Stickleback Males Increase Red Coloration and Courtship Behaviours in the Presence of a Competitive Rival

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Abstract

In species where females preferentially select the most colourful males, males may strategically invest in courtship and nuptial colour according to the presence of rivals. In this experimental study, we tested this in the three-spined stickleback (*Gasterosteus aculeatus*) in which mature males exhibit carotenoid-based red coloration to attract mates and defend their territories against male competitors. We challenged experimental males with either a red-ornamented dummy male or a non-ornamented dummy for five min per day in six-d experimental trial, which was repeated twice during the breeding season. We found that the males presented with a coloured rival exhibited more frequent courtship behaviours (i.e. fanning and gluing) to females than those presented with a non-coloured intruder during the second experimental trial. At the end of each trial, the experimental males also showed a significantly larger area of red coloration in the presence of a coloured intruder. Our findings suggest that male sticklebacks regulate mating effort according to the presence of competitive rivals by increasing their investment in costly signals when successful mating and territory defence is at risk.

Introduction

Secondary sexual traits of males, such as courtship behaviours and ornaments, have evolved for many reasons, including female choice, male contests, female/male game and mate stimulation (Darwin 1871; Grafen 1990; Maynard-Smith 1991; Andersson 1994; Wachtmeister 2001; Servedio et al. 2013). A prevalent theory suggests that secondary sex traits act as an 'honest signal', indicating high fitness in context other than mating ('handicap principle': Zahavi 1975, 1977), and females are selected to discriminate the differences of males in such signals, which are correlated with selective advantage, by increasing the expected number of their own genes in future generations (Kodric-Brown & Brown 1984; Grafen 1990). One classic assumption of the handicap model is that, to be honest, the expression of signals must be costly and dependent on genetic or phenotypic quality (Maynard-Smith & Harper 2003), although signals can also be honest by design and convention (Hasson 1997; Harper 2006) or need not be costly depending

on their biological details (Számadó 2011). A number of empirical studies have demonstrated costs and condition dependence of courtship displays and ornaments despite several confounding problems (reviewed in Kotiaho 2001; Cotton et al. 2004). However, sexual signalling is a component of reproductive effort (Getty 2006), and thus, in iteroparous organisms, life-history trade-offs affect the amount of resources invested into current sexual advertisement (Kokko 1997; Hunt et al. 2004; Getty 2006). Therefore, sexual signalling effort will depend on both future breeding opportunities and the current level of mating competition, that is, signalling effort of other males in the population (Lindström et al. 2009).

In natural populations, mating conditions are often variable and natural selection should favour phenotypic plasticity in mating effort (e.g. Wong & Svensson 2009). Similarly to sperm competition games, males should increase signalling effort in the presence of competitive male rivals because the risk of unsuccessful mating increases. However, above a certain level of male competition, the optimal strategy is to reduce

mating effort (Bretman et al. 2011). Theoretical and empirical studies of sexual selection often assumed that sexual traits are fixed throughout the mating season, but in some species, males may dynamically modulate signalling effort over the breeding season according to breeding conditions (e.g. Velando et al. 2006; Gautier et al. 2008). In particular, males may adjust their nuptial colours (in fish), song and courtship displays (in birds) to the presence of other male competitors (e.g., Galeotti et al. 1997; Candolin 1999a). Secondary sexual traits function both in male/male competition and as cues for female choice in many species (reviewed in Berglund et al. 1996). For example, male nuptial colours in many territorial fish species may be used for both attracting females and threatening rivals. Studies of the three-spined stickleback (*Gasterosteus aculeatus*) showed that the presence of male competition influences the expression of red nuptial coloration that females use as criteria for mate choice (Candolin 1999a, 2000a; Wong et al. 2007). Therefore, males should increase their fitness by adjusting the expression of costly sexual traits to the level of competition for mating.

In this experimental study, we tested whether males are able to adjust their signalling effort, that is nuptial coloration and courtship display, to attractiveness of a dummy rival in their territories by using the three-spined stickleback as a study system. In this species, females base their mate choice mainly on the intensity of the males' red nuptial coloration (Rowland 1994), which is an honest sexual trait based on carotenoid pigments signalling their ability to resist parasites, disease or oxidative stress and longevity (Pike et al. 2007). Males defend a territory, build a nest in which one or more females will deposit eggs and provide intensive parental care (i.e., fanning of the eggs and guarding of the fry). As stickleback males (as well as females) can breed several times within a single breeding season, but reproduction is costly, they should allocate their resource and energy between current reproduction and condition (Chellappa et al. 1989; Poizat et al. 1999; Candolin 2000b; Pike et al. 2007). Given this breeding system, there may be selection pressure on males to allocate their resource and energy in sexual traits at optimal level. A previous study showed that after mating competition with other males, subordinate males reduce nuptial coloration probably due to the socially imposed costs of signalling, that is, risk of fights (Candolin 1999a). In our experiment, by using a dummy, we avoid social costs associated with male-male competition and study if red coloration in male rival is used as a cue to adjust signalling effort.

Here, we challenged sexually mature males with a territory and nest with either an attractive and competitive rival (dummy with a red nuptial colour) or a non-coloured dummy across two mating events in the season. We predict that males with an attractive and competitive rival will invest more in courtship effort and red coloration if they adjust mating strategy in social context. As future breeding opportunities decrease as the breeding season progresses, we expect an increased signalling effort and a stronger effect of competitive rivals in the second mating events.

Methods

Fish Collection and Rearing Conditions

We collected sexually immature three-spined sticklebacks with handnets from three nearby localities at Rias Baixas (Rio Vigo, Rio Umia and Rio Tamuxe), Galicia, Spain during February 2012. Most Galician populations are annual populations, and the majority of fish seem to reproduce a single spawning season (S.-Y. Kim and A. Velando, personal observation). The three-spined stickleback is a locally abundant species at the capture sites. A total of 70 fish were collected, of which 22 eventually mature males and 23 mature females were used in this study. Up to 30 fish at a time were transported in a 25 l tank with an air stone to a laboratory at the University of Vigo. After transport, which took <2 h, fish were acclimated to the laboratory temperature before releasing in holding aquaria. All fish were healthy on arrival to the laboratory and during acclimatization. Captured fish were housed in three 100 l holding aquaria, each containing <30 fish with a water filter, an air stone and several artificial plants, at 13–19°C (natural range of seasonal water temperature in the sampling sites) under natural photoperiod. The laboratory was air-conditioned if necessary to avoid heat stress. Fish were fed daily *ad libitum* with bloodworms. To prevent the risk of whitespot infection, the salinity was adjusted with sea salt to approx. 1.5 ppt. Wild fish were sampled with permission by the Xunta de Galicia (015/2012). This study complied with current laws of Spain and Animal Ethics Guidelines of the University of Vigo. After the experiment, study fish were retained in an artificial pond with suitable living conditions as breeding stock for future experiments.

When males began to develop blue eye coloration, which indicates sexual maturation, they were allocated in individual aquaria (33 × 18 × 19 cm) and maintained under the same photoperiod, temperature, water and diet conditions as the holding aquaria.

Each individual aquarium contained a sponge filter, an artificial plant and a Petri dish filled with sand for nesting. One hundred 5 cm long strands of green polyester thread were provided as nesting materials (see also Pike et al. 2007). Males were not in visual contact with each other.

Experimental Procedures

As soon as a pair of males from the same locality had completed nest building (May-Jul), we randomly assigned one to the rival present group and the other to the rival absent group then begun daily treatment and behavioural observation, which lasted during 6 d (rival absent: n = 11 males; rival present: n = 11 males). Therefore, the dates of maturation and nest building did not differ between the two experimental groups (t_{20} = −0.302, p = 0.766; t_{20} = −0.069, p = 0.946). In the morning, males of the rival present group were challenged with a dummy stickleback with red colour on cheeks and belly, simulating an attractive and competitive rival whereas males of the other group were presented with a dummy stickleback of the same size and morphology without red colour. The dummies were made of white clay and painted with manicure for colour (bright red for the nuptial colour area) and waterproofing then attached to a thin stick for handling. Lateral body area and red colour area of the dummy were 392 mm^2 and 41 mm^2, respectively, and those of study males ranged between 182 and 337 mm^2 and 0.02–25 mm^2, respectively. Note that body area of fish measured in this study excluded area of fins, and thus the size of dummies was actually similar to that of male stickle-backs, including body and fin areas (for methods, see below; Frischknecht 1993; Candolin 1999b). Mean colour intensity of the red nuptial colour area of the red-ornamented dummy was 51.5, slightly brighter than our study males in which the red colour intensity ranged 56.7–134.0. Thus, the area and colour intensity of red ornament of the dummy was slightly exaggerated, but within the natural range, to make it sure that experimental males perceive it as a competitive rival (S.-Y. Kim & A. Velando, own data). We simulated territorial invasion by moving the dummy within 5 cm proximity (without physical contact) and visual range of each focal fish during 5 min and counted number of attacks against the dummy. As the non-coloured dummy had no sexual ornament, it was probably not perceived as a rival for mating, but as a potential egg predator or nest destructor, by male sticklebacks in the rival absent group.

In the afternoon, the paired males from the two treatment groups in their own individual aquaria were presented with the same gravid female during 5 min and their courtship behaviours to attract the female to their nests were observed. During the courtship test, most focal males immediately responded to the appearance of a gravid female by approaching her, exhibiting the 'zig-zag dance' and even biting and then made intensive ventilating movements over his nest ('fanning') and in addition glued kidney secretion to the nest ('gluing'), which imitate parental care for eggs and nest building (Tinbergen & Van Iersel 1946; Rowland 1994). In this study, we quantified fanning and gluing behaviours by counting numbers of fanning displays (i.e. a bout of continuous and intense movements of pectoral fins over the nest) and gluing episodes in each courtship test. Individual males were allowed direct contact with females to induce them to court normally, but the females never spawned. The order of aggression test with a dummy and courtship test with a female were altered every-day between the paired males from the rival present and absent groups.

On the sixth day, immediately after courtship test each focal male was weighed with a digital balance (to the nearest 0.01 g) and photographed for colour analysis. The protocol for photography and image analysis corresponds to that described previously (Frischknecht 1993; Candolin 1999b) with minor modifications. Each focal fish was placed into a small transparent plastic box containing water and a black sponge that fixed the sample on its lateral side (either left or right to reduce handling time) and served as a non-reflective background. The sample was illuminated with a LED lamp from the above while photographing with a digital camera (Nikon D90). The positions of photographer, camera, lamp and fish were always the same. The whole procedure of weighing and photographing took <1 min.

We measured the whole body area and the area and intensity of the red nuptial coloration from the digital images by using IMAGE analysis software (analySIS FIVE, Olympus). We determined the whole body area of fish except fins as the region of interest then selected areas that ranged in colour between yellow and red (hue: 1–60 and 340–359; saturation: 50–255; intensity: 0–255). The size of the red area and mean colour intensity of the area were recorded.

Approximately, 10 d after the completion of the first experimental trial, fish were moved to new clean aquaria then allowed again to build nests. When both the paired males from the rival present and absent groups built their new nests we repeated the same

experimental procedure during another 6 d (control: n = 8 males; experimental: n = 8 males). Sample size was reduced in the second trial as some males did not build a new nest. The experimental treatment of each focal male in the second trial was the same as the first trial. The study males were weighed and photographed again after the second trial.

As soon as the entire experimental procedure during 6 d was completed, the half of randomly selected control (n = 6) and experimental (n = 5) males were allowed to breed by introducing a fully gravid female into the individual aquaria for up to 1 h. This was to see whether our experimental treatment influences their reproductive performances. However, as only four males from the rival absent group and three males from the rival present group succeed in spawning it was impossible to make a meaningful comparison.

Statistical Analysis

We explored factors and covariates associated with body mass, aggressive response to the dummy, courtship behaviours (i.e. fanning and gluing) and red nuptial coloration of focal males by using linear and generalized linear mixed-effect models (LMEs and GLMMs), including male identity nested within the paired rival present/absent group as a random effect, to account for the non-independence of focal individuals presented with the same female on the same day. Prior to the analysis, effects of the population of origin, date of maturation and date of test were explored, but never found to be significant. Therefore, these variables were not included in subsequent analyses to avoid over-parameterization. All statistical analyses were performed using the R (version 2.15.2, R Development Core Team 2012). The LMEs and GLMMs were implemented using lme function of the R package nlme (Pinheiro et al. 2013) and lmer function of the lme4 package (Bates et al. 2013), respectively.

We used GLMMs with Poisson error distribution and a log link function to test whether challenging males with a coloured rival influences their territorial (number of attacks toward the dummy rival, n = 228 observations) and courtship behaviours (number of fanning and number of gluing, n = 228 observations). In all GLMMs, treatment, trial, day (1–6 d), body mass and treatment × trial interaction were included as fixed effects. We excluded other interactions, which were not of direct interest, to avoid over-parameterization due to the small sample size. The full model was reduced by removing fixed effects sequentially based on Akaike information criterion (AIC) (Crawley 2007).

To examine whether the experimental treatment influences investment of males in their sexual ornaments, body mass, area and intensity of red coloration on the last (sixth) day of daily treatment across two experimental trials were analysed using LMEs. Treatment (coloured rival present/absent), trial (first/second) and their interaction were included as fixed effects in all LMEs. Body mass and size were included as additional fixed effects in the LME fitted to the size of red area. In the analysis for the intensity of red area, body mass and the size of red area were included as additional fixed effects. Non-significant fixed effects were dropped sequentially from the full model by using deletion tests to assess the significance of the change in the likelihood ratio that resulted when a given term was removed from the LME using maximum likelihood. Then, the final LME model was fitted using the default restricted maximum likelihood to obtain estimates and significance levels of fixed effects (Crawley 2007).

Besides, to test whether area of red coloration honestly represents individual condition across different experimental treatments and trials we analysed the interacting effects of body condition, treatment and trial on this trait in an additional model. Body condition of each focal fish on the day of photographing, that is, the sixth day of treatment in each trial, was calculated as the residual from a linear regression of body mass against total body area ($r = 0.922$, $p < 0.001$; see Schulte-Hostedde et al. 2005).
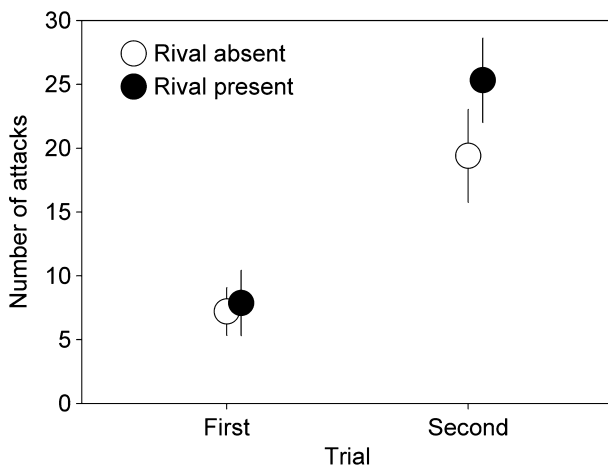
Results

The level of aggressiveness of experimental male sticklebacks toward a dummy was low during the first experimental trial then increased during the second trial in both coloured rival present and absent groups, but the difference between the two experimental periods was greater in the rival present group (significant interacting effect, treatment × trial, in Table 1, Fig. 1). Therefore, fish challenged with a coloured rival turned more aggressive than fish from the rival absent group in the second trial. The level of aggression progressively increased during 6 d of daily treatment (Table 1). Interestingly, larger (heavier) males were less aggressive toward a dummy rival than smaller males (Table 1).

The levels of two different courtship behaviours, fanning and gluing, showed similar results (Table 2, Fig. 2). Males from the both treatment groups courted more frequently to the presented females during the second trial than the first trial. During the first 6-d experimental trial, the levels of courtship behaviours

Table 1: Results from generalized linear mixed models with Poisson error distribution and a log link on the number of attacks against a dummy

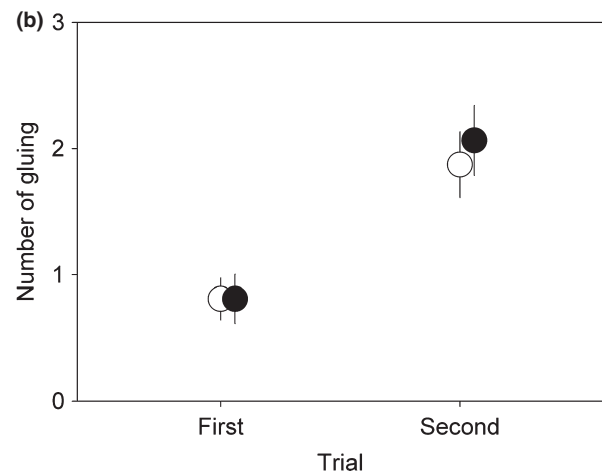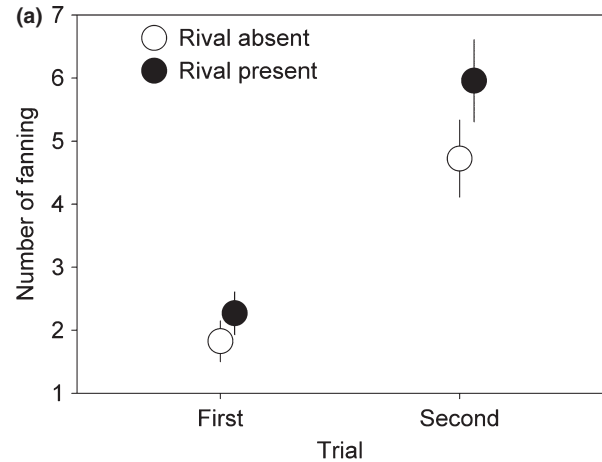| Fixed effects	Est $\pm$ SE	z	p
Intercept	3.041 $\pm$ 0.985	3.089	0.002
Treatment (competitive rival)	0.398 $\pm$ 1.108	0.359	0.720
Trial (second)	1.160 $\pm$ 0.070	16.650	<0.001
Day	0.052 $\pm$ 0.011	4.868	<0.001
Body mass	−3.079 $\pm$ 0.573	−5.374	<0.001
Treatment $\times$ trial	0.922 $\pm$ 0.115	7.983	<0.001

Fixed effect parameters in the final models are presented. Full model: attacks = treatment + trial + day + body mass + treatment $\times$ trial, random effect: male identity (paired group).

Fig. 2: Number of courtship behaviours, (a) fanning and (b) gluing, of males during 5 min according to the experimental treatment and trial.

Fig. 1: Number of attacks towards a dummy rival during 5 min according to the experimental treatment and trial.

showed little difference between the two experimental groups, but during the second trial, the males from the rival present group courted more frequently than those presented with a non-coloured dummy. Larger males tend to court more frequently, and the day of treatment did not influence the courtship behaviours.

Body mass of male sticklebacks was not influenced by treatment, trial and their interaction (LME:

treatment: $t_{10} = 1.519$, p = 0.160; trial: $t_{15} = 0.091$, p = 0.928; treatment $\times$ trial: $t_{14} = −0.512$, p = 0.617). Fish challenged with a coloured dummy rival had significantly larger areas of red nuptial coloration than the others across the two experimental trials (Table 3, Fig. 3a). However, when we looked at the interacting effect of treatment and trial on the colour

Table 2: Results from generalized linear mixed models with Poisson error distribution and a log link on the courtship behaviours

Fixed effects	(a) Number of fanning			(b) Number of gluing		
	Est $\pm$ SE	z	p	Est $\pm$ SE	z	p
Intercept	−1.765 $\pm$ 0.748	−2.359	0.018	−3.513 $\pm$ 1.108	−3.169	0.002
Treatment (competitive rival)	−0.014 $\pm$ 0.406	−0.033	0.973	−0.426 $\pm$ 0.528	−0.806	0.420
Trial (second)	0.826 $\pm$ 0.120	6.894	<0.001	0.763 $\pm$ 0.184	4.144	<0.001
Body mass	1.946 $\pm$ 0.652	2.987	0.003	2.747 $\pm$ 0.977	2.813	0.005
Treatment $\times$ trial	0.485 $\pm$ 0.175	2.778	0.005	0.844 $\pm$ 0.305	2.773	0.006

Fixed effect parameters in the final models are presented. Full model: fanning/gluing = treatment + trial + day + body mass + treatment $\times$ trial, random effect: male identity (paired group).

Table 3: Results from linear mixed models on the effect of experimental treatment and trial (first/second) on the size and intensity of red areas in three-spined stickleback males

| Fixed effects	Est $\pm$ SE	t	p
(a) Size			
Intercept	3.946 ± 1.523	2.591	0.020
Treatment (competitive rival)	4.991 ± 2.153	2.317	0.043
(b) Intensity			
Intercept	42.214 ± 13.786	3.062	0.009
Treatment (competitive rival)	0.471 ± 5.973	0.079	0.939
Trial (second)	8.886 ± 6.375	1.394	0.187
Body mass	36.215 ± 12.601	2.874	0.013
Treatment $\times$ trial	-21.406 ± 8.897	-2.406	0.032

Fixed effect parameters in the final models are presented. Full model: size = treatment + trial + body mass + body size + treatment $\times$ trial; intensity = treatment + trial + body mass + size of red area + treatment $\times$ trial, random effect: male identity (paired group).

intensity of the red area, we found that the colour intensity value decreased from the first 6-d trial to the second trial in experimental males, whereas the opposite pattern was observed in males from the rival absent group (Table 3, Fig. 3b). A low value of intensity indicates redder coloured fish because red colour reduces the amount of light reflected by silvery skin of sticklebacks (see also Candolin 1999b). Therefore, males challenged with a competitive rival had brighter red areas after the second 6-d trial than after the first trial.

Size of red colour area depended on body condition calculated as mass-size residuals, but this effect differed between the experimental treatments and trials (LME: effect of treatment: $t_{10} = 1.727$, p = 0.115; trial: $t_{10} = 0.087$, p = 0.932; body condition: $t_{10} = 1.506$, p = 0.163; treatment $\times$ trial: $t_{10} = -0.243$, p = 0.813; treatment $\times$ body condition: $t_{10} = -2.655$, p = 0.024; trial $\times$ body condition: $t_{10} = -1.139$, p = 0.281; treatment $\times$ trial $\times$ body condition: $t_{10} = 2.763$, p = 0.020; Fig. 4). In accordance with the hypothesis that honest sexual signal represents the bearer's body condition, our results suggest that size of red colour area was an honest sexual signal of condition only in males presented with a non-coloured dummy during the first experimental trial and in males challenged with a coloured rival during the second trial (Fig. 4).

Discussion

The results of our experimental study on three-spined sticklebacks support the hypothesis that males adjust their allocation in secondary sexual signals, depending on the presence of a rival. Behavioural effort for
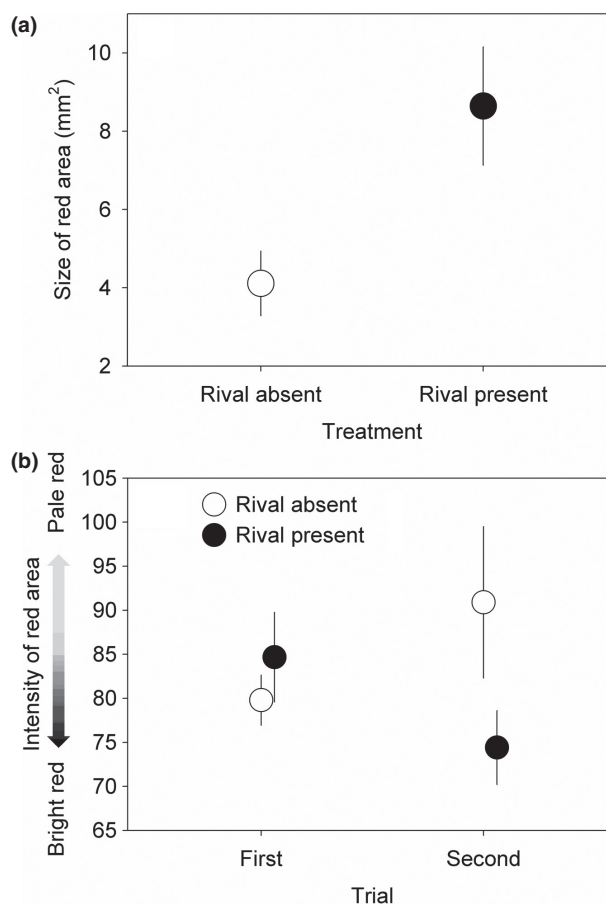
Fig. 3: (a) Size of red area in males challenged with a non-coloured dummy and coloured dummy rival. (b) Colour intensity of red area according to the experimental treatment and trial.

territory defence and courtship increased greatly from the first to second 6-d experimental trial, particularly in fish challenged with a coloured rival. In the presence of a rival with a strong secondary sexual signal, males increased their signalling effort by enlarging and brightening red nuptial area, particularly in the second experimental trial, which was performed later the season. Our results suggest that the influence of mating competition on the signalling strategy of males may depend on the temporal dynamics. The increased signalling effort in the presence of an attractive rival became more evident in the later mating trial.

In this species, early in the season, males establish a territory, which is aggressively defended against intruders (reviewed in Wootton 1984). Aggressive behaviour is important not only for territory maintenance but also for reducing paternity losses by sneaking males (Wootton 1984; Goldschmidt et al. 1992; Candolin & Vlieger 2013). In this species, the function of red colour of intruders as releaser of aggression has
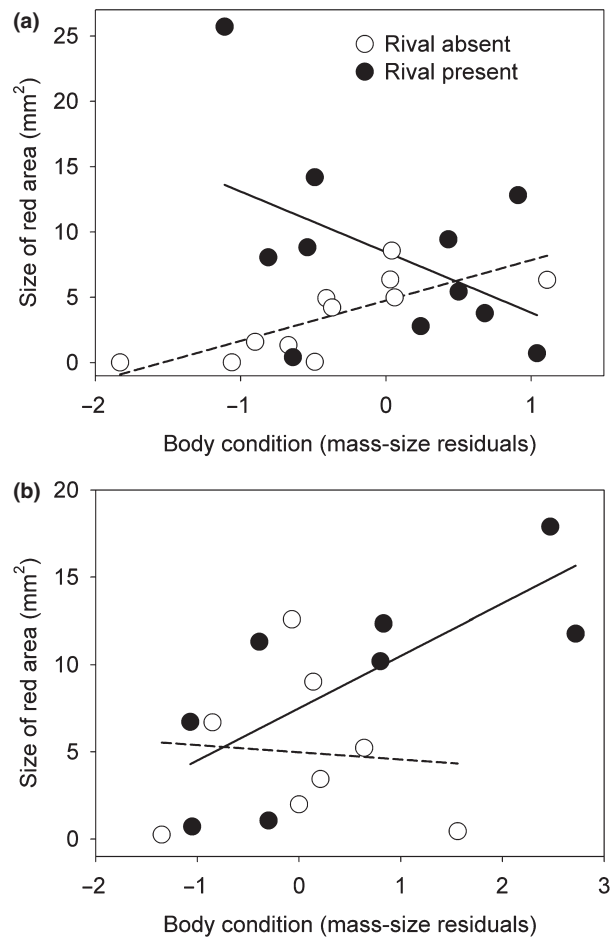
Fig. 4: Relationship between size of red area and body condition, calculated as mass-size residuals, of males from the coloured rival present and absent groups after (a) first and (b) second 6-d experimental trials.

been subject to an intense debate. In an early study, ter Pelkwijk & Tinbergen (1937) reported that male sticklebacks attacked more to dummies with red nuptial coloration than dummies without red patch. Nevertheless, some posterior studies in this species did not support that the red patch functions as a simple releaser of aggression (e.g. Peeke et al. 1969; Wootton 1971; Rowland 1982). The effect of red coloration may be context-dependent, thus for example red coloration elicited more aggression from territorial than non-territorial males (Bolyard & Rowland 1996). This suggests that territorial males are more motivated to defend their nests against a competitive rival. In our study, we found that during the first experimental trial red coloration in the dummy intruder did not influence male aggressiveness, but it gave rise to more attacks during the second trial when males increased their reproductive effort in territory defence and courtship.

Our results suggest that mating effort (i.e. carotenoid-based red coloration and courtship) was rapidly modulated according to a social stimulus (i.e. the presence of colourful rivals in their territory). Both courtship and colour are costly sexual signals in animals, including the three-spined stickleback. Courtship and territorial behaviours can be energetically costly (Vehrencamp et al. 1989; Hack 1997; Basolo & Alcaraz 2003; but see Bennett & Houck 1983). Vigorous courtship behaviours may increase risk of drawing attention of predators and mating competitors and constrain energy for self-maintenance and other activities (Candolin 1997; Kotiaho 2001). Increased allocation of carotenoid pigments in sexual ornaments may restrain the signaller's physiological condition by reducing this limiting resource, which also plays an important role as antioxidants and immunosimulants (Pike et al. 2007). Therefore, the presence of a coloured rival influenced territorial males to increase allocation of carotenoids and energy in their current reproductive opportunity especially during the later experimental trial. An increasing number of studies across diverse taxa, including sticklebacks, also showed that males adjust ejaculate size and quality according to the presence of competitors and the risk of sperm competition (Zbinden et al. 2003; Kilgallon & Simmons 2005). Altogether, these results suggest that males may assess their competence at breeding territory and increase resources allocated to mating/fertilization when mating success is at risk (Bretman et al. 2011).

Sticklebacks breed several times within a single breeding season, and the future breeding opportunities decrease as the season progresses. In our study, the presence of a coloured rival at the breeding territory affected mating effort especially in the second mating trial. In a previous study, it was observed that the honesty of the sexual signal varies over a lifetime and this has been interpreted as a dynamic signalling tactic that depends on future reproductive opportunities (Candolin 2000b). Accordingly, in our study, red carotenoid-based red colouration was not always an honest sexual signal that represents bearer's condition measured as mass-size residuals across the two experimental trials over the season.

In the males that were presented to a non-coloured rival, red coloration honestly indicated condition in the first experiment but not in the second trial performed later the season, probably because when low future opportunities are expected males should invest heavily in the current sexual signals ('terminal investment'; Candolin 1999b; Nielsen & Holman 2012). However, the opposite effect of condition and trial on the sexual signal was observed when males faced a

coloured rival. The experimental males in worse condition invested heavily in red nuptial coloration in the first experimental trial, resulting in a negative correlation between condition and red size area, but in the second trial the signalling tactic changed to be honest. In the first trial of our study, fish in bad condition could have increased their investment in the current mating to compete with the coloured rival, but their condition might constrain such dishonest signalling strategy in the later experimental trial. These results suggest that male-male competition may enforce honesty of sexual signal in late life (Lindström et al. 2009; see also Candolin 2000a; Wong et al. 2007).

Our finding demonstrates that stickleback males adjusted their sexual signalling effort in behaviours and colour in response to the presence of a rival. This flexible signalling strategy would have a fitness consequence if males that reduce sexual signalling effort in the absence of severe mating competition prolong reproductive lifespan and increase their investment in paternal care in both current and future reproductions. On the other hand, in the presence of severe mating competition, there would be selection pressure on males to increase mating success by investing heavily in sexual signals, particularly when future expectation for additional mating opportunities is reduced with time.

Acknowledgements

Literature Cited

Andersson, M. 1994: Sexual Selection. Princeton Univ. Press, Princeton, NJ.

Basolo, A. L. & Alcaraz, G. 2003: The turn of the sword: length increases male swimming costs in swordtails. Proc. R. Soc. B **270**, 1631—1636.

Bates, D., Maechler, M. & Bolker, B. 2013: lme4: Linear Mixed-effects Models Using S4 Classes. R Package Version 0.999999–2. http://lme4.r-forge.r-project.org/.

Bennett, A. F. & Houck, L. D. 1983: The energetic cost of courtship and aggression in a plethodontid salamander. Ecology **64**, 979—983.

Berglund, A., Bisazza, A. & Pilastro, A. 1996: Armaments and ornaments: an evolutionary explanation of traits of dual utility. Biol. J. Linn. Soc. **58**, 385—399.

Bolyard, K. J. & Rowland, W. J. 1996: Context-dependent response to red coloration in stickleback. Anim. Behav. **52**, 923—927.

Bretman, A., Gage, M. J. G. & Chapman, T. 2011: Quick-change artists: male plastic behavioural responses to rivals. Trends Ecol. Evol. **26**, 467—473.

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

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

Candolin, U. 1999b: The relationship between signal quality and physical condition: is sexual signalling honest in the three-spined stickleback? Anim. Behav. **58**, 1261—1267.

Candolin, U. 2000a: Increased signalling effort when survival prospects decrease: male–male competition ensures honesty. Anim. Behav. **60**, 417—422.

Candolin, U. 2000b: Changes in expression and honesty of sexual signalling over the reproductive lifetime of sticklebacks. Proc. R. Soc. B **267**, 2425—2430.

Candolin, U. & Vlieger, L. 2013: Should attractive males sneak: the trade-off between current and future offspring. PLoS One **8**, e57992.

Chellappa, S., Huntingford, F. A., Strang, R. H. C. & Thomson, R. Y. 1989: Annual variation in energy reserves in male 3-spined stickleback, *Gasterosteus aculeatus* L. (Pisces: Gasterosteidae). J. Fish Biol. **35**, 275—286.

Cotton, S., Fowler, K. & Pomiankowski, A. 2004: Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? Proc. R. Soc. B **271**, 771—783.

Crawley, M. J. 2007: The R Book. John Wiley & Sons, Chichester.

Darwin, C. 1871: The Descent of Man, and Selection in Relation to Sex. John Murray, London.

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

Galeotti, P., Saino, N., Sacchi, R. & Moller, A. P. 1997: Song correlates with social context, testosterone and body condition in male barn swallows. Anim. Behav. **53**, 687—700.

Gautier, P., Barroca, M., Bertrand, S., Eraud, C., Gaillard, M., Hamman, M., Motreuil, S., Sorci, G. & Faivre, B. 2008: The presence of females modulates the expression of a carotenoid-based sexual signal. Behav. Ecol. Sociobiol. **62**, 1159—1166.

Getty, T. 2006: Sexually selected signals are not similar to sports handicaps. Trends Ecol. Evol. **21**, 83—88.

Goldschmidt, T., Foster, S. A. & Sevenster, P. 1992: Inter-nest distance and sneaking in the three-spined stickleback. Anim. Behav. **44**, 793—795.

Grafen, A. 1990: Sexual selection unhandicapped by the Fisher process. J. Theor. Biol. **144**, 473—516.

Hack, M. A. 1997: The energetic costs of fighting in the house cricket, *Acheta domesticus* L. Behav. Ecol. **8**, 28—36.

Harper, D. G. C. 2006: Maynard Smith: amplifying the reasons for signal reliability. J. Theor. Biol. **239**, 203—209.

Hasson, O. 1997: Towards a general theory of biological signalling. J. Theor. Biol. **185**, 139—156.

Hunt, J., Brooks, R., Jennions, M. D., Smith, M. J., Bentsen, C. L. & Bussière, L. F. 2004: High-quality male field crickets invest heavily in sexual display but die young. Nature **432**, 1024—1027.

Kilgallon, S. J. & Simmons, L. W. 2005: Image content influences men's semen quality. Biol. Lett. **1**, 253—255.

Kodric-Brown, A. & Brown, J. H. 1984: Truth in advertising. The kinds of traits favored by sexual selection. Am. Nat. **124**, 309—323.

Kokko, H. 1997: Evolutionarily stable strategies of age-dependent sexual advertisement. Behav. Ecol. Sociobiol. **41**, 99—107.

Kotiaho, J. S. 2001: Costs of sexual traits: a mismatch between theoretical considerations and empirical evidence. Biol. Rev. **76**, 365—376.

Lindström, J., Pike, T. W., Blount, J. D. & Metcalfe, N. B. 2009: Optimization of resource allocation can explain the temporal dynamics and honesty of sexual signals. Am. Nat. **174**, 515—525.

Maynard-Smith, J. 1991: Theories of sexual selection. Trends Ecol. Evol. **6**, 146—151.

Maynard-Smith, J. & Harper, D. 2003: Animal Signals. Oxford Univ. Press, Oxford.

Nielsen, M. L. & Holman, L. 2012: Terminal investment in multiple sexual signals: immune-challenged males produce more attractive pheromones. Funct. Ecol. **26**, 20—28.

Peeke, H. V. S., Wyers, E. J. & Herz, M. J. 1969: Waning of the aggressive response to male models in the three-spined stickleback (*Gasterosteus aculeatus* L.). Anim. Behav. **17**, 224—228.

ter Pelwijk, J. J. & Tinbergen, N. 1937: Eine reizbiologische analyse einiger verhaltensweisen von *Gasterosteus aculeatus* L. Z. Tierpsychol. **1**, 193—200.

Pike, T. W., Blount, J. D., Bjerkeng, B., Lindström, J. & Metcalfe, N. B. 2007: Carotenoids, oxidative stress and female mating preference for longer lived males. Proc. R. Soc. B **274**, 1591—1596.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & The R Core team. 2013: nlme: Linear and Nonlinear Mixed Effects Models. R Package Version 3.1-109. http://cran.r-project.org/web/packages/nlme/index.html.

Poizat, G., Rosecchi, E. & Crivelli, A. J. 1999: Empirical evidence of a trade-off between reproductive effort and expectation of future reproduction in female three-spined sticklebacks. Proc. R. Soc. B **266**, 1543—1548.

R Development Core Team. 2012: R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org.

Rowland, W. J. 1982: The effects of male coloration on stickleback aggression: a reexamination. Behaviour **80**, 118—126.

Rowland, W. J. 1994: Proximate determinants of stickleback behaviour: an evolutionary perspective. In: The Evolutionary Biology of the Threespine Stickleback, (Bell, M. A. & Foster, S. A., eds). Oxford Univ. Press, Oxford, pp. 297—344.

Schulte-Hostedde, A. I., Zinner, B., Millar, J. S. & Hickling, G. J. 2005: Restitution of mass-size residuals: validating body condition indices. Ecology **86**, 155—163.

Servedio, M. R., Price, T. D. & Lande, R. 2013: Evolution of displays within the pair bond. Proc. R. Soc. B **280**, 1471—2954.

Számadó, S. 2011: The cost of honesty and the fallacy of the handicap principle. Anim. Behav. **81**, 3—10.

Tinbergen, N. & Van Iersel, J. J. A. 1946: "Displacement reactions" in the three-spined stickleback. Behaviour **1**, 56—63.

Vehrencamp, S. L., Bradbury, J. W. & Gibson, R. M. 1989: The energetic cost of display in male sage grouse. Anim. Behav. **38**, 885—896.

Velando, A., Beamonte, R. & Torres, R. 2006: Pigment-based skin colour in the blue-footed booby: an honest signal of current condition used by females to adjust reproductive investment. Oecologia **149**, 543—552.

Wachtmeister, C. A. 2001: Display in monogamous pairs: a review of empirical data and evolutionary explanations. Anim. Behav. **61**, 861—868.

Wong, B. B. M. & Svensson, P. A. 2009: Strategic male signalling effort in a desert-dwelling fish. Behav. Ecol. Sociobiol. **63**, 543—549.

Wong, B. B. M., Candolin, U. & Lindström, K. 2007: Environmental deterioration compromises socially enforced signals of male quality in three-spined sticklebacks. Am. Nat. **170**, 184—189.

Wootton, R. J. 1971: Measures of the aggression of parental male three-spined sticklebacks. Behaviour **40**, 228—262.

Wootton, R. J. 1984: A Functional Biology of Sticklebacks. Croom Helm, London.

Zahavi, A. 1975: Mate selection-a selection for a handicap. J. Theor. Biol. **53**, 205—214.

Zahavi, A. 1977: The cost of honesty (further remarks on the handicap principle). J. Theor. Biol. **67**, 603—605.

Zbinden, M., Mazzi, D., Künzler, R., Largiadèr, C. R. & Bakker, T. C. M. 2003: Courting virtual rivals increase ejaculate size in sticklebacks (*Gasterosteus aculeatus*). Behav. Ecol. Sociobiol. **54**, 205—209.