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An increase in pH boosts olfactory communication in sticklebacks

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Human-induced eutrophication is a serious environmental problem that constrains visual communication and influences the mate choice process in fishes. Eutrophication also changes the chemical environment and the pH of the water, which could influence the use of olfactory cues in mate choice. Here, we show that an increase in pH enhances the use of male olfactory cues in mate choice in three-spined sticklebacks, *Gasterosteus aculeatus*. In a laboratory choice experiment, gravid females were more attracted to male olfactory cues when pH was raised. This could compensate for impaired visual communication in eutrophied waters and facilitate adaptive mate choice.

Keywords: anthropogenic disturbance; environmental change; multiple cues; mate preference; olfaction; sexual selection

1. INTRODUCTION

Recent research shows that anthropogenic disturbances can influence evolutionary processes through effects on sexual selection (Weatherhead 2005; Rankin & Kokko 2006). A serious human-induced problem in aquatic habitats is eutrophication, which promotes excessive plant growth and phytoplankton blooms (Smith 2003). This reduces visibility (Utne-Palm 2002) and influences the mate choice process of fishes (Candolin 2004; Järvenpää & Lindström 2004; Candolin *et al.* 2007; Wong *et al.* in press). This could have serious consequences for population viability by increasing the cost of sexual selection (Kokko & Brooks 2003).

Cues other than visual ones, such as olfactory cues, could increase in importance when visibility deteriorates. The value of olfactory cues depends, however, on environmental factors, such as water turbulence (Weissburg *et al.* 2002) and the pH value of the water (Brown *et al.* 2002). Since increased photosynthesis augments pH by reducing the amount of dissolved carbon dioxide (Reddy 1981; Stumm & Morgan 1981), eutrophication could influence olfactory communication.

Here, we investigated if an increase in pH value influences the use of olfactory cues in mate choice in three-spined sticklebacks (*Gasterosteus aculeatus*). Sticklebacks in the Baltic Sea are spawning in an increasingly eutrophied environment where visibility is reduced. This has decreased the honesty of visual cues as indicators of mate quality (Wong *et al.* in press) and diminished their use in mate choice (Candolin *et al.* 2007; Engström-Öst & Candolin 2007). A pH-dependent use of olfactory cues could have a major impact on sexual selection, since the pH of eutrophied waters can increase to over 9 during daytime (Reddy 1981; Perus & Bonsdorff 2004; Olsonen 2007), and olfactory cues play a crucial role in MHC-dependent mate choice (Reusch *et al.* 2001; Milinski *et al.* 2005) and in species recognition (Rafferty & Boughman 2006).

2. MATERIAL AND METHODS

Adult three-spined sticklebacks were caught with minnow traps from the shores of the Baltic Sea (59°50′ N, 23°15′ E). Males were housed in individual flow-through aquaria (10 l) under natural light conditions. They were given a nesting dish (11 cm in diameter) filled with sand and filamentous algae (*Chladophora* sp.) for nest construction. A gravid female enclosed in a transparent Plexiglas cylinder was presented daily to each male for 30 min to initiate nest building and courtship behaviour.

Two days after the male had finished nest building (Reusch *et al.* 2001), the water flow was stopped and a female was presented to the male as described above. Three litres of water was carefully extracted within 5 cm of the male, filtered through a plankton net (mesh size 62 μm) and bottled. The pH of the water was either left at its natural level of 8.0 ± 0.2 s.d. (male) or increased to 9.5 (male pH+) by carefully adding a 0.025 M NaOH (figure 1). The pH value rises from 8 to over 9 under high primary production in brackish water (J. Heuschele 2006, personal observation). To test whether an increased pH level alone triggers a difference in female behaviour, filtered water (62 μm plankton net) from a holding aquarium without a male was extracted and manipulated according to the above procedures, resulting in water containing no male olfactory cues and either increased (pH+) or natural (C) pH levels. An equimolar solution of dissolved NaCl ($1461\ g\,l^{-1}\ M = 58.44\ g\,mol^{-1}$) was added to the natural pH treatments (male and C), matching the amount of added Na^+ ions in high pH treatments (male pH+ and pH+), to keep the ion activity constant in all treatments.

The three treatments (male pH+, male, pH+) were tested against the control (C), one at a time. An experimental aquarium (50 l) was divided intro three sections with the help of two marks on the outside of the aquarium; a neutral section in the middle (18×30 cm) and two choice sections on each side (16×30 cm). A gravid female was placed in the neutral section. After 10 min of habituation, the stimulus and the control seawater (C) were simultaneously dripped into the aquarium at a rate of 1.5 drops per second (figure 1).

The aquarium was video recorded from the front side and the video tapes analysed using the software JWatcher v. 1.0. Preliminary tests with coloured water showed that water dripping into the aquarium formed a clear upper layer in the water column; therefore recordings of activity were restricted to the upper 8 cm. The time spent in each section, treatment t_T, control t_C and neutral t_N, was recorded over a 10 min period. The relative time spent with the treatment was calculated as $t_T/(t_T+t_C)$, where t_T is time spent in the treatment section and t_C in the control section. Odour preferences of gravid females have been found reliable to predict mate preferences (Milinski *et al.* 2005).

Each female, in turn, was subjected to all the three treatments within a 4 h period, in random order to control potential order effects. After each run, the tank was cleaned using dissolved hydrogen peroxide (McLennan 2005). To avoid observer bias, the bottles with the stimulus water were coded by a third person. The mean pH value of the water in the test aquarium and in the holding aquaria was 8.0 (± 0.2 s.d.).

A total of 20 females were tested. The odour cues used were produced by 12 males. The data were checked for normality and homogeneity of variance before analyses.

3. RESULTS

The proportion of time that females were associated with the treatment water compared to the control water varied among the three treatments (figure 2; linear mixed model ANOVA with treatment as fixed factor and male as random factor; $F=4.846$, $p=0.012$). Pairwise comparisons of the treatments showed that females

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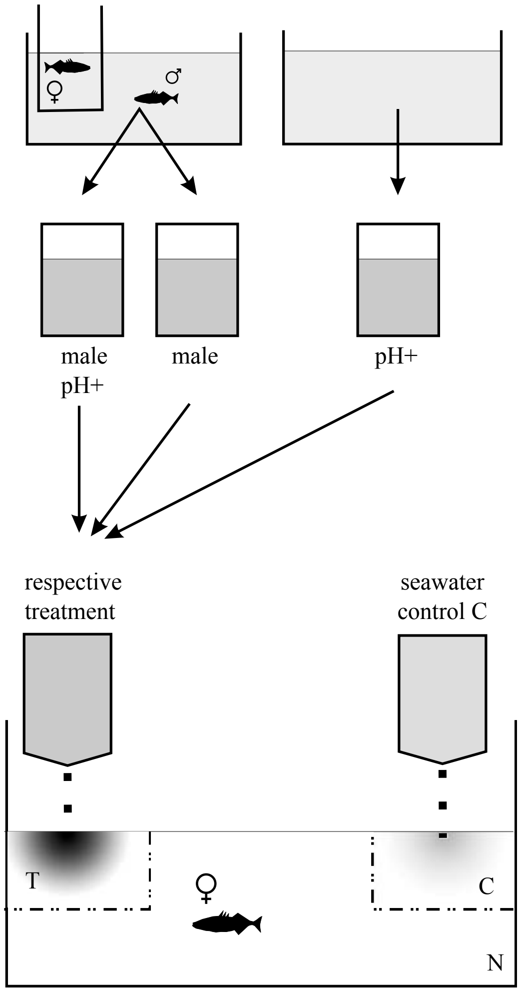

Figure 1. The experimental setup and procedure. T, C and N indicate the treatment, control and neutral sections.

spent more time in the section with both male olfactory cues and increased pH (male pH+) than in the sections containing only male olfactory cues (male) (Fisher test; $p=0.024$) or only having an increased pH value (pH+) ($p=0.005$).

4. DISCUSSION

An increase in pH enhanced the signal value of male scent. Females were more attracted to male olfactory cues when pH was raised. This was not due to females being attracted to water with increased pH, since pure water with increased pH did not attract females. Since odour preferences of gravid females have been demonstrated to reflect mate preferences (Milinski *et al.* 2005), the results suggest that an increased pH of the water facilitates odour-based mate assessment.

The enhanced interest in male scent under increased pH levels could be due to either an improvement of the transmission of the chemical cue or an increase in olfactory sensitivity towards the cue. Male scent consists of different compounds, like hormones, MHC peptides and amino acids (Sorensen & Stacey 2004; Milinski *et al.* 2005; Sorensen *et al.* 2005; Yambe *et al.* 2006). An increase in pH could facilitate the binding or transportation of olfactory cues to olfactory receptors, for example by changing the three-dimensional structure of the peptides involved. This occurs, for example, in pheromone-binding proteins in a moth (Zubkov *et al.* 2005). Alternatively, changed pH could influence the chemical cues themselves. Low pH values cause irreversible disruptions of fish alarm cues, through alterations in the structure of the transmitting molecules (Brown *et al.* 2002; Leduc *et al.* 2004). It is possible that an increase in pH could prevent the disruption of chemical cues.

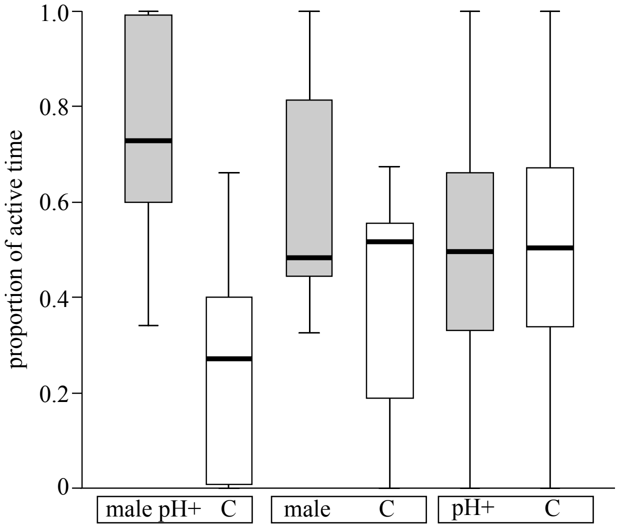

Figure 2. Proportional amount of time that female sticklebacks spent with the treatment and the respective control. Box plots show 25th, 50th (median) and 75th percentiles, whiskers the minimum and maximum range of the data.

Low pH has been found to have a negative effect on olfactory communication in salmon (*Salmo salar* L.) by hampering the detection of pheromones released by females (Moore 1994). Humic acids, again, bind pheromones in water and leave them undetectable to chemoreceptors (Hubbard *et al.* 2002), which hinders intraspecific communication based on olfactory cues in the swordtail fish (*Xiphophorus birchmanni*; Fisher *et al.* 2006). This suggests that high pH could have the opposite positive effect on olfactory communication.

Regardless of the exact mechanisms behind the pH-related enhancement of olfactory cues, eutrophication-related pH increases could enhance the importance of olfaction in mate search and mate choice, and compensate for impaired visual communication (Candolin *et al.* 2007; Engström-Öst & Candolin 2007). The honesty of visual displays is reduced in turbid water, since male–male competition that promotes honesty is relaxed under poor visibility (Candolin 1999; Wong *et al.* in press). An increased use of olfactory cues in eutrophied waters could facilitate adaptive mate choice, particularly since olfactory cues give information on the allele diversity at MHC genes and indirect genetic benefits (Reusch *et al.* 2001).

This study suggests that human-induced environmental change may affect organisms in ways that are not immediately apparent. Besides the obvious effect on sexual selection through impaired visibility, eutrophication may also affect olfactory communication. The consequences that this may have at the population level, through effects on adaptive mate choice, remains to be determined.

The experimental procedures were approved by the Animal Care Committee of the University of Helsinki (86–06).

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