ORIGINAL ARTICLE

Freshwater Biology WILEY

An experimental test of intra- and inter-specific competition between invasive western mosquitofish (*Gambusia affinis*) and native plains topminnow (*Fundulus sciadicus*)

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Funding information
Denver Audobon Society; Colorado Water Conservation Board

Abstract

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

2. In this study, we conducted a laboratory experiment to test for the relative strength of interference competition between native plains topminnow (*Fundulus sciadicus*) and invasive western mosquitofish (*Gambusia affinis*) at three experimental temperatures. Intra- and inter-specific competition was quantified using an isodar approach, which assumes that animals are ideally distributed to maximise their fitness. Thus, their distributions measure the quality and quantity of habitat patches. This was supplemented by behavioural observations of intra- and inter-specific competition.

3. Contrary to our predictions, we 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 interference competition was significantly stronger than inter-specific competition at all temperature levels. Behavioural observations corroborated this analysis of habitat selection that aggression was most frequent among plains topminnow in sympatry.

4. This study shows that the widely perceived aggression of adults might not be the only key mechanism of global invasion success by mosquitofish. Other ecological traits, such as rapid reproduction, environmental tolerance, and interactions with early life stages of native species, might also be responsible for their invasion success. Additional investigations are warranted to determine whether

1 | INTRODUCTION

Biological invasions are a major threat in many ecosystems with deleterious effects on biodiversity and ecosystem functions (Mack et al., 2000). Freshwater ecosystems are especially susceptible to invaders (Strayer, 2010), due to widespread habitat degradation and simplification, which favour generalist invaders (Dudgeon et al., 2006; Moorhouse & Macdonald, 2015). In addition, fish are the most introduced aquatic animals globally (Gozlan et al., 2010). Introduced species have been cited as a global causal factor of many freshwater fish extinctions (Dias et al., 2017; Miller et al., 1989). Aquatic invasive species affect native species via multiple mechanisms such as predation (Zaret & Paine, 1973), hybridisation (Huxel, 1999), and disease transmission (Peeler et al., 2011). However, competition is a common mechanism where invasive species outcompete native species for habitat or food resources (Carmona-Catot et al., 2013). These ecological interactions are often asymmetrical from invasive species to native species (Riley et al., 2008), jeopardising their coexistence in local aquatic habitats.

The relative strength of intra- versus inter-species competition is a key factor in determining the coexistence of ecologically similar species using similar habitats and resources. Species coexistence is facilitated when intra-specific competition is stronger than inter-specific competition (Chesson, 2000). This *stabilising* mechanism allows species to recover from low abundance and limits 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 the survival of individuals then depends on stochastic factors without favouring one species over the other (Fausch et al., 2021; McPeek & Siepielski, 2019). Species cannot 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 (*equalising mechanism*; Chesson, 2000). Accordingly, characterising competition of sympatric species is critical for advancing our understanding of invasion ecology. However, rigorous tests of intra- versus inter-specific competition are few between aquatic invasive and native species (Britton et al., 2018), with even fewer examining the influence of abiotic factors on these mechanisms (Carmona-Catot et al., 2013).

Abiotic factors shape fundamental niche space, species distributions, and inter-specific competition (Jackson et al., 2001). The outcomes of species interactions between freshwater fish species depend on a variety of factors including water temperature (Carmona-Catot et al., 2013; Taniguchi et al., 1998; Taniguchi & Nakano, 2000), water velocity (Morita et al., 2016), salinity (Alcaraz et al., 2008), and turbidity (Hazelton & Grossman, 2009). Knowledge of temperature-dependent inter-specific competition is particularly important for freshwater biodiversity conservation as climate change accelerates and other anthropogenic activities such as dams, groundwater extraction, and riparian management affect the thermal regime of freshwater habitats (Poole & Berman, 2001; Roth et al., 2010; Sinokrot et al., 1995). Such knowledge informs management the management of aquatic invasive species because complete eradications of highly invasive species is often unattainable; instead, the control of invasive species should focus on identifying and managing environmental conditions that minimise their proliferation and consequently their ecological damage (Dunham et al., 2020).

The western mosquitofish (MSQ), *Gambusia affinis*, is a small-bodied fish (maximal length of females = 7 cm and males = 4 cm) that, since the early 1900s, has been introduced as a biological mosquito control agent on every continent except Antarctica (Pyke, 2008). Native to the Mississippi River basin, MSQ thrives in lentic habitats and 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 & Kats, 1999), and invertebrates (Leyse et al., 2004; Tsang & Dudgeon, 2021). Given their major ecological impacts globally, they are listed as one of IUCN's 100 worst invasive alien species in the world (Simberloff & Rejmanek, 2019). Mosquitofish tolerate a wide range of abiotic conditions, and the early maturation and high fecundity of females (Vondracek et al., 1988) allow them to proliferate once introduced in a new habitat. Their establishment is facilitated as MSQ outcompetes many small-bodied fish, and much of its success as an invader has been attributed to MSQ's fierce competitive aggression through interference competition (Laha & Mattingly, 2006; Meffe, 1985; Schaefer et al., 1994; Sutton et al., 2013; Thompson et al., 2012). Mosquitofish have invaded Colorado's eastern plains aquatic ecosystems extensively and have encroached on the habitats once used by native plains fish species. Invasions of MSQ have coincided with marked declines in the plains topminnow (PTM), *Fundulus sciadicus*, listed as a tier one species of greatest conservation need in Colorado (Colorado Parks and Wildlife, 2015). Plains topminnow currently occupy small, isolated habitat patches (Pasbrig et al., 2012). Both species occupy water-column habitats of slow-moving water bodies and are omnivores feeding in the water column (Pyke, 2008; Schumann et al., 2015; Thiessen et al., 2018). Moreover, MSQ's critical thermal maxima (40–43°C; Otto, 1974) and tolerance to salinity (11.54 g/L; Newman & Aplin, 1992) are greater than PTM's critical thermal maxima (37°C; Smale & Rabeni, 1995) and salinity

tolerances (0.2 g/L; Griffith, 1974). The ability of MSQ to withstand extremes in abiotic stressors may further contribute to its invasive success over native species.

In this paper, we used experimental stream units in the laboratory to quantify the relative strength of intra- versus inter-specific competition between the native PTM and invasive MSQ adults along a temperature range (16, 22, and 28°C) and to determine mechanisms of species interactions that prevent their coexistence in the wild. The strength of competition was measured by habitat selection between preferred low-velocity habitats and marginal high-velocity habitats in allopatry versus sympatry based on the isodar theory (Morris, 1988) and behavioural observations of aggression between the two species. Isodar theory is based on the concept of ideal free distributions, which assumes that animals are spatially distributed to maximise their fitness, and thus, their distributions reflect differences in habitat quantity or quality (Morris, 1988). Although local extirpations of PTM populations have been documented in areas invaded by MSQ in Colorado, this spatial pattern of species distributions alone is insufficient to establish MSQ as a cause of PTM declines (Pasbrig et al., 2012). Plains aquatic ecosystems in this arid region have experienced extensive habitat loss and fragmentation, water quality degradation, and periodic and long-term drying due to anthropogenic activities related primarily to agriculture (Dodds et al., 2004; Falke et al., 2011; Perkin et al., 2017). A question remains whether MSQ invasion caused precipitous declines in PTM populations or whether MSQ is simply an opportunistic invader of highly degraded aquatic ecosystems (Bauer, 2012; MacDougall & Turkington, 2005). Addressing this question informs management of this global invader in Colorado and elsewhere. We predicted that competitive interactions are asymmetrical from the invasive MSQ to the native PTM based on a plethora of studies that documented behavioural aggression of adult MSQ (Laha & Mattingly, 2006; Meffe, 1985; Schaefer et al., 1994; Sutton et al., 2013; Thompson et al., 2012). Contrary to our prediction, we report that intraspecific competition in PTM was stronger than the interspecific competition and discuss its implications on mechanisms of species coexistence and invasive species management.

2 | MATERIALS AND METHODS

2.1 | Theoretical framework

We 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 maximise their fitness. Thus, their distributions reflect differences in habitat quantity or quality (i.e., ideal free distributions). In a single-species, two-habitat system composed of equal quantity and a more suitable Habitat 1, and a less suitable Habitat 2, isodars are lines of equal fitness between the two habitats and are represented by linear regression:

$$N_{A,1} = C + b N_{A,2} \qquad (1)$$

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:

$$N_{A,1} = C - \alpha N_{B,1} + b\left(N_{A,2} + \beta N_{B,2}\right) \qquad (2)$$

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, α, 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; Rodríguez, 1995).

In addition, we applied the single species isodar approach (Equation 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) and a sympatric situation in which individuals of a second species have replaced half of the population. 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 slope would be steeper, relative to allopatry (Figure 1a). In contrast, if inter-specific competition is stronger than the intra-specific competition, fewer proportions of individuals would occupy the suitable habitat patch and the isodar slope would become less steep in sympatry (Figure 1c). A third scenario is when intra- and inter-specific competition is equally strong, and isodars would not shift (Figure 1b). This framework allows one to statistically compare isodar slopes between allopatry and sympatry to infer the relative strength of intra- and inter-specific competition. It was used in this study to compare between allopatric and sympatric isodars of native PTM and non-native MSQ in two-habitat systems, where one habitat simulated a slow-velocity plains habitat (i.e., a more suitable habitat) and the other a high-velocity habitat (i.e., a less suitable habitat) with the minimum cost of movement between the two habitats. We focus on comparing isodar slopes instead of intercepts because the former measures habitat selection as habitat space becomes more crowded, a situation suitable to measure competition. In contrast, the latter quantifies habitat differences at very low abundances (Morris, 1988).

2.2 | Experimental stream units

Three replicates of recirculating experimental stream units were constructed to evaluate the competition between MSQ and PTM. An experimental stream unit consisted of three fibreglass circular
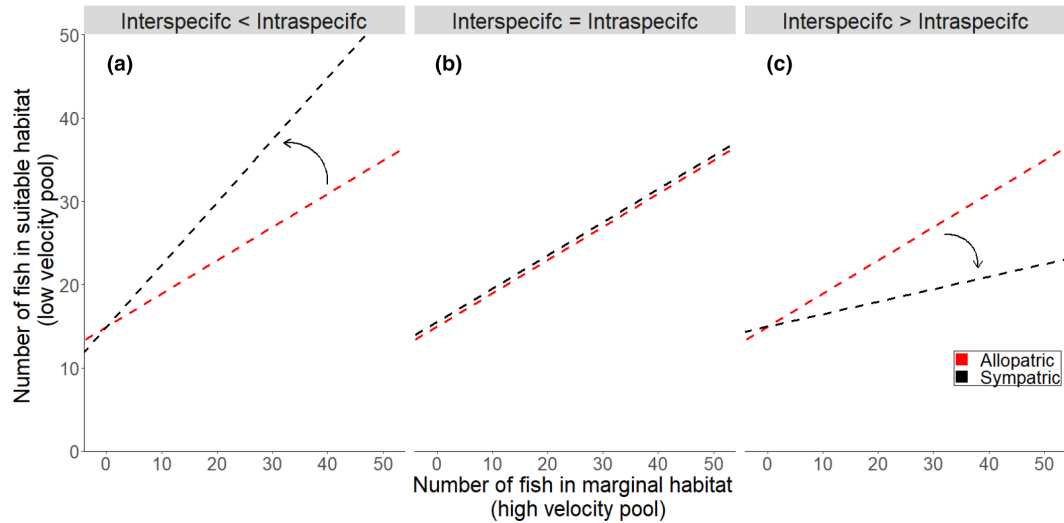
FIGURE 1 Diagram representing the expected shift in isodar slopes when a second species is introduced dependent on the strength of competition. If intra-specific competition is stronger than inter-specific competition, addition, and replacement by individuals of a second species would relax overall competition allowing more individuals to occupy the suitable habitat shifting sympatric isodar upward, relative to allopatry (a). In contrast, inter-specific competition is stronger than intra-specific competition, the isodar would shift downward in sympatry (c). If intra- and inter-specific competition is equally strong, the isodars would not shift (b).
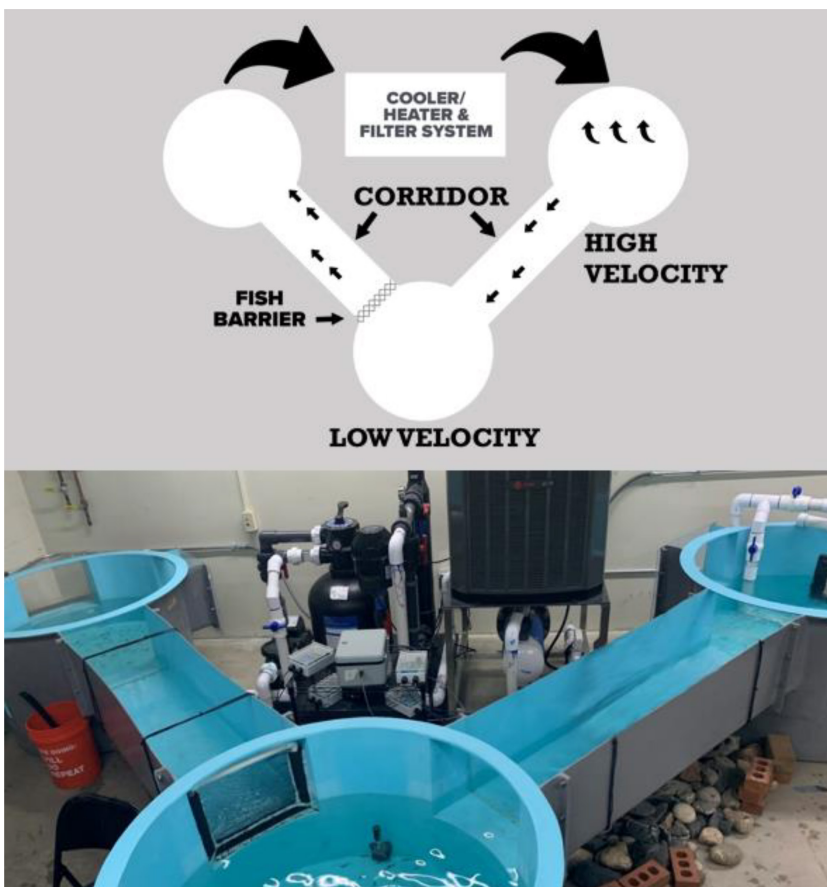

FIGURE 2 Diagram (a) and photograph (b) of a recirculating experimental stream unit with arrows indicating the flow direction. Water was reintroduced at the bottom of the upstream pool using a white PVC tube; slits on its upper and lateral sides allowed water circulation and created a high-velocity environment in the upstream pool. Three experimental stream units were replicated to test three temperature levels (16, 22, and 28°C) simultaneously. Fish were counted in each pool six times daily to test for intra- and inter-specific competition using the two-habitat isodar theory.

tanks (*pools*, Model FCT-235) and two straight corridors (Red Ewald LLC, Karnes City, TX, U.S.A.), a bead filtration system equipped with UV sterilisers (Model 930084, Pentair Aquatic Eco-Systems Inc., Apopka, FL, U.S.A.), and an air-cooled heat pump for water

temperature control (Model Titan HP-2, Aqua Logic Inc., San Diego, CA, U.S.A.; Figure 2). 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 2300 L of water. Water was reintroduced at the bottom of the upstream pool using an 80-cm long PVC tube with three 15-cm long slits located at the upper and lateral sides of the PVC tube to circulate water and increase turbulence and velocity in the upstream pool. Experimental stream units were constructed on a level floor and high velocity was created solely due to the water circulation in the upstream pool.

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, Colorado, over a year. The summer water temperatures along the lower South Platte stretch reach a mean monthly temperature of 28–30°C, with a mean yearly temperature of 17.7°C (Otto, 1974; Watt, 2003). We are unaware of any previous study on PTM thermal preference and tolerance, but MSQ thermal preference is under genetic control with preferences ranging from 28 to 31°C depending on the origin of the population (Winkler, 1979). During the study period, water temperature in experimental units was monitored hourly using loggers (U22-001, HOBO Onset Computer Corp.). Pool 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. The heat pumps had an upper control limit of 28°C.

Lights in the room were automated to simulate a summer photoperiod. The sunrise started at 06:00 daily at the lowest colour temperature (2,700 K) and brightness (1%), and colour and brightness increased by approximately 10% in 5-min increments until they reached maximum colour temperature (6,500 K) and brightness (100%, or 538 lm) at 07:00. The maximum colour temperature and brightness were maintained from 07:00 until 21:00. The sunset started at 21:00 and lasted until 22:00 by decreasing colour and brightness by approximately 10% every 5 min. The room was dark between 22:00 and 06:00. All data collections were conducted during daytime hours (07:00–17:00).

2.3 | Fish collection

MSQ and PTM rarely coexist in high abundance, so they were collected from two separate locations in the South Platte River basin, Colorado. Mosquitofish were collected from Running Deer Natural Area in Fort Collins, and PTM were collected from Pawnee National Grassland, where MSQ has yet to invade, from May to July of 2021. Both field sites were characterised by large pond complexes in the floodplain areas with dense submerged aquatic vegetation. Fish were collected using seine and dip nets. We collected 550 MSQ and 450 PTM, and their body size was matched as much as possible to remove body size effects on the outcome of inter-specific competition. Female MSQs were collected because males are smaller and less aggressive than females in this species (Priddis et al., 2009; Rincón et al., 2002). Sexual dimorphism is less apparent in PTM, and males and females were included in the study. Based on measurements of randomly selected individuals in the sample, the total length of

MSQ (mean = 43 mm, $SD = 3.6$) was significantly smaller than that of PTM (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 37% formaldehyde solution for 1 hr to treat parasites. Fish were then acclimated to experimental streams for 36 hr preceding data collection and housed in the downstream most pool of the experimental stream units, separated with a permanent barrier from the other two pools (Figure 2). This barrier created a two-habitat system composed of an upstream, high-velocity pool with an average water column flow of 0.3 m/s, and a downstream, low-velocity pool with 0 m/s. The high-velocity pool was maintained using an upwelling of recirculated water introduced from a PVC pipe placed on the pool bed (Figure 2).

2.4 | Data collection

We conducted a 9-week experiment between May and July 2021 to record fish habitat choices between hypothesised preferred low-velocity pools and less preferred high-velocity pools when: (1) MSQ alone (allopatric) were housed at different abundances (40, 60, 80, 100, and 120 individuals per experimental stream unit); (2) PTM alone (allopatric) were housed at these different abundances; and (3) 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 3 weeks for each patry scenario by randomly assigning a temperature level (16, 22, or 28°C) to each of the three experimental units weekly and rotating temperature assignments randomly, so that each unit was subject to all three temperature levels over 3 weeks to remove unintended unit effects on habitat choices. Starting on Monday every week, fish abundance was set at 40 total individuals in each experimental unit and increased by 20 fish daily until 120 individuals were reached on Friday. The fish in the allopatric trials were also used in the sympatric trials. However, individuals were randomised between trials across the three experimental stream units. Water quality was tested every 3 days for nitrate, nitrite, ammonia, and pH levels, and a third of the water in each experimental unit was changed weekly to maintain fish health.

Six times per day, we counted the number of individuals in each pool at 90-min intervals. A sequence of counts collected at the same time of the day from Monday through Friday was treated as a replicate. As a result, we obtained six replicates for isodar regression weekly for 3 weeks for 18 replicates for each patry scenario and temperature level. Before fish were counted, a temporary barrier was placed in the corridor between the two pools so that fish could

not move between them during counting. Observers then counted the number of fish visually 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 in low abundances (40, 60, and 80 fish), then, when identification of species became difficult at higher abundances, observers netted and counted individuals. For the last count of the day, observers netted all fish to ensure species identification and redistributed them in equal densities among the high- and low-flow velocity pools. Fish were fed once daily after the final counts with thawed bloodworms at 10% of the total weight of all individuals in each experimental unit. Food was distributed equally between the pools in each unit to not provide a spatial cue on food availability and affect fish habitat choice.

In a secondary experiment, we characterised behavioural aggressions in sympatry via observations to complement the experiment on habitat choices. Total fish abundance was increased daily over 3 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 to 16, 22, or 28°C over the 3-day period. Temperature was held constant in each experimental unit to ensure that fish were acclimated to each temperature during these short-duration behavioural observations. Observers counted aggressions at 90-min intervals six times daily for 10 min to record counts of aggressive behaviours from one fish to another. These aggressive actions included chases, bites, jolts/thrusts, and posturing (Matthews & Wong, 2015). Observers used step ladders to ensure a view of the entire pool. They positioned themselves as far as possible from the pool while maintaining a view of fish, standing still to reduce behavioural alterations from observer presence. PTM and MSQ were reliably identified from above due to differences in visual characteristics (Figure S1).

2.5 | Data analysis

2.5.1 | Isodar models

Gaussian 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 ($N_{A,1}$) and the predictor was the count of fish in the less preferred high-velocity habitat ($N_{A,2}$; Equation 1). In sympatry, fish counts were pooled between the two species in each habitat. Isodar intercepts and slopes (Equation 1) were inferred by averaging across 18 replicates for allopatric PTM, allopatric MSQ, and sympatric trials at each temperature level. Specifically, we developed models with random intercepts and slopes, where $C_i \sim$ Normal $\left(\widehat{C}, \sigma_C \right)$ and $b_i \sim$ Normal $\left(\widehat{b}, \sigma_b \right)$ for ith replicate ($i = 1, ..., 18$), $\widehat{C}$ is the overall intercept, $\widehat{b}$ 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 $\widehat{b}$ 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 $\widehat{b}$ between allopatry and sympatry were above or below 0. Prior to analysis, a small integer value (2) was added to fish count in the low-velocity pool ($N_{A,1}$) and high-velocity pool ($N_{A,2}$) to facilitate model convergence.

We further used the two-species, two-habitat isodar model (Equation 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. Our primary interest was to evaluate the competitive effect of invasive MSQ on native PTM, but we evaluated the competitive effect of PTM on MSQ for comparisons. Therefore, two models were developed where the response ($N_{A,1}$) was PTM count in the preferred low-velocity habitat ($N_{A,2}$ = PTM count in high-velocity pool, $N_{B,1}$ = MSQ count in low-velocity pool, and $N_{B,2}$ = MSQ count in high-velocity pool) in one model (Equation 2), and the response ($N_{A,1}$) was MSQ count in the preferred low-velocity habitat ($N_{A,2}$ = MSQ count in high-velocity pool, $N_{B,1}$ = PTM count in low-velocity pool, and $N_{B,2}$ = PTM count in high-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 $\left(\widehat{\alpha}, \sigma_\alpha \right)$ and $\beta_i \sim$ Normal $\left(\widehat{\beta}, \sigma_\beta \right)$ for ith replicate ($i = 1, ..., 18$), $\widehat{\alpha}$ is the average competitive effect of one individual of Species B on A in the low-velocity habitat, $\widehat{\beta}$ is the average competitive effect of one individual of Species B on A in the high-velocity habitat, and σ_α and σ_β are their respective standard deviation. For model convergence, we used posterior mean values of allopatric isodar intercept ($\widehat{C}$) and slope ($\widehat{b}$) (Equation 1) in two-species isodar models (Equation 2). Statistical significance of inter-specific coefficient, $\widehat{\alpha}$ and $\widehat{\beta}$, was declared if its 95% posterior samples was below 1 (inter-specific competition < intra-specific competition) or above 1 (inter-specific competition > intra-specific competition). Prior to data analysis, we added a small integer value (2) to count of each species in each habitat.

We also fit Poisson hierarchical linear models in the isodar analyses because the response variable was count of individuals in the low-velocity pool. Ecological inferences based on the Poisson models were identical to those in the Gaussian models, as described above. We report our results based on the Gaussian models because these models were used in the seminal paper on the isodar analysis (Morris, 1988).

2.5.2 | Behavioural aggression

We tested whether frequency of behavioural 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 behaviour per 10 min of observations in sympatry, and the predictors were

temperature with three levels (16, 22, and 28°C) and species pair exhibiting aggression with four levels (MSQ–MSQ, MSQ–PTM, PTM–PTM, and PTM–MSQ). The Poisson distribution was used to model count data and let variance to scale with mean. We used 22°C as the reference temperature and the PTM–PTM interaction as the reference species pair in the Poisson ANOVA and tested for significant differences between other temperature levels and species pairs relative to the reference levels. We considered behavioural 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 analysed with a Bayesian approach using a Markov chain Monte Carlo method in JAGS (Plummer, 2012), called from R (R Core Team, 2022) with the jagsUI package. Diffuse priors were used for all parameters. Posterior distributions of parameters were drawn from 30,000 iterations of three chains after a burn-in period of 5,000 iterations and a thinning rate of 10, for a total of 9,000 posterior samples. Model convergence was checked by visually examining plots of the Markov chain Monte Carlo chains for good mixture as well as ensuring that the R-hat statistic was less than 1.1 for all model parameters (Gelman & Hill, 2006). A modified Bayesian r^2 was calculated to assess model fit for the isodar linear regression analyses (Gelman et al., 2019).

3 | RESULTS

As expected for plains fishes occupying lentic habitats and sluggish streams, MSQ and PTM used the low-velocity pool more frequently than the high-velocity pool. On average, 51%–84% of individuals used the low-velocity pool in sympatry and allopatry across different fish abundance and temperature levels (Figure S2). The use of the preferred slow-velocity pool depended on temperature,

with proportionally more individuals using this pool at 16°C compared to 22 and 28°C, indicating that behavioural interactions were temperature-dependent (Table S1). Fish abundance (40, 60, 80, 100, 120 individuals per experimental unit) affected habitat use differently depending on species composition (Table S1).

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 (Figure 3). The allopatric slope for PTM ($\hat{b}$ in Equation 1) was 0.34 (95% credible interval [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 ($\hat{b}$) 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 ($\hat{b}$) 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. These results demonstrated that intraspecific competition was consistently stronger than interspecific competition (Figure 1). Bayesian r^2 values were 0.08 for allopatric PTM, 0.17 for allopatric MSQ, and 0.25 for sympatric models.

Interspecific competition coefficients in the two-species isodar analysis (Equation 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 pools, 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 pools (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 pools at 16°C.
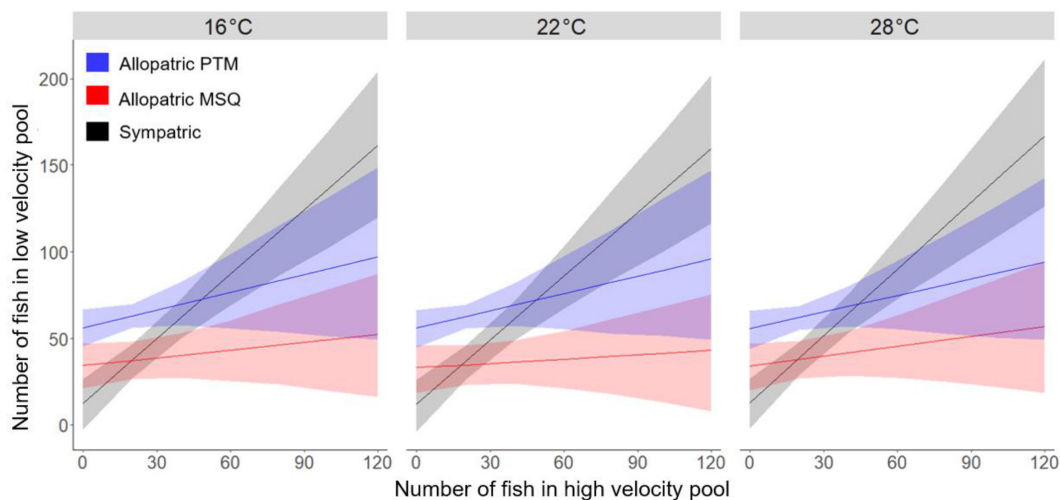

FIGURE 3 Isodars of allopatric plains topminnow (PTM; blue), allopatric mosquitofish (MSQ; red), and sympatric pooled species (black) at 16, 22, and 28°C. All isodars indicate a statistically significant (>95%) difference in the proportion of posterior samples between the allopatric slope and the sympatric slope. The mean predicted isodar is represented by the solid-coloured line and the 95% highest posterior density interval are represented by the coloured bands.

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.

| 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]

Abbreviation: CI, credible interval.

Note: The coefficients represent the average competitive effect of one individual of one species on the other species in low-velocity or high-velocity pool. Coefficients <1 indicate that interspecific competition is weaker than intraspecific competition, and those >1 indicate that interspecific competition is stronger than intraspecific competition. Asterisks (*) indicate a statistically significant (>95%) difference from 1 in the proportion of posterior samples.
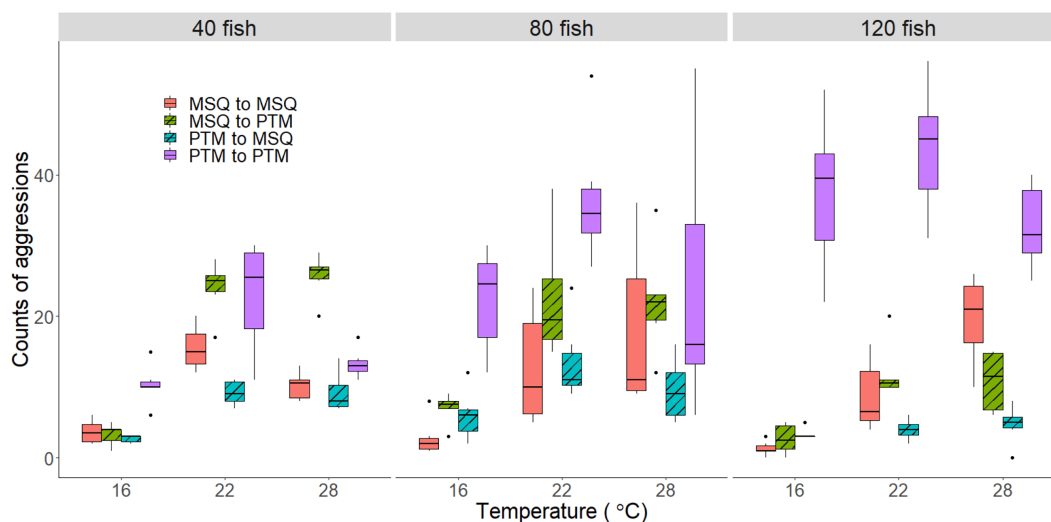

FIGURE 4 Counts of aggressive behaviours in sympatry at 40, 80, and 120 individuals and at 16, 22, and 28°C, categorised by species pair. The line inside the box represents the median, the borders represent the interquartile range (IQR), the whiskers represent (±) 1.5*IQR, and the dots are outliers. Hatched bars indicate interspecific aggressions and solid bars indicate intraspecific aggressions.

Specifically, the mean effect of MSQ on PTM ($\hat{\beta}$ in Equation 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 high-velocity pools at 16°C.

Behavioural aggression was most frequent among individuals of PTM, and we did not find evidence that MSQ was more aggressive than PTM (Figure 4, Table S2). Interspecific aggression from MSQ to PTM was significantly less frequent than intraspecific aggression from PTM to PTM when abundance per pool was 80 individuals (posterior mean = −0.53 [95% CI: −0.91, −0.16]) and 120 individuals (−1.64 [−2.01, −1.29]). Damage from fin nipping from conspecifics was prevalent among PTM (Figure S3). At all abundance levels, behavioural aggression was significantly less at 16°C relative to 22°C.

4 | DISCUSSION

Neither the isodar nor aggression analyses demonstrated competitive effects of invasive MSQ on native PTM at any temperature level, despite previous studies showing evidence of interference

competition by this global invader (Laha & Mattingly, 2006; Meffe, 1985; Schaefer et al., 1994; Sutton et al., 2013; Thompson et al., 2012). It is unlikely that our unique findings are due to experimental or analytical artefacts for several reasons. First, this is the first study on competitive effects of MSQ on PTM to our knowledge, but previous studies documented agonistic interactions of MSQ to other species in the same genus *Fundulus* (Laha & Mattingly, 2006; Sutton et al., 2013), which are similar to PTM in ecological and habitat requirements. Thus, species traits of PTM are not a plausible reason for the unexpected results. Second, body size of PTM was approximately 10% larger than that of MSQ. Although body size differences affect the outcome of competitive interactions in freshwater fish, this range of differences should not disadvantage smaller fish (i.e., MSQ) in behavioural aggression (Nakano, 1995; Sanches et al., 2012) and previous studies also used female MSQ in measuring their behavioural aggression (Laha & Mattingly, 2006; Thompson et al., 2012). Third, we collected data for 9 weeks for habitat selection and an additional week for behavioural observations of aggression, whereas some previous

studies collected data for much shorter periods (c. 24 hr) (Laha & Mattingly, 2006; Meffe, 1985). Our robust data on habitat selection were analysed using the isodar theory, which has been widely applied to study competition among terrestrial fauna (Morris et al., 2000; Ovadia & Abramsky, 1995; Tarjuelo et al., 2017) and shown to be an effective tool for analysing competition among freshwater fauna (Morita et al., 2004; Rodríguez, 1995). Accordingly, this study represents one of the most thorough evaluations of MSQ's competitive interactions with native freshwater fishes. Finally, our previous study similarly reported lack of aggressive behaviour by MSQ toward northern redbelly dace *Chrosomus eos* in a different laboratory setting (Ciepiela et al., 2021), indicating that behavioural aggression of MSQ may not be as ubiquitous as currently perceived. This study provides important insights into the competitive interactions between MSQ and PTM, as its unique findings are supported by various factors including the similarity of PTM to other studied species, the lack of effect in body size difference between PTM and MSQ, and the thorough data collection and analysis of MSQ's behaviour toward native freshwater fish. Understanding these species interactions is crucial for predicting the impacts of non-native species on native ecosystems and for developing effective management strategies to mitigate potential negative impacts.

Intraspecific competition in native PTM was the most frequent type of behavioural interactions, which was particularly evident in the behavioural observations of aggression. The species coexistence theory posits that stronger intraspecific competition than interspecific competition is one criterion that permits coexistence of ecologically similar species (Chesson, 2000). Despite our finding, distribution patterns of the two study species in eastern Colorado show that MSQ populations have replaced PTM populations, and they rarely coexist in high abundance locally (Colorado Parks and Wildlife, unpublished data). How do we reconcile this gap between the theoretical prediction and empirical pattern? Notably, we found that intraspecific competition in PTM was more prevalent than intraspecific competition in MSQ. However, theoretical work on species coexistence and competition does not always distinguish the strength of intraspecific competition between species of interest (Chesson, 2000; Morris, 1988). In our species pair, it is plausible that stronger intraspecific competition in PTM than in MSQ would make their local coexistence less likely because competition regulates population dynamics of PTM more readily but the population growth of MSQ is not limited by such density-dependent demographic processes. These mechanisms would expedite local extirpations of PTM while facilitating establishment and growth of invading MSQ populations. Thus, this case study points to a need for integrating potential differences in intraspecific competition in predicting species coexistence in general.

Lack of behavioural aggression by MSQ in this study urges us to consider other mechanisms and ecological traits that make it such a successful invader globally. Invasive success of MSQ has been attributed to their high propagule pressure (Woodford et al., 2013), reproductive ability (Vondracek et al., 1988), and tolerance to a wide range of abiotic factors (Alcaraz et al., 2008; Chervinski, 1983; Otto, 1974). Propagule pressure in MSQ populations has been documented to exhibit exponential growth (Woodford et al., 2013), which may be combined with aspects of their demography to explain their reproductive dominance. The average brood size of MSQ and PTM is comparable with 40–50 fry per brood for MSQ and 30–50 eggs for PTM (Pasbrig, 2010). However, MSQ can have up to seven broods annually (Haynes & Cashner, 1995) and rear live-beared young (Vondracek et al., 1988), allowing them to propagate quickly once introduced. In contrast, PTM are restricted to producing one brood per year and require aquatic vegetation on which to lay their eggs (Kaufmann & Lynch, 1991). An array of anthropogenic mechanisms and ecological traits of MSQ, along with findings of our study, indicate that behavioural aggression would not be the only key reason why MSQ has managed to invade lotic and lentic habitats globally.

Intra- and inter-specific competition depended on water temperature, where the use of the less-preferred high-velocity pools was more common and behavioural aggression was more frequent at 22 and 28°C, compared to 16°C. This result is not surprising based on temperature preferences of our study species (Watt, 2003), and their activities should increase with temperature to some degree. However, it indicates that species interactions would probably become more frequent and consequently a more important determinant of fish assemblage composition as climate change warms surface water temperatures globally (Barbarossa et al., 2021). It is particularly concerning that MSQ aggressions toward PTM also increased with temperature, although once again this interspecific aggression was less frequent than the intraspecific aggression among PTM. In addition, in many systems droughts will occur more frequently under climate change, thus crowding fish in confined spaces and increasing encounter rates and behavioural interactions between MSQ and native fish species (Beatty et al., 2022). In the case of PTM confined to shrinking habitats, this increased competition may lead to increased aggression among individuals and declines in their native fish populations. Predicting persistence of freshwater fish populations and assemblages requires knowledge on context-dependent biotic interactions, and our study advances this frontier by quantifying the relative strength of intra- and inter-specific competition along a temperature range.

We acknowledge three limitations of this study. First, this laboratory study was conducted in controlled environments that deviate from their natural habitats. For example, we did not provide physical cover to maximise encounters of individuals (Casterlin & Reynolds, 1977) and measure their competition and behavioural aggression. Natural habitats of PTM and MSQ are often characterised with dense cover of aquatic macrophytes, which can mitigate negative impacts of species interactions (Magellan & García-Berthou, 2016; Sutton et al., 2013). Second, we focused on competition and behavioural aggression at the adult stage of these species, but such dynamics can occur across life stages (Laha & Mattingly, 2006; Sutton et al., 2013; Taylor et al., 2001), as MSQ have been documented to cannibalise their young and prey on

juveniles of other species (Laha & Mattingly, 2006). Finally, this study focused on the aggressive interactions between the species at an equal proportion of abundances. Rates of intraspecific aggression could be lower when species are rare compared to the other competitor and across different stages of invader abundance (Bradley et al., 2019; Geange & Stier, 2009). Additional research is warranted to develop a more complete understanding of MSQ effects on other life stages and abundances of native small-bodied species.

Despite these limitations, our study cautions us to more carefully examine the dominant view that MSQ are a driver of native species declines (Ennen et al., 2021; Meffe, 1985; Sutton et al., 2013). Aquatic invasive species are widely regarded as one of the direct causes of biodiversity loss (Moorhouse & Macdonald, 2015; Moyle & Leidy, 1992). However, much of the supporting evidence is based on distributional correlations between species invasion and native species decline in degraded systems (Williams-Subiza & Epele, 2021). Our data indicate that interference competition between adult MSQ and PTM may not the be the only key mechanism of global invasion success by mosquitofish, and perhaps MSQ might be characterised as an opportunistic invader that has proliferated in degraded aquatic ecosystems (Bauer, 2012; MacDougall & Turkington, 2005). Additional investigations are much needed to determine whether MSQ are a driver of biodiversity loss, or a passenger of ecosystem change because this information fundamentally changes management approaches to managing this species. If MSQ is indeed a cause of native fish declines, management actions to control their populations or minimise their ecological damage need to be prioritised (Dunham et al., 2020). Alternatively, if MSQ is an opportunistic invader of degraded aquatic ecosystems, managers may opt to focus on addressing the root causes of ecological degradation instead of population control of MSQ. From the management perspective, it is equally important to elucidate a range of environmental conditions that allow or prevent coexistence of invasive and native aquatic species, especially if managers have an ability to manipulate the environmental conditions (e.g., flow and thermal regime) (Freeman et al., 2022; Poole & Berman, 2001).

AUTHOR CONTRIBUTIONS

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data are available from the corresponding author on reasonable request.

ETHICS STATEMENT

All applicable international, national, and institutional guidelines for the use of animals were followed. The study was conducted in accordance with procedures approved by the Colorado State University Institutional Animal Care and Use Committee (KR Protocol Number 1573).

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