Water turbidity by algal blooms causes mating system breakdown in a shallow-water fish, the sand goby *Pomatoschistus minutus*

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Eutrophication as a result of human activity has resulted in increased algal blooms and turbidity in aquatic environments. We investigated experimentally the effect of algal turbidity on the mating system and sexual selection in the sand goby, *Pomatoschistus minutus* (Pallas), a marine fish with a resource-defence mating system and paternal care. Owing to male–male competition and female choice, large males can monopolize multiple mates, while some males do not achieve mating at all. We show that the number of eggs laid was the same in both turbid and clear tanks but that mating success was more evenly distributed among males in turbid than in clear water. The opportunity for sexual selection was lower in turbid conditions. In turbid conditions mating success was less skewed towards large males. Our results suggest that increased turbidity can change mating systems and decrease the opportunity for sexual selection as well as selection intensity.

Keywords: eutrophication; turbidity; sexual selection; breeding system; sand goby; *Pomatoschistus*

1. INTRODUCTION

Animal breeding systems are affected by various environmental factors. Resource distribution has been shown to be an especially important determinant of mating systems (Emlen & Oring 1977; Lindström & Seppä 1996). Also, human-induced changes in the environment can have strong effects on the reproduction of animals (Guillette *et al.* 1994) and hence possibly also their breeding systems.

Many aquatic environments have become increasingly eutrophic as a result of human activity. Eutrophication leads to increased growth of planktonic algae (Larsson *et al.* 1985). As a consequence, both turbidity and silting organic material increases. Increased breakdown of organic material consumes oxygen in the water column and causes changes in the constitution of the bottom substrate (Larsson *et al.* 1985). Algal turbidity leads to a decrease in light intensity and a narrowing of the light spectrum below the surface (Seehausen & van Alphen 1998).

Increased silting, low oxygen concentrations and altered light conditions have all been shown to affect the reproductive behaviour of fishes (Potts *et al.* 1988; Seehausen *et al.* 1997; Jones & Reynolds 1999a; Hale *et al.* 2003; Lissåker *et al.* 2003). In the sea stickleback Potts *et al.* (1988) found that following silt addition parental behaviour (nest pushing, nest inspection) increased as males attempted to clear the nest of silt. In the common goby parental males increased both the time spent fanning and fanning tempo, as well as the frequency of egg-directed activities under conditions of low oxygen (Jones & Reynolds 1999b). As a consequence, males breeding under low oxygen lost more weight than control males. They were also more likely to abandon brood care during consecutive brood cycles (Jones & Reynolds 1999a). More importantly, females who normally prefer mated males in the

common goby reversed their preference for egg-guarding males under low oxygen conditions (Reynolds & Jones 1999).

A dramatic example of human-induced effects on mating systems comes from the Lake Victoria rocky shore cichlids. Owing to eutrophication the turbidity of Lake Victoria has increased. Fewer sympatric cichlid species are found in areas of increased water turbidity, and fish from more turbid waters are duller in coloration (Seehausen *et al.* 1997). According to Seehausen *et al.* (1997) species are lost owing to hybridization when turbidity masks the species-specific male colour signals and hence prevents females from mating with males of their own species. In aquaria Seehausen & van Alphen (1998) have shown that females of the sympatric red/blue cichlid species pair, *Haplochromis nyererei*, prefer conspecific over heterospecific males under broad-spectrum illumination but mate indiscriminately under monochromatic light where colour differences are masked. There are, however, few experimental studies on the direct effects of algal turbidity on fish behaviour (e.g. Cobcroft *et al.* 2001; Utne-Palm 2002) and none of these studies has been concerned with the possible effects of algal turbidity on the mating system of fishes.

The Baltic Sea is one of the most polluted seas in the world (Elmgren 1989) and the effects of eutrophication are substantial in many parts of it (Cederwall & Elmgren 1990). Significant increases in total nitrogen and phosphorus, as well as dissolved inorganic nitrate and phosphate concentrations, have been recorded from most of the Baltic Sea between the 1970s and 1990s (Sandén & Rahm 1993; Perttilä *et al.* 1995). The abundance of planktonic algae in the Baltic Sea has increased, leading to a significant decrease in Secchi-depths throughout the twentieth century (Sandén & Håkansson 1996) and to increased occurrence and duration of hypoxia and anoxia (Elmgren 1989).

Studies in the Baltic have focused on indirect effects of algal growth such as decreased oxygen levels caused by the

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decomposing algae (Karlson *et al.* 2002). Bonsdorff *et al.* (1995) were able to demonstrate dramatic changes in the community structure of zoobenthos and fishes mediated partly through behavioural alterations. Decomposing processes under drifting mats of decaying filamentous algae consume oxygen, which causes benthic animals to move away from these sites and hence become more strongly exposed to predation.

We investigated experimentally the effects of algal turbidity on the mating system and opportunity for sexual selection in the sand goby, *Pomatoschistus minutus*, a marine fish with a resource-defence mating system and male parental care. Males defend nests and attract females who spawn in the nest by attaching the eggs in a single layer to the ceiling of the nest. The male then guards the eggs until they hatch (Hesthagen 1977). The male fans and cleans the eggs and removes dead and infected eggs from the nest. Fanning aerates the eggs and can also prevent sediment from accumulating in the nest. If the nest is big, the male can guard the eggs of several females simultaneously (Lindström 1988).

A male in spawning condition develops a dark, bluish-black coloration on the pectoral fin and a bright, blue spot lined by white on the first dorsal fin. Sand goby females favour large, actively courting and colourful males with suitable nest sites and high paternal quality (Forsgren 1992, 1997a,b). However, in the study population male-male competition for nest sites is intense and a large male with a high resource holding potential can usually monopolize multiple mates (Lindström & Seppä 1996).

As mentioned previously, modified light spectra and lowered oxygen levels have both been shown to change female mate choice and hence mating system. Our goal in this study was to test if turbidity caused by increased algal density would change the mating system of the sand goby. Our expectation was that if turbidity interferes with sexual-selection processes then mating success should be more evenly distributed in turbid than clear water conditions.

2. MATERIAL AND METHODS

This study was carried out at the Tvärminne zoological station, southern Finland, from late May to early July 2001. Fish were caught by hand trawl at a nearby breeding area. In the laboratory, males and females were housed separately in large tanks with continuously renewed seawater and fed *ad libitum* with live *Mysis* spp. and frozen chironomid larvae. After the experiment all fish were released back to their natural breeding area.

We conducted the experiment outdoors to guarantee natural light conditions. Each replicate of the experimental set-up involved four glass aquaria, each with a bottom area of 80 cm × 80 cm and a water level maintained at 30 cm. Two of the tanks were designated to be clear-water control tanks whereas the other two tanks were made turbid by adding water containing planktonic algae. Natural water temperature (11–18 °C) was maintained by placing the tanks in wading pools (diameter 180 cm) with a continuous flow-through of seawater. The set-up was repeated six times and for each replicate the treatment and control tanks were switched diagonally. Hence the total number of replicates for each treatment was 12.

We used *Brachiomonas submarina*, a unicellular planktonic alga 10–15 µm in size, to produce algal turbidity. The initial culture was obtained from pure monocultures maintained by Tvärminne

zoological station. The algae were grown in white uncovered 50 l buckets with aeration in natural light. We used seawater filtered through a 20 µm sieve to eliminate most of the grazers. Nitrogen and phosphorus, 0.046 and 0.036 g l⁻¹, respectively, were added to the cultures to ensure sufficient nutrients for algal growth.

Algae were added to the treatment tanks 24 h after starting the replicate (see later). This was done by first decreasing the water level in treatment and control tanks to 10 cm and then adding to the treatment tanks a mixture of water from the individual cultures until a water level of 30 cm was reached. The turbidity was visually made to match intense algal blooms that occur naturally in the Baltic Sea during the sand goby breeding season. Control tanks similarly received clear seawater. Throughout the experiments we monitored turbidity by sampling the treatment and control tanks every 12 h and analysing the absorption spectrum on a Shimadzu UV-2101 PC-spectrophotometer. Total absorption was obtained by summing the absorbance values from 380 to 800 nm (0.5 nm interval). These values were used to obtain a mean absorbance from the five measurements taken from each aquarium. The absorbance (mean ± s.d.) in control tanks (0.9 ± 0.476, *n* = 12) was much lower than in the turbid tanks (14.8 ± 7.634, *n* = 12; *t*-test, *t*₂₂ = 3.420, *p* < 0.001).

The bottoms of the experimental tanks were covered with *ca.* 4 cm of fine sand. Each aquarium had four halved clay flowerpots, 9 cm in diameter, as nest sites, located in the four corners of tanks with the openings facing towards the centre. This nest size could accommodate eggs from four or more females. We placed a piece of transparent film, cut to the shape of the nest, into the ceiling of each nest. Any eggs spawned in the nests would be attached to the sheet.

At the start of each replicate, we added four males of different size to each aquarium. Males in experimental and control tanks were similar in size (*t*-test, weight: *t*₂₂ = 0.092, *p* = 0.927; total length: *t*₂₂ = 0.171, *p* = 0.866). Males were then allowed to build nests for 24 h after which the first female was added. We then added a ripe female every 12 h until a total of four females had been introduced. Experimental and control tanks received females of the same size (*t*-test, weight: *t*₂₂ = 0.261, *p* = 0.796; total length: *t*₂₂ = 0.041, *p* = 0.967).

Before each female addition we recorded the nest construction stage, and the presence and number of eggs. The degree of nest building was ranked according to 1: no construction, 2: some sand piled on the nest, 3: nest completely covered with sand. Spawns were checked by removing the piece of transparent film. Egg masses were photographed using a Minolta RD175 digital camera fitted with a 50 mm macro lens. After photography, the transparent film was replaced in the nest. Replicates were finished 12 h after adding the fourth female.

We used four different measures of sexual selection. First, we counted the number of mated males. Second, opportunity for sexual selection (Arnold & Wade 1984) on males was estimated by the coefficient of variation (CV) in the number of eggs received by individual males. Third, the selection differential was estimated as the size difference between mated and unmated males. Fourth, the size of the male receiving the first mating was used as a measure of sexual selection.

To compare the size of mated and unmated males we used repeated-measures ANOVA to take into account the paired nature of this comparison. In this analysis we excluded pools in which males had died (3) and in which we were unable to unequivocally assign male identity to mating status (1). Hence sample sizes in this comparison differ from the analyses of distribution of spawns.

3. RESULTS

Males equally constructed nests in clear tanks (building index: median = 3, $n = 12$) and in turbid tanks (median = 3, $n = 12$; Mann–Whitney U -test, $U_{12,12} = 64$, $p = 0.544$). The first mating (mean $\pm$ s.d.) appeared in turbid and in clear tanks approximately simultaneously (clear tanks 1.42^{nd} observation ± 0.67 , $n = 12$, turbid tanks, $1.5^{\text{th}} \pm 0.67$, $n = 12$; Mann–Whitney U -test, $U_{12,12} = 66.5$, $p = 0.710$). The number of spawns (clear tanks: 2.6 ± 1.1 , $n = 12$; turbid tanks: 2.8 ± 0.7 , $n = 12$) as well as the number of eggs laid (figure 1a) were the same in both treatments (t -test, $t_{22} = 0.666$, $p = 0.512$, and $t_{22} = 1.146$, $p = 0.264$, respectively). In turbid tanks mating success was more evenly distributed as more males received spawns than in clear tanks (Mann–Whitney U -test, $U_{12,12} = 28$, $p = 0.004$; figure 1b). Consequently the opportunity for sexual selection was lower in turbid tanks (Mann–Whitney U -test, $U_{12,12} = 114$, $p = 0.008$; figure 1c).

Mating success was less skewed towards large males in turbid tanks. Mated males were, on average, larger than unmated males in both treatments (repeated-measures ANOVA, within-subjects effect of mating status, $F_{1,18} = 14.415$, $p = 0.001$). However, the size difference between mated and unmated males was greater in clear than in turbid tanks (within-subject mating status and treatment interaction, $F_{1,18} = 10.774$, $p = 0.004$; figure 2). The length of the first male to receive eggs was bigger in clear (53.54 ± 3.91 , $n = 11$) than in turbid tanks (48.92 ± 5.73 , $n = 12$; two-sample t -test, $t_{21} = 2.24$, $p = 0.036$).

4. DISCUSSION

The sand goby mating system was clearly different in turbid and clear water conditions. In turbid water, mating success was distributed more evenly among males and the size difference between mated and unmated males was smaller. The opportunity for sexual selection and the selection differential will both diminish in turbid water. Consequently, traits that are currently maintained by sexual selection will have a smaller selection advantage and may eventually disappear because of the overall relaxation in selection pressure. In the current study population the sand goby is sexually size dimorphic, with males being larger than females (Forsgren *et al.* 1996; Lehtonen & Lindström 2004). In our study area turbid conditions frequently occur during a breeding season (personal observation). If these conditions became more prevalent this might lead to the disappearance of size dimorphism. It is likely that this would also involve changes in many other traits of the sand goby, although this study cannot identify those.

It is important to realize that the change in mating system observed was not due to fecundity differences between the treatments, as the total number of eggs spawned was the same in both treatments. We found that the size difference between mated and unmated males was smaller in turbid tanks. This is of course expected to happen as more males receive eggs. One could, therefore, argue that some of the change in selection differentials observed here is a simple consequence of this and not a result of a diminished role of body size in turbid conditions. We did, however, also show that in turbid pools the first mated male was smaller than in control pools. Had the decrease in mean

Figure 1. A comparison of the distribution of mating success in clear and turbid water. (a) Total number of eggs spawned; (b) number of nests containing eggs; and (c) coefficient of variation in male mating success (based on number of eggs) in the two treatments. Error bars indicate ± 1 s.e.m.

mated male size merely been a consequence of the above concern then first-mated males should not differ in size between treatments. We thus think that the size difference observed is a consequence of real changes in the selection pressures and that the change in breeding system was a real consequence of how females mated with males. This could be due to a change in female preference or their ability to express their preferences or because of changes in male–male competition or mate attraction effort.

Male body size is an important trait in determining mating success in the sand goby. Bigger males are more successful in the competition for nests (Lindström 1988, 1992) and they are also preferred by females (Forsgren 1992). Hence, if turbidity hampers the assessment of male body size in either mate choice or male–male competition,

Figure 2. The mean body size of mated (filled bars) and unmated (open bars) males. The means are based on pool-wise means of males guarding eggs and nesting males without eggs. Error bars indicate ± 1 s.e.m.

then one may expect that the large male size advantage decreases in turbid conditions. However, body size in fishes is a feature that probably can be assessed without visual information using, for example, the lateral line system (Pitcher 1993). It is therefore likely that males can assess dominance relationship without visual information although this may require some additional behavioural interactions. Similarly, females could use similar methods for assessing males. Furthermore, sand goby males produce courtship sounds after they have attracted the female into the nest (Lindström & Lugli 2000). Several features of the sound produced depend on body size (Lindström & Lugli 2000) and thus provide reliable information about male size.

Turbidity prevented the species-specific sexual signalling system from working in Lake Victoria cichlids, resulting in hybridization (Seehausen *et al.* 1997; Seehausen & van Alphen 1998). Did the disappearance of colour signals interfere with female mate choice in the current study? Sand goby females favour colourful males (Forsgren 1992). Sand gobies have blue coloration on the first dorsal fin and the anal fin. These may not be visible under algal turbidity because primarily blue light is filtered out. Hence mate choice based on colour signals may be curbed. In the sand goby this may interfere with the attempts of a female to choose a male of high quality, resulting in a more even distribution of mating success among males. However, it is clear that this study cannot determine whether the loss of signal information is the reason for a decrease in sexual selection opportunity but this question will have to be addressed in future work.

Turbidity may affect mate choice through the cost of mate sampling and assessment. If costs are high, females can become less discriminating (Kodric-Brown 1990). For example, in the presence of predators, females are less choosy (Forsgren 1992; Berglund 1993) and when they have to expend more energy on mate sampling (Milinski & Bakker 1992). Male sailfin mollies (*Poecilia latipinna* Lesueur) have been found to be less discriminating in turbid than clear water conditions (K. Heubel, unpublished data). In fishes, many biological, chemical and physical factors cause stress that they often respond to by changing

their behaviour. It is possible that owing to low visibility, mechanical irritation or other reasons, abundant planktonic algae induced stress in females, causing them to be less selective.

From the above discussion it is clear that there are several potential reasons for the observed change in mating system under algal turbidity. All of these alternatives could, by themselves, explain the observed change but they could also operate in concert. Hence it is important to examine each of them in future studies. In conclusion, whatever the reasons for the change in mating system, this change may have considerable influences on sand goby populations if turbid conditions occur frequently enough in nature. The most notable long-term effect would be a breakdown of the sexual-selection process and an ensuing change in the distribution of traits currently under sexual selection, such as male body size and coloration.

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REFERENCES

- Arnold, S. J. & Wade, M. J. 1984 On the measurement of natural and sexual selection: theory. *Evolution* **38**, 709–719.
- Berglund, A. 1993 Risky sex: male pipefishes mate at random in the presence of a predator. *Anim. Behav.* **46**, 169–175.
- Bonsdorff, E., Norkko, A. & Sandberg, E. 1995 Structuring zoobenthos: the importance of predation, siphon cropping and physical disturbance. *J. Exp. Mar. Biol. Ecol.* **192**, 125–144.
- Cederwall, H. & Elmgren, R. 1990 Biological effects of eutrophication in the Baltic Sea, particularly the coastal zone. *Ambio* **19**, 109–112.
- Cobcroft, J. M., Pankhurst, P. M., Hart, P. R. & Battaglene, S. C. 2001 The effects of light intensity and algae-induced turbidity on feeding behaviour of larval striped trumpeter. *J. Fish Biol.* **59**, 1181–1197. (doi:10.1006/jfbi.2001.1729)
- Elmgren, R. 1989 Man's impact on the ecosystem of the Baltic Sea: energy flows today and at the turn of the century. *Ambio* **18**, 326–332.
- Emlen, S. T. & Oring, L. W. 1977 Ecology, sexual selection, and the evolution of mating systems. *Science* **197**, 215–223.
- Forsgren, E. 1992 Predation risk affects mate choice in a gobiid fish. *Am. Nat.* **140**, 1041–1049.
- Forsgren, E. 1997a Female sand gobies prefer good fathers over dominant males. *Proc. R. Soc. Lond. B* **264**, 1283–1286. (doi:10.1098/rspb.1997.0177)
- Forsgren, E. 1997b Mate sampling in a population of sand gobies. *Anim. Behav.* **53**, 267–276.
- Forsgren, E., Kvarnemo, C. & Lindström, K. 1996 Mode of sexual selection determined by resource abundance in two sand goby populations. *Evolution* **50**, 646–654.
- Guillette, L. J., Gross, T. S., Masson, G. R., Matter, J. M., Percival, H. F. & Woodward, A. R. 1994 Developmental abnormalities of the gonad and abnormal sex-hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. *Environ. Hlth Perspect.* **102**, 680–688.
- Hale, R. E., St Mary, C. M. & Lindström, K. 2003 Parental responses to changes in costs and benefits along an environmental gradient. *Environ. Biol. Fish.* **67**, 107–116.

- Hesthagen, I. H. 1977 Migrations, breeding and growth in *Pomatoschistus minutus* (Pallas) (Pisces, Gobiidae) in Oslo-fjorden, Norway. *Sarsia* **63**, 17–26.
- Jones, J. C. & Reynolds, J. D. 1999a Costs of egg ventilation for male common gobies breeding in conditions of low dissolved oxygen. *Anim. Behav.* **57**, 181–188.
- Jones, J. C. & Reynolds, J. D. 1999b Oxygen and the trade-off between egg ventilation and brood protection in the common goby. *Behaviour* **136**, 819–832.
- Karlson, K., Rosenberg, R. & Bonsdorff, E. 2002 Temporal and spatial large-scale effects of eutrophication and oxygen deficiency on benthic fauna in Scandinavian and Baltic waters—a review. *Oceanogr. Mar. Biol.* **40**, 427–489.
- Kodric-Brown, A. 1990 Mechanisms of sexual selection: insights from fishes. *Ann. Zool. Fenn.* **27**, 87–100.
- Larsson, U., Elmgren, R. & Wulff, F. 1985 Eutrophication and the Baltic Sea: causes and consequences. *Ambio* **14**, 9–14.
- Lehtonen, T. & Lindström, K. 2004 Habitat specific resource distribution affects sexual selection and habitat preferences in sand gobies. *Oikos* **104**, 327–335.
- Lindström, K. 1988 Male-male competition for nest sites in the sand goby, *Pomatoschistus minutus*. *Oikos* **53**, 67–73.
- Lindström, K. 1992 The effect of resource holding potential, nest size, and information about resource quality on the outcome of intruder-owner conflicts in the sand goby. *Behav. Ecol. Sociobiol.* **30**, 53–58.
- Lindström, K. & Lugli, M. 2000 A quantitative analysis of the courtship acoustic behaviour and sound patterning in male sand goby, *Pomatoschistus minutus*. *Environ. Biol. Fish.* **58**, 411–424.
- Lindström, K. & Seppä, T. 1996 The environmental potential for polygyny and sexual selection in the sand goby, *Pomatoschistus minutus*. *Proc. R. Soc. Lond. B* **263**, 1319–1323.
- Lissåker, M., Kvarnemo, C. & Svensson, O. 2003 Effects of a low oxygen environment on parental effort and filial cannibalism in the male sand goby, *Pomatoschistus minutus*. *Behav. Ecol.* **14**, 374–381.
- Milinski, M. & Bakker, T. C. M. 1992 Costs influence sequential mate choice in sticklebacks, *Gasterosteus aculeatus*. *Proc. R. Soc. Lond. B* **250**, 229–233.
- Perttilä, M., Niemistö, L. & Mäkelä, K. 1995 Distribution, development and total amount of nutrients in the Gulf of Finland. *Esuar. Coast. Shelf. Sci.* **41**, 345–360.
- Pitcher, T. J. 1993 *Behavior of teleost fishes*. London: Chapman & Hall.
- Potts, G. W., Keenleyside, M. H. A. & Edwards, J. M. 1988 The effect of silt on the parental behavior of the sea stickleback, *Spinachia spinachia*. *J. Mar. Biol. Assoc. UK* **68**, 277–286.
- Reynolds, J. D. & Jones, J. C. 1999 Female preference for preferred males is reversed under low oxygen conditions in the common goby (*Pomatoschistus microps*). *Behav. Ecol.* **10**, 149–154.
- Sanden, P. & Håkansson, B. 1996 Long-term trends in Secchi depths in the Baltic Sea. *Limnol. Oceanogr.* **41**, 346–351.
- Sanden, P. & Rahm, L. 1993 Nutrient trends in the Baltic Sea. *Environmetrics* **4**, 75–103.
- Seehausen, O. & van Alphen, J. J. M. 1998 The effect of male coloration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis nyererei* complex). *Behav. Ecol. Sociobiol.* **42**, 1–8.
- Seehausen, O., van Alphen, J. J. M. & Witte, F. 1997 Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science* **277**, 1808–1811.
- Utne-Palm, A. C. 2002 Visual feeding of fish in a turbid environment: physical and behavioural aspects. *Mar. Freshwat. Behav. Physiol.* **35**, 111–128.