation by mosquitofish on juvenile and larval fishes (Meffe 1985; Belk and Lydeard 1994; Taylor et al. 2001; Laha and Mattingly 2007). In the current study, predation on juvenile fish was pronounced even in treatments where eastern mosquitofish density was low. The strong effect of eastern mosquitofish on population size structure and recruitment of prey was further demonstrated by the control treatments where platyfish increased their numbers by producing additional young. It is presumed that eastern mosquitofish in other treatments did not reduce fecundity and reproduction of platyfish, but no juveniles were observed because eastern mosquitofish consumed the neonates (Meffe 1985). Swordtails did not produce additional recruits in these experiments, likely due to high temperatures during the summer in west-central Florida when the experiments were conducted (C. A. Watson, University of Florida, personal communication).

Much of the mortality among the nonindigenous poeciliids in our experiments resulted from eastern mosquitofish harassment, not directed predation (encounter competition, *sensu* Schoener 1983). There were few significant differences among treatments in sublethal damage to nonindigenous species with surviving fish usually having little damage whereas dead fish had substantial damage to the caudal region. Our data on mortalities and fin damage as well as qualitative observations suggest that eastern mosquitofish initially focus aggression on a few fish, attacking already injured fish until killed, and only later do the eastern mosquitofish attack new individuals. Across all experiments and replicates only one adult platyfish and three swordtails showed signs of significant consumption with substantial amounts of tissue missing outside of the caudal region. However, the near absence of consumption by eastern mosquitofish on dead adults may be an experimental artifact from the frequent removal of dead individuals. Attacks by eastern mosquitofish on juveniles were likely direct predation rather than aggression. Although exceeding gape size, juveniles in all cases were completely consumed.

Our results show the potential for native eastern mosquitofish to resist multiple stages of invasion of

introduced platyfish and swordtails. By reducing adult survival, eastern mosquitofish may reduce the likelihood of establishment, or limit their range and expansion (Maron and Vila 2001; Kolar and Lodge 2002). Given the results of this study and previous experiments (Belk and Lydeard 1994; Taylor et al. 2001; Mills et al. 2004), the strength of biotic resistance is positively correlated with eastern mosquitofish density. Although within the range of observed natural densities (Trexler et al. 2005), the high densities of eastern mosquitofish used in this study are more typical of seasonally-contacted pools and other dry-season refugia where fishes are concentrated during low water levels. These medium and high densities also may be reached in permanent marshes or littoral zones of lakes and canals of eutrophic status (Turner et al. 1999; Chimney and Jordan 2008). Therefore the strongest effects of eastern mosquitofish on introduced fishes may be limited to specific times of the year or types of habitats. Furthermore, native predators can be overwhelmed by propagule pressure, a factor not evaluated in our experiments (Von Holle and Simberloff 2005; Hollebone and Hay 2007).

Data from our stage-structured population experiments suggest that biotic resistance by eastern mosquitofish through predation on juveniles is probably of greater importance in natural systems compared to predation on adults. Mosquitofish have reduced or extirpated endemic populations of small-bodied fishes through recruitment failure where introduced (Meffe 1985; Belk and Lydeard 1994) and platyfish and swordtails are species that would be highly vulnerable to extirpation through brief periods of recruitment failure because they are short-lived species with low population storage capacity (Secor 2007). While there have been no studies conducted to monitor wild populations of introduced poeciliids in Florida, available evidence indicates that introduced populations of platyfish in the state are localized (Fuller et al. 1999) and found in small streams where eastern mosquitofish are usually uncommon (Nico unpublished data; Hill unpublished data).

Abiotic factors can also be an important contributing factor in determining success of freshwater fish invasions (Kolar and Lodge 2002). Our experiments revealed that habitat complexity at an introduction site might influence invasion success of platyfish and swordtails in the presence of eastern mosquitofish. The

declining survival of swordtails at high stem density in Experiment 3 was an unexpected result. Increasing stem densities have been shown to reduce predation effects of large, predatory fish such as largemouth bass and northern pike *Exox lucius* (Savino and Stein 1982; Anderson 1984; Eklov and VanKooten 2001). In our study it was noted that eastern mosquitofish closely associated with the simulated vegetation and as habitat complexity increased, the swordtails were found more in the vegetated areas. Although it is possible that eastern mosquitofish predation efficiency may decrease with increasing habitat complexity (Baber and Babbitt 2004), encounters with prey may increase even at high stem densities due to greater use of vegetated habitat by prey fish. Encounter rate is a fundamental component in determining predation rates (Osenberg and Mittlebach 1989) and thus may be the cause of the lower swordtail survival at high stem density in this experiment.

Prey species may vary in ability to alter behavior and habitat use patterns as an anti-predator response (Savino and Stein 1989; Bean and Winfield 1995). Baber and Babbitt (2004) demonstrated the inability of a species of tadpole to use habitat as a predation refuge as effectively as a morphologically similar species when preyed upon by eastern mosquitofish. Differing anti-predatory responses may explain the seemingly differential predation rates between platyfish and swordtails by eastern mosquitofish. The increased use of the vegetated area by the swordtails at high stem density despite potentially increasing encounter rates and subsequent higher mortality rates suggests an inability for swordtails to appropriately respond to predation threats. Although platyfish were found more commonly in the vegetation at high stem density during Day 1, fish moved out of the vegetation by Day 3 and mortality was not significantly different among stem densities for this species. Swordtails had higher mortality rates, over shorter time, compared to the platyfish in Experiments 1 and 3 even though they are the larger of the two introduced species. Qualitative observations suggest that swordtails moved more than platyfish, potentially increasing encounters with eastern mosquitofish or eliciting more aggression. Interestingly, platyfish are locally established in Florida and swordtail is not (USGS 2007; Shafland et al. 2008).

The behavior experiments indicate the potential for eastern mosquitofish to have negative, indirect effects

on swordtails. Biotic resistance may be increased if eastern mosquitofish aggression and predation threat excludes swordtails from structurally complex habitat. In these experimental systems with one predator, the swordtails could separate themselves and decrease interaction with the eastern mosquitofish by avoiding the complex habitat. In natural systems, larger piscivorous species readily feed on small-bodied fish if they cannot refuge in shallow or vegetated areas. Harvey et al. (2004) experimentally demonstrated that two native fish species excluded a nonindigenous fish from refuge habitat and exposed it to an open water predator. In addition to direct predation and harassment of small nonindigenous fish, our observations imply that native eastern mosquitofish may drive platyfish and swordtails out of cover thereby exposing them to other predators.

Although the present study focused on impacts of eastern mosquitofish on platyfish and swordtail, there are many other small-bodied fish species in ornamental aquaculture and the aquarium trade in Florida (e.g., Hill and Yanong 2002), but, with one exception, none are widely established (Fuller et al. 1999; Nico and Fuller 1999). Mosquitofish, based on the results of this study and previous research, may harass and prey upon these species and augment the ability of aquatic communities to resist their establishment. Investigating resident predator and introduced prey interactions is necessary to increase prediction and assess risks of introduced species. While there is a growing body of literature describing characteristics of successful fish invasions based on the introduced species biology and native range and the abiotic characteristics of the introduction site (Kolar and Lodge 2002; Marchetti et al. 2004; Ruesink 2005), few include measures of biotic resistance. More studies of biotic effects are needed to understand the factors that contribute to the failure or success of introductions.

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