

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  $17 \pm 1$  °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  $\alpha$  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.

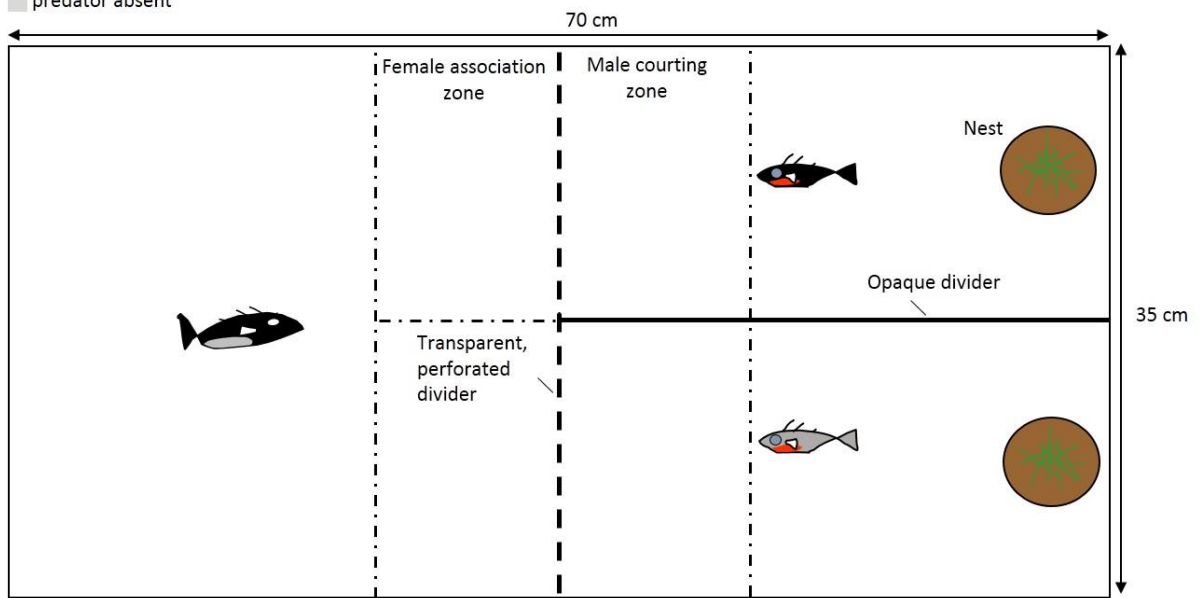
611

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:

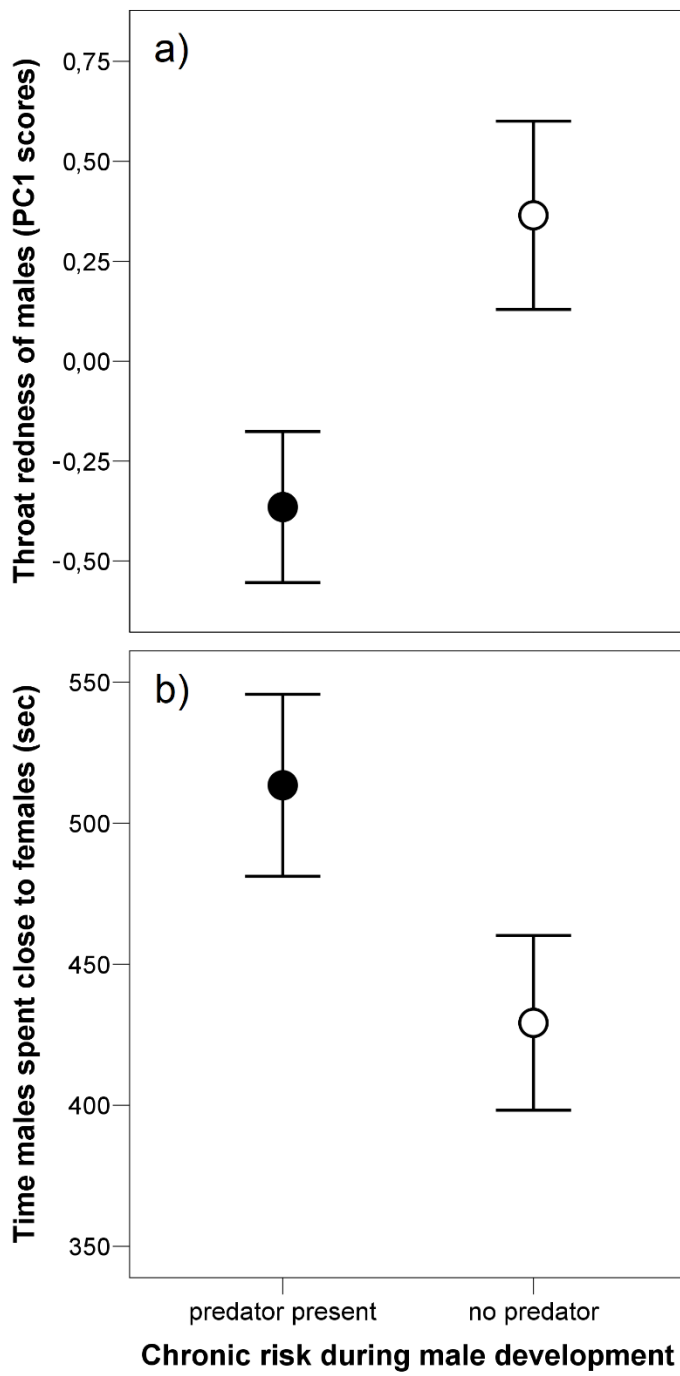
- predator present
- predator absent



616

617

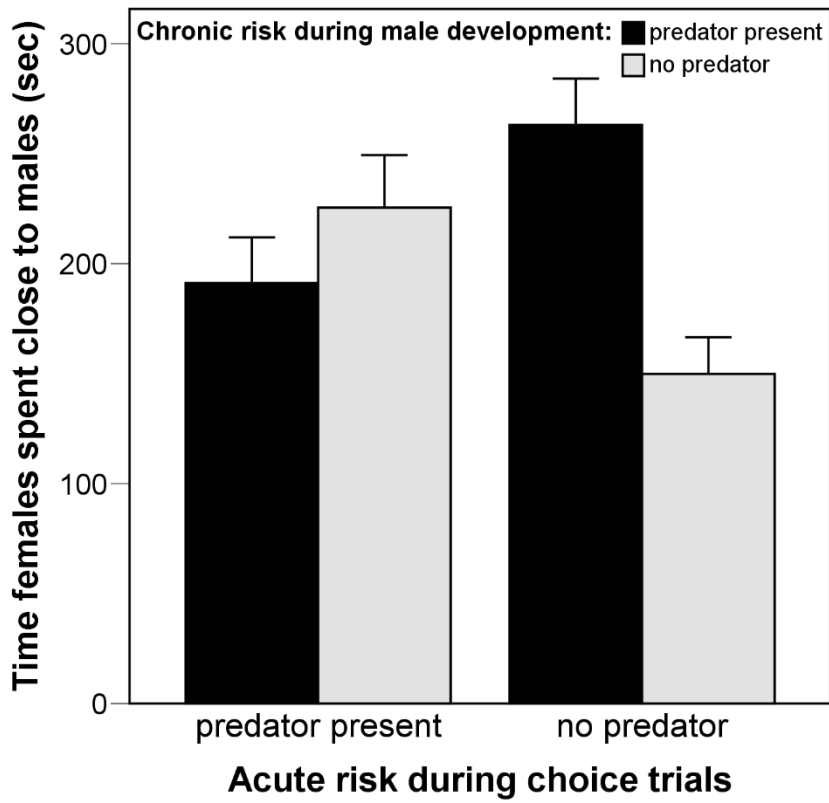




619

620

621 Fig. 3



622

623