Final Report on a Lois Webster Fund Project: "Experimental test of condition-specific competition between native plains topminnow and non-native mosquitofish"

November 1, 2021

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Abstract

The western mosquitofish (*Gambusia affinis,* MSQ), considered one of the 100 worst invasive species in the world, has been implied in declines of native fish populations including the plains topminnow (*Fundulus sciadicus,* PTM). However, little is known about why their invasions are so successful in some cases but not others. We performed experiments to identify how competition between PTM and MSQ is regulated at three temperatures of 16° C, 22° C, and 28° C and at varying densities of 40, 60, 80, 100, and 120 individuals, and recorded fish habitat preference between high and low velocity tanks. We found that both fish species preferred the low velocity habitat that represented plains streams and wetlands more closely. However, in sympatry, temperature and density played a key role in mediating habitat use and consequently inter-specific competition. Contrary to published information, MSQ was not aggressive toward PTM. Instead, agonistic interactions were most frequently observed between individuals of PTM (i.e., intra-specific competition > inter-specific competition). Overall, these findings suggest that PTM may be in decline due not solely to aggressive behavior by MSQ and environmental change will likely affect species coexistence.

Introduction

Biological invasions are a threat in almost every ecosystem, with consequences on native species persistence and ecosystem function (Mack, 2000). The western mosquitofish (MSQ, Fig. 1a), *Gambusia affinis*, is a small-bodied fish whose native range lies in the Mississippi River basin. Mosquitofish have been introduced as a biological mosquito control agent and have been documented on every continent except Antarctica (Pyke, 2008). As an aggressive competitor, MSQ outcompete native species with a niche overlap (e.g., food) and tolerate a wide range of abiotic conditions (Pasbrig et al., 2012; Sutton et al., 2013). This highly invasive species appears on the list of 100 worst invasive alien species in the world (Simberloff and Rejmanek, 2019).

MSQ has been spreading in plains streams of Colorado in the last few decades (Fig. 1), and we witnessed a rapid spread of this invasive species when we surveyed the Arikaree River of the Republican River basin in 2019 as a project funded by the Lois Webster Fund. Spread of mosquitofish appears to coincide with local extirpations of native plains fish. In particular, plains topminnow (PTM), *Fundulus sciadicus* (Fig. 1b), appears hardest hit by mosquitofish invasion based on consultations with fisheries biologists at the Colorado Parks and Wildlife. PTM is a species of special concern in five states it is found (Grunau, 2015), and is a Tier 1 species of greatest conservation need in Colorado (Pasbrig et al., 2012). However, MSQ invasion stops at the Front Range and there are streams along the Front Range where PTM populations still persist (Fig. 1), sometimes co-existing with MSQ. This pattern of species distributions suggests that invasion success of MSQ and population persistence of PTM depend on environmental conditions. In this study, we tested our prediction that inter-specific competition is determined by water temperature and flow conditions by experimentally manipulating these abiotic conditions in laboratory stream mesocosms.

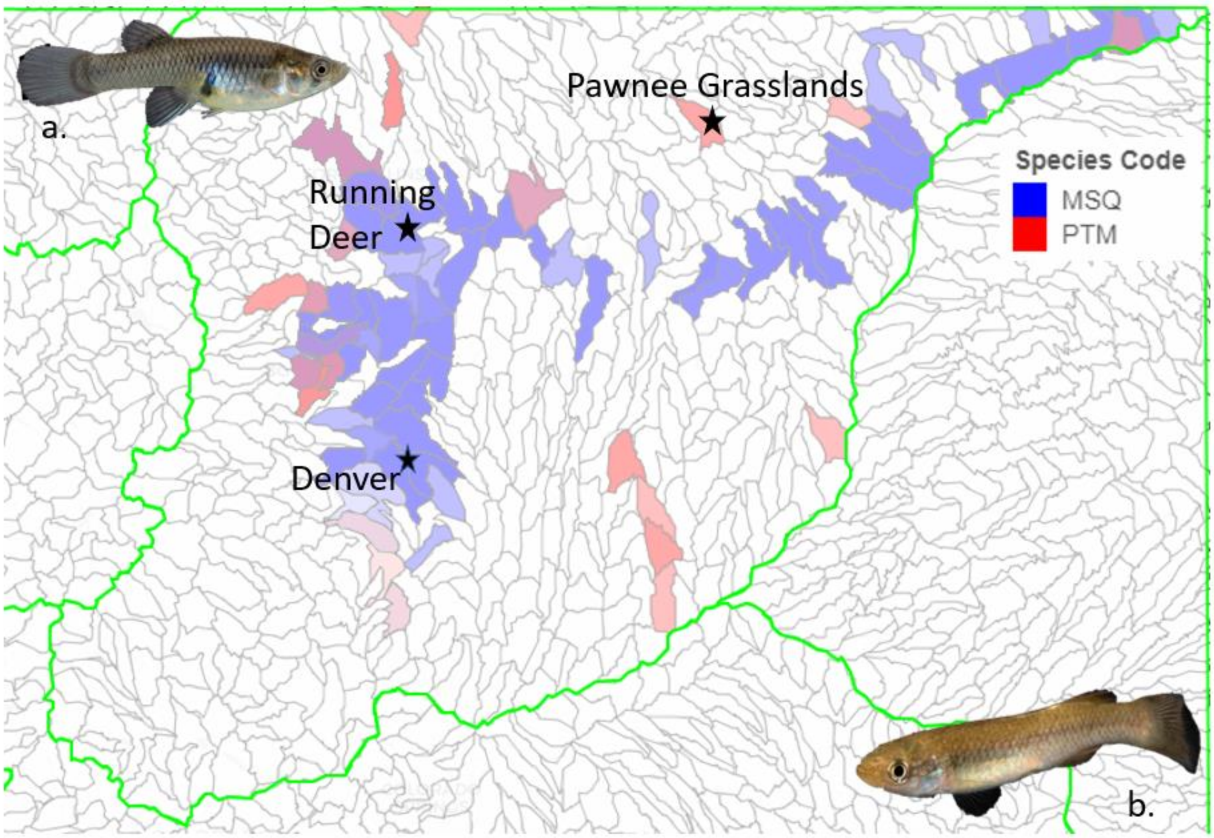

Figure 1. HUC12 level presence/absence data of MSQ (a) and PTM (b) in Colorado's South Platte River basin.

Study Objectives

The objectives of this study were three-fold:

(1) How do temperature and flow mediate competition between MSQ and PTM?
(2) Does the habitat use depend not only on fish density but also on species composition?
(3) Is competition (behavioral interactions) stronger between individuals of the same species or different species?

Species Collection, Experimental Setup, and Data Collection

Fish for this study were collected from two sites during the summer of 2021. Plains topminnow were collected from West Willow Creek on the Pawnee National Grasslands (Fig. 1, Fig. 2a). Western mosquitofish were collected from Running Deer Natural area in Fort Collins (Fig. 1, Fig. 2b) where their range overlaps with PTM, but greatly outnumber PTM. Fish were transported to the Anatomy Zoology building on Colorado State University's campus where they were quarantined and acclimated for three days before data collection began in the experimental stream units (Fig. 2c).

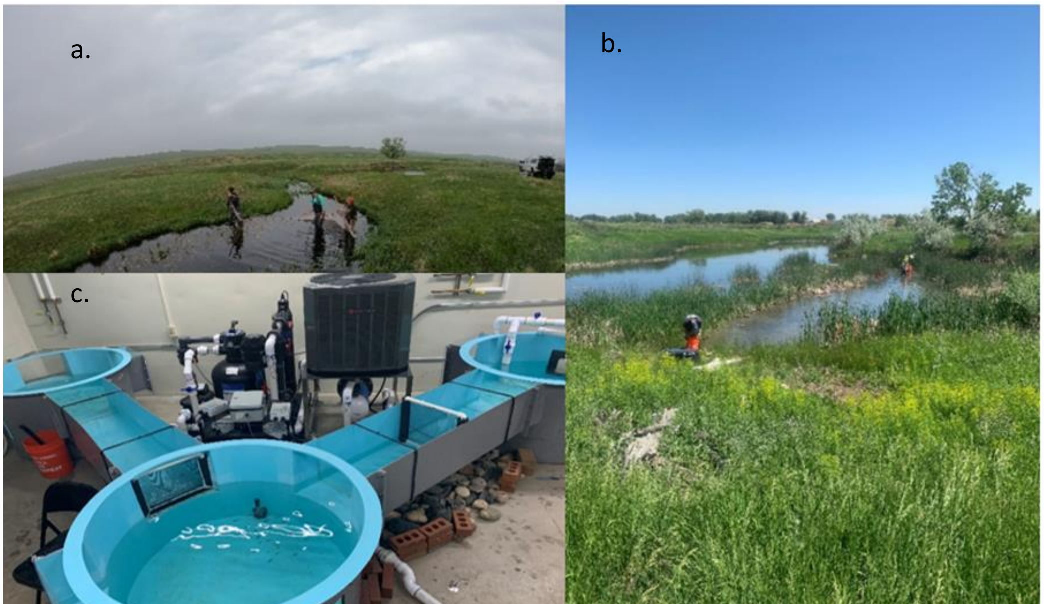

Figure 2. (a) Collection of Plains Topminnow from Pawnee National Grasslands, (b) Mosquitofish collection from Running Deer Natural Area, (c) An experimental stream unit at Colorado State University

We set up three replicated experimental stream units, with each unit composed of two circular tanks (i.e., simulating pools) connected by a straight corridor (Fig. 3). Water recirculated through each mesocosm consisted of the upstream pool having a faster water velocity and the downstream pool having a slower water velocity. The chiller/heater unit controlled water temperature and we maintained three temperatures of 16^{o}, 22^{o}, and 28^{o} C. Fish were exposed to a photoperiod of 14 hours. Lighting for the lab turned on at 6 am at the lowest color temperature (2700K) and brightness (1%). It then proceeded in 5-minute increments increasing in approximately 10% steps of color and brightness until it reached a maximum color temperature (6500K) and brightness (100%- 50 footcandles) at 7 am. This max color temperature and brightness was maintained from 7am to 9pm. At 9 pm, the reverse process happened in 5-minute increments until total darkness was achieved at 10 pm.

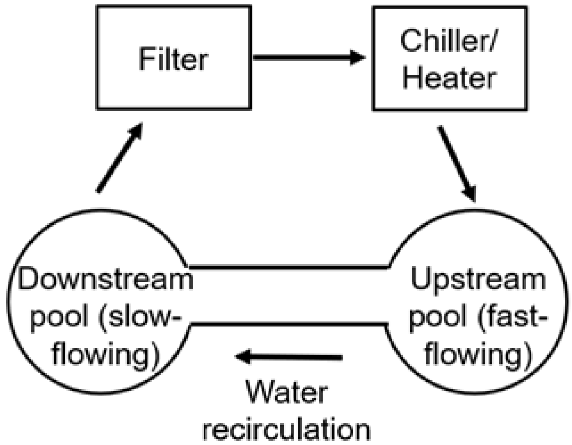

Figure 3. A simplified sketch of the mesocosm. Velocity is faster in the upstream pool due to upwelling. Pools are 3 feet in diameter, connected by a 4-feet straight corridor.

We conducted a 9-week experiment to record fish habitat choices, (1) when mosquitofish alone (allopatric) were housed in the mesocosm at different densities (40, 60, 80, 100, and 120 individuals), (2) when topminnow alone (allopatric) were housed in the mesocosm at different densities (40, 60, 80, 100, and 120 individuals), and (3) when the two species were housed together (sympatric) at different total densities (40, 60, 80, 100, and 120 individuals) at an equal ratio of MSQ:PTM (i.e. 20 PTM:20MSQ for a total of 40 fish). This set of experiments took a total of 3 weeks requiring fish count by pool 6 times daily from Monday through Friday. We repeated this set of experiments at 3 different temperatures (20^o, 24^o, and 28 °C) for a total of 9 weeks. These temperatures were selected to represent a range of temperatures MSQ and PTM experience in their natural habitat along the lower South Platte River. To this date no study of topminnow thermal preference has been conducted and MSQ thermal preference has ranged 28-31^o C (Bacon *et al.*, 1968; Winkler, 1979). The summer water temperatures along a stretch of the lower South Platte reached an average of 28-30^o C, with a mean yearly temperature of 17.7 C (Watt, 2003). Thus our temperatures of 16^o, 22^o, and 28^o C were selected to represent a range of temperatures that MSQ and PTM would experience naturally over the course of a year.

One additional week of experimentation was spent to examine the behavioral interactions (i.e. fin nipping and chasing) directed from MSQ to MSQ, MSQ to PTM, PTM to MSQ, and PTM to PTM. Observers counted interactions at 10-minute intervals (Fig. 3b). Specifically, we recorded aggressive behavioral interactions from one fish to another. These aggressive actions were chases, bites, jolts/thrusts, and aggressive posturing as described in Matthews and Wong (2015). Any of these four actions were counted as an interaction and the direction of aggression was recorded. Total fish density was increased daily over the course of three days and interactions were recorded at densities of 40, 80, and 120 individuals. All fish were restricted to the downstream (low velocity) pool for the interaction experiment while a different temperature was held constant in each of the three mesocosms (16^o, 22^o, and 28^o C).

Figure 4 (a) Mosquitofish (left) and Plains Topminnow (right). Visual differences can be observed by looking for the light bar found along the dorsal side of the Plains Topminnow and the two light bars behind the head of the Mosquitofish. (b) Thomas Wallace (left) and Kelley Sinning (right), undergraduate research assistants performing counts of fish interactions.

Results and Discussion

<u>Objective 1 & 2: How do temperature and flow mediate competition between mosquitofish and plains topminnow? Does the habitat use depend not only on fish density but also on species composition?</u>

Given that these two species occupy slow-moving plains streams, the allopatric experiments (Fig. 5a) confirmed our hypothesis that both PTM and MSQ prefer the slow-moving downstream pool. This trend was observed across temperatures in the allopatric treatments. Similarly, as densities of fish were increased in allopatry, we observed no changes in the preference for the low velocity habitat for MSQ, but there is a slight decline in the proportion of individuals in the downstream pool as PTM densities increase, indicative of competition due to crowding.

The sympatric experiments (Fig. 5b) showed a change in proportion of PTM in the downstream preferred habitat at both different densities and temperatures. In the coolest treatment (16^{o} C), MSQ were typically located in the downstream pool at the lowest densities. At the highest density, MSQ and PTM exhibit an almost equal proportion of individuals in the upstream and downstream pools, while PTM remained constant. At the middle temperature (22^{o} C), there appeared to be a constant but slightly higher proportion of MSQ in the downstream pool compared to PTM where the proportion remained close to half and half. In the warmest treatment (28^{o} C), the proportion of MSQ and PTM in the downstream pool remained similar for all densities greater than 40 which resulted in more PTM in the downstream pool.

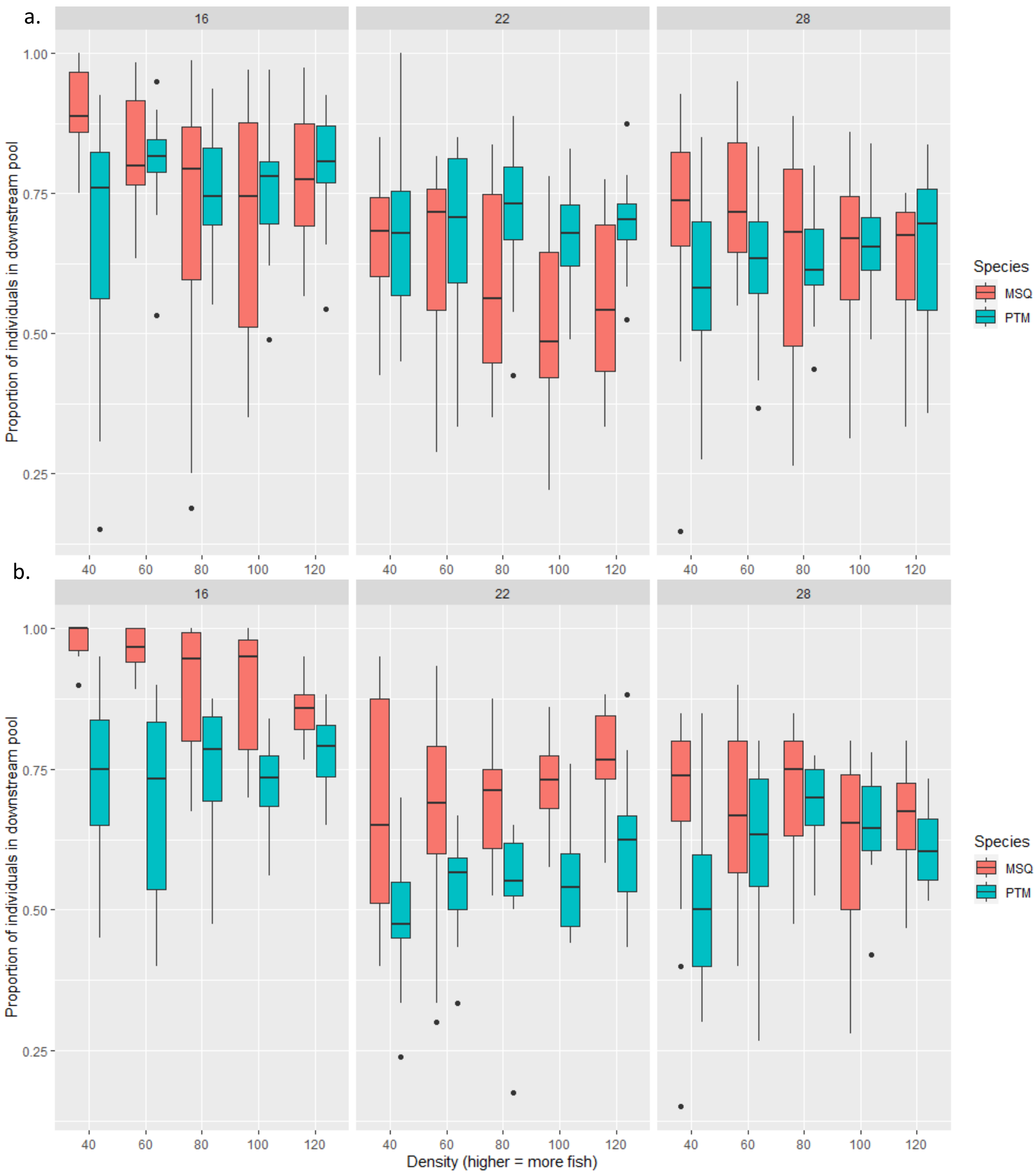

Figure 5. Box and whisker plot showing the proportion of individuals in the upstream high flow pool of MSQ and PTM in allopatry (a) and sympatry (b) at three different temperatures of 16°, 22°, and 28° Celsius. The box shows the interquartile range from first quartile (Q1) to the third quartile (Q3), while the middle hinge indicates the median. The whiskers indicate the maximum and minimum values within 1.5(Interquartile Range) with points outside of the whiskers being outliers, or outside of the 1.5*IQR.*

Objective 3: Is competition (behavioral interactions) stronger between individuals of the same species or different species?

Our results indicated that PTM to PTM interactions were the most frequent interaction at all densities and all temperatures, except for the MSQ to PTM interaction at the warmest temperature (28° C) and lowest density (40). Number of interactions was largely dependent on the temperature, and the coldest treatment (16° C) exhibited the smallest number of interactions relative to total fish. The interactions of PTM to PTM greatly exceeded the interactions of PTM to MSQ suggesting that intraspecific interactions in PTM are stronger than interspecific interactions. The frequency of these interactions decreased with increasing density, suggesting that higher densities of fish may dissuade agonistic interactions between conspecifics and among competitors. Agonistic behaviors are a mechanism that has often been claimed to be a strong factor in the competitive success of MSQ. Previous studies have shown MSQ aggression towards native species in the laboratory (Barrier & Hicks, 1994; Laha & Mattingly, 2007; for topminnows see Sutton *et al.*, 2013). We did observe some agonistic behaviors from MSQ towards PTM in the lowest fish density (40), however these aggressive interactions quickly diminished at larger fish densities. In contrast, we observed PTM exhibiting the most frequent agonistic behaviors at all fish densities, specifically towards conspecifics, suggesting that intraspecific competition was stronger than interspecific competition.

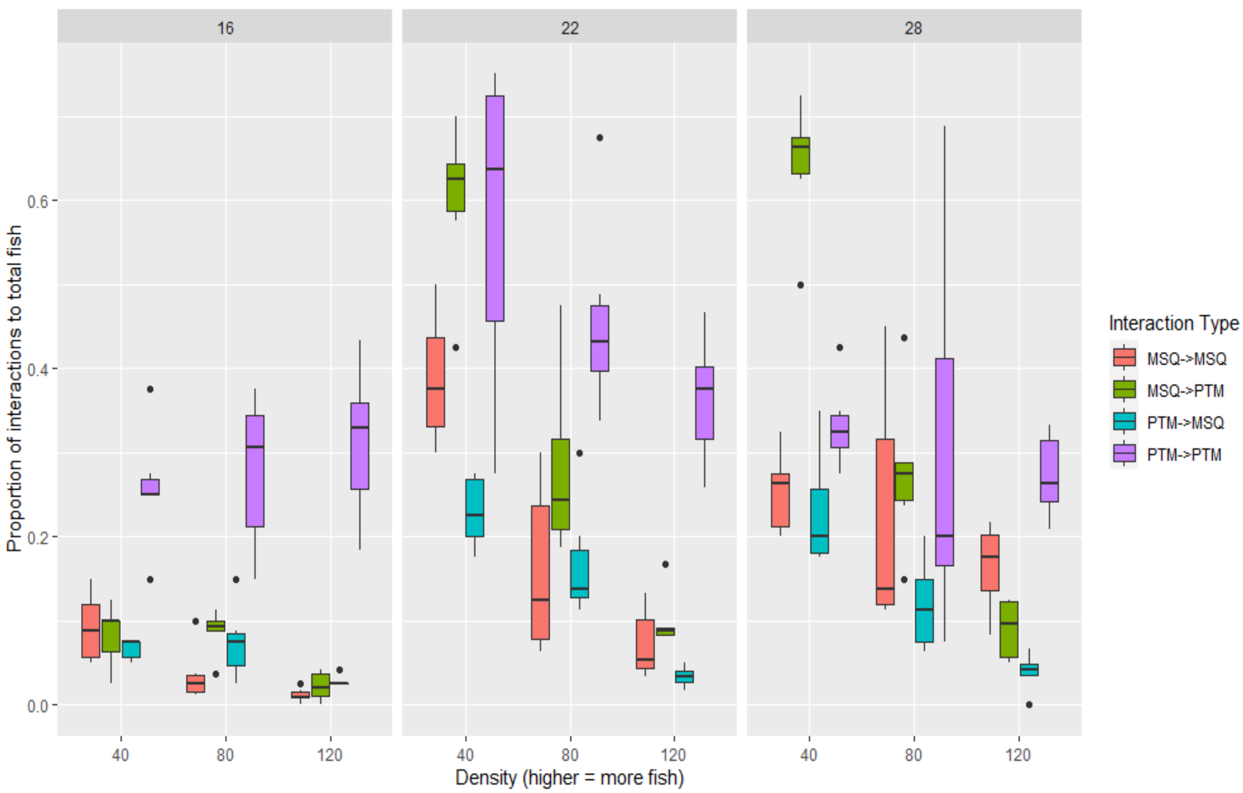

Figure 6. Box and whisker plot showing the proportion of interactions to total number of fish between MSQ and PTM at three different temperatures of 16°, 22°, and 28° Celsius. The box shows the interquartile range from first quartile (Q1) to the third quartile (Q3), while the middle hinge indicates the median. The whiskers indicate the maximum and minimum values within 1.5(Interquartile Range) with points outside of the whiskers being outliers, or outside of the 1.5*IQR.*

We observed changes in fish habitat preference and competition at each of the different temperatures (16º, 22º, and 28º C), flows (high and low), and fish densities (40, 80, 60, 100, and 120) suggesting that habitat preference and competition are mediated by all these factors and that environmental change will likely affect species coexistence. We found that higher densities of fish reduce interspecific interactions and particularly the aggressive tendencies of MSQ towards other species that is so prevalent in the literature was found to be almost entirely absent in this study. Further research on how demographic parameters are affected by these abiotic factors is necessary to determine at what temperatures and flow PTM and MSQ can coexist. The invasion of MSQ provides a stark example of the ongoing need for prevention, early detection, and rapid response to freshwater invaders. This study demonstrates that habitat choice and competition depend on temperature, flow, density, and that furthering our understanding of these dynamics can untangle the complexities of coexistence between MSQ and PTM, assisting in native species conservation.

Acknowledgement

We are grateful for the financial assistance provided by the Audubon Society of Greater Denver through the Lois Webster Fund. We would like to thank Matt Fairchild at the US Forest Service Boyd Wright at Colorado Parks and Wildlife, and Aran Meyer from the City of Fort Collins Natural areas for assisting with the logistics for fish collection. Kelley Sinning and Thomas Wallace, undergraduate students at Colorado State University, played a critical role in collecting data. Experimental data and fish were collected in accordance with a protocol (#1573) approved by the Institutional Animal Care and Use Committee, Colorado State University.

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