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How not to be seen: does eutrophication influence three-spined stickleback *Gasterosteus aculeatus* sneaking behaviour?

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Experiments done in aquaria under control and either turbid or densely vegetated conditions indicated that three-spined stickleback *Gasterosteus aculeatus* sneaking behaviour is affected by decreased underwater visibility. Success of sneaking behaviour decreased significantly under increased turbidity but not increased vegetation density. The total number of sneaking attempts, including unsuccessful ones, was not affected by either form of decreasing visibility.

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Key words: alternative mating tactics; sexual selection; sneak fertilization; turbidity; vegetation density; visibility.

INTRODUCTION

Human-induced eutrophication of marine systems is an environmental issue that has received increasing attention in recent decades (Cloern, 2001). Over-enrichment with nutrients stimulates growth of underwater plants, typically resulting in episodes of low or no oxygen availability when these decay (Pollock *et al*., 2007), algal blooms (De Jonge *et al*., 2002), decreased biodiversity and shifts in faunal assemblages, often at the expense of specialists (Karlson *et al*., 2002). One particularly affected ecosystem is the Baltic Sea in northern Europe. Its narrow link to the North Sea and resulting long retention times of water, combined with a densely populated catchment area, make it vulnerable to eutrophication. Algal blooms have caused a decrease in underwater visibility, due to both blooms of planktonic algae that increase turbidity and blooms of filamentous algae that increase vegetation density (Sandén & Håkansson, 1996; Bonsdorff *et al*., 2002).

Many fish species rely on vision to forage and choose mates. Foraging behaviour can either be enhanced or be compromised by turbidity. An important factor seems to be prey size and consequently distance over which prey and predator interact (De Robertis *et al*., 2003). Mate choice is principally reported to suffer from decreased visibility, examples being the Lake Victoria cichlids (Seehausen *et al*., 1997), or the three-spined stickleback *Gasterosteus aculeatus* L. (Engström-Öst &

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Candolin, 2007; Wong *et al*., 2007). Male *G. aculeatus* establish territories, build nests, attract females with ritualised courtship behaviour and red nuptial colouration and care for the eggs (Van den Assem, 1967). Males can show alternative mating tactics (Oliveira *et al*., 2008) and Van den Assem (1967) gave a detailed account of this so-called sneaking behaviour; a male sneaks into a territory, intrudes on a courting couple and tries to mate with the female and fertilize her eggs before the resident male can do so.

In many organisms, alternative mating tactics are performed by individuals that would otherwise not breed, allowing a larger part of the population to reproduce. This can undermine sexual selection due to female mate choice (Oliveira *et al*., 2008).

In *G. aculeatus*, however, sneaking behaviour is not performed exclusively by non-nesting males who would otherwise not reproduce (*cf.* 'best-of-a-bad-job'; Dawkins, 1980). It seems to be an opportunistic tactic, shown by nesting and non-nesting males alike (Van den Assem, 1967; Goldschmidt *et al*., 1992; Jamieson & Colgan, 1992; Rico *et al*., 1992; De Fraipont *et al*., 1993; Le Comber, 2003). This would reduce variance in male reproductive success, although determining to what degree requires genetic data.

Candolin (2004) noted that increased vegetation density led to a more even distribution of eggs among nests. Possibly this was due to an increase in the frequency or success of sneaking behaviour and subsequent egg stealing, although this was not measured (Candolin, 2004). Van den Assem (1967) described how sneakers quickly assume drab colouration when intruding, suggesting they could benefit from decreased underwater visibility by becoming harder to detect for nesting males, and thus be more successful. Sneakers, however, might find it harder to detect opportunities to begin with, resulting in a decrease in the frequency of attempts. In this study, underwater visibility was decreased in two different ways to test if and how sneaking behaviour changes. The working hypothesis is that decreasing underwater visibility will decrease the frequency of sneaking behaviour but increase its success.

MATERIALS AND METHODS

Adult *G. aculeatus* were collected using minnow traps from early May until the end of June 2008 in Långskär Bay in the Baltic Sea in southern Finland (60° N; 23° E). Fish were housed in an outdoor facility at Tvärminne Zoological Station under natural light and temperature conditions in aquaria (150 l) with a continuous flow of fresh sea water, at a density of 0·25 fish per litre. They were separated by sex by regular inspection for development of eggs or nuptial colouration. Males were transferred to individual aquaria (10 l) and given a plastic plant saucer (Ø 12·5 cm) filled with sand and some filamentous algae (*Cladophora* sp.) for nest construction. A gravid female in a glass jar with perforated lid was presented to each male daily for 30 min to encourage nest building and courtship behaviour. All fish were fed *ad libitum* with frozen chironomid *Chironomus* sp. larvae twice daily, sometimes supplemented with live mysid shrimps. After the experiments, all fish were returned to their natural breeding area.

Experimental aquaria (70 cm × 40 cm × 30 cm) contained a thermometer, an air stone supplying a constant airflow and were surrounded by curtains to prevent interactions with the outside environment. Small holes in the front curtain allowed visual observation while minimizing disturbance. Each aquarium contained two plexiglass dividers, one transparent and perforated (divider 1) and one opaque (divider 2), creating three compartments [Fig. 1(a)] that

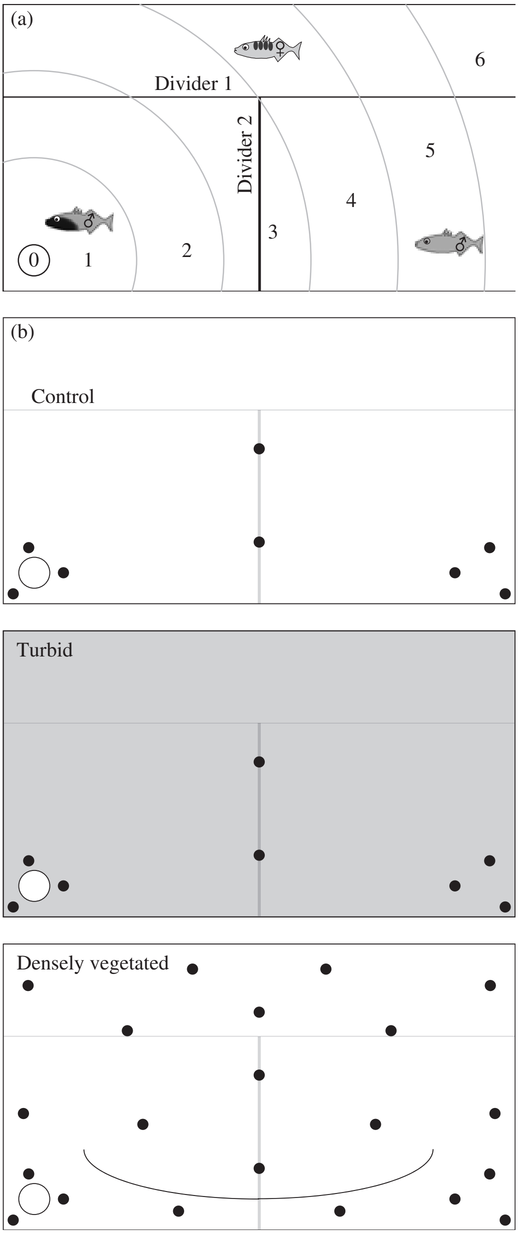

Fig. 1. The experimental setup. (a) Tank division and zones, fish not drawn to scale. The bottom left and right compartments contained one male each, here left the nesting male and right the potential sneaker, separated by an opaque plexiglass divider (divider 2). The top compartment contained the female and was separated from the male compartments by a perforated transparent plexiglass divider (divider 1). Each aquarium stood on a wooden board that had two series of concentric circles drawn on it, each series originating at the position where a nesting dish could be placed, with circles drawn at 10 cm intervals. For simplicity, only one set of concentric circles is shown here. Numbers 0–6 refer to the different zones, 0 being the nesting dish. (b) Experimental treatments: control (eight plants), turbid (eight) and densely vegetated (22). Dots, and the curved line in the densely vegetated treatment, indicate the position of artificial plants.

were not airtight, allowing exchange of olfactory cues. A mirror stood on top of the aquarium at an angle such that, when seated behind the curtain, an observer had a top-down view of the aquarium and the wooden board on which it was standing. Each of these boards had two series of concentric circles drawn on it, each series originating at the position where a nesting dish would be placed, with circles drawn at 10 cm intervals [Fig. 1(a)]. When viewed *via* the mirror, these circles allowed a quick estimation of the distance of fish to the nesting dish. Aquaria were filled to a depth of 20 cm with sea water and assigned at random to control, turbid or densely vegetated treatments.

Algal turbidity was produced using the fast-growing non-toxic flagellate alga *Isochrysis* sp. that is part of the phytoplankton community of the Baltic Sea. Algae were grown outdoors in uncovered containers (40–80 l) under natural light by day and artificial light by night, in filtered (20 μm) sea water and aquatic fertilizer (Kemira GrowHow, puutarhan hydrolannos 1; Kemira; www.kemira.com) at a concentration of 0·75%. Cultures were continuously aerated with air stones and stirred using a filter pump and, to stimulate growth, heated to *c*. 18° C using aquarium tube heaters. For the experiments, algal culture was added until a turbidity of *c*. 7 nephelometric turbidity units (NTU) was reached. Turbidity levels were measured with a portable nephelometer (Hach 2100P; Hach Company; www.hach.com). Mean ± S.D. turbidity was 1·44 ± 0·44 in control replicates, 6·81 ± 1·39 in turbid replicates and 1·41 ± 0·48 in densely vegetated replicates. Turbidity in the Baltic Sea can vary between 0·5 and 45·0 NTU but rarely exceeds 30 NTU (Granqvist & Mattila, 2004). The values chosen here for the turbid treatment were the highest possible without seriously hindering behavioural observations.

Artificial algae were made from two 15 cm long unravelled bunches of green polypropylene strings attached to a cement foot and, for the densely vegetated treatment, an additional piece of more complex vegetation made from a 30 cm long piece of curved metal wire with strings attached along its length. In control and turbid replicates, a baseline level of vegetation was present, consisting of eight plants, three around each of two possible nest positions and two in the middle. In densely vegetated replicates, 22 plants were placed, spaced throughout the aquarium in a fixed pattern [Fig. 1(b)]. This corresponded to 28·6 v. 78·6 plants m^{-2} for control *v*. densely vegetated treatments. The latter value is conservative because the complex plant offered more cover than a single polypropylene plant.

Two males, one with a completed nest, the other without a nest but showing signs of nuptial colouration, were picked at random from the individual aquaria. Standard body length (L_S, cm) and wet body mass (M, mg) was measured and Fulton's condition factor (K) was calculated afterwards as $K = 100\ ML_S^{-3}$ (Nash *et al*., 2006). Fish were marked individually by clipping the tip of the first or the second of the two large dorsal spines and then introduced into the experimental aquarium. The male with the completed nest was assigned the role of nesting male and was placed at random in the left or right compartment with his nesting dish. The other male was assigned the role of potential sneaking male and was placed without his nesting dish in the other compartment. One hour after introduction, the opaque divider (divider 2) separating the males was removed. Fish were left overnight to allow them to establish dominance relationships.

Mate choice in *G. aculeatus* will sometimes take several hours (pers. obs.). To determine if the potential sneaker would intrude when the female spawned, video recordings were made. A webcam was placed next to the aquarium, on the side of the nesting male, offering a close-up view of the nesting dish and its direct surroundings. Up to four webcams were connected to the Universal Serial Bus (USB) 2.0 port of a computer running the open source webcam application Dorgem 2.1.0 (Fesevur, 2005) under Windows XP. A self-powered USB hub was used to ensure sufficient power to each of the webcams. Different models of webcams were used to avoid driver conflicts when connecting more than one webcam of the same model. Screenshots were made at one frame s^{-1} and a resolution of 352 × 288 pixels whenever motion was detected. Preliminary observations showed that this system was highly sensitive and detected even small movements such as a nesting male hovering over his nest. As the nesting dish was positioned in a corner of the aquarium, directly against the glass wall, visibility was only minimally affected from the point of view of the camera, even under turbid conditions. This ensured that any attempt at sneaking would be detected and recorded.

Just before introducing the female, temperature and turbidity were measured and airflow to the air stone was stopped to ensure a smooth water surface and so enable observations *via* the mirror. A gravid female was taken from the holding aquaria, measured and weighed as above, and placed behind the transparent divider (divider 1), separated from the males. The fish were given 5 min to acclimatize after which divider 1 was carefully removed. Observations started as soon as fish resumed activity.

During behavioural observations, the following was recorded: number of dashes (defined as the nesting male dashing at the potential sneaker but not touching it), attacks (defined as the nesting male shortly pushing or biting the potential sneaker, involves physical contact) and fight and chase bouts (defined as an extended form of attacks, lasting >3 s, usually involving the nesting male chasing the potential sneaker through the aquarium at high speed).

For each observation, the zone where the behaviour took place, or started for fight and chase bouts, was noted as observed *via* the mirror. For fight and chase bouts, bout duration was measured with a stopwatch. Observations lasted for 30 min or until the female spawned, whichever came first. In the former case, the camera was left running until termination. Video recordings were checked regularly and the experiment was stopped if the female had not spawned within 9 h of introduction, or 30 min postspawning, whichever came first. Waiting until 30 min postspawning should allow the potential sneaker sufficient time to intrude and fertilize eggs. Bakker *et al*. (2006) found that egg clutches were not completely fertilized after 10 min by the sperm of one male, although they mentioned that their *in vitro* protocols may decrease fertilization efficiency. Sperm in the ejaculate of one male is numerous enough to fertilize all eggs in a clutch (Zbinden *et al*., 2001; I. Mayer, pers. comm.) so it seems highly unlikely there are unfertilized eggs left 30 min post-fertilization that a sneaker could fertilize. Van den Assem (1967) also mentions 30 min as the length of the period postspawning in which eggs can be fertilized. Upon termination, all fish and the nest were collected, and the female was weighed a second time if she had spawned. The aquarium was emptied and aquarium and artificial plants were rinsed.

Video recordings were watched afterwards to determine: (1) if sneaking attempts took place. An attempt was deemed successful if the sneaker male crept through the nest after the female had spawned. Time until sneak fertilization was measured as time between these two events. (2) The number and duration of intrusions by the sneaker male into zone 0, the nesting dish, before and after spawning, duration defined as time between the sneaker male entering and exiting the nesting dish. (3) The time to spawning (starting at introduction of the female), spawning duration (defined as the time between a female entering and exiting the nest) and time to fertilization (defined as the time between the female exiting the nest and the nesting male entering to fertilize the eggs). Counted observations were transformed into rates by calculating the number of observations per min.

Statistical analyses were done in R 2.7.2 (R Development Core Team, 2008). Data on sneaking attempts were analysed using G-tests. In some cases, potential sneakers took over the nest. As difference in M between the two males was expected to also be of influence, data on nest takeovers were analysed using a logistic regression. Treatment and relative difference in M were taken as input variables, the latter calculated as $(M_{\text{sneaker}} - M_{\text{nester}})M^{-1}_{\text{sneaker}}$. Distribution of distances to the nest at which defensive behaviours took place was analysed using a general linear model (GLM) from the package NLME (Pinheiro *et al*., 2008). Replicate number was added as a random factor because behaviours were observed more than once in most replicates. All other data were analysed using one-way ANOVAs or, where data did not meet assumptions of normality and no suitable transformations could be found, Wilcoxon rank-sum tests. Experiments were designed with the idea of comparing control conditions with conditions of decreased visibility, either an increase in turbidity or an increase in vegetation density. A direct comparison between the effects of turbidity and vegetation density yields results that are not straightforward to interpret from a biological perspective and was not the intention of these experiments. Data have been analysed for all three treatments simultaneously though, which includes comparing turbid and densely vegetated treatments. This yielded very similar results that have not been reported here.

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RESULTS

In 22% (control, $n = 9$; turbid, $n = 8$; densely vegetated, $n = 11$) of all replicates (control, $n = 43$; turbid, $n = 41$; densely vegetated, $n = 44$) roles reversed overnight, before introduction of the female, and the potential sneaker had taken control of the nest, keeping the original nest owner at a distance. There was no difference between control and turbid (logistic regression, d.f. = 1 and 82, $P > 0{\cdot}05$) or control and densely vegetated (logistic regression, d.f. = 1 and 84, $P > 0{\cdot}05$) replicates in the number of takeovers observed nor was there a significant difference in relative mass difference between replicates with and without nest takeovers for control and turbid (logistic regression, d.f. = 1 and 82, $P > 0{\cdot}05$) or control and densely vegetated treatments (logistic regression, d.f. = 1 and 84, $P > 0{\cdot}05$).

In many replicates, there were no or few interactions between the potential sneaker and the nesting male in the first 30 min after female introduction. Potential sneakers often hid from the courting couple and stayed in the area where they had been introduced. Attack rates, fight and chase bout rates (Wilcoxon rank-sum test, d.f. = 1, $P > 0{\cdot}05$ for all) and fight and chase bout durations (ANOVA, d.f. = 1 and 27, $P > 0{\cdot}05$ for both) did not differ between control and turbid or control and densely vegetated replicates. Dash rates, however, decreased significantly in both turbid and densely vegetated replicates compared with the control (Wilcoxon rank-sum test, d.f. = 1, $P < 0{\cdot}05$ for both) (data not shown). For the distance to the nest at which defensive behaviours were observed, no differences were found for dashes (GLM; control *v*. turbid d.f. = 1 and 13, control *v*. densely vegetated d.f. = 1 and 12, $P > 0{\cdot}05$ for both), attacks (GLM; control *v*. turbid d.f. = 1 and 18, control *v*. densely vegetated d.f. = 1 and 20, $P > 0{\cdot}05$ for both) or fight and chase bouts (GLM; control *v*. turbid d.f. = 1 and 17, control *v*. densely vegetated d.f. = 1 and 23, $P > 0{\cdot}05$ for both).

Time to spawning, spawning duration and time to fertilization were all skewed towards small values. The first two variables did not differ between control and turbid or control and vegetated replicates (Wilcoxon rank-sum test, d.f. = 1, $P > 0{\cdot}05$ for all). Time to fertilization, however, was significantly shorter in turbid compared with control replicates (Wilcoxon rank-sum test, d.f. = 1, $P < 0{\cdot}05$) but did not differ between control and densely vegetated compared replicates (Wilcoxon rank-sum test, d.f. = 1, $P > 0{\cdot}05$) (data not shown).

Sneaking attempts were observed in 22% of all replicates (control, $n = 11$; turbid, $n = 6$; densely vegetated, $n = 10$) and there were no differences between control and turbid replicates (*G*-test, d.f. = 1, $P > 0{\cdot}05$) or control and densely vegetated replicates (*G*-test, d.f. = 1, $P > 0{\cdot}05$) in the observed numbers. Power analyses indicated that to find a statistically significant difference given these proportions of sneaking attempts, and using a statistical power of 0·80, the sample size per group would have had to be 210 replicates per treatment for the comparison control *v*. turbid, and 3530 replicates for the comparison control *v*. densely vegetated. The minimum detectable difference given 11 sneaking attempts in the control would be two or less, or 20 or more sneaking attempts in the experimental treatment. For the replicates where sneaking was attempted, sneakers and nesters did not differ in L_S, M or K between control and turbid conditions (ANOVA, d.f. 1 and 15, $P > 0{\cdot}05$ for all comparisons) or control and densely vegetated conditions (ANOVA, d.f. 1 and 19, $P > 0{\cdot}05$ for all comparisons).

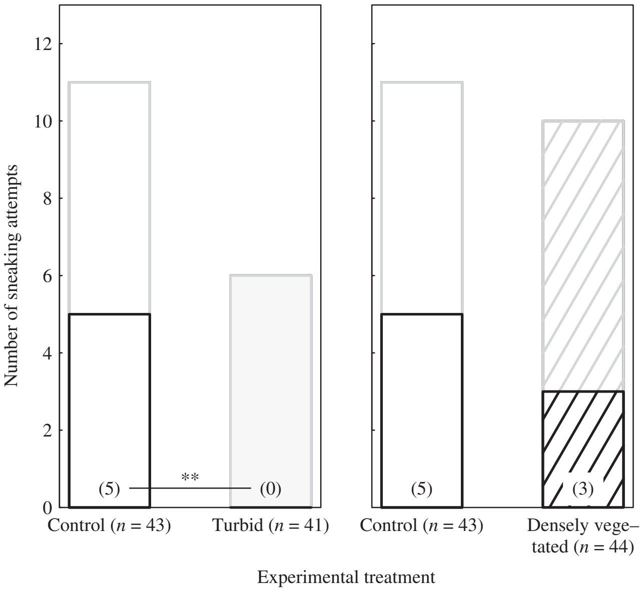

FIG. 2. The total number of sneaking attempts (☐) and the number of successful attempts (☐, numbers are indicated at the base of columns). Sample sizes are given in parentheses following each treatment. For turbid replicates, no sneaking attempts were successful and this differed significantly from control replicates (G-test, **, $P < 0.01$).

Successful sneaking attempts were observed in only eight replicates (control, $n = 5$; turbid, $n = 0$; densely vegetated, $n = 3$). In all cases, the sneaker only entered the nest after the female had left. The number of successful sneaking attempts differed significantly between control and turbid replicates (G-test, d.f. $= 1$, $P < 0{\cdot}01$), but not between control *v*. densely vegetated replicates (G-test, d.f. $= 1$, $P > 0{\cdot}05$) (Fig. 2). For the latter comparison to be statistically significant, given these ratios and a power of 0·80, the sample size per group would have had to be 567 replicates per treatment. The minimum detectable difference given five successful sneak fertilizations would have to be 14 or more successful cases in the densely vegetated treatment. The sneakers and nesters did not differ between successful and failed sneaking attempts for L_S, M or K between control and turbid conditions (ANOVA, d.f. 1 and 15, $P > 0{\cdot}05$ for all comparisons) or control and densely vegetated conditions (ANOVA, d.f. 1 and 19, $P > 0{\cdot}05$ for all comparisons).

No differences were found between control and turbid or control and densely vegetated replicates for the frequencies or the durations of intrusions into zone 0, the nesting dish, before or after spawning (Wilcoxon rank-sum test, d.f. $= 1$, $P > 0{\cdot}05$ for all comparisons).

DISCUSSION

Potential sneakers were expected to attempt sneaking less when visibility decreased, but be more successful at it. Contrary to expectations, the number of sneaking attempts did not decrease, and in turbid water the success of sneaking attempts even decreased (Fig. 2). Work done by others showed that intruders have a harder time locating nests when visibility decreases. Both Dzieweczynski & Rowland (2004; *G. aculeatus*) and Hibler & Houde (2006; guppies *Poecilia reticulata* Peters) decreased visibility by placing opaque screens in their tanks. The former showed that male *G. aculeatus* prefer to court in concealed areas when a competitor was present, the latter that there were fewer courtship intrusions by other *P. reticulata*. Using opaque screens completely blocks visibility, whereas turbidity and increased vegetation density only hinder it. This might be an explanation why a significant decrease in the number of sneaking attempts was not found in this study. Another explanation could be that the sneakers had become sufficiently familiar with their surroundings to know where the nest was located, despite reduced visibility. This is an unfortunate artefact of working within the confines of an aquarium. Acclimatizing the fish overnight was necessary to allow them to establish dominance relationships. In pilot experiments, males were introduced only shortly before the female. This resulted in males ignoring the female and fighting instead, leading to an unacceptably high proportion of failed replicates when females shed their eggs prematurely.

Other research on the effects of decreased visibility on courtship intrusions shows a mixed picture. Sargent & Gebler (1980) gave male *G. aculeatus* flowerpots to nest in. These males suffered fewer sneak fertilizations and territorial encounters than nesting males without flowerpots. Mori (1995), however, studying *G. aculeatus* in a Japanese stream, showed a positive correlation between degree of nest cover and probability of nest raiding and argued that sneakers were harder to detect when visibility decreased. Stoltz & Neff (2006), in a study on bluegill sunfish *Lepomis macrochirus* Rafinesque, found no influence of peripheral vegetation on intrusion frequency by sneakers, although their study was not specifically designed to address this question.

More circumstantial evidence for decreased visibility resulting in reduced courtship intrusion and sneak fertilization probability comes from Wong *et al*. (2007). They showed that male *G. aculeatus* reduced courtship in the presence of a competitor, but did so less under turbid conditions. Kraak *et al*. (2000) showed that male *G. aculeatus*, especially large and red ones, prefer to nest in dense vegetation when given a choice, and suggest that such nests are better defendable against sneakers. In an earlier study, Kraak *et al*. (1999) already showed that nests in dense vegetation had a higher egg survival rate than exposed ones. Candolin (2004) showed that vegetation cover reduces the variation in egg numbers between *G. aculeatus* nests, although the contribution of egg stealing and sneak fertilization to this was unknown. The overall picture emerging from these studies is that decreasing visibility usually leads to reduced intrusion by other males.

Once a potential sneaker detects an opportunity and attempts sneaking, his success depends on remaining undetected by the nesting male. Intuitively decreased visibility would be expected to benefit the sneaker at this point, but results showed a significant decrease in sneaking success for turbid replicates, where no single attempt was successful (Fig. 2). Le Comber (2003) showed that the detection of a sneaker lowers the probability of the female spawning. A female aborting spawning due to an intrusion is another way in which a sneaking attempt can fail, and this was observed twice in control conditions, but not under turbid or densely vegetated conditions.

Are nesting males more defensive when visibility decreases? No differences were found between treatments for rates of attacks and fight and chase bouts, nor the duration of the latter. Dash rates decreased significantly under decreased visibility. Furthermore, time to fertilization was significantly shorter in turbid compared with control replicates. This could be a direct consequence of the reduced interactions with intruders, as shown by the reduced dash rates, although a decrease was not found when comparing control and densely vegetated replicates where dash rates were similarly reduced. Finally, considering that neither the number of sneaking attempts nor the distance at which potential sneakers and nesters interacted changed between treatments, the notion of nesting males being more defensive under decreased visibility does not seem supported.

Another possibility is that turbidity increases contrast between a sneaker and his background, facilitating detection by the nesting male. Indeed, several authors have observed how moderate turbidity enhances foraging success, and attribute this to increased contrast between prey and their surroundings (Boehlert & Morgan, 1985; Miner & Stein, 1993; Utne-Palm, 2002).

It is also possible that nesting males shift to other sensory modalities, such as olfaction or mechanoreception, to detect intruders when visibility decreases. Increased turbidity leads to increased use of olfactory cues by *G. aculeatus* during foraging (Webster *et al*., 2007) and mate choice (Heuschele, 2009).

Nesting males will probably benefit from decreased sneaking, as courtship intrusions are costly to them. Several authors describe how female *G. aculeatus* delay or abort spawning when courtship is interrupted (Goldschmidt *et al*., 1992), also because nesting males increase aggression (Van den Assem, 1967) and decrease courtship rate (Le Comber *et al*., 2003). Similar observations have been reported for other fish species (Taborsky, 1994; Willmott & Foster, 1995), although in some species females prefer to spawn when sneakers are present (Taborsky, 2008). In general, however, reduced sneaking will increase a nesting male's reproductive output (Alonzo & Warner, 2000), and this would be an unexpected benefit of decreased visibility due to eutrophication.

A decrease in sneak fertilizations could also have evolutionary consequences. Work by both Järvenpää & Lindström [2004; sand gobies *Pomatoschistus minutus* (Pallas), turbidity] and Candolin (2004; *G. aculeatus*, vegetation density) shows that decreased visibility results in a more even distribution of eggs among nests, decreasing the opportunity for sexual selection. Other indications that decreased visibility breaks down the mating system come from Candolin *et al*. (2007; vegetation density) and Engström-Öst & Candolin (2007; turbidity), who show that *G. aculeatus* spent more time and energy on courtship and mate choice when visibility decreases. Wong *et al*. (2007) also argue that decreased visibility reduces sexual selection by diminishing the effectiveness of sexual displays and weakening signals of male quality.

Given this, what would a decrease in sneaking behaviour effect? In mating systems where males either sneak or nest, a decrease in sneaking could result in intensified sexual selection if a smaller proportion of the population now reproduces. If these sneakers become breeders, however, a larger proportion of the population reproduces, weakening sexual selection. Indeed, Candolin (2004) observed that density of breeding males increases in dense vegetation. This could complicate matters, as this means that the number of nests where sneaking can be attempted increases. Another complication of the *G. aculeatus* breeding system is that both nesting and non-nesting males can sneak, as outlined in the introduction. It is currently unknown if both groups respond similarly, only the response of non-nesting males was addressed here. If it is mainly nesting males that sneak, and the most attractive nesters that have the highest sneaking success, then sneaking could skew mating success towards fewer males and intensify sexual selection.

If decreased sneaking behaviour intensifies sexual selection, this could compensate for relaxing sexual selection due to a breakdown of the mating system as discussed by Wong *et al*. (2007). Sneak fertilization, however, is not the main mode of reproduction. It occurred in 22% of replicates in the experiments reported here, although this could be higher under natural conditions where nesting males can also sneak. The net result, even under natural conditions, would probably still be a relaxation of sexual selection. If decreased sneaking behaviour weakens sexual selection, it will add to above-mentioned mechanisms and lead to a further relaxation of sexual selection still.

It is worth pointing out that these considerations specifically apply to resource defence mating systems. Given the wide variety in fish mating systems (Taborsky, 1994), it remains to be determined what the effects of decreased visibility due to eutrophication would be in, for example, group spawning fishes, where many individuals participate in spawning simultaneously and males do not monopolize females.

To summarize, decreased underwater visibility due to eutrophication might interfere with alternative mating tactics. Success of sneaking behaviour significantly decreased under increased turbidity but not increased vegetation density.

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