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

Jennifer S. Rehage

Earth and Environment Department, Southeast Environmental Research Center, Florida International University,
rehagej@fiu.edu

Katherine L. Dunlop

Oceanographic Center, Nova Southeast Environmental Research Center, Florida International University

William F. Loftus

U.S. Geological Survey, Florida Integrated Science Center, Everglades National Park Field Station

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1 Antipredator responses by native mosquitofish to non-native cichlids: an examination of the role
2 of prey naiveté

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5 Jennifer S. Rehage¹ (corresponding author), Katherine L. Dunlop² and William F. Loftus³

6
7

8 ¹ Earth and Environment Department, Southeast Environmental Research Center, Florida

9 International University

10 University Park, ECS 337, 11200 S.W. 8th Street, Miami, FL 33199

11 rehagej@fiu.edu, (305) 348-3804, (305) 348-6137 Fax

12

13 ² Oceanographic Center, Nova Southeastern University

14 8000 N. Ocean Drive, Dania Beach, FL 33004

15 kdunlop@nova.edu

16

17 ³ U.S. Geological Survey, Florida Integrated Science Center

18 Everglades National Park Field Station, 40001 State Road 9336, Homestead, FL 33034

19 bill_loftus@usgs.gov

20

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24 **Abstract**

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

46 prey experience, if prey rely on general antipredator responses or predation cues, and/or show
47 neophobic responses.

48 **Key words:** predation, evolutionary naiveté, antipredator behavior, fish, invasion

49 **Introduction**

50 Species invasions that cause high impact to invaded communities often result from novel
51 intertrophic interactions such as predation (Kats & Ferrer 2003; Gurevitch & Padilla 2004; Salo
52 et al. 2007). By novel, we mean interactions where the predator has no common evolutionary
53 history with native prey, resulting in prey that are evolutionarily naive to the introduced
54 predators (Diamond & Case 1986; Cox & Lima 2006). An extreme form of novelty is illustrated
55 by the introduction of predators to oceanic islands (and Australia), where predation itself may be
56 novel or the predator archetype is absent (Ogutu-Ohwayo 1990; Fritts & Rodda 1998; Blackburn
57 et al. 2004). A less severe and perhaps more common form of novelty stems from variation in
58 predator archetypes, where native and non-native predators exhibit varying behavioral and
59 morphological adaptations for prey capture (Cox & Lima 2006). For instance, variation in
60 predator hunting mode (Schmidt 2007) among native and non-native predators could result in
61 prey experiencing some degree of predator novelty. Under either scenario, the evolutionary
62 naiveté of native taxa can result in the failure of prey to recognize predation threats, in
63 inappropriate antipredator responses or in appropriate but ineffective responses (Diamond &
64 Case 1986; Banks & Dickman 2007).

65 Prey naiveté is hypothesized to be partly responsible for the strong negative effects of
66 introduced aquatic predators (Cox & Lima 2006). Dispersal by aquatic top predators is relatively
67 low and isolation at intercontinental and regional scales can be high in freshwater systems,
68 resulting in significant variation in predator archetypes and regimes among and within water
69 bodies. For instance, hydrological gradients can result in small-scale variation in predation
70 regimes (Wellborn et al. 1996) that should generate prey naiveté to predators that are allopatric
71 along the gradient (Cox & Lima 2006). In the Florida Everglades, the recurrent pattern of

72 seasonal dry-down limits the abundance of large-bodied predators both temporally and spatially
73 (Chick et al. 2004; Trexler et al. 2005), which may result in gradients in naiveté that accompany
74 hydrological gradients across the landscape (i.e., long vs. short hydroperiod marshes), although
75 this remains untested.

76 Another and perhaps more important source of gradients in prey naiveté is the patchiness
77 in the distribution of non-native predators. The African jewelfish, *Hemichromis letourneuxi*, one
78 of the most recent invaders of the Everglades, is presently limited to the southern and western
79 regions of the ecosystem (Shafland et al. 2008). Jewelfish were first detected in Everglades
80 National Park (ENP) in 2000 (J. Kline pers. comm.) and are presently undergoing a rapid range
81 expansion into longer hydroperiod marshes and mangrove regions (Shafland et al. 2008, Rehage
82 et al. unpubl. data), but have not yet colonized marshes in the Water Conservation Areas in the
83 northern Everglades. They are piscivores, with fish accounting for 70-80% of their diet (Loftus et
84 al. 2006). Their small size allows them to invade shallower habitats, where larger invaders have
85 previously been excluded, and thus they are an especially concerning invader. Among the more
86 established invaders of the Everglades is the Mayan cichlid, *Cichlasoma urophthalmus*, first
87 detected in 1983 in ENP and presently widespread throughout the system (Loftus & Kushlan
88 1987; Fuller et al. 1999; Shafland et al. 2008). Mayan cichlids can be a dominant component of
89 the fish community of certain habitats. In particular, they can account for up to 40% of fish
90 abundance in the mangrove zone (Trexler et al. 2001). They are also predators, with fish being a
91 dominant prey item in their diets (Bergmann & Motta 2005). These two cichlids invaders are
92 currently the two most abundant fish invaders in ENP out of about 14 established species
93 (Shafland et al. 2008; J. Kline pers. comm.), and thus have the potential to have a high impact on
94 invaded aquatic communities. How native Everglades prey, particularly small-fish taxa, cope and

95 respond to these predation threats that vary in the degree of novelty is currently unexplored.
96 Furthermore, although prey naiveté is invoked as a major mechanism for the high impact of
97 introduced predators, few studies have examined it directly (Cox & Lima 2006).

98 In this study, we used laboratory assays to examine the effect of predator novelty and
99 prey naiveté on predator-prey interactions between native Everglades prey and non-native cichlid
100 predators. Specifically, we quantified the antipredator behavior of native Eastern mosquitofish
101 (*Gambusia holbrooki*), and the hunting mode, habitat domain and lethality of the Mayan cichlid
102 and African jewelfish. Mosquitofish are the most ubiquitous fish species in the Everglades
103 (Trexler et al. 2005), and should be readily encountered and consumed by both predators. In fact,
104 stomach analyses of African jewelfish from our study sites in ENP show that mosquitofish are
105 the most abundant prey item (Loftus et al. 2006). Predation by non-native predators on
106 ubiquitous prey such as mosquitofish may lead to invaders having wide-ranging impacts.
107 Moreover, we expect ubiquitous prey to have important functional roles throughout the system,
108 and if non-native predators are able to significantly decrease their abundance, this could also
109 contribute to high impacts in the invaded system. We manipulated the degree of novelty in
110 predator-prey interactions by using these two predator species that varied in the time since
111 invasion, and mosquitofish populations that varied in experience with them. We hypothesized
112 that variation in the hunting modes and habitat domain of the predators would make jewelfish a
113 relatively novel predation threat. Due to the greater naiveté of Everglades prey with African
114 jewelfish, we expected predation rates to be higher by this novel predator. We hypothesized that
115 due to greater experience, the antipredator responses of prey would be stronger to the Mayan
116 cichlid. We compared mosquitofish populations with the expectation that prey from jewelfish-
117 invaded areas would exhibit greater and/or more effective antipredator responses to jewelfish

118 than naive prey from areas where jewelfish are absent, and thus jewelfish predation would be
119 higher on the naive population.

120

121 **Methods**

122 To examine the predatory behavior and effect of the cichlids species and the antipredator
123 response of mosquitofish, we conducted behavioral assays in laboratory aquaria. In a 4 x 2
124 factorial design, we observed the effects of four predation treatments on two mosquitofish prey
125 populations. Predation treatments consisted of predator pairs in a replacement series design
126 where predator density remained constant (Sih et al. 1998). Treatments included: (JJ) 2 African
127 jewelfish, (MM) 2 Mayan cichlids, (MJ) 1 African jewelfish + 1 Mayan cichlid, and (NP) no
128 predators. Mosquitofish were collected from two populations that varied in their naiveté to
129 African jewelfish, but had similar levels of experience with Mayan cichlids. The ENP
130 mosquitofish population was considered ‘experienced’ since African jewelfish have been present
131 and abundant for close to a decade. A second prey population from northern Water Conservation
132 Area 3A (WCA3A) was considered naive to African jewelfish since despite repeated sampling
133 by ourselves and colleagues over the past few years, they have never been collected there or that
134 far north in the inner Everglades ecosystem. Both populations should have similar levels of
135 experience with Mayan cichlids, which have been present and abundant throughout since the
136 1980’s.

137 Trials were conducted in two blocks in July 27-30, 2007 and March 3-8, 2008,
138 corresponding to the wet and dry seasons in the Everglades respectively (hereafter referred to as
139 the season effect). A minor objective of our study was to examine whether predator motivation
140 and antipredator behavior would vary seasonally in response to dry-down and the expected

141 physiological stress associated with it (i.e., reduced prey abundance and poor condition).
142 Cichlids were collected from the Rocky Glades region of ENP using unbaited minnow traps
143 deployed overnight in June-July 2007 and January-February 2008. Mosquitofish were collected
144 using dip nets at a WCA3A site (N 26.147, W 80.57134) and at the same ENP Rocky Glades
145 locations where predators were collected. Predator species were size-matched in trials, but
146 because of species-specific size differences, African jewelfish were adults (51.7 ± 0.9 mm
147 standard length), while Mayan cichlids were juveniles of approximately 65.6 ± 1.8 mm standard
148 length. Bergmann & Motta (2005) showed that fish remain the primary prey item for Mayan
149 cichlids throughout development. All prey used in the study were juveniles (13.1 ± 0.2 mm
150 standard length). Prior to the experiment, we held predators and prey in 795-l outdoor tanks and
151 fed them a combination of live prey, including mosquitofish from both populations. We fed prey
152 flakes *ad libitum*.

153 In both seasons, trials were conducted over 4 consecutive days. Each day, we tested a
154 single replicate of the 8 treatment by population combination (4 treatments x 2 prey populations
155 x 4 days x 2 seasons = 64 experimental units). To minimize inter-individual variation in predator
156 motivation, randomly-assembled predator pairs were used repeatedly with the two prey
157 populations. Pairs were randomly assigned to days 1 or 2 of the block and then used again in day
158 3 and 4 respectively with a different prey population. For instance, a predator pair that
159 experienced the ENP prey population on day 1, was assigned to the WCA3A mosquitofish
160 population on day 3, and similar for day 2 and 4. Trials were not conducted on consecutive days
161 in order to obtain overnight prey mortality rates and then standardize hunger levels prior to the
162 next trial. This protocol was repeated with a new set of predators in the dry season, for a total
163 number of 24 jewelfish and 24 Mayan predators used in the study.

164 For each trial, behavioral data were collected on a group of 6 mosquitofish (6 prey x 4
165 treatments x 2 populations x 8 replicates = 384 prey). Mosquitofish groups from both
166 populations were isolated in 5.7-l containers the evening prior and then randomly assigned to
167 treatments on the day of trials. Similarly, predators were isolated in 5.7-l containers the evening
168 before trials and between trials. To standardize hunger levels, all feeding was suspended 24 h
169 before trials, as well as between trials for the predators (e.g., no feeding on day 2 for a predator
170 used on days 1 and 3).

171 Trials were conducted in 8 56.8-l aquaria (50 x 24.5 x 40 cm height) covered on all 4
172 sides with white vinyl. Artificial vegetation was used to provide structural complexity for both
173 predators and prey (16 x 16 cm, covering approximately 1/3 of tank area). This artificial
174 vegetation consisted of black plastic strips (4 x 22 cm) attached to a weighted plastic grid that
175 rested on the bottom and to one side of each tank. To minimize observer effects, observations
176 were conducted through mirrors placed at 45° angles above tanks. At the beginning of each trial,
177 prey were released and allowed to acclimate for 15 min before predators were added. The first
178 observation was taken 5 min after predator release.

179 Prey and predator behavior was assessed through spot-check observations conducted by
180 two observers, one taking data on the predators and the other on the prey (Martin & Bateson
181 2007). Observers spent 20-60 s per tank accounting for all individuals and noting their activity,
182 microhabitat use, and the shoaling behavior of prey. Ten spot-check observations were
183 conducted per tank, one every 10-12 min for a total trial duration of approximately 2 h. All
184 observations were conducted between 10 AM-1 PM. Activity was scored as active if there was
185 movement that resulted in a change in position (e.g., movement of fins was scored as inactivity).
186 For microhabitat use, we noted vertical distribution within the tank (top, middle or bottom one

187 third of the water column), and whether predators and prey were in or out of the habitat structure.
188 For these three variables, we calculated the proportion of predators and prey engaged in each
189 behavior over the 10 observations and then averaged them. Shoaling behavior by mosquitofish
190 was scored as a 1 if prey were aggregated in a social group of at least 4 individuals (within
191 approximately 4 body lengths of each other); otherwise it was scored as a 0, and then scores were
192 averaged over the 10 observations. At the end of the spot-check observations, tanks were
193 observed continually for 5 min to obtain count data on the attacks on prey and predator
194 inspections. Rapid approaches by predators to the prey with or without contact were considered
195 attacks. Predator inspections consisted of cautious approaches by prey, followed by a rotation or
196 retreat of the prey while still visually fixated on the predator. Actual predation events during this
197 observation period occurred in only 3 of the 64 trials for a total of 9 prey consumed.

198 At the end of all behavioral observations, we assessed mortality rates of the prey in the
199 same observation tanks. In order to avoid prey depletion, an additional 6 prey (of the same size
200 and population) were added to each tank. In the few cases where prey were consumed during the
201 observation period, we replaced them in order to begin all replicates with 12 mosquitofish. We
202 left predators and prey in covered tanks overnight, and between 7-8 AM on the following day,
203 we uncovered tanks and counted the number of surviving prey. Photoperiod over the study was
204 set to 14L:10D, and water temperature averaged 25.7 ± 0.18 ° C.

205 Statistical analyses

206 Population differences among predator treatments were examined with linear models. In
207 addition to population and treatment main effects, we tested the effects of the population by
208 treatment interaction, of season (the blocking factor), and of predator pair nested within season to
209 account for the repeated used of predators. These same effects were tested in two MANOVAs

210 ran prior to the ANOVAs. A MANOVA was run for the five focal prey variables for which data
211 were collected in all treatments: the proportion of prey active, at the top of the water column and
212 using the habitat structure, the occurrence of prey shoals, and prey mortality. A second
213 MANOVA was run for the remaining five focal variables that involved predators and for which
214 data were collected only in the three predation treatments: the proportion of predators active, at
215 the top of the water column, and using the habitat structure, and the number of predator attacks
216 and inspections. Preliminary analyses also examined the effect of predator sequence (day 1 vs. 3,
217 and day 2 vs. 4), and of the sequence by season interaction on all response variables and found
218 little effect; therefore, these factors were removed from final analyses reported here. Predator
219 sequence only affected two of the predator variables and none of the prey variables, and the
220 effect was seen only in the dry season, in which predators spent more time at the top of the water
221 column and less time in the habitat structure on day 3 and 4 relative to day 1 and 2.

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

228

229 **Results**

230 *Prey behavior*

231 The antipredator behavior of mosquitofish varied to a greater extent as a function of
232 predator treatments than populations. Little variation in antipredator behavior was detected

233 between jewelfish-naive (WCA3A) and jewelfish-experienced (ENP) populations. The exception
234 was prey activity, which was high overall, and relatively higher in the ENP population (98%
235 active relative to 92% in WCA3A population, Fig. 1A), but was unaffected by predator treatment
236 (Table 1).

237 The presence of predators resulted in shifts in microhabitat use by prey. Mosquitofish
238 were found higher in the water column if the predators were African jewelfish or mixed (Tukey
239 pairwise comparisons: JJ vs. MM and NP, $p < 0.0001$; MJ vs. MM and NP, $p < 0.0004$). Over
240 80% of prey were observed in the top 1/3 of the water column in JJ and MJ treatments compare
241 to only 48% in MM and 30% in NP treatments (Fig. 1B). Prey tended to use the habitat structure
242 more if predators were absent than if predators were the Mayan cichlid pair since Mayan cichlid
243 use of the structure was relatively high (Table 1, Fig. 1C).

244 Mosquitofish shoaled more in the presence of predators (NP vs. JJ, MM and MJ, $p <$
245 0.0248 , Table 1). Shoals were also more common with the jewelfish pair (JJ vs. MM, $p =$
246 0.0058). The occurrence of shoals averaged 63% with the jewelfish pair, 41% with the Mayan
247 pair, and only 18% in the no predator treatment (Fig. 2). Shoaling rates did not vary between the
248 single and mixed predator treatments. Shoaling was the only variable that varied between blocks
249 (higher in the dry season, Table 1). Despite the fact that mosquitofish appeared to respond more
250 strongly to jewelfish predators with their shoaling behavior and greater use of the top of the
251 water column, predator inspections by both prey populations were higher on the less novel
252 Mayan predators (MM vs. JJ and MJ, $p < 0.0335$, Fig. 3).

253

254 *Predator behavior*

255 African jewelfish and Mayan cichlids varied in their activity and microhabitat use,
256 suggesting variation in hunting modes and habitat domains. Their behavior was also unaffected
257 by the degree of novelty of the prey; predator behavior was similar toward the ENP and WCA3A
258 mosquitofish populations (Table 1). Predator activity was highest for the jewelfish pair,
259 intermediate for the mixed predator treatment and lowest for the Mayan pair (Table 1, all
260 pairwise comparisons, $p < 0.0064$; Fig. 1A). Jewelfish pairs also spent more time in the upper
261 water column and less time in habitat structure relative to the Mayan pairs (JJ vs. MM, $p <$
262 0.0005 for both comparisons; Figs. 1B and C). The vertical distribution of predators was affected
263 by predator identity (Table 1). Certain predator pairs spend more time high in the water column
264 than others.

265

266 *Prey mortality*

267 Despite variation in predator behavior and the prey response, predator voracity and
268 lethality were similar among predator combinations. The number of attacks on prey at the end of
269 trials was low, on average one attack per 5-minute observation period, and did not differ among
270 treatments (Table 1). Similarly, overnight predation rates were comparable across predator
271 combinations and between the two prey populations (Table 1). On average, predators consumed
272 8 mosquitofish relative to zero mortality in the control tanks (Fig. 4).

273

274 **Discussion**

275 The prey naiveté hypothesis suggests that the high impact of aquatic predators relates to
276 prey's limited ability to detect and respond to novel predation threats posed by non-native
277 piscivores (Cox & Lima 2006). Our results did not find support for this notion. First, predator

278 avoidance responses by mosquitofish appeared stronger to the more novel predation threat, the
279 African jewelfish, relative to those exhibited toward Mayan cichlids. Mosquitofish responded by
280 altering their microhabitat use, increasing shoaling, and examining predators. Despite prey
281 engaging in these behaviors in the presence of jewelfish, mortality rates were similar between the
282 two predators. No variation in attack rates and overnight predation rates was detected, although
283 predator microhabitat use and activity varied. Little variation was found between the seasons
284 (blocks) suggesting that the characteristic seasonal hydrological variation of the Everglades
285 ecosystem may have little effect on the predator and prey behaviors examined here, although our
286 power to detect this effect was likely low.

287 Second, the amount of naiveté of mosquitofish populations did not appear to affect their
288 antipredator response. The response to jewelfish was as strong by the naive WCA3A
289 mosquitofish population, which had no experience with jewelfish, than by the ENP population,
290 where jewelfish occur and pose a significant predation threat to mosquitofish (Loftus et al.
291 2006). Confirmation of this result with a larger number of experienced and naive prey
292 populations is needed. Examination of this question with other prey species is also needed. Our
293 own examination of the response of other Everglades prey to novel African jewelfish shows that
294 antipredator responses are species specific (Dunlop & Rehage, unpubl. data) and may result in
295 variation in prey vulnerability. Nannini and Belk (2006) found similar variation for the response
296 of two minnow species to introduced trout.

297 Our experimental design using free-ranging predators and prey allowed us to examine the
298 response of predators and prey spatially. Prey typically try to avoid areas with high predation
299 risk, while predators concentrate efforts in areas with more prey. Most studies cage or otherwise
300 restrict predator movement (Lima 2002), limiting one's ability to examine this behavioral

301 response race (Sih 2005). Mosquitofish did not reduce activity in the presence of predators, but
302 altered the use of tank microhabitats. In the presence of African jewelfish, prey moved higher in
303 the water column. Changes in the vertical distribution of mosquitofish in response to predation
304 risk have been noted in previous studies (Garcia et al. 1992; Smith & Belk 2001). Since both
305 predators were found relatively low in the water column, this change in microhabitat use likely
306 reduced their spatial coincidence with predators. With Mayan cichlids, prey minimized
307 encounters by reducing use of habitat structure in their presence, because Mayan pairs used
308 cover to the greatest extent. Mosquitofish also increased shoaling behavior in response to
309 jewelfish but not Mayan cichlids. Shoaling is known to function largely as a defense behavior
310 since it typically enhances vigilance and predator confusion and abates attacks, allowing for
311 coordinated evasion and risk dilution (reviewed by Pitcher & Parrish 1993).

312 Prey often engage in the visual inspection of potential predators as a means of assessing
313 predator identity and motivation (Lima & Dill 1990; Dugatkin & Godin 1992; Brown 2003). In
314 this study, prey inspections were directed towards Mayan cichlids more than African jewelfish.
315 This result agrees with previous work showing that experienced prey inspect more than relatively
316 naive prey (Magurran & Seghers 1990; Kelley & Magurran 2003; but see Brown & Warburton
317 1999). It is also possible that prey engaged in higher inspections with Mayans cichlids because
318 Mayans were perceived to be the lower-risk predator. Since inspections involve approaches to
319 the predator, they can be riskier than other antipredator behaviors (Dugatkin 1992), and prey may
320 afford to engage in inspection only with relatively low-risk predators (Smith & Belk 2001).
321 Mayan cichlids were also less active than jewelfish, and previous work shows that all else being
322 equal, prey are more likely to inspect stationary rather than moving threats (Pitcher et al. 1986;
323 Dugatkin & Godin 1992). Further, in the presence of the highly-active jewelfish, it may be

324 unnecessary for mosquitofish to engage in inspection to assess risk, as microhabitats with active
325 predators can become ‘cue-saturated’ (Preisser et al. 2007).

326 Prey seemed to respond to the two cichlid predators with both different antipredator
327 tactics and different magnitudes of response. The increase in shoaling and use of the upper water
328 column shown only with African jewelfish suggest to us that both mosquitofish populations
329 perceived jewelfish to be the riskier predators, despite their variable experience with them. Prey
330 altered microhabitat use (either to the top of the water column or out of the structure) when faced
331 with both predators, but the magnitude of the response (e.g., behavior without predators –
332 behavior with predators) was much greater in the presence of jewelfish, suggesting higher risk.
333 The same is seen in the shoaling behavior, shoal sizes are greater in the presence of jewelfish
334 than in the presence of Mayans. We expect prey to modulate their response to match the
335 predation threat (i.e., threat-sensitive predator avoidance hypothesis; Helfman 1989; Chivers et
336 al. 2001; Mirza et al. 2006; Botham et al. 2008) or perception of such risk (Sih 1992; Brown
337 2003; Lima & Steury 2005).

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

347 experiments that have manipulated predator movement show that prey exhibit stronger
348 antipredator responses to moving rather than stationary predation threats (Brown & Warburton
349 1997; Brown & Warburton 1999; Wisenden & Harter 2001).

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

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

370 predation risk for prey in nearby marsh habitats, perhaps allowing prey to develop strong general
371 antipredator behaviors.

372 Third, naive prey may be able to detect jewelfish as a threat despite their novelty, if they
373 rely on general predation cues for predator detection and recognition. General cues include
374 chemical cues associated with predator diet, disturbance cues associated with stressed/startled
375 prey, and damage-released alarm cues associated with a predator attack (reviewed by Chivers &
376 Smith 1998; Brown 2003; Wisenden & Chivers 2006). These alarm signals can effectively
377 'label' potential predators as such. In contrast, native species that rely on specific cues (e.g., the
378 scent or vocalization of a particular predator) may be unable to recognize and respond to novel
379 predators (Jones et al. 2004; Smith et al. 2008). Although mosquitofish are known to respond to
380 the release of conspecific skin extract (Garcia et al. 1992), only a very small number of predation
381 events occurred, making it unlikely that alarm cues were important. Instead, predators were fed
382 mosquitofish prior to trials (along with other native prey) and at least closely-related western
383 mosquitofish (*Gambusia affinis*) can detect predator dietary cues (Smith & Belk 2001).

384 Finally, we suggest that general visual cues could also be used in predator detection
385 instead or in addition to chemical cues. Prey may be responding to the presence of any novel,
386 large (above a certain threshold) and moving object (Dill 1974; Brown & Warburton 1997;
387 Wisenden & Harter 2001). In a sense, this constitutes a neophobic response. Neophobia refers to
388 the fear of novelty, and is typically characterized by aversion, hesitation or caution (Greenberg
389 2003). Neophobia may be adaptive when predation risk is very high and/or predator diversity is
390 low (Brown & Chivers 2005). Under these circumstances, a large moving individual encountered
391 by prey is likely to be a potential predator, and prey should exhibit antipredator behavior in
392 response regardless of predator identity. Both of these conditions could apply to our WCA3A

393 prey. As mentioned earlier, marshes nearby canals may experience high predation regimes, and
394 at same time, the diversity of piscivores in Everglades habitats is relatively low, usually
395 dominated by seven to eight taxa (Chick et al. 2004; Rehage & Trexler 2006).

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

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413

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420

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Figure legends

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

569

570 **Fig. 2:** Shoaling behavior of mosquitofish across predator treatments (JJ= 2 African jewelfish,
571 MM = 2 Mayan cichlids, MJ = African jewelfish + Mayan cichlid, and NP = no predator) and
572 prey populations (WCA3A = naive and ENP = experienced to jewelfish-both are experienced
573 with Mayan cichlids). Groups of 4, 5 or 6 prey were considered a shoal and scored as 1's;
574 smaller groups were scored as 0's. Shown are means \pm SE.

575

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

581

582 **Fig. 4:** Overnight mosquitofish mortality rates across treatments (JJ = 2 African jewelfish, MM =
583 2 Mayan cichlids, MJ = African jewelfish + Mayan cichlid, and NP = no predator) and prey
584 populations (WCA3A = naive and ENP = experienced with African jewelfish-both are
585 experienced with Mayan cichlids). Shown are means \pm SE.

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600 **Table 1:** Result of ANOVAs and MANOVA's (p-values and R²) testing predation treatment, population, interaction, **season (or**
 601 **block)**, and predator pair effects. MANOVA1 contains the 5 variables measured in all treatments, and MANOVA2 contains the 5
 602 variables measured in the 3 predator treatments only.

		P values for effects				
Variables	R ²	Treatment	Population	Treatment x Population	Season	Predator pair (Season)
MANOVA1 Wilks' Lambda		0.0001	0.0945	0.9248	0.0001	0.4322
MANOVA2 Wilks' Lambda		0.0001	0.9567	0.5999	0.6798	0.0522
<i>Prey</i>						
Activity	0.21	0.4318	0.0380	0.4814	0.0932	0.5437
Vertical distribution	0.63	0.0001	0.1262	0.8297	0.5850	0.0992
Use of habitat structure	0.18	0.0571	0.8561	0.7119	0.2182	0.7063
Shoaling behavior	0.64	0.0001	0.4871	0.3419	0.0001	0.5835
Predator inspections	0.32	0.0112	0.7997	0.3269	0.1741	0.1380
Mortality	0.64	0.0001	0.5741	0.9609	0.9655	0.4663

615							
616	<i>Predators</i>						
617	Activity	0.56	0.0001	0.8481	0.2298	0.8780	0.2126
618	Vertical distribution	0.48	0.0001	0.3185	0.7373	0.4942	0.0192
619	Use of habitat structure	0.38	0.0008	0.6445	0.2574	0.7646	0.1892
620	Attacks	0.14	0.2009	0.7829	0.6451	0.3486	0.5315
