

THESIS

AN EXPERIMENTAL TEST OF INTRA- AND INTER-SPECIFIC COMPETITION
BETWEEN INVASIVE WESTERN MOSQUITOFISH (*GAMBUSIA AFFINIS*) AND
NATIVE PLAINS TOPMINNOW (*FUNDULUS SCIADICUS*)

Submitted by

Samuel Lewis

Department of Fish, Wildlife, and Conservation Biology

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Fall 2022

Master's Committee:

Advisor: Yoichiro Kanno

Jonathan D. Salerno

John S. Sanderson

Daniel L. Preston

Copyright by Samuel Timothy Lewis 2022

All Rights Reserved

ABSTRACT

AN EXPERIMENTAL TEST OF INTRA- AND INTER-SPECIFIC COMPETITION BETWEEN INVASIVE WESTERN MOSQUITOFISH (*GAMBUSIA AFFINIS*) AND NATIVE PLAINS TOPMINNOW (*FUNDULUS SCIADICUS*)

1. Invasive species are a major threat to freshwater conservation. Species coexistence in invaded habitats depends on the relative strength of intra- versus inter-specific competition, where inter-specific competition from invasive species to native species is often stronger than intra-specific competition, jeopardizing their coexistence.

2. In this study, I conducted a laboratory experiment to test for the relative competitive strength between native plains topminnow (*Fundulus Sciadicus*) and invasive western mosquitofish (*Gambusia affinis*) at 16, 22, and 28 °C. Data were analyzed using the isodar theory, which assumes that animals are ideally distributed to maximize their fitness and thus their distributions measure the quality and quantity of habitat patches. This was supplemented by behavioral observations of intra- and inter-specific competition.

3. Contrary to my predictions, I did not find evidence that competition was asymmetrical from the invasive mosquitofish to the native plains topminnow. Instead, more individuals occupied their shared preferred habitat (a slow-moving pool) in sympatry compared to allopatry, and the isodar analysis demonstrated that intra-specific competition was significantly stronger than inter-specific competition at all temperature levels. This analysis of habitat selection was corroborated by behavioral observations that aggression was most frequent between plains topminnow in sympatry.

4. This study indicates that aggression might not always be the key mechanism of invasion, even for one of the most successful aquatic invasive species widely known for their aggressive behavior. In mosquitofish, other ecological traits such as fast reproductive cycle and tolerance to a wide range of environmental conditions might be responsible for their invasion success globally. This suggests that mosquitofish may not be a driver in native species declines, but rather an opportunistic invader in degrading ecosystems.

ACKNOWLEDGEMENTS

I would like to thank my advisor Yoichiro Kanno for his guidance in experimental planning, writing, editing and overall support. My committee members Jon Salerno, John Sanderson, and Dan Preston have been instrumental in expanding the scope of my research topics. This study would not have been possible without the financial support of the Colorado Water Conservation Board. I want to thank Harry Crockett, Matthew Fairchild, Ryan Fitzpatrick, Aran Meyer, and Boyd Wright for their logistical support for fish collections in the field. Audrey Harris, Kelley Sinning, George Valentine, and Thomas Wallace provided field and laboratory assistance. Finally, I want to thank my wife Megan for her support in the pursuit of my degree.

TABLE OF CONTENTS

ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	iv
LIST OF TABLES.....	vi
LIST OF FIGURES.....	vii
Chapter 1 - An experimental test of intra- and inter-specific competition between invasive western mosquitofish (<i>Gambusia affinis</i>) and native plains topminnow (<i>Fundulus Sciadicus</i>).....	1
Introduction.....	1
Materials and Methods.....	4
Theoretical framework.....	4
Experimental stream units.....	6
Fish collection.....	8
Data collection.....	9
Data analysis.....	11
Isodar models.....	11
Behavioral aggression.....	13
Results.....	14
Habitat use.....	14
Isodar slopes.....	15
Coefficients of competition.....	15
Behavioral aggression.....	16
Discussion.....	18
REFERENCES.....	23
APPENDIX.....	31

LIST OF TABLES

Table 1 – Isodar coefficients of competition	16
Table S1 – ANOVA results for habitat preference	31
Table S2 – ANOVA results for behavioral aggression.....	32

LIST OF FIGURES

Figure 1 – Theoretical framework of isodar theory	6
Figure 2 – Experimental stream diagram.....	9
Figure 3 – Isodar slopes	15
Figure 4 – Boxplot of fish aggressions	17
Figure S1 – Species comparison.....	33
Figure S2 – Damage from fin nipping.....	33
Figure S3 – Boxplots of fish habitat use.....	34
Figure S4 – Density of Posterior Distributions for α	35
Figure S5 – Density of Posterior Distributions for β	36
Figure S6 – Density of Posterior Distributions for b	37

AN EXPERIMENTAL TEST OF INTRA- AND INTER-SPECIFIC COMPETITION BETWEEN INVASIVE WESTERN MOSQUITOFISH (*GAMBUSIA AFFINIS*) AND NATIVE PLAINS TOPMINNOW (*FUNDULUS SCIADICUS*)

Introduction

Biological invasions are a major threat in many ecosystems with deleterious effects to biodiversity and ecosystem function (Mack et al. 2000). Freshwater ecosystems are not only especially susceptible to invaders (Strayer 2010) but have suffered greatly from introductions of non-native species as fishes are the most introduced aquatic animals globally (Gozlan et al. 2010). Introduced species have been cited as a causal factor of 68% of freshwater fish extinctions in North America (Miller et al. 1989) and the cause for 21% of declines in aquatic species richness globally (Mollot et al., 2017). Aquatic invasive species affect native species via multiple mechanisms such as predation (Zaret and Paine 1973), hybridization (Huxel 1999), and disease transmission (Peeler et al. 2011), but competition is a common mechanism where invasive species outcompete native species for habitat or food resources (Carmona-Catot et al., 2013). These biological interactions are often asymmetrical from invasive species to native species (Riley et al., 2008), jeopardizing their coexistence in local aquatic habitats.

Relative strength of intra- versus inter-species competition is a key factor in determining whether ecologically similar species competing for resources can coexist. Species coexistence is facilitated when intra-specific competition is stronger than inter-specific competition (Chesson 2000). This “stabilizing” mechanism allows species to recover from low abundance but also limit their population growth (Chesson 2000). In less common cases, species may coexist when they are ecologically equivalent, or when intra- and inter-specific competition is equally strong, because survival of individuals then depends on stochastic factors without favoring one species

over the other in a systematic manner (McPeck & Siepielski 2019; Fausch et al. 2021). Species don't coexist when inter-species competition is stronger than intra-specific competition, unless a competitively inferior species has mechanisms to overcome this disadvantage such as higher fecundity or immigration rates ("equalizing mechanism"; Chesson 2000). Accordingly, characterizing competition of sympatric species is critical for advancing our understanding in invasion ecology. However, rigorous tests of intra- versus inter-specific competition are few between aquatic invasive and native species (Britton et al., 2017), with even less examining the influence of abiotic factors on these mechanisms (Carmona-Catot et al., 2013).

Western mosquitofish (MSQ), *Gambusia affinis*, is a small-bodied fish (maximal length of females = 7 cm and males = 4 cm) that since the early 1900's has been introduced as a biological mosquito control agent on every continent except Antarctica (Pyke, 2008). Native to the Mississippi River basin, MSQ thrive in slow moving pools of rivers and streams (Krumholz 1948). Studies have revealed the negative effects of MSQ on a variety of taxa ranging from small native fish (Pasbrig 2010), amphibians (Goodsell and Kats 1999) and invertebrates (Leyse et al. 2004). Given their major ecological impacts globally, they are listed as one of IUCN's 100 worst invasive alien species in the world (Simberloff and Rejmanek, 2019). The early maturation and high fecundity of female MSQ (Vondracek et al., 1988) allow them to proliferate once introduced in a new habitat. Their establishment is facilitated as MSQ outcompete many small-bodied native fish species through niche overlap and through its tolerance of a wide range of abiotic conditions. In addition, much of its success as an invader has been attributed to MSQ's fierce competitive aggression through interference competition (Meffe, 1985; Shaefer et al., 1994; Laha & Mattingly, 2006; Thompson et al., 2012; Sutton et al., 2013).

Mosquitofish have been documented in Colorado's eastern plains stream ecosystems since the 1990's and have greatly expanded its range encroaching on the pools used by many native eastern plains fish. Colorado's eastern plains stream ecosystems are highly productive with lots of aquatic vegetation and have historically supported diverse communities of native fish serving as a crucial aquatic food source for many terrestrial organisms (Fausch and Bestgen 1997). However, extreme temperatures changes, increasing salinity concentrations, and periodic and long-term drying (Dodds et al., 2004), coinciding with the introduction of invasive species has seen marked declines in many of Colorado's native plains fish. The conservation of these species has been further complicated by the intense competition for water between ecological and anthropogenic uses facilitated by decreased water availability (Perkin et al., 2017). The proliferation of introduced species, decreases in water availability, and declines in native fish species necessitates research that considers the complexities of changing abiotic factors.

The native plains topminnow (PTM), *Fundulus Sciadicus*, have declined in their distributions and abundance in areas that overlap with MSQ expansion causing their listing as a tier 1 species of greatest conservation need in Colorado. Declines in PTM have been attributed to habitat fragmentation, degradation of habitat quality, and introduction of nonnative species (Pasbrig et al., 2012). Marked declines have been observed in almost every instance where MSQ have been introduced and little is understood about what mechanism is driving these declines in Colorado (Pasbrig et al., 2012). Temperature and hence altitude seems to mediate competition among cyprinids (Taniguchi et al., 1998) and there are streams along in Colorado's South Platte basin where PTM populations persist and coexist with MSQ. It is necessary to study the abiotic factors such as temperature that may play a role in mediating coexistence.

In this study, I used a laboratory experiment to (1) determine habitat preference of PTM and MSQ between two different habitats (high and low velocity) in allopatry. Due to both PTM and MSQ's preference of slow-moving pools I expect their preferences to be similar in allopatry. I then (2) identified how habitat selection shifted when in sympatry with response to the strength of inter- and intra-specific interference competition, to identify any displacement that may be occurring due to MSQ. I (3) calculated the coefficients of competition for MSQ and PTM in sympatry using an isodar analysis and evaluated how different temperatures influenced these competitive effects. Finally, I tested (4) how the behavioral aggressions of MSQ and PTM in sympatry were influenced by temperature and abundance.

Materials and Methods

Theoretical framework

I tested the relative strength of intra- and inter-specific competition between native PTM and non-native MSQ based on the isodar theory (Morris 1988), which assumes that animals are spatially distributed to maximize their fitness and thus their distributions reflect differences in habitat quantity or quality (i.e., ideal free distributions). In a single-species, two-habitat system composed of a less suitable Habitat 1 and a more suitable Habitat 2, isodars are lines of equal fitness between the two habitats and are represented by linear regression:

$$N_{A,2} = C + b N_{A,1} \quad (\text{Eq. 1})$$

where $N_{A,1}$ and $N_{A,2}$ are the count of species A in Habitat 1 and 2, respectively, with an isodar intercept C and slope b . The isodar analysis can be extended to sympatric situations (Morris 1988), and isodar regression in a two-species, two-habitat is represented by:

$$N_{A,2} = C - \alpha N_{B,2} + b (N_{A,1} + \beta N_{B,1}) \quad (\text{Eq. 2})$$

where α is the average competitive effect of one individual of Species B on A in Habitat 1 and β is the same effect in Habitat 2. The former is of particular interest because it measures the competitive effect in the more suitable habitat patch. The isodar theory was originally developed in systems where animals compete for resources and habitats via exploitation (Morris 1988) but has since been applied to those involving interference competition including stream fish assemblages (Morita et al. 2004).

In addition, I applied the single species isodar approach (Eq. 1) in a unique manner by pooling counts of both species in each habitat to test for the strength of intra- and inter-specific competition (Figure 1). Assume that an isodar has been developed for a single-species, two-habitat system (i.e., allopatry). Furthermore, assume a sympatric situation, in which half of the population has been replaced by individuals of a second species. Here, total counts of individuals are constant between allopatry and sympatry to isolate the effects of intra- versus inter-specific competition from those due to changes in total counts (Fausch 1988). If intra-specific competition is stronger than inter-specific competition, replacement by individuals of a second species would relax overall competition and more individuals would occupy the suitable habitat patch. As a result, the isodar would shift upward in sympatry, relative to allopatry (Figure 1a). In contrast, if intra-specific competition is weaker than inter-specific competition, fewer proportions of individuals would occupy the suitable habitat patch and the isodar would shift downward in sympatry (Figure 1c). A third scenario is when intra- and inter-specific competition is equally strong, and isodars would not shift (Figure 1b). Since the isodar intercepts represent the abundance of individuals that the preferred habitat can accommodate, if both species prefer the same habitat we can infer competition by focusing on the isodar slopes. This framework allows one to then statistically compare isodar slopes between allopatry and sympatry to infer the

relative strength of intra- and inter-specific competition. This framework was used in my study by comparing allopatric and sympatric isodars of native PTM and non-native MSQ in two-habitat systems, where one habitat simulated a slow-velocity plains stream (i.e., a more suitable habitat) and the other a more turbulent stream (i.e., a less suitable habitat) with the minimum cost of movement between the two systems.

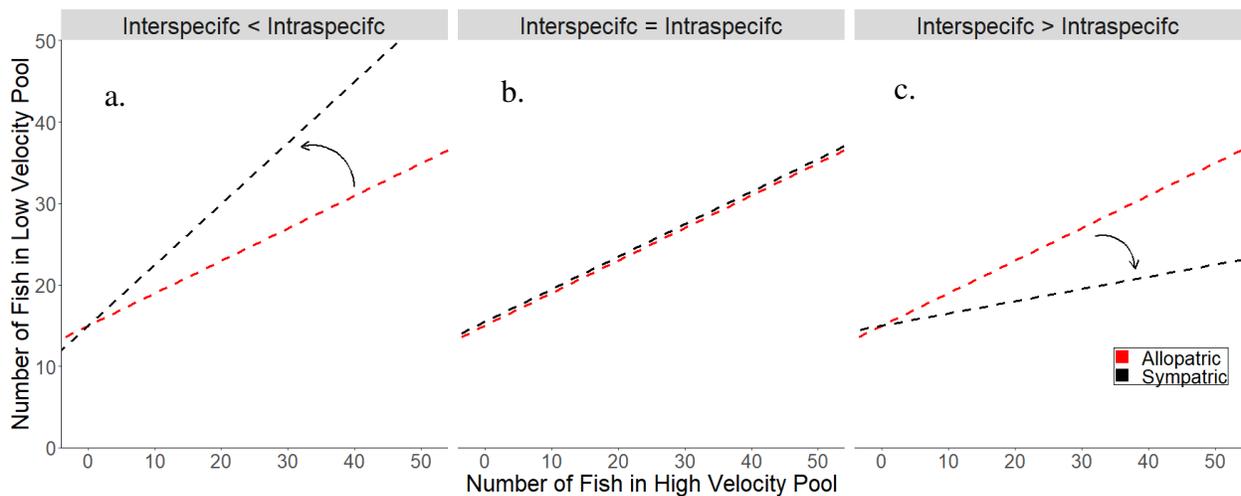


Figure 1. Diagram representing the expected shift in isodar slopes when a second species is introduced dependent on the strength of competition. If intra-specific competition is stronger than inter-specific competition, addition, and replacement by individuals of a second species would relax overall competition allowing more individuals to occupy the suitable habitat shifting sympatric isodar down, relative to allopatry (Figure 1a). In contrast, intra-specific competition is weaker than inter-specific competition, the isodar would shift downward in sympatry (Figure 1c). If intra- and inter-specific competition is equally strong the isodars would not shift (Figure 1b).

Experimental stream units

Three replicates of recirculating experimental stream units were constructed to evaluate competition between MSQ and PTM in the Anatomy/Zoology Building on the Colorado State University main campus, Fort Collins, Colorado. An experimental stream unit consisted of three fiberglass circular tanks (“pools”, Model FCT-235) and two straight corridors (Red Ewald LLC, Karnes City, Texas, USA), a bead filtration system equipped with UV sterilizers (Model 930084,

Pentair Aquatic Eco-Systems Inc., Apopka, Florida, USA), and an air-cooled heat pump for water temperature control (Model Titan HP-2, Aqua Logic Inc., San Diego, California, USA). Pools were 122 cm in diameter, with water filled up to approximately 60 cm deep. Corridors were 180 cm long, 43 cm wide, and 15 cm deep. Each experimental stream unit recirculated approximately 2,300 liters of water.

Three experimental temperatures (16, 22, and 28 °C) were selected to represent a range of temperatures MSQ and PTM experience in their natural habitat along the lower South Platte River over the course of a year. The summer water temperatures along a stretch of the lower South Platte reach an average of 28-30° C, with a mean yearly temperature of 17.7° C (Watt, 2003). To this date no study of PTM thermal preference has been conducted, but the water temperature MSQ congregate in has been largely dependent on the source of the fish with a range of 28-31° C (Winkler, 1979). Water temperature in experimental units was monitored hourly using loggers (U22-001, HOBO Onset Computer Corp.) during the study period, and tank temperature remained consistent over time at 16.0 °C (SD = 0.20) for the 16 °C experiment, 21.7 °C (SD = 0.24) for the 22 °C experiment, and 27.2 °C (SD = 0.37) for the 28 °C experiment. 28 °C was the maximum capacity of the water heaters and was thus selected to represent the maximum temperature effect for this study.

Lights in the room were automated to simulate a summer photoperiod. The sunrise started at 6 am daily at the lowest color temperature (2,700 K) and brightness (1 %), and color and brightness increased by approximately 10 % in 5-minute increments until they reached maximum color temperature (6,500 K) and brightness (100 %, or fifty foot-candles) at 7 am. The maximum color temperature and brightness was maintained from 7 am until 9 pm. The sunset started at 9 pm and lasted until 10 pm by decreasing color and brightness by approximately 10 % every 5

minutes. The room was dark between 10 pm and 6 am. All data collections were conducted during the day-time hours (7 am – 5 pm).

Fish collection

Because MSQ and PTM rarely coexist in high abundance, they were collected from two separate locations in Colorado. Mosquitofish were collected from Running Deer Natural Area in Fort Collins, and PTM were collected from Pawnee National Grassland from May through July of 2021. Fish were collected using seine and dip nets. I collected 550 individuals of MSQ and 450 individuals of PTM, and their body size was matched as much as possible to remove body size effects on the outcome of inter-specific competition. Female MSQ were collected because males are smaller than females in this species. Based on measurements of randomly selected individuals, total length of MSQ (mean = 43 mm, SD = 3.6) was significantly smaller than that of PTM in my study (mean 47 mm, SD = 7.0) (t-test: $t = -3.042$, $df = 59.14$, $P = 0.003$).

However, the difference in mean total length was less than 10% of the mean total length of the smaller species, MSQ. In addition, weight did not differ significantly between MSQ (mean = 1.03 g, SD = 0.30) and PTM (mean = 0.99 g, SD = 0.38) ($t = 0.829$, $df = 69.49$, $P = 0.41$).

Upon collection in the field, fish were immediately transported in oxygenated and insulated coolers to the experimental stream units on the Colorado State University main campus, where fish were immersed in a formalin solution for one hour to treat parasites. Fish were then acclimated to experimental streams for 36 hours preceding experimentation and housed in the downstream most pool of the experimental set up separated with a permanent barrier from the experimental pools (Fig 2). This restricted access to the most downstream pool for the duration of the experiments, creating a two-habitat system composed of an upstream, higher velocity pool, with an average water column flow of 0.3 m/s and a downstream, lower

velocity, pool with 0 m/s. The higher velocity pool was maintained using an upwelling of recirculated water introduced at the head of the high velocity tank.

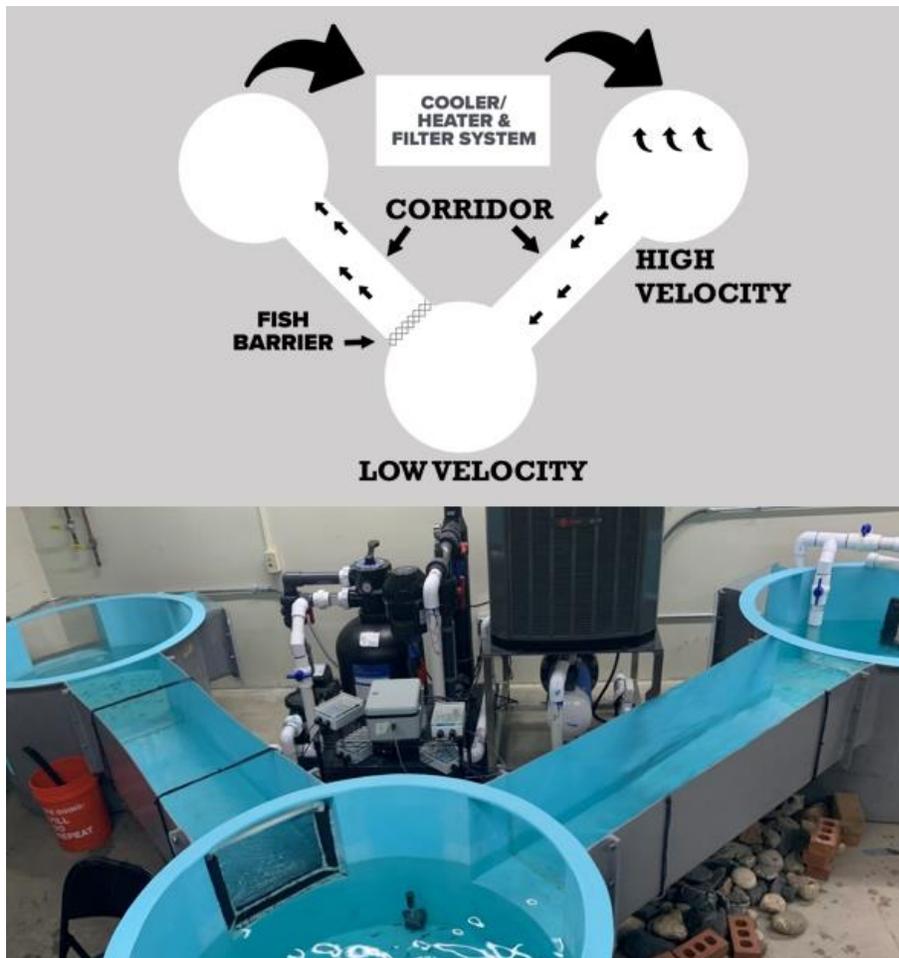


Figure 2. Diagram (A) and photograph (B) of a recirculating experimental stream unit with arrows indicating the flow direction. Fish were counted in each pool six times daily to test for intra- and inter-specific competition using the two-habitat isodar theory.

Data collection

I conducted a nine-week experiment between May and July 2021 to record fish habitat choices between hypothesized preferred low-velocity pools and less preferred high velocity pools when (1) MSQ alone (allopatric) were housed in the experimental units at different abundances (40, 60, 80, 100, and 120 individuals), (2) when PTM alone (allopatric) were housed in the

experimental units at these different abundances, and (3) when the two species were housed together (sympatric) at different total abundances (40, 60, 80, 100, and 120 individuals) at an equal ratio between PTM and MSQ. Data were collected for three weeks for each patry scenario by randomly assigning a temperature level (16, 22, or 28 °C) to each of three experimental units weekly and rotating random temperature assignments, so that each unit was subject to all three temperature levels over three weeks to remove unintended unit effects on habitat choices. Starting on Monday every week, fish abundance was set at 40 total individuals across units and increased by twenty fish daily until a total of 120 individuals were reached on Friday. Water quality was tested every three days to ensure nitrate, nitrite, ammonia, and pH levels were safe for fish, and a third of the water in each experimental unit was changed at the end of the week to maintain tank and fish health.

Six times a day, I counted number of individuals in each pool at 90-minute intervals. A sequence of count collected at the same time of the day from Monday through Friday was treated as a replicate. As a result, I obtained six replicates for isodar regression weekly for three weeks for a total of 18 replicates for each patry scenario, temperature level, and abundance. Before fish were counted, a temporary barrier was placed in the corridor between the two pools so that fish could not move between the two habitats during counting. Observers then counted the number of fish visually without disturbing fish in allopatry, repeating counts three times per pool to ensure accuracy, with an average of the three counts recorded as the observed fish count. In sympatry observers counted fish without disturbance in low abundances (40, 60, and 80 fish), then when identification of species became difficult, observers netted individuals to get an accurate count. For the last count of the day, observers netted all fish to ensure species identification and redistributed in equal densities among the high and low flow velocity pools to expose fish to both

habitats. Fish were fed once daily after the final counts with thawed bloodworms at 10% of total weight of all individuals in each experimental stream. Food was distributed equally between the pools in each unit so as not to provide a spatial cue on food availability and affect fish habitat choice.

One additional experiment was performed that examined behavioral aggressions in sympatry to complement strength of intra- and inter-specific competition inferred from habitat choices. Total fish abundance was increased daily over the course of three days and aggressions were recorded at abundances of 40, 80, and 120 individuals, with a 1:1 ratio of PTM and MSQ. All fish were restricted to the low-velocity pool by barriers for the aggression experiment, and each of the three experimental units was assigned with 16°, 22°, or 28° C over the three-day period. Temperature was held constant in each experimental unit to ensure fish were acclimated to each temperature as to reduce alterations to fish behavior due to rapid temperature change. Observers counted aggressions at 90-minute intervals six times daily for 10-minute durations to record counts of aggressive behaviors from one fish to another. These aggressive actions were chases, bites, jolts/thrusts, and aggressive posturing as described in Matthews and Wong (2015). Observers used step ladders to ensure a view of the entire pool and positioned themselves as far as possible and from the pool while maintaining a view of fish, standing still to reduce behavioral alterations from observer presence. PTM and MSQ were reliably identified from above due to differences in visual characteristics (Fig. S1) as PTM have a light bar located anterior to the dorsal fin and the males have bright orange highlights on their caudal and dorsal fins.

Data analysis

Isodar models

Hierarchical linear regression models were used to compare the relative strength of intra- and inter-specific competition between PTM and MSQ (Figure 1). The response was the count of fish in the preferred low-velocity habitat ($N_{A,2}$) and the predictor was the count of fish in the less preferred high-velocity habitat ($N_{A,1}$) (Eq. 1). In sympatry, fish counts were pooled between the two species in each habitat. Isodar intercepts and slopes (Eq. 1) were inferred by averaging across replicates for allopatric PTM, allopatric MSQ, and sympatric trials at each temperature level. Specifically, I developed models with random intercepts and slopes, where $C_i \sim Normal(\hat{C}, \sigma_C)$ and $b_i \sim Normal(\hat{b}, \sigma_b)$ for i^{th} replicate ($i = 1, \dots, 18$), \hat{C} is the overall intercept, \hat{b} is the overall slope across replicates, σ_C is the intercept standard deviation, and σ_b is the slope standard deviation. At each temperature level, posterior samples of \hat{b} were compared between allopatric PTM and sympatry to evaluate the relative strength of intra-specific competition of PTM versus inter-specific competition, and between allopatric MSQ and sympatry to evaluate the relative strength of intra-specific competition of MSQ versus inter-specific competition (Figure 1). Statistical significance was declared if 95% of the posterior differences in \hat{b} between allopatry and sympatry were above or below 0. Prior to analysis, a small integer value (2) was added to fish count in the high velocity pool ($N_{A,1}$) and low-velocity pool ($N_{A,2}$) to facilitate model convergence.

I further used the two-species, two-habitat isodar model (Eq. 2) to infer the inter-specific competition coefficient in the preferred low-velocity habitat (α) and the less preferred high velocity habitat (β), where the former is of particular interest. My primary interest was to evaluate the competitive effect of invasive MSQ on native PTM, but I evaluated the competitive effect of PTM on MSQ for comparisons. Therefore, two models were developed where the response ($N_{A,2}$) was PTM count in the preferred low-velocity habitat ($N_{A,1}$ = PTM count in high-

velocity pool, $N_{B,1}$ = MSQ count in high-velocity pool, and $N_{B,2}$ = MSQ count in low-velocity pool) in one model (Eq. 2), and the response ($N_{A,2}$) was MSQ count in the preferred low-velocity habitat ($N_{A,1}$ = MSQ count in high-velocity pool, $N_{B,1}$ = PTM count in high-velocity pool, and $N_{B,2}$ = PTM count in low-velocity pool) in the other model. Inter-specific competition coefficients were inferred at each temperature level by again specifying random intercepts and slopes, so that $\alpha_i \sim Normal(\hat{\alpha}, \sigma_\alpha)$ and $\beta_i \sim Normal(\hat{\beta}, \sigma_\beta)$ for i^{th} replicate ($i = 1, \dots, 18$), $\hat{\alpha}$ is the average competitive effect of Species B on A in the high-velocity habitat, $\hat{\beta}$ is the average competitive effect of Species B on A in the low-velocity habitat, and σ_α and σ_β are their respective standard deviation. For model convergence, I used posterior mean values of allopatric isodar intercept (\hat{C}) and slope (\hat{b}) (Eq. 1) in two-species isodar models (Eq. 2). Statistical significance of inter-specific coefficient, $\hat{\alpha}$ and $\hat{\beta}$, was declared if its 95% posterior samples was below 0 (inter-specific competition < intra-specific competition) or above 0 (inter-specific competition > intra-specific competition). Prior to data analysis, I added a small integer value (2) to count of each species in each habitat.

Behavioral aggression

I tested whether frequency of behavioral aggression depended on water temperature and species pair at each abundance level (40, 80, and 120 fish) using two-way Poisson Analysis of Variance (ANOVA) models. The response was count of aggressive behavior per 10 minutes of observations in sympatry, and the predictors were temperature with three levels (16, 22, and 28 °C) and species pair with four levels (MSQ to MSQ, MSQ to PTM, PTM to PTM, and PTM to MSQ). The Poisson distribution was used to model count data and let variance to scale with mean. I considered behavioral aggression significantly different between levels of water

temperature and species pair if 95% of pair-wise differences in posterior samples were smaller or greater than zero.

All models were analyzed with a Bayesian approach using a Markov chain Monte Carlo (MCMC) method in Program JAGS (Plummer 2018) called from Program R (R Core Team 2022) with the jagsUI package. Diffuse priors were used for all parameters. Posterior distributions of parameters were estimated from 35,000 iterations of three chains after a burn-in period of 5,000 iterations and a thinning rate of 10, for a total of 6,000 posterior samples. Model convergence was checked by visually examining plots of the MCMC chains for good mixture as well as ensuring that the R-hat statistic was less than 1.1 for all model parameters (Gelman and Hill 2007).

Results

Habitat Use

As expected for plains fishes occupying sluggish streams, MSQ and PTM used the low-velocity tank more frequently than the high-velocity tank. On average, 51 – 84 % of individuals used the slow-velocity tank in sympatry and allopatry across different fish abundance and temperature levels (Fig. S3). The use of the preferred slow-velocity tank depended on temperature, with proportionately more individuals using this tank at 16 °C compared to 22 and 28 °C, indicating that competition was temperature-dependent (Table S1). Fish abundance (40, 60, 80, 100, 120 individuals per experimental unit) had equivocal effects on habitat use (Table S1).

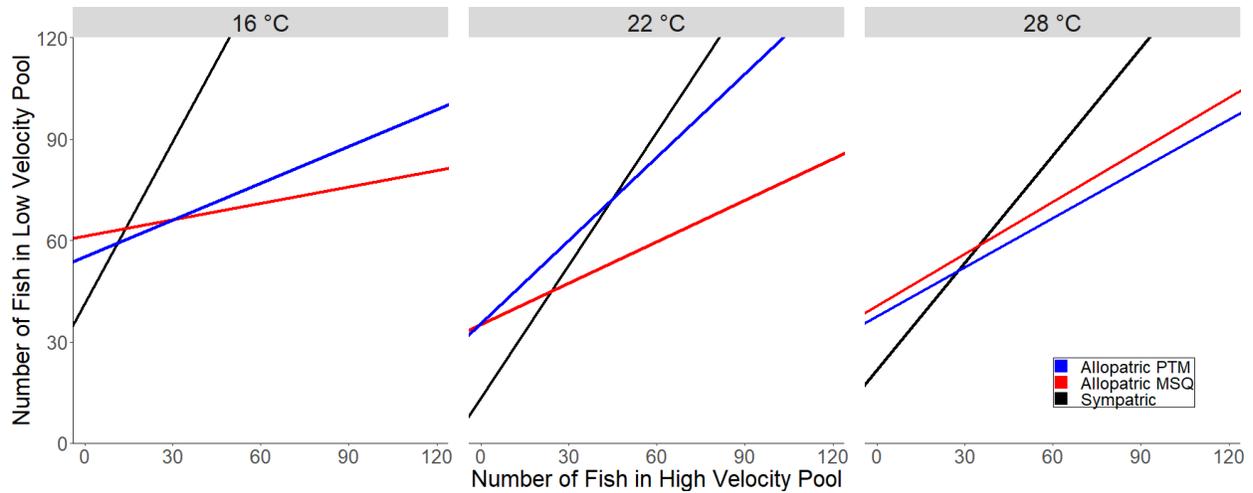


Figure 3. Isodar slope of allopatric PTM (β), in blue, allopatric MSQ (ϕ), in red, and sympatric pooled species (ϵ), in black at the three different experimental temperatures of 16, 22, and 28 degrees Celsius. All isodars indicate a statistically significant (> 95%) difference in the proportion of posterior samples (PPS) between the allopatric slope and the sympatric slope.

Isodar slopes

Intraspecific competition was inferred to be stronger than interspecific competition across all temperatures for both PTM and MSQ when isodar slopes were compared between allopatry and sympatry (Fig. 3). The allopatric slope for PTM ($\hat{\beta}$ in Eq. 1) was 0.34 (95% CI: [-0.05, 0.81]) at 16°C, 0.81 [0.49, 1.20] at 22°C, and 0.48 [0.12, 0.84] at 28°C. The allopatric slope for MSQ ($\hat{\phi}$) was 0.17 (-0.21, 0.60) at 16°C, 0.44 [0.09, 0.81] at 22°C, and 0.54 [0.19, 0.95] at 28°C. The pooled sympatric isodar slope ($\hat{\epsilon}$) was 1.62 [1.14, 2.17] at 16°C, 1.30 [0.91, 1.72] at 22°C, and 1.09 [0.73, 1.44] at 28°C, and was significantly greater than allopatric isodar slopes of PTM and MSQ at all temperatures. Based on my conceptual framework (Fig. 1), my data demonstrated that intraspecific competition was consistently stronger than interspecific competition.

Coefficients of competition

Interspecific competition coefficients in the two species isodar analysis (Eq. 2) similarly showed that intraspecific competition was stronger than interspecific competition in most cases (Table 1). Importantly, in all six cases of interspecific competition in their preferred low-velocity

tanks, 95% CI of interspecific competition coefficients were less than 1, indicating that the average competitive effect of one individual of one species on the other species was smaller than its intraspecific competition. Interspecific competition was significantly weaker than intraspecific competition in four of the six cases in the less preferred high-velocity tanks (Table 1). In the other two cases, 95% CI of interspecific competition coefficients overlapped 1 (i.e., interspecific = intraspecific competition), and these results may be due to imprecise estimates of the coefficients arising from infrequent use of the high-velocity tanks at 16 °C. Specifically, the mean effect of MSQ on PTM ($\hat{\beta}$ in Eq. 2) was 1.57 (95% CI [0.17, 4.74]), and the mean effect of PTM on MSQ was 0.31 [0.01, 1.20] in the low-velocity tanks at 16 °C.

Table 1– Inter-specific competition coefficients between mosquitofish (MSQ) and plains topminnow (PTM) at 16, 22, and 28 °C, based on two-species, two-habitat isodar models. The coefficients represent the average competitive effect of one individual of one species on the other species in faster-moving (upstream) or slower-moving (downstream) pool. Coefficients < 1 indicate that interspecific competition is weaker than intraspecific competition, and those > 1 indicate that interspecific competition is stronger than intraspecific competition. Starred values indicate a statistically significant (> 95%) difference from 1 in the proportion of posterior samples (PPS).

Temperature	$\hat{\alpha}$ [95% CI] Low velocity		$\hat{\beta}$ [95% CI] High velocity	
	MSQ to PTM	PTM to MSQ	MSQ to PTM	PTM to MSQ
16 °C	0.70* [0.58,0.90]	0.59* [0.48,0.71]	1.56[0.17,4.74]	0.31 [0.01,1.20]
22 °C	0.69* [0.58,0.83]	0.23* [0.07,0.40]	0.07* [0.02,0.27]	0.17* [0.01,0.53]
28 °C	0.59* [0.43,0.73]	0.61* [0.46,0.75]	0.26* [0.01,0.70]	0.14* [0.01,0.47]

Behavioral aggressions

Behavioral aggression was most frequent between individuals of PTM, and I did not find evidence that MSQ was more aggressive than PTM (Fig. 4, Table S2). Intraspecific aggression from one individual PTM to another was significantly more frequent than interspecific aggression from MSQ to PTM when abundance per tank was 80 individuals (posterior mean = 0.53 [95% CI: 0.18, 0.87]) and 120 individuals (1.64 [1.29, 2.03]). Damage from fin nipping from conspecifics was prevalent among PTM (Fig. S2). In addition, intraspecific aggression between PTM was significantly more frequent than intraspecific aggression between MSQ when per-tank abundance was 80 individuals (-0.52 [-0.91,-0.73]) and 120 individuals (-1.64 [-2.01,-1.29]). At all abundance levels, behavioral aggression was significantly fewer at 16 °C relative to 22 °C.

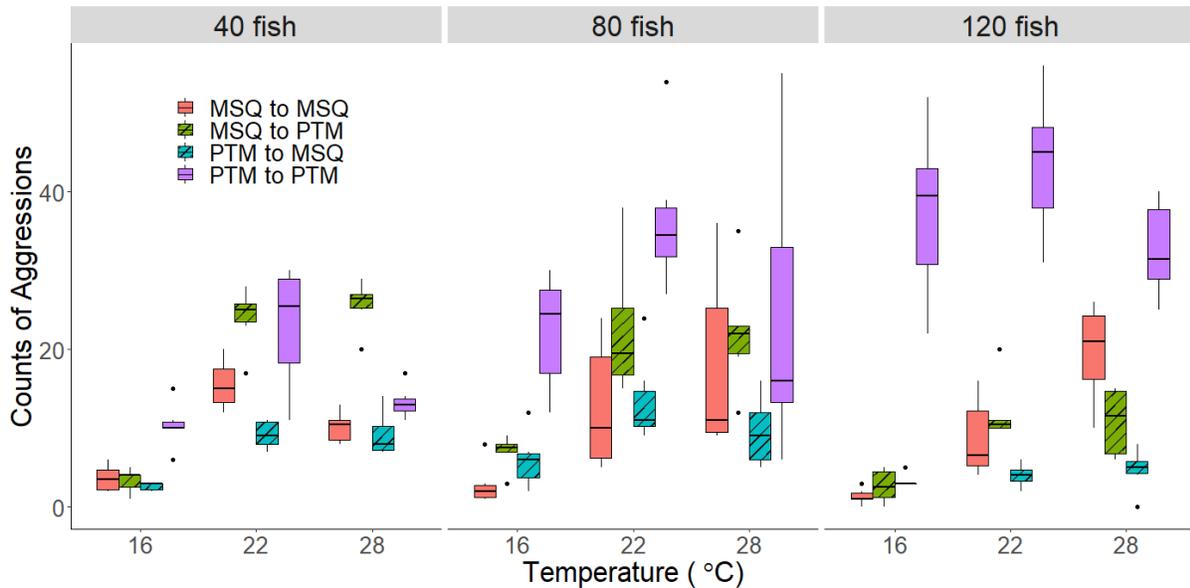


Figure 4. Counts of aggressive behaviors to total fish in sympatry at abundances of 40, 80, and 120 individuals and at the three different experimental temperatures of 16, 22, and 28 degrees Celsius, categorized by aggression type. The line inside the box plot represents the median, the borders represent the interquartile range (IQR), the whiskers represent (+/-) 1.5*IQR while the dots are outliers. Hatched bars indicate interspecific aggressions while solid bars indicate intraspecific aggressions.

Discussion

Mosquitofish's aggression was found to be weaker than intraspecific competition among plains topminnow in both the isodar analyses and behavioral observations, which was supported across three different tests including my theoretical framework, isodar analysis for the coefficients of competition, and the tests of behavioral aggression. My conceptual framework (Fig. 1) which demonstrated that intraspecific competition was consistently stronger than interspecific competition supported these finding first. These results support the practice of pooling species observations to infer overall strength of competition of sympatric species. The two species isodars identified the strength at which intraspecific competition regulates competition in MSQ and PTM populations at varying river velocities and temperature gradients. Finally, the behavioral observations of aggressions provided further support for the strength of PTM intraspecific competition with less chasing and nips occurring at the coldest temperatures which contrasted the increasing MSQ initiated aggressions as temperatures increased. The lack of aggression from MSQ to PTM indicates that other mechanisms (i.e., demography and environmental tolerances) may be responsible for invasive MSQ's success.

Mosquitofish's overwhelming aggression has been well established in experimental laboratory mesocosms (Meffe, 1985; Shaefer et al., 1994; Laha & Mattingly, 2006; Thompson et al., 2012; Sutton et al., 2013), with their strengthened agonistic behaviors documented towards other *Fundulus spp.* and among conspecifics. The striking contrast of my results may be linked to slight differences in my experimental set up which was designed to estimate competitive coefficients of competition among populations of PTM and MSQ. Laha and Mattingly (2006) and Meffe (1985) both recorded aggressions within an hour of introducing a second species and observed a decline in MSQ aggressions over the following 24-hour period. In my experiment

individuals of both species were allowed to cohabitate for 48 hours prior to counts of aggressive behaviors and species habitat preferences, mirroring natural conditions of species coexistence of an established invader. Furthermore, Sutton et al. (2013) observed that while overall aggression of both MSQ and *Fundulus spp.* was increased in instances where vegetation was absent, there was little to no aggression from *Fundulus spp.* in the presence of vegetation. Since my experimental set up simulated the pelagic conditions most common for these species to interact (Casterlin and Reynolds, 1977), my higher aggression rates were as expected. The lack of vegetation and prolonged introductory period in sympatry may provide strong evidence for the results of this study to have strong ecological inference to natural conditions.

The invasive success of MSQ has also been attributed to their widespread introductions (Simberloff and Rejmanek, 2019), reproductive ability (Vondracek et al., 1988), and resilience to changing abiotic factors (Alcaraz et al., 2008). My trial ranges of 40 to 120 fish abundances were selected to analyze the competition of coexisting populations where large numbers of MSQ have been introduced. Many previous aggression studies of MSQ have examined differing ratios of invaders to native fish (Laha & Mattingly 2006, Sutton et al. 2013), however often introduce less than 10 individuals. It is necessary to study both the large abundances, addressed in this study, and simulated varying levels of invaders to capture the nuances that lead to MSQ invasive success. MSQ invasive superiority may be explained further aspects of their demography. Factors such as their multiple spawnings and livebearing young (Vondracek et al., 1988) may facilitate MSQ's invaders as PTM are restricted to a single spawning season with obligate egg laying on aquatic vegetation (Kaufmann and Lynch, 1991). Moreover, the abiotic factors (i.e., temperature and salinity) that MSQ exhibit resilience towards need further study on how they control invasion success. Increasing salinity levels have been found to reduce MSQ aggression

(Alcaraz et al. 2008), but the extent to what levels of salinity many small bodied native fish and MSQ can endure are largely unknown. My study's results on temperature's influence on competition and aggression provide concern for continued exacerbation of intraspecific effects among native fish.

Few studies have provided evidence of coexistence with native small-bodied fishes (Barrier and Hicks, 1994; Magellan and Berthou, 2016) and even less contradict the prevalence of MSQ's aggressive behaviors (Ciepela et al., 2021). Female MSQ may exhibit greater aggression than males, as many studies, including or own, through attempted body size matching exclude the smaller male MSQ (Laha & Mattingly 2006). Exploring this introduced bias may offer insight to how native fish coexist with heterogenous populations of female and male MSQ. Furthermore, identifying instances where MSQ aggression is secondary to intraspecific competition among adult fish may offer insights for specific drivers that can promote the coexistence of MSQ. Artificial refugia can create novel habitat space for cyprinodontoid use, decreasing aggression to conspecifics (Magellan and Berthou, 2016), which could reduce PTM stress by reducing the strength of intraspecific competition. Barrier and Hicks (1994) associated several mechanisms through which demographic asynchrony and resilience to periodic drought conditions have allowed native species to coexist with MSQ. Furthermore, temperature may be an important driver mediating aggressive behaviors as cooler temperatures decreased overall aggressions in my study, while Ciepela et al., (2021) observed condition specific interspecific aggressions were facilitated by thermal preference. Identification of both the demographic parameters and resilience of native species to changing abiotic conditions (i.e., temperature and salinity) is a crucial step in understanding the mechanisms facilitating coexistence.

Native species coexistence with MSQ is further threatened by climate uncertainties that predict flashier flood events increasing river flows (Li et al., 2022) and increasing global surface water temperatures (Barbarossa et al., 2021). The concerns of these issues can be highlighted by the results of this study as I observed increased temperatures caused an increasing trend in MSQ aggressions towards PTM. Future climate scenarios with warmer temperatures could see displacement of fish from their native range as invading MSQ resilient to changes dominate assemblages through increased aggression. Similarly increased flood events and flow velocities (Lennox et al., 2019) could also promote greater interspecific competition from invading MSQ. As observed in the uncertainty around the strength of competition in high flow environments, the $\hat{\beta}$ at the coldest temperatures indicate some evidence for interspecific competition to be more impactful on native fish.

Isodars has been widely applied to examine competition among terrestrial fauna (Ovadia and Abramsky, 1995; Morris et al., 2000, Tarjuelo et al., 2017), however few studies have used this method to quantify competition among fish assemblages (Morita et al., 2004). Even though rivers and streams are continuous, so they can be easily classified into discrete habitat systems, either pools or riffles. This allowed me to estimate coefficients of competition depending on the habitat of interest and tested accordingly using isodar methods. In the laboratory the high and low velocity habitats reflect these natural distinctions found in the riverscape. Some unexpected limitations to the traditional generalized linear model used in isodar methods were experienced in the laboratory setting as issues with a singular fit and Simpson's paradox in the collected habitat use data arose. Both issues were resolved using a hierarchical Bayesian model (Qian et al., 2019; Singmann and Kellen, 2019) that grouped trials together allowing me to identify the

underlying linear associations of the data influenced by temperature and abundance and ultimately quantifying the coefficients of competition.

My study challenges the dominant paradigm that MSQ are a driver of native species decline. Invasive species are widely accepted as one of the leading direct causes of biodiversity loss. However, much of the evidence for this contention is based on simple correlations between exotic dominance and native species decline in degraded systems. My results suggest that MSQ may be better viewed as an opportunistic invader that has proliferated in degraded ecosystems. While the idea of invaders as passengers of change has been documented in plant systems (MacDougall and Turkington, 2005; Bauer 2012) it is novel for the management of MSQ. The combined use of isodar methods with behavioral observations revealed the lack of MSQ aggression towards PTM providing clear inference that coexistence of these species requires restoration for the benefit of native species.

REFERENCES

- Alcaraz, C., Bisazza, A., & García-Berthou, E. (2008). Salinity mediates the competitive interactions between invasive mosquitofish and an endangered fish. *Oecologia*, *155*(1), 205–213. <https://doi.org/10.1007/s00442-007-0899-4>
- Barbarossa, V., Bosmans, J., Wanders, N., King, H., Bierkens, M. F. P., Huijbregts, M. A. J., & Schipper, A. M. (2021). Threats of global warming to the world’s freshwater fishes. *Nature Communications*, *12*(1), Article 1. <https://doi.org/10.1038/s41467-021-21655-w>
- Barrier, R. F. G., & Hicks, B. J. (1994). Behavioral interactions between black mudfish (*Neochanna diversus* Stokell, 1949: Galaxiidae) and mosquitofish (*Gambusia affinis* Baird & Girard, 1854)a. *Ecology of Freshwater Fish*, *3*(3), 93–99. <https://doi.org/10.1111/j.1600-0633.1994.tb00110.x>
- Bauer, J. T. (2012). Invasive species: “Back-seat drivers” of ecosystem change? *Biological Invasions*, *14*(7), 1295–1304. <https://doi.org/10.1007/s10530-011-0165-x>
- Britton, J. R., Ruiz-Navarro, A., Verreycken, H., & Amat-Trigo, F. (2018). Trophic consequences of introduced species: Comparative impacts of increased interspecific versus intraspecific competitive interactions. *Functional Ecology*, *32*(2), 486–495. <https://doi.org/10.1111/1365-2435.12978>
- Carmona-Catot, G., Magellan, K., & García-Berthou, E. (2013). Temperature-Specific Competition between Invasive Mosquitofish and an Endangered Cyprinodontid Fish. *PLOS ONE*, *8*(1), e54734. <https://doi.org/10.1371/journal.pone.0054734>
- Casterlin, M. E., & Reynolds, W. W. (1977). Aspects of habitat selection in the mosquitofish *Gambusia affinis*. *Hydrobiologia*, *55*(2), 125–127. <https://doi.org/10.1007/BF00021053>

- Chesson, P. (2000). General Theory of Competitive Coexistence in Spatially-Varying Environments. *Theoretical Population Biology*, 58(3), 211–237.
<https://doi.org/10.1006/tpbi.2000.1486>
- Ciepiela, L. R., Fitzpatrick, R. M., Lewis, S. T., & Kanno, Y. (2021). Behavioral Interactions between a Native and an Invasive Fish Species in a Thermally Heterogeneous Experimental Chamber. *Fishes*, 6(4), Article 4. <https://doi.org/10.3390/fishes6040075>
- Dodds, W. K., Gido, K., Whiles, M. R., Fritz, K. M., & Matthews, W. J. (2004). Life on the Edge: The Ecology of Great Plains Prairie Streams. *BioScience*, 54(3), 205–216.
[https://doi.org/10.1641/0006-3568\(2004\)054\[0205:LOTETE\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2004)054[0205:LOTETE]2.0.CO;2)
- Fausch, K. D. (1988). Tests of Competition between Native and Introduced Salmonids in Streams: What Have We Learned? *Canadian Journal of Fisheries and Aquatic Sciences*, 45(12), 2238–2246. <https://doi.org/10.1139/f88-260>
- Fausch, K. D., & Bestgen, K. R. (1997). Ecology of Fishes Indigenous to the Central and Southwestern Great Plains. In F. L. Knopf & F. B. Samson (Eds.), *Ecology and Conservation of Great Plains Vertebrates* (pp. 131–166). Springer.
https://doi.org/10.1007/978-1-4757-2703-6_6
- Fausch, K. D., Nakano, S., Kitano, S., Kanno, Y., & Kim, S. (2021). Interspecific social dominance networks reveal mechanisms promoting coexistence in sympatric charr in Hokkaido, Japan. *Journal of Animal Ecology*, 90(2), 515–527. <https://doi.org/10.1111/1365-2656.13384>
- Gelman, A., & Hill, J. (2006). *Data Analysis Using Regression and Multilevel/Hierarchical Models*. Cambridge University Press.

- Goodsell, J. A., & Kats, L. B. (1999). Effect of Introduced Mosquitofish on Pacific Treefrogs and the Role of Alternative Prey. *Conservation Biology*, 13(4), 921–924.
<https://doi.org/10.1046/j.1523-1739.1999.98237.x>
- Gozlan, R. E., Britton, J. R., Cowx, I., & Copp, G. H. (2010). Current knowledge on non-native freshwater fish introductions. *Journal of Fish Biology*, 76(4), 751–786.
<https://doi.org/10.1111/j.1095-8649.2010.02566.x>
- Huxel, G. R. (1999). Rapid displacement of native species by invasive species: Effects of hybridization. *Biological Conservation*, 89(2), 143–152. [https://doi.org/10.1016/S0006-3207\(98\)00153-0](https://doi.org/10.1016/S0006-3207(98)00153-0)
- Kaufmann, S., & Lynch, J. (1991). Courtship, eggs, and development of the plains topminnow in Nebraska (Actinopterygii: Fundulidae). *Prairie Naturalist*, 23(1), 41–45.
- Krumholz, L. A. (1948). Reproduction in the Western Mosquitofish, *Gambusia affinis affinis* (Baird & Girard), and Its Use in Mosquito Control. *Ecological Monographs*, 18(1), 1–43.
<https://doi.org/10.2307/1948627>
- Laha, M., & Mattingly, H. T. (2006). Ex situ evaluation of impacts of invasive mosquitofish on the imperiled Barrens topminnow. *Environmental Biology of Fishes*, 78(1), 1–11.
<https://doi.org/10.1007/s10641-006-9040-5>
- Lennox, R. J., Crook, D. A., Moyle, P. B., Struthers, D. P., & Cooke, S. J. (2019). Toward a better understanding of freshwater fish responses to an increasingly drought-stricken world. *Reviews in Fish Biology and Fisheries*, 29(1), 71–92. <https://doi.org/10.1007/s11160-018-09545-9>
- Leyse, K. E., Lawler, S. P., & Strange, T. (2004). Effects of an alien fish, *Gambusia affinis*, on an endemic California fairy shrimp, *Linderiella occidentalis*: Implications for conservation

of diversity in fishless waters. *Biological Conservation*, 118(1), 57–65.

<https://doi.org/10.1016/j.biocon.2003.07.008>

Li, Z., Gao, S., Chen, M., Gourley, J. J., Liu, C., Prein, A. F., & Hong, Y. (2022). The conterminous United States are projected to become more prone to flash floods in a high-end emissions scenario. *Communications Earth & Environment*, 3(1), Article 1.

<https://doi.org/10.1038/s43247-022-00409-6>

MacDougall, A. S., & Turkington, R. (2005). Are Invasive Species the Drivers or Passengers of Change in Degraded Ecosystems? *Ecology*, 86(1), 42–55. <https://doi.org/10.1890/04-0669>

Magellan, K., & García-Berthou, E. (2016). Experimental evidence for the use of artificial refugia to mitigate the impacts of invasive *Gambusia holbrooki* on an endangered fish.

Biological Invasions, 18(3), 873–882. <https://doi.org/10.1007/s10530-016-1057-x>

Matthews, S. A., & Wong, M. Y. L. (2015). Temperature-dependent resolution of conflict over rank within a size-based dominance hierarchy. *Behavioral Ecology*, 26(3), 947–958.

<https://doi.org/10.1093/beheco/arv042>

McPeck, M. A., & Siepielski, A. M. (2019). Disentangling ecologically equivalent from neutral species: The mechanisms of population regulation matter. *Journal of Animal Ecology*,

88(11), 1755–1765. <https://doi.org/10.1111/1365-2656.13072>

Meffe, G. K. (1985). Predation and Species Replacement in American Southwestern Fishes: A Case Study. *The Southwestern Naturalist*, 30(2), 173–187. <https://doi.org/10.2307/3670732>

Miller, R. R., Williams, J. D., & Williams, J. E. (1989). Extinctions of North American Fishes During the past Century. *Fisheries*, 14(6), 22–38. [https://doi.org/10.1577/1548-](https://doi.org/10.1577/1548-8446(1989)014<0022:EONAFD>2.0.CO;2)

[8446\(1989\)014<0022:EONAFD>2.0.CO;2](https://doi.org/10.1577/1548-8446(1989)014<0022:EONAFD>2.0.CO;2)

- Mollot, G., Pantel, J. H., & Romanuk, T. N. (2017). Chapter Two - The Effects of Invasive Species on the Decline in Species Richness: A Global Meta-Analysis. In D. A. Bohan, A. J. Dumbrell, & F. Massol (Eds.), *Advances in Ecological Research* (Vol. 56, pp. 61–83). Academic Press. <https://doi.org/10.1016/bs.aecr.2016.10.002>
- Morita, K., Tsuboi, J.-I., & Matsuda, H. (2004). The impact of exotic trout on native charr in a Japanese stream. *Journal of Applied Ecology*, *41*(5), 962–972. <https://doi.org/10.1111/j.0021-8901.2004.00927.x>
- Morris, D. W. (1988). Habitat-dependent population regulation and community structure. *Evolutionary Ecology*, *2*(3), 253–269. <https://doi.org/10.1007/BF02214286>
- Morris, D. W., Davidson, D. L., & Krebs, C. J. (2000). Measuring the ghost of competition: Insights from density-dependent habitat selection on the co-existence and dynamics of lemmings. *Evolutionary Ecology Research*, *2*(1), 69–80.
- Ovadia, O., & Abramsky, Z. (1995). Density-Dependent Habitat Selection: Evaluation of the Isodar Method. *Oikos*, *73*(1), 86–94. <https://doi.org/10.2307/3545729>
- Pasbrig, C. A., Koupal, K. D., Schainost, S., & Hoback, W. W. (2012). Changes in range-wide distribution of plains topminnow *Fundulus sciadicus*. *Endangered Species Research*, *16*(3), 235–247. <https://doi.org/10.3354/esr00400>
- Peeler, E. J., Oidtmann, B. C., Midtlyng, P. J., Miossec, L., & Gozlan, R. E. (2011). Non-native aquatic animals introductions have driven disease emergence in Europe. *Biological Invasions*, *13*(6), 1291–1303. <https://doi.org/10.1007/s10530-010-9890-9>
- Perkin, J. S., Gido, K. B., Falke, J. A., Fausch, K. D., Crockett, H., Johnson, E. R., & Sanderson, J. (2017). Groundwater declines are linked to changes in Great Plains stream fish

- assemblages. *Proceedings of the National Academy of Sciences*, 114(28), 7373–7378.
<https://doi.org/10.1073/pnas.1618936114>
- Qian, S. S., Stow, C. A., Nojavan A., F., Stachelek, J., Cha, Y., Alameddine, I., & Soranno, P. (2019). The implications of Simpson’s paradox for cross-scale inference among lakes. *Water Research*, 163, 114855. <https://doi.org/10.1016/j.watres.2019.114855>
- Riley, L. A., Dybdahl, M. F., & Hall, R. O. (2008). Invasive species impact: Asymmetric interactions between invasive and endemic freshwater snails. *Journal of the North American Benthological Society*, 27(3), 509–520. <https://doi.org/10.1899/07-119.1>
- Russell, J. C., Sataruddin, N. S., & Heard, A. D. (2014). Over-invasion by functionally equivalent invasive species. *Ecology*, 95(8), 2268–2276.
- Schaefer, J. F., Heulett, S. T., & Farrell, T. M. (1994). Interactions between Two Poeciliid Fishes (*Gambusia holbrooki* and *Heterandria formosa*) and Their Prey in a Florida Marsh. *Copeia*, 1994(2), 516–520. <https://doi.org/10.2307/1447002>
- Simberloff, D., & Rejmanek, M. (Eds.). (2019). 100 of the World’s Worst Invasive Alien Species: A Selection From The Global Invasive Species Database. In *Encyclopedia of Biological Invasions* (pp. 715–716). University of California Press.
<https://doi.org/10.1525/9780520948433-159>
- Singmann, H., & Kellen, D. (2019). An Introduction to Mixed Models for Experimental Psychology. In *New Methods in Cognitive Psychology* (pp. 4–31). Routledge.
- Strayer, D. L. (2010). Alien species in fresh waters: Ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology*, 55(s1), 152–174.
<https://doi.org/10.1111/j.1365-2427.2009.02380.x>

- Sutton, T. M., Zeiber, R. A., & Fisher, B. E. (2013). Agonistic behavioral interactions between introduced western mosquitofish and native topminnows. *Journal of Freshwater Ecology*, 28(1), 1–16. <https://doi.org/10.1080/02705060.2012.688492>
- Taniguchi, Y., Rahel, F. J., Novinger, D. C., & Gerow, K. G. (1998). Temperature mediation of competitive interactions among three fish species that replace each other along longitudinal stream gradients. *Canadian Journal of Fisheries and Aquatic Sciences*, 55(8), 1894–1901. <https://doi.org/10.1139/f98-072>
- Tarjuelo, R., Traba, J., Morales, M. B., & Morris, D. W. (2017). Isodars unveil asymmetric effects on habitat use caused by competition between two endangered species. *Oikos*, 126(1), 73–81. <https://doi.org/10.1111/oik.03366>
- Thompson, K. A., Hill, J. E., & Nico, L. G. (2012). Eastern mosquitofish resists invasion by nonindigenous poeciliids through agonistic behaviors. *Biological Invasions*, 14(7), 1515–1529. <https://doi.org/10.1007/s10530-012-0176-2>
- Vondracek, B., Wurtsbaugh, W. A., & Cech, J. J. (1988). Growth and reproduction of the mosquitofish, *Gambusia affinis*, in relation to temperature and ration level: Consequences for life history. *Environmental Biology of Fishes*, 21(1), 45–57. <https://doi.org/10.1007/BF02984442>
- Watt, J. T. (2002). *Water quality changes at a streamflow augmentation project, Lower South Platte River, Colorado* [Text, Colorado State University]. <https://mountainscholar.org/handle/10217/1838>
- Winkler, P. (1979). Thermal Preference of *Gambusia affinis affinis* as Determined under Field and Laboratory Conditions. *Copeia*, 1979(1), 60–64. <https://doi.org/10.2307/1443729>

Zaret, T. M., & Paine, R. T. (1973). Species Introduction in a Tropical Lake. *Science*, 182(4111), 449–455. <https://doi.org/10.1126/science.182.4111.449>

APPENDIX

Table S1 - Effects parameterization for two-way binomial ANOVA results of temperature (16, 22, and 28 degrees Celsius) and abundance (40, 60, 80, 100, and 120 individuals) effecting proportion of individuals in low-velocity (downstream pools) for allopatric PTM, allopatric MSQ, and pooled species in sympatry. All coefficients are reported on the logit scale, except for the overall mean. The middle abundance of 80 fish and temperature 22°C were used as reference levels.

	Mean [95% CI]		
	Allopatric PTM	Allopatric MSQ	Pooled Species
Overall Mean	0.7 [0.68,0.71]	0.55 [0.53,0.57]	0.66 [0.64,0.67]
Abundance			
40	-0.26 [-0.37,-0.15]	0.58 [0.46,0.7]	-0.18 [-0.3,-0.07]
60	0.04 [-0.6,0.14]	0.43 [0.33,0.53]	-0.12 [-0.22,-0.02]
80	0	0	0
100	-0.05 [-0.14,0.04]	-0.16 [-0.24,-0.08]	-0.09 [-0.18,0]
120	0.04 [-0.05,0.12]	0.01 [-0.07,0.09]	0.01 [-0.07,0.1]
Temperature			
16°C	0.39 [0.32,0.46]	0.91 [0.84,0.99]	0.94 [0.86,1.02]
22°C	0	0	0
28°C	-0.24 [-0.31,-0.17]	0.34 [0.27,0.41]	0.02 [-0.05,0.08]

Table S2 - Mean parameterization of two-way Poisson ANOVA results for temperature (16, 22, and 28 degrees Celsius) and fish aggression types (MSQ-MSQ, MSQ-PTM, PTM-MSQ, PTM-PTM) effecting counts of aggressive behaviors by abundance of 40, 80, and 120 individuals. All coefficients are reported on the log scale. The PTM-to-PTM aggression and temperature 22°C were used as reference levels.

	Mean [95% CI]		
	40 fish	80 fish	120 fish
Overall Mean	3.2 [3.01,3.37]	3.04 [2.73,3.35]	2.15 [1.8,2.5]
Aggression			
MSQ to MSQ	-0.49 [-0.73,-0.27]	-1.09 [-1.48,-0.73]	-1.6 [-1.99,-1.24]
MSQ to PTM	0.08 [-0.13,0.29]	-0.52 [-0.91,-0.16]	-1.64 [-2.01,-1.29]
PTM to MSQ	-0.82 [-1.06,-0.58]	-1.08 [-1.47,-0.7]	-2.32 [-2.72,-1.94]
PTM to PTM	0	0	0
Temperature			
16°C	-1.29 [-1.53,-1.06]	-0.93 [-1.28,-0.6]	-0.73 [-1.08,-0.38]
22°C	0	0	0
28°C	-0.21 [-0.39,-0.03]	-0.15 [-0.48,0.15]	0.14 [-0.19,0.47]
Overdispersion Term	0.17 [0.04,0.29]	0.49 [0.38,0.63]	0.48 [0.35,0.62]



Figure S1. Aerial view of a side-by-side comparison of MSQ (left) and PTM (right). Characteristics used to distinguish PTM from MSQ include the light bar located anterior to the dorsal fin, the bright orange highlights on the caudal and dorsal fin of PTM, and the olive-green coloration of PTM.



Figure S2. Evidence of fin nipping damage on an adult male PTM's caudal fin. Damage can be seen highlighted within the blue circle.

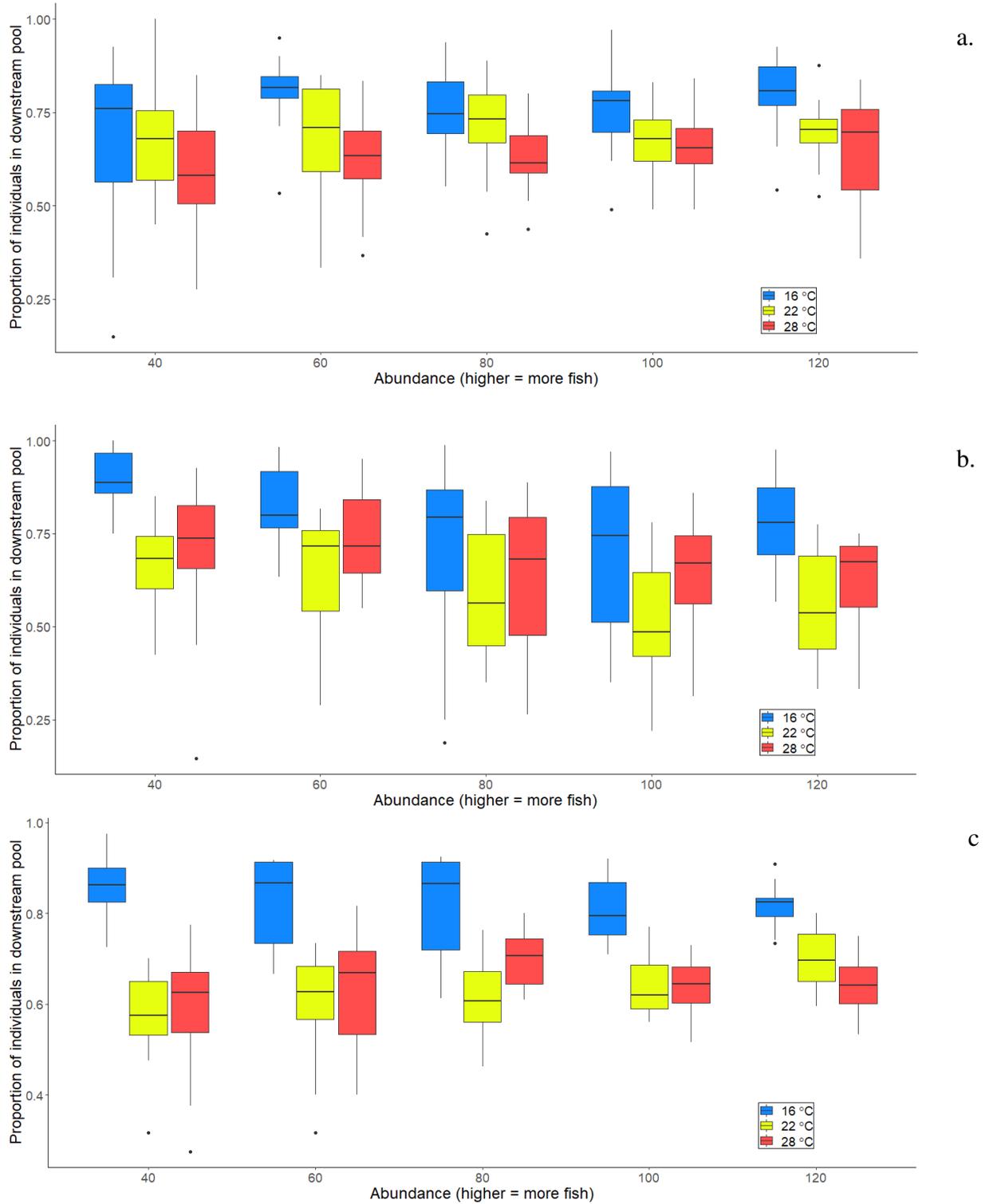


Figure S3. Proportion of individuals in the low-velocity pool at 40, 60, 80, 100, and 120 individuals at 16, 22, and 28 °C for PTM (a.), MSQ (b.) and in sympatry (c.). The line inside the box plot represents the median, the borders represent the interquartile range (IQR), the whiskers represent (+/-) 1.5*IQR while the dots are outliers.

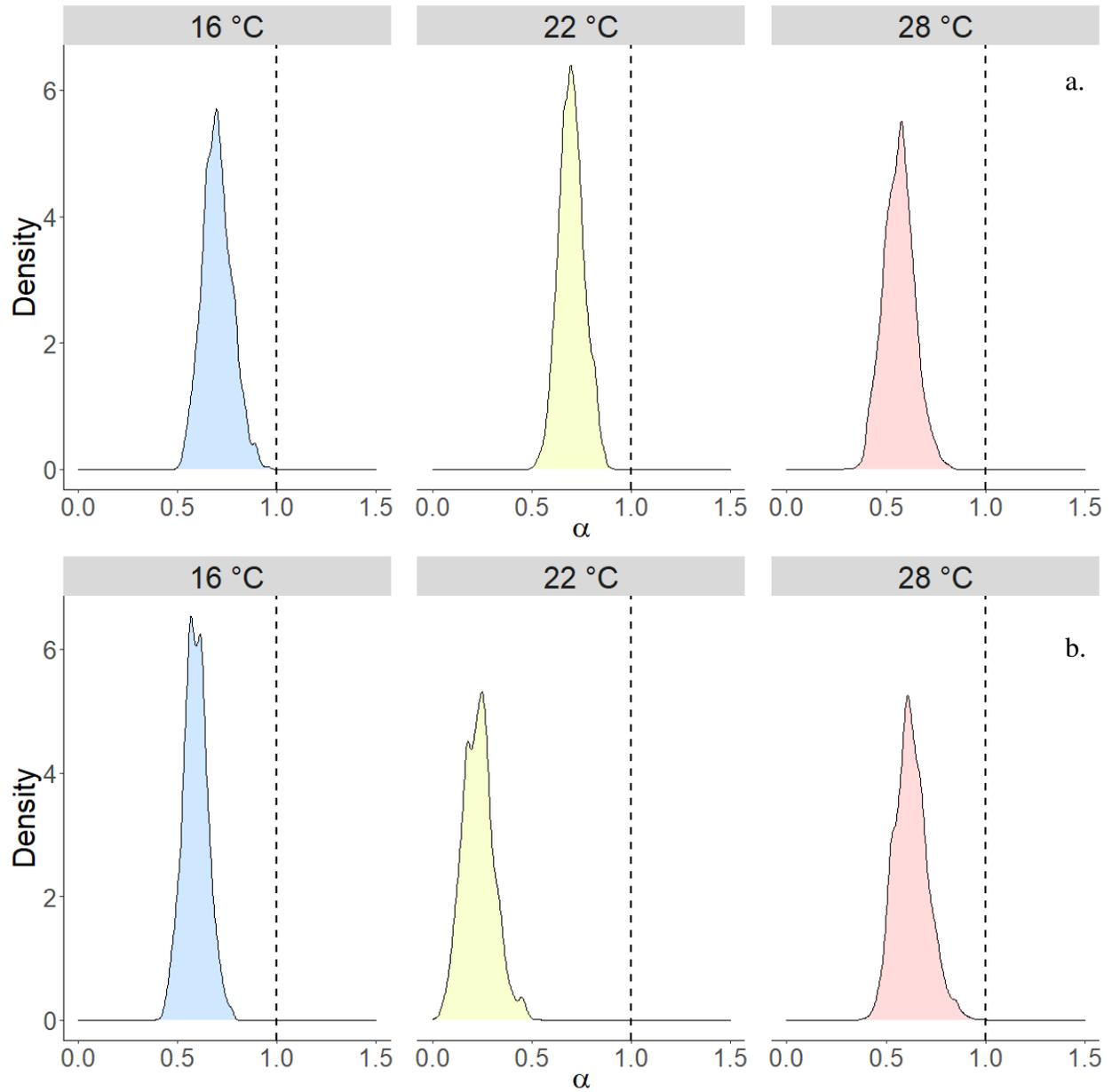


Figure S4. Density plots of the posterior distributions for the coefficients of competition in the preferred habitat (α) for the effect of mosquitofish on plains topminnow (a) and the effect of plains topminnow on mosquitofish (b) across three experimental temperatures (16 °C, 22 °C, and 28 °C).

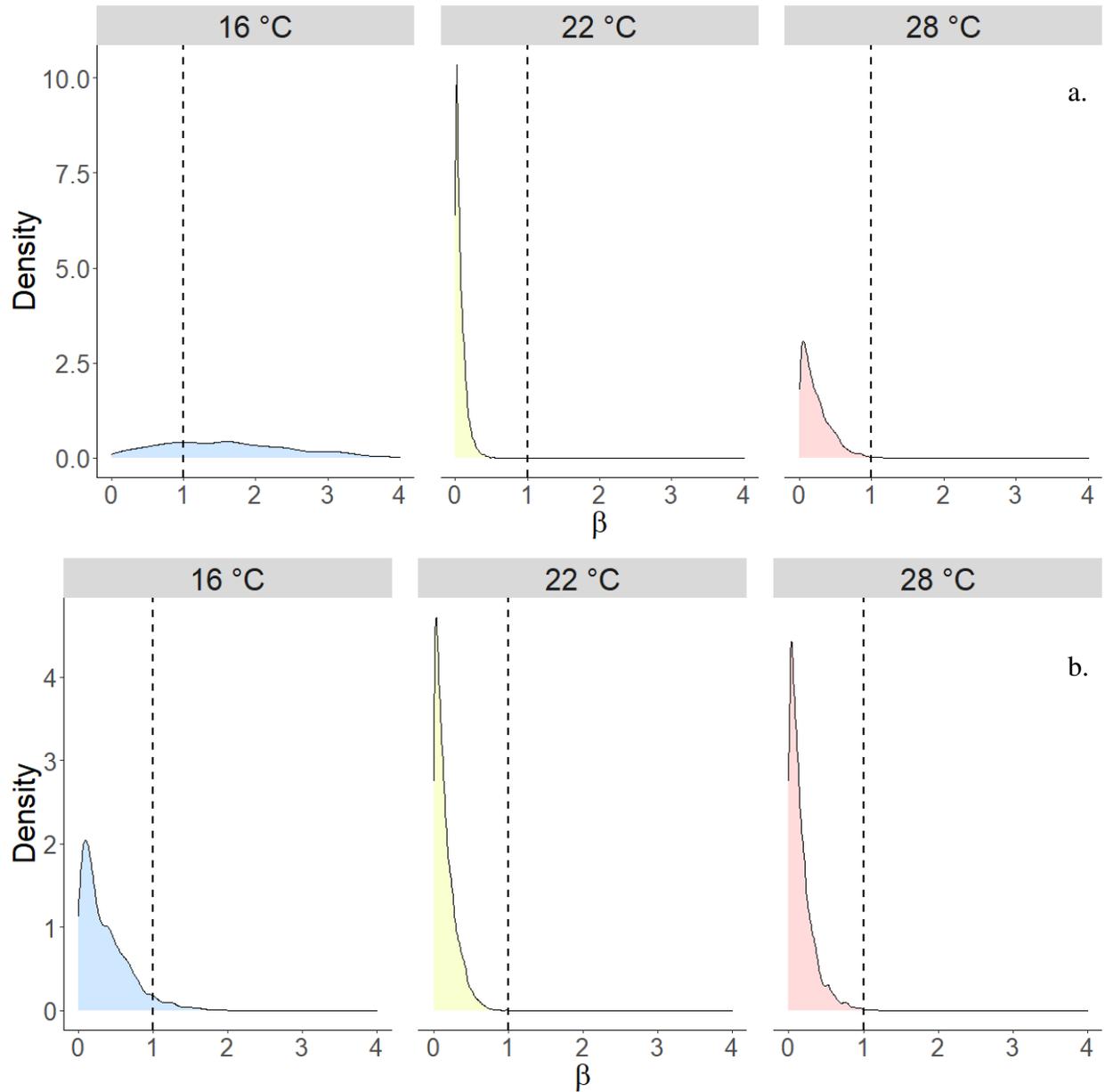


Figure S5. Density plots of the posterior distributions for the coefficients of competition in the unpreferred habitat (β) for the effect of mosquitofish on plains topminnow (a) and the effect of plains topminnow on mosquitofish (b) across three experimental temperatures (16 °C, 22 °C, and 28 °C).

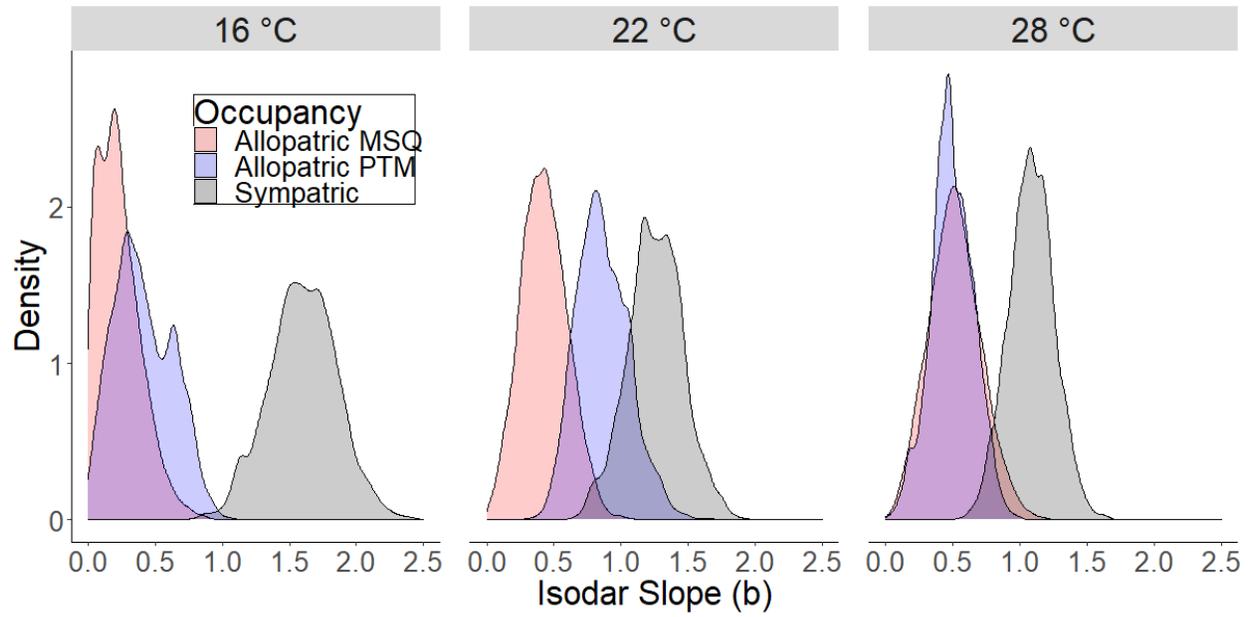


Figure S6. Density plots of the posterior distributions for the isodar slopes (b) across three experimental temperatures (16 °C, 22 °C, and 28 °C) for allopatric mosquitofish, allopatric plains topminnow, and in sympatry.