Antipredator Responses by Native Mosquitofish to Non-Native Cichlids: An Examination of the Role of Prey Naiveté

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Abstract

The strong impact of non-native predators in aquatic systems is thought to relate to the evolutionary naiveté of prey. Due to isolation and limited dispersal, this naiveté may be relatively high in freshwater systems. In this study, we tested this notion by examining the antipredator response of native mosquitofish, *Gambusia holbrooki,* to two non-native predators found in the Everglades, the African jewelfish, *Hemichromis letourneuxi,* and the Mayan cichlid, *Cichlasoma urophthalmus*. We manipulated prey naiveté by using two mosquitofish populations that varied in their experience with the recent invader, the African jewelfish, but had similar levels of experience with the longer-established Mayan cichlid. Specifically, we tested these predictions: (1) predator hunting modes differed between the two predators, (2) predation rates would be higher by the novel jewelfish predator, (3) particularly on the naive population living where jewelfish have not invaded yet, (4) antipredator responses would be stronger to Mayan cichlids due to greater experience and weaker and/or ineffective to jewelfish, and (5) especially weakest by the naive population. We assayed prey and predator behavior, and prey mortality in lab aquaria where both predators and prey were free-ranging. Predator hunting modes and habitat domains differed, with jewelfish being more active search predators that used slightly higher parts of the water column and less of the habitat structure relative to Mayan cichlids. In disagreement with our predictions, predation rates were similar between the two predators, antipredator responses were stronger to African jewelfish (except for predator inspections), and there was no difference in response between jewelfish-savvy and jewelfish-naive populations. These results suggest that despite the novelty of introduced predators, prey may be able to respond appropriately if non-native predator archetypes are similar enough to those of native predators, if prey rely on general antipredator responses or predation cues, and/or show neophobic responses.

Introduction

Species invasions that cause high impact to invaded communities often result from novel intertrophic interactions such as predation (Kats & Ferrer 2003; Gurevitch & Padilla 2004; Salo et al. 2007). By novel, we mean interactions where the predator has no common evolutionary history with native prey, resulting in prey that are evolutionarily naive to the introduced predators (Diamond & Case 1986; Cox &

Lima 2006). An extreme form of novelty is illustrated by the introduction of predators to oceanic islands (and Australia), where predation itself may be novel or the predator archetype is absent (Ogutu-Ohwayo 1990; Fritts & Rodda 1998; Blackburn et al. 2004). A less severe and perhaps more common form of novelty stems from variation in predator archetypes, where native and non-native predators exhibit varying behavioral and morphological adaptations for prey capture (Cox & Lima 2006). For instance, variation in predator hunting mode (Schmitz 2007) among native and non-native predators could result in prey experiencing some degree of predator novelty. Under either scenario, the evolutionary naiveté of native taxa can result in the failure of prey to recognize predation threats, in inappropriate antipredator responses or in appropriate but ineffective responses (Diamond & Case 1986; Banks & Dickman 2007).

Prey naiveté is hypothesized to be partly responsible for the strong negative effects of introduced aquatic predators (Cox & Lima 2006). Dispersal by aquatic top predators is relatively low and isolation at intercontinental and regional scales can be high in freshwater systems, resulting in significant variation in predator archetypes and regimes among and within water bodies. For instance, hydrological gradients can result in small-scale variation in predation regimes (Wellborn et al. 1996) that should generate prey naiveté to predators that are allopatric along the gradient (Cox & Lima 2006). In the Florida Everglades, the recurrent pattern of seasonal dry-down limits the abundance of large-bodied predators both temporally and spatially (Chick et al. 2004; Trexler et al. 2005), which may result in gradients in naiveté that accompany hydrological gradients across the landscape (i.e., long vs. short hydroperiod marshes), although this remains untested.

Another and perhaps more important source of gradients in prey naiveté is the patchiness in the distribution of non-native predators. The African jewelfish, *Hemichromis letourneuxi,* one of the most recent invaders of the Everglades, is presently limited to the southern and western regions of the ecosystem (Shafland et al. 2008). Jewelfish were first detected in Everglades National Park (ENP) in 2000 (J. Kline, pers. comm.) and are presently undergoing a rapid range expansion into longer hydroperiod marshes and mangrove regions (Shafland et al. 2008; Rehage et al. unpublished data), but have not yet colonized marshes in the Water Conservation Areas in the northern Everglades. They are piscivores, with fish accounting for 70–80% of their diet

(Loftus et al. 2006). Their small size allows them to invade shallower habitats, where larger invaders have previously been excluded, and thus they are an especially concerning invader. Among the more established invaders of the Everglades is the Mayan cichlid, *Cichlasoma urophthalmus,* first detected in 1983 in ENP and presently widespread throughout the system (Loftus 1987; Fuller et al. 1999; Shafland et al. 2008). Mayan cichlids can be a dominant component of the fish community of certain habitats. In particular, they can account for up to 40% of fish abundance in the mangrove zone (Trexler et al. 2001). They are also predators, with fish being a dominant prey item in their diets (Bergmann & Motta 2005). These two cichlids invaders are currently the two most abundant fish invaders in ENP out of about 14 established species (Shafland et al. 2008; J. Kline, pers. comm.), and thus have the potential to have a high impact on invaded aquatic communities. How native Everglades prey, particularly small-fish taxa, cope and respond to these predation threats that vary in the degree of novelty is currently unexplored. Furthermore, although prey naiveté is invoked as a major mechanism for the high impact of introduced predators, few studies have examined it directly (Cox & Lima 2006).

In this study, we used laboratory assays to examine the effect of predator novelty and prey naiveté on predator–prey interactions between native Everglades prey and non-native cichlid predators. Specifically, we quantified the antipredator behavior of native Eastern mosquitofish, *Gambusia holbrooki,* and the hunting mode, habitat domain and lethality of the Mayan cichlid and African jewelfish. Mosquitofish are the most ubiquitous fish species in the Everglades (Trexler et al. 2005), and should be readily encountered and consumed by both predators. In fact, stomach analyses of African jewelfish from our study sites in ENP show that mosquitofish are the most abundant prey item (Loftus et al. 2006). Predation by non-native predators on ubiquitous prey such as mosquitofish may lead to invaders having wide-ranging impacts. Moreover, we expect ubiquitous prey to have important functional roles throughout the system, and if non-native predators are able to significantly decrease their abundance, this could also contribute to high impacts in the invaded system. We manipulated the degree of novelty in predator–prey interactions by using these two predator species that varied in the time since invasion, and mosquitofish populations that varied in experience with them. We hypothesized that

variation in the hunting modes and habitat domain of the predators would make jewelfish a relatively novel predation threat. Due to the greater naiveté of Everglades prey with African jewelfish, we expected predation rates to be higher by this novel predator. We hypothesized that due to greater experience, the antipredator responses of prey would be stronger to the Mayan cichlid. We compared mosquitofish populations with the expectation that prey from jewelfish-invaded areas would exhibit greater and/or more effective antipredator responses to jewelfish than naive prey from areas where jewelfish are absent, and thus jewelfish predation would be higher on the naive population.

Methods

To examine the predatory behavior and effect of the cichlids species and the antipredator response of mosquitofish, we conducted behavioral assays in laboratory aquaria. In a 4×2 factorial design, we observed the effects of four predation treatments on two mosquitofish prey populations. Predation treatments consisted of predator pairs in a replacement series design where predator density remained constant (Sih et al. 1998). Treatments included: (JJ) two African jewelfish, (MM) two Mayan cichlids, (MJ) one African jewelfish + one Mayan cichlid, and (NP) no predators. Mosquitofish were collected from two populations that varied in their naiveté to African jewelfish, but had similar levels of experience with Mayan cichlids. The ENP mosquitofish population was considered 'experienced' since African jewelfish have been present and abundant for close to a decade. A second prey population from northern Water Conservation Area 3A (WCA3A) was considered naive to African jewelfish since despite repeated sampling by ourselves and colleagues over the past few years, they have never been collected there or that far north in the inner Everglades ecosystem. Both populations should have similar levels of experience with Mayan cichlids, which have been present and abundant throughout since the 1980s.

Trials were conducted in two blocks between July 27–30, 2007 and March 3–8, 2008, corresponding to the wet and dry seasons in the Everglades, respectively (hereafter referred to as the season effect). A minor objective of our study was to examine whether predator motivation and antipredator behavior would vary seasonally in response to dry-down and the expected physiological stress associated with it (i.e., reduced prey abundance and poor condition). Cichlids were collected from the Rocky

Glades region of ENP using unbaited minnow traps deployed overnight between June–July 2007 and January–February 2008. Mosquitofish were collected using dip nets at a WCA3A site (N 26.147, W 80.57134) and at the same ENP Rocky Glades locations where predators were collected. Predator species were size-matched in trials, but because of species-specific size differences, African jewelfish were adults (51.7 ± 0.9 mm standard length), while Mayan cichlids were juveniles of approximately 65.6 ± 1.8 mm standard length. Bergmann & Motta (2005) showed that fish remain the primary prey item for Mayan cichlids throughout ontogeny. All prey used in the study were juveniles (13.1 ± 0.2 mm standard length). Prior to the experiment, we held predators and prey in 795-l outdoor tanks and fed them a combination of live prey, including mosquitofish from both populations. We fed prey flakes *ad libitum*.

In both seasons, trials were conducted over four consecutive days. Each day, we tested a single replicate of the eight treatment by population combination (four treatments × two prey populations × four days × two seasons = 64 experimental units). To minimize interindividual variation in predator motivation, randomly assembled predator pairs were used repeatedly with the two prey populations. Pairs were randomly assigned to days 1 or 2 of the block and then used again in days 3 and 4, respectively, with a different prey population. For instance, a predator pair that experienced the ENP prey population on day 1 was assigned to the WCA3A mosquitofish population on day 3, and similarly for days 2 and 4. Trials were not conducted on consecutive days to obtain overnight prey mortality rates and then standardize hunger levels prior to the next trial. This protocol was repeated with a new set of predators in the dry season, for a total number of 24 jewelfish and 24 Mayan predators used in the study.

For each trial, behavioral data were collected on a group of six mosquitofish (six prey × four treatments × two populations × eight replicates = 384 prey). Mosquitofish groups from both populations were isolated in 5.7-l containers the evening prior and then randomly assigned to treatments on the day of trials. Similarly, predators were isolated in 5.7-l containers the evening before trials and between trials. To standardize hunger levels, all feeding was suspended 24 h before trials, as well as between trials for the predators (e.g., no feeding on day 2 for a predator used on days 1 and 3).

Trials were conducted in 8 56.8-l aquaria ($50 \times 24.5 \times 40$ cm height) covered on all four sides

with white vinyl. Artificial vegetation was used to provide structural complexity for both predators and prey (16 × 16 cm, covering approximately one-third of tank area). This artificial vegetation consisted of black plastic strips (4 × 22 cm) attached to a weighted plastic grid that rested on the bottom and to one side of each tank. To minimize observer effects, observations were conducted through mirrors placed at a 45° angle above tanks. At the beginning of each trial, prey were released and allowed to acclimate for 15 min before predators were added. The first observation was taken 5 min after predator release.

Prey and predator behavior was assessed through spot-check observations conducted by two observers, one taking data on the predators and the other on the prey (Martin & Bateson 2007). Observers spent 20–60 s per tank accounting for all individuals and noting their activity, microhabitat use, and the shoaling behavior of prey. Ten spot-check observations were conducted per tank, one every 10–12 min for a total trial duration of approximately 2 h. All observations were conducted between 10 am and 1 pm. Activity was scored as active if there was movement that resulted in a change in position (e.g., movement of fins was scored as inactivity). For microhabitat use, we noted vertical distribution within the tank (top, middle or bottom one third of the water column), and whether predators and prey were in or out of the habitat structure. For these three variables, we calculated the proportion of predators and prey engaged in each behavior over the 10 observations and then averaged them. Shoaling behavior by mosquitofish was scored as a 1 if prey were aggregated in a social group of at least four individuals (within approximately four body lengths of each other); otherwise it was scored as a zero, and then scores were averaged over the 10 observations. At the end of spot-check observations, tanks were observed continually for 5 min to obtain count data on the attacks on prey and predator inspections. Rapid approaches by predators to the prey with or without contact were considered attacks. Predator inspections consisted of cautious approaches by prey, followed by a rotation or retreat of the prey while still visually fixated on the predator. Actual predation events during this observation period occurred in only three of the 64 trials for a total of nine prey consumed.

At the end of all behavioral observations, we assessed mortality rates of the prey in the same observation tanks. To avoid prey depletion, an additional six prey (of the same size and population)

were added to each tank. In the few cases where prey were consumed during the observation period, we replaced them to begin all replicates with 12 mosquitofish. We left predators and prey in covered tanks overnight, and between 7 and 8 am on the following day, we uncovered tanks and counted the number of surviving prey. Photoperiod over the study was set to 14L:10D, and water temperature averaged 25.7 ± 0.18°C.

Statistical Analyses

Population differences among predator treatments were examined with linear models. In addition to
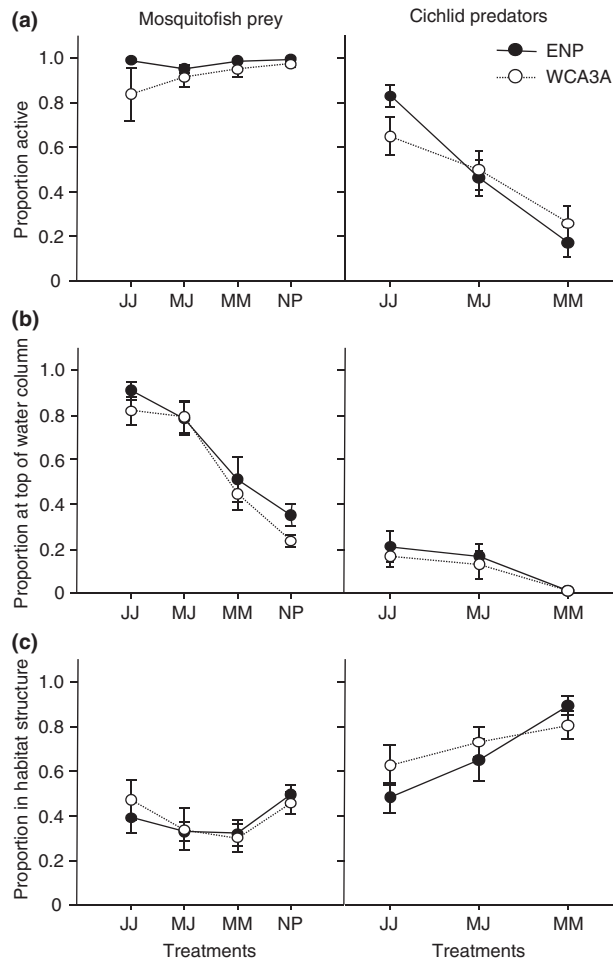

Fig. 1: Effects of predator treatment (JJ = 2 African jewelfish, MM = 2 Mayan cichlids, MJ = African jewelfish + Mayan cichlid, and NP = no predator) and prey population (WCA3A = naïve and ENP = experienced with African jewelfish-both are experienced with Mayan cichlids) on the (a) activity level, (b) water column distribution, and (c) use of habitat structure of both predators and prey. All variables represent the proportion of fish in each behavior ($\bar{x} \pm$ SE).

population and treatment main effects, we tested the effects of the population by treatment interaction, of season (the blocking factor), and of predator pair nested within season to account for the repeated used of predators. These same effects were tested in two MANOVAs ran prior to the ANOVAs. A MANOVA was run for the five focal prey variables for which data were collected in all treatments: the proportion of prey active, at the top of the water column, and using the habitat structure, the occurrence of prey shoals, and prey mortality. A second MANOVA was run for the remaining five focal variables that involved predators and for which data were collected only in the three predation treatments: the proportion of predators active, at the top of the water column, and using the habitat structure, and the number of predator attacks, and inspections. Preliminary analyses also examined the effect of predator sequence (day 1 vs. 3, and day 2 vs. 4), and of the sequence by season interaction on all response variables and found little effect; therefore, these factors were removed from final analyses reported here. Predator sequence only affected two of the predator variables and none of the prey variables, and the effect was seen only in the dry season, in which predators spent more time at the top of the water column and less time in the habitat structure on days 3 and 4 relative to days 1 and 2.

To meet parametric test assumptions, we examined the behavior of residuals and transformed variables where evidence of non-normality and variance heterogeneity was found, which included all variables except prey mortality (Kery & Hatfield 2003). Angular transformations were applied to proportions and log transformations (Ln of observed value + 1) to counts. Tukey's pairwise comparisons were used to compare treatment and treatment by population means. All statistical tests were conducted using the GLM procedure in SAS® 9.1.3.

Results

Prey Behavior

The antipredator behavior of mosquitofish varied to a greater extent as a function of predator treatments than populations. Little variation in antipredator behavior was detected between jewelfish-naive (WCA3A) and jewelfish-experienced (ENP) populations. The exception was prey activity, which was high overall, and relatively higher in the ENP population (98% active relative to 92% in WCA3A population, Fig. 1a), but was unaffected by predator treatment (Table 1).

The presence of predators resulted in shifts in microhabitat use by prey. Mosquitofish were found higher in the water column if the predators were African jewelfish or mixed (Tukey's pairwise comparisons: JJ vs. MM and NP, p < 0.0001; MJ vs. MM and NP, p < 0.0004). Over 80% of prey were observed in the top one-third of the water column in JJ and MJ treatments compare to only 48% in

Table 1: Result of ANOVAs and MANOVAs (p values and R^2) testing treatment, population, season, and predator pair effects (significant effects are in bold)

| Variables | R^2 | P values for effects | | | | |
		Treatment	Population	Treatment × Population	Season	Predator pair (Season)
MANOVA 1 Wilks' Lambda		**0.0001**	0.0945	0.9248	**0.0001**	0.4322
MANOVA 2 Wilks' Lambda		**0.0001**	0.9567	0.5999	0.6798	**0.0522**
Prey						
Activity	0.21	0.4318	**0.0380**	0.4814	0.0932	0.5437
Vertical distribution	0.63	**0.0001**	0.1262	0.8297	0.5850	0.0992
Use of habitat structure	0.18	0.0571	0.8561	0.7119	0.2182	0.7063
Shoaling behavior	0.64	**0.0001**	0.4871	0.3419	**0.0001**	0.5835
Predator inspections	0.32	**0.0112**	0.7997	0.3269	0.1741	0.1380
Mortality	0.64	**0.0001**	0.5741	0.9609	0.9655	0.4663
Predators						
Activity	0.56	**0.0001**	0.8481	0.2298	0.8780	0.2126
Vertical distribution	0.48	**0.0001**	0.3185	0.7373	0.4942	**0.0192**
Use of habitat structure	0.38	**0.0008**	0.6445	0.2574	0.7646	0.1892
Attacks	0.14	0.2009	0.7829	0.6451	0.3486	0.5315

MANOVA 1 contains the five variables measured in all treatments, and MANOVA 2 contains the 5 variables measured in the three predator treatments only.
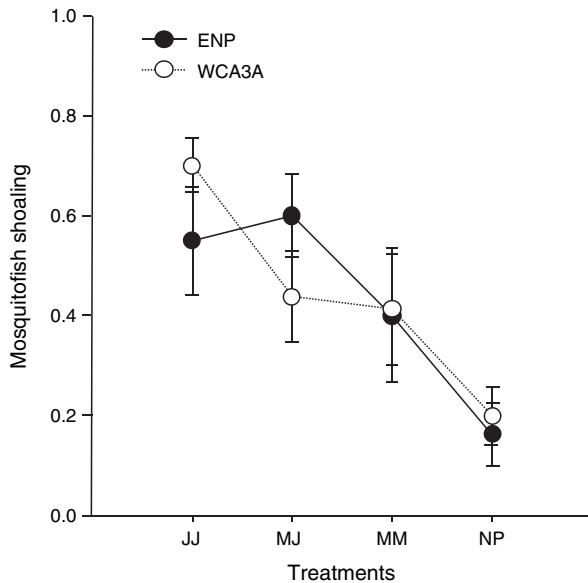
Fig. 2: Shoaling behavior of mosquitofish across predator treatments (JJ = 2 African jewelfish, MM = 2 Mayan cichlids, MJ = African jewelfish + Mayan cichlid, and NP = no predator) and prey populations (WCA3A = naive and ENP = experienced to jewelfish-both are experienced with Mayan cichlids). Groups of 4, 5 or 6 prey were considered a shoal and scored as 1s; smaller groups were scored as 0s. Shown are x̄ ± SE.
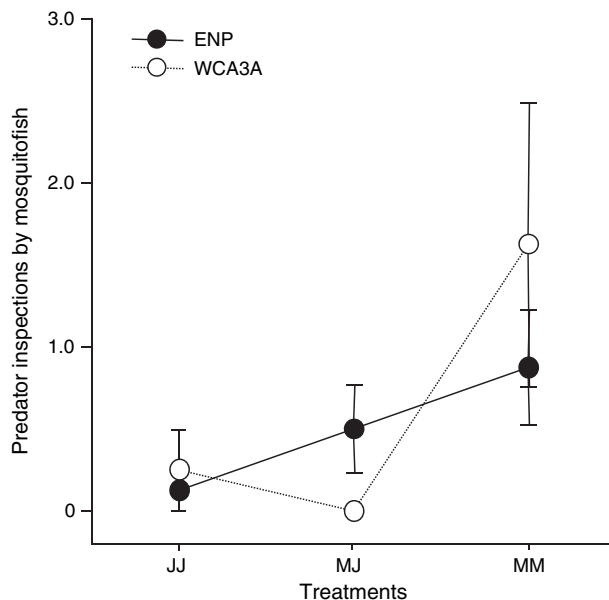

Fig. 3: Counts of predator inspections by mosquitofish across predation treatments (JJ = 2 African jewelfish, MM = 2 Mayan cichlids, MJ = African jewelfish + Mayan cichlid, and NP = no predator) and mosquitofish populations (WCA3A = naive and ENP = experienced with African jewelfish-both are experienced with Mayan cichlids) over a 5-min continuous observation period at the end of trials. Shown are x̄ ± SE.

MM and 30% in NP treatments (Fig. 1b). Prey tended to use the habitat structure more if predators were absent than if predators were the Mayan cichlid pair since Mayan cichlid use of the structure was relatively high (Table 1, Fig. 1c).

Mosquitofish shoaled more in the presence of predators (NP vs. JJ, MM and MJ, p < 0.0248, Table 1). Shoals were also more common with the jewelfish pair (JJ vs. MM, p = 0.0058). The occurrence of shoals averaged 63% with the jewelfish pair, 41% with the Mayan pair, and only 18% in the no predator treatment (Fig. 2). Shoaling rates did not differ between the single and mixed predator treatments. Shoaling was the only variable that varied between blocks (higher in the dry season, Table 1). Despite the stronger response of mosquitofish to jewelfish with their shoaling behavior and greater use of the top of the water column, predator inspections by both prey populations were higher on the less novel Mayan predators (MM vs. JJ and MJ, p < 0.0335, Fig. 3).

Predator Behavior

African jewelfish and Mayan cichlids varied in their activity and microhabitat use, suggesting variation in hunting modes and habitat domains. Their behavior was also unaffected by the degree of novelty of the prey; predator behavior was similar toward the ENP and WCA3A populations (Table 1). Predator activity was highest for the jewelfish pair, intermediate for the mixed predator treatment and lowest for the Mayan pair (Table 1, all pairwise comparisons, p < 0.0064; Fig. 1a). Jewelfish pairs were more likely to be found in the upper water column and spent less time in habitat structure relative to Mayans (JJ vs. MM, p < 0.0005 for both comparisons; Fig. 1b,c). The vertical distribution of predators was affected by predator identity (Table 1). Certain predator pairs spend more time high in the water column than others.

Prey Mortality

Despite variation in predator behavior and prey response, predator voracity and lethality were similar among predator combinations. The number of attacks on prey at the end of trials was low, on average one attack per 5-min observation period, and did not differ among treatments (Table 1). Similarly, overnight predation rates were comparable across predator combinations and between the two prey populations (Table 1). On average, predators consumed eight mosquitofish relative to zero mortality in the control tanks (Fig. 4).
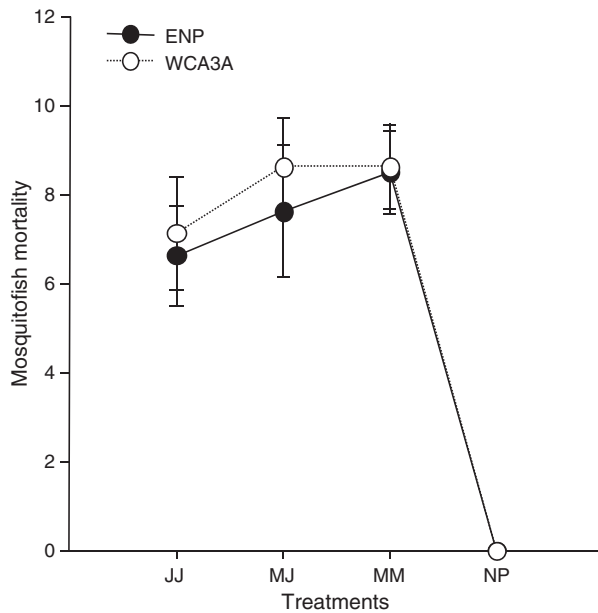
Fig. 4: Overnight mosquitofish mortality rates across treatments (JJ = 2 African jewelfish, MM = 2 Mayan cichlids, MJ = African jewelfish + Mayan cichlid, and NP = no predator) and prey populations (WCA3A = naive and ENP = experienced with African jewelfish-both are experienced with Mayan cichlids). Shown are $\bar{x} \pm$ SE.

Discussion

The prey naiveté hypothesis suggests that the high impact of aquatic predators relates to the prey's limited ability to detect and respond to novel predation threats posed by non-native piscivores (Cox & Lima 2006). Our results did not find support for this notion. First, predator avoidance responses by mosquitofish appeared stronger to the more novel predation threat, the African jewelfish, relative to those exhibited toward Mayan cichlids. Mosquitofish responded by altering their microhabitat use, increasing shoaling, and examining predators. Despite prey engaging in these behaviors in the presence of jewelfish, mortality rates were similar between the two predators. No variation in attack rates and overnight predation rates was detected, although predator microhabitat use and activity varied. Little variation was found between the seasons (blocks) suggesting that the characteristic seasonal hydrological variation of the Everglades ecosystem may have little effect on the predator and prey behaviors examined here, although our power to detect this effect was likely low.

Second, the amount of naiveté of mosquitofish populations did not appear to affect their antipredator response. The response to jewelfish was as strong

by the naive WCA3A mosquitofish population, which had no experience with jewelfish, than by the ENP population, where jewelfish occur and pose a significant predation threat to mosquitofish (Loftus et al. 2006). Confirmation of this result with a larger number of experienced and naive prey populations is needed. Examination of this question with other prey species is also needed. Our own examination of the response of other Everglades prey to novel African jewelfish shows that antipredator responses are species specific (Dunlop & Rehage, unpublished data) and may result in variation in prey vulnerability. Nannini & Belk (2006) found similar variation for the response of two minnow species to introduced trout.

Our experimental design using free-ranging predators and prey allowed us to examine the response of predators and prey spatially. Prey typically try to avoid areas with high predation risk, while predators concentrate efforts in areas with more prey. Most studies cage or otherwise restrict predator movement (Lima 2002), limiting one's ability to examine this behavioral response race (Sih 2005). Mosquitofish did not reduce activity in the presence of predators, but altered the use of tank microhabitats. In the presence of African jewelfish, prey moved higher in the water column. Changes in the vertical distribution of mosquitofish in response to predation risk have been noted in previous studies (Garcia et al. 1992; Smith & Belk 2001). Since both predators were found relatively low in the water column, this change in microhabitat use likely reduced their spatial coincidence with predators. With Mayan cichlids, prey minimized encounters by reducing use of habitat structure in their presence, because Mayan pairs used cover to the greatest extent. Mosquitofish also increased shoaling behavior in response to jewelfish but not Mayan cichlids. Shoaling is known to function largely as a defense behavior since it typically enhances vigilance and predator confusion and abates attacks, allowing for coordinated evasion and risk dilution (reviewed by Pitcher & Parrish 1993).

Prey often engage in the visual inspection of potential predators as a means of assessing predator identity and motivation (Lima & Dill 1990; Dugatkin & Godin 1992; Brown 2003). In this study, prey inspections were directed towards Mayan cichlids more than African jewelfish. This result agrees with previous work showing that experienced prey inspect more than relatively naive prey (Magurran & Seghers 1990; Kelley & Magurran 2003; but see Brown & Warburton 1999). It is also possible that prey engaged in higher inspections with Mayans

cichlids because Mayans were perceived to be the lower-risk predator. Since inspections involve approaches to the predator, they can be riskier than other antipredator behaviors (Dugatkin 1992), and prey may afford to engage in inspection only with relatively low-risk predators (Smith & Belk 2001). Mayan cichlids were also less active than jewelfish, and previous work shows that all else being equal, prey are more likely to inspect stationary rather than moving threats (Pitcher et al. 1986; Dugatkin & Godin 1992). Further, in the presence of the highly active jewelfish, it may be unnecessary for mosquitofish to engage in inspection to assess risk, as microhabitats with active predators can become 'cue-saturated' (Preisser et al. 2007).

Prey seemed to respond to the two cichlid predators with both different antipredator tactics and different magnitudes of response. The increase in shoaling and use of the upper water column shown only with African jewelfish suggest to us that both mosquitofish populations perceived jewelfish to be the riskier predators, despite their variable experience with them. Prey altered microhabitat use (either to the top of the water column or out of the structure) when faced with both predators, but the magnitude of the response (e.g., behavior without predators – behavior with predators) was much greater in the presence of jewelfish, suggesting higher risk. The same is seen in the shoaling behavior, shoal sizes are greater in the presence of jewelfish than in the presence of Mayans. We expect prey to modulate their response to match the predation threat (i.e., threat-sensitive predator avoidance hypothesis; Helfman 1989; Chivers et al. 2001; Mirza et al. 2006; Botham et al. 2008) or perception of such risk (Sih 1992; Brown 2003; Lima & Steury 2005).

We suspect that the perception of higher risk by jewelfish may relate to the disparity in predator behavior and predation cue intensity. Brown & Chivers (2005) suggest that predator movement is a primary visual cue used by prey to distinguish between relevant and irrelevant threats. Jewelfish were significantly more active than Mayan cichlids and spent more time out in the open water suggesting an 'active' hunting mode (Preisser et al. 2007; Schmitz 2007), which could have been perceived, even by the inexperienced WCA3A prey, as a more imminent threat (i.e., a more motivated predator). In contrast, Mayans were less active and remained low in the water column and in the habitat structure suggesting a 'sit and wait' or 'sit and pursue' predator mode (Schmitz 2007), at least in the daytime

hours when data were collected. In agreement, experiments that have manipulated predator movement show that prey exhibit stronger antipredator responses to moving rather than stationary predation threats (Brown & Warburton 1997, 1999; Wisenden & Harter 2001).

The fact that naive and experienced prey populations had similarly strong antipredator responses toward African jewelfish is one of the most interesting results. Their responses seem to indicate that both prey populations deemed jewelfish to be the riskier predator; but how did they arrive to this same perception if WCA3A prey have no experience with jewelfish? We suggest at least four possible explanations that merit consideration. First, although jewelfish are a new predator in the Everglades, their predator archetype may not be novel and instead it resembles common predators mosquitofish encounter enough (i.e., native centrarchids) to allow for prey recognition and response. However, our data show that their hunting mode is at least different from one other common non-native predator. Jewelfish are also considerably more active, social and aggressive than at least one of the abundant Everglades centrarchids examined so far (*Lepomis gulosus*) (Dunlop & Rehage, unpublished data; Schofield et al. 2007). Whether the variation in predator hunting mode and habitat domain seen here generates sufficient predator novelty to cause prey to fail to respond or respond inappropriately or ineffectively deserves further study.

Second, it is plausible that the WCA3A mosquitofish are exhibiting a general antipredator response (e.g., multi-predator hypothesis; Blumstein 2006), whereby exposure to high-risk environments allows prey to develop heightened antipredator responses regardless of whether or not prey have had experience with particular predators. WCA3A mosquitofish were collected from a marsh adjacent to the I75 canal. Canals bisecting Everglades marshes provide key habitat for large-bodied fishes (Rehage & Trexler 2006) and may act to locally increase predation risk for prey in nearby marsh habitats, perhaps allowing prey to develop strong general antipredator behaviors.

Third, naive prey may be able to detect jewelfish as a threat despite their novelty, if they rely on general predation cues for predator detection and recognition. General cues include chemical cues associated with predator diet, disturbance cues associated with stressed/startled prey, and damage-released alarm cues associated with a predator attack (reviewed by Chivers & Smith 1998; Brown 2003;

Wisenden & Chivers 2006). These alarm signals can effectively 'label' potential predators as such. In contrast, native species that rely on specific cues (e.g., the scent or vocalization of a particular predator) may be unable to recognize and respond to novel predators (Jones et al. 2004; Smith et al. 2008). Although mosquitofish are known to respond to the release of conspecific skin extract (Garcia et al. 1992), only a very small number of predation events occurred, making it unlikely that alarm cues were important. Instead, predators were fed mosquitofish prior to trials (along with other native prey), and at least closely related western mosquitofish (*Gambusia affinis*) can detect predator dietary cues (Smith & Belk 2001).

Finally, we suggest that general visual cues could also be used in predator detection instead or in addition to chemical cues. Prey may be responding to the presence of any novel, large (above a certain threshold) and moving object (Dill 1974; Brown & Warburton 1997; Wisenden & Harter 2001). In a sense, this constitutes a neophobic response. Neophobia refers to the fear of novelty, and is typically characterized by aversion, hesitation or caution (Greenberg 2003). Neophobia may be adaptive when predation risk is very high and/or predator diversity is low (Brown & Chivers 2005). Under these circumstances, a large moving individual encountered by prey is likely to be a potential predator, and prey should exhibit antipredator behavior in response regardless of predator identity. Both of these conditions could apply to our WCA3A prey. As mentioned earlier, marshes nearby canals may experience high predation regimes, and at same time, the diversity of piscivores in Everglades habitats is relatively low, usually dominated by seven to eight taxa (Chick et al. 2004; Rehage & Trexler 2006).

In conclusion, we note that this study examines the first level of prey naiveté, which relates to predator detection and recognition (Banks & Dickman 2007) and shows that prey may be able to overcome it. Cox & Lima (2006) suggest that this may be the most damaging form of prey naiveté, but we suggest otherwise. A large body of literature shows that prey, particularly aquatic prey that often lack innate responses to sympatric predators, can learn to recognize novel predators very quickly and effectively (i.e. after a single exposure) (Brown & Warburton 1999; Brown 2003; Brown & Chivers 2005; Mirza et al. 2006), and may be able to generalize this recognition to related predators (i.e., in the same family; Ferrari et al. 2007). Although not yet explored greatly, we expect that experience and learning are likely to be key mechanisms allowing for novel predator detection in invasion scenarios. Instead, the ability of prey to show appropriate and effective antipredator responses once predators are detected may be more important in determining large invader impacts. Here, prey are limited by their arsenal of behavioral responses and other forms of phenotypic plasticity, and this may be a larger obstacle to overcome than recognition (e.g., Banks et al. 2008). Additional studies are needed to elucidate the ability of native prey to respond to non-native predators, and the role played by different levels of prey naiveté in invasive predator impact.

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