

Research Article

Complex interactions between native and invasive species: investigating the differential displacement of two topminnows native to Nebraska

David A. Schumann^{1a*}, W. Wyatt Hoback^{1b} and Keith D. Koupal²

¹University of Nebraska at Kearney, Department of Biology, 2401 11th Avenue, Kearney, Nebraska 68849, USA

²Nebraska Game and Parks Commission, Kearney Field Office, 1617 1st Avenue, Kearney, Nebraska 68847, USA

^aCurrent address: Natural Resource Management, South Dakota State University, Box 2140B, SNP 138, Brookings, South Dakota 57007-1696, USA

^bCurrent address: Entomology and Plant Pathology, Oklahoma State University, 127 Noble Research Center, Stillwater, Oklahoma 74078-3033, USA

*Corresponding author

E-mail: david.schumann@sdstate.edu

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Abstract

The establishment of nonnative species and subsequent replacement of native species is among the greatest threats to freshwater biodiversity worldwide. However, little is known of the effects that invasive species have on individual species and specific mechanisms by which species displacement occurs. The expansion of western mosquitofish *Gambusia affinis* beyond its native range has commonly been thought to cause shifts in species assemblages throughout the United States. Although plains topminnow *Fundulus sciadicus* populations have declined as the range of western mosquitofish in Nebraska has expanded, long-term co-existence of mosquitofish with the closely related northern plains killifish *Fundulus kansae* has been observed within the same systems. To investigate the potential mechanisms by which differential displacement of the native topminnow species may have occurred, biotic interactions and spatial distribution of both conspecific and heterospecific groups of plains topminnow, plains killifish, and western mosquitofish were quantified in microcosms for three life stages (larval, juvenile, and adult). Interactions between adult western mosquitofish and the native species were complex and dependent upon life stage, but collectively suggested that populations of both plains topminnow and plains killifish are vulnerable to mosquitofish establishment. Detrimental effects towards fundulid species were primarily mediated through predation of larvae and aggressive interactions directed towards juvenile life stages which caused significant mortality. In addition, simultaneous alterations of behavior, as quantified by activity patterns and microhabitat use, were apparent in both native species in response to western mosquitofish presence. Because similar impacts of western mosquitofish were observed for plains killifish and plains topminnow, the persistence of plains killifish in areas occupied by western mosquitofish may be linked to species attributes not assessed in the current analysis. Subtle differences in microhabitat selection between the topminnow species and adult western mosquitofish could potentially explain observed differential displacement in the environment. These results have augmented our understanding of the impacts that western mosquitofish has on the at-risk plains topminnow, and have further elucidated the potential mechanisms for impact via competitive interactions.

Key words: species displacement, competition, invasive species, aggressive interactions, western mosquitofish, plains topminnow, plains killifish

Introduction

Despite the potential negative effects that invasive species may have on native communities, relatively little is known about the mechanisms by which species displacement occurs (Meffe 1985; Lydeard and Belk 1993; Goldsworthy and Bettoli 2006; Laha and Mattingly 2007). Evidence of the widespread disruption of native fish communities by invasive species is largely anecdotal and rarely has involved rigorous quantification although invasive species can impact assemblage structure

(Gurevitch et al. 2000; Olden and Poff 2005). Generally native species similar to nonnative invaders in terms of ecological role and life history are at increased risk as a result of complex biotic interactions (Barrier and Hicks 1994; Mills et al. 2004; Laha and Mattingly 2007); however, few introduced species have had a measureable effect on native species (Tyus and Saunders 2000; Jeschke and Strayer 2005). When observed, negative impacts by invasive fishes are commonly considered to be the result of interference and exploitative competition, predation, introduced disease, and

hybridization (Meffe 1985; Mills et al. 2004; Laha and Mattingly 2007). However, invasive fishes may negatively influence native fishes through multiple biotic interactions that collectively influence coexistence potential (Mills et al. 2004; Henkanaththegedara and Stockwell 2014). Determination of the principle mechanisms by which species displacement occurs has proven difficult. Despite the challenge, the identification of negative effects that nonnative species have on native species and the manner in which they are mediated are necessary to accurately estimate the impact of nonnative species introductions and establishment (Laha and Mattingly 2007).

The introduction and spread of the western mosquitofish (WMF) *Gambusia affinis* (Baird and Girard, 1853) has caused shifts in species assemblages through predation, competition, and stress (Meffe 1985; Laha and Mattingly 2007), having had particularly great impacts on small native fishes (Courtenay and Meffe 1989; Galat and Robinson 1992; Chipps and Wahl 2004; Mills et al. 2004). Introductions of WMF have been sporadic but have resulted in a nearly global distribution of the species and its consideration as one of the most widely distributed freshwater fish in the world (Lynch 1988; Courtenay and Meffe 1989). In the United States, WMF have been introduced to 36 states beyond their native range and has been described as the most destructive species in the American West (Meffe 1985; Fuller et al. 1999; Olden and Poff 2005; Westhoff et al. 2013). High rates of consumption and continued anthropogenic demand emphasizes the likelihood for the continued spread and negative impacts on native populations from this species (Chipps and Wahl 2004; Pyke 2006; Westhoff et al. 2013).

Although, WMF act as a benign component of complex stream fish communities within its native range, they are considered a detriment in introduced systems (Matthews and Marsh-Matthews 2011). Establishment of WMF has commonly resulted in declines in local abundance and changes to distribution of native fishes (Meffe 1985; Goldsworthy and Bettoli 2006; Pasbrig et al. 2012; Westhoff et al. 2013) often mediated by predation of early life stages and subsequent disruption of native species recruitment (Galat and Robinson 1992; Barrier and Hicks 1994; Laha and Mattingly 2007; Sutton et al. 2013). Competitive interactions (Meffe et al. 1983; Haas 2005; Alcaraz et al. 2008), aggressive behaviors causing physical harm to juveniles and adults (Meffe 1984; Marsh and Minckley 1990; Barrier and Hicks 1994;

Sutton et al. 2013), or multiple interactions among two or more of these mechanisms have been documented (Mills et al. 2004).

The introduction and subsequent expansion of WMF in Nebraska has been recently implicated in the reduction of plains topminnow (PTM) *Fundulus sciadicus* (Cope, 1865) populations because of perceived competitive interactions (Lynch 1988; Lynch and Roh 1996; Rahel and Thel 2004; Haas 2005; Fischer and Paukert 2008; Pasbrig et al. 2012). Concurrent with PTM declines, the range of WMF in Nebraska has expanded (Haas 2005; Pasbrig et al. 2012). Observations of the local replacement of PTM by WMF in nine lotic systems provide anecdotal evidence of the detrimental effects of this species in Nebraska (Haas 2005; Pasbrig et al. 2012). However, the closely related plains killifish (PKF) *Fundulus kansae* (Garman, 1895) continues to succeed in the presence of WMF at more than 60 localities (NGPC unpub. data). The differential persistence of these two species in the same genus directly contrasts expected outcomes as both species commonly inhabit small, vegetated shallow bodies of water with little to no current, are surface-orientated feeders, and are diurnally active (Casterlin and Reynolds 1977; Rahel and Thel 2004a, b; Haas 2005).

The potential differential displacement of PTM and PKF observed in Nebraska lotic systems suggests that mechanisms negatively affecting PTM persistence may be less severe to PKF in the same systems. A laboratory examination of the interactions between the nonnative WMF and the two native fundulids was undertaken to investigate the observed differences in coexistence. The objectives of this study were (1) to quantify predation of PTM and PKF larvae by WMF, (2) to document the amount of intra- and interspecific aggressive interactions among species, and (3) to investigate the effect that aggressive interactions have on the spatial distribution of each species in replicate environments.

Methods

Fish collection and husbandry.—Plains topminnow were collected from a pond constructed to house a refuge population and mimic natural environments (Schumann et al. 2012) that was located near Wilcox, South-central, Nebraska. Pond produced PTM were used because of concern for the status of native populations. Western mosquitofish were captured at two Platte River sites near Kearney, Nebraska that did not have either fundulid species present and PKF were collected

from Sweet Creek, near Ravenna, Nebraska at locations in which fish are locally abundant. None of the fundulid individuals were collected from local systems in which mosquitofish were present and all sites were within 100 km of Kearney, Nebraska. All species were collected using a 4-mm mesh dip-net or seine (6.4 m × 1.2 m × 6.4-mm mesh). Captured individuals were transported to the University of Nebraska at Kearney campus in treated municipal well water that was aerated. For predation trials, WMF females were separated and allowed to give birth due to inability to capture small-juvenile WMF. Larval (< 15 mm) PTM and PKF were readily collected in the field during the spring. Prior to experimental trials, fish species were housed in separate mesh cages (capacity, 125-l) placed in the same 1,900-l recirculating system. Fish were fed dried bloodworms (*Chironomus* spp.) in excess twice daily and allowed to acclimate to laboratory conditions for a minimum of one week prior to trials.

Western mosquitofish predation.—Three treatments were conducted to determine the relative predation by adult WMF on small-juvenile WMF and larval PTM and PKF. Adult WMF used for this study were female (30–50 mm TL) and all trial fish were selected randomly from available specimens. All individuals from each species were measured to TL (mm) prior to each trial using a digital camera elevated to 30 cm above the water in a clear pan with a ruler secured to the bottom (Mills et al. 2004). One experimental trial was conducted in each of thirty 9.46-l aquaria with aeration. For each replicate ($N = 10$), a single adult WMF and 10 larvae PTM or PKF individuals or small-juvenile WMF were housed together for 24 hours. A plastic divider was used to initially separate study individuals for an acclimation period of two hours. Study fish were fed in excess twice daily in an attempt to limit predation to typical behaviors rather than those resultant of starvation (Meffe 1985; Sutton et al. 2013). After the trial period the number of consumed individuals was recorded as well as any mortality of adult mosquitofish. Comparisons among treatment groups were made using a Kruskal-Wallis one-way analysis of variance on ranks and, if significance was observed, multiple pairwise comparisons were made using a Tukey HSD test to isolate the source of variation (Zar 2010).

Behavioral interactions.—Aggressive interactions among species were investigated using methods described by Barrier and Hicks (1994) and modified

by Laha and Mattingly (2007). Aggressive behaviors were evaluated for two size classes of PTM and PKF: juvenile (< 30 mm TL) and adult (≥ 30 mm TL) to identify potentially vulnerable size classes. Adult WMF used for this study were female (30–50 mm TL) and were selected randomly from available specimens. All individuals from each species were measured to TL (mm) prior to each trial using a digital camera as before (Mills et al. 2004). Trials were conducted in 37.85-l aquariums which included a plastic divider to initially separate study individuals for an acclimation period of two hours. A single sponge filter attached to an aerator was used in each aquarium and provided the only source of cover. Study fish were fed in excess twice daily in an attempt to limit interactions resulting from hunger (Meffe 1985; Sutton et al. 2013). Control trials ($N = 10$) were conducted in which conspecific groups consisting of four adult and four juvenile PTM and PKF individuals or eight adult, female WMF. A substitutive design was employed and the density of eight individuals per tank was maintained during heterospecific treatments where four individuals were paired with four of another species in the following communities: adult WMF with juvenile or adult PTM or PKF. A total of 10 replicates were completed for each combination using different fish in each trial.

Survival and the number of inter- and intra-specific aggressive interactions of conspecific and heterospecific groups of PTM, PKF, and WMF were quantified. At 0 h, the plastic divider was removed allowing fish to interact for 24 h with a 13 h light and 11 h dark period. Each replicate was digitally recorded for a 25 min period at the trial's initiation and, after 24 hours, a second 25 minute period was recorded. The first 5 min of each video was discarded because in pilot trials fish were affected by human presence when activating cameras. The resulting 40 minutes of video footage was reviewed and the initiator, target, and movement type were assessed. Aggressive interactions were categorized as intention movements, chases, or nips (Barrier and Hicks 1994; Laha and Mattingly 2007). Intention movements were situations in which an individual orientated in the direction of another or initiated a short movement towards another which lasted less than 0.5 seconds. Chases were active pursuits which lasted longer than 0.5 seconds and nips were circumstances in which physical contact between individuals occurred. All types of aggressive interactions were pooled prior to analysis.

Table 1. Size and mean \pm SE number of larval plains topminnow and plains killifish consumed by a single female western mosquitofish (WMF) that was not constrained by a gape limitation during a 24 hour trial period ($N = 10$ larvae per trial; 10 replicate trials). No significant difference (ANOVA, $P = 0.65$) in mean number consumed was observed.

Prey Species	Mean TL (mm) \pm SE		
	WMF Predators	Larval or Young Prey	Mean No. Consumed
Western Mosquitofish	43.7 \pm 1.93	8.7 \pm 0.37	3.7 \pm 0.80
Plains Topminnow	44.0 \pm 1.71	10.4 \pm 0.54	5.0 \pm 1.37
Plains Killifish	44.1 \pm 1.55	11.4 \pm 0.37	4.5 \pm 0.62

Mann-Whitney Rank Sum tests comparing survival among each species were conducted by size class (Zar 2010) to determine the species and size classes that were most susceptible to WMF aggression. The sum of aggressive behaviors initiated by WMF was counted and compared among all PTM and PKF treatments. Size specific aggressive interactions initiated by WMF targeting PTM and PKF were compared within and between species. Comparisons were made using Kruskal-Wallis one-way analysis of variance on ranks. If significance was observed, post-hoc pairwise multiple comparisons were made using a Tukey HSD test to isolate the source of variation.

Interference competition.—During each behavioral trial, the number of individuals occupying the open water areas of the aquaria and actively swimming versus sedentary, benthic behaviors were recorded at two minute increments from the 24 h footage. A single 5 \times 10 cm sponge filter attached to an aerator was established in the same corner of each aquarium and provided the lone cover. The mean number of individuals occupying each horizontal area (i.e., water-column vs. benthic) was compared for all conspecific and heterospecific trials to measure the influence that WMF had on the distribution of each size class of PTM and PKF. Comparisons were made using Kruskal-Wallis one way analysis of variance on ranks and if significance was observed pairwise multiple comparisons were made using a Tukey HSD test to isolate the source of variation.

For all analyses significance was determined at $\alpha = 0.05$ and analysis was completed using SAS version 9.3 (SAS Institute Inc., Cary, North Carolina).

Results

Western mosquitofish predation.—Western mosquitofish consumed 37, 50, and 45 percent of small-juvenile WMF, larval PTM, and larval

PKF, respectively (Table 1). Although significant differences in mean total length of young individuals existed ($H = 12.86$, $P = 0.002$), in which larval PKF were significantly larger than small-juvenile WMF (Tukey HSD, $P < 0.05$), no significant differences were found in the number of individuals of each species consumed ($H = 0.64$, $P = 0.73$) (Table 1). There was no significant difference in total length of WMF adults used as predators ($H = 0.05$, $P = 0.97$) and throughout all trials no mortality of WMF predators occurred (Table 1).

Survival.—Mortality of both native species increased, regardless of size class evaluated, when subjected to the aggressive behaviors of WMF (Figure 1). Mean \pm standard error (SE) percent survival during single species control groups was high ranging from 98 \pm 3.9 to 100 percent (Figure 1) and was not significantly different among species ($H = 2.0$, $P = 0.37$). Survival of juvenile PTM and PKF was 83 \pm 16.9 percent for both species when housed with WMF (Figure 1). Survival of PTM juveniles was similar to adult survival when housed with WMF (Mann-Whitney $U = 33.5$, $P = 0.17$). However, juvenile PKF suffered significantly more mortality than adults when introduced to WMF (Mann-Whitney $U = 24.5$, $P = 0.02$). Adult survival of PTM (92 \pm 12.1%) and PKF (98 \pm 7.9%) in the presence of WMF was not significantly different (Mann-Whitney $U = 40.0$, $P = 0.30$).

Behavioral interactions.—Adult WMF were consistently the most aggressive species in all behavioral trials (Figure 2) and, at all times, WMF initiated more aggressive behaviors during conspecific control trials than the other species examined ($H = 21.7$, $P < 0.001$). The number of aggressive actions observed during conspecific control trials for WMF was up to 60 times more than the conspecific interactions observed for PKF (Tukey HSD, $P < 0.05$) and PTM (Tukey HSD, $P < 0.05$). Plains topminnow were the recipient of at least two times more aggressive

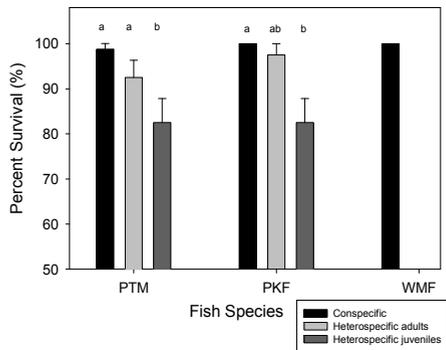


Figure 1. Mean (\pm SE) survivorship during behavioral trials of juvenile (15-30 mm TL) and adult ($>$ 30 mm TL) plains topminnow (PTM), plains killifish (PKF), and western mosquitofish (WMF) during heterospecific ($N = 10$) and conspecific ($N = 10$) trials. Significant differences ($P < 0.05$), Kruskal-Wallis ANOVA are denoted by different letters.

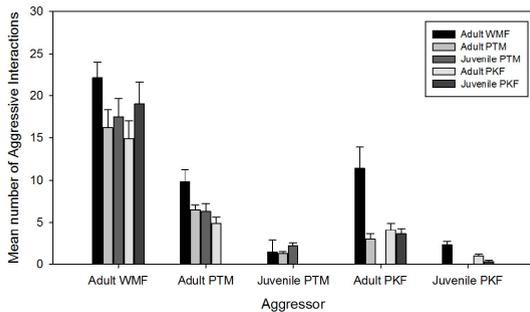


Figure 2. Mean (\pm S.E.) number of aggressive actions from the fish species initiating the aggressive interaction towards either conspecific ($N = 8$ individuals) or heterospecific adult ($N = 8$ individuals) or juvenile ($N = 8$ individuals). Western mosquitofish (WMF), plains topminnow (PTM), and plains killifish (PKF).

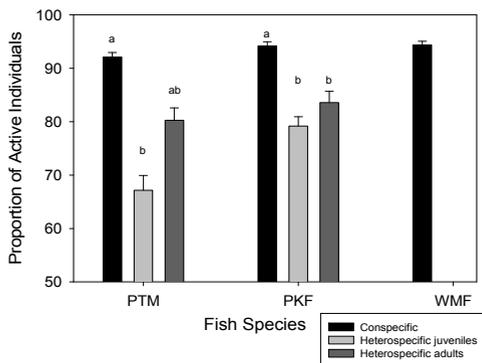


Figure 3. Mean percentage (\pm SE) of actively swimming individuals among conspecific ($N = 8$) and heterospecific ($N = 8$) treatment groups of western mosquitofish (WMF), plains topminnow (PTM), and plains killifish (PKF). Significant differences ($P < 0.05$), Kruskal-Wallis ANOVA are denoted by different letters.

interactions when housed with adult WMF than during conspecific control trials ($H = 12.3$, $P = 0.002$). Similarly, PKF were at least three times more likely to be subjects to aggressive behaviors when WMF were introduced than during conspecific control trials ($H = 18.1$, $P < 0.001$). When introduced to adult WMF, adult PTM (Tukey HSD, $P < 0.05$), juvenile PTM (Tukey HSD, $P < 0.05$), adult PKF (Tukey HSD, $P < 0.05$), and juvenile PKF (Tukey HSD, $P < 0.05$) were subjected to more aggressive actions than when with single-species conspecific control groups.

The number of aggressive actions initiated by WMF did not differentiate between the size class of heterospecific taxa or fundulid species evaluated. The number of interactions initiated by WMF and directed towards adult and juvenile PTM (Mann-Whitney $U = 45.0$, $P = 0.73$), as well as adult and juvenile PKF (Mann-Whitney $U = 37.0$, $P = 0.34$) were similar during heterospecific trials. When comparing the total number of interspecific aggressive actions directed towards both native species and initiated by western mosquitofish, no significant difference was observed during adult (Mann-Whitney $U = 43.0$, $P = 0.62$) or juvenile (Mann-Whitney $U = 44.5$, $P = 0.70$) trials (Figure 2).

Interference competition.—No significant difference ($H = 4.1$, $P = 0.13$) in the proportion of active individuals using open water areas was observed during conspecific trials for all species (Figure 3). The mean proportion of active individuals was found to be 0.92 ± 0.01 for PKF and 0.94 ± 0.01 for both WMF and PTM control groups (Figure 3). Both juvenile (Tukey HSD, $P < 0.05$) and adult (Tukey HSD, $P < 0.05$) PTM spent significantly less ($H = 70.6$, $P < 0.001$) time actively swimming in open water when WMF were introduced (Figure 2). Juvenile PTM spent significantly less time in open water areas than adult PTM (Tukey HSD, $P < 0.05$) when introduced to WMF. As with PTM, the proportion of both juvenile (Tukey HSD, $P < 0.05$) and adult (Tukey HSD, $P < 0.05$) PKF using open water was significantly reduced ($H = 10.56$, $P = 0.005$) in the presence of WMF, however, no difference existed between the activity of juvenile and adult PKF. The proportion of individuals actively using open water areas in the presence of WMF was significantly different between PTM and PKF during juvenile heterospecific trials (Mann-Whitney $U = 3232.5$, $P < 0.001$) with a greater proportion of PKF occupying open water areas than PTM (Figure 3).

Discussion

In laboratory trials, larval PTM and PKF were equally susceptible to adult WMF predation which caused substantial mortality in a relatively short trial period. Western mosquitofish have traits typical of piscivorous fishes that include strong, conical teeth and a short intestine (Pyke 2006), and are known to be efficient predators of young-of-year fishes (Chipps and Wahl 2004) with the ability to limit recruitment of several fish species (Meffe et al. 1983; Taylor et al. 2001; Johnson and Bettoli 2003; Goldsworthy and Bettoli 2006; Laha and Mattingly 2006; Laha and Mattingly 2007). Despite growth to the juvenile size class resulting in the native species obtaining a size refuge from vulnerability to mosquitofish predation, aggressive interactions initiated by WMF continue through each life stage and were found to negatively affect survival of juvenile individuals of both fundulid species. For juvenile fundulids, mortality exceeded 20 percent in 24 hours. Similar results have been observed, in which survival of larval and juvenile Barrens topminnow *Fundulus julisia* (Williams and Etnier 1982; Laha and Mattingly 2007), least chub *Lotichthys phlegethontis* (Cope, 1874) (Mills et al. 2004), and black mudfish *Neochanna diversus* (Stokell, 1949) (Barrier and Hicks 1994) decreased in the presence of WMF. The high level of mortality incurred in the short timeframe of these experiments indicate that adult WMF are efficient predators of larval individuals and aggressive competitors of juvenile fundulids under laboratory conditions despite frequent provision of food.

Although the threat of predation and mortality declined with growth, the effects of aggression continued through each life stage for both species evaluated. Similar studies concluded that WMF impact juveniles of least chub, black mudfish, and Barrens topminnow by reducing their body condition (Barrier and Hicks 1994; Mills et al. 2004; Laha and Mattingly 2007). The presence of WMF and resultant aggressive actions directed towards adult individuals of both native species, however, may not have compromised the physiological well-being of various species that fed and grew normally, suggesting a minor role in species replacement such as previously described for adult Barrens topminnow (Goldsworthy and Bettoli 2006; Laha and Mattingly 2007) and Sonoran topminnow *Poeciliopsis occidentalis* (Girard, 1859) (Meffe 1985). The impact of these aggressive behaviors among WMF and the native species on co-occurrence in natural settings remains unknown

as both species were equally impacted by the introduction of WMF in replicate environments.

Western mosquitofish displayed more aggressive behaviors towards conspecifics as well as towards both fundulids. These results agree closely with results of previous research in which WMF demonstrated more intra- and interspecific aggression than four other members of the genus *Fundulus* (Sutton et al. 2013) and exhibited rates of nearly 1.25 aggressive acts per minute per fish (Martin 1975). Compared with WMF, PTM and PKF exhibited relatively few aggressive behaviors during conspecific tank trials and intraspecific aggressive interactions were reduced when in the presence of WMF. This contrasts with the characterization of PTM as an aggressive species with a pugnacious temperament (Propst and Carlson 1986; Rahel and Thel 2004).

The presence of WMF caused a change in behavior in mixed species trials resulting in altered water column distributions for juveniles and adults of both fundulids. Previous research has demonstrated similar effects of WMF presence in which least chub remained stationary for long periods (Mills et al. 2004), four topminnow species sought refuge (Sutton et al. 2013), and juvenile black mudfish assumed a benthic position (Barrier and Hicks 1994). Conversely, several studies have found limited effects on the distribution and behavior of related species including adult black mudfish (Barrier and Hicks 1994), Sonoran topminnow (Meffe 1985), and Barrens topminnow also fed and moved freely despite the presence of WMF (Laha and Mattingly 2007). In our study, a greater proportion of adult PKF were unaffected in terms of activity patterns than adult PTM. Changed behavior likely reduces foraging opportunities leading to reduced growth and reproductive potential (Mills et al. 2004; Sutton et al. 2013) and potentially providing a mechanism for differential persistence of PTM and PKF in environmental populations.

Interactions between adult WMF and both fundulids are complex and depend on life stage. Adult WMF directly contribute to mortality of larval PTM and PKF through predation, and adult WMF aggressively dominate juvenile and adult size classes resulting in changed behaviors in both species. Our results indicate that adult WMF are capable of altering behavior of fundulid species in a lentic setting, however the extent to which each species evaluated in our study utilizes refuge habitats in the environment to avoid WMF aggression requires further study. In the field, the persistence of PKF in areas occupied by

WMF may be a result of tolerance to higher flows. Although PKF occur more frequently in habitats with water velocities lower than 12 cm/s, they can utilize habitats with greater velocities (Woodling 1985; Cross and Collins 1995; Conklin et al. 1996). The results of behavioral research conducted in laboratory settings, including these results, are often difficult to apply to natural systems due to a multitude of abiotic and biotic factors that may have direct or indirect association with observed interactions in the environment. It is possible that such behavioral experiments may not characterize or potentially even exaggerate natural behaviors (Laha and Mattingly 2007; Sutton et al. 2013). It is also plausible that the lack of exposure to piscivorous predators from the PTM used in these experiments may have altered results. However, general support for our conclusions is available as similar microcosm studies have repeatedly confirmed the aggressive and insatiable predatory behavior of WMF towards small native fishes (Laha and Mattingly 2007), albeit without complexities of habitat available.

Our laboratory data suggest that replacement of PTM by WMF is primarily due to predation on larvae and aggressive interactions that result in substantial mortality of juveniles, with altered habitat use of adults potentially playing a role. Because of similar interactions of WMF towards both species of fundulids in laboratory settings, the persistence of PKF in areas occupied by WMF must be linked to differences not measured in our study. It is likely that niche partitioning between PTM and PKF as well as limited spatial and temporal overlap between adult WMF and PKF explain the observed persistence of this species after WMF introduction. Because PKF spawn in areas that are likely less densely occupied by WMF than PTM, their larvae are potentially subjected to less predation (Rahel and Thel 2004 a, b). Overlap between PTM spawning and adult habitat with adult WMF habitat is likely an important factor in the decline of PTM observed in Nebraska. Moreover, as PKF grow they are often captured in areas of greater velocity than both PTM and WMF (Rahel and Thel 2004 a, b). This characteristic likely allows them to escape aggressive interactions and thus, interference observed with this species during our study may be exaggerated compared to natural conditions. Utilizing laboratory tests to assess the effects of an invasive species on closely-related native species in this study has led to a better understanding of mechanisms involved in the persistence of native fundulids.

The development of conservation strategies for PTM and other threatened small fishes will require additional field trials to distinguish how PKF and other small fishes persist despite occupying similar ecological space as the invasive WMF.

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