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

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21 **Summary:**

22 Novel predator introductions are thought to have a high impact on native prey, especially
23 in freshwater systems. Prey may fail to recognize predators as a threat, or show
24 inappropriate or ineffective responses. The ability of prey to recognize and respond
25 appropriately to novel predators may depend on the prey's use of general or specific cues
26 to detect predation threats. We used laboratory experiments to examine the ability of
27 three native Everglades prey species (Eastern mosquitofish, flagfish and riverine grass
28 shrimp) to respond to the presence, as well as to the chemical and visual cues of a native
29 predator (warmouth) and a recently introduced nonnative predator (African jewelfish).
30 We used prey from populations that had not previously encountered jewelfish. Despite
31 this novelty, the native warmouth and nonnative jewelfish had overall similar predatory
32 effects, except on mosquitofish, which suffered higher warmouth predation. All three
33 prey species showed surprisingly consistent and strong responses to the nonnative
34 jewelfish, which were similar in magnitude to the responses exhibited to the native
35 warmouth. Fish prey responded largely to chemical cues, while shrimp showed no
36 response to either chemical or visual cues. Overall, responses by mosquitofish and
37 flagfish to chemical cues indicated low differentiation among cue types, with similar
38 responses to general and specific cues. The fact that antipredator behaviors were similar
39 toward native and nonnative predators suggests that the susceptibility to predation by a
40 novel fish predator is similar to that of native fishes, and prey may overcome predator
41 novelty, at least for confamilial predators.

42 **Introduction**

43 The susceptibility of prey to predation risk is strongly influenced by the prey's
44 ability to detect and respond to predation threats (Hoare et al., 2007; Ramo-Jiliberto et al.,
45 2007; Smith et al., 2008a). If the predation threat is novel, the ability of prey to both
46 recognize and respond to predators may be limited (Gamradt & Kats, 1996). For instance,
47 a lack of evolutionary history between a nonnative predator and native prey may cause
48 prey to be naïve to a nonnative predator's threat (i.e., naïve prey hypothesis; Smith et al.,
49 2008b; Sih et al., 2010). **Even if nonnative predators are similar to native predators (e.g.,**
50 **both are fish), differences in predator archetypes due to variation in morphological and**
51 **behavioral foraging adaptations can result in strong naiveté for the prey** (Cox & Lima,
52 2006). This naiveté can contribute to the high consumptive effects of nonnative predators
53 introduced to isolated ecosystems such as islands and freshwater systems (Vermeij, 1991;
54 Cox & Lima, 2006; Nannini & Belk, 2006; Wohlfahrt et al., 2006; Salo et al., 2007; Sih
55 et al., 2010). Thus, in order to better understand the overall effects of nonnative
56 predators, we must gain a mechanistic understanding of how prey recognize and respond
57 to new threats and may overcome predator novelty.

58 Prey naïveté toward nonnative predators may arise from three sequential
59 mechanisms: (a) the failure of prey to detect or recognize novel predators as a threat, (b)
60 their inability to respond appropriately, and/or (c) their inability to effectively evade
61 novel predators despite their appropriate response (Banks & Dickman, 2007). For
62 instance, the lack of experience with predators among island-endemic species often

63 means that prey altogether lack behavioral responses to introduced predators (Wiles et al.,
64 2002; Blackburn et al., 2004). In other cases, prey recognized the predator as a threat, but
65 show the wrong responses (e.g., crypsis against scent-hunting cursorial predators; Banks
66 & Dickman, 2007). Thirdly, prey may recognize and respond with appropriate behaviors,
67 but these are not effective against novel predators. Prey may increase use of higher cover
68 habitats, but predation may still be high (Kinnear et al., 2002). Cox & Lima (2006)
69 suggest that a lack of novel predator recognition may be the most damaging form of prey
70 naiveté. A prey's failure to recognize a novel predator may inhibit its antipredator
71 responses, or weaken such defenses if recognition is delayed (Cox & Lima, 2006, but see
72 Rehage et al., 2009).

73 Predator recognition hinges on the sensory information used to assess risk, which
74 ~~is often~~ visual, chemical or a combination of the two (Hartman & Abrahams, 2000;
75 Mathis & Vincent, 2000; Chivers et al., 2001; Wisenden et al., 2004; Smith et al., 2008b).
76 Cues used in predator detection may also vary from general to specific (Brown, 2003;
77 Webb et al., 2009). Specific cues can effectively label a predation threat by revealing the
78 predator's identity (i.e., a predator's particular odor or specific shape, Magurran &
79 Girling, 1986; Kats & Dill, 1998; Wisenden & Chivers, 2006), while general cues are
80 produced by a relatively broad range of information, and are not linked to a specific
81 predator (i.e., damage or diet cues, habitat cues, broad visual cue – large moving object,
82 Dill, 1974; Sih, 1986; Garcia et al., 1992; Gelowitz et al., 1993; Orrock et al., 2004).
83 Specific cues allow prey to moderate antipredator responses by minimizing the use of
84 costly antipredator behaviors against low-risk predators (Ramos-Jiliberto et al., 2007). At

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85 the same time prey that rely on specific cues may be at a disadvantage when faced with
86 novel, nonnative predators not previously encountered (Sih et al., 2010). Here, their
87 ability to overcome predator novelty will be strongly dependent on cue association and
88 rapid learning (e.g., Ferrari et al., 2007).

89 In our study, we compared the mechanisms of cue utilization, predator
90 recognition, and antipredator response among native taxa faced with either a sympatric
91 native predator or an allopatric nonnative predation threat. Our intent was to gain a better
92 understanding of the risk posed by novel, nonnative predators, and of the variation in the
93 susceptibility of native prey to these newly-arrived predators. In three laboratory
94 experiments, we compared predation rates, antipredator behaviors, and cue use by three
95 Everglades taxa in response to the threat of nonnative African jewelfish, *Hemichromis*
96 *letourneuxi*, and that of a common native centrarchid predator, the warmouth, *Lepomis*
97 *gulosus*. The small-body size, piscivorous diet and aggressive behavior of the jewelfish
98 make it a likely competitor to native centrarchids, which are the dominant
99 mesoconsumers in the system (Loftus & Kushlan, 1987; Heymans et al., 2002; Rehage &
100 Trexler, 2006; Schofield et al., 2007). With Everglades National Park (ENP) currently
101 home to fourteen nonnative fishes species, many of them predators (Loftus et al., 2000;
102 Trexler et al., 2000; Shafland et al., 2008), there is a need to better understand
103 interactions among native and nonnative taxa. To date, few studies have documented any
104 significant ecological effects from fish introductions in ENP, which has lead to
105 conflicting perspectives on the overall impact of nonnative aquatic taxa across the
106 Greater Everglades ecosystem (Shafland, 1996; Trexler et al., 2000).

107 We focused on the African jewelfish because, due to the recentness of the
108 invasion in ENP (since 2000, J. Kline, pers. comm.; Courtenay et al., 1974; Shafland et
109 al., 2008), we are able to track its spread; and its current patchy distribution creates
110 heterogeneity in prey naiveté throughout the landscape. Thus, we are able to examine
111 interactions among jewelfish and native Everglades prey that have not previously
112 encountered them in nature, and are thus ‘naïve’ to their threat. Further, the majority of
113 the nonnative taxa in the Everglades are cichlids, and thus there is an interest in learning
114 how novel of a threat newly-arrived unfamiliar predators are. Ferrari et al. (2007)
115 showed that prey may be able to generalize their antipredator response to closely-related
116 predators in the absence of experience. At the same time, variation in predator hunting
117 behavior and habitat domain even among closely-related predators can create some level
118 of predator novelty (Rehage et al., 2009). Here, we focused on three common native prey
119 species: Eastern mosquitofish, *Gambusia holbrooki*, flagfish, *Jordanella floridae*, and
120 riverine grass shrimp, *Palaemonetes paludosus*. These three species are widely-
121 distributed in the Everglades, co-occur, and are among the most abundant prey of
122 freshwater marshes (Turner et al., 1999; Trexler et al., 2001; Rehage & Trexler, 2006).
123 They are also readily consumed by both nonnative jewelfish (Rehage et al., 2009;
124 Whitaker et al., 2011) and native warmouth (W.F. Loftus, unpubl. data), but little is
125 known about prey-specific vulnerability to piscine predators.

126 In the three experiments, we address four key questions: (1) Is the predation threat
127 posed by nonnative jewelfish similar to that posed by the native warmouth? (2) How do
128 nonnative predators and native predators interact to affect prey mortality? (3) Do prey

129 exhibit the same antipredator responses to native and nonnative predators? (4) What
130 predator cues are prey using to detect these predators? In the first experiment, we
131 examined the antipredator behavior of each prey species to the presence of predators, as
132 well as predator behavior and predation rates. We expected weaker antipredator
133 responses by all three taxa to the novel jewelfish predator, and thus higher predation rates
134 by the nonnative predator. We also expected to see variation in the vulnerability of the
135 prey taxa to both predators, which we hypothesized would relate to their antipredator
136 behavior, habitat domain overlap with predators (Schmitz, 2007), and thus encounter
137 rates. For instance, since both predators tend to be found low in the water column, we
138 expected demersal prey (shrimp and flagfish) to experience higher predation by both the
139 predator types (Rehage et al. 2009; Whitaker et al. 2010). In experiments 2 and 3, we
140 assessed the prey's use of chemical and visual cues, both general and specific. We
141 expected that the antipredator response of prey would relate to the use of general or
142 specific predation cues in predator detection. We expected native prey to respond to the
143 cues of the native predator more strongly than those of the nonnative predator. Further,
144 we hypothesize that if prey are unable to smell or recognize African jewelfish visually as
145 a predator, they could still respond appropriately if they relied on general cues for
146 predator detection (i.e., conspecifics damage cues). From these experiments, we hoped to
147 gain new insights into the mechanisms underlying variation in the vulnerability of
148 Everglades aquatic taxa to recent invasions.

149

150 **Methods**

151 *Study organisms*

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

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Comment [reviewer1]: Kate add a statement on holding periods for both predators and prey

165

166 *Experiment design*

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167 In each of the three experiments conducted in the study, we used a 3x4 factorial
168 design (3 species x 4 experimental treatments) to compare prey antipredator responses to
169 the presence, chemical, and visual cues of native and nonnative predators. When
170 predators were present, we also quantified predator behavior and predation rates.
171 Experiment 1 compared predations rates, and predator and prey behavior, while

172 experiments 2 and 3 examined prey behavior in response to chemical and visual cues
173 respectively. In all three experiments, data was collected on each prey species separately,
174 and on a randomly-assembled group of six similarly-size individuals from each prey
175 species (Rehage et al. 2009). For each experiment, we randomly selected a new group of
176 six prey, such that prey were only used once. Three key prey behaviors were repeatedly
177 assessed in the three experiments separately for each prey species: activity, grouping and
178 use of habitat structure. Previous research shows that these are behaviors typically
179 affected by predation risk (ref.).

Comment [reviewer2]: Kate do you have some refs you can add here?

180 All trials were conducted in 12 56.8-L aquaria (50 x 24.5 x 40 cm height) at a
181 water depth of 33 cm using dechlorinated tap water with a temperature of approximately
182 25.7 °C. Each tank was provided with structural complexity in the form of artificial
183 vegetation covering a bit more than a 1/3 of the tank area. The artificial vegetation
184 consisted of black plastic strips (4 x 22 cm) attached to a weighted plastic grid (20 x 25
185 cm), which sat on the bottom and to one side of the tank. This amount of structure
186 corresponds to a plant stem density of approximately 484 stems/m², which falls within
187 the range found in Everglades marshes (18 to 677 stems/m²; Jordan et al., 1997). To
188 minimize observer effects in the first experiment, tanks were covered on all four sides
189 with a white vinyl covering, and observations were conducted through mirrors positioned
190 above tanks. For the later cue experiments, tanks were covered on three sides only, and
191 observations were conducted laterally from behind a blind.

Comment [reviewer3]: kate, can you double check that the grid was 8 x 10 inches, which would be equivalent to 20 x 25 cm? Thanks,

192 Twelve hours prior to the start of each experiment, all feeding was suspended in
193 order to standardize hunger levels, and six prey of each species were randomly selected

194 | from stock tanks, and isolated into groups in 5.7-L containers separately by species.
195 | Fifteen minutes before trials, the prey group was randomly assigned to a treatment and
196 | replicate tank. Prey sizes, based on a random sample from the three experiments (n = 15
197 | for each spp) averaged (± standard errors) 13.26 ± 0.50 mm standard length (SL) for
198 | mosquitofish, 19.09 ± 0.65 mm SE SL for flagfish, and 8.69 ± 0.34 mm carapace length
199 | (CL) for grass shrimp.

200 | Behavioral observations were conducted through a series of discrete spot-checks
201 | by a single observer positioned approximately one meter in front of each tank (Mathis &
202 | Smith, 1993b). For experiment 1, 10 spot-check observations were conducted in rounds,
203 | with the observer observing all tanks over a period of 15-20 minutes, then returning to the
204 | first tank for another round, and repeating this for 10 rounds (approximately xx hours of
205 | total observation). For the cue experiments, the 12 spot-check observations were done
206 | consecutively with the observer performing all observations at one tank and then moving
207 | to the next tank; 6 were conducted pre- and 6 post-cue addition. Here, observations were
208 | conducted approximately every 2 minutes, except observations 6 and 7, which were
209 | conducted immediately pre- and post-cue addition (within 1 min.). Total observation
210 | periods for experiments 2 and 3 were approximately 12 minutes. For all observations, we
211 | recorded three key prey behaviors of interest: activity, microhabitat use (use of habitat
212 | structure and water column), and group size. At each spot check, we scored the activity
213 | and microhabitat use of each individual in the group, and then averaged the score for the
214 | group. Activity was scored as '0' if immobile, '1' = slow, '2' = medium, and '3' = high.
215 | We considered high activity to be a darting or active escape response at high speed from

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Comment [reviewer4]: Kate can you add range of hours of observations

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216 a predator. Medium activity was a continuous uninterrupted swimming pattern (longer
217 than 3 seconds), while slow swimming involved a cautious ‘stop and go’ swimming
218 behavior. We assessed two components of microhabitat use: the prey’s vertical
219 distribution in the water column, and the use of structure. To determine vertical
220 distribution, we divided the water column into equal-sized horizontal layers (top = ‘2’,
221 middle = ‘1’ and bottom = ‘0’), recorded the location of each fish at each spot check and
222 averaged for the 6 fish in the group. Marks on each corner of tanks, which divided the 33
223 cm water column into three 11-cm zones, aided the observer in scoring use of the water
224 column (these were clearly visible from a top view in experiment 1). To quantify habitat
225 structure use, we counted the number of prey within the structure at each spot check.
226 Lastly, for the schooling or grouping behavior, we recorded the occurrence of a group at
227 each observation (group present= 1, group absent = 0). Prey were considered to be in a
228 social group if at least four of the six individuals were closer than 2 body lengths (Rehage
229 et al., 2009). All observations were conducted between 11AM and 2 PM.

230

231 *Experiment 1: Predator-prey interactions*

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232 Here, we crossed the three prey species with four treatments in a replacement
233 series design (Sih et al., 1998): (NP) a no predator control, (WW) two warmouth, (JJ) two
234 jewelfish, and (WJ) one warmouth + one jewelfish. Trials were conducted in two time
235 blocks (March 31-April 4, 2008; and April 10-14, 2008). For both blocks, a single
236 replicate was tested each day over the five-day period (4 treatments x 3 prey spp x 5
237 replicates per block x 2 blocks = 120 experimental units). Each predator was used once

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238 during each block, returned to stock tanks, randomized, and then used again in the second
239 block (9 predators x 2 predators spp x 5 replicates = 90 total predators). Prey species
240 were tested only once (120 experimental units x 6 individuals/group = 720 total prey).

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241 Previous studies have shown that prey are capable of responding to dietary cues
242 released by predators that have consumed conspecifics (Mathis & Smith, 1993a; Chivers
243 & Mirza, 2001). To eliminate the effects of these cues in the experiment, predators were
244 maintained on a diet consisting solely of commercial earthworms for five days prior to
245 the start of trials (Gelowitz et al., 1993; Mathis & Smith, 1993b). Previous studies have
246 shown that digestions rates for piscivorous and crustacean-consuming predators are less
247 than 48 hours when waters temperatures are approximately 22.7°C (Kitchell & Windell,
248 1968). Temperatures within the holding tank average across the three experiments.
249 Following this five-day diet flushing period, predators (warmouth: 65.56 ± 1.66 SE mm
250 SL, n = 45, and jewelfish 56.67 ± 1.01 SE mm SL n = 45) were randomly selected and
251 isolated in 5.7-L containers the evening before trials. We were careful to conduct water
252 changes during this feeding period, and not transfer any of the water of the predator stock
253 tank or isolation container to experimental tanks.

254 In addition to the prey's behavior, we recorded predator activity and microhabitat
255 use using the same scoring used for the prey. At the beginning of trials, prey groups were
256 released into aquaria first, allowed 15 minutes to acclimate, and then predators were
257 added. Observations started 10 minutes after predator release. At the conclusion of all
258 behavioral observations on trial days, we assessed overnight prey mortality. To prevent
259 prey depletion in tanks, an additional six prey individuals of the same species and size

260 were added, for a total of 12 prey individuals per tank. Rehage et al. (2009) showed
261 overnight predation rates of 7 mosquitofish using a similar setup. Prey were added
262 following the observation period (2-3 PM), and mortality checks were done the following
263 morning (7-8 AM). If any of the original prey were consumed during the behavioral
264 observations (only 40 of 720 prey were consumed over the observation period), prey
265 were replaced before assessing overnight mortality, but not during the observation period
266 (Rehage et al., 2009).

Comment [reviewer5]: Kate, can you check that these times sound OK to you?

267

268 *Experiment 2: Prey responses to chemical cues*

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

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

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

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

Comment [reviewer6]: but there is no block in this experiment right?

Deleted: We prepared the cue twice during each block to assure its freshness
()

304

305 *Experiment 3: Prey responses to visual cues*

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

Deleted: over a five-day period

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315 For the predator visual cues, we used three predators of each species in all trials.
316 Similar to the prey, the three warmouth and three jewelfish were isolated for a 12-hour
317 period in the 5.7-L containers prior to the experiment. In the day of trials, the prey group
318 and the predator were placed in adjacent glass tanks (broad side, covered by a removable
319 barrier), and allowed to acclimate for 15 minutes. We conducted trials in two adjacent
320 56.8-L aquaria (one containing the six focal individuals of a prey species and one
321 containing a single live predator or predator model). For the no predator control, the tanks
322 adjacent to the prey did not contain a visual stimulus, but we removed the barrier at the
323 beginning of each trial as done in predator treatments. Six spot check observations were
324 conducted pre and six post removal of the barrier (observations 6 and 7 were conducted
325 within a minute of barrier removal).

326 For the general predator cue, we used a predator model that consisted of a wooden
327 dowel shaped in the form of a fish of similar size as the focal predators (60 mm SL,
328 Figure 1). The use of models as predator stimuli has been found to be an effective tool for
329 examining antipredator behavior (Rowland, 1999; Corkum, 2002). The model was
330 suspended in the bottom third of the water column (11 cm from tank bottom) with
331 monofilament line from a pulley system (Figure 1). During trials, we used a lever
332 attached to the pulley system to move the model at approximately 0.25m/s, along the
333 broad side of the tank, from one end of the tank to the other.

334

335 *Statistical analyses*

336 We used general linear models to examine variation in prey behavior, predator
337 behavior, and prey mortality. Across the three experiments, we consistently examined
338 variation in four prey behaviors (activity, vertical distribution, habitat use, and grouping)
339 with factorial MANOVAs and ANOVAs that tested for species, treatment, species x
340 treatment effects (and a time blocking factor when appropriate). These analyzes were
341 performed using prey group means that were averaged over trial duration (i.e., the mean
342 of all observations, Rehage et al., 2009). For the cue experiments, we calculated the
343 difference between post and pre-stimulus behaviors (average of 6 post-cue spot checks
344 minus average of 6 pre-cue spot checks), and performed analyses on these differences.
345 Since prey were only used once, behaviours are averaged to obtain group means, and the
346 measured behaviors are not mutually exclusive, we consider the behaviours measured to
347 be independent (Martin & Bateson, 2007).

348 For experiment 1, we also conducted ANOVAs to compare prey mortality
349 (factorial: prey species and predator treatment effects) and predator behavior (one-way:
350 predator treatment). The number of predators active, at the top of the water column, and
351 in structure were averaged for each trial and compared across treatments. To satisfy
352 normality assumptions, we examined residuals in all models, and transformed variables
353 (\sqrt{y} -transformations for counts and $\arcsin(\sqrt{y})$ -transformations for proportions) that
354 showed evidence of non-normality or heteroscedacity (Kery & Hatfield, 2003). LSD
355 pairwise comparisons were used in posthoc tests, and significance at the 0.05 level is
356 denoted with letters in bar graphs. All analyses were performed using SAS 9.1 (SAS
357 Institute Inc., Cary, NC, USA).

358

359 **Results**

360 *Experiment 1: Prey responses to predator presence & predation rates*

361 The three native prey species varied in activity and grouping behavior, but show
362 similar microhabitat use. Overall, grass shrimp were less active and less likely to form
363 groups than either mosquitofish or flagfish. Across predator treatments, the behavioral
364 response of the three species was surprisingly similar (Table 1). For three of the four
365 behaviors measured, we recorded consistent responses to the presence of predators,
366 regardless of predator identity. All three prey species decreased activity, moved higher in
367 the water column, and increased grouping in treatments in the presence of predators

368 (Figures 2 & 3). Thus, contrary to expectations, prey responses to the native vs. the
369 nonnative predators were similar in strength and direction for all prey.

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

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

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

389 consumed in mixed predator treatment, and 29% in the jewelfish treatment (Figure 4).
390 Consumption rates of flagfish and grass shrimp did not differ significantly among the
391 treatments, but mortality of mosquitofish was higher in the presence of the native
392 warmouth pair than in the other two predator treatments.

393

394 *Experiment 2: Prey responses to chemical cues*

395 Overall, prey responses to chemical cues ~~relatively~~ weak, showing more prey-
396 specific responses, and low differentiation among cue types (Table 2, Figure 5). For
397 instance, grass shrimp did not respond to any of the chemical cues presented.
398 Mosquitofish shifted activity and grouping behavior when chemical cues were present,
399 but few to no differences were detected among cue types. Mosquitofish became less
400 active with the scent of warmouth and jewelfish, and increased grouping indiscriminately
401 to both the general and the two specific chemical cues (Figure 5A&C). Flagfish became
402 less active in response to all cue types, including the scent of novel jewelfish (Figure
403 5A&B). They moved lower in the water column with the conspecific cue and the
404 jewelfish scent, but not the warmouth scent.

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405

406 *Experiment 3: Prey responses to visual cues*

407 Overall, prey behavior in response to visual cues only did not vary strongly
408 among prey, or more importantly among cue types, with two exceptions (Figure 5).

409 Mosquitofish increased grouping in the presence of the fish model, and flagfish decrease
410 activity strongly when warmouth were present in the adjacent tank (Figure 5A&C). There
411 were some behavioral differences between pre and post cue delivery, but these
412 differences were generally consistent across treatments including in the control tank,
413 where no predator nor predator model was present. Activity was lower across all three
414 prey in the post-cue observations, and prey tended to move lower in the water column.

415

416 **Discussion**

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

430 general antipredator behavior (e.g., reduced activity) that are known to increase survival
431 (e.g., Skelly, 1994).

432 Because of the naiveté of prey, introduced predators may have greater
433 consumptive effects relative to non-consumptive effects when compared to native
434 predators (Sih et al., 2010). These greater consumptive effects may explain the boom and
435 bust cycles we often see associated with invasions (e.g., Bohn et al., 2008). In our trials,
436 however, jewelfish had similar or lower consumptive effects to those of a similar-sized
437 native centrarchid. Foraging rates were similar on the two demersal prey, grass shrimp
438 and flagfish, but varied for the top-dwelling mosquitofish. Jewelfish consumed less
439 mosquitofish, despite the fact that mosquitofish are a major component of jewelfish diets
440 (W. Loftus, unpub. data), and jewelfish consume them readily in the lab (Rehage et al.,
441 2009). This is surprising given that both predators had similar microhabitat use in the
442 lower water column, and would typically be expected to forage more effectively on prey
443 that share the same habitat domain (Schmitz, 2007).

444 The shared prey and similarity in habitat use between the native warmouth and
445 the nonnative jewelfish supports the notion that native centrarchids, which are common
446 mesoconsumers throughout Everglades habitats (Chick et al., 2004; Rehage & Trexler,
447 2006) are likely to compete for resources with nonnative jewelfish (Schofield et al.,
448 2007), as they do with other nonnative cichlids (Brooks & Jordan, 2010). However, we
449 did not see any evidence of interference that would lead to risk enhancement or risk
450 reduction when both predators were present (Sih et al., 1998; Schmitz, 2007). Predation

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451 rates in the mixed predator treatments were similar to those in single predator treatment,
452 except for the lower predation rate on mosquitofish when predators were mixed.

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

464 Alternatively, it may be possible that the similarity in the behavioral responses
465 observed in our trials are due to constraints provided by the experimental setup, which
466 caused the prey to exhibit heightened and common generalized responses to a 'pulse' in
467 predation risk (Lima & Bednekoff, 1999; Reylea, 2003; Schmitz, 2007). The
468 effectiveness of antipredator behavior is dependent not only on the identity of the
469 predator and its foraging tactics, but also on the type of habitat where the predator is
470 encountered (Brown & Smith, 1997). It may be possible that in the constrained space of
471 lab aquaria, prey use generalized and stronger antipredator tactics to evade heightened
472 predation risks since predator avoidance is limited (Hickman et al., 2004). Shifts in

473 habitat use to predator-free environments will be limited under these lab conditions
474 (Crowl & Covich, 1994). However, we believe our experimental setup had elements of
475 reality. Most tank predation studies cage and restrict predator movements, which
476 generates limited behavioral responses, and restrict our ability to examine how predators
477 and prey interact in space (Lima, 2002; Sih, 2005). By employing a free-ranging predator
478 experimental design, we were able to observe predator-prey encounters at close
479 proximity, and quantify the behavioral response of prey given an encounter, but as in
480 other studies, sacrificed the ability of prey to exhibit other spatial responses.

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

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494 greater chance of survival with novel predators than predator naïve prey, due to their
495 fixed behavioral responses.

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

504 Prey exhibited antipredator behavior in response to both general and specific cues,
505 but mostly when these cues were chemical. A number of studies have documented the
506 use of chemical cues in predator recognition (Mathis et al., 1993a ; Mathis & Vincent,
507 2000), including those produced by nonnative taxa (Pearl et al., 2003). Chemical cues
508 likely provide an early warning of predation threats, which may be refined with the
509 introduction of visual cues (Kats & Dill, 1998; Chivers et al., 2001). The low response to
510 the visual cues used in our experiment, may be due to the fact that the visual cues used
511 did not provide enough information for prey to correctly identify the predator threat
512 (Wisenden, 2004), or they did not reflect a high risk encounter to merit a response
513 (Corkum, 2002).

514 Prey often show a greater reliance on chemical cues when visual cues are
515 diminished, such as in turbid waters, in heavily-vegetated habitats, or with cryptic

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Deleted: Despite being introduced at a short-distance, the visual cues may have been inadequate for fine-scale discrimination of the threat (Mathis & Vincent, 2000), or deemed meaningless in the absence of accompanying chemical information

516 predators (Hartman & Abrahams, 2000; Mathis & Vincent, 2000; Amo et al., 2004).
517 Because of the high density of emergent grasses (Gunderson & Loftus, 1993), the high
518 biomass of periphyton (Turner et al., 1999), and the presence of flocculent material atop
519 the benthos (Rehage & Trexler, 2006), the structural complexity of Everglades marsh
520 habitats is relatively high. Under these conditions, prey may be expected to rely more
521 intensely on chemical information as seen in our study (Mathis & Vincent, 2000).
522 Similarly, several of the common native predators use a sit and wait hunting strategy, for
523 which, it is more advantageous for prey to use chemical cues in predator recognition
524 (Amos et al., 2004); especially if prey are able to recognize not previously encountered
525 predators as a threat when they are closely related to known predators (i.e., confamiliar
526 predators; Ferrari et al., 2007). Both mosquitofish and flagfish showed a significant
527 decrease in activity and increase in vertical distribution to the isolated scents of jewelfish
528 and warmouth. Often, the strength of a prey species' antipredator response will depend
529 on dietary cues, and whether the predator has consumed conspecific or heterospecific
530 prey (Wohlfahrt et al., 2006), but we removed these cues from our experiment. Instead,
531 we suggest that the fish species may be relying on chemical kairomones for predator
532 recognition and response. Kairomones are prominent chemical cues that are similar
533 across freshwater fish families and are believed to be a partial metabolite of fish-
534 associated bacteria (Dicke & Sabelis, 1988; Elert & Phonert, 2000). Previous work shows
535 that prey use these cues in predator recognition (Gelowitz et al., 1993; Kats & Dill,
536 1998). Kusch et al. (2004) showed that fathead minnow populations exhibited intense
537 behavioral responses to increasing concentrations of northern pike odor, *Esox lucius*, and

538 were able to recognize the size of the predators that generated the cues. The recognition
539 of predator kairomones by prey can occur very quickly under natural conditions
540 (Wisenden & Chivers, 2006). It may be possible that the prey's prior experience with
541 other cichlid predators may have allowed particularly the fish prey to respond to
542 nonnative jewelfish. Ferrari et al. (2007) showed that fathead minnows trained to
543 recognized the scent of a particular salmonid predator, also exhibited antipredator
544 responses to the scent of two other salmonid species, despite no experience with them.

545 While chemical cues appear to be a primary source of information in predator-
546 prey interactions in our trials, the antipredator responses observed during the cue
547 experiments were weaker than those observed in the first experiment where predators
548 were present. This suggests that prey may need multiple cues to identify a predation risk,
549 and determine the degree of risk-sensitive behavior to exhibit (i.e. threat sensitivity
550 hypothesis, Amo et al., 2004; Botham et al., 2008). For instance, the relatively weak
551 response of shrimp observed in the chemical cue trials may be due to the fact that they
552 require other cue types, such as tactile cues. Crowl and Covich (1994) found that
553 chemical cues elicit a partial response from freshwater shrimp, but when coupled with the
554 physical presence of the predator the intensity of the responses increases. Mosquitofish
555 similarly increase avoidance behavior when both the chemical and visual cues of
556 predatory fish are present (Smith & Belk, 2001).

557

558 **Conclusions**

559 Introduced predators are a major concern for the Everglades, and have been
560 implicated in fish population declines elsewhere in freshwater systems (Cox & Lima,
561 2006). With the continued invasion of new species, the probability for synergistic effects
562 among fish predators that could drastically alter the way nonnative species interact with
563 natives and thus their impact (e.g., O’Dowd et al., 2003) becomes a concern. Our data
564 show that a newly- arrived predator may have similar predatory effects and elicit similar
565 antipredator behavior from native prey. Thus, the vulnerability of Everglades prey to new
566 predators does not seem to vary among taxa, and may be less than expected based on the
567 novelty of the interaction, perhaps because of the experience of Everglades prey with
568 cichlid predators. If predation rates and prey risk to nonnative cichlids are similar, we
569 would expect nonnative predators to function in a similar matter as native predators.
570 However, we do not know if the addition of nonnative cichlids to the system is increasing
571 overall predation regimes, with important consequences for the transfer of energy
572 throughout food webs and ecosystem components, or replacing them. Further work is
573 needed to distinguish between the two, and better assess the consequences of multiple
574 invasions in the long-term.

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582

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780 Figure 1. Diagram of the model used for the visual cue experiment. The predator model
781 consisted of a wooden dowel shaped like a fish (60 mm SL), suspended in the water
782 column, at a depth of approximately of 11 cm, and moved using a pulley system. The tank
783 containing the model was positioned adjacent to the prey tank and separated with a
784 removable barrier, similar to the other treatments.

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786 Figure 2. Mean predator and prey activity, vertical distribution, and structure use (± 1
787 SE) for the first experiment across predator treatments (NP = no predators, JJ= 2
788 jewelfish, WW = 2 warmouth, WJ = 1 jewelfish + 1 warmouth). Activity was scored 0-3
789 (0 = not active), vertical distribution was scored as 0-2 (0 = bottom), and structure use
790 reflect counts of the number of prey individuals within the structure averaged over the
791 observation period. Significant pairwise differences ($P \leq 0.05$) are indicated with
792 lettering above bars.

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

798 Figure 4. Mean predation rate (± 1 SE) on all prey across treatments (NP = no predators,
799 JJ= 2 jewelfish, WW = 2 warmouth, WJ = 1 jewelfish + 1 warmouth). Letters indicate
800 significant pairwise differences at $P \leq 0.05$.

801 Figure 5. Mean predator and prey activity, vertical distribution, and habitat use (± 1 SE)
802 in the two cue experiments across predator treatments (NP = no predators, JJ= 2
803 jewelfish, WW = 2 warmouth, WJ = 1 jewelfish + 1 warmouth). Activity was scored 0-3
804 (0 = not active), vertical distribution was scored as 0-2 (0 = bottom), and the occurrence
805 of prey groups was scored as 0-1 (0 = group absent, 1 = group present). Significant
806 pairwise differences ($P \leq 0.05$) are indicated by different uppercase letters.

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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).

	Predator treatment		Prey species		Predator treatment x prey species		Block		R ²
	F (df)	p	F (df)	p	F (df)	p	F (df)	p	
<i>Predator-Prey Experiment</i>									
Prey Mortality	157.4 (3, 96)	<0.001	9.5 (2, 96)	<0.001	3.3 (6, 96)	0.005	0.3 (1, 96)	0.581	0.8
Predator behavior									
Multivariate Analysis	4.1 (8, 138)	<0.001					0.5 (4, 69)	0.733	
Activity	4.5 (2, 72)	0.015					0.5 (1, 72)	0.504	0.2
Vertical Distribution	1.2 (2, 72)	0.310					0.2 (1, 72)	0.684	0.1
Use of habitat structure	0.7 (2, 72)	0.490					0.3 (1, 72)	0.585	0.0
Predator-predator interactions	7.0 (2, 72)	0.002					0.0 (1, 72)	0.878	0.3
Prey behavior									
Multivariate Analysis	14.4 (12, 246)	<0.001	28.9 (8, 186)	<0.001	1.6 (24, 326)	<0.044	0.5 (4, 93)	0.766	
Activity	20.8 (3, 96)	<0.001	50.5 (2, 96)	<0.001	1.8 (6, 96)	0.102	0.1 (1, 96)	0.759	0.7
Vertical Distribution	40.0 (3, 96)	<0.001	17.1 (2, 96)	<0.001	2.2 (6, 96)	0.005	1.1 (1, 96)	0.289	0.7
Use of habitat structure	5.6 (3, 96)	0.001	1.0 (2, 96)	0.372	0.2 (6, 96)	0.968	0.0 (1, 96)	0.958	0.2
Grouping	9.8 (3, 96)	<0.001	131.2 (2, 96)	<0.001	2.4 (6, 96)	0.034	0.2 (1, 96)	0.657	0.8

Table 2. Results of ANOVAs and MANOVAs (F values, degrees of freedom, p values, and R²) testing treatment, species, and block effects for the two cue experiments (significant effects are in bold).

	Predator Treatment		Prey Species		Predator treatment x prey species		Block		R ²
	F (df)	p	F (df)	p	F (df)	p	F (df)	p	
<i>Chemical Cue Experiment</i>									
Prey behavior									
Multivariate Analysis	2.0 (12, 278)	0.023	3.7 (8, 210)	<0.001	2.0 (24, 367)	0.006			
Activity	5.9 (2, 108)	0.009	2.4 (3, 108)	0.095	2.5 (6, 108)	0.024			0.3
Vertical Distribution	2.0 (2, 108)	0.106	9.3 (3, 108)	0.002	1.5 (6, 108)	0.200			0.2
Use of habitat structure	1.4 (2, 108)	0.253	0.1 (3, 108)	0.893	1.6 (6, 108)	0.143			0.1
Grouping	4.0 (2, 108)	0.449	2.1 (3, 108)	0.022	1.4 (6, 108)	0.388			0.1
<i>Visual Cue Experiment</i>									
Prey behavior									
Multivariate Analysis	1.4 (15, 254)	0.170	5.6 (10, 184)	<0.001	1.0 (30, 370)	0.551	0.9 (5, 92)	0.460	
Activity	1.2 (2, 96)	0.318	22.1 (3, 96)	<0.001	0.7 (6, 96)	0.600	3.0 (1, 96)	0.086	0.4
Vertical Distribution	0.9 (2, 96)	0.446	7.3 (3, 96)	0.001	0.5 (6, 96)	0.834	2.1 (1, 96)	0.148	0.2
Use of habitat structure	1.5 (2, 96)	0.231	2.6 (3, 96)	0.800	1.6 (6, 96)	0.150	0.0 (1, 96)	0.935	0.2
Grouping	0.2 (2, 96)	0.866	2.8 (3, 96)	0.069	1.2 (6, 96)	0.340	0.4 (1, 96)	0.538	0.2