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

2   of prey naiveté

3

4

5   Jennifer S. Rehage[1] (corresponding author), Katherine L. Dunlop[2] and William F. Loftus[3]

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

21

22   Running title: mosquitofish naiveté to introduced cichlids

23   Total words: 7,250

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

Introduction

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

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

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

7

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 45º 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 $25.7 \pm 0.18\,°C$.

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.

421 **Literature Cited**

422 Banks, P. B. & Dickman, C. R. 2007: Alien predation and the effects of multiple levels of prey

423   naivete. Trends Ecol. Evol. **22**, 229-230.

424 Banks, P. B., Nordström, M., Ahola, M., Salo, P., Fey, K. & Korpimäki, E. 2008: Impacts of

425   alien mink predation on island vertebrate communities of the Baltic Sea Archipelago:

426   review of a long-term study. Boreal Environ Res. **13**, 3-16.

427 Bergmann, G. T. & Motta, P. J. 2005: Diet and morphology through ontogeny of the

428   nonindigenous Mayan cichlid *Cichlasoma (Nandopsis) urophthalmus* (Günter 1862) in

429   southern Florida. Env. Biol. Fish. **72,** 205-211

430 Blackburn, T. M., Cassey, P., Duncan, R. P., Evans, K. L. & Gaston, K. J. 2004: Avian

431   extinction and mammalian introductions to ocean islands. Science **305,** 1955-1958.

432 Blumstein, D. T. 2006: The multi-predator hypothesis and the evolutionary persistence of

433   antipredator behavior. Ethology **112**: 209-217.

434 Botham, M. S., Hayward, R. K., Morrell L. J., Croft D. P., Ward, R., Ramnarine I. and Krause, J.

435   2008: Risk-sensitive antipredator behavior in the Trinidadian guppy, *Poecilia reticulata*.

436   Ecology **89,** 3174-3185.

437 Brown, C. & Warburton, K. 1997: Predator recognition and anti-predator responses in the

438   rainbowfish *Melanotaenia eachamensis.* Behav. Ecol. Sociobiol. **41,** 61-68.

439 Brown, C. & Warburton, K. 1999.:Differences in timidity and escape responses between

440   predator-naive and predator-sympatric rainbowfish populations. Ethology **105,** 491-502.

441 Brown, G. E. 2003: Learning about danger: chemical alarm cues and local risk assessment

442   in prey fishes. Fish Fish. **4,** 227-234.

443 Brown, G. E. & Chivers, D. P. 2005: Learning as an Adaptive Response to Predation. In:

444   Ecology of Predator-Prey Interactions (Barbosa, P. & Castellanos, I., eds). Oxford

445   University Press, New York, NY, pp. 34-54.

446 Chick, J. H., Ruetz, C. R. III & Trexler, J. C. 2004: Spatial scale and abundance patterns of large

447   fish communities in freshwater marshes of the Florida Everglades. Wetlands **24,** 652–

448   664.

449 Chivers, D. P. & Smith, R. F. 1998: Chemical alarm signaling in aquatic predator-prey systems:

450   a review and prospectus. Ecoscience **5,** 338-352.

451 Chivers, D. P., Mirza, R. S., Bryer P. J., Kiesecker J. M. 2001: Threat-sensitive predator

452   avoidance by slimy sculpins; understanding the importance of visual **vs.** chemical

453   information. Can. J. Zool. **79,** 867-873.

454 Cox, J. G., Lima S. L. 2006: Naiveté and an aquatic-terrestrial dichotomy in the effects of

455   introduced predators. Trends Ecol. Evol. **21,** 674-680.

456 Diamond, J., Case T. J. 1986: Overview: Introductions, Extinctions, Exterminations and

457   Invasions. In: Community ecology (Diamond, J. & Case, T. J., eds). Harper and Row,

458   New York, NY, pp. 65-79.

459 Dill, L. M. 1974: The escape response of the zebra danio (*Brachydanio rerio*) I. The

460   stimulus for escape. Animal Behav. **22,** 711-722.

461 Dugatkin, L. A. 1992: Tendency to inspect predators predicts mortality risk in the guppy

462   (*Poecilia reticulata*). Behav. Ecol. **3,** 124-27.

463 Dugatkin, L. A. & Godin, J. G. J. 1992: Predator inspection, shoaling and foraging under

464   predation hazard under predation threat in the Trinidadian guppy *Poecilia reticulata*.

465   Env. Biol. Fish. **34,** 265-276.

466   Ferrari, M. C. O., Gonzalo, A., Messier, F. & Chivers, D. P. 2007: Generalization of learned

467   predator recognition: an experimental test and framework for future studies. Proc. R. Soc.

468   Lond., Ser. B, Biol. Sci. **274,** 1853-1859.

469   Fritts, T. J. & Rodda, G. H. 1998: The role of introduced species in the degradation of island

470   ecosystems: a case history of Guam. Annu. Rev. Ecol. Syst. **29,** 113-140.

471   Fuller, P. L., Nico, L. G. & Williams, J. D. 1999: Nonindigenous Fishes Introduced into Inland

472   Waters of the United States. Am. Fish. Soc., Bethesda, MD.

473   Garcia, C., Rolan-Alvarez, E. & Sanchez, L. 1992: Alarm reaction and alert state in *Gambusia*

474   *affinis* (Pisces, Poeciliidae) in response to chemical stimuli from injured conspecifics. J.

475   Ethol. **10,** 41-46.

476   Greenberg, R. 2003: The role of Neophobia and Neophilia in the Development of Innovative

477   Behaviour of Birds. In: Animal Innovation (Reader, S. M. & Laland, K. N., eds). Oxford

478   University Press, Oxford, pp. 175–196.

479   Gurevitch, J. & Padilla, D. 2004: Are invasive species a major cause of extinctions? Trends

480   Ecol. Evol. **19,** 470-474.

481   Helfman, G. S. 1989: Threat-sensitive predator avoidance in damselfish-trumpetfish interactions.

482   Behav. Ecol. Sociobiol. **24,** 47-58.

483   Jones, M. E., Smith, G. C. & Jones, S. M. 2004: Is anti-predator behavior in Tasmanian eastern

484   quolls (*Dasyurus viverrinus*) effective against introduced predators? Anim. Conserv. **7,**

485   155-160.

486   Kats, L. B. & Ferrer, R. P. 2003: Alien predators and amphibian declines: review of two decades

487   of science and the transition to conservation. Divers. and Distrib. **9,** 99-110.

488 Kelley, J. L. & Magurran, A. E. 2003: Effects of relaxed predation pressure on visual predator

489   recognition in the guppy. Behav. Ecol. Sociobiol. **54,** 225.232.

490 Kery, M. & Hatfield, J. S. 2003: Normality of raw data in general linear models: the most

491   widespread myth in statistics. Bull. Ecol. Soc. Am. **84,** 92-94.

492 Lima, S. L. 2002: Putting predators back into behavioural predator-prey interactions.

493   Trends Ecol. Evol. **17**, 70-75.

494 Lima, S. L. & Dill, L. M. 1990: Behavior decisions made under the risk of predation: a review

495   and prospectus. Can. J. Zool. **68,** 619-640.

496 Lima, S. L. & Steury, T. D. 2005: Perception of Predation Risk: the Foundation of Nonlethal

497   Predator-Prey Interactions. In: Ecology of Predator-Prey Interactions (Barbosa, P. &

498   Castellanos, I., eds). Oxford University Press, New York, NY, pp. 166-188.

499 Loftus, W. F. & Kushlan, J. A. 1987: Freshwater fishes of southern Florida. Bull.

500   Fla. State Mus. Biol. Sci. **31,** 147–344.

501 Loftus, W. F., Trexler J. C., Dunker, K., Liston, S. E. & Rehage, J. S. 2006: Introduced

502   fishes in short-hydroperiod wetlands: evaluation of sampling, status, and potential effects.

503   Final Report from USGS to Everglades NP for Agreement # CESI IA F5284-04-0039.

504   U.S. Geological Survey, Homestead, FL.

505 Magurran, A. E. & Seghers, B. H. 1990: Population differences in predator recognition and

506   attack cone avoidance in the guppy *Poecilia reticulata*. Anim. Behav. **40,** 443-432.

507 Martin, P. & Bateson, P. 2007: Measuring Behavior: an Introductory Guide, 3rd edn. Cambridge

508   University Press, Cambridge.

509 Mirza, R. S., Ferrari, M. C. O., Kiesecker, J. M. & Chivers, D. P. 2006: Responses of American

510   toad tadpoles to predation cues: behavioural response thresholds, threat-sensitivity and

511   acquired predation recognition. Behaviour **143,** 877-889.

512   Nannini, M. A. & Belk, M. C. 2006. Antipredator responses of two native stream fishes

513   to an introduced predator: does similarity in morphology predict similarity in behavioural

514   response? Ecol of FW Fish **15**, 453-463.

515   Ogutu-Ohwayo, R. 1990: The decline of native fishes of Lakes Victoria and Kioga (East

516   Africa) and the impact of introduced species, especially the Nile perch, *Lates niloticus*,

517   and the Nile tilapia, *Oreochromis niloticus*. Env. Biol. Fish. **27,** 81-96.

518   Pitcher, T. J., Green, D. A. & Magurran, A. E. 1986: Dicing with death: predator inspection

519   behaviour in minnow shoals. J. Fish Biol. **28,** 439-448.

520   Pitcher, T. J. & Parrish, J. K. 1993: Functions of shoaling behaviour in teleosts. In: Behaviour of

521   Teleost Fishes (Pitcher, T. J., ed). Chapman & Hall, London, pp. 363–439.

522   Preisser, E. L., Orrock, J. L. & Schmitz, O. J. 2007: Predator hunting mode and habitat domain

523   alters non-consumptive effects in predator-prey interactions. Ecology **88,** 2744-2751.

524   Rehage, J. S. & Trexler, J. C. 2006: Assessing the net effect of anthropogenic disturbance on

525   aquatic communities in wetlands: community structure relative to distance from canals.

526   Hydrobiologia **569,** 359-373.

527   Salo, P., Korpimäki, E., Banks, P. B., Nordström, M. & Dickman, C. R. 2007: Alien predators

528   are more dangerous than native predators to prey populations. Proc. R. Soc. Lond., Ser.

529   B, Biol. Sci. **274,** 1237-1243.

530   Schmitz, O. J. 2007: Predator diversity and trophic interactions. Ecology **88,** 2415-2426.

531   Schofield P., Loftus W.F. & Brown M. 2007: Hypoxia tolerance of two centrarchid sunfishes

532   and an introduced cichlid from karstic Everglades wetlands of southern Florida, U.S.A. J.

533   Fish Biol., **41:** 87-99.

534   Shafland, P. L., Gestring, K. B. & Stanford, M. S. 2008: Florida's exotic freshwater fishes. Fla.

535   Sci. **71**: 220-245.

536   Sih, A., Englund, G. & Wooster, D. 1998: Emergent impacts of multiple predators on prey.

537   Trends Ecol. Evol. **13,** 350-355.

538   Sih, A. 1992: Prey uncertainty and the balancing of antipredator and feeding needs. Am. Nat.

539   **139,** 1052-1069.

540   Sih, A. 2005: Predator-prey space use as an emergent outcome of a behavioral response

541   race. In: Ecology of Predator-Prey Interactions (Barbosa, P. & Castellanos, I., eds).

542   Oxford University Press, New York, NY, pp. 240-255.

543   Smith, G. R., Boyd, A., Der, C. B. & Winter, K. E. 2008: Behavioral response of American toad

544   and bullfrog tadpoles to the presence of cues from the invasive fish, *Gambusia affinis.*

545   Biol. Invasions **10,** 743-748.

546   Smith, M. E. & Belk, M. C. 2001: Risk assessment in western mosquitofish (*Gambusia affinis*):

547   do multiple cues have additive effects? Behav. Ecol. Sociobiol. **51,** 101-107.

548   Trexler, J. C., Loftus, W. F., Jordan, F., Lorenz, J. J., Chick J. H. & Kobza, R. M. 2001:

549   Empirical assessment of fish introductions in a subtropical wetland: an evaluation of

550   contrasting views. Biol. Invasions **2,** 265–277.

551   Trexler, J. C., Loftus, W. F. & Perry, S. 2005: Hydrological limitation of Everglades fish

552   communities by a twenty-five year intervention study. Oecologia **145,** 140-142.

553   Wellborn, G. A., Skelly, D. K. & Werner, E. E. 1996: Mechanisms creating community structure

554   across a freshwater habitat gradient. Annu. Rev. Ecol. Syst. **7,** 337-363.

555   Wisenden, B. D. & Harter, K. R. 2001: Motion, not shape, facilitates association of

556   predation risk with novel objects by fathead minnows (*Pimephales promelas*). Ethology

557   **107,** 357-364.

558   Wisenden, B. D. & Chivers, D. P. 2006: The role of public chemical information in antipredator

559   behaviour. In: Fish Communication (Ladich, F., Collins, S. P., Moller, P. & Kapoor, B.

560   G., eds). Science Publisher, Enfield, NH, pp. 259-278.

561

Figure legends

563

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 ± 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 ± 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 ± 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 ± SE.

586

587

588

589

590

591

592

593

594

595

596

597

598

599

Table 1: Result of ANOVAs and MANOVA's (p-values and R^2) testing predation treatment, population, interaction, **season (or block),** and predator pair effects. MANOVA1 contains the 5 variables measured in all treatments, and MANOVA2 contains the 5 variables measured in the 3 predator treatments only.

603

Variables	R^2	P values for effects				
		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**
Prey						
Activity	0.21	0.4318	**0.0380**	0.4814	0.0932	0.5437
Vertical distribution	0.63	**0.0001**	0.1262	0.8297	0.5850	0.0992
Use of habitat structure	0.18	0.0571	0.8561	0.7119	0.2182	0.7063
Shoaling behavior	0.64	**0.0001**	0.4871	0.3419	**0.0001**	0.5835
Predator inspections	0.32	**0.0112**	0.7997	0.3269	0.1741	0.1380
Mortality	0.64	**0.0001**	0.5741	0.9609	0.9655	0.4663

615							
616	*Predators*						
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

621