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1 **Effects of chronic and acute predation risk on sexual ornamentation**
2 **and mating preferences**

3 Running title: Effects of predation risk on sexual selection

4 Abstract

5 Phenotypic plasticity is wide-spread in animals, but how plastic responses to predation threat affect
6 traits under sexual selection and influence mating preferences is not well understood. Here, we
7 examined how chronic predation risk during development and acute predation risk during mate choice
8 affect the expression of male secondary sexual traits and female mating preference in the three-spined
9 stickleback, *Gasterosteus aculeatus*. Males reared under chronic predation risk developed less intense
10 red breeding colouration but showed higher courtship activity than males that grew up in a predator-
11 free environment. Acute predation risk during mate choice did not influence male behaviour or
12 ornamentation. Predation risk experienced during development did not affect female mating
13 preferences, while acute predator presence induced a switch in preferences for male courtship activity.
14 Male body size and eye colouration influenced the intensity of female mating preferences, while the
15 trait changing most in response to predation risk during development (red colouration) had no
16 significant impact. The observed interplay between developmental plasticity in male ornamental traits
17 and environment-dependent female mating preferences may lead to dynamic processes altering the
18 strength and direction of sexual selection depending on both the chronic and acute risk of predation.
19 These processes may contribute to the maintenance of within- and among-population variation in
20 secondary sexual traits, and may, ultimately, facilitate speciation.

21 Introduction

22 Natural and sexual selection are significant drivers of animal phenotypic diversity (Darwin, 1859, 1871),
23 but they often operate in opposite directions (Dunn et al., 2015; Kotiaho et al., 2001). In many species,
24 sexual selection leads to the evolution of conspicuous ornaments or courtship behaviour in males,
25 serving to attract females or signal dominance towards rivals (Andersson, 1994). Choosy females
26 preferring males based on the extent of their ornamentation are assumed to obtain direct or indirect
27 fitness benefits (Kokko et al., 2003). Because highly ornamented males achieve higher reproductive
28 success, directional sexual selection is expected to favour the spread of genes underlying conspicuous
29 phenotypes, and decreasing genetic variability for these traits within populations (Falconer and
30 Mackay, 1996; Fisher, 1930). However, the emergence of genes and alleles encoding conspicuous
31 ornaments can be constrained by natural selection via lowered survival probabilities (Ercit and
32 Gwynne, 2015; Kotiaho et al., 2001; Woods Jr et al., 2007), and by development and maintenance costs
33 of striking phenotypes (Roberts et al., 2004). Both will lead to a decreased residual reproductive value
34 of highly ornamented individuals. Such varying selection pressures resulting from the interplay
35 between natural and sexual selection may contribute to the maintenance of genetic variation (Chaine
36 and Lyon, 2008; Cornwallis and Uller, 2010; Kuijper et al., 2012; Lewontin, 1974; Robinson et al., 2012),
37 and may also drive diversification processes (Maan and Seehausen, 2011).

38 Understanding the causes underlying phenotypic variation in sexual ornamentation and mating
39 behaviour within a population is crucial for a comprehensive understanding of the evolution and
40 diversification of secondary sexual traits (Foster et al., 2015a). One mechanism contributing to
41 variation is phenotypic plasticity, i.e. a single genotype's ability to produce different phenotypes in
42 response to environmental variation (DeWitt and Scheiner, 2004; West-Eberhard, 2003). Phenotypic
43 plasticity has been proposed to facilitate population persistence under varying or unfavourable
44 environmental conditions and contribute to the establishment of populations in new habitats, thereby
45 leading to genetic divergence (Foster et al., 2015b; Pfennig et al., 2010). It may also contribute to
46 variation in sexual ornamentation and mate choice (Cornwallis and Uller, 2010; Griffith and Sheldon,

47 2001; Ingleby et al., 2010; Price, 2006), and, thus, may affect the outcome of sexual selection and
48 potentially promote speciation processes (West-Eberhard, 2003).

49 Predation risk is an important driver of variation in behavioural, ornamental, and morphological
50 phenotypes (e.g. Endler, 1995; Ferrari et al., 2010; Groenewoud et al., 2016; Hettyey et al., 2015). It is
51 a potent selective force acting against conspicuous phenotypes and favouring the maintenance of
52 cryptic appearance (Ekanayake et al., 2015; Ryan et al., 1982), as well as the emergence of anti-
53 predator phenotypes (Brock et al., 2015; Price et al., 2015). Accordingly, in predation-exposed natural
54 populations, reduced ornamentation (Endler, 1980; Lindholm et al., 2014; Outomuro and Johansson,
55 2015), and cryptic mating behaviour (Engqvist et al., 2015; Koga et al., 1998) occur regularly.
56 Furthermore, life-history theory predicts the evolution of different investment strategies in
57 reproductive traits to depend on predation risk (Candolin, 1998; Lima and Bednekoff, 1999; Wolf et
58 al., 2007). Adaptations to risk might either be genetically fixed or phenotypically plastic. While
59 predator-induced developmental plasticity is known to alter several characteristics of living organisms,
60 including behaviour, morphology, development rate, or toxin production (e.g. Hettyey et al., 2019;
61 Relyea, 2001; Tollrian and Harvell, 1999), relatively few studies examined its impact on sexually
62 selected traits. In guppies (*Poecilia reticulata*) and a riverine cichlid (*Pelvicachromis taeniatus*), the
63 exposure to chemical predator cues delayed the development of conspicuous male colouration
64 (Meuthen et al., 2018; Ruell et al., 2013). Furthermore, in guppies and palmate newts (*Lissotriton*
65 *helveticus*), males remained less colourful when chronically exposed to such cues (Ruell et al., 2013;
66 Winandy and Denoel, 2015).

67 In the presence of predators, females have repeatedly been shown to be less selective and show
68 reduced or altered preferences (e.g. Forsgren, 1992; Godin and Briggs, 1996; Pilakouta et al., 2017).
69 Under risky conditions choosing less conspicuous male phenotypes may be beneficial, as flamboyant
70 sexual patterns might attract predators (Magnhagen, 1991; Zuk and Kolluru, 1998). This can lead to
71 changes or inversion of mating preferences, e.g. from a preference for conspicuous traits to their
72 avoidance (Bierbach et al., 2011; Godin and Dugatkin, 1996; Pilakouta and Alonzo, 2014). Thus,

73 predation risk can alter the strength and sometimes even the direction of sexual selection. However,
74 little is known about how chronic predator exposure during ontogeny affects the development of
75 ornamental and behavioural traits of males, how it affects female choice, and how the interplay
76 between altered male phenotypes and female preferences ultimately shape mating patterns (Ruell et
77 al., 2013; Winandy and Denoel, 2015).

78 In the present study, we examined how variation in chronic predation risk during ontogeny and
79 variation in acute predation risk during mate choice affect traits under sexual selection in three-spined
80 stickleback (*Gasterosteus aculeatus*), which are ideal for studying predator-induced plasticity in
81 sexually selected characteristics. They are an important food source for a wide range of fishes and
82 birds (e.g. Johnson and Candolin, 2017; Kemper, 1995; Maitland, 1965; Moodie, 1972; Reimchen,
83 1980) and their name-giving spines represent morphological adaptations against predation (Gross,
84 1978; Hoogland et al., 1956; Moodie, 1972; Reimchen, 1994). During the reproductive phase, males
85 develop a conspicuous nuptial body colouration, including a strikingly red belly and bright blue eyes,
86 which plays a role in intra- as well as in intersexual selection (e.g. Bakker and Milinski, 1993; Bakker
87 and Mundwiler, 1994; Flamarique et al., 2013; Milinski and Bakker, 1990; Rowland, 1984). This
88 conspicuous colouration, however, makes them more vulnerable to predation (Johnson and Candolin,
89 2017; Maitland, 1965; Moodie, 1972; Whoriskey and Fitzgerald, 1985). Like many other fishes
90 possessing a non-structural colouration, three-spined stickleback can extenuate this colouration within
91 a short timeframe (Candolin, 1999; Hiermes et al., 2016; Kim and Velando, 2014). Populations facing
92 high levels of predation differ from predator-free populations in colouration (Gygax et al., 2018;
93 Moodie, 1972), morphology, (Bell and Foster, 1994; Marchinko, 2009; Reimchen, 1980), and anti-
94 predator behaviour (Dingemanse et al., 2007; Foster et al., 2015b; Huntingford et al., 1994; Wund et
95 al., 2015). Apart from some contribution of random genetic drift, such among-population differences
96 mainly result from local adaptation (Moodie, 1972; Peichel et al., 2001; Reimchen and Nosil, 2004) or
97 phenotypic plasticity (Ab Ghani et al., 2016; Candolin, 1997; Candolin, 1998; Frommen et al., 2011;
98 Gygax et al., 2018; Kozak and Boughman, 2012; Stein and Bell, 2014). We performed a comprehensive

99 test of predator-induced phenotypic changes in sexually selected traits, i.e. male ornamental traits and
100 female mating preferences, in the three-spined stickleback. We examined the effects of (i) chronic
101 predation risk experienced during individual development, and (ii) acute predation risk during
102 reproductive interactions on (a) male ornament expression and (b) mating behaviour, as well as on (c)
103 female mating preferences.

104 Material and Methods

105 *Experimental subjects*

106 The laboratory-reared population used in this study represents the F1 offspring of fish caught from an
107 anadromous, genetically heterogeneous population from the Island of Texel, the Netherlands (Heckel
108 et al., 2002). The parental generation was collected during their spring migration in April 2009 and
109 brought to the laboratory at the XXX (*deleted in order to anonymize*). Here, they were housed under
110 standardized summer light conditions (16h light, 8h dark) at a temperature of 17 ± 1 °C to simulate the
111 start of the breeding season. Fish were fed daily with frozen *Chironomus* larvae in excess. The breeding
112 procedures followed the protocol of Frommen et al. (2008). Briefly, males that showed signs of nuptial
113 colouration were isolated in 10 l tanks filled with aerated tap water and provided with sand on the
114 bottom and green cotton wool threads as nest-building material. Once a male had finished building his
115 nest, a gravid female was introduced into his tank to allow mating. In total, we used the offspring of
116 eight unrelated stickleback pairs. Eggs were removed from the nest and divided into equal-sized sibling
117 groups of approx. 20 to 30 eggs, which were then transferred to small 1 l tanks (16 x 10 x 6 cm, l x w x
118 h) aerated by air stones. To simulate predation risk, we used European perch (*Perca fluviatilis*) that
119 occur in sympatry with *G. aculeatus* in large parts of Europe, including the Texel population (J.
120 Hottentot, pers. communication) and that readily prey on juvenile and adult stickleback (Gross, 1978;
121 Hoogland et al., 1956). We added perch-conditioned water (see below) daily to the tank of one sibling
122 group. The other group received aged tap water as a control. Previous studies showed that already
123 larval three-spined sticklebacks readily recognize predatory perch solely based on chemical cues
124 (Frommen et al., 2011; Lehtiniemi, 2005). At the age of 3-4 months, we transferred juvenile fish into

125 larger tanks (50 x 30 x 30 cm) filled with the respective treatment water (perch-water or tap water)
126 and aerated through an internal filter. One-third of the tank water was replaced by treatment water
127 daily.

128 Predator water production followed the protocol established by Frommen et al. (2011). Perch
129 measured between 75 and 83 mm standard length (SL) and were kept in tanks (50 x 30 x 30 cm) each
130 housing one individual. These tanks were equipped the same way as the tanks containing stickleback.
131 We produced control water in identical tanks that differed from the predator treatment only by the
132 lack of a perch. The tanks providing treatment water were aerated through internal filters and were
133 placed on shelves above the stickleback tanks. We connected each treatment tank to the tanks holding
134 the experimental fish through a hose-and-tap system, which facilitated water exchange. Perch were
135 fed daily with frozen *Chironomus* larvae in excess. Each perch water and tap water tank provided
136 stimulus water for two unrelated sibling groups of stickleback. Experimental fish were kept under these
137 conditions for approximately one year, when they reached sexual maturity.

138 *Experimental procedure*

139 Males that showed developing nuptial colouration were removed from the group tank and isolated in
140 22 x 25 x 48 cm plastic tanks, filled to a level of 15 cm with aerated water from the home-tank
141 treatment. They were equipped with a Petri dish filled with sand and 1 g of green wool cut in small
142 pieces as nesting material. We stimulated males to build a nest by presenting a randomly chosen gravid
143 female from the stock population held in a net cage for 5 minutes every day (Frommen and Bakker,
144 2006). During this time, water was exchanged daily as described above and replaced by either perch-
145 conditioned water or tap water. As soon as nests were completed in the Petri dish and males were
146 courting the stimulus females vigorously they were considered ready for the experimental trials.

147 To assess female preferences during mate choice, we employed a dichotomous set-up and
148 quantified the time females allocated towards two reproductively active males reared in different
149 treatments (Fig. 1). The experimental tank (70 x 35 x 40 cm) was divided transversally into two equal-
150 sized compartments (35 x 35 x 40 cm) by a transparent plastic partition with holes to allow water

151 circulation. The partition was temporarily covered with an opaque, black plastic divider that was lifted
152 at the beginning of the trial (Fig. 1). One of the two compartments was further divided longitudinally
153 in half by an opaque divider, creating the two male compartments. The undivided compartment
154 constituted the female section and contained a plastic plant in the centre to offer refuge and facilitate
155 acclimatization. The whole apparatus was wrapped with a dark plastic foil and placed behind a black
156 curtain to prevent disturbance during trials. We positioned a webcam above each tank to record fish
157 behaviour. A neon lamp placed 60 cm above the water level in the centre of each tank ensured uniform
158 illumination.

159 We tested the preference of females (either reared under perceived predation risk or without
160 predation risk) in the presence of two simultaneously provided males (one raised under predation risk
161 and one without predation risk). Four fish formed one experimental unit. The testing of a given unit
162 lasted for two days. To examine the effect of acute predation risk on mating behaviour, we tested each
163 female twice with the same stimulus males, once in perch-conditioned water and once in tap-water.
164 On the first day, we used one of the two females along with two males. On the second day, the same
165 two males were used again but exposed to the female originating from the other development
166 environment. The experiment consisted of 19 units, i.e. we tested 19 females reared under chronic
167 predation risk and 19 females that developed in clear tap water. Males and females within
168 experimental units were unrelated to each other to avoid relatedness affecting mating decisions.

169 On the day of the respective mate choice trials, we filled all compartments of the experimental
170 tanks either with perch-conditioned water or with tap water to a level of 13 cm. Next, we moved the
171 two males (one male from each environment) together with their nests into the experimental tank.
172 Males were matched in SL to the nearest 2 mm within pairs. Stickleback males readily re-accepted
173 their nests after the movement as indicated by nest repair behaviour and show-fanning (Mehlis et al.,
174 2009; Rick and Bakker, 2008b). After a 30-minute acclimatization period, we added a gravid focal
175 female originating either from the predator-exposed development treatment or from the control

176 group to the female compartment. Females ready to spawn can easily be recognized by their swollen
177 abdomen and by eggs shining through the skin near the cloaca (Frommen et al., 2012).

178 After 5 minutes, we lifted the opaque divider, allowing the female to evaluate both males. Once
179 the female had inspected both males, as indicated by entering the respective choice zone in front of
180 each male, we recorded all individuals' behaviour for 15 minutes. At the end of the trial, we lowered
181 the divider. Two minutes later, we removed the males and immediately photographed them in a water-
182 filled photo box (10 x 5 x 5.5 cm) under standardized light conditions in front of a uniform black
183 background and illuminated with a Volpi Intralux 6000 fibre optic light source (see Bakker and
184 Mundwiler, 1994; Frommen et al., 2008 for details). We took photographs using a Canon Eos 400D
185 Camera equipped with an EFS 18-50 mm lens. To correct for potential illumination differences between
186 pictures, the standardized white side of a Novoflex Zebra Grey Card was visible on each image (Bakker
187 and Mundwiler, 1994). We took photos of the ventral and the left lateral side of males.

188 Subsequently, we performed the same behavioural test with the same three individuals in a
189 second experimental tank filled with the opposite treatment water, following the same protocol as
190 before. To control for any side bias, we inverted the positions of the two males. After the trial, we
191 photographed males again and measured the SL and mass of both males and the female. Based on
192 these measurements, we calculated the body condition factor for each male following Bolger and
193 Conolly (1989). Subsequently, we placed males back into their individual tanks with their nest. Females
194 were placed in a tank of a nest-tending male that did not take part in the experiment. All females
195 spawned with that male within 24 hours after termination of their trial, ensuring that we only analysed
196 data of reproductively ripe and receptive females (Frommen and Bakker, 2006). On the following day,
197 the same two males were tested together again following the experimental protocol described above,
198 but with a female reared in the other treatment group. As the order of the acute exposure treatments
199 was inverted, we could examine their effects on male ornamental colouration. We changed the water
200 in the experimental tanks after each trial. Between experimental units, treatment order and the
201 positions of individuals in the tanks were randomized.

202 *Video and photo analyses*

203 From each photo, we measured male colouration at ten standardized points on the throat and six
204 points on the eye following the protocol described in Frommen et al. (2008). Before measurement, we
205 corrected photographs for any differences in brightness (L^*) using the Novoflex Zebra Grey Card as a
206 white standard (Bakker and Mundwiler, 1994). We measured colouration with Photoshop in the CIE
207 (L^* , a^* , b^*) colour space, which has the advantage of being more device-independent than the RGB
208 colour space (Stevens and Cuthill, 2005). The “ a^* ” axis represents the colour spectrum ranging from
209 green (negative values) to magenta (positive values), and the “ b^* ” axis represents the range from blue
210 (negative values) to yellow (positive values). We took measurements at the predefined spots
211 measuring 5 x 5 pixels with the colour sampler tool CS3 in Adobe Photoshop. Three indices per picture
212 were calculated by averaging the ten values of a^* and b^* for the throat and the six values of b^* for the
213 eye, subsequently called redness A, redness B, and blueness, respectively. Because average estimates
214 of redness A and B were correlated (Pearson’s $r=0.37$, $N=76$, $P=0.001$), we combined these two
215 variables via a PCA. The first component explained 68.36 % of the variance, and both original variables
216 loaded strongly and positively on it (both $r=0.83$). We used component scores on PC 1 as overall
217 measures of redness in further analyses.

218 We analysed videos blindly with respect to fish identity, the chronic treatment fish were taken
219 from, and acute predation risk. For video-analysis, we used the “Observer” software by Noldus
220 (Wageningen, Netherlands). We measured the time females spent in the choice zone in front of the
221 respective male compartments (Fig. 1). Time spent close to a male stimulus has been shown to reliably
222 predict mating probability in this species (McLennan and McPhail, 1990; Milinski et al., 2005). We also
223 aimed at measuring male courtship activity. Studies on free-ranging male three-spined stickleback
224 often use the number or duration of zig-zag courtship dances to measure male sexual activity (e.g.
225 Head et al., 2017; Kraak and Bakker, 1998; McGhee et al., 2015). However, these dances are performed
226 over considerable distances. As our set-up restricted male movements to a maximum distance of 35
227 cm (the total length of male compartments) males remained courting close to the clear divider and

228 were highly active during trials. Previous studies showed that under such conditions, time being close
229 to a gravid female is in strong positive correlation with the amount of zig-zag dances (Kraak and Bakker,
230 1998; Rowland et al., 1991). Therefore, we used the time the respective male spent in a given courtship
231 zone (11.5 x 11.5 cm, Fig. 1) bordering on the female compartment as a proxy for male courtship
232 activity (c.f. Frommen et al., 2009b; Kraak and Bakker, 1998; Rick and Bakker, 2008b; Rowland et al.,
233 1991). We judged experimental fish to have entered a zone once their head and pectoral fins crossed
234 the zone boundary.

235 *Statistical analyses*

236 Statistical analyses were performed using IBM SPSS Statistics 25. To examine whether chronic
237 predation risk during development or acute predation risk during mate choice trials affected male
238 colouration (eye blueness and throat redness) or behaviour (time spent close to the female), we ran
239 repeated-measures multivariate general linear models (RMMGLM). Throat redness, eye blueness, and
240 time spent close to the female were the dependent variables, acute predation risk (present/absent)
241 was the within-subjects factor, and chronic predation risk the between-subjects factor. We also
242 entered the interaction between chronic and acute predation risk into the model. In addition, we
243 analysed the effects of chronic predation risk on male SL and body condition using a multivariate
244 general linear model (MGLM). We log-transformed SL data to meet the assumptions of homogeneity
245 of variances and normality of model residuals. We entered SL and body condition as dependent
246 variables and chronic predation risk as a fixed factor. Because we measured SL and mass of males twice
247 (see above), we calculated averages for SL and condition to simplify the analysis and avoid pseudo-
248 replication.

249 We analysed the effects of the males' and females' development environment, acute predation
250 risk and male traits on female mating preference using linear mixed-effects modelling procedures
251 (LMM). We entered the time females spent close to a male as the dependent variable, male and female
252 development environment (chronic predation risk present/absent), and acute predation risk
253 (present/absent) as fixed effects, and trial ID as a repeated-measures random variable. To assess male

254 phenotypic traits' effects on female preference, we entered residual values of male body length, male
255 body condition, eye blueness, throat redness and the time males spent close to the female as
256 covariates. We used residual values to account for male traits' potential dependence on chronic and
257 acute predation risk, thereby avoiding interdependence among explanatory variables. Residual values
258 were standardized scores of male phenotypic traits calculated separately for the four combinations of
259 the male development environment and acute predation risk during choice trials. Because both males
260 and females were used repeatedly within a given experimental unit, we also entered male ID and
261 female ID as random effects. We entered the three two-way interactions among the fixed effects into
262 the model. We applied backward stepwise removal procedures (Grafen and Hails, 2002) for model
263 simplification. Removed variables were re-entered one by one to the final model to obtain relevant
264 statistics. We retained random effects in LMMs to avoid pseudo-replication and the resulting inflation
265 of the degrees of freedom. We confirmed that the fitted models fulfilled requirements by plot
266 diagnosis. All tests were two-tailed, with α set to 0.05.

267 Results

268 *Effects of chronic and acute predation risk on male traits*

269 Chronic exposure to chemical cues of predators during development affected the development of
270 secondary sexual traits and courtship behaviour of males (RMMGLM; $F_{3,34}=4.15$, $P=0.013$): males
271 reared in the absence of chronic predation risk had higher values of throat redness ($F_{1,36}=5.84$, $P=0.021$;
272 Fig. 2a) and tended to spend less time close to the female ($F_{1,36}=3.66$, $P=0.064$; Fig. 2b) than those
273 reared in the presence of predators, while a similar effect on eye blueness was not apparent ($F_{1,36}=1.25$,
274 $P=0.27$). Acute predation risk or its interaction with chronic predation risk neither affected male
275 ornamentation nor behaviour (acute risk: $F_{3,34}=0.22$, $P=0.89$; acute risk \times chronic risk: $F_{3,34}=0.88$,
276 $P=0.46$). Males in the two chronic exposure treatments did not differ in SL or body condition (MGLM;
277 $F_{2,35}=0.21$, $P=0.81$).

278 *Effects of chronic and acute predation risk and male traits on female mating preference*

279 Female preference was affected by the interaction between chronic predation risk experienced by
280 males during development and acute predation risk (LMM; $F_{1,68.8}=14.65$, $P<0.001$): in mate choice trials
281 performed under acute predation risk, males reared under chronic predation risk were less attractive
282 to females than males reared in the absence of predator cues (Fig. 3). This pattern was reversed when
283 cues of predation risk were absent during mate choice trials. The male development environment's
284 main effect was also significant ($F_{1,67.8}=4.17$, $P=0.045$), while acute predation risk during choice trials
285 was non-significant ($F_{1,68.8}=0.01$, $P=0.92$). Importantly, the development environment of females did
286 not affect their preference ($F_{1,66.9}=0.16$, $P=0.69$). The interactions between the male and female
287 development environments and the female development environment and acute predation risk were
288 also non-significant (both $P>0.36$). Residual values of male SL and eye blueness positively affected the
289 time females spent close to a male (LMM; SL: $F_{1,67.8}=10.28$, $B=1.23$, $SE=0.38$, $P=0.002$; eye blueness:
290 $F_{1,94.9}=7.71$, $B=1.02$, $SE=0.37$, $P=0.007$). However, female preference was not affected by the residual
291 values of the time males spent close to the divider ($F_{1,132.1}=0.11$, $P=0.74$), male throat redness
292 ($F_{1,76.1}=0.37$, $P=0.55$) or male body condition ($F_{1,67}=0.83$, $P=0.36$).

293 Discussion

294 In the present study, we show that predator-induced changes shape male and female traits that play
295 central roles in sexual selection. Chronic predation risk experienced during development (from the egg
296 stage to reaching sexual maturity) and acute predation risk experienced during mate choice affected
297 male ornamental traits and female mating preferences. These effects were sex-specific, as chronic
298 exposure to predation risk during development only affected male traits, but not female preference,
299 while acute predation risk experienced during mate choice did not affect male phenotype but
300 influenced female choice.

301 Chronic exposure to chemical cues of predators resulted in a weaker expression of red throat
302 colouration in males and tended to increase courtship activity as compared to males that developed
303 in the absence of predation risk. Plastic responses to predation risk can result in more cryptic nuptial

304 colouration in prey species (Meuthen et al., 2018; Ruell et al., 2013; Winandy and Denoel, 2015). The
305 bright red colouration of stickleback's throat has been shown to increase the risk of predator-attacks
306 (Johnson and Candolin, 2017; Maitland, 1965; Moodie, 1972). Hence, a reduction in the red
307 ornamentation's conspicuousness is likely to result in lowered predation risk when predators are
308 present in the environment. Enhanced activity in the presence of females may, on the other hand, only
309 temporarily lead to increased risk of detection by predators. At the same time, it may be necessary to
310 attract females despite the less conspicuous colouration. This result is in accordance with life-history
311 theory, predicting that individuals should take higher risks during reproduction, especially if their
312 chance of reproducing again is low (Candolin, 1998). Comparable to many other populations (Wootton,
313 1976) the breeding season of three-spined sticklebacks on Texel lasts only a few weeks in early
314 summer, and adults die afterwards (Kemper, 1995). Thus, opportunities to reproduce are limited to
315 one short period, which should lead to an increased willingness to accept higher predation risk,
316 especially during the phase of active courtship, which usually last only for a few minutes per mating
317 (Tinbergen, 1952). Indeed, reproductively active male and female three-spined stickleback are more
318 risk-prone in the presence of a predator than non-reproductive ones (Frommen et al., 2009a). In
319 contrast to throat colour, eye colour was not influenced by chronic predator exposure. This suggests
320 that these different colour signals are expressed independently from each other and may provide
321 females with multiple cues to assess male quality (Flamarique et al., 2013; Frischknecht, 1993).

322 Different than to male's throat colouration and courtship intensity female preference was not
323 affected by chronic predation risk experienced during development. As males exhibiting more intense
324 red throat colouration are usually preferred over duller males by females (Bakker and Milinski, 1993),
325 and more intensely courting males are preferred over less active males under predator free testing
326 conditions (Candolin, 1997; Rowland, 1995), the observed lack of preference might be explained by a
327 differential preference for male colour and activity depending on acute predation risk (Candolin, 1997).
328 Indeed, several studies on female preferences for male nuptial colouration and courtship activity
329 showed that under certain circumstances, the red colouration (Candolin et al., 2007; Engström-Öst and

330 Candolin, 2007; McDonald et al., 1995) and courtship activity (Künzler and Bakker, 2001; Rowland,
331 1995) can lose its significance for mate choice, while other indicators of quality can gain importance
332 instead (Boughman, 2001; Flamarique et al., 2013; Heuschele et al., 2009; Rick and Bakker, 2008a). In
333 line with this argument, acute risk during choice trials and male development environment
334 interactively influenced female preference, indicating that preferences may shift when acute
335 predation risk changes. These results are in accordance with recent studies on three-spined stickleback
336 suggesting that female and male mate choice is affected by the presence of predators (Kozak and
337 Boughman, 2015; McGhee et al., 2015), and highlight that incorporating natural threat stimuli can
338 change the outcome of studies on sexual selection and mate choice. Therefore, our results call for the
339 application of more natural settings and planned experimental co-testing of potentially important
340 ecological factors when investigating sexual preferences.

341 In our experiment, males with more intensely coloured blue eyes and larger SL were preferred
342 over duller-coloured and smaller males (cf. Flamarique et al., 2013; Rowland, 1989), and this was
343 independent of acute predation risk. Interestingly, male traits that appeared responsive to chronic
344 predation risk (i.e., throat redness and male courtship behaviour) were less important for female
345 choice in our study. In contrast, male traits that were less affected by chronic predation threat (i.e.,
346 eye blueness) or where variation was kept minimal per experimental design (SL) seemed to be highly
347 relevant for female choice, independent of predators' acute presence. These findings may help explain
348 the maintenance of variation in sexually selected traits, leaving different male traits free to respond to
349 sexual selection depending on the chronic and acute risk of predation.

350 In conclusion, we show that chronic and acute predation risk can influence the expression of
351 sexually selected traits in three-spined stickleback. Such phenotypic plastic effects may contribute to
352 speciation processes if male sexual ornaments and female choice develop into the same direction (i.e.,
353 females prefer male phenotypes expressed in the same predator-environment as themselves).
354 However, in our study population, plastic antipredator responses in male sexual ornaments and female
355 preferences did align with each other. Hence, the interactive effect of chronic and acute predation risk

356 on male sexual ornaments and female mating preference may lead to dynamic processes that can alter
357 the strength and even the direction of sexual selection, resulting in temporal and spatial variation in
358 secondary sexual traits.

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594

595 Figure legends

596 Fig. 1: The experimental set-up viewed from above. In the three-compartment tank, a female (left
597 compartment) was given a choice between two stimulus males: one reared under chronic predation
598 risk (upper right compartment) and the other one raised in the absence of risk (lower right
599 compartment). Focal females were also either reared under chronic predation risk (in the present case)
600 or in a risk-free environment. The same experimental triad (i.e., the focal female and the two stimulus
601 males) were tested twice, once under acute predation risk (i.e., in tap water conditioned with predator
602 cues) and once without acute predation risk (i.e., in clear tap water). The two males were then
603 presented to a further female from the other development environment. We estimated female mating
604 preferences for the respective males by measuring the time females spent in the association zones in
605 front of the males. Male courtship activity was assessed by measuring the time males spent in the
606 courtship zones. Zone boundaries were drawn on the bottom of the tank with a black marker.

607

608 Fig. 2: (a) Throat redness of males and (b) the time males spent close to the female in relation to the
609 development environment of males (presence or absence of chronic predation risk). Mean values (\pm SE)
610 are shown.

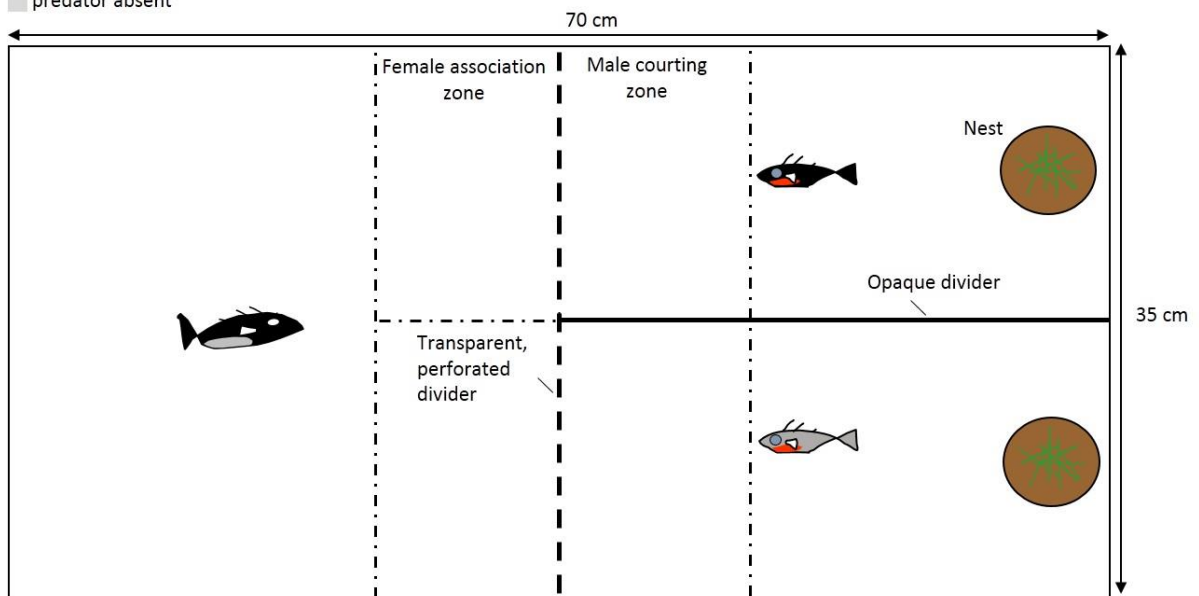
611

612 Fig. 3: Female preferences measured as the time females spent close to males reared in different
613 environments (presence or absence of chronic predation risk) under different experimental conditions
614 (presence or absence of acute predation risk). Mean values (\pm SE) are shown.

615 Fig. 1

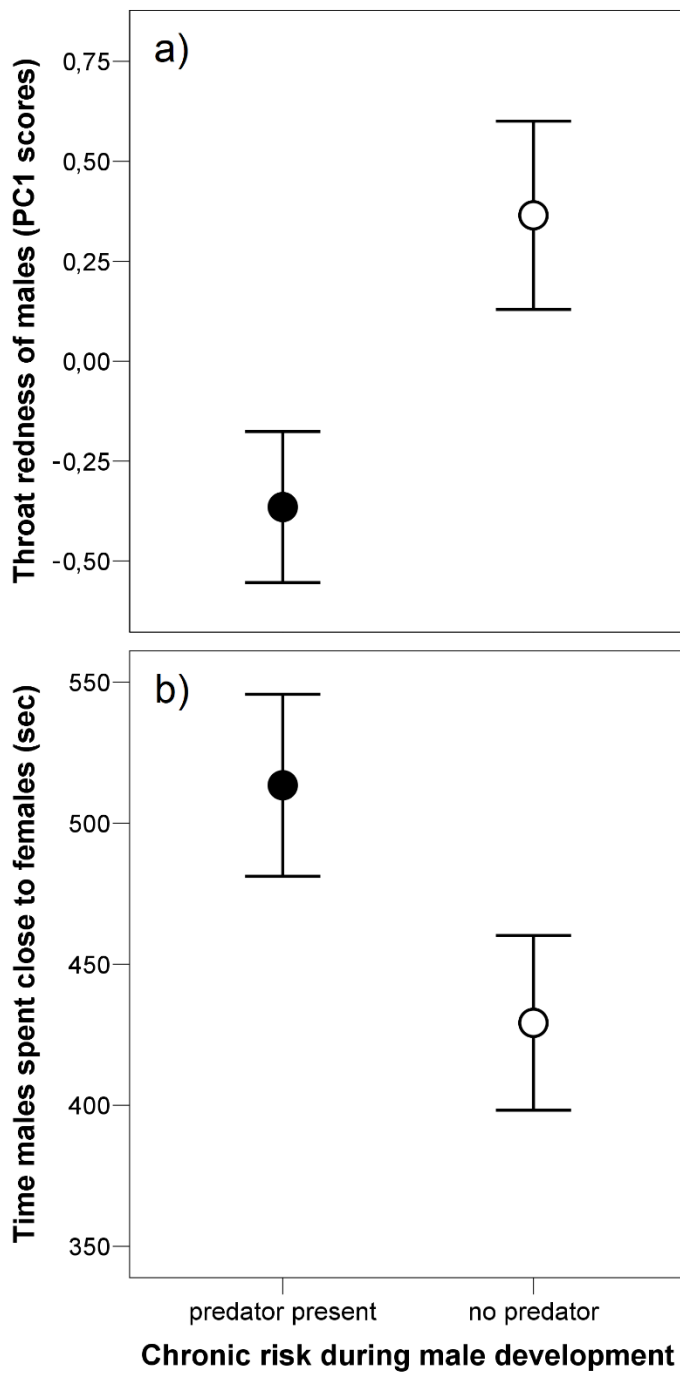
Rearing environment:

- predator present
- predator absent



616

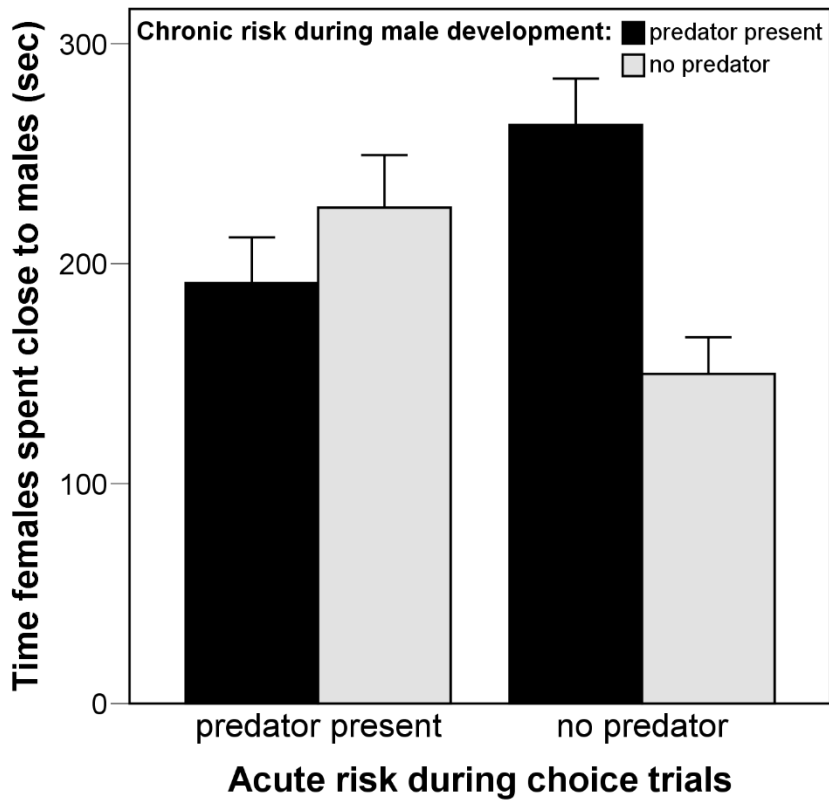
617



619

620

621 Fig. 3



622

623