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

Running title: Effects of predation risk on sexual selection

Abstract

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

Introduction

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

Understanding the causes underlying phenotypic variation in sexual ornamentation and mating behaviour within a population is crucial for a comprehensive understanding of the evolution and diversification of secondary sexual traits (Foster et al., 2015a). One mechanism contributing to variation is phenotypic plasticity, i.e. a single genotype's ability to produce different phenotypes in response to environmental variation (DeWitt and Scheiner, 2004; West-Eberhard, 2003). Phenotypic plasticity has been proposed to facilitate population persistence under varying or unfavourable environmental conditions and contribute to the establishment of populations in new habitats, thereby leading to genetic divergence (Foster et al., 2015b; Pfennig et al., 2010). It may also contribute to variation in sexual ornamentation and mate choice (Cornwallis and Uller, 2010; Griffith and Sheldon, 2001; Ingleby et al., 2010; Price, 2006), and, thus, may affect the outcome of sexual selection and potentially promote speciation processes (West-Eberhard, 2003).

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

In the presence of predators, females have repeatedly been shown to be less selective and show reduced or altered preferences (e.g. Forsgren, 1992; Godin and Briggs, 1996; Pilakouta et al., 2017). Under risky conditions choosing less conspicuous male phenotypes may be beneficial, as flamboyant sexual patterns might attract predators (Magnhagen, 1991; Zuk and Kolluru, 1998). This can lead to changes or inversion of mating preferences, e.g. from a preference for conspicuous traits to their avoidance (Bierbach et al., 2011; Godin and Dugatkin, 1996; Pilakouta and Alonzo, 2014). Thus, predation risk can alter the strength and sometimes even the direction of sexual selection. However, little is known about how chronic predator exposure during ontogeny affects the development of ornamental and behavioural traits of males, how it affects female choice, and how the interplay between altered male phenotypes and female preferences ultimately shape mating patterns (Ruell et al., 2013; Winandy and Denoel, 2015).

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

Material and Methods

Experimental subjects

The laboratory-reared population used in this study represents the F1 offspring of fish caught from an anadromous, genetically heterogeneous population from the Island of Texel, the Netherlands (Heckel et al., 2002). The parental generation was collected during their spring migration in April 2009 and brought to the laboratory at the XXX (*deleted in order to anonymize*). Here, they were housed under standardized summer light conditions (16h light, 8h dark) at a temperature of 17 ± 1 °C to simulate the start of the breeding season. Fish were fed daily with frozen *Chironomus* larvae in excess. The breeding procedures followed the protocol of Frommen et al. (2008). Briefly, males that showed signs of nuptial colouration were isolated in 10 l tanks filled with aerated tap water and provided with sand on the bottom and green cotton wool threads as nest-building material. Once a male had finished building his nest, a gravid female was introduced into his tank to allow mating. In total, we used the offspring of eight unrelated stickleback pairs. Eggs were removed from the nest and divided into equal-sized sibling 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 h) aerated by air stones. To simulate predation risk, we used European perch (*Perca fluviatilis*) that occur in sympatry with *G. aculeatus* in large parts of Europe, including the Texel population (J. Hottentot, pers. communication) and that readily prey on juvenile and adult stickleback (Gross, 1978; Hoogland et al., 1956). We added perch-conditioned water (see below) daily to the tank of one sibling group. The other group received aged tap water as a control. Previous studies showed that already larval three-spined sticklebacks readily recognize predatory perch solely based on chemical cues (Frommen et al., 2011; Lehtiniemi, 2005). At the age of 3-4 months, we transferred juvenile fish into larger tanks (50 x 30 x 30 cm) filled with the respective treatment water (perch-water or tap water) and aerated through an internal filter. One-third of the tank water was replaced by treatment water daily.

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

Experimental procedure

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

To assess female preferences during mate choice, we employed a dichotomous set-up and quantified the time females allocated towards two reproductively active males reared in different treatments (Fig. 1). The experimental tank (70 x 35 x 40 cm) was divided transversally into two equal-sized compartments (35 x 35 x 40 cm) by a transparent plastic partition with holes to allow water circulation. The partition was temporarily covered with an opaque, black plastic divider that was lifted at the beginning of the trial (Fig. 1). One of the two compartments was further divided longitudinally in half by an opaque divider, creating the two male compartments. The undivided compartment constituted the female section and contained a plastic plant in the centre to offer refuge and facilitate acclimatization. The whole apparatus was wrapped with a dark plastic foil and placed behind a black curtain to prevent disturbance during trials. We positioned a webcam above each tank to record fish behaviour. A neon lamp placed 60 cm above the water level in the centre of each tank ensured uniform illumination.

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

On the day of the respective mate choice trials, we filled all compartments of the experimental tanks either with perch-conditioned water or with tap water to a level of 13 cm. Next, we moved the two males (one male from each environment) together with their nests into the experimental tank. Males were matched in SL to the nearest 2 mm within pairs. Stickleback males readily re-accepted their nests after the movement as indicated by nest repair behaviour and show-fanning (Mehlis et al., 2009; Rick and Bakker, 2008b). After a 30-minute acclimatization period, we added a gravid focal female originating either from the predator-exposed development treatment or from the control group to the female compartment. Females ready to spawn can easily be recognized by their swollen abdomen and by eggs shining through the skin near the cloaca (Frommen et al., 2012).

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

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

Video and photo analyses

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

We analysed videos blindly with respect to fish identity, the chronic treatment fish were taken from, and acute predation risk. For video-analysis, we used the "Observer" software by Noldus (Wageningen, Netherlands). We measured the time females spent in the choice zone in front of the respective male compartments (Fig. 1). Time spent close to a male stimulus has been shown to reliably predict mating probability in this species (McLennan and McPhail, 1990; Milinski et al., 2005). We also aimed at measuring male courtship activity. Studies on free-ranging male three-spined stickleback often use the number or duration of zig-zag courtship dances to measure male sexual activity (e.g. Head et al., 2017; Kraak and Bakker, 1998; McGhee et al., 2015). However, these dances are performed over considerable distances. As our set-up restricted male movements to a maximum distance of 35 cm (the total length of male compartments) males remained courting close to the clear divider and were highly active during trials. Previous studies showed that under such conditions, time being close to a gravid female is in strong positive correlation with the amount of zig-zag dances (Kraak and Bakker, 1998; Rowland et al., 1991). Therefore, we used the time the respective male spent in a given courtship zone (11.5 x 11.5 cm, Fig. 1) bordering on the female compartment as a proxy for male courtship activity (c.f. Frommen et al., 2009b; Kraak and Bakker, 1998; Rick and Bakker, 2008b; Rowland et al., 1991). We judged experimental fish to have entered a zone once their head and pectoral fins crossed the zone boundary.

Statistical analyses

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

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

Results

Effects of chronic and acute predation risk on male traits

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

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

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

Discussion

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

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

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

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

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

In conclusion, we show that chronic and acute predation risk can influence the expression of sexually selected traits in three-spined stickleback. Such phenotypic plastic effects may contribute to speciation processes if male sexual ornaments and female choice develop into the same direction (i.e., females prefer male phenotypes expressed in the same predator-environment as themselves). However, in our study population, plastic antipredator responses in male sexual ornaments and female preferences did align with each other. Hence, the interactive effect of chronic and acute predation risk on male sexual ornaments and female mating preference may lead to dynamic processes that can alter the strength and even the direction of sexual selection, resulting in temporal and spatial variation in secondary sexual traits.

References

Ab Ghani NI, Herczeg G, Merilä J, 2016. Effects of perceived predation risk and social environment on the development of three-spined stickleback (*Gasterosteus aculeatus*) morphology. Biol J Linn Soc 118:520-535.

Andersson M, 1994. Sexual selection. Princeton: Princeton University Press.

Bakker TCM, Milinski M, 1993. The advantages of being red - sexual selection in the stickleback. Mar Behav Physiol 23:287-300.

Bakker TCM, Mundwiler B, 1994. Female mate choice and male red coloration in a natural three-spined stickleback (*Gasterosteus aculeatus*) population. Behav Ecol 5:74-80.

Bell MA, Foster SA, 1994. The evolutionary biology of the threespine stickleback. Oxford: Oxford University Press.

Bierbach D, Schulte M, Herrmann N, Tobler M, Stadler S, Jung CT, Kunkel B, Riesch R, Klaus S, Ziege M, Indy JR, Arias-Rodriguez L, Plath M, 2011. Predator-induced changes of female mating preferences: innate and experiential effects. BMC Evol Biol 11:190.

Bolger T, Connolly PL, 1989. The selection of suitable indices for the measurement and analysis of fish condition. J Fish Biol 34:171-182.

Boughman JW, 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. Nature 411:944-948.

Brock KM, Bednekoff PA, Pafilis P, Foufopoulos J, 2015. Evolution of antipredator behavior in an island lizard species, *Podarcis erhardii* (Reptilia: Lacertidae): the sum of all fears? Evolution 69:216-231.

Candolin U, 1997. Predation risk affects courtship and attractiveness of competing threespine stickleback males. Behav Ecol Sociobiol 41:81-87.

Candolin U, 1998. Reproduction under predation risk and the trade-off between current and future reproduction in the threespine stickleback. Proc R Soc B 265:1171-1175.

Candolin U, 1999. Male–male competition facilitates female choice in sticklebacks. Proc R Soc B 266:785-789.

Candolin U, Salesto T, Evers M, 2007. Changed environmental conditions weaken sexual selection in sticklebacks. J Evol Biol 20:233-239.

Chaine AS, Lyon BE, 2008. Adaptive plasticity in female mate choice dampens sexual selection on male ornaments in the lark bunting. Science 319:459-462.

Cornwallis CK, Uller T, 2010. Towards an evolutionary ecology of sexual traits. Trends Ecol Evol 25:145-152.

Darwin C, 1859. On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. London: John Murray.

Darwin C, 1871. The descent of man, and selection in relation to sex. London: John Murray.

DeWitt T, Scheiner S, 2004. Phenotypic variation from single genotypes. In: DeWitt T, Scheiner S, editors. Phenotypic plasticity: functional and conceptual approaches Ney ork: Oxford University Press. p. 1-9.

Dingemanse NJ, Wright J, Kazem AJN, Thomas DK, Hickling R, Dawnay N, 2007. Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. J Anim Ecol 76:1128-1138.

Dunn PO, Armenta JK, Whittingham LA, 2015. Natural and sexual selection act on different axes of variation in avian plumage color. Sci Adv 1:e1400155.

Ekanayake KB, Weston MA, Nimmo DG, Maguire GS, Endler JA, Küpper C, 2015. The bright incubate at night: sexual dichromatism and adaptive incubation division in an open-nesting shorebird. Proc R Soc B 282:20143026.

Endler JA, 1980. Natural selection on color patterns in *Poecelia reticulata*. Evolution 34:76-91.

Endler JA, 1995. Multiple-trait coevolution and environmental gradients in guppies. Trends Ecol Evol 10:22-29.

Engqvist L, Cordes N, Reinhold K, 2015. Evolution of risk-taking during conspicuous mating displays. Evolution 69:395-406.

Engström-Öst J, Candolin U, 2007. Human-induced water turbidity alters selection on sexual displays in sticklebacks. Behav Ecol 18:393-398.

Ercit K, Gwynne DT, 2015. Darwinian balancing selection: predation counters sexual selection in a wild insect. Evolution 69:419-430.

Falconer DS, Mackay TFC, 1996. Introduction to quantitative genetics. Harlow, Essex: Longmans Green.

Ferrari MCO, Wisenden BD, Chivers DP, 2010. Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. Canad J Zool 88:698-724.

Fisher RA, 1930. The genetical theory of natural selection. Oxford: Oxford University Press.

Flamarique IN, Bergstrom C, Cheng CL, Reimchen TE, 2013. Role of the iridescent eye in stickleback female mate choice. J Exp Biol 216:2806-2812.

Forsgren E, 1992. Predation risk affects mate choice in a gobiid fish. Am Nat 140:1041-1049.

Foster S, Wund M, Graham M, Earley R, Gardiner R, Kearns T, Baker J, 2015a. Iterative development and the scope for plasticity: contrasts among trait categories in an adaptive radiation. Heredity 115:335-348.

Foster SA, Wund MA, Baker JA, 2015b. Evolutionary influences of plastic behavioral responses upon environmental challenges in an adaptive radiation. Integr Comp Biol 55:406-417.

Frischknecht M, 1993. The breeding coloration of male three-spined sticklebacks (*Gasterosteus aculeatus*) as an indicator of energy investment in vigor. Evol Ecol 7:439-450.

Frommen JG, Bakker TCM, 2006. Inbreeding avoidance through non-random mating in sticklebacks. Biol Lett 2:232-235.

Frommen JG, Bakker TCM, Proscurcin LC, Mehlis M, 2012. Gravidity-associated shoaling decisions in three-spined sticklebacks (*Gasterosteus aculeatus*). Ethology 118:1149-1156.

Frommen JG, Herder F, Engqvist L, Mehlis M, Bakker TCM, Schwarzer J, Thünken T, 2011. Costly plastic morphological responses to predator specific odour cues in three-spined sticklebacks (*Gasterosteus aculeatus*). Evol Ecol 25:641-656.

Frommen JG, Luz C, Mazzi D, Bakker TCM, 2008. Inbreeding depression affects fertilization success and survival but not breeding coloration in threespine sticklebacks. Behaviour 145:425-441.

Frommen JG, Mehlis M, Bakker TCM, 2009a. Predator-inspection behaviour in female three-spined sticklebacks *Gasterosteus aculeatus* is associated with status of gravidity. J Fish Biol 75:2143-2153.

Frommen JG, Rahn AK, Schroth SH, Waltschyk N, Bakker TCM, 2009b. Mate-choice copying when both sexes face high costs of reproduction. Evol Ecol 23:435-446

Godin J-GJ, Briggs SE, 1996. Female mate choice under predation risk in the guppy. Anim Behav 51:117-130.

Godin J-GJ, Dugatkin LA, 1996. Female mating preference for bold males in the guppy, *Poecilia reticulata*. Proc Natl Acad Sci USA 93:10262-10267.

Grafen A, Hails R, 2002. Modern statistics for the life sciences. Oxford: Oxford University Press.

Griffith SC, Sheldon BC, 2001. Phenotypic plasticity in the expression of sexually selected traits: neglected components of variation. Anim Behav 61:987-993.

Groenewoud F, Frommen JG, Josi D, Tanaka H, Jungwirth A, Taborsky M, 2016. Predation risk drives social complexity in cooperative breeders. Proc Natl Acad Sci USA 113:4104-4109.

Gross HP, 1978. Natural selection by predators on the defensive apparatus of three-spined stickleback, *Gasterostrus aculeatus* L. Canad J Zool 56:398-413.

Gygax M, Rentsch AK, Rudman SM, Rennison DJ, 2018. Differential predation alters pigmentation in threespine stickleback (*Gasterosteus aculeatus*). J Evol Biol 31:1589-1598.

Head ML, Fox RJ, Barber I, 2017. Environmental change mediates mate choice for an extended phenotype, but not for mate quality. Evolution 71:135-144.

Heckel G, Zbinden M, Mazzi D, Kohler A, Reckeweg G, Bakker TCM, Largiadèr CR, 2002. Microsatellite markers for the three-spined stickleback (*Gasterosteus aculeatus* L.) and their applicability in a freshwater and an anadromous population. Conservation Genetics 3:79-81.

Hettyey A, Tóth Z, Thonhauser KE, Frommen JG, Penn DJ, Van Buskirk J, 2015. The relative importance of prey-borne and predator-borne chemical cues for inducible antipredator responses in tadpoles. Oecologia 179:699-710.

Hettyey A, Üveges B, Móricz ÁM, Drahos L, Capon RJ, Van Buskirk J, Tóth Z, Bókony V, 2019. Predator-induced changes in the chemical defence of a vertebrate. J Anim Ecol 88:1925-1935.

Heuschele J, Mannerla M, Gienapp P, Candolin U, 2009. Environment-dependent use of mate choice cues in sticklebacks. Behav Ecol 20:1223-1227.

Hiermes M, Rick IP, Mehlis M, Bakker TCM, 2016. The dynamics of color signals in male threespine sticklebacks *Gasterosteus aculeatus*. Curr Zool 62:23-31.

Hoogland R, Morris D, Tinbergen N, 1956. The spines of sticklebacks (*Gasterosteus* and *Pygosteus*) as means of defence against predators (*Perca* and *Esox*). Behavior 10:205-236.

Huntingford FA, Lazarus J, Barrie BD, Webb S, 1994. A dynamic analysis of cooperative predator inspection in sticklebacks. Anim Behav 47:413-423.

Ingleby FC, Hunt J, Hosken DJ, 2010. The role of genotype-by-environment interactions in sexual selection. J Evol Biol 23:2031-2045.

Johnson S, Candolin U, 2017. Predation cost of a sexual signal in the threespine stickleback. Behav Ecol 28:1160-1165.

Kemper JH, 1995. Role of the three-spined stickleback *Gasterosteus aculeatus* L. in the food ecology of the spoonbill *Platalea leucorodia*. Behaviour 132:1285-1299.

Kim SY, Velando A, 2014. Stickleback males increase red coloration and courtship behaviours in the presence of a competitive rival. Ethology 120:502-510.

Koga T, Backwell PRY, Jennions MD, Christy JH, 1998. Elevated predation risk changes mating behaviour and courtship in a fiddler crab. Proc R Soc B 265:1385-1390.

Kokko H, Brooks R, Jennions MD, Morley J, 2003. The evolution of mate choice and mating biases. Proc R Soc B 270:653-664.

Kotiaho JS, Simmons LW, Tomkins JL, 2001. Towards a resolution of the lek paradox. Nature 410:684-686.

Kozak GM, Boughman JW, 2012. Plastic responses to parents and predators lead to divergent shoaling behaviour in sticklebacks. J Evol Biol 25:759-769.

Kozak GM, Boughman JW, 2015. Predator experience overrides learned aversion to heterospecifics in stickleback species pairs. Proc R Soc B 282:20143066.

Kraak SBM, Bakker TCM, 1998. Mutual mate choice in sticklebacks: attractive males choose big females, which lay big eggs. Anim Behav 56:859-866.

Kuijper B, Pen I, Weissing FJ, 2012. A guide to sexual selection theory. Ann Rev Eco Evol Syst 43:287-311.

Künzler R, Bakker TCM, 2001. Female preference for single and combined traits in computer animated stickleback males. Behav Ecol 12:681-685.

Lehtiniemi M, 2005. Swim or hide: predator cues cause species specific reactions in young fish larvae. J Fish Biol 66:1285-1299.

Lewontin RC, 1974. The genetic basis of evolutionary change. New York: Columbia University Press.

Lima SL, Bednekoff PA, 1999. Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. Am Nat 153:649-659.

Lindholm AK, Head ML, Brooks RC, Rollins LA, Ingleby FC, Zajitschek SRK, 2014. Causes of male sexual trait divergence in introduced populations of guppies. J Evol Biol 27:437-448.

Maan ME, Seehausen O, 2011. Ecology, sexual selection and speciation. Ecol Lett 14:591-602.

Magnhagen C, 1991. Predation risk as a cost of reproduction. Trends Ecol Evol 6:183-185.

Maitland PS, 1965. The feeding relationshios of salomon, trout, minnows, stone loach and 3-spined sticklebacks in the river Endrick, Scotland. J Anim Ecol 34:109-133.

Marchinko KB, 2009. Predation's role in repeated phenotypic and genetic divergence of armor in threespine stickleback. Evolution 63:127-138.

McDonald CG, Reimchen TE, Hawryshyn CW, 1995. Nuptial colour loss and signal masking in *Gasterosteus*: an analysis using video imaging. Behaviour 132:963-977.

McGhee KE, Feng S, Leasure S, Bell AM, 2015. A female's past experience with predators affects male courtship and the care her offspring will receive from their father. Proceedings of the Royal Society B 282:20151840.

McLennan DA, McPhail JD, 1990. Experimental investigations of the evolutionary significance of sexually dimorphic nuptial colouration in *Gasterosteus aculeatus* (L.): the relationship between male colour and female behaviour. Canad J Zool 68:482-492.

Mehlis M, Bakker TCM, Langen K, Frommen JG, 2009. Cain and Abel reloaded? Kin recognition and male-male aggression in three-spined sticklebacks *Gasterosteus aculeatus* L. J Fish Biol 75:2154-2162.

Meuthen D, Baldauf SA, Bakker TC, Thünken T, 2018. Neglected patterns of variation in phenotypic plasticity: age-and sex-specific antipredator plasticity in a cichlid fish. Am Nat 191:475-490.

Milinski M, Bakker TCM, 1990. Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. Nature 344:330-333.

Milinski M, Griffiths S, Wegner KM, Reusch TBH, Haas-Assenbaum A, Boehm T, 2005. Mate choice decisions of stickleback females predictably modified by MHC peptide ligands. Proc Natl Acad Sci USA 102:4414-4418.

Moodie GEE, 1972. Predation, natural selection and adaptation in an unusual 3-spine stickleback. Heredity 28:155-167.

Outomuro D, Johansson F, 2015. Bird predation selects for wing shape and coloration in a damselfly. J Evol Biol 28:791-799.

Peichel CL, Nereng KS, Ohgi KA, Cole BLE, Colosimo PF, Buerkle CA, Schluter D, Kingsley DM, 2001. The genetic architecture of divergence between threespine stickleback species. Nature 414:901-905.

Pfennig DW, Wund MA, Snell-Rood EC, Cruickshank T, Schlichting CD, Moczek AP, 2010. Phenotypic plasticity's impacts on diversification and speciation. Trends Ecol Evol 25:459-467.

Pilakouta N, Alonzo SH, 2014. Predator exposure leads to a short-term reversal in female mate preferences in the green swordtail, *Xiphophorus helleri*. Behav Ecol 25:306-312.

Pilakouta N, Correa MA, Alonzo SH, 2017. Predation risk reduces a female preference for heterospecific males in the green swordtail. Ethology 123:95-104.

Price SA, Friedman ST, Wainwright PC, 2015. How predation shaped fish: the impact of fin spines on body form evolution across teleosts. Proc R Soc B 282:20151428.

Price TD, 2006. Phenotypic plasticity, sexual selection and the evolution of colour patterns. J Exp Biol 209:2368-2376.

Reimchen TE, 1980. Spine deficiency and polymorphism in a population of *Gasterosteus aculeatus*: an adaption to predators? Can J Zool 58:1232-1244.

Reimchen TE, 1994. Predators and morphological evolution in threespine stickleback. In: Bell MA, Foster SA, editors. The evolutionary biology of the threespine stickleback Oxford: Oxford University Press. p. 240-276.

Reimchen TE, Nosil P, 2004. Variable predation regimes predict the evolution of sexual dimorphism in a population of threespine stickleback. Evolution 58:1274-1281.

Relyea RA, 2001. Morphological and behavioral plasticity of larval anurans in response to different predators. Ecology 82:523-540.

Rick IP, Bakker TCM, 2008a. Color signaling in conspicuous red sticklebacks: do ultraviolet signals surpass others? BMC Evol Biol 8:189.

Rick IP, Bakker TCM, 2008b. UV wavelengths make female three-spined sticklebacks (*Gasterosteus aculeatus*) more attractive for males. Behav Ecol Sociobiol 62:439-445.

Roberts ML, Buchanan KL, Evans MR, 2004. Testing the immunocompetence handicap hypothesis: a review of the evidence. Anim Behav 68:227-239.

Robinson MR, van Doorn GS, Gustafsson L, Qvarnstrom A, 2012. Environment-dependent selection on mate choice in a natural population of birds. Ecol Lett 15:611-618.

Rowland W, 1989. Mate choice and the supernormality effect in female sticklebacks (*Gasterosteus aculeatus*). Behav Ecol Sociobiol 24:433-438.

Rowland WJ, 1984. The relationship among nuptial coloration, aggression, and courtship of male three-spined sticklebacks, *Gasterosteus aculeatus*. Canad J Zool 62:999-1004.

Rowland WJ, 1995. Do female stickleback care about male courtship vigour? Manipulation of display tempo using video playback. Behaviour 132:951-961.

Rowland WJ, Baube CL, Horan T, 1991. Signalling of sexual receptivity by pigmentation pattern in female sticklebacks. Anim Behav 42:243-249.

Ruell EW, Handelsman CA, Hawkins CL, Sofaer HR, Ghalambor CK, Angeloni L, 2013. Fear, food and sexual ornamentation: plasticity of colour development in Trinidadian guppies. Proc R Soc B 280:20122019.

Ryan MJ, Tuttle MD, Rand AS, 1982. Bat predation and sexual advertisement in a neotropical anuran. Am Nat 119:136-139.

Stein LR, Bell AM, 2014. Paternal programming in sticklebacks. Anim Behav 95:165-171.

Stevens M, Cuthill IC, 2005. The unsuitability of HTML-based colour charts for estimating animal colours - a comment on Berggren and Merilä (2004). Front Zool 2:14.

Tinbergen N, 1952. The curious behavior of the stickleback. Scientific American 187:22-27.

Tollrian R, Harvell CD, 1999. The ecology and evolution of inducible defenses. New Jersey: Princeton University Press.

West-Eberhard MJ, 2003. Developmental plasticity and evolution. Oxford: Oxford University Press.

Whoriskey FG, Fitzgerald GJ, 1985. The effect of bird predation on an estuarine stickleback (Pisces, Gasterosteidae) community. Canad J Zool 63:301-307.

Winandy L, Denoel M, 2015. Expression of sexual ornaments in a polymorphic species: phenotypic variation in response to environmental risk. J Evol Biol 28:1049-1056.

Wolf M, van Doorn GS, Leimar O, Weissing FJ, 2007. Life-history trade-offs favour the evolution of animal personalities. Nature 447:581-584.

Woods Jr WA, Hendrickson H, Mason J, Lewis SM, 2007. Energy and predation costs of firefly courtship signals. Am Nat 170:702-708.

Wootton RJ, 1976. The biology of the sticklebacks. London: Academic Press.

Wund MA, Baker JA, Golub JL, Foster SA, 2015. The evolution of antipredator behaviour following relaxed and reversed selection in Alaskan threespine stickleback fish. Anim Behav 106:181-189.

Zuk M, Kolluru GR, 1998. Exploitation of sexual signals by predators and parasitoids. Quart Rev Biol 73:415-438.

Figure legends

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

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

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

Fig. 1

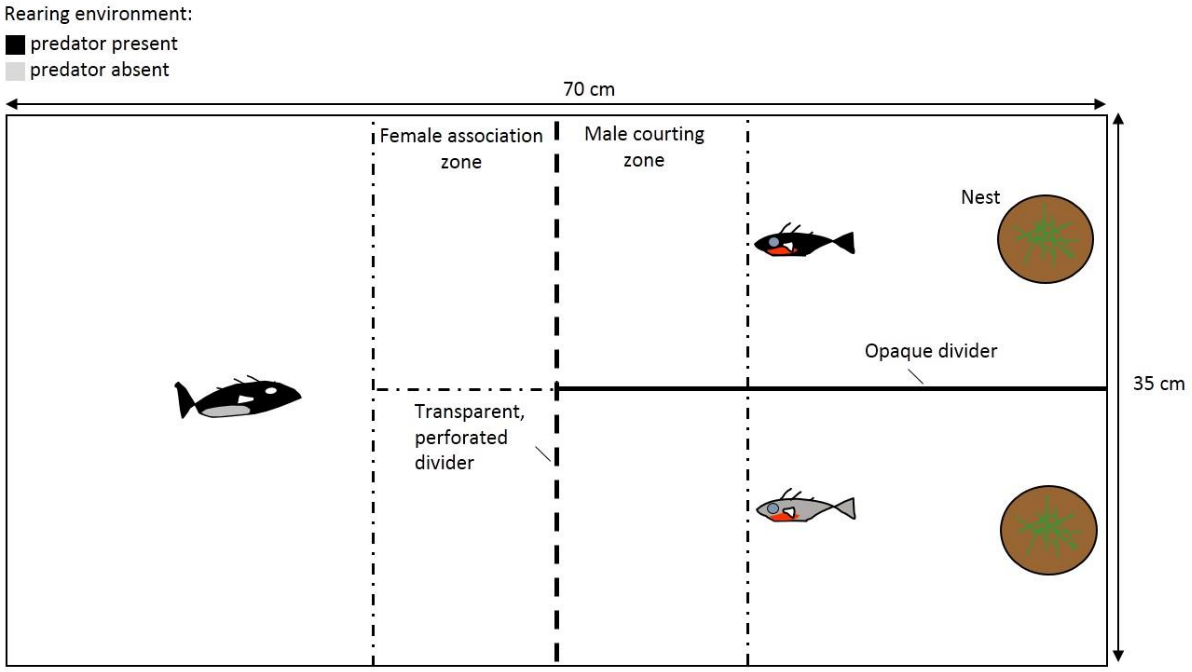

Rearing environment:
■ predator present
▨ predator absent
70 cm
Female association zone
Male courting zone
Nest
Opaque divider
35 cm
Transparent, perforated divider

Fig. 2

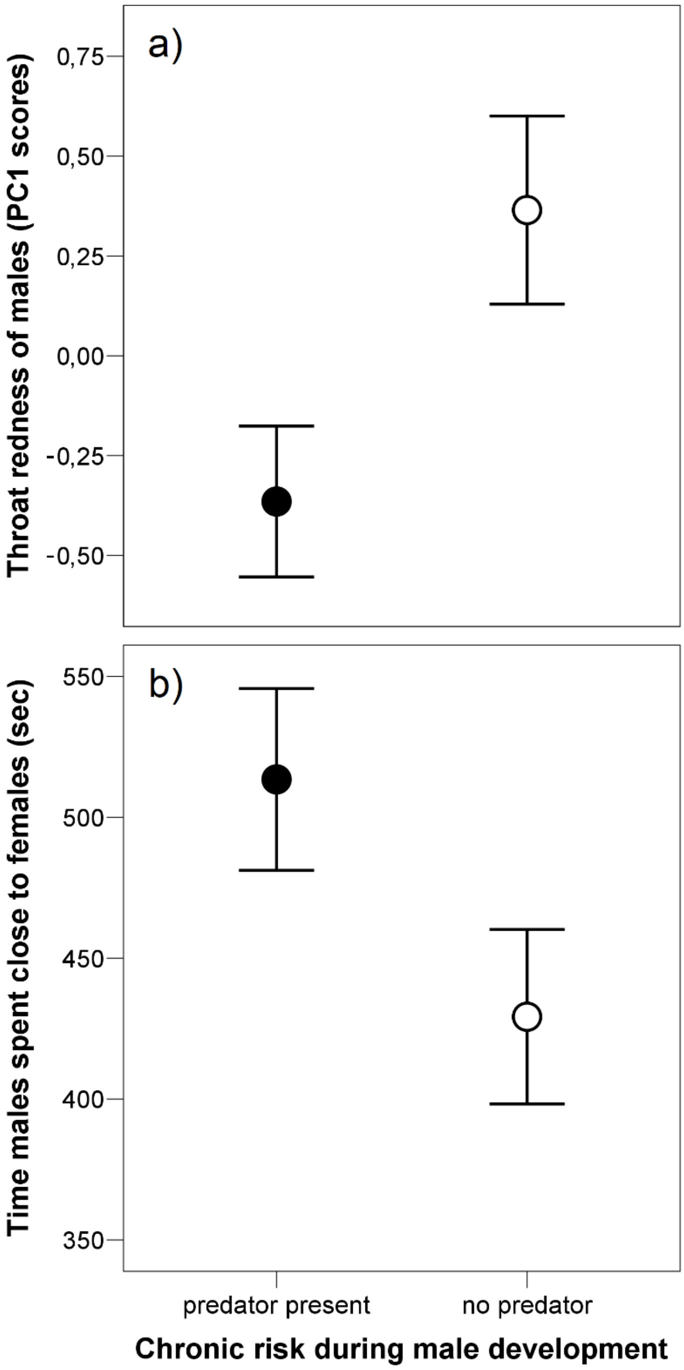
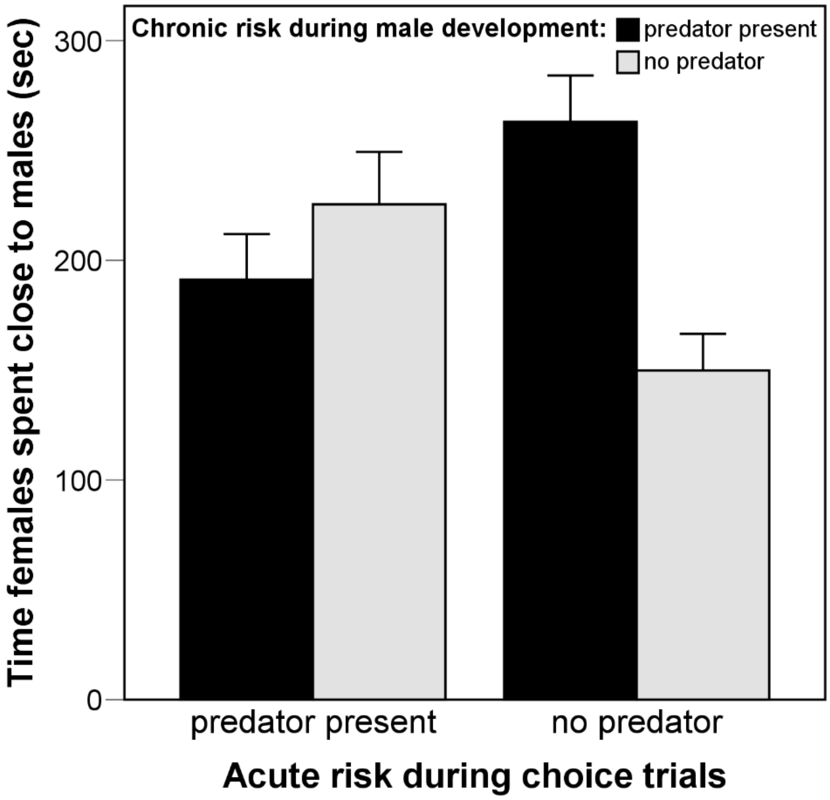

a)
Throat redness of males (PC1 scores)
0,75
0,50
0,25
0,00
-0,25
-0,50
b)
Time males spent close to females (sec)
550
500
450
400
350
predator present
no predator
Chronic risk during male development

Fig. 3


Chronic risk during male development:
predator present
no predator
Time females spent close to males (sec)
300
200
100
0
predator present
no predator
Acute risk during choice trials