Invasive mosquitofish become more aggressive in the presence of native pike young-of-year: implications for native predator recruitment

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT 5 from OpenAI in order to review the language (English). After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Abstract

Invasive species often exhibit aggressive behaviour, boldness, and high foraging activity, which contribute to their establishment success and impact on native ecosystems. The mosquitofish (*Gambusia holbrooki*), one of the world's most invasive fish species, is known for its aggressive nature, which threatens the survival of native species. Lake littoral zones, critical for juvenile fish development, are particularly vulnerable to such invasions. This study investigates the interactions between mosquitofish and young-of-the-year (YOY) cisalpine pike (*Esox cisalpinus*), a native top predator reliant on these habitats. We conducted a controlled laboratory experiment over five days, observing behavioural interactions across different density treatments. Both species exhibited high levels of intraspecific aggression, with mosquitofish forming dominance hierarchies, where a single dominant individual accounted for over 84% of interactions. In interspecific treatments, mosquitofish aggression intensified, leading to the direct mortality of ~92% of pike through fin nipping. This aggression was considered primarily social, aimed at maintaining dominance and competing for food, while pike aggression was driven by cannibalistic predation. The vulnerability of pike to mosquitofish aggression was linked to fin morphology, as mosquitofish targeted the caudal fin, impairing pike mobility and survival. Although laboratory conditions lack the complexity of natural ecosystems, the findings highlight the potential impact of mosquitofish on native species, particularly at early life stages. Enhanced aggression during interspecific interactions may be a key mechanism behind mosquitofish dispersal and invasion success. While the effects observed here may be less pronounced in natural environments, the ability of mosquitofish to dominate and outcompete native species, combined with their adaptability and rapid reproduction, poses a significant ecological threat.

Key words: *Esox cisalpinus*; *Esox* sp.; *Gambusia holbrooki*; aggressive behaviour; interactions; dominance; Invasive Alien Species (IAS)

1. Introduction

Invasive species typically exhibit higher levels of aggressiveness, foraging activity, exploratory behaviour, and boldness than native species, traits that significantly enhance their establishment success (Chapple et al., 2012). These behavioural characteristics influence how invasive species interact with other species and the environment, often exacerbating their impacts (Juette et al., 2014). Freshwater ecosystems are particularly susceptible to biological invasions, where human activities such as habitat modification and degradation, and pollution further amplify these threats (Bryers, 2002; Crooks et al., 2011).

The widespread introduction of fish into freshwater habitats, often for purposes such as their use as food, recreational fishing, and their potential for controlling insect populations, exemplifies this global issue (Gozlan et al., 2010). The significant impact of introduced fish arises from various direct interactions, including predation (e.g., Weis, 2011), competition for resources (e.g., Caiola & Sostoa, 2005), and hybridization with native species (e.g., D'Amato et al., 2007). Additionally, indirect effects such as habitat alteration and the introduction of diseases, combined with the invasive species ability to achieve high population densities, contribute to increase their impacts. These consequences can lead to both local and global species extinctions, as well as disruptions in ecosystem functioning (e.g., Capps and Flecker, 2013).

The mosquitofish (*Gambusia holbrooki*) is a social fish introduced worldwide, primarily to control mosquito populations (Clavero and Garcia-Berthou, 2005). Mosquitofish live in mixed-sex groups and establish dominance hierarchies determined by intraspecific aggressiveness and body size (Matthews & Wong, 2015; Flood and Wong 2017). While their primary diet consists of aquatic insects, mosquitofish display high dietary plasticity, consuming phytoplankton, micro and macro-invertebrates, and small amphibians and fish (e.g., Pen & Potter, 1991; Blanco et al., 2004). Mosquitofish, listed among the 100 most invasive species worldwide (Lowe et al., 2000), is known for its high invasiveness and have been associated with significant shifts in native community composition and ecosystem functioning (Alcaraz et al., 2008; MacDonald et al., 2012; Ruiz-Navarro et al., 2013). Despite their small size (< 5cm), particular attention has been given to their aggressive nature (e.g., Caiola & Sostoa, 2005; Pyke, 2008). Field studies focused on mosquitofish effects on other species have observed to produce alterations on reproduction periods (Howe et al., 1997), spatial distribution (García-Berthou et al., 2005; Pike 2008), and movement patterns (Bool et al. 2011) of other species. These impacts are often amplified by the diverse range of habitats colonized by mosquitofish (Pyke, 2008). Mosquitofish inhabit the littoral zones of lakes and rivers, which hold significant importance for fish stocks as they provide crucial spawning and nursery habitats for many fish species, thereby reducing fry predation risk and increasing access to diverse food resources (Winfield, 2004; Pyke, 2008). Consequently, the effects of mosquitofish might extend beyond competing larvivorous species, impacting juveniles of species at higher trophic levels, such as the pike.

The cisalpine pike (*Esox cisalpinus*) is endemic to Italy and southern Switzerland and was recently described as a distinct species from the northern pike (*Esox lucius*), based on morphological and genetic differences (Bianco & Delmastro, 2011; Lucentini et al, 2011). Currently classified as "Vulnerable" on the IUCN Red List, the cisalpine pike faces multiple threats, including the degradation of spawning habitats, hybridization with the non-native northern pike, and competition from other introduced species such as the largemouth bass (*Micropterus salmoides*) and wels catfish (*Silurus glanis*; Lorenzoni et al., 2002 a, b; Vejřík et al., 2017). Although historically misidentified as northern pike, the cisalpine pike appears to share similar ecological traits. It is a top predator in rivers, lakes and ponds and can grow up, on average, to 100 cm in length (Pompei et al., 2017). Many of its biological and ecological characteristics are assumed to be similar to those of its closest relative, the northern pike (Miliardi & Castaldelli, 2018). Pike is a phytophilic species, spawning in shallow waters by releasing eggs on submerged vegetation and plant roots. The larvae remain attached to vegetation until the yolk sac is fully absorbed. Like other pike species, it is a specialist predator whose diet shifts ontogenetically, from zooplankton during early life stages to fish as it matures (Morrow et al., 1997; Byström et al., 2007; Craig, 2008). To reduce predation risk from larger organisms and better exploit food resources, young-of-the-year (YOY) pike inhabit lake littoral zones (Laffaille et al., 2001). Cannibalism is common among YOY pike and serves as a mechanism to regulate intraspecific competition, favouring the survival of the fittest and most aggressive individuals (Craig, 2008). As a top predator, pike plays a crucial role in structuring lake ecosystems. Its decline, due to factors such as reduced spawning habitat and pressure from recreational fisheries (Oele et al., 2019; Bergström et al., 2022), may lead to significant changes in lake ecosystem dynamics (Berg et al., 1997; Byström et al., 2007).

Pike larvae and juveniles can co-occur with mosquitofish in shallow areas of lakes, ponds and canals, yet there is no information on the mechanisms, strength and consequences of their interactions. To address this gap, we conducted a laboratory experiment observing species behaviour and interactions over five days across a density gradient. Individuals of similar size were used to minimize predatory attacks (Keiller et al., 2021; Lewis et al., 2024). By studying two aggressive species, our aim was to highlight the behavioural effects of mosquitofish on a native top-predator. Given that pike cannibalism is influenced by density and environmental stressors (Giles et al., 1986; Craig, 2008), we hypothesize that the high voracity and aggressive behaviour of mosquitofish (Pyke, 2008) will increase intraspecific aggression among pike, potentially leading to higher rates of cannibalism. Furthermore, when mosquitofish dominate the assemblage, we predict that their voracious feeding behaviour may lead to resource depletion, indirectly causing pike mortality through starvation.

2. Methods

2.1 Species collection and acclimatisation

We collected mosquitofish (*Gambusia holbrooki*) from Lake Comabbio (NW Italy, 45°45'45.2" N, 8°41'33.3" E) using electrofishing techniques. Cisalpine pike fry were first-generation offspring obtained from wild individuals captured in Lake Candia (NW Italy, 45°32'31.3" N, 7°90'68.1" E) grown in the aquarium facilities of the Institute of Water Research of National Research Council of Italy (IRSA-CNR) located in Pallanza. The two species were housed separately in two holding tanks (217 × 40 × 17 cm) and maintained under constant water conditions: temperature of 18-20 °C, salinity of 1.7 ppt, and photoperiod of 12 hours light and 12 hours darkness. Before the start of the experiment, fish were acclimated to the laboratory conditions for two weeks and fed daily with *Artemia* spp. and black fly larvae (Diptera, Simuliidae).

2.2 Experimental design

The experiment was conducted in 33 plastic tanks (16 x 16 x 14 cm), each containing 1L of water, under constant conditions of temperature (18-20 °C) and salinity (1.7 ppt). The experiment was performed over a five-day period in July 2021 at the IRSA-CNR laboratories. Feeding was stopped 24 hours prior to the start of the experiment. To minimize mortality due to size differences (Skov et al., 2003), 48 individuals from each species were selected and distributed among the treatment tanks based on their similar body size (mean total length ± SD: mosquitofish 24.6 ± 1.9 mm; pike 25.6 ± 1.7 mm; Table S1). All mosquitofish used were males.

To assess the effects of mosquitofish on pike under different density conditions, we established three interspecific treatments with a total density of 4 individuals$\cdot$L^{-1}, corresponding to pike:mosquitofish ratios of: 3:1, 2:2, and 1:3. Additionally, four intraspecific control treatments per species were established, with densities from 4 to 1 ind$\cdot$L^{-1}. Each treatment was replicated three times. The experiment lasted five consecutive days. Dead individuals were removed from the tanks every morning and inspected for external injuries. Fish were fed to apparent satiation once daily in the morning with *Artemia* spp. and black fly larvae (Diptera, Simuliidae).

2.3 Behavioural characterization

To quantify fish behaviour and interactions, we recorded each tank for 1h starting at feeding period using action cameras (GoPro Hero, Qumox SJ4000, and Apexcam 4K/20MP). For each individual, we measured feeding time and voracity by analysing the first 10 minutes after food release. Additionally, we assessed individual aggressiveness and activity by analysing a 10-minute period starting 30 minutes after the food release, resulting in a total of 26.6 observation hours per species.

During the food consumption period, we observed that aggressive interactions between individuals (i.e., approach, charge, bite, and pursuit) were rare. Thus, we defined the feeding time as the duration of this low-aggression period per tank by representing the accumulative number of aggressive interactions (see example in Fig. S1). Feeding voracity for each individual was determined by: (i) time before first consumption, (ii) prey consumed first (i.e., black fly larva or *Artemia* spp.), and (iii) number of successful and unsuccessful consumptions per food type.

Aggressive interactions per individual were determined using six metrics: (i) approach, when one fish swims towards another; (ii) charge, when one fish rapidly swims towards another; (iii) butting or bite, forceful impact with the snout or bite; (iv) pursuit, a rapid chase of one fish by another; (v) escape, when one fish is forced by another to retreat (Caiola & Sostoa, 2005); and (vi) predation (including cannibalism).

Fish activity was quantified as the proportion of time each individual spent: (i) motionless (inactivity); (ii) swimming slowly; and (iii swimming rapidly (e.g., during pursuit).

2.4 Data analyses

Generalized linear mixed models (GLMM), followed by a Tukey's HSD pairwise multiple comparisons test, were performed using "glmmTMB" package (Brooks et al., 2017) on R (version 4.4.2; R Core Team, 2024) to determine any significant differences in the average of behavioural variables measured between treatments. The model included density and treatment as fixed effects, with tank ID and day as crossed random effects. The assumptions of the GLMM were checked using Levene's test for homogeneity, the Shapiro–Wilk test, and visual inspection of QQ plots for normality of residuals. For aggressive behaviour, the best-fitting family model (Poisson, negative binomial, or quasi-Poisson) was selected based on Akaike's information criterion (AIC; Table S2). Negative binomial and quasi-Poisson distributions were applied when overdispersion in count data was detected. For activity, the models were run using a binomial family. Species dominance, defined as the interactions performed by a single dominant individual per tank, was calculated using Simpson's dominance index.

3. Results

3.1. Cisalpine pike intraspecific treatments

Cisalpine pike mortality on intraspecific treatments increased with density ($R^2 = 0.607$, $F_{1,7} = 13.36$, p-value < 0.01), stabilising the density per tank to 2 ind·L^{-1} (Fig. 1, Table 1 & S3). One individual in the treatments with a starting density of 1 ind·L^{-1} died on the second day of the experiment, apparently due to a random event (Table S3).

One cannibalistic event was observed *in situ*, during which a pike consumed up to half of individualist conspecific. Other dead individuals showed bite marks along the body and severe fin damage consistent with conspecific attacks. The total number of aggressive interactions and the number of approaches per individual (which represented 86.7 ± 9.1 % of total aggressive interactions) were higher at higher intraspecific densities (GLMM, p-value > 0.1; Table 1, S4 & S5). The number of bites performed per individual (9.9 ± 7.8 % of total aggressive interactions) was greatest in the treatment starting with 4 ind·L^{-1} (p-value > 0.1; Table 1 & S5). The treatment starting with 3 ind·L^{-1} showed the lowest aggression levels, with no bites observed in two of the tanks (Table S5). The 65.7 ± 24.5 % of all agonistic interactions per tank were performed by a single dominant individual, without differences among density treatments (dominance index: 0.57 - 0.72; p-value > 0.1; Table 1, S4 & S5). Time spent swimming was maximum in the treatment starting with 2 ind·L^{-1} (p-value > 0.1; Table 1 & S4), where individuals were active during 19.4 ± 9.9 % of the observation time. Individuals with fin damage in treatments starting with 3 and 4 ind·L^{-1} showed no mobility or feeding activity and died within 1-2 days (Table S3 & S6). Time spent feeding in all intraspecific pike treatments exceeded the observation period (i.e., ≥10 minutes; Table S6). In one observation, a single individual in the 2 ind·L^{-1} treatment did not feed.

3.2. Mosquitofish intraspecific treatments

One mosquitofish died in each of the tanks started with a density of 4 ind·L^{-1} (Fig. 1; Table S3). The deceased fish did not have fatal injuries on their bodies. Time spent feeding was shortest in the treatment starting with 4 ind·L^{-1} (p-value < 0.05; Table 1 & S4).

The treatment starting with 3 ind·L^{-1} exhibited the lowest number of aggressive interactions per individual (p-value > 0.05; Table S5). In all treatments, one dominant individual performed 91.3 ± 13.8 % of the aggressive interactions, with no significant differences according to density treatments (dominance index ≥ 0.84; p-value > 0.1; Table 1, S4 & S5). Bites accounted for the 63.4 % ± 10.6 % of the aggressive interactions (Table 1). The highest frequency of pursuit and approach interactions were in the treatment starting with 2 ind·L^{-1} (p-value < 0.05 and p-value < 0.1, respectively; Table 1 & S4). Mosquitofish density did not have an impact on the time spent swimming (p-value > 0.1; Table S4), but it did increase with the overall aggressiveness of the tanks (GLMM, p-value < 0.01; Table S4). On two and three observations during feeding time, respectively, one individual did not feed in the treatments starting with 2 ind·L^{-1} and 3 ind·L^{-1} respectively. In all observations of the treatment starting with 4 ind·L^{-1}, one individual did not feed until the density was reduced to 3 ind·L^{-1} by mortality (Table S6).

3.3. Mosquitofish – Cisalpine pike interactions

The presence of mosquitofish increased pike mortality by 66.6 ± 23.0 % (Fig. 1; Table S3). By the end of the experiment, just one pike YOY remained alive in two of the interspecific tanks that started with 3 pike:1 mosquitofish ind·L^{-1}. Dead pike presented fin damage, mainly on posterior fin, that conditioned pike to assume an upside-down position generally during one day before the death. No mosquitofish died in the interspecific treatments (Fig. 1; Table S3). In treatments where mosquitofish were more abundant (1:3 ind·L^{-1}), the single pike continued to feed, while one mosquitofish was consistently excluded from feeding until the pike death (Table S6). In the remaining treatments, mosquitofish fed actively, and pike only stopped feeding when injured and positioned ventrally.

Pike agonistic behaviour and activity did not differ significantly among treatments (p-value > 0.05; Table S4). However, pike spent more time swimming and feeding in treatments with higher conspecific density (3:1 and 2:2 ind·L^{-1}) than in 1:3 ind·L^{-1} (p-value < 0.05; Tab. 1 & S4). Mosquitofish were significantly more aggressive in treatments with higher pike abundance (i.e., 2:2 and 3:1 ind·L^{-1}; $p < 0.05$; Table S4), with over 78% of their bites directed toward conspecifics ($p < 0.01$; Table 1 & S4). As in intraspecific treatments, 91.3 ± 13.8 % of all agonistic interactions per tank were performed by a single dominant mosquitofish (Table 1). A total of 3 bites from pike towards mosquitofish were observed during the observation time (Table 1). Mosquitofish spent more time swimming in 2:2 and 3:1 ind·L^{-1} treatments than in the intraspecific controls (p-value < 0.05; Fig. 1, Table 1 & S4). For both species, time spent swimming increased according to the number of aggressive interactions performed in each tank (p-value < 0.01; Table S4).

4. Discussion

The results of this laboratory study show that mosquitofish aggressive behaviour has strongly reduces the survival of cisalpine pike young-of-the-year (YOY). As expected, both species exhibited high levels of aggressiveness towards conspecifics in intraspecific treatments. Mosquitofish established a hierarchical structure where a dominant individual accounted for over 84 % of the interactions. This high aggressiveness led to the death of one individual per tank in the 4 ind·L^{-1} treatment, primarily due to feeding exclusion and persistent attacks. Individuals with low mobility were also observed on treatments with lower density, all characterized by darker coloration, that might be related with stress conditions of these submiss individuals. Although our observations were made in small tanks, such aggressive interactions could represent precursors for dispersal events, where dominant individuals force subordinates to leave the group (Cote et al., 2011). According to our observations, mosquitofish interactions, mainly bites, were carried out to maintain hierarchical status or to compete for food and territory (i.e., social aggressivity). In contrast, pike interactions in intraspecific treatments were mainly approaches and bites to predate their conspecifics. This was expected, as cannibalism is common on larval pike (Greszkiewicz et al., 2020).

Feeding strategies differed between the two species. Mosquitofish are opportunistic surface feeders, primarily consuming invertebrates (Pen et al., 1993; Stoffels & Humphries, 2003). In our trials, mosquitofish actively patrolled and captured mobile prey in their path.. Pike displayed a predator-catch behaviour, as well its relative northern pike (Greszkiewicz et al., 2020), approaching their prey before a swift capture. These differences in predation strategies may have allowed pike to feed in interspecific treatments, despite the time for food availability has been reduced by mosquitofish.

In interspecific treatments, mosquitofish caused the mortality of ~92 % of pike YOY through fin-nipping. Furthermore, our results show that mosquitofish increased their aggressive behaviour in interspecific treatments. Similar patterns have been reported when mosquitofish interacted with the Pacific blue-eye (*Pseudomugil signifer*; Keiller et al., 2021). Surprisingly, we found that mosquitofish was more aggressive towards conspecifics. In hierarchical species, where niche overlap is higher among conspecifics, the introduction of a second species can significantly alter population dynamics and increase conspecific agonistic interactions (Forrester et al., 2006; Munday, 2004). Aggression among conspecifics may increase to maintain or change dominance hierarchies and as a response to increased competition for resources. These findings highlight the territorial nature of mosquitofish, where intraspecific aggression may promote dispersal of subordinate individuals. As seen in another mosquitofish, *Gambusia affinis*, the more aggressive individuals are more likely to disperse, and subordinate individuals can be forced to leave established groups (Cote et al., 2011). Thus, this behaviour can facilitate mosquitofish dispersal and colonization of new habitats. In natural systems, mosquitofish often share littoral habitats with other species such as pumpkinseed (*Lepomis gibbosus*), which might prey upon small mosquitofish (Horth, 2004). Although such multi-species interactions remain poorly studied, our results suggest that increased aggression within mixed assemblages could further promote mosquitofish dispersal and colonisation of new habitats.

In our study, we observed that the pike caudal fin was particularly vulnerable to mosquitofish bites. In several cases, a single mosquitofish bite was sufficient to remove the caudal fin, leading to loss of balance and subsequent death. The morphology of the pike caudal fin is crucial for its rapid movements to catch preys. Given that the caudal fin is essential for rapid propulsion and prey capture in pike, fin damage significantly reduced predatory efficiency and escape ability, ultimately leading to mortality as observed in our experiment. Bodies of dead pike showed no additional bites from mosquitofish, and living but inverted individuals were no longer attacked, suggesting that mosquitofish aggression was not predatory. Similar fin-nipping behaviour has been observed towards other species such as Iberian killifish, *Aphanius iberus*, without leading to their death. Given that mosquitofish have a propensity for fin nipping (Beatty et al., 2022), which was observed as the most frequent form of agonistic interaction in this study, the fin morphology and resistance of a second species may determine their vulnerability to mosquitofish aggression.

Sinclair et al., 2011, studied the effects of fin resistance in mosquitofish intraspecific attacks, concluding that individuals with larger fins can withstand more fin damage before experiencing detrimental effects, such as reduced swimming speed. While fin-nipping alone may not always cause mortality, resulting skin damages could facilitate secondary infections (Esteban, 2012). These attacks might also induce antipredator behaviours, such as increased refuge use, reduced foraging time, and displacement to suboptimal habitats, which can hinder growth and heighten competition with other species (Mills et al., 2004; Pyke, 2008; Sutton et al., 2013). Contrary to our expectations, the direct bites and high mobility of mosquitofish limited our ability to detect indirect behavioural effects on pike (e.g., increased cannibalism due to competition). Understanding such species interactions is crucial for predicting the impact of non-native species on ecosystems and for developing effective strategies to mitigate them. Our results support the main hypothesis aggressive behaviour is a key trait underlying the ecological impact and invasion success of mosquitofish. However, not all the studies found agonistic interactions between mosquitofish and other species (Ciepiela et al., 2021; Lewis et al., 2024), suggesting that behavioural aggression, while widespread, is context-dependent.

Conclusions

Although laboratory experiments lack the full complexity of natural environments, the effects of mosquitofish on colonized habitats have been widely studied. By studying behavioural interactions between mosquitofish and cisalpine pike YOY, this study provides evidence that mosquitofish exhibit higher levels of aggression toward conspecifics in interspecific treatments. Aggressiveness is frequently cited as a trait of successful invasive species, and our results suggest that increased aggression during interspecific interactions may represent a mechanism behind mosquitofish dispersal and boldness. This study highlights that mosquitofish aggression could significantly affect the survival of the native species, such as *Esox cisalpinus*, at early life stages. Together with their high pollution tolerance, rapid reproduction, and competitive behaviour, the intraspecific aggressiveness shown by mosquitofish might be a driving factor facilitating their invasiveness. In our laboratory experiments, mosquitofish showed fewer interspecific interactions than conspecifics ones. Therefore, the effects observed in this study may be less pronounced in natural environments. However, individual dominance and aggression were independent of treatment density, suggesting that aggressive behaviour is an intrinsic species trait that may persist regardless of habitat size or complexity.

Mosquitofish have the potential to decimate native fish populations with restricted habitats, such as the ornate rainbowfish (*Rhadinocentrus ornatus*) in Australian coastal streams (Keller & Brown, 2008) and the Iberian killifish (*Aphanius iberus*) in Iberian salt marshes (Ruiz-Navarro et al., 2013). In our case, pike and other species that reproduce in littoral lake zones may also be affected by mosquitofish; however, the magnitude of these effects might depend on habitat size and natural barriers limiting mosquitofish dispersal. While habitat modification, degradation, pollution, and other anthropogenic or climatic factors play a major role in determining the conservation status of pike and other native freshwater fishes, these disturbances can also facilitate the establishment of non-native species (Mor et al., 2022). Our results suggest that the presence of mosquitofish could negatively affect cisalpine pike populations in areas where the two species coexist.

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7. Tables

Table 1. Summary of behavioural and survival metrics for pike (*Esox cisalpinus*) and mosquitofish (*Gambusia holbrooki*) across intraspecific (densities of 1–4 ind·L^{-1}) and interspecific (pike:mosquitofish ratios of 1:3, 2:2, and 3:1) treatments.

		Intraspecific (ind·L^{-1})				**Interspecific (Pike:Mosquitofish ind·L^{-1})**		
		1	**2**	**3**	**4**	**1:3**	**2:2**	**3:1**
Mean death (%)	Pike	33.3 ± 57.7	0. 0 ± 0.0	33.3 ± 33.3	58.3 ± 14.4	100. 0 ± 0.0	100. 0 ± 0.0	77.8 ± 19.2
	Mosquitofish	0. 0 ± 0.0	0. 0 ± 0.0	0. 0 ± 0.0	25.0 ± 0.0	0. 0 ± 0.0	0. 0 ± 0.0	0. 0 ± 0.0
Feeding metrics								
Time spend feeding (s)	Pike		600	600	600	264 ± 147.5	473.2 ± 157.5	536 ± 140.7
	Mosquitofish		328 ± 181.2	290 ± 169.4	128 ± 59.4			
Time first predation (s)	Pike		22.5 ± 29.6	22.5 ± 34.6	61.8 ± 116.7	17.8 ± 32.2	55.3 ± 74.2	33.5 ± 34.6
	Mosquitofish		13.2 ± 12.6	10.6 ± 14.7	9.3 ± 22.5	19.1 ± 35.6	16.5 ± 47.9	13.2 ± 13.6
Agonistic interactions								
Agonistic interactions (nº/minute)	Pike		1.3 ± 0.6	2.2 ± 0.6	3.3 ± 2.4	0.1 ±0.1	0.4 ± 0.4	1.3 ± 0.8
	Mosquitofish		34.0 ± 24.8	23.1 ± 15.5	46.8 ± 20.8	34.3 ± 21.4	48.1 ± 35.7	7.1 ± 8.5
Approach (%)	Pike		81.1 ± 5.4	96.5 ± 6.1	82.5 ± 7.2	0. 0 ± 0.0	40.5 ± 36.7	79.6 ± 26.6
	Mosquitofish		11.3 ± 1.4	3.9 ± 1.0	12.3 ± 7.5	18.7 ± 13.6	13.9 ± 7.8	56.1 ± 3.6
Charge (%)	Pike		2.8 ± 4.8	0. 0 ± 0.0	7.3 ± 8.2	16.7 ± 28.9	0. 0 ± 0.0	0. 0 ± 0.0
	Mosquitofish		13.8 ± 8.3	21.5 ± 15.9	12.2 ± 12.3	18.9 ± 5.6	17.0 ± 4.9	2.9 ± 1.0
Bite (%)	Pike		16.1 ± 8.3	3.5 ± 6.1	10.2 ± 4.2	16.7 ± 28.9	26.2 ± 25.1	20.4 ± 25.6
	Mosquitofish		57.0 ± 9.2	64.1 ± 13.1	69.1 ± 9.2	50.5 ± 14.7	53.1 ± 9.4	37.1 ± 5.0
Pursuit (%)	Pike		0. 0 ± 0.0	0. 0 ± 0.0	0. 0 ± 0.0	0. 0 ± 0.0	0. 0 ± 0.0	0. 0 ± 0.0
	Mosquitofish		17.9 ± 2.4	10.5 ± 8.1	6.4 ± 4.0	11.9 ± 5.6	16.0 ± 4.5	3.9 ± 4.2
Total interspecific bites (number - %)	Pike					1 (100 ± 0)	2 (50.0 ± 70.7)	0 (0)
	Mosquitofish					24 (14.1 ± 12.5)	54 (21.7 ± 7.3)	79 (100 ± 0)
Dominance index	Pike		0.72 ± 0.23	0.69 ± 0.27	0.57 ± 0.23	0.86 ± 0.22	0.88 ± 0.21	0.83 ± 0.23
	Mosquitofish		0.94 ± 0.11	0.85 ± 0.20	0.84 ± 0.20			
Dominance (%)	Pike		78.2 ± 18.9	73.9 ± 23.6	65.3 ± 21.4	90.3 ± 15.9	91.1 ± 15.4	89.4 ± 13.3
	Mosquitofish		96.6 ± 7.0	89.3 ± 15.2	88.6 ± 16.2			
Activity								
Time spent swimming	Pike	0.16±0.17	0.19±0.10	0.16±0.12	0.12±0.13	0.05±0.93	0.12±0.15	0.15±0.17
	Mosquitofish	0.19±0.15	0.21±0.17	0.11±0.16	0.12±0.17	0.21±0.20	0.40±0.20	0.39±0.17

8. Figures

Figure 1. Survival (%) of mosquitofish (*Gambusia holbrooki*, gray bars) and cisalpine pike (*Esox cisalpinus*, orange bars) under: (a) intraspecific interactions across increasing starting densities (1–4 ind·L^{-1}) for each species, and (b) interspecific interactions across three treatment ratios (mosquitofish:pike = 1:3, 2:2, 3:1). Error bars indicate the standard error of the mean.