Environmental Deterioration Compromises Socially Enforced Signals of Male Quality in Three-Spined Sticklebacks

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ABSTRACT: Social costs are often important in promoting the honesty of sexually selected traits. What happens, then, when social costs are relaxed? In species that breed in shallow coastal waters, increases in the frequency and severity of phytoplankton blooms may undermine the value of visual signals by reducing visibility and, in so doing, lead to dishonest signaling by relaxing the social consequences of high signaling effort for poor-quality individuals. Here, we experimentally test the effects of algally induced water turbidity on the role of male-male competition in facilitating reliable sexual displays in three-spined sticklebacks. We found that males in poor condition reduced their courtship effort in the presence of competition in turbid water. This reduction, however, was to a much lesser extent than that observed in clear water. Thus, courtship under conditions of algal turbidity did not reflect male condition as honestly as courtship in clear water. Algal turbidity also influenced breeding coloration, with males in poor condition reducing their area of red nuptial coloration in turbid conditions. Our findings suggest that anthropogenic disturbance to the signaling environment can potentially reduce the evolutionary potential of sexual selection by diminishing the efficacy of visual displays and weakening socially enforced signals of male quality.

Keywords: environmental disturbance, eutrophication, sexual selection, signal honesty, turbidity, *Gasterosteus aculeatus*.

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The honesty of sexually selected traits is often socially enforced (Candolin 1999a, 2000a, 2000b; Berglund and Rosenqvist 2001). In many taxa, signals of mate attraction also function as badges of status that are used by males to communicate dominance to rivals (Berglund et al. 1996). In a competitive setting, faking quality can have dire consequences for poor-quality males because by increasing their signaling effort in an attempt to attract females, such individuals also risk serious injury by provoking potential rivals (Candolin 2000b). In this way, social costs can play an important role in maintaining signal honesty (Tibbetts and Dale 2004; Wong and Candolin 2005). What happens, then, when social costs are relaxed?

Human-induced changes to the environment, such as changes to visibility or habitat complexity, can potentially ameliorate social constraints by reducing the intensity of male-male competition (Candolin 2004; Järvenpää and Lindström 2004). It remains to be tested, however, whether this, in turn, might diminish the overall strength of sexual selection by constraining the value of signal expression and/or by promoting dishonest signaling. Eutrophication, in this regard, is having an alarming effect on the Baltic Sea and its inhabitants. In the Baltic, an increase in nutrient input is promoting the growth of filamentous algae and phytoplankton, and this, in turn, has caused changes to both habitat structure and water clarity (Larsson et al. 1985; Raateoja et al. 2005). These changes have had dire consequences for the mating systems of several Baltic species of marine fish that breed in shallow coastal waters (Candolin 2004; Järvenpää and Lindström 2004; Candolin et al. 2007). One such species, the three-spined stickleback *Gasterosteus aculeatus*, appears to be a model candidate for investigating how environmental deterioration might affect the expression of sexually selected traits.

Male three-spined sticklebacks establish territories close to the shore, where they build and defend nests constructed from plant material. Males entice females to the nest by performing elaborate and conspicuous courtship displays (Rowland 1994). The male's red breeding coloration also plays a pivotal role in female mate choice and

is believed to communicate a host of male traits ranging from good genes to superior male parental abilities (Barber et al. 2001). The latter is especially important because males alone are responsible for looking after the developing embryos and defending the brood from potential predators. Candolin (1999*b*) previously showed, however, that male sexual signals are not always reliable indicators of male quality. Specifically, because of a life-history trade-off between present and future signaling effort, males in poor physical condition tend to invest heavily in current signaling when future prospects of survival and reproduction are grim (Candolin 1999*b*). Such males make poor choices for females because they are also more likely to cannibalize the female's eggs (Candolin 2000*a*). Candolin (1999*a*, 2000*a*), however, demonstrated that male-male competition facilitates female choice by preventing poor-quality suitors from dishonestly signaling their condition to females. In the Baltic Sea, the socially imposed costs responsible for maintaining signal honesty are likely to be compromised by a deterioration of the stickleback's visual environment as a result of water turbidity from algal blooms. Specifically, because males display close to their nests, turbidity may, in turn, make it more difficult for males to see one another, thus allowing poor-quality individuals to establish territories and to signal dishonestly. In light of this, we predicted that algal turbidity, by reducing visibility, should relax male competition and, in so doing, promote dishonest sexual signaling and reduce the evolutionary potential of sexual selection (Järvenpää and Lindström 2004; Candolin et al. 2007).

Methods

Collecting, Maintenance, and Conditioning

Sticklebacks were collected from the Baltic Sea in southern Finland and housed in separate-sex aquaria under natural light and temperature conditions at the Tvärminne Zoological Research Station. Females were maintained on frozen chironomid larvae. Males, on the other hand, were assigned randomly to one of two feeding regimes (fed and unfed) for 3 weeks to manipulate condition (Candolin 1999*b*, 2000*a*). It is worth noting that male sticklebacks, in this regard, often encounter long periods of food deprivation in nature: males often do not feed for up to 3 weeks at a time (e.g., during the parental phase), and energy reserves often decrease naturally during the course of the breeding season (Candolin 1999*b*). At the end of the 3-week-long conditioning period, males were allowed to construct nests in individual aquaria containing a shallow sand-filled nesting dish and tufts of *Cladophora* as nesting material. After they had finished constructing their nests, males were measured and weighed to quantify body

condition using the Fulton index (body mass/length^3; Ricker 1975) and spine-clipped for identification (Candolin 2000*a*, 2000*b*).

Experimental Setup

One fed and one starved male (size matched to within 1 mm standard length) were randomly assigned, along with their nests, to each of two adjoining compartments of an experimental aquarium. Males were separated by an opaque partition to prevent visual and olfactory contact. Stimulus females were housed in a third compartment separated from the two males.

The experiment consisted of two treatments: trials were run either in clear water (control treatment, $n = 23$) or in water that was made turbid to simulate the algal blooms that occur in the Baltic Sea during the stickleback breeding season (algal treatment, $n = 23$). The latter was achieved by adding *Brachiomonas submarina*, a unicellular planktonic alga, to the treatment tanks, as done by Järvenpää and Lindström (2004). Experimental aquaria were randomly assigned to control and algal treatments. The standard length (mean ± SE) and body mass (mean ± SE) of males did not differ between treatments (length of males in control = 51.64 ± 0.30 mm, algal = 51.63 ± 0.42 mm; *t*-test: $t = 0.02$, df = 22, $P = .98$; body mass of males in control = 1.68 ± 0.037 g, algal = 1.70 ± 0.057 g; $t = 0.29$, df = 22, $P = .77$). In the algal treatment, a mixture of algae and water was added 1 day after males were introduced into the experimental aquaria to achieve the desired turbidity. To control for any possible effects caused by introducing water into the treatment tanks, an equivalent amount of clear seawater was also added to control tanks (Järvenpää and Lindström 2004). Turbidity, measured in nephelometric turbidity units (NTU; mean ± SE), was higher in algal tanks (10.35 ± 0.99 NTU, $n = 23$) than in control tanks (0.66 ± 0.24 NTU, $n = 23$; two-sample *t*-test: $t = 9.78$, df = 22, $P < .0001$). These levels are well within the range recorded in the Baltic Sea during the stickleback breeding season (which may vary between 0.5 and 45 NTU; Granqvist and Mattila 2004). Turbidity did not differ significantly between the 2 days that male courtship and color were measured (control treatment: $t = 0.69$, df = 22, $P = .49$; algal treatment: $t = 0.22$, df = 22, $P = .83$).

Male Signaling Effort in Absence of Competition

One day after adding algae to the treatment tanks and clear sea water to the control tanks, a gravid female was placed into a clear Plexiglas tube (diameter of 10 cm) positioned in the middle of the female compartment in front of the opaque divider separating the two males. Con-

trol and treatment tanks received females of the same length (control = 55.20 ± 0.72 mm, algal = 54.61 ± 0.64 mm; $t = 0.61$, df = 22, $P = .54$) and body mass (control = 2.26 ± 0.087 g, algal = 2.26 ± 0.085 g; two-sample t-test: $t = 0.01$, df = 22, $P = .99$). We then recorded, over a 10-min period, the number of times each male attempted to lead the female to the nest to spawn, which, in this population, has previously been shown to reflect male courtship intensity and attractiveness to females (Candolin 1997). We also measured total courtship activity by tallying the number of courtship behaviors observed over the same 10-min period (i.e., zigzag movements, leads, bites, fanning displays, and swimming through the nest; sensu Candolin 1997). Male nuptial coloration was measured immediately after female exposure by photographing his left lateral side under standardized conditions described by Candolin (1999*b*). After photography, the male was returned immediately to the aquarium. The area of red coloration was later determined using the image analysis software ImageJ, version 1.34s (http://rsb.info.nih.gov/ij/). Because the color vision of sticklebacks does not differ greatly from that of humans (reviewed by Frischknecht [1993]), we used a system for defining color space in ImageJ that was based on human visual perception, namely, the YUV color model, which decomposes color into three components: Y = luminance, U = blue chroma, and V = red chroma. For the purpose of color analyses, we first cropped each image so that the fish was presented against a white background. All areas that ranged in color from yellow to red to purple were then selected in YUV mode (corresponding to the following YUV values: Y = 32–255, U = 0–143, and V = 141–255). Following this, the image was converted to 8-bit gray scale, and a color threshold was applied (using the plug-in "Threshold_Colour," available at http://www.dentistry.bham.ac.uk/landinig/software/software.html). This was done to remove from the image all pixels that were outside the selected values (i.e., the nonred areas on the male's body). The area of the remaining pixels was then measured, and this, in relation to the total area of the male's body in the original image, was used to determine the percent of the lateral side that was red colored, yielding a red-area value.

Male Signaling Effort in Presence of Competition

When both males had been photographed and returned to their respective compartments, the opaque divider was removed so that males could interact. A clump of artificial vegetation positioned in the middle of the aquarium provided a boundary marker separating the two male territories (Candolin 1999*a*). After the partition was removed, both males continued to take care of, and defend, their nests. One day after the partition was removed, we again

measured the courtship activity and color of males exposed to a gravid female using procedures identical to the ones described above. Stimulus females used here were size matched (± 1 mm standard length) with those presented to males in the absence of competition.

Within each male pair, the relative amount of courtship activity and area of red coloration was calculated as $a/(a + b)$, where a is the value for the poor-condition male and b the value for the good-condition male. All data were checked for normality and homogeneity of variances before analyses.

Results

Male Signaling Effort in Absence of Competition

On the basis of their lower body condition index (paired t-test: $t = 6.99$, df = 45, $P < .0001$), food-deprived males were clearly in poorer physical condition than their well-fed counterparts. Despite this, in the absence of competition, food-deprived males actually courted more intensively than well-fed males (repeated-measures ANOVA, leads: $F = 5.48$, df = 1, 45, $P = .024$; total activity: $F = 4.28$, df = 1, 45, $P = .044$; fig. 1).

In the absence of competition, food-deprived males in clear water also expressed relatively larger areas of red nuptial coloration than did well-fed males (repeated-measures ANOVA: $F = 7.70$, df = 1, 22, $P = .011$; fig. 2). By contrast, the difference in red area between good- and poor-condition males was reduced under turbid conditions (within-subjects interaction between male condition and water turbidity: $F = 4.35$, df = 1, 44, $P = .043$; fig. 2).

Male Signaling Effort in Presence of Competition

In clear water, males in poor condition decreased the intensity of their courtship activity when interacting with good-condition males. As a result, males in good condition ended up courting more (repeated-measures ANOVA on the effect of competition on the difference between males in leads: $F = 30.76$, df = 1, 22, $P < .001$; total activity: $F = 25.06$, df = 1, 22, $P < .001$; fig. 1*A*). The effect of competition on difference in courtship activity, however, was weakened under turbid conditions (within-subjects interaction between water turbidity and competition on difference in leads: $F = 3.85$, df = 1, 44, $P = .056$; total activity: $F = 5.21$, df = 1, 44, $P = .027$; fig. 1).

The difference in red color expression between paired males was unaffected by the presence of competition (within-subjects effects: $F = 0.99$, df = 1, 45, $P = .32$). This was true irrespective of whether males were tested under clear or turbid conditions (within-subjects inter-
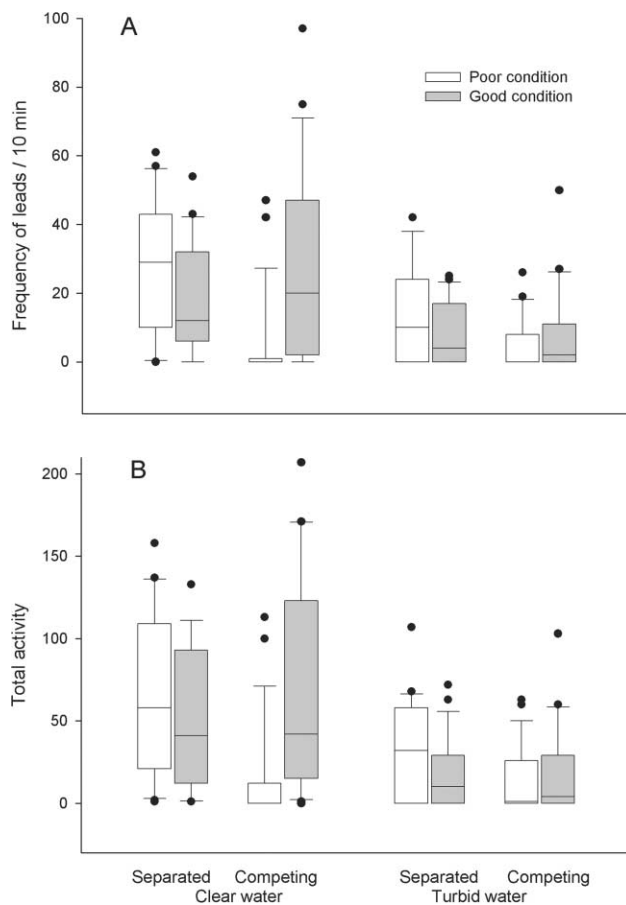
Figure 1: Number of (*A*) leads and (*B*) total courtship behaviors exhibited by good- and poor-condition males in the presence and absence of competition under turbid ($n = 23$) and clear ($n = 23$) water conditions. Box plots show tenth, twenty-fifth, fiftieth (median), seventy-fifth, and ninetieth percentiles with horizontal lines and all data points outside this range.

action between water turbidity and competition: $F = 2.02$, df $= 1, 44$, $P = .16$). However, when the two male groups were analyzed separately, we found that the presence of algae reduced the red color expression of poor-condition males (between-subject effects in repeated-measures ANOVA: $F = 4.35$, df $= 1, 44$, $P = .043$) but had no effect on the color expression of males in good condition ($F = 0.11$, df $= 1, 44$, $P = .74$; fig. 2) regardless of whether a competitor was present (within-subject effects for males in poor condition: $F = 0.11$, df $= 1, 44$, $P = .74$; good condition: $F = 1.82$, df $= 1, 44$, $P = .18$; fig. 2).

Discussion

The results of our study suggest that any regulatory effects that competition may have on the honesty of male sexual

displays in sticklebacks may be compromised under turbid conditions. Although males in poor condition reduced their courtship effort in the presence of competition in turbid water, this reduction was to a much lesser extent than that observed in clear water. Consequently, there was no difference in courtship effort between poor-condition males and those in good condition. Thus, courtship under conditions of algal turbidity did not reflect male condition as honestly as did courtship in clear water. This is likely due to a reduction in visibility that would otherwise permit males to inhibit one another's courtship attempts. As a result, increased water turbidity seems to weaken some of the social costs that keep courtship displays honest.

Algal turbidity also appears to influence red breeding coloration. Although the area of red displayed by good-condition males did not differ between turbid and clear water conditions, males in poor condition expressed less red coloration in turbid water. This was true irrespective of whether a competitor was present. It would seem that poor-condition males are more sensitive to the lower payoffs of expressing large areas of red breeding coloration under algal turbidity. Decreased visibility, in this regard, likely diminishes the effectiveness of visual signals. Earlier studies have previously shown, for example, that female preference for red males disappears under green light, which masks the color difference between prospective suitors (Milinski and Bakker 1990), and under dense growth of filamentous algae, which reduces visibility (Candolin et al. 2007).

The results of our study have important implications for the reliability of sexual signals in human-altered environments. Increased turbidity of coastal waters in the
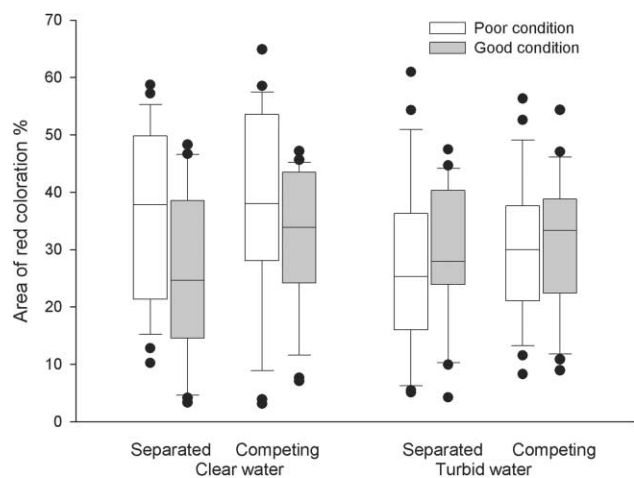

Figure 2: Area of red breeding coloration displayed by good- and poor-condition males in the presence and absence of competition under turbid ($n = 23$) and clear ($n = 23$) conditions.

breeding areas of sticklebacks could allow males in poor condition to signal at a higher level than they would otherwise. Previous studies have demonstrated that poor-quality males with a high signaling effort are also more likely to cannibalize their own broods (Candolin 2000*a*). Thus, increased turbidity is likely to have dire consequences for females relying on the honesty of these displays to select high-quality suitors with superior parental qualities.

In the longer term, it is important to bear in mind that some degree of dishonesty is permitted in an evolutionarily stable signaling system (Johnstone and Grafen 1993; Kokko 1997). However, if dishonesty becomes pervasive under algal turbidity, the current signaling system of Baltic sticklebacks could become unstable. If this were to occur, traits that yield very little, if any, benefit under turbid conditions could, eventually, be diminished or lost. Such a situation appears to have occurred among the rift-valley cichlids of Lake Victoria, with males living under turbid conditions expressing duller coloration than those living in clearer waters (Seehausen et al. 1997).

It remains to be seen whether Baltic sticklebacks can adapt to the increasingly turbid conditions afflicting their environment. Certainly, from an evolutionary perspective, sticklebacks appear to be highly adaptable organisms, with evidence from several North American populations suggesting a proclivity for exploiting novel habitat (Schluter and McPhail 1992; Boughman 2001; McKinnon et al. 2004). Adjustment to turbidity could involve a switch to alternate cues that more accurately convey male condition under reduced visibility. On the other hand, if algal turbidity leads to more random matings because of increased difficulties in discriminating between potential mates and/or a relaxation of social costs that keep signals honest, it is conceivable that human-induced changes to the signaling environment might lead to a decrease in population viability (Fisher et al. 2006).

Literature Cited

Barber, I., S. A. Arnott, V. A. Braithwaite, J. Andrew, and F. A. Huntingford. 2001. Indirect fitness consequences of mate choice in sticklebacks: offspring of brighter males grow slowly but resist parasitic infections. Proceedings of the Royal Society B: Biological Sciences 268:71–76.

Berglund, A., and G. Rosenqvist. 2001. Male pipefish prefer dominant over attractive females. Behavioral Ecology 12:402–406.

Berglund, A., A. Bisazza, and A. Pilastro. 1996. Armaments and ornaments: an evolutionary explanation of traits of dual utility. Biological Journal of the Linnean Society 58:385–399.

Boughman, J. W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. Nature 411:944–948.

Candolin, U. 1997. Predation risk affects courtship and attractiveness of competing threespine stickleback males. Behavioral Ecology and Sociobiology 41:81–87.

———. 1999*a*. Male-male competition facilitates female choice in sticklebacks. Proceedings of the Royal Society B: Biological Sciences 266:785–789.

———. 1999*b*. The relationship between signal quality and physical condition: is sexual signalling honest in the three-spined stickleback? Animal Behaviour 58:1261–1267.

———. 2000*a*. Increased signalling effort when survival prospects decrease: male-male competition ensures honesty. Animal Behaviour 60:417–422.

———. 2000*b*. Male-male competition ensures honest signaling of male parental ability in the three-spined stickleback (*Gasterosteus aculeatus*). Behavioral Ecology and Sociobiology 49:57–61.

———. 2004. Effects of algae cover on egg acquisition in male three-spined stickleback. Behaviour 141:1389–1399.

Candolin, U., T. Salesto, and M. Evers. 2007. Changed environmental conditions weaken sexual selection in sticklebacks. Journal of Evolutionary Biology 20:233–239.

Fisher, H. S., B. B. M. Wong, and G. G. Rosenthal. 2006. Alteration of the chemical environment disrupts communication in a freshwater fish. Proceedings of the Royal Society B: Biological Sciences 273:1187–1193.

Frischknecht, M. 1993. The breeding colouration of male three-spined sticklebacks (*Gasterosteus aculeatus*) as an indicator of energy investment in vigour. Evolutionary Ecology 7:439–450.

Granqvist, M., and J. Mattila. 2004. The effect of turbidity and light intensity on the consumption of mysids by juvenile perch (*Perca fluviatilis* L.). Hydrobiologia 514:93–101.

Järvenpää, M., and K. Lindström. 2004. Water turbidity by algal blooms causes mating system breakdown in a shallow-water fish, the sand goby *Pomatoschistus minutus*. Proceedings of the Royal Society B: Biological Sciences 271:2361–2365.

Johnstone, R. A., and A. Grafen. 1993. Dishonesty and the handicap principle. Animal Behaviour 46:759–764.

Kokko, H. 1997. Evolutionarily stable strategies of age-dependent sexual advertisement. Behavioral Ecology and Sociobiology 41:99–107.

Larsson, U., R. Elmgren, and F. Wulff. 1985. Eutrophication and the Baltic Sea: causes and consequences. Ambio 14:9–14.

McKinnon, J. S., S. Mori, B. K. Blackman, L. David, D. M. Kingsley, L. Jamieson, J. Chou, and D. Schluter. 2004. Evidence for ecology's role in speciation. Nature 429:294–298.

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

Raateoja, M., J. Seppälä, H. Kuosa, and K. Myrberg. 2005. Recent changes in the trophic state of the Baltic Sea along SW coast of Finland. Ambio 34:188–191.

Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Bulletin of the Fisheries Research Board of Canada 191:1–382.

Rowland, W. J. 1994. Proximate determinants of stickleback behaviour: an evolutionary perspective. Pages 297–344 *in* M. A. Bell and S. A. Foster, eds. The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Schluter, D., and J. D. McPhail. 1992. Ecological character displacement and speciation in sticklebacks. American Naturalist 140:85–108.

Seehausen, O., J. M. van Alphen, and F. Witte. 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. Science 277:1808–1811.

Tibbetts, E. A., and J. Dale. 2004. A socially enforced signal of quality in a paper wasp. Nature 432:218–222.

Wong, B. B. M., and U. Candolin. 2005. How is female mate choice affected by male competition? Biological Reviews of the Cambridge Philosophical Society 80:559–571.