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1 **Predation risk reduces a female preference for heterospecific males in the**
2 **green swordtail**

3

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13 Running title: Predators influence female heterospecific preferences

14

15 **Abstract**

16 The presence of a predator can result in the alteration, loss, or reversal of mate preference. Under
17 predation risk, females may change their initial preference for conspicuous males, favouring less
18 flashy males, to reduce the risk of being detected by predators. Previous studies on predator-
19 induced plasticity in mate preferences have given females a choice between more and less
20 conspicuous conspecific males. However, in species that naturally hybridize, it is also possible
21 that females might choose an inconspicuous heterospecific male over a conspicuous conspecific
22 male under predation risk. Our study addresses this question using the green swordtail
23 (*Xiphophorus helleri*) and the southern platyfish (*X. maculatus*), which are sympatric in the wild.
24 We hypothesized that *X. helleri* females would prefer the sworded conspecific males in the
25 absence of a predator but favour the less conspicuous, swordless, heterospecific males in the
26 presence of a predator. Contrary to this, females associated more with the heterospecific male
27 than the conspecific male in the control treatment, and they were non-choosy in the predator
28 treatment. This might reflect that females were attracted to the novel male phenotype in the
29 control treatment but became more neophobic after predator exposure. Regardless of the
30 underlying mechanism, our results suggest that predation pressure may affect female preferences
31 for conspecific versus heterospecific males. We also found striking within-population, between-
32 individual variation in behavioural plasticity; females differed in the strength and direction of
33 their preferences, as well as in the extent to which they altered their preferences in response to
34 changes in perceived predation risk. Such variation in female preferences for heterospecific
35 males might lead to temporal and spatial variation in hybridization rates in the wild.

36

37 Keywords: behavioural reaction norms, mate choice, neophilia, phenotypic plasticity, predation
38 risk, *Xiphophorus*

39

40 **Introduction**

41 Sexual selection through mate choice is an important evolutionary force contributing to
42 morphological and behavioural diversity within and among species (Andersson 1994). Female
43 preference for conspicuous ornaments and intense courtship displays has been well documented
44 in birds (e.g. Andersson 1982), fishes (e.g. Basolo 1990a), reptiles (e.g. Sigmund 1983),
45 amphibians (e.g. Malacarne & Cortassa 1983), and insects (e.g. Wilkinson & Reillo 1994). This
46 behaviour may be adaptive for females, due to a sexy sons or good genes benefit if these traits
47 are honest signals of heritable male quality (Weatherhead & Raleigh 1979). However, there is
48 increasing evidence for among-population, within-population, as well as within-individual
49 variation in mate preferences (Endler & Houde 1995; Jennions & Petrie 1997; Brooks & Endler
50 2001; Kodric-Brown & Nicoletto 2001; Coleman *et al.* 2004; Lynch *et al.* 2005; Bailey & Zuk
51 2008; Chaine & Lyon 2008; Pilakouta & Alonzo 2014). Thus, a particular male may not be
52 preferred by all females, and an individual female may not always prefer the same type of male.
53 Such variation should not be surprising, given that mate choice is influenced by several factors,
54 including individual experience and condition, inherent perceptual biases, and the environmental
55 conditions under which a mating decision is made (Mays & Hill 2004; Hunt *et al.* 2005; Walling
56 *et al.* 2008; Amcoff *et al.* 2013; Ryan & Cummings 2013).

57 Predator presence, in particular, can play an important role in mate choice, resulting in the
58 alteration, loss, or reversal of mate preference. For example, we might expect females to become
59 less choosy in the presence of predators, because of higher mate searching costs (Crowley *et al.*

60 1991). In cases where association with a certain male phenotype itself incurs a potential cost to
61 the female, females may instead change the direction of their preference (Pilakouta & Alonzo
62 2014). Since conspicuous males are more likely to be attacked and thus experience a higher
63 mortality risk (Godin & McDonough 2003; Hernandez-Jimenez & Rios-Cardenas 2012), females
64 associating with these males may also incur an increased risk of predation (Pocklington & Dill
65 1995). Additionally, if the male trait is heritable, male offspring from such matings will also be
66 conspicuous and will potentially experience a higher mortality rate.

67 Predator-induced plasticity in mate preferences has received little attention, with a focus to
68 date on fishes and insects. For example, female sand gobies (*Pomatoschistus minutus*; Forsgren
69 1992) and male pipefishes (*Syngnathus typhle*; Berglund 1993) become less choosy in the
70 presence of predators. Female crickets (*Gryllus integer*) typically prefer long-bout male calls but
71 are more likely to mate with males with less conspicuous, short-bout calls when the risk of
72 predation increases (Hedrick & Dill 1993). In guppies (*Poecilia reticulata*), female bias for
73 brightly coloured and larger males decreases after predator exposure (Godin & Briggs 1996;
74 Gong & Gibson 1996). These studies demonstrate a plastic female preference for inconspicuous
75 males under predation risk, but they all gave females a choice between different phenotypes of
76 conspecific males. It is still unclear whether females might choose an inconspicuous
77 heterospecific male over a conspicuous conspecific male after exposure to a direct cue of
78 predation risk. Such predator-induced mate preferences for heterospecifics might occur in
79 sympatric species that naturally hybridize. If predator presence indeed influences mate
80 preferences for heterospecifics, it will affect when and how often hybridization occurs and thus
81 have an effect on species maintenance.

82 Our study addresses this question using the green swordtail (*Xiphophorus helleri*) and the
83 southern platyfish (*X. maculatus*). Heterospecific mating preferences in the *Xiphophorus* genus
84 (Family Poeciliidae) have been studied extensively (e.g. Ryan & Wagner 1987; Hankison &
85 Morris 2002; Hankison & Morris 2003; Wong & Rosenthal 2006; McLennan & Ryan 2008;
86 Willis *et al.* 2011; Willis *et al.* 2012), making this group particularly suitable for our study. This
87 genus comprises two swordtail clades and two platyfish clades (Basolo 1995). In most swordtail
88 species, males have 'swords,' which are ventral caudal fin extensions, whereas none of the
89 platyfish species have this secondary sexual trait. Ryan and Wagner (1987) showed that *X.*
90 *pygmaeus* females prefer larger, sworded heterospecific males (*X. nigrensis*) to smaller
91 conspecific males without swords. Female *X. pygmaeus* also prefer the larger *X. cortezi* males to
92 conspecific males (Hankison & Morris 2002). Lastly, female *X. maculatus* and *X. variatus* prefer
93 heterospecific sworded males to conspecific swordless males (Basolo 1990b; Haines & Gould
94 1994). These heterospecific mating preferences are consistent with recent studies suggesting that
95 hybridisation in the genus *Xiphophorus* is not only possible but probable: hybridisation has been
96 very widespread in the evolutionary history of this group, with many of these species being
97 interfertile (Hyoun Kang *et al.* 2013; Cui *et al.* 2013). It is worth noting that this earlier work on
98 heterospecific mating preferences focused on females choosing between ornamented
99 heterospecifics and dull or less ornamented conspecifics, whereas we are investigating female
100 preferences for ornamented conspecifics versus inconspicuous heterospecifics.

101 For our study, we chose to use female *X. helleri* because (i) preference for conspicuous males
102 with long swords has been well established in this species (e.g. Basolo 1990a, 1998; Trainor &
103 Basolo 2000) and (ii) females reverse the direction of their preference, favouring less
104 conspicuous males with short swords after predator exposure (Johnson & Basolo 2003; Pilakouta

105 & Alonzo 2014). We chose *X. maculatus* as the less conspicuous, swordless heterospecific
106 species, because *X. helleri* and *X. maculatus* occur sympatrically throughout most of their range
107 and can produce viable offspring under laboratory conditions (e.g. Meyer *et al.* 2006; Scharl *et*
108 *al.* 2013). A recent study also reported that there is strong evidence of historical hybridization
109 between *X. helleri* and *X. maculatus* and that it is possible that gene flow between these species
110 is ongoing (Schumer *et al.* 2013).

111 We conducted dichotomous choice tests to evaluate the mate preferences of *X. helleri*
112 females for conspicuous *X. helleri* males and the less conspicuous *X. maculatus* males in the
113 presence and absence of a predation threat. Our hypothesis was that females would prefer
114 conspecific males in the control (no predator) treatment but favour heterospecific males in the
115 predator treatment because of the risk of associating with the conspicuous conspecific under
116 predation threat. It is important to better understand how direct cues of predation risk may
117 influence the likelihood of interspecific mating and thus play a role in hybridisation rates in
118 natural populations. If a predator encounter causes females to choose an inconspicuous
119 heterospecific male over a conspicuous conspecific male, then predator abundance could
120 influence the rate of hybridisation and gene flow between species.

121

122 **Methods**

123 *Study Animals*

124 All male and female *X. helleri* used in this study were first-generation descendants of wild-
125 caught swordtails collected from Princess Margaret Creek in Belize by a private breeder. *X.*
126 *maculatus* and several species of piscivorous fishes have been observed in this creek, including
127 the Neotropical cichlid *Petenia splendida* (Basolo & Wagner 2004). The individuals used in this
128 study came from the same population as those in Pilakouta & Alonzo (2014), where we showed

129 that females prefer long-sworded conspecific males in the absence of a predator but prefer short-
130 sworded conspecific males in the presence of a predator.

131 Females were between 12 and 18 months old. All females had previously interacted with
132 conspecific males and were non-virgins, but they were not gravid at the time of the experiment.
133 We chose to use non-virgin females, because they tend to be more responsive when they
134 encounter a male (Basolo 1990). These females had no previous experience with predators or *X.*
135 *maculatus* males before this experiment. Male *X. maculatus* were acquired at 12 months old from
136 Texas State University's *Xiphophorus* Genetic Stock Center. They were reared from the Belize
137 Play II strain, with their ancestors originating from Kate's Lagoon in Belize.

138 The fish were reared at 22-24°C and a pH of 7.5-8 on a 12:12 h light:dark photoperiod. They
139 were fed daily between 10 am and 11 am. Their diet consisted of commercial micropellets and
140 frozen bloodworms on alternate days. Females were kept in groups of three to five individuals in
141 45-L and 90-L tanks. Males were kept individually in 10-L tanks. All tanks included a sponge
142 filter and artificial plants for cover. Approximately 30-40% of the water was changed weekly.

143

144 *Video Playback Design*

145 For the mate choice trials, we used videos of non-courting males actively swimming in a tank (as
146 in Trainor & Basolo 2000; Pilakouta & Alonzo 2014). We recorded three *X. helleri* and three *X.*
147 *maculatus* males individually, using a digital camera (Panasonic Lumix DMC-TS10) on a
148 Sunpak tripod. All of the males we used were between 12 and 18 months old. For each mate
149 choice trial, we randomly selected one of the *X. helleri* and one of the *X. maculatus* videos, such
150 that there were nine possible combinations of conspecific and heterospecific videos. For the
151 control treatment, we recorded a video of a tank containing artificial plants and a bubbler but no

152 fish. For the predator treatment, we used a video of *P. splendida* swimming around in a tank with
153 some plants (as in Pilakouta & Alonzo 2014). *P. splendida* is a common predator for swordtails
154 and occurs sympatrically with both species (Basolo & Wagner 2004). All videos were adjusted
155 such that the videos being displayed reflected the fish's true size. While there was no significant
156 variation in male body size within species, there was inevitably a difference between species, as
157 *Xiphophorus maculatus* males are naturally smaller than *X. helleri*.

158 Using video playback allowed us to repeatedly present multiple females with the same males.
159 This method eliminates confounding factors, such as temporal variation in male motivational
160 state, display rate, and appearance (Kodric-Brown & Nicoletto 1997), making any observed
161 differences among individuals and between treatments more robust. Video playback has been
162 used successfully in previous studies on mating behaviour in this species (e.g. Trainor & Basolo
163 2000; Basolo & Trainor 2002; Johnson & Basolo 2003; Pilakouta & Alonzo 2014).

164 The experimental setup consisted of a 40-L tank (61 × 23 × 33 cm) with three adjacent flat-
165 screen monitors (Fig. 1). The two side monitors (Dell 2007WFPb) projected the *X. helleri* and *X.*
166 *maculatus* male videos, and the rear monitor (Lenovo 9227-AC1) projected the control or
167 predator video. We marked two vertical lines on the front of the tank to divide it into three equal-
168 sized compartments. The apparatus was illuminated by fluorescent lighting situated
169 approximately 1 m above the experimental set up. We recorded all mate choice trials using a
170 digital camera on a tripod placed about 1 m in front of the experimental setup.

171

172 *Experimental Protocol*

173 Mate choice trials were conducted between 1 pm and 5 pm. Water temperature and other
174 parameters, including pH, KH (carbonate hardness), and GH (general hardness), in the

175 experimental tank were matched to those in the holding tanks. For each trial, we placed a single
176 female in the tank and let her acclimate for 20 minutes. Nothing was displayed on the monitors
177 during this period. We then presented the female with a looped 5-minute sequence of either the
178 predator video or control video on the rear monitor. After turning off the rear monitor, videos of
179 a conspecific male and a heterospecific male were displayed on the two side monitors. We
180 randomized which of the two males videos (conspecific/heterospecific) was displayed on the left
181 versus the right monitor at the start of each mate choice trial. To ensure that the female had a fair
182 choice between the two males, she was given time to investigate the two sides of the tank before
183 we started recording behavioural data. The 20-minute mate choice trial started if the female had
184 inspected both sides and returned to the neutral zone within 2 minutes (as in Johnson & Basolo
185 2003). Any females that showed a bias in this initial period ($n=1$) were excluded from the mate
186 choice trials. To avoid any potential side biases, male videos were switched between the two side
187 monitors halfway through the trial. We used the same conspecific and heterospecific male videos
188 for the whole duration of a given mate choice trial.

189 During each trial, we recorded the following variables: association time with each male
190 (defined below), time spent directly interacting with each male (e.g. gliding: swimming in a tight
191 circle using only the pectoral fins with the genital pore exposed; Liley, 1966), and the number of
192 times the female crossed into the neutral zone from one of the two sides. Association time was
193 the amount of time spent on each of the two sides, which is a good indicator of female mate
194 choice in this species (Trainor & Basolo 2000; Walling *et al.* 2010). Direct interaction time was a
195 subset of association time.

196 We used a paired design for this experiment, meaning that we observed each of 20 females in
197 both the control and predator treatments, with 7 to 14 days between the two trials. Females were

198 presented with the same conspecific and heterospecific male videos in both treatments, and the
199 order of the two treatments was determined randomly for each female. This design allowed us to
200 assess the effect of predation risk on mate choice behaviour on an individual level.

201

202 *Data Analysis*

203 To examine differences in female mate preferences within and between treatments, we used
204 general linear mixed models (LMM). We ran two separate models with association time and
205 direct interaction time as the response variables. Both models included the following fixed
206 effects: treatment (control or predator), the species of the male (conspecific or heterospecific),
207 and the interaction between these two factors. A statistically significant interaction would
208 indicate a change in the strength and/or direction of female preference between treatments. We
209 also assigned female identity, conspecific male identity, and heterospecific male identity as
210 random effects. These analyses were ran in R version 3.2.0, using the 'lmer' function in the 'lme4'
211 package (Bates *et al.* 2013). All models were fitted using maximum likelihood methods.

212 To compare individual-level female preferences within and between treatments, we
213 calculated a measure of association bias, by subtracting the total time each female spent on the
214 side of the tank closer to the heterospecific male from the total time spent on the side of the tank
215 closer to the conspecific male. These differences were calculated separately for each treatment.
216 Large positive values suggested a strong preference for the conspecific male, large negative
217 values suggested a strong preference for the heterospecific male, and values close to zero
218 suggested the female was not choosy.

219 To determine whether females changed their level of activity in response to predator
220 exposure, we used a two-tailed paired t-test to compare the number of times each female crossed

221 into the centre of the tank between the two treatments. A two-tailed paired t-test was also used to
222 compare the amount of time spent in the neutral zone in the two treatments. This was done to
223 look for between-treatment differences in the total amount of time females spent associating with
224 the two males, as a measure of sexual responsiveness.

225

226 *Ethical Note*

227 The protocols used in this experiment were conducted in accordance with the Association for the
228 Study of Animal Behaviour guidelines. All procedures were approved by the Animal Care and
229 Use Committee of Yale University (IACUC protocol #2011-10908).

230

231 **Results**

232 Treatment (control or predator) and the species of the male (conspecific or heterospecific) did
233 not have an overall effect on female association time (LMM *Treatment*: LR $\chi^2=0.36$, $P=0.55$;
234 *Male species*: LR $\chi^2=2.73$, $P=0.10$). However, there was a statistically significant effect of the
235 interaction between treatment and the species of the male on female association time (LMM: LR
236 $\chi^2=7.37$, $P=0.007$). This interaction reflects that females spent more time associating with the
237 heterospecific male in the control treatment, but they did not show a preference for either male
238 after being exposed to the predator video (Fig. 2). The amount of time a female spent directly
239 interacting with a male did not depend on the species of the male (LMM: LR $\chi^2=0.30$, $P=0.58$),
240 the treatment (LMM: LR $\chi^2=0.81$, $P=0.37$), or the interaction between these two factors (LMM:
241 LR $\chi^2=0.23$, $P=0.63$). None of the random effects included in our models were statistically
242 significant.

243 Female activity was overall unaffected by exposure to the predator video. The total amount
244 of time females spent in the centre of the tank did not differ between treatments (paired t test:
245 $t=1.2$, $P=0.24$). Moreover, the number of times females moved from the sides into the centre of
246 the tank was the same in the control and predator treatments (paired t test: $t=1.3$, $P=0.20$).

247 On average, females seemed to spend more time associating with the heterospecific male in
248 the control treatment but showed no strong preference between the heterospecific and
249 conspecific male in the predator treatment (Fig. 2). Nevertheless, when looking at preferences on
250 an individual level, there was substantial variation among females in terms of which male they
251 preferred within each treatment, as well as the magnitude and direction in which their preference
252 changed between treatments (Fig. 3).

253

254 **Discussion**

255 *X. helleri* females preferred the less conspicuous *X. maculatus* males over the sworded *X. helleri*
256 males in the control treatment. This preference did not persist after predator exposure: on
257 average, females were non-choosy in the predator treatment (Fig. 2). We also documented
258 striking between-individual variation in the degree of behavioural plasticity in response to
259 predator exposure, using females from a single population. Within each of the two treatments,
260 different females preferred different males and also varied in the strength of their preference,
261 with some females being non-choosy. Between treatments, there was variation in both the degree
262 and direction of plasticity in female preferences, as indicated by the slopes of the behavioural
263 reaction norms (Fig. 3). Overall, we find that predation risk causes a switch from preferring
264 heterospecific males in the absence of predators to exhibiting no preference between sworded
265 conspecifics and swordless heterospecifics in the presence of predators. Yet, we also find

266 substantial variation in the strength of heterospecific mate preference among females in the
267 absence of a predator and in the extent of the predator-induced change in preference.

268 'Foreign-mate preferences', such as the one reported here, are not uncommon and have been
269 observed in various taxa, including the jumping spider *Habronattus pugillis* (Hebets & Maddison
270 2005) and the Blue Waxbill, *Uraeginthus angolensis* (Collins & Luddem 2002). In fact,
271 preferences for heterospecifics may partly explain the finding that a large proportion of the
272 genomes of most *Xiphophorus* species is derived from hybridisation (Cui *et al.* 2013). However,
273 our results are surprising because females typically prefer conspicuous males and avoid them
274 when there is a high risk of predation (e.g. Johnson & Basolo 2003; Pilakouta & Alonzo 2014).
275 We thus expected *X. helleri* females to prefer the swordless heterospecific male over the sworded
276 conspecific male in the predator treatment, not in the control treatment.

277 Instead, we found that female preference for the heterospecific male was reduced after
278 exposure to the predator video. Even though this pattern was unexpected, it is important to note
279 that we used the same methodology and individuals from the same population as in Pilakouta
280 and Alonzo (2014). In that study, females expressed preferences that were consistent with earlier
281 work (Gong & Gibson 1996; Johnson & Basolo 2003): females preferred conspicuous
282 conspecifics in the absence of a predator and less conspicuous conspecifics in the presence of a
283 predator (Pilakouta & Alonzo 2014). Therefore, our findings are unlikely to be due to the
284 experimental setup or the conditions of the study in general.

285 One potential explanation for the heterospecific preference in the control treatment is that
286 females were showing a bias for the novel male phenotype. Neophilia, the attraction to novel
287 stimuli, is one of the hypotheses proposed to explain the maintenance of male colour
288 polymorphism and female preference for novel male colour patterns in guppies, *Poecilia*

289 *reticulata* (Hughes *et al.* 1999; Hughes *et al.* 2013), which are in the same family (Poeciliidae) as
290 *Xiphophorus*. In addition, Verzijden *et al.* (2012) showed that although female familiarity with a
291 certain male phenotype can lead to a positive preference bias for that phenotype, it can also
292 induce aversion as in the case of the swordtail *X. malinche*. In zebra finches (*Taeniopygia*
293 *guttata*), variation in neophilia among females in the context of approach to novel objects
294 reflects variation in reproductive strategies (Schielzeth *et al.* 2011). Lastly, in some cases, novel
295 phenotypes arising from hybridization may be more attractive to females of the parental species
296 (Rosenthal 2013).

297 After exposure to the predator video, there was a reduction in the females' preference for the
298 heterospecific male. We suggest that females might have become more neophobic because of the
299 risk of predation. There is evidence that neophobia (i.e., the generalized avoidance response to
300 novel stimuli) is phenotypically plastic and that predation risk plays a role in this plasticity: one
301 way to avoid predation is to be frightened of anything that is new (Brown *et al.* 2013). In our
302 experiment, some females may have changed their preference due to risk-induced neophobia,
303 thus favouring the conspecific because of familiarity.

304 Regardless of its underlying cause, the observed predator-induced decline in female
305 preferences for heterospecific males could have important implications for ecological and
306 evolutionary dynamics. Accumulating evidence suggests that environmental disturbances can
307 break down hybridization barriers, potentially leading to introgression into parental lineages, the
308 merging of gene pools, or hybrid speciation (Rosenthal 2013). Thus, if the trend we have
309 documented accurately represents a pattern occurring in natural populations, predation pressure
310 may affect the likelihood of interspecific mating and consequently play a role in hybridisation
311 rates and the maintenance of species.

312 Lastly, when looking at mate preferences on an individual level, there was substantial
313 among-female variation in association bias in the two treatments (Fig. 2). Within-population
314 variation in behavioural plasticity among individuals has been a topic of increasing interest in
315 recent years (Jennions & Petrie 1997; Wagner 1998; Wolf *et al.* 2008; Coppens *et al.* 2010;
316 Dingemanse *et al.* 2010; Westneat *et al.* 2011; Mathot *et al.* 2011; Mathot *et al.* 2012;
317 Dingemanse & Wolf 2013; Han & Brooks 2013), but variation in mate preference plasticity has
318 been largely ignored. We are not aware of any other studies reporting individual behavioural
319 reaction norms in the context of mating preferences across environments (e.g., low versus high
320 risk of predation). Here, we show that individuals from the same population differ in their
321 preference for heterospecifics versus conspecifics in the absence of a predator, as well as in the
322 extent to which they alter their preferences in response to changes in perceived predation risk.
323 Variation in behavioural plasticity can result from additive and interactive effects of past
324 experiences, current individual condition, and genetics (Dingemanse & Wolf 2013). In our study,
325 all individuals were exposed to similar environmental conditions in the laboratory, so it is more
326 likely that the variation we observed was due to intrinsic differences among females. If variation
327 in propensity to hybridize is heritable, interspecific matings will involve a nonrandom set of
328 individuals in the population (Schielzeth *et al.* 2009), which can have important consequences
329 for the evolutionary trajectory of hybrid lineages (Rosenthal 2013).

330 Our findings raise interesting questions for future studies to address, as it is still unclear what
331 the proximate and ultimate causes of the among-individual variation in mate preference plasticity
332 are, whether this variation is adaptive, and how selection acts to maintain it. The presence or
333 absence of this variation, as well as whether or not it is heritable, can have significant
334 implications for ecological and evolutionary dynamics (Dingemanse & Wolf 2013). Another

335 suggestion for future research is to investigate whether predation risk reduces the preference for
336 conspicuous heterospecific males in species in which females have been shown to prefer
337 sworded heterospecifics, such as *X. pygmaeus* (Ryan & Wagner 1987), *X. maculatus* (Basolo
338 1990), and *X. variatus* (Haines & Gould 1994). If that is indeed the case, the frequency of
339 encounters between females and predators may contribute to variation in the frequency of
340 interspecific mating between populations with different predator abundances.

341 In summary, it has been well established that predators can have important nonconsumptive
342 effects on prey populations by causing changes in prey behaviour (Lima & Dill 1990; Peckarsky
343 *et al.* 2008; Schmitz *et al.* 2008), such as mate choice for conspecific males (e.g. Hedrick & Dill
344 1993; Godin & Briggs 1996; Johnson & Basolo 2003; Pilakouta & Alonzo 2014). Here, we
345 provide novel insights into how predation risk may also influence female preference for
346 heterospecific males. Our findings suggest that predation pressure may affect the likelihood of
347 interspecific mating, which could have important implications for ecological and evolutionary
348 dynamics. Moreover, the observed variation among females in their mating preference for
349 conspecifics versus heterospecifics and in how they change their preferences in response to
350 predation risk could lead to temporal and spatial differences in hybridization rates in natural
351 populations.

352

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357

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510 **Figure Legends**

511

512 **Fig. 1** Experimental set up for dichotomous choice test, consisting of three monitors and a 40-L
513 tank. The rear monitor displayed the control or predator video, which was followed by the
514 conspecific and heterospecific male videos on the two side monitors.

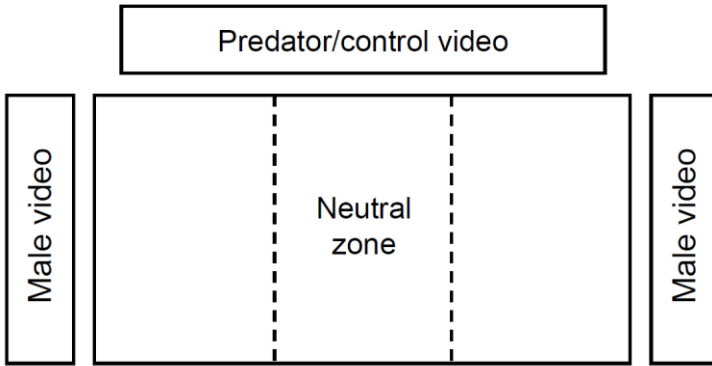
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516 **Fig. 2** Amount of time (mean \pm SE) females spent associating with the conspecific (grey) and
517 heterospecific (white) male in the control and predator treatment during a 30-min mate choice
518 trial. Females spent more time associating with heterospecific males in the control treatment, but
519 after predator exposure, they associated with conspecific and heterospecific males equally.

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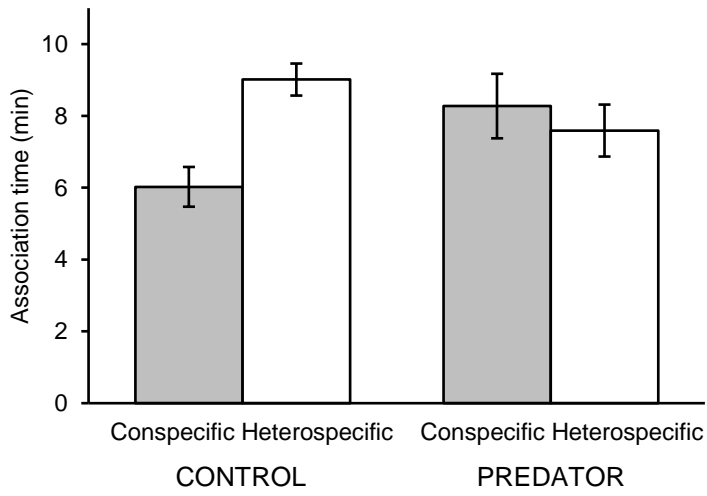
521 **Fig. 3** Behavioural reactions norms for individual females, where the environmental gradient is
522 presence or absence of a predation threat, and the response variable (behavioural trait) is
523 association bias. Positive values suggest a preference for the conspecific male, and negative
524 values suggest a preference for the heterospecific male. The majority of females spent less time
525 on the side closest to the heterospecific in the predator treatment than they did in the control
526 treatment (black). However, some females associated more with the heterospecific in the
527 predator treatment than they did in the control treatment (grey).

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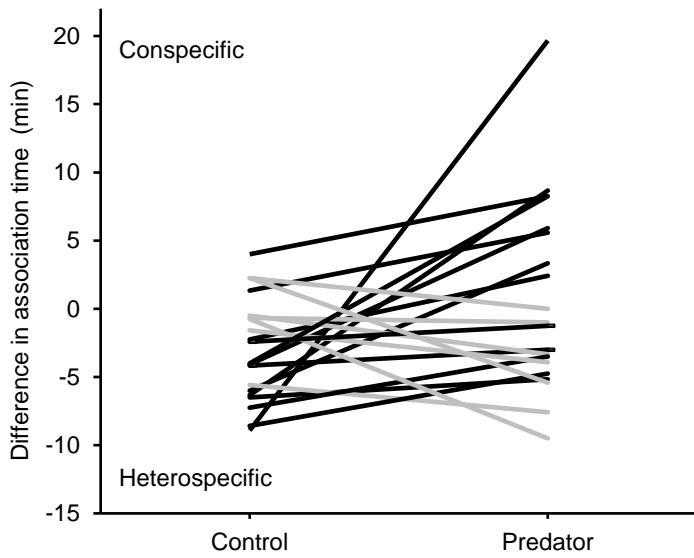
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