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Antipredator behavior and cue recognition by multiple Everglades prey to a novel cichlid predator

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Running head: Prey cue use and response to a novel predator
Summary:

Novel predator introductions are thought to have a high impact on native prey, especially in freshwater systems. Prey may fail to recognize predators as a threat, or show inappropriate or ineffective responses. The ability of prey to recognize and respond appropriately to novel predators may depend on the prey's use of general or specific cues to detect predation threats. We used laboratory experiments to examine the ability of three native Everglades prey species (Eastern mosquitofish, flagfish and riverine grass shrimp) to respond to the presence, as well as to the chemical and visual cues of a native predator (warmouth) and a recently introduced nonnative predator (African jewelfish).

We used prey from populations that had not previously encountered jewelfish. Despite this novelty, the native warmouth and nonnative jewelfish had overall similar predatory effects, except on mosquitofish, which suffered higher warmouth predation. All three prey species showed surprisingly consistent and strong responses to the nonnative jewelfish, which were similar in magnitude to the responses exhibited to the native warmouth. Fish prey responded largely to chemical cues, while shrimp showed no response to either chemical or visual cues. Overall, responses by mosquitofish and flagfish to chemical cues indicated low differentiation among cue types, with similar responses to general and specific cues. The fact that antipredator behaviors were similar toward native and nonnative predators suggests that the susceptibility to predation by a novel fish predator is similar to that of native fishes, and prey may overcome predator novelty, at least for congeneric predators.
Introduction

The susceptibility of prey to predation risk is strongly influenced by the prey’s ability to detect and respond to predation threats (Hoare et al., 2007; Ramo-Jiliberto et al., 2007; Smith et al., 2008a). If the predation threat is novel, the ability of prey to both recognize and respond to predators may be limited (Gamradt & Kats, 1996). For instance, a lack of evolutionary history between a nonnative predator and native prey may cause prey to be naïve to a nonnative predator’s threat (i.e., naïve prey hypothesis; Smith et al., 2008b; Sih et al., 2010). Even if nonnative predators are similar to native predators (e.g., both are fish), differences in predator archetypes due to variation in morphological and behavioral foraging adaptations can result in strong naïveté for the prey (Cox & Lima, 2006). This naïveté can contribute to the high consumptive effects of nonnative predators introduced to isolated ecosystems such as islands and freshwater systems (Vermeij, 1991; Cox & Lima, 2006; Nannini & Belk, 2006; Wohlfahrt et al., 2006; Salo et al., 2007; Sih et al., 2010). Thus, in order to better understand the overall effects of nonnative predators, we must gain a mechanistic understanding of how prey recognize and respond to new threats and may overcome predator novelty.

Prey naïveté toward nonnative predators may arise from three sequential mechanisms: (a) the failure of prey to detect or recognize novel predators as a threat, (b) their inability to respond appropriately, and/or (c) their inability to effectively evade novel predators despite their appropriate response (Banks & Dickman, 2007). For instance, the lack of experience with predators among island-endemic species often
means that prey altogether lack behavioral responses to introduced predators (Wiles et al.,
2002; Blackburn et al., 2004). In other cases, prey recognized the predator as a threat, but
show the wrong responses (e.g., crypsis against scent-hunting cursorial predators; Banks
& Dickman, 2007). Thirdly, prey may recognize and respond with appropriate behaviors,
but these are not effective against novel predators. Prey may increase use of higher cover
habitats, but predation may still be high (Kinnear et al., 2002). Cox & Lima (2006)
suggest that a lack of novel predator recognition may be the most damaging form of prey
naïveté. A prey’s failure to recognize a novel predator may inhibit its antipredator
responses, or weaken such defenses if recognition is delayed (Cox & Lima, 2006, but see
Rehage et al., 2009).

Predator recognition hinges on the sensory information used to assess risk, which
is often visual, chemical or a combination of the two (Hartman & Abrahams, 2000;
Mathis & Vincent, 2000; Chivers et al., 2001; Wisenden et al., 2004; Smith et al., 2008b).
Cues used in predator detection may also vary from general to specific (Brown, 2003;
Webb et al., 2009). Specific cues can effectively label a predation threat by revealing the
predator’s identity (i.e., a predator’s particular odor or specific shape, Magurran &
Girling, 1986; Kats & Dill, 1998; Wisenden & Chivers, 2006), while general cues are
produced by a relatively broad range of information, and are not linked to a specific
predator (i.e., damage or diet cues, habitat cues, broad visual cue – large moving object,
Dill, 1974; Sih, 1986; Garcia et al., 1992; Gelowitz et al., 1993; Orrock et al., 2004).
Specific cues allow prey to moderate antipredator responses by minimizing the use of
costly antipredator behaviors against low-risk predators (Ramos-Jiliberto et al., 2007).
the same time prey that rely on specific cues may be at a disadvantage when faced with novel, nonnative predators not previously encountered (Sih et al., 2010). Here, their ability to overcome predator novelty will be strongly dependent on cue association and rapid learning (e.g., Ferrari et al., 2007).

In our study, we compared the mechanisms of cue utilization, predator recognition, and antipredator response among native taxa faced with either a sympatric native predator or an allopatric nonnative predation threat. Our intent was to gain a better understanding of the risk posed by novel, nonnative predators, and of the variation in the susceptibility of native prey to these newly-arrived predators. In three laboratory experiments, we compared predation rates, antipredator behaviors, and cue use by three Everglades taxa in response to the threat of nonnative African jewelfish, *Hemichromis letourneuxi*, and that of a common native centrarchid predator, the warmouth, *Lepomis gulosus*. The small-body size, piscivorous diet and aggressive behavior of the jewelfish make it a likely competitor to native centrarchids, which are the dominant mesoconsumers in the system (Loftus & Kushlan, 1987; Heymans et al., 2002; Rehage & Trexler, 2006; Schofield et al., 2007). With Everglades National Park (ENP) currently home to fourteen nonnative fishes species, many of them predators (Loftus et al., 2000; Trexler et al., 2000; Shafland et al., 2008), there is a need to better understand interactions among native and nonnative taxa. To date, few studies have documented any significant ecological effects from fish introductions in ENP, which has lead to conflicting perspectives on the overall impact of nonnative aquatic taxa across the Greater Everglades ecosystem (Shafland, 1996; Trexler et al., 2000).
We focused on the African jewelfish because, due to the recentness of the invasion in ENP (since 2000, J. Kline, pers. comm.; Courtenay et al., 1974; Shafland et al., 2008), we are able to track its spread; and its current patchy distribution creates heterogeneity in prey naïveté throughout the landscape. Thus, we are able to examine interactions among jewelfish and native Everglades prey that have not previously encountered them in nature, and are thus ‘naïve’ to their threat. Further, the majority of the nonnative taxa in the Everglades are cichlids, and thus there is an interest in learning how novel of a threat newly-arrived conframiliar predators are. Ferrari et al. (2007) showed that prey may be able to generalize their antipredator response to closely-related predators in the absence of experience. At the same time, variation in predator hunting behavior and habitat domain even among closely-related predators can create some level of predator novelty (Rehage et al., 2009). Here, we focused on three common native prey species: Eastern mosquitofish, *Gambusia holbrooki*, flagfish, *Jordanella floridae*, and riverine grass shrimp, *Palaemonetes paludosus*. These three species are widely-distributed in the Everglades, co-occur, and are among the most abundant prey of freshwater marshes (Turner et al., 1999; Trexler et al., 2001; Rehage & Trexler, 2006). They are also readily consumed by both nonnative jewelfish (Rehage et al., 2009; Whitaker et al., 2011) and native warmouth (W.F. Loftus, unpubl. data), but little is known about prey-specific vulnerability to piscine predators.

In the three experiments, we address four key questions: (1) Is the predation threat posed by nonnative jewelfish similar to that posed by the native warmouth? (2) How do nonnative predators and native predators interact to affect prey mortality? (3) Do prey
exhibit the same antipredator responses to native and nonnative predators? (4) What
predator cues are prey using to detect these predators? In the first experiment, we
examined the antipredator behavior of each prey species to the presence of predators, as
well as predator behavior and predation rates. We expected weaker antipredator
responses by all three taxa to the novel jewelfish predator, and thus higher predation rates
by the nonnative predator. We also expected to see variation in the vulnerability of the
prey taxa to both predators, which we hypothesized would relate to their antipredator
behavior, habitat domain overlap with predators (Schmitz, 2007), and thus encounter
rates. For instance, since both predators tend to be found low in the water column, we
expected demersal prey (shrimp and flagfish) to experience higher predation by both the
 predator types (Rehage et al. 2009; Whitaker et al. 2010). In experiments 2 and 3, we
assessed the prey’s use of chemical and visual cues, both general and specific. We
expected that the antipredator response of prey would relate to the use of general or
specific predation cues in predator detection. We expected native prey to respond to the
cues of the native predator more strongly than those of the nonnative predator. Further,
we hypothesize that if prey are unable to smell or recognize African jewelfish visually as
a predator, they could still respond appropriately if they relied on general cues for
predator detection (i.e., conspecifics damage cues). From these experiments, we hoped to
gain new insights into the mechanisms underlying variation in the vulnerability of
Everglades aquatic taxa to recent invasions.
Methods

Study organisms

For all experiments, native and nonnative predators were collected from freshwater marshes in ENP and southern Big Cypress National Preserve where jewelfish and native centrarchids co-occur. The three prey species were collected exclusively in northern Water Conservation Area 3A (WCA3A), where jewelfish have not yet invaded. Additional warmouth were also collected at this site. We collected predators and prey using unbaited minnow traps deployed overnight (2.5-cm openings, 3-mm mesh), in addition to D-frame dip nets used for collecting prey (1-mm mesh). Prior to the experiments, predators were kept separately at approximately equal densities in 795 L outdoor tanks at Nova Southeastern University Oceanographic Center, Dania, FL. During this holding period, predators were fed a combination of live prey (including experimental prey), and earthworms obtained commercially. Prey species were kept separately by species in and at similar densities in 795-L tanks prior to trials, and fed commercial flakes ad libitum.

Experiment design

In each of the three experiments conducted in the study, we used a 3x4 factorial design (3 species x 4 experimental treatments) to compare prey antipredator responses to the presence, chemical, and visual cues of native and nonnative predators. When predators were present, we also quantified predator behavior and predation rates. Experiment 1 compared predation rates, and predator and prey behavior, while
experiments 2 and 3 examined prey behavior in response to chemical and visual cues respectively. In all three experiments, data was collected on each prey species separately, and on a randomly-assembled group of six similarly-size individuals from each prey species (Rehage et al. 2009). For each experiment, we randomly selected a new group of six prey, such that prey were only used once. Three key prey behaviors were repeatedly assessed in the three experiments separately for each prey species: activity, grouping and use of habitat structure. Previous research shows that these are behaviors typically affected by predation risk (ref.).

All trials were conducted in 12 56.8-L aquaria (50 x 24.5 x 40 cm height) at a water depth of 33 cm using dechlorinated tap water with a temperature of approximately 25.7 °C. Each tank was provided with structural complexity in the form of artificial vegetation covering a bit more than a 1/3 of the tank area. The artificial vegetation consisted of black plastic strips (4 x 22 cm) attached to a weighted plastic grid [20 x 25 cm], which sat on the bottom and to one side of the tank. This amount of structure corresponds to a plant stem density of approximately 484 stems/m², which falls within the range found in Everglades marshes (18 to 677 stems/m²; Jordan et al., 1997). To minimize observer effects in the first experiment, tanks were covered on all four sides with a white vinyl covering, and observations were conducted through mirrors positioned above tanks. For the later cue experiments, tanks were covered on three sides only, and observations were conducted laterally from behind a blind.

Twelve hours prior to the start of each experiment, all feeding was suspended in order to standardize hunger levels, and six prey of each species were randomly selected...
from stock tanks, and isolated into groups in 5.7-L containers separately by species.

Fifteen minutes before trials, the prey group was randomly assigned to a treatment and replicate tank. Prey sizes, based on a random sample from the three experiments (n = 15 for each spp) averaged \( (\pm \text{standard errors}) 13.26 \pm 0.50 \) mm standard length (SL) for mosquitofish, \( 19.09 \pm 0.65 \) mm SE SL for flagfish, and \( 8.69 \pm 0.34 \) mm carapace length (CL) for grass shrimp.

Behavioral observations were conducted through a series of discrete spot-checks by a single observer positioned approximately one meter in front of each tank (Mathis & Smith, 1993b). For experiment 1, 10 spot-check observations were conducted in rounds, with the observer observing all tanks over a period of 15-20 minutes, then returning to the first tank for another round, and repeating this for 10 rounds (approximately \( xx \) hours of total observation). For the cue experiments, the 12 spot-check observations were done consecutively with the observer performing all observations at one tank and then moving to the next tank; 6 were conducted pre- and 6 post-cue addition. Here, observations were conducted approximately every 2 minutes, except observations 6 and 7, which were conducted immediately pre- and post-cue addition (within 1 min.). Total observation periods for experiments 2 and 3 were approximately 12 minutes. For all observations, we recorded three key prey behaviors of interest: activity, microhabitat use (use of habitat structure and water column), and group size. At each spot check, we scored the activity and microhabitat use of each individual in the group, and then averaged the score for the group. Activity was scored as ‘0’ if immobile, ‘1’ = slow, ‘2’ = medium, and ‘3’ = high. We considered high activity to be a darting or active escape response at high speed from...
a predator. Medium activity was a continuous uninterrupted swimming pattern (longer than 3 seconds), while slow swimming involved a cautious ‘stop and go’ swimming behavior. We assessed two components of microhabitat use: the prey’s vertical distribution in the water column, and the use of structure. To determine vertical distribution, we divided the water column into equal-sized horizontal layers (top = ‘2’, middle = ‘1’ and bottom = ‘0’), recorded the location of each fish at each spot check and averaged for the 6 fish in the group. Marks on each corner of tanks, which divided the 33 cm water column into three 11-cm zones, aided the observer in scoring use of the water column (these were clearly visible from a top view in experiment 1). To quantify habitat structure use, we counted the number of prey within the structure at each spot check. Lastly, for the schooling or grouping behavior, we recorded the occurrence of a group at each observation (group present= 1, group absent = 0). Prey were considered to be in a social group if at least four of the six individuals were closer than 2 body lengths (Rehage et al., 2009). All observations were conducted between 11AM and 2 PM.

Experiment 1: Predator-prey interactions

Here, we crossed the three prey species with four treatments in a replacement series design (Sih et al., 1998): (NP) a no predator control, (WW) two warmouth, (JJ) two jewelfish, and (WJ) one warmouth + one jewelfish. Trials were conducted in two time blocks (March 31-April 4, 2008; and April 10-14, 2008). For both blocks, a single replicate was tested each day over the five-day period (4 treatments x 3 prey spp x 5 replicates per block x 2 blocks = 120 experimental units). Each predator was used once To minimize the habituation of the predators to test conditions, e
during each block, returned to stock tanks, randomized, and then used again in the second block (9 predators x 2 predators spp x 5 replicates = 90 total predators). Prey species were tested only once (120 experimental units x 6 individuals/group = 720 total prey).

Previous studies have shown that prey are capable of responding to dietary cues released by predators that have consumed conspecifics (Mathis & Smith, 1993a; Chivers & Mirza, 2001). To eliminate the effects of these cues in the experiment, predators were maintained on a diet consisting solely of commercial earthworms for five days prior to the start of trials (Gelowitz et al., 1993; Mathis & Smith, 1993b). Previous studies have shown that digestion rates for piscivorous and crustacean-consuming predators are less than 48 hours when waters temperatures are approximately 22.7°C (Kitchell & Windell, 1968). Temperatures within the holding tank average across the three experiments.

Following this five-day diet flushing period, predators (warmouth: 65.56 ± 1.66 SE mm SL, n = 45, and jewelfish 56.67 ± 1.01 SE mm SL n = 45) were randomly selected and isolated in 5.7-L containers the evening before trials. We were careful to conduct water changes during this feeding period, and not transfer any of the water of the predator stock tank or isolation container to experimental tanks.

In addition to the prey’s behavior, we recorded predator activity and microhabitat use using the same scoring used for the prey. At the beginning of trials, prey groups were released into aquaria first, allowed 15 minutes to acclimate, and then predators were added. Observations started 10 minutes after predator release. At the conclusion of all behavioral observations on trial days, we assessed overnight prey mortality. To prevent prey depletion in tanks, an additional six prey individuals of the same species and size
were added, for a total of 12 prey individuals per tank. Rehage et al. (2009) showed overnight predation rates of 7 mosquitofish using a similar setup. Prey were added following the observation period (2-3 PM), and mortality checks were done the following morning (7-8 AM). If any of the original prey were consumed during the behavioral observations (only 40 of 720 prey were consumed over the observation period), prey were replaced before assessing overnight mortality, but not during the observation period (Rehage et al., 2009).

Experiment 2: Prey responses to chemical cues

The three native Everglades prey species were tested in four chemical cue treatments: (NP) a no-cue control, (G) a general cue consisting of the odor of injured conspecifics, (W) specific chemical cues from the native warmouth, and (J) specific chemical cues from the nonnative jewelfish. Chemical cue trials were conducted over a 5-day period with 2 replicates per day (4 treatments x 3 prey species x 2 replicates per day x 5 days = 240 experimental units). Trials were conducted between August 23 and September 4, 2008. Each aquaria was provided with the same structural complexity described earlier, sodium zeolite chips placed at the bottom of the tank to remove ammonia, as well as aeration (vinyl tubing and an airstone) used for the cue release (Mathis & Smith, 1993b; Chivers & Smith, 1998). This airstone apparatus was positioned in the lower third of the water column at the opposite end of the tank from the habitat structure. We injected 60 mL of chemical cue into the vinyl tubing with a syringe for diffusion into the tank, and conducted observations 6 and 7 of the 12 observations within

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a minute pre- and post-cue addition (Mathis & Smith, 1993b; Brown & Smith, 1997; Chivers et al., 2001).

For the specific cues, six randomly selected predators of each species were used to prepare predator odors. As in the first experiment, predators were maintained on a diet consisting solely of commercial earthworms for five days prior to the stimulus collection in order to remove dietary cues. On the fifth day of feeding, each predator was transferred to 5.7-L clear plastic containers containing 1.2 L of new dechlorinated tap water. These chambers contained a single air stone but had no filtration system. After 2.5 days, the predators were removed and water samples were collected from each predator chamber, and frozen into separate 120-mL units at –20°C for later use (Gelowitz et al., 1993; Brown & Godin, 1999; Kusch et al., 2004). Predator cues were not mixed and cue preparation was done twice over the five days of trials.

The general chemical cue was obtained from conspecific skin extracts. Thirty donors were randomly selected from each prey spp, and humanely sacrificed with a blow to the head. For the fishes, we removed the skin and ground it up using a pestle and mortar to release the alarm signaling club cells (Pfieffer, 1977; Wisenden, 2000). Because grass shrimp do not possess these alarm cells, muscle tissue from beneath the carapace and tail was used instead (Magurran et al., 1996). Fish skin and shrimp tissue were diluted to 0.5g/500 mL with distilled water, and the suspension was filtered and separated into 18 120 mL-units and frozen at –20°C (Magurran et al., 1996). Following Mathis & Smith, (1993b), we prepared the cue every xx days. For the control, 60 mL aliquots of distilled water were frozen, and injected in a similar manner as chemical cues.
Experiment 3: Prey responses to visual cues

Similar to the chemical cue experiment, treatments for the last experiment included: (NP) a no cue control, (G) general visual cues from a predator model, (W) specific visual cues from the native warmouth, and (J) specific visual cues from the nonnative jewelfish. Trials were conducted over two five-day time blocks (October 29–November 1, 2008; and November 10- November 14, 2008). For both blocks, a single replicate of each treatment by species combination was tested each day (4 treatments x 3 prey species x 5 replicates per block x 2 blocks = 120 experimental units). Predators were used only once in each block, returned to stock tanks, randomized, and then used again in the second block (a total of 30 jewelfish and 30 warmouth).

For the predator visual cues, we used three predators of each species in all trials. Similar to the prey, the three warmouth and three jewelfish were isolated for a 12-hour period in the 5.7-L containers prior to the experiment. In the day of trials, the prey group and the predator were placed in adjacent glass tanks (broad side, covered by a removable barrier), and allowed to acclimate for 15 minutes. We conducted trials in two adjacent 56.8-L aquaria (one containing the six focal individuals of a prey species and one containing a single live predator or predator model). For the no predator control, the tanks adjacent to the prey did not contain a visual stimulus, but we removed the barrier at the beginning of each trial as done in predator treatments. Six spot check observations were conducted pre and six post removal of the barrier (observations 6 and 7 were conducted within a minute of barrier removal).
For the general predator cue, we used a predator model that consisted of a wooden
dowel shaped in the form of a fish of similar size as the focal predators (60 mm SL,
Figure 1). The use of models as predator stimuli has been found to be an effective tool for
examining antipredator behavior (Rowland, 1999; Corkum, 2002). The model was
suspended in the bottom third of the water column (11 cm from tank bottom) with
monofilament line from a pulley system (Figure 1). During trials, we used a lever
attached to the pulley system to move the model at approximately 0.25m/s, along the
broad side of the tank, from one end of the tank to the other.

Statistical analyses

We used general linear models to examine variation in prey behavior, predator
behavior, and prey mortality. Across the three experiments, we consistently examined
variation in four prey behaviors (activity, vertical distribution, habitat use, and grouping)
with factorial MANOVAs and ANOVAs that tested for species, treatment, species x
treatment effects (and a time blocking factor when appropriate). These analyzes were
performed using prey group means that were averaged over trial duration (i.e., the mean
of all observations, Rehage et al., 2009). For the cue experiments, we calculated the
difference between post and pre-stimulus behaviors (average of 6 post-cue spot checks
minus average of 6 pre-cue spot checks), and performed analyses on these differences.
Since prey were only used once, behaviours are averaged to obtain group means, and the
measured behaviors are not mutually exclusive, we consider the behaviours measured to
be independent (Martin & Bateson, 2007).
For experiment 1, we also conducted ANOVAs to compare prey mortality (factorial: prey species and predator treatment effects) and predator behavior (one-way: predator treatment). The number of predators active, at the top of the water column, and in structure were averaged for each trial and compared across treatments. To satisfy normality assumptions, we examined residuals in all models, and transformed variables (√y-transformations for counts and arcsin(√y)-transformations for proportions) that showed evidence of non-normality or heteroscedacity (Kery & Hatfield, 2003). LSD pairwise comparisons were used in posthoc tests, and significance at the 0.05 level is denoted with letters in bar graphs. All analyses were performed using SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Experiment 1: Prey responses to predator presence & predation rates

The three native prey species varied in activity and grouping behavior, but show similar microhabitat use. Overall, grass shrimp were less active and less likely to form groups than either mosquitofish or flagfish. Across predator treatments, the behavioral response of the three species was surprisingly similar (Table 1). For three of the four behaviors measured, we recorded consistent responses to the presence of predators, regardless of predator identity. All three prey species decreased activity, moved higher in the water column, and increased grouping in treatments in the presence of predators.
(Figures 2 & 3). Thus, contrary to expectations, prey responses to the native vs. the nonnative predators were similar in strength and direction for all prey.

The only exception was a differential response to predator treatments in the vertical distribution of prey (Figure 2). Mosquitofish moved higher in the water column regardless of predator treatment, but the response was dependent on predator identity for flagfish and grass shrimp, shrimp showed a stronger response when predators were mixed, while flagfish showed equally high responses with mixed or warmouth predators, but a lesser response when the predators were the jewelfish pair (Figure 2B). Little variation in use of the habitat structure was seen across treatments for shrimp, but a slight decrease was detected for the fish prey when predators were present (Figure 2C). However, overall use of the structure was low; on average only one of the six individuals was found in the structure across treatments.

The predator pairs varied in activity, but showed similar patterns of microhabitat use in our experimental tanks (Table 1, Figure 2). Warmouth pairs were the least active, while average activity levels were similar for the jewelfish pair and the mixed predator treatment. Across pair types, predators remained low in the water column and on average, one of the predators spent the trial duration in the more complex artificial vegetation.

Predation rates varied as a function of predator treatments, prey species, and the predator treatments by prey species interaction (Table 1). As may be expected, mortality was higher in predator treatments (zero in the absence of predators), but highest in the warmouth treatment; 38% of prey were consumed in warmouth treatment relative to 33%
consumed in mixed predator treatment, and 29% in the jewelfish treatment (Figure 4).

Consumption rates of flagfish and grass shrimp did not differ significantly among the treatments, but mortality of mosquitofish was higher in the presence of the native warmouth pair than in the other two predator treatments.

Experiment 2: Prey responses to chemical cues

Overall, prey responses to chemical cues relatively weak, showing more prey-specific responses, and low differentiation among cue types (Table 2, Figure 5). For instance, grass shrimp did not respond to any of the chemical cues presented.

Mosquitofish shifted activity and grouping behavior when chemical cues were present, but few to no differences were detected among cue types. Mosquitofish became less active with the scent of warmouth and jewelfish, and increased grouping indiscriminately to both the general and the two specific chemical cues (Figure 5A&C). Flagfish became less active in response to all cue types, including the scent of novel jewelfish (Figure 5A&B). They moved lower in the water column with the conspecific cue and the jewelfish scent, but not the warmouth scent.

Experiment 3: Prey responses to visual cues

Overall, prey behavior in response to visual cues only did not vary strongly among prey, or more importantly among cue types, with two exceptions (Figure 5).
Mosquitofish increased grouping in the presence of the fish model, and flagfish decrease activity strongly when warmouth were present in the adjacent tank (Figure 5A&C). There were some behavioral differences between pre and post cue delivery, but these differences were generally consistent across treatments including in the control tank, where no predator nor predator model was present. Activity was lower across all three prey in the post-cue observations, and prey tended to move lower in the water column.

Discussion

Nonnative predator effects are expected to be higher than those of native predators due to the lack of experience of the prey with the new predator, its foraging tactics, and cues (Cox & Lima, 2006; Banks & Dickman, 2007; Sih et al., 2010). Our experimental results with African jewelfish and Everglades prey, however, do not support this notion. First and contrary to expectations, the nonnative jewelfish did not have a greater predatory effect on the three focal prey species tested relative to the native centrarchid predator. Second, our prey showed antipredator responses to nonnative jewelfish that were generally similar in magnitude and direction as those exhibited toward the native warmouth. Lastly, two of the three prey species tested appeared to be able to detect and respond to olfactory cues from novel African jewelfish, despite having not encountered these olfactory cues before. These results suggest that although prey may be faced with new predators, if these predators are somewhat similar to existing predation threats (i.e., other fish predators, or confamilial predators), prey may be able to exhibit
general antipredator behavior (e.g., reduced activity) that are known to increase survival 
(e.g., Skelly, 1994).

Because of the naiveté of prey, introduced predators may have greater 
consumptive effects relative to non-consumptive effects when compared to native 
predators (Sih et al., 2010). These greater consumptive effects may explain the boom and 
bust cycles we often see associated with invasions (e.g., Bohn et al., 2008). In our trials, 
however, jewelfish had similar or lower consumptive effects to those of a similar-sized 
native centrarchid. Foraging rates were similar on the two demersal prey, grass shrimp 
and flagfish, but varied for the top-dwelling mosquitofish. Jewelfish consumed less 
mosquitofish, despite the fact that mosquitofish are a major component of jewelfish diets 
(W. Loftus, unpub. data), and jewelfish consume them readily in the lab (Rehage et al., 
2009). This is surprising given that both predators had similar microhabitat use in the 
lower water column, and would typically be expected to forage more effectively on prey 
that share the same habitat domain (Schmitz, 2007).

The shared prey and similarity in habitat use between the native warmouth and 
the nonnative jewelfish supports the notion that native centrarchids, which are common 
mesoconsumers throughout Everglades habitats (Chick et al., 2004; Rehage & Trexler, 
2006) are likely to compete for resources with nonnative jewelfish (Schofield et al., 
2007), as they do with other nonnative cichlids (Brooks & Jordan, 2010). However, we 
did not see any evidence of interference that would lead to risk enhancement or risk 

reduction when both predators were present (Sih et al., 1998; Schmitz, 2007). Predation
rates in the mixed predator treatments were similar to those in single predator treatment, except for the lower predation rate on mosquitofish when predators were mixed.

Prey responded to the presence of predators with typical generalized antipredator behavior (i.e., decreases in activity and increases in grouping, Sih et al., 2010), and these responses were similar to the native and nonnative predators, and similar for the two fish and shrimp prey. All prey became less active, moved higher in the water column, and increased aggregation in the presence of predators. Due to their different morphologies and habitat domains, we expected to see more variation in prey antipredator behavior. Even congeneric species of similar morphology and ecology show markedly different behavioral responses (Nannini & Belk, 2006). Antipredator responses typically relate to a species’ history of exposure to predation risk and should influence their vulnerability to predators. Our results suggest that these species may experience similar predation risk in the field, and may be equally vulnerable to novel predation threats.

Alternatively, it may be possible that the similarity in the behavioral responses observed in our trials are due to constraints provided by the experimental setup, which caused the prey to exhibit heightened and common generalized responses to a ‘pulse’ in predation risk (Lima & Bednekoff, 1999; Reylea, 2003; Schmitz, 2007). The effectiveness of antipredator behavior is dependent not only on the identity of the predator and its foraging tactics, but also on the type of habitat where the predator is encountered (Brown & Smith, 1997). It may be possible that in the constrained space of lab aquaria, prey use generalized and stronger antipredator tactics to evade heightened predation risks since predator avoidance is limited (Hickman et al., 2004). Shifts in
habitat use to predator-free environments will be limited under these lab conditions 
(Crowl & Covich, 1994). However, we believe our experimental setup had elements of 
reality. Most tank predation studies cage and restrict predator movements, which 
generates limited behavioral responses, and restrict our ability to examine how predators 
and prey interact in space (Lima, 2002; Sih, 2005). By employing a free-ranging predator 
experimental design, we were able to observe predator-prey encounters at close 
proximity, and quantify the behavioral response of prey given an encounter, but as in 
other studies, sacrificed the ability of prey to exhibit other spatial responses. 

Yet, all else being equal, we expected to see differential behavior toward the 
native and the nonnative predator. We suggest three possible mechanisms for the 
similarity in response across the three prey types. First, we suggest that an adaptive 
evolutionary history with multiple predators may have allowed the prey to develop 
nonplastic behavioral traits in response to any predator threats (i.e. multiple predator 
hypothesis, Sih, 1986; Blumstein, 2006; Wolfahrt et al., 2006). In general, fixed 
antipredator behavioral responses are expected to occur when predation risks are 
continuously high (Wolfahrt et al., 2006). In the Everglades, recurrent seasonal dry-down 
forces prey to live or move into deeper habitats where larger-bodied fishes are abundant 
and predation regimes are expected to be relatively high (Loftus & Eklund, 1994; Rehage 
& Trexler, 2006; Rehage & Loftus, 2007). This co-occurrence with predators may allow 
prey to exhibit similar anti-predator responses to multiple threats, including those they 
have not encountered before. Sih (1986) found that predator-experienced prey had a
greater chance of survival with novel predators than predator naïve prey, due to their fixed behavioral responses.

Second, prey species could be exhibiting a neophobic response, whereby they are responding to all things novel with aversion, hesitation, or caution (Greenberg, 2003). These responses are expected to be adaptive in high predation risk environments, where larger fish, although not recognized, are likely to be a predator and elicit a response (Brown & Chivers, 2005). Thirdly, despite the fact that the prey used in our experiments were ‘naïve’ to jewelfish, since they had not previously encountered them in nature, jewelfish may not have represented a novel nor unfamiliar threat, such that prey responded in similar magnitude as to a known predator.

Prey exhibited antipredator behavior in response to both general and specific cues, but mostly when these cues were chemical. A number of studies have documented the use of chemical cues in predator recognition (Mathis et al., 1993a; Mathis & Vincent, 2000), including those produced by nonnative taxa (Pearl et al., 2003). Chemical cues likely provide an early warning of predation threats, which may be refined with the introduction of visual cues (Kats & Dill, 1998; Chivers et al., 2001). The low response to the visual cues used in our experiment, may be due to the fact that the visual cues used did not provide enough information for prey to correctly identify the predator threat (Wisenden, 2004), or they did not reflect a high risk encounter to merit a response (Corkum, 2002). Prey often show a greater reliance on chemical cues when visual cues are diminished, such as in turbid waters, in heavily-vegetated habitats, or with cryptic
predators (Hartman & Abrahams, 2000; Mathis & Vincent, 2000; Amo et al., 2004).

Because of the high density of emergent grasses (Gunderson & Loftus, 1993), the high biomass of periphyton (Turner et al., 1999), and the presence of flocculent material atop the benthos (Rehage & Trexler, 2006), the structural complexity of Everglades marsh habitats is relatively high. Under these conditions, prey may be expected to rely more intensely on chemical information as seen in our study (Mathis & Vincent, 2000).

Similarly, several of the common native predators use a sit and wait hunting strategy, for which, it is more advantageous for prey to use chemical cues in predator recognition (Amos et al., 2004); especially if prey are able to recognize not previously encountered predators as a threat when they are closely related to known predators (i.e., confamiliar predators; Ferrari et al., 2007). Both mosquitofish and flagfish showed a significant decrease in activity and increase in vertical distribution to the isolated scents of jewelfish and warmouth. Often, the strength of a prey species’ antipredator response will depend on dietary cues, and whether the predator has consumed conspecific or heterospecific prey (Wohlfahrt et al., 2006), but we removed these cues from our experiment. Instead, we suggest that the fish species may be relying on chemical kairomones for predator recognition and response. Kairomones are prominent chemical cues that are similar across freshwater fish families and are believed to be a partial metabolite of fish-associated bacteria (Dicke & Sabelis, 1988; Elert & Phonert, 2000). Previous work shows that prey use these cues in predator recognition (Gelowitz et al., 1993; Kats & Dill, 1998). Kusch et al. (2004) showed that fathead minnow populations exhibited intense behavioral responses to increasing concentrations of northern pike odor, *Esox lucius*, and
were able to recognize the size of the predators that generated the cues. The recognition of predator kairomones by prey can occur very quickly under natural conditions (Wisenden & Chivers, 2006). It may be possible that the prey’s prior experience with other cichlid predators may have allowed particularly the fish prey to respond to nonnative jewelfish. Ferrari et al. (2007) showed that fathead minnows trained to recognized the scent of a particular salmonid predator, also exhibited antipredator responses to the scent of two other salmonid species, despite no experience with them. While chemical cues appear to be a primary source of information in predator-prey interactions in our trials, the antipredator responses observed during the cue experiments were weaker than those observed in the first experiment where predators were present. This suggests that prey may need multiple cues to identify a predation risk, and determine the degree of risk-sensitive behavior to exhibit (i.e. threat sensitivity hypothesis, Amo et al., 2004; Botham et al., 2008). For instance, the relatively weak response of shrimp observed in the chemical cue trials may be due to the fact that they require other cue types, such as tactile cues. Crowl and Covich (1994) found that chemical cues elicit a partial response from freshwater shrimp, but when coupled with the physical presence of the predator the intensity of the responses increases. Mosquitofish similarly increase avoidance behavior when both the chemical and visual cues of predatory fish are present (Smith & Belk, 2001).

Conclusions
Introduced predators are a major concern for the Everglades, and have been implicated in fish population declines elsewhere in freshwater systems (Cox & Lima, 2006). With the continued invasion of new species, the probability for synergistic effects among fish predators that could drastically alter the way nonnative species interact with natives and thus their impact (e.g., O’Dowd et al., 2003) becomes a concern. Our data show that a newly-arrived predator may have similar predatory effects and elicit similar antipredator behavior from native prey. Thus, the vulnerability of Everglades prey to new predators does not seem to vary among taxa, and may be less than expected based on the novelty of the interaction, perhaps because of the experience of Everglades prey with cichlid predators. If predation rates and prey risk to nonnative cichlids are similar, we would expect nonnative predators to function in a similar matter as native predators. However, we do not know if the addition of nonnative cichlids to the system is increasing overall predation regimes, with important consequences for the transfer of energy throughout food webs and ecosystem components, or replacing them. Further work is needed to distinguish between the two, and better assess the consequences of multiple invasions in the long-term.
Acknowledgements

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References


30


Wohlfahrt, B. Mikolajewski, D.J. Joop, G. & Suhling, F. 2006. Are behavioral traits in prey sensitive to the risk imposed by predatory fish? Freshwater Biol. 51: 76-84.
Figure 1. Diagram of the model used for the visual cue experiment. The predator model consisted of a wooden dowel shaped like a fish (60 mm SL), suspended in the water column at a depth of approximately of 11 cm, and moved using a pulley system. The tank containing the model was positioned adjacent to the prey tank and separated with a removable barrier, similar to the other treatments.

Figure 2. Mean predator and prey activity, vertical distribution, and structure use (± 1 SE) for the first experiment across predator treatments (NP = no predators, JJ= 2 jewelfish, WW = 2 warmouth, WJ = 1 jewelfish + 1 warmouth). Activity was scored 0-3 (0 = not active), vertical distribution was scored as 0-2 (0 = bottom), and structure use reflect counts of the number of prey individuals within the structure averaged over the observation period. Significant pairwise differences (P ≤ 0.05) are indicated with lettering above bars.

Figure 3. The mean occurrence of prey groups for the first experiment (± 1 SE) across predator treatments (NP = no predators, JJ= 2 jewelfish, WW = 2 warmouth, WJ = 1 jewelfish + 1 warmouth). Prey grouping was scored as 0-1 (0 = group absent, 1 = group present). Significant pairwise differences (P ≤ 0.05) are indicated by different uppercase letters.

Figure 4. Mean predation rate (± 1 SE) on all prey across treatments (NP = no predators, JJ= 2 jewelfish, WW = 2 warmouth, WJ = 1 jewelfish + 1 warmouth). Letters indicate significant pairwise differences at P ≤ 0.05.
Figure 5. Mean predator and prey activity, vertical distribution, and habitat use (± 1 SE) in the two cue experiments across predator treatments (NP = no predators, JJ = 2 jewelfish, WW = 2 warmouth, WJ = 1 jewelfish + 1 warmouth). Activity was scored 0-3 (0 = not active), vertical distribution was scored as 0-2 (0 = bottom), and the occurrence of prey groups was scored as 0-1 (0 = group absent, 1 = group present). Significant pairwise differences ($P \leq 0.05$) are indicated by different uppercase letters.
Table 1. Results of ANOVAs and MANOVAs (F values, degrees of freedom, p values, and R²) testing treatment, species, and block effects for the first predator-prey experiment (significant effects are in bold).

<table>
<thead>
<tr>
<th></th>
<th>Predator treatment</th>
<th>Prey species</th>
<th>Predator treatment x prey species</th>
<th>Block</th>
<th>R²</th>
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<tr>
<td></td>
<td>F (df) p</td>
<td>F (df) p</td>
<td>F (df) p</td>
<td>F (df) p</td>
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<tr>
<td>Preditor-Prey Experiment</td>
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<tr>
<td>Prey Mortality</td>
<td>157.4 (3, 96) &lt;0.001</td>
<td>9.5 (2, 96) &lt;0.001</td>
<td>3.3 (6, 96) 0.005</td>
<td>0.3 (1, 96)</td>
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<td>Predator behavior</td>
<td></td>
<td></td>
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<tr>
<td>Multivariate Analysis</td>
<td>4.1 (8, 138) &lt;0.001</td>
<td></td>
<td></td>
<td>0.5 (4, 93)</td>
<td>0.733</td>
</tr>
<tr>
<td>Activity</td>
<td>4.5 (2, 72) 0.015</td>
<td></td>
<td></td>
<td>0.5 (1, 72)</td>
<td>0.504</td>
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<tr>
<td>Vertical Distribution</td>
<td>1.2 (2, 72) 0.310</td>
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<td>0.2 (1, 72)</td>
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<tr>
<td>Use of habitat structure</td>
<td>0.7 (2, 72) 0.490</td>
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<td>0.3 (1, 72)</td>
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<td>Predator-predator interactions</td>
<td>7.0 (2, 72) 0.002</td>
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<td>0.0 (1, 72)</td>
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<tr>
<td>Multivariate Analysis</td>
<td>14.4 (12, 246) &lt;0.001</td>
<td>28.9 (8,186) &lt;0.001</td>
<td>1.6 (24, 320) &lt;0.044</td>
<td>0.5 (4, 93)</td>
<td>0.766</td>
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<td>Activity</td>
<td>20.8 (1, 96) &lt;0.001</td>
<td>50.5 (2, 96) &lt;0.001</td>
<td>1.8 (6,96) 0.102</td>
<td>0.1 (1, 96)</td>
<td>0.759</td>
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<td>Vertical Distribution</td>
<td>40.0 (1, 96) &lt;0.001</td>
<td>17.1 (2, 96) &lt;0.001</td>
<td>2.2 (6,96) 0.005</td>
<td>1.1 (1, 96)</td>
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<td>Use of habitat structure</td>
<td>5.6 (2, 96) 0.001</td>
<td>1.0 (2, 96) 0.372</td>
<td>0.2 (6,96) 0.968</td>
<td>0.0 (1, 96)</td>
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<td>Grouping</td>
<td>9.8 (3, 96) &lt;0.001</td>
<td>131.2 (2, 96) &lt;0.001</td>
<td>2.4 (6,96) 0.034</td>
<td>0.2 (1, 96)</td>
<td>0.657</td>
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Table 2. Results of ANOVAs and MANOVAs (F values, degrees of freedom, p values, and $R^2$) testing treatment, species, and block effects for the two cue experiments (significant effects are in bold).

<table>
<thead>
<tr>
<th></th>
<th>Predator Treatment</th>
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<th>Predator treatment x prey species</th>
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<td>$F_{(df)}$</td>
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<td>$F_{(df)}$</td>
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<tr>
<td>Multivariate Analysis</td>
<td>2.0 (12, 278)</td>
<td><strong>0.023</strong></td>
<td>3.7 (8, 210)</td>
<td>&lt;0.001</td>
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<td>Activity</td>
<td>5.9 (2, 108)</td>
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<td>2.4 (3, 108)</td>
<td>0.095</td>
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<td>Vertical Distribution</td>
<td>2.0 (2, 108)</td>
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<td>9.3 (3, 108)</td>
<td><strong>0.002</strong></td>
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<td><strong>Visual Cue Experiment</strong></td>
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<td>Multivariate Analysis</td>
<td>1.4 (15, 254)</td>
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<td>5.6 (10, 184)</td>
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<td>Activity</td>
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<td>22.1 (3, 96)</td>
<td>&lt;0.001</td>
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<td>Grouping</td>
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<td>2.8 (3, 96)</td>
<td>0.069</td>
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41