MESOCOSM EVALUATION OF WESTERN MOSQUITOFISH IMPACTS ON NORTHERN STARHEAD TOPMINNOWS

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ABSTRACT.   Western mosquitofish, stocked frequently throughout Indiana, are highly aggressive and often negatively impact native fishes that occupy a similar ecological niche. Because northern starhead topminnows are the most vulnerable of the Indiana topminnows to western mosquitofish aggression, we conducted a 110-day outdoor mesocosm experiment from June through October 2006 to examine changes in abundance, biomass, and wet weight per individual fish at different topminnow and mosquitofish densities. Control mesocosms (three replicates per treatment) contained either 30 northern starhead topminnows or 30 western mosquitofish, while experimental treatments (three replicates per treatment) contained both species at one of the following density levels: (1) 20 topminnows and 10 mosquitofish; (2) 15 individuals of each species; and (3) 10 topminnows and 20 mosquitofish. During the experiment, western mosquitofish exhibited successful reproduction while northern starhead topminnows declined in mean abundance, biomass, and wet weight per individual, indicating that the presence of western mosquitofish had a negative impact on topminnows. However, northern starhead topminnows did not have any negative impact on western mosquitofish. Based on these results, we do not recommend stocking western mosquitofish into Indiana waterways.

Keywords: Western mosquitofish, northern starhead topminnow, abundance, biomass, mesocosm

Non-native species have become established in aquatic systems worldwide (Li & Moyle 1999; McKinney 2002; Casal 2006). Although past attitudes regarding the stocking of non-native fishes have been largely favorable, recent research has demonstrated the potential for negative impacts (Schoenherr 1981; Fausch & White 1986; Moyle & Light 1996; Li & Moyle 1999; Gale et al. 2004; Rieman et al. 2006). As a consequence of these introductions, non-native fishes can lead to large-scale changes in aquatic systems and population declines of native species (Moyle 1986; Everett & Sherfy 2001).

Competition for limited resources and agonistic behaviors or predation can result in negative impacts by introduced fishes on native

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species. In laboratory coexistence studies, Sonoran topminnows (*Poeciliopsis occidentalis*) did not successfully produce offspring and exhibited caudal fin damage in the presence of mosquitofish (Meffe 1985). Early life stages of the Barrens topminnow (*Fundulus julisia*) were preyed upon by western mosquitofish (*Gambusia affinis*) in the laboratory, while adult topminnows suffered physical impairment due to fin nips (Laha & Mattingly 2007). In Indiana waters, western mosquitofish and native topminnows (genus *Fundulus*) have been observed to consume similar prey items, indicating high diet overlap and potential trophic competition (Clem & Whitaker 1995; Zeiber 2007). Western mosquitofish also chased and attacked topminnows in laboratory microcosm studies which, in some cases, resulted in the mortality of individual topminnows (Zeiber 2007).

Behavioral responses of native fishes to introduced species may also lead to a suite of indirect negative effects from a subsequent shift in niche use (Savino & Stein 1989). Smaller fish are often restricted to habitats that offer protection from predators, but those habitats may have fewer resources and could lead to

increased intraspecific competition (Mittelbach 1986). For example, small bluegills (*Lepomis macrochirus*) are often restricted to locations in natural lakes which contain aquatic vegetation due to the risk of open-water predation by largemouth bass (*Micropterus salmoides*) (Werner et al. 1983), and a decrease in foraging rates may occur when fish are restricted to particular habitats. In contrast, bluegills utilized open-water habitats and consumed less-preferred, smaller prey items when a larger, more-aggressive species such as green sunfish (*Lepomis cyanellus*) were present (Werner & Hall 1977). However, the impacts on survivorship and reproduction from a shift in habitat and/or prey resource use for most fishes are often unknown (Marchetti 1999).

In terms of non-native fish introductions, limited coordinated or inconsistent management actions coupled, with a general lack of recognition of the problem, can result in inadequate measures to protect ecosystem balance (Koehn & MacKenzie 2004). For example, the Indiana Department of Natural Resources (IDNR) currently does not allow the stocking of mosquitofish within state public waters. However, mosquitofish can be stocked in private waters and, consequently, have escaped to public aquatic systems. Although all topminnow species in Indiana are potentially vulnerable to western mosquitofish introductions, Zeiber (2007) found that the northern starhead topminnow (*Fundulus dispar*) was the most vulnerable killifish species to western mosquitofish aggression in laboratory microcosm experiments. As a result, we conducted an outdoor mesocosm study to examine changes in abundance, biomass, and wet weight per individual of northern starhead topminnow at different mosquitofish densities. The results of this research will fill a critical need to understand the impacts of western mosquitofish on native topminnows in Indiana waters.

METHODS

Field collections.—Adult western mosquitofish were collected from Martell Forest Pond (Tippecanoe County, Indiana), while northern starhead topminnows were captured from Pine Lake and Upper Fish Lake (Laporte County, Indiana). Both species were collected during May 2006, and sampling was conducted using a 3.18 mm knotless mesh seine (length = 3.05 m; depth = 1.22 m) and 3.18 mm knotless mesh

dip net (diameter = 40 mm; Memphis Net and Twine, Memphis, Tennessee). These nets were effective during collections because most fish were located at water depths less than 15 cm. A range of fish sizes was collected, including males and females of both species; however, pregnant female mosquitofish were not included in the study. Following collections, fish were placed into 8.93 l (~ 8 gallons) buckets of water containing aquatic vegetation and transported to the Purdue University Cunningham Forest complex (Lafayette, Indiana).

Mesocosm experiment.—A 110-day outdoor mesocosm study was used to examine changes in abundance and body size of western mosquitofish and northern starhead topminnows. Fifteen 416 l plastic mesocosms were placed in a 10 × 10 m area in a location that allowed partial shade during the day. Mesocosms were filled with 379 l of groundwater, inoculated with 38 l of pond water containing duckweed (*Lemna* spp., to provide overhead visual cover), phytoplankton, zooplankton, and other aquatic macroinvertebrates to simulate natural pond conditions, and allowed to colonize for four weeks prior to the introduction of the fish. Each mesocosm also contained four clusters of artificial vegetation: two clusters were composed of sixteen 45 cm long sections of green plastic tarp (to simulate submerged vegetation), while the other two clusters were composed of sixteen 90 cm long sections of tarp (to simulate floating vegetation and provide shade at the surface). For each vegetation cluster, plastic strips were attached to a 3.18 cm hex nut which held the cluster secure on the bottom. These vegetation clusters simulated the dense aquatic vegetation that both species prefer as feeding and nursery areas, and also provided a substrate for female northern starhead topminnows to deposit their eggs (Balon 1981; Becker 1983).

Control mesocosms contained either 30 northern starhead topminnows or 30 western mosquitofish, while the mesocosms for the experimental treatments contained both fish species at one of the following three density combinations: (1) 20 northern starhead topminnows and 10 western mosquitofish; (2) 15 individuals of each species; and (3) 10 northern starhead topminnows and 20 western mosquitofish. Thus, there were two control groups and three density treatments, each with three replicates. For both species, the sex ratio in each mesocosm was 50:50 males:females. At the

Table 1.—Fish densities for control and treatment groups used during the mesocosm experiments, including northern starhead topminnows (NST) and western mosquitofish (WMF), mean number of fish at the end of the experiment and mean biomass (g) and mean wet weight (g) of individual fish before and after the experiment.

Experiment	Number of fish	Mean number of fish after experiment	Mean biomass (g) before experiment	Mean biomass (g) after experiment	Wet weight (g) before experiment	Wet weight (g) after experiment
Control	30 NST	4	36.94	2.57	1.23	0.62
		(3 – 5)	(29.46 – 48.30)	(2.32 – 2.73)	(0.98 – 1.61)	(0.53 – 0.77)
	30 WMF	27	12.30	3.68	0.41	0.14
		(17 – 38)	(10.13 – 15.41)	(2.83 – 4.77)	(0.34 – 0.51)	(0.11 – 0.20)
Treatment	20 NST	<1	23.80	0.12	1.19	0.12
	10 WMF	(0 – 1)	(20.31 – 25.78)	(0 – 0.36)	(1.02 – 1.29)	(0 – 0.36)
		29	3.83	4.13	0.38	0.16
		(16 – 46)	(3.72 – 3.93)	(3.16 – 5.25)	(0.37 – 0.40)	(0.11 – 0.20)
	15 NST	0	20.75	0.00	1.38	0.00
	15 WMF	(0)	(16.21 – 23.15)	(0)	(1.08 – 1.54)	(0)
		32	10.68	5.90	0.71	0.19
		(28 – 35)	(5.45 – 14.27)	(5.04 – 6.65)	(0.36 – 0.95)	(0.15 – 0.24)
	10 NST	0	11.31	0.00	1.13	0.00
	20 WMF	(0)	(7.91 – 13.84)	(0)	(0.79 – 1.38)	(0)
		19	12.85	4.65	0.64	0.25
		(16 – 20)	(5.19 – 20.69)	(4.02 – 5.47)	(0.26 – 1.04)	(0.22 – 0.27)

onset of the experiment (June 2006), fish of each species were counted to their assigned density and the combined wet weight of individuals for each species introduced into each mesocosm was recorded to the nearest 0.01 g. Following fish stocking, a 3.18 mm knotless mesh covering was secured over the mesocosms to prevent fish from escaping and outside predators from removing fish. Water-quality parameters, which included water temperature (°C), pH, specific conductance (μS/cm), dissolved oxygen (mg/l), and turbidity (nephalometric turbidity units [NTU]), were recorded weekly in each mesocosm using a Hydrolab Quanta (Hach-Hydrolab Company, Loveland, Colorado). Supplemental food was not provided during the study period to simulate natural pond conditions. At the termination of the experiment (October 2006), fish were collected from each mesocosm, identified to species, enumerated, and the combined wet weight of fish for each species was recorded to the nearest 0.01 g. Data were analyzed by comparing the mean number of western mosquitofish and/or northern starhead topminnows, total biomass (g), and wet weight (g) per individual fish at the beginning and end of the study to assess changes during the experimental period.

RESULTS

The number of northern starhead topminnows and western mosquitofish in each treatment declined during the experiments, with the exception of western mosquitofish at the two lowest density treatments (Table 1; Fig. 1). Northern starhead topminnows in control mesocosms declined from an initial mean density of 30 to a final mean density of 4 fish (−87%). The mean density of western mosquitofish also declined in control mesocosms, albeit to a lesser extent over the experimental period (30 to 27 fish; −10%). In mesocosms with an initial density of 20 northern starhead topminnows and 10 western mosquitofish, the mean density was different at the end of the experiment for northern starhead topminnows (one fish; −95%), but not different for western mosquitofish (29 fish; +195%). At equal densities (15 fish of each species), the mean density at the end of the experiment was also different at zero fish for northern starhead topminnows (−100%) and 32 individuals for western mosquitofish (113%). In mesocosms that contained 10 northern starhead topminnows and 20 western mosquitofish, there was a decline in mean northern starhead topminnow density to zero fish (−100%), but the decline in western mosquitofish density to 19 fish (−5%) was not
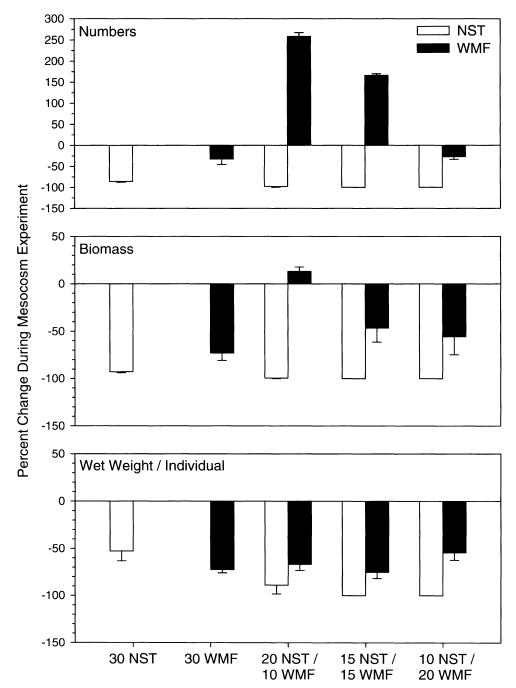
Figure 1.—Mean percent changes in the number of individuals, total mesocosm biomass, and wet weight per individual northern starhead topminnow (NST) and western mosquitofish (WMF) during the mesocosm experiment for each experimental treatment.

as large a change from the initial density (t = 1.0, P = 0.211).

The biomass of northern starhead topminnows and western mosquitofish in each meso-

cosm declined during the experiments, with the exception of western mosquitofish biomass at the lowest density (Table 1; Fig. 1). Northern starhead topminnow biomass in control meso-

cosms declined from a mean of 36.9 g to 2.57 g (−93%), and biomass of western mosquitofish in control mesocosms also declined from a mean of 12.29 g to 3.68 g (−70%). In mesocosms with an initial density of 20 northern starhead topminnows and 10 western mosquitofish, the mean biomass for northern starhead topminnows declined from 20.8 g to 0.12 g (−9%), but the change in western mosquitofish biomass from 3.83 g to 4.13 g represented an increase in biomass (+8%). At equal densities (15 fish of each species), the biomass of northern starhead topminnows declined from 20.75 g to 0.00 g (−100%), but the decline in western mosquitofish biomass from 10.68 g to 5.90 g was not nearly as large (−45%; $t = 2.2$, $P = 0.082$). In mesocosms that contained 10 northern starhead topminnows and 20 western mosquitofish, there was a decline in biomass of northern starhead topminnows from 11.31 g to 0.00 g (−100%) and western mosquitofish from 12.85 g to 4.65 g (−64%).

The wet weight per individual fish of northern starhead topminnows and western mosquitofish in each mesocosm declined during the experiment (Table 1; Fig. 1). Northern starhead topminnow mean weight per individual in control mesocosms declined from 1.23 g to 0.62 g (−50%). However, the mean weight per individual for western mosquitofish in control mesocosms declined from 0.41 g to 0.14 g (−66%). In mesocosms with 20 northern starhead topminnows and 10 western mosquitofish initially, the mean weight per individual declined for northern starhead topminnows from 1.19 g to 0.12 g (−90%) and western mosquitofish from 0.38 g to 0.16 g (−58%). At equal densities (15 fish of each species), the mean weight per individual declined for northern starhead topminnows from 1.38 g to 0.00 g (−100%) and western mosquitofish from 0.71 g to 0.19 g (−73%). In mesocosms that initially contained 10 northern starhead topminnows and 20 western mosquitofish, the mean weight per individual of topminnows declined from 1.13 g to 0.00 g (−100%), but the decline in western mosquitofish weight per individual from 0.64 g to 0.25 g was not as large (−61%).

DISCUSSION

The potential impacts of stocking western mosquitofish into systems containing northern starhead topminnows were simulated in a 110-day mesocosm experiment. Although there was a decline in the mean abundance, biomass, and wet weight per individual for topminnows in all treatments, the declines were more pronounced in mesocosms also containing mosquitofish. Topminnows did survive in control mesocosms throughout the experiment, so the presence of western mosquitofish had an impact on this species. Rogowski & Stockwell (2006) showed that when western mosquitofish were kept in aquaria with White Sands pupfish (*Cyprinodon tularosa*), growth rates and biomass were lower than when the latter species was maintained alone. Eastern mosquitofish predation on smaller least killifish (*Heterandria formosa*) caused a shift in size structure of the latter species in mesocosms, leaving only larger female topminnows (Schaefer et al. 1994). In field coexistence studies with western mosquitofish and Sonoran topminnows, all topminnows exhibited fin or body damage within two weeks, and had died and were consumed by mosquitofish within three weeks (Meffe 1985).

Western mosquitofish were not impacted by northern starhead topminnows during the mesocosm experiment. For most mesocosms, there was typically a higher mosquitofish density at the end of the experiment than were initially stocked. Although both mean biomass and wet weight per individual fish in mesocosms for mosquitofish declined during the experiment, most of the fish at the end of the experiment were juveniles, indicating that there was successful reproduction. This occurred in both control and experimental mesocosms, so the presence of topminnows did not influence mosquitofish reproduction. In previous studies, eastern mosquitofish exhibited a 50% increase in overall biomass in outdoor mesocosms, which was due to an increase in density from reproduction rather than increased fish size (Sepúlveda et al. 2005). Schaefer et al. (1984) showed that the density, biomass, and mean length per individual of eastern mosquitofish in outdoor microcosms in a Florida marsh were not influenced by least killifish. Similarly, western mosquitofish exhibited increases in mean biomass and wet weight per individual, regardless of the presence or absence of threespine stickleback (*Gasterosteus aculeatus*) (Offill & Walton 1999).

Although few northern starhead topminnows survived in mesocosms that also contained mosquitofish, there was also a large decline in the number of northern starhead topminnows

in control mesocosms. As a result, the observed declines in mean abundance, biomass, and wet weight per individual may not have been caused solely by western mosquitofish. The upper thermal tolerance of northern starhead topminnows is unknown, but water temperatures in mesocosms ranged from 10–27°C, which is within the known spawning temperature range for this species (17°–30°C; Taylor & Burr 1997). These mesocosm temperatures were also within the upper thermal tolerance limit (38°C) and spawning temperature range for mosquitofish (Otto 1974; Becker 1983). Therefore, water temperature was probably not a factor that contributed to the differential success of mosquitofish relative to topminnows.

Both western mosquitofish and northern starhead topminnows spawn during spring and summer months (Becker 1983; Taylor & Burr 1997). Female western mosquitofish have a protracted spawning period, produce two to six broods throughout the summer, and the number of live offspring produced per brood ranges from 14–218 (Krumholz 1948; Haynes & Cashner 1995). In contrast, northern starhead topminnows only spawn from mid-April to mid-July, produce only two egg clutches during this period, and the number of eggs per clutch ranges from 7–30 (Taylor & Burr 1997). Consequently, these differences may have contributed to the differential reproduction success of northern starhead topminnows (no reproduction) and western mosquitofish (successful reproduction).

The declines in the number of northern starhead topminnows in experimental mesocosms may also be due to food limitations. Although the initial pond-water inoculation contained zooplankton and other aquatic macroinvertebrates, the density of these prey resources may not have been sufficient to support both topminnows and mosquitofish in the same mesocosm. Zeiber (2007) found that these two species in Indiana had nearly identical diets that consisted of zooplankton, culicid (mosquito) larvae, other aquatic macroinvertebrates, and terrestrial insects. Competition for limited prey resources may have allowed only one species to receive adequate nutrition, and due to the aggressive nature of mosquitofish, this species was most likely able to outcompete topminnows. However, this does not explain why topminnow numbers declined in control mesocosms.

Northern starhead topminnows and, to a lesser extent, western mosquitofish may also have experienced a natural die-off over the summer in the control and experimental mesocosms. The mean longevity for northern starhead topminnows is two years, with some individuals surviving to age 3 (Becker 1983; Taylor & Burr 1997). In contrast, few western mosquitofish survive past one year, and the maximum lifespan for females is 1.5 years (Daniels & Felley 1992; Haynes & Cashner 1995). Therefore, the fish used in the mesocosm study may have been near the end of their life span and, consequently, were unable to survive the entire experimental period. While Zeiber (2007) successfully maintained both species in the laboratory at similar environmental conditions, those fish were almost strictly juveniles or young adults and not senescent. Regardless of the reason(s) for the topminnow declines, it is likely that the negative effects that occurred in the experimental mesocosms were exacerbated by the presence of western mosquitofish.

Due to the impacts of western mosquitofish on northern starhead topminnows observed during this study, we recommend that the former species not be stocked into any aquatic systems in Indiana. Unfortunately, western mosquitofish may already inhabit systems that support northern starhead topminnows. For example, western mosquitofish were found for the first time in 2006 in Loomis Lake, Indiana, a system that has historically supported a large population of northern starhead topminnows. Therefore, additional research is required to determine the potential long-term impacts of western mosquitofish on northern starhead topminnows in systems where they co-occur.

ACKNOWLEDGMENTS

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