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When the Invader Becomes the Invaded: Temporal Variation of *Gambusia affinis* and Centrarchid Sunfish in Two Small Ponds

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Abstract - Piscivory, intraguild predation, and competition by native fish can limit the ability of non-native fish to invade or persist in invaded aquatic ecosystems. *Gambusia affinis* (Western Mosquitofish) is one of the most invasive species of freshwater fishes; however, the presence of native piscivorous or facultatively piscivorous fish might negatively affect their populations. We examined the effect of an “invasion” of native *Lepomis megalotis* (Longear Sunfish) on a non-native population of Western Mosquitofish in a small pond in central Ohio (Olde Minnow Pond). We also monitored fish populations in a nearby pond (Wood Duck Pond) that had small populations of non-native Western Mosquitofish and native *Lepomis macrochirus* (Bluegill Sunfish) throughout the study period. The abundance of Western Mosquitofish in minnow traps in Olde Minnow Pond was greatly reduced after the “invasion” of Longear Sunfish. In contrast, Wood Duck Pond showed no decline in Western Mosquitofish numbers in traps over the same period. In addition, the proportion of male Western Mosquitofish in Olde Minnow Pond was greater after the “invasion” of Longear Sunfish, but no such change was observed in Wood Duck Pond. In general, male and female body size did not differ between the pre- and post-invasion periods. Our results suggest that the invasion, abundance, and persistence of populations of non-native Western Mosquitofish can be limited by the presence of native *Lepomis*.

Introduction

Biotic resistance, either through native competitors or predators, can explain some failures of invasive species to establish (Hill 2016, Tuckett et al. 2021, Zenni and Nuñez 2013). In addition, populations of non-native species that have established themselves in an ecosystem can sometimes dramatically decline, with these declines likely the result of the introduction of competitors (Simberloff and Gibbons 2004) or the reintroduction or recovery of native predators (Flaherty and Lawton 2019, Sheehy and Lawton 2014). It is also possible for declines in non-native species to be associated with abiotic factors operating on their own or interacting with other factors (e.g., Glowacki et al. 2021, Pearson et al. 2022, Rose and Todd 2017). In aquatic ecosystems, facultative piscivory, intraguild predation, and competition by native fish can limit the ability of non-native fish to invade (Britton 2012, Tuckett et al. 2021). Indeed, intraguild predators can present particularly strong biotic resistance to invasion because they are acting as both predators and competitors (e.g.,

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Deacon et al. 2023), especially if the effects are asymmetrical (Tuckett et al. 2021). However, even in simple aquatic habitats, native predator fish may not be able to eliminate non-native invasive fish (e.g., Beaune et al. 2019). Ultimately, more study of the cause of population declines in non-native species is needed (Hill 2016).

Gambusia affinis (Baird and Girard) (Western Mosquitofish) is one of the most invasive species of freshwater fish (Copp et al. 2009, Fryxell et al. 2022, Pyke 2008) and has been shown to have numerous negative effects on native fishes, and other aquatic species in ecosystems where they have been introduced or have invaded (Pyke 2008). For non-native Western Mosquitofish, the presence of a larger native piscivorous or facultatively piscivorous fish could negatively affect their abundance and their ability to persist in an invaded aquatic ecosystem. For example, populations of Western Mosquitofish in ponds without fish predators typically had higher abundances than in ponds with fish predators (Fryxell et al. 2019). In addition, native intraguild predators that compete with Western Mosquitofish but are also large enough to consume mosquitofish may limit the successful invasion of Western Mosquitofish and allow coexistence with native species (e.g., Henkanaththegedara and Stockwell 2013, 2014). For example, the presence of native *Glossogobius callidus* (Smith) (River Goby), an opportunistic predator, reduced the abundance of Western Mosquitofish (Howell et al. 2013). In addition, if native fish or other taxa acting as predators or intraguild predators preferentially prey upon male vs. female or small vs. large Western Mosquitofish, there could be consequences for their population dynamics. For example, *Ardea cinerea* L. (Grey Heron) prey preferentially on larger (female) Western Mosquitofish, resulting in altered sex ratios that are much more male biased than usual (Britton and Moser 1982). The presence of fish predators can also affect the sex ratio of *Gambusia* spp. (mosquitofish) populations (e.g., *G. manni* Hubbs [Bahamas Mosquitofish]; Krumholz 1963). Such alterations of the sex ratio of mosquitofish populations can influence their community- or ecosystem-level effects (e.g., Fryxell et al. 2015) and their population dynamics (Thresher et al. 2013). Removal of Western Mosquitofish individuals from populations by predation can also alter the size of the individuals in the population (e.g., removal of male Western Mosquitofish affected male size; Hughes 1985).

Fishes in the genus *Lepomis* (sunfish) may be able to influence the abundance or presence of Western Mosquitofish. In some areas, *Lepomis* spp. and Western Mosquitofish coexist in the same body of water (e.g., Landress 2016, Lynch 1988, Moyle and Nichols 1973). However, sunfish can consume other fish, particularly small fish, and fish eggs (Feiner et al. 2013, Marsh-Matthews et al. 2013, Miller et al. 2015), including Western Mosquitofish (Fisher et al. 2012, Simkins and Belk 2017). They may also be competitors with Western Mosquitofish (Blaustein 1991) since their diets can be similar (Fisher et al. 2012). Thus, it is possible for native sunfish to act as predators or competitors for invasive Western Mosquitofish, with potential consequences for the abundance of Western Mosquitofish populations.

We examined the effect of an “invasion” of native *Lepomis megalotis* (Rafinesque) (Longear Sunfish) on a non-native population of Western Mosquitofish in a small pond in central Ohio. to the best of our knowledge, Olde Minnow Pond had

only non-native Western Mosquitofish in its fish community since they were introduced in the late 1960s or early 1970s. We initially monitored the Western Mosquitofish population in Olde Minnow Pond during the summers of 2009 and 2010. In the fall of 2014, we discovered that Olde Minnow Pond had been colonized by Longear Sunfish, probably due to flooding and transport of fish from an upstream pond. While we did not conduct systematic surveys of Olde Minnow Pond between the summer of 2010 and fall 2014, we did do ad hoc collecting of Western Mosquitofish from Olde Minnow Pond using dipnets and minnow traps in the summers of 2011, 2012, and 2013 to provide individuals for other experiments. During these ad hoc sampling events, we never captured or observed any sunfish in Olde Minnow Pond. We subsequently monitored Western Mosquitofish and Longear Sunfish populations in Olde Minnow Pond in the summers of 2015 and 2016. In addition to monitoring fish populations in Olde Minnow Pond, we monitored fish populations in nearby (~200 m) Wood Duck Pond in the summers of 2010, 2015, and 2016. As far as we know, Wood Duck Pond has likely had populations of non-native Western Mosquitofish and native *Lepomis macrochirus* Rafinesque (Bluegill Sunfish) since the late 1960s or early 1970s. Wood Duck Pond suffered a winterkill in the winter of 2000 that reduced populations of both species, which continue to be low (see Smith et al. 2005). By monitoring the fish populations in these 2 ponds before and after the “invasion” of Olde Minnow Pond by Longear Sunfish in 2014, we can examine the potential effects of an “invasion” of a pond by native sunfish on Western Mosquitofish abundance, sex ratio, and body size, while using Wood Duck Pond to evaluate whether any observed changes in Olde Minnow Pond might be explained by broader climatic or environmental conditions rather than by the arrival of the sunfish.

Field-site Description

We studied populations of Western Mosquitofish in Olde Minnow Pond and Wood Duck Pond on the Denison University Biological Reserve, Licking County, OH (40.0833°N, 82.5180°W). Olde Minnow Pond is a 0.60-ha pond with vegetation primarily composed of *Chara* sp. (muskgrass) and *Elodea* sp. (waterweed) and with no other fish species other than Western Mosquitofish present prior to 2014 and both Western Mosquitofish and Longear Sunfish occurring together after 2014 (J.J. Arrington and J.E. Rettig unpubl. data; M. Johnston and J.E. Rettig, unpubl. data; Schultz and Mick 1998; Surace and Smith 2016). Wood Duck Pond is a 0.86-ha pond with well-established beds of *Ceratophyllum* sp. (coontail), waterweed, and *Myriophyllum* sp. (water milfoil), and both Western Mosquitofish and Bluegill Sunfish present throughout the study period (J.J. Arrington and J.E. Rettig unpubl. data; Schultz and Mick 1998; Smith et al. 2005; Surace and Smith 2016).

Methods

We used unbaited minnow traps (41.5 cm long, 19 cm diameter at ends, 22 cm diameter in middle, 2 cm diameter at opening, 4-mm mesh; Gee Exotic Minnow Trap, Tackle Factory, Filmore, NY) to monitor fish abundances in each pond.

Minnow traps, such as those used in our study, capture juvenile sunfish (Blaustein 1989), and we found juvenile sunfish readily entered the minnow traps. We trapped ponds either weekly (2009, 2015, 2016) or every other week (2010) from mid-June to late July (2009), mid-May to mid-July (2010), and late May to mid-July (2015, 2016). In the summer of 2023, we trapped for fish in both ponds on a limited basis (3 times between late-May and late-June). We deployed traps in the morning and checked them after 24 hours. We placed traps throughout the ponds, including both open water in the center of the ponds and vegetated habitats along the shallower edges of the ponds. In Olde Minnow Pond, we placed 18 traps per sampling day in 2009 and 2010 (except 16 June 2009, $n = 16$) and 10 traps in 2015 and 2016. In Wood Duck Pond, we placed 18 traps per sampling day in 2010 and 10 traps in 2015 and 2016. We identified, sexed, and counted all fish captured in a trap. In addition, we measured the total length of 10–15 randomly selected male and female Western Mosquitofish from each trap.

We initially visualized the abundance of Western Mosquitofish, Longear Sunfish, and Bluegill Sunfish captured in traps on each capture date by pond and created smoothed lines for each year to facilitate visualizing the trends in fish abundances (see Fig. 1). Because there was some variability in the frequency and timing of trapping sessions among years, we further analyzed abundances in traps by limiting our analyses to trapping sessions from similar dates (i.e., ± 2 –3 d) across all years. We analyzed ponds separately given the differences in the number of years sampled pre-invasion (Olde Minnow Pond: 2009 and 2010; Wood Duck Pond: 2010). In addition, our intent with including the Wood Duck Pond analyses is to determine if the patterns of fish abundance were qualitatively (rather than quantitatively) similar or different and not to compare abundances directly between ponds. We used generalized linear models (GLMs) to examine the effect of invasion period (pre and post), year (nested in invasion period), sampling date (mid-June, late June, early July), and the interaction between invasion period and sampling date on the abundance of Western Mosquitofish and sunfish, the proportion of male Western Mosquitofish (arcsine-square root transformed), and the mean total length of male and female Western Mosquitofish. For the analyses of fish abundance, we used a Poisson distribution with log link for the GLM. We used either pairwise Wilcoxon post-hoc tests (for abundance analyses) or Tukey–Kramer HSD post hoc tests (for proportion of male and size analyses) to further examine significant effects. We did not include the 2023 data in these analyses given the limited nature of the sampling but report the mean number of fish caught per trap.

Results

Olde Minnow Pond

Figure 1 provides the trapping data for all dates and traps for both Western Mosquitofish and Longear Sunfish for all 4 years. These data indicate that Western Mosquitofish abundance in traps in Olde Minnow Pond was higher in 2009 and 2010 and much lower in 2015 and 2016 (Fig. 1A). Longear Sunfish were absent from traps in 2009 and 2010, while the highest number per trap was found in 2016 (Fig. 1B).

In the more limited sampling in 2023, Western Mosquitofish were only captured in Olde Minnow Pond in 1 trap in late June, with Longear Sunfish captured at higher levels (Table 1). The following analyses only consider the pooled dates for 2009, 2010, 2015, and 2016 (i.e., similar dates across all 4 years).

The number of Western Mosquitofish caught per trap in Olde Minnow Pond was greater pre-invasion than post-invasion (invasion period: $\chi^2_1 = 6185.9$, $P < 0.0001$; Fig. 2A). Number of Western Mosquitofish per trap was highest in 2010 followed by 2009 and was drastically lower in 2015 and 2016 (year[invasion]: $\chi^2_2 = 320.8$, $P < 0.0001$; see Fig. 1A, Table 2). The number of trapped Western Mosquitofish was greatest in mid-June compared to late June and early July (sampling date: $\chi^2_2 = 99.6$, $P < 0.0001$; Fig. 2A). The difference in the mean number of Western Mosquitofish caught per trap between pre- and post-invasion increased over the season (invasion x sampling date interaction: $\chi^2_2 = 727.4$, $P < 0.0001$; Fig. 2A).

The proportion of male Western Mosquitofish in Olde Minnow Pond was greater post-invasion compared to pre-invasion (invasion period: $\chi^2_1 = 32.2$, $P < 0.0001$; Fig. 3A). The difference in the proportion of male Western Mosquitofish in the

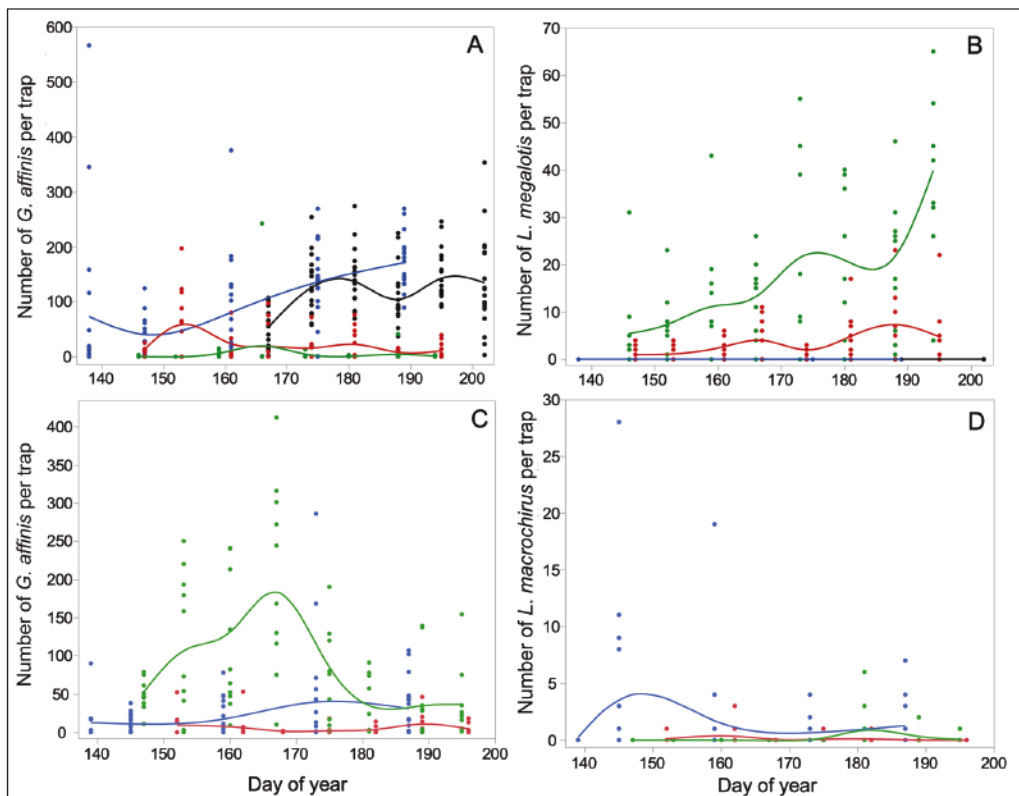


Figure 1. Trapping data showing the number of fish captured per trap for (A) non-native *Gambusia affinis* (Western Mosquitofish) and (B) native *Lepomis megalotis* (Longear Sunfish) in Olde Minnow Pond, and (C) Western Mosquitofish and (D) native *L. macrochirus* (Bluegill Sunfish) in Wood Duck Pond. 2009 (black), 2010 (blue), 2015 (red), and 2016 (green).

trapped samples among years in Olde Minnow Pond only approached statistical significance, with the ratio of males to females tending to increase from 2009 to 2016 (year[invasion]: $\chi^2_2 = 5.78$, $P = 0.056$; Table 2). On average, sampling date did not affect the proportion of males (sampling date: $\chi^2_2 = 1.55$, $P = 0.46$; Fig. 3A). The difference in the mean proportion of male Western Mosquitofish between the pre-invasion and post-invasion periods increased from mid-June to early July (invasion period x sampling date: $\chi^2_1 = 20.5$, $P < 0.0001$; Fig. 3A).

Table 1. Mean (± 1 SE) number of *Gambusia affinis* (Western Mosquitofish) and sunfish (*Lepomis megalotis* [Longear Sunfish] or *Lepomis macrochirus* [Bluegill Sunfish]) per trap in Olde Minnow Pond and Wood Duck Pond in central Ohio in the early summer of 2023. $n = 10$ traps per pond.

	May 17	June 1	June 27
Olde Minnow Pond			
Longear Sunfish	3.7 $\pm$ 1.2	16.6 $\pm$ 5.2	13.3 $\pm$ 2.7
Western Mosquitofish	0.0	0.0	0.2 $\pm$ 0.2
Wood Duck Pond			
Bluegill Sunfish	0.5 $\pm$ 0.2	0.7 $\pm$ 0.4	0.4 $\pm$ 0.4
Western Mosquitofish	0.0	0.0	0.0

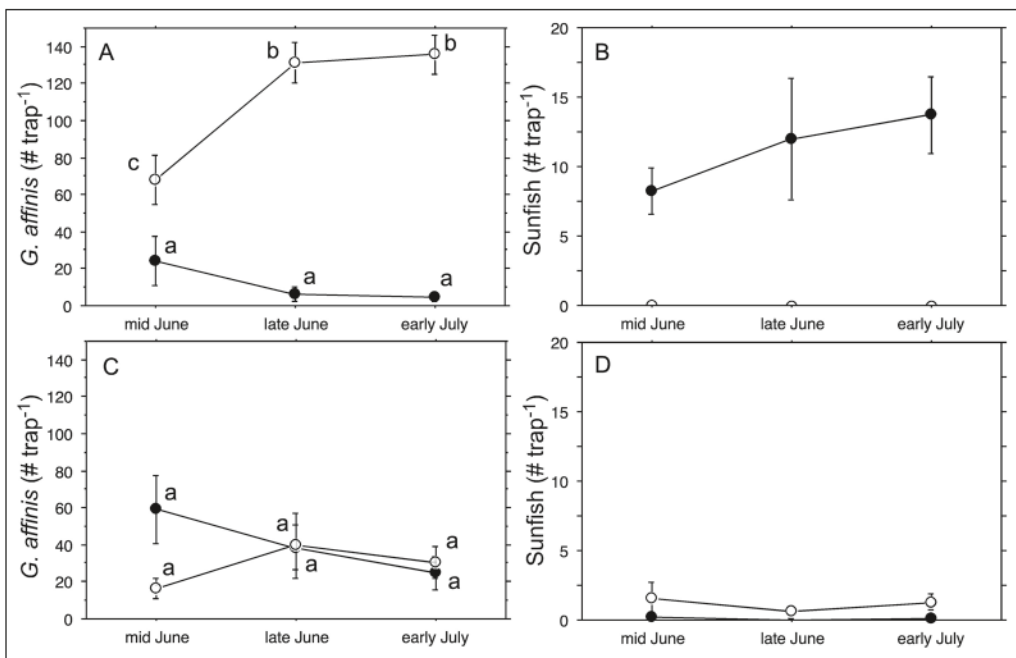
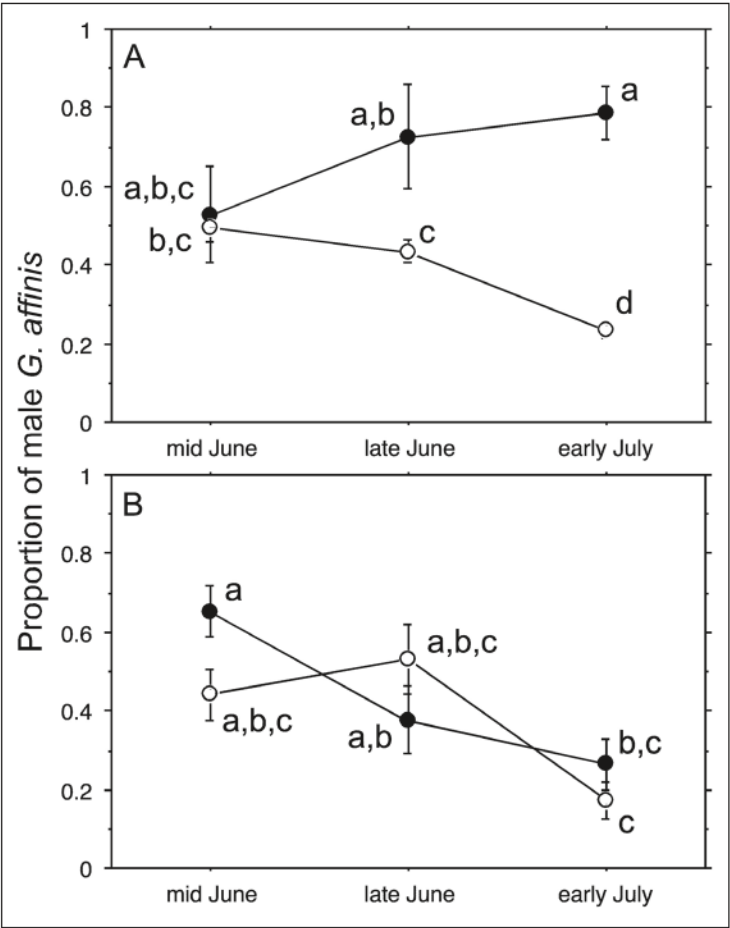


Figure 2. The mean (± 1 S.E.) number of individual fish caught per trap on 3 sampling dates during the pre-invasion (open circles) and post-invasion (closed circles) periods: (A) *Gambusia affinis* (Western Mosquitofish) in Olde Minnow Pond, (B) *Lepomis megalotis* (Longear Sunfish) in Olde Minnow Pond, (C) Western Mosquitofish in Wood Duck Pond, and (D) *L. macrochirus* (Bluegill Sunfish) in Wood Duck Pond. Means sharing letters are not significantly different (pairwise Wilcoxon post-hoc tests on significant invasion period x sampling date interactions; for data in panels B and D, ANOVA showed no significant effects).

Table 2. Mean (± 1 SE) number of *Gambusia affinis* (Western Mosquitofish) per trap, proportion of male Western Mosquitofish, total length (TL; mm) of male and female Western Mosquitofish, and number of sunfish (*Lepomis megalotis* [Longear Sunfish] or *Lepomis macrochirus* [Bluegill Sunfish]) per trap in each year of the study for Olde Minnow Pond and Wood Duck Pond. For variables with a significant overall analysis, means of each variable within a pond that share a letter are not significantly different (pairwise Wilcoxon post hoc: number per trap; Tukey–Kramer HSD: all others). *n* is given in parentheses.

	<i>G. affinis</i> trap ⁻¹	Proportion of male <i>G. affinis</i>	Male TL	Female TL	<i>Lepomis</i> trap ⁻¹
Olde Minnow					
2009 (52)	95.4 $\pm$ 8.5 ^A	0.33 $\pm$ 0.02 ^A	26.5 $\pm$ 0.09 ^A	37.3 $\pm$ 0.08	0.0 ^A
2010 (52)	129.2 $\pm$ 11.1 ^B	0.43 $\pm$ 0.02 ^{a,B}	27.9 $\pm$ 0.09 ^B	38.1 $\pm$ 0.18	0.0 ^A
2015 (30)	13.2 $\pm$ 4.8 ^C	0.72 $\pm$ 0.06 ^C	27.4 $\pm$ 0.36	38.7 $\pm$ 0.89	4.6 $\pm$ 1.0 ^B
2016 (30)	10.3 $\pm$ 8.1 ^C	0.63 $\pm$ 0.15 ^{B,C}	27.8 $\pm$ 0.70 ^B	39.3 $\pm$ 0.60	18.0 $\pm$ 3.0 ^C
Wood Duck					
2010 (54)	29.0 $\pm$ 6.7 ^A	0.38 $\pm$ 0.05	26.3 $\pm$ 0.14 ^A	36.9 $\pm$ 0.41 ^A	1.2 $\pm$ 0.4 ^A
2015 (30)	7.1 $\pm$ 2.5 ^{A,B}	0.44 $\pm$ 0.10	27.0 $\pm$ 0.15 ^B	37.8 $\pm$ 0.23 ^A	0.2 $\pm$ 0.11 ^B
2016 (30)	74.6 $\pm$ 13.5 ^B	0.42 $\pm$ 0.05	25.9 $\pm$ 0.18 ^A	34.2 $\pm$ 0.22 ^B	0.1 $\pm$ 0.07 ^B

Figure 3. The mean (± 1 SE) proportion of male *Gambusia affinis* (Western Mosquitofish) per trap on 3 sampling dates during the pre-invasion (open circles) and post-invasion (closed circles) periods in (A) Olde Minnow Pond and (B) Wood Duck Pond. Means sharing letters are not significantly different (Tukey HSD post-hoc tests on significant invasion period x sampling date interactions).



Mean TL of male Western Mosquitofish was slightly smaller pre-invasion than post-invasion (invasion period: $\chi^2_1 = 4.03$, $P = 0.045$; Fig. 4A). Mean male TL varied among years, with 2009 having the smallest males (year[invasion]: $\chi^2_1 = 58.9$, $P < 0.0001$; Table 2). Male TL did not differ among sampling dates (sampling date: $\chi^2_2 = 0.79$, $P = 0.67$; Fig. 4A). The difference between the pre- and post-invasion periods decreased across sampling dates (invasion period x sampling date: $\chi^2_2 = 7.08$, $P = 0.029$; Fig. 4A).

Mean TL of female Western Mosquitofish in Olde Minnow Pond did not differ between the pre- and post-invasion periods (invasion period: $\chi^2_1 = 1.53$, $P = 0.22$; Fig. 4B). Year also had no effect on mean female TL (year[invasion]: $\chi^2_2 = 1.71$, $P = 0.42$; Table 2). Female TL did not differ among sampling dates (sampling date: $\chi^2_2 = 1.93$, $P = 0.38$; Fig. 4B). The interaction between invasion period and sampling date was not significant (invasion period x sampling date interaction: $\chi^2_2 = 3.76$, $P = 0.15$; Fig. 4B).

The mean number of Longear Sunfish per trap in Olde Minnow Pond was greater in the post-invasion period than in the pre-invasion period (invasion period: $\chi^2_1 = 931.9$, $P < 0.0001$; Fig. 2B). The mean number of Longear Sunfish captured per trap was highest in 2016, with 2015 having some individuals, and 2009 and 2010 having none (year[invasion]: $\chi^2_2 = 257.5$, $P < 0.0001$; see Fig. 1B, Table 2). Sampling date

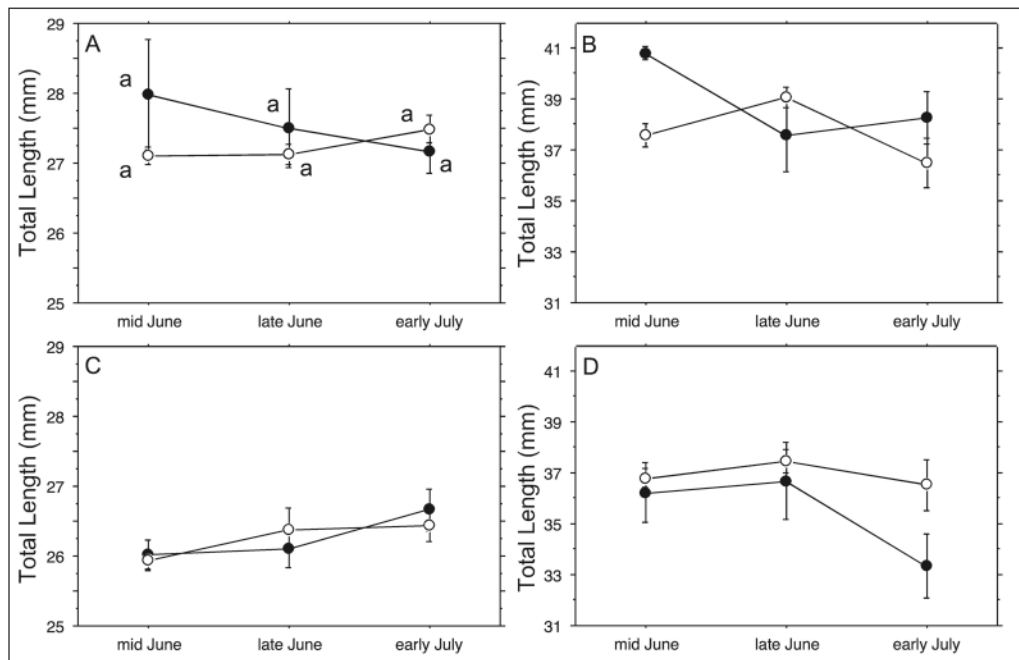


Figure 4. The mean (± 1 SE) total length of *Gambusia affinis* (Western Mosquitofish) in Olde Minnow Pond (A: males, B: females) and Wood Duck Pond (C: males, D: females) at each sampling dates in the pre-invasion (open circles) and post-invasion (closed circles) periods. Means sharing letters are not significantly different (pairwise Wilcoxon post-hoc tests on significant invasion period x sampling date interactions; for data in panels B–D, ANOVA showed no significant effects).

had no effect on number of Longear Sunfish captured (sampling date: $\chi^2_2 < 0.0001$, $P = 1.0$; Fig. 2B). There was no significant interaction between invasion period and sampling date ($\chi^2_2 < 0.0001$, $P = 1.0$; Fig. 2B).

Wood Duck Pond

Figure 1 provides the trapping data for all dates and traps for both Western Mosquitofish and Bluegill Sunfish for all 3 years during which Wood Duck Pond was sampled. The numbers of Western Mosquitofish in traps were low in 2010, even lower in 2015, but highest in 2016 (Fig. 1C). The abundance of Bluegill Sunfish in traps in Wood Duck Pond was generally low but had peaks of abundance in 2010 (Fig. 1D). In 2023, very few fish of either sunfish or Western Mosquitofish were captured (Table 1). The following analyses only consider the pooled dates for 2010, 2015, and 2016 (i.e., similar dates across all 3 years).

The mean number of Western Mosquitofish captured per trap in Wood Duck Pond was higher in the post-invasion period than in the pre-invasion period (invasion: $\chi^2_1 = 26.9$, $P < 0.0001$; Fig. 2C). The number of Western Mosquitofish captured per trap was 3 times higher in 2016 than in 2010 which was 4 times greater than in 2015 (year[invasion]: $\chi^2_1 = 1,947.9$, $P < 0.0001$; see Fig. 1C, Table 2). The number of Western Mosquitofish caught in Wood Duck Pond was higher in mid-June and late June than early July (sampling date: $\chi^2_2 = 80.6$, $P < 0.0001$; Fig. 2C). The number of Western Mosquitofish caught per trap appeared higher in the post-invasion period than the pre-invasion period in mid-June, but appears to not differ during the late June and early July sampling dates (invasion period x sampling date: $\chi^2_2 = 369.2$, $P < 0.0001$; Fig. 2C).

The mean proportion of male Western Mosquitofish in Wood Duck Pond did not differ between the pre- and post-invasion periods (invasion period: $\chi^2_1 = 0.42$, $P = 0.52$; Fig. 3B). Proportion of male Western Mosquitofish in trapped samples did not differ among years (year[invasion]: $\chi^2_1 = 0.33$, $P = 0.57$; Table 2). Proportion of males declined across the sampling dates (sampling date: $\chi^2_2 = 14.3$, $P = 0.0008$; Fig. 3B). There was a significant interaction between invasion period and sampling date; however, there was no discernible pattern to the significant interaction (invasion period x sampling date interaction, $\chi^2_2 = 6.60$, $P = 0.037$; Fig. 3B).

Mean TL of male Western Mosquitofish in Wood Duck Pond did not differ between the pre- and post-invasion periods (invasion period: $\chi^2_1 = 0.88$, $P = 0.35$; Fig. 4C). Mean male TL did vary among years with mean TL being larger in 2015 relative to 2010 and 2016 (year[invasion]: $\chi^2_1 = 13.20$, $P = 0.0003$; Table 2). Male TL did not differ among sampling dates (sampling date: $\chi^2_2 = 4.62$, $P = 0.10$; Fig. 4C). The interaction between invasion period and sampling date was not significant (invasion period x sampling date: $\chi^2_2 = 1.03$, $P = 0.60$; Fig. 4C).

Mean female TL of Western Mosquitofish in Wood Duck Pond did not differ between the pre- and post-invasion periods (invasion period: $\chi^2_1 = 0.63$, $P = 0.43$; Fig. 4D). Female TL did differ among years with 2016 having the smallest mean TL (year[invasion]: $\chi^2_1 = 15.8$, $P < 0.0001$; Table 2). Female TL visually appeared to decrease in early July compared to the June sampling dates (sampling date: $\chi^2_2 = 8.73$, $P = 0.013$; Tukey–Kramer HSD: all $P > 0.05$; Fig. 4D). The interaction

between invasion period and sampling date was not significant (invasion period x sampling date interaction: $\chi^2_2 = 5.26$, $P = 0.07$; Fig. 4D).

In Wood Duck Pond, we caught fewer Bluegill Sunfish during the pre-invasion period than the post-invasion period (invasion period: $\chi^2_1 = 54.0$, $P < 0.0001$; Fig. 2D). Number of Bluegill Sunfish captured did not differ among years (year[invasion]: $\chi^2_1 = 1.33$, $P = 0.25$; see Fig.1D, Table 2). Number of Bluegill Sunfish captured per trap did not change over the course of the trapping season ($\chi^2_2 = 4.86$, $P = 0.088$; Fig. 2D). The interaction between invasion period and sampling date was not significant ($\chi^2_2 = 0.36$, $P = 0.84$; Fig. 2D).

Discussion

Our data show that the abundance of Western Mosquitofish in traps in Olde Minnow Pond was greatly reduced after the “invasion” of Longear Sunfish, with evidence of increasing numbers of Western Mosquitofish during the season in the pre-invasion period and no increase in the post-invasion period. In contrast, Wood Duck Pond showed slightly higher numbers of Western Mosquitofish during the post-invasion period than in the pre-invasion period. In addition, the sex ratio of Western Mosquitofish in Olde Minnow Pond appeared to be affected by the arrival of Longear Sunfish, with the proportion of males in the population being greater post-invasion than pre-invasion. In general, no such shifts in sex ratio were observed in Wood Duck Pond. There were limited differences in male and female size before and after the invasion. Our limited trapping in the summer of 2023 continued to show very low abundances of Western Mosquitofish in Olde Minnow Pond and substantial numbers of Longear Sunfish, whereas in Wood Duck Pond, both Bluegill Sunfish and Western Mosquitofish were very rare. An attempted restoration of Wood Duck Pond was made in the winter of 2018, resulting in the draining and dredging of the pond, likely having a major negative effect on the fish populations in Wood Duck Pond. These results, when taken together, suggest the decrease in the numbers of Western Mosquitofish captured in traps and the shift in the proportion of males in Olde Minnow Pond during the post-invasion period was likely due to the arrival of Longear Sunfish.

While minnow traps appear to provide good estimates of the density of Western Mosquitofish populations (e.g., Blaustein 1989, Schooley and Page 1984, Stewart and Miura 1985), they may capture larger Western Mosquitofish better than small Western Mosquitofish and juvenile *Lepomis cyanellus* Rafinesque (Green Sunfish) better than either size class of Western Mosquitofish (Blaustein 1989). Also, their efficiency can be affected by the relative trapability of a species, as well as potential interactions between species (He and Lodge 1990). However, additional anecdotal observations of Olde Minnow Pond before, during, and after the trapping study indicate that the change in the abundance of Western Mosquitofish in traps in Olde Minnow Pond was a reflection of an overall change in the population of Western Mosquitofish in the pond and not simply avoidance of traps containing Longear Sunfish (e.g., Blake and Gabor 2016). First, prior to 2014, we regularly saw large, very dense schools of Western Mosquitofish throughout Olde Minnow Pond but

have only rarely seen such schools after 2014. Second, when we collected Western Mosquitofish for experiments prior to 2014 we easily and quickly captured >100 fish in less than 10 sweeps, but after 2014, we required many more sweeps to collect similar numbers of Western Mosquitofish. We therefore conclude that the decline in Western Mosquitofish abundance in Olde Minnow Pond is real and not simply an artifact of changes in trapability of Western Mosquitofish with the arrival of Longear Sunfish (although we recognize that changes in trapability may contribute to our observations).

The cause of the decline in Western Mosquitofish abundance in Olde Minnow Pond with the arrival of Longear Sunfish may be predation and/or competition (i.e., Longear Sunfish are likely an intraguild predator). In particular, there appears to be a potential reduction of successful recruitment and survival by Western Mosquitofish in Olde Minnow in the post-invasion period relative to the pre-invasion period, as suggested by the seasonal increase in numbers caught pre-invasion compared to the constant or even decline in numbers across the season post-invasion. The effect on recruitment and survival appears to be stronger on females than males as evidenced by the higher proportion of males post-invasion than pre-invasion, with no such shift in sex ratio evident in Wood Duck Pond. In 2 mesocosm experiments, we found that juvenile Bluegill Sunfish had negative effects on the recruitment of Western Mosquitofish, essentially eliminating the production of offspring, probably due to consumption (J.E. Rettig, E.P. Tristano, A.C. Burger, and G.R. Smith, unpubl. data). Opportunistic angling and seining in Olde Minnow Pond during the summers of 2015 and 2016 found Longear Sunfish with standard lengths up to 182 mm, with several >170 mm (J.E. Rettig, K.D. Rose, A.J. Baird, and Z.D. Baird, unpubl. data) suggesting Longear Sunfish in Olde Minnow Pond are large enough to consume at least juvenile and male Western Mosquitofish. Other studies have also shown that Western Mosquitofish populations can be negatively affected by predatory fishes (e.g., Henkanaththegedara and Stockwell 2013, 2014; Howell et al. 2013; Rehage et al. 2014), including sunfish (Fisher et al. 2012, Simkins and Belk 2017). In addition, the presence of a predator (*Micropterus salmoides* [Lacépède] [Largemouth Bass]) reduced reproduction in the *Gambusia holbrooki* Girard (Eastern Mosquitofish) (Mukherjee et al. 2014). It is also possible that Longear Sunfish reduced the Western Mosquitofish population through competition. We found that Western Mosquitofish grew less in mesocosms with juvenile Bluegill Sunfish than in mesocosms without them (J.E. Rettig, E.P. Tristano, A.C. Burger, and G.R. Smith, unpubl. data), and diets of Western Mosquitofish from Olde Minnow Pond showed shifts between 2010 and 2015/2016, whereas no similar shifts were observed in Wood Duck Pond (Rettig et al. 2023). These observations support a potential role of competition between Western Mosquitofish and Longear Sunfish in Olde Minnow Pond. Other studies also suggest such competition is possible and likely to negatively affect Western Mosquitofish population. For example, Green Sunfish and Western Mosquitofish compete with each other (Blaustein 1991), and Western Mosquitofish can be negatively affected by competition from other species of fish (e.g., *Cyprinella lutrensis* (Baird and Girard) [Red Shiner]; Rehage et

al. 2020). In addition, juvenile Bluegill Sunfish may behaviorally inhibit foraging and resource use by Western Mosquitofish, especially males (Clemmer and Rettig 2019). Thus, the apparent negative effect of Longear Sunfish in Olde Minnow Pond is likely due to the consumption of Western Mosquitofish, especially larvae and juveniles, as well as interspecific competition. Indeed, Tsurui-Sato et al. (2019) found that introduced populations of Western Mosquitofish can be controlled by newly invaded non-native fish that interfere with their successful reproduction.

In conclusion, our observations on the changes of abundance and proportion of males in the Western Mosquitofish population in Olde Minnow Pond after the arrival of Longear Sunfish suggest the invasion and persistence of the non-native Western Mosquitofish may be limited by the presence of native sunfish. Whether the population of Western Mosquitofish will continue to decline to extirpation or will reach an equilibrium (a possibility suggested by the long-term coexistence of Bluegill Sunfish and Western Mosquitofish at very low abundances in Wood Duck Pond) is not clear and will be the subject of future monitoring of these ponds. Indeed, it is possible that there will be shifts in the morphology and ecology of Western Mosquitofish in response to the presence of a centrachid predator that may facilitate coexistence (see Fryxell et al. 2019; Wood et al. 2022a, b), although in the short-term we did not observe any substantial shifts in body size immediately following the invasion.

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