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Subordinate brown trout exaggerate social signalling in turbid conditions

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Visual displays allow animals to communicate information about social status and to minimize costs of dangerous fighting. However, in aquatic animals, visual signals may be seriously affected by increased turbidity. In juvenile salmonids, subordinates signal defeat through a darkening in coloration and in doing so reduce further attacks from dominant individuals. We examined the behaviour and physiology of socially interacting brown trout, *Salmo trutta*, dyads in clear water, low turbidity and high turbidity. Overall, the characteristic aggression associated with socially competing salmonids was reduced in turbid conditions and visual displays of subordinates were exaggerated. It has been suggested that darkening of subordinate salmonids is primarily a result of increased stress, and acts secondarily in communication. However, although plasma cortisol was highest in subordinates, turbidity did not affect cortisol concentrations. In conclusion, exaggeration of subordinate visual signals in turbid conditions appears to be a response to alterations in environment, rather than a secondary stress effect.

Fighting over resources can be costly and competitors benefit if contests can be resolved through communication and avoidance of dangerous fighting (Hurd 1997; Oliveira et al. 1998; Briffa & Sneddon 2006). While a large amount of research has demonstrated the existence of signals of relative fighting ability, which may stem escalation of a contest to injurious fighting (Hammerstein & Parker 1982), animals may also signal defeat in an attempt to end a contest. In fish, rapid changes in colour are often associated with competitive encounters (Stacey & Chiszar 1977; Rosenthal & Lobel 2006) with individuals of different social status displaying different colour patterns. In juvenile salmonids, darkening of body and sclera colour during social contests is believed to signal subordination, eliciting fewer aggressive attacks from dominant individuals (O'Connor et al. 1999; Suter & Huntingford 2002).

The darker coloration of subordinate salmonids has been documented in a variety of studies (e.g. Keenleyside & Yamamoto 1962) and is believed to be mediated by stress-induced elevation of α-melanocyte-stimulating hormone (α-MSH; Höglund et al. 2000). Indeed, elevated α-MSH is one of many physiological changes associated with subordination in salmonid fish, which also include increased plasma cortisol and brain serotonergic activity, breakdown of hepatic glycogen stores and impaired ionoregulation (Sloman & Armstrong 2002; Johnsson et al. 2006). Darkening of body and sclera colour does not appear to prevent the onset of aggression but accompanies defeat and reduces further attacks (O'Connor et al. 1999). Therefore, it is possible that darkening is primarily associated with the stress of subordination and secondarily acts as an indicator of subordination.

For visual signals within the aquatic environment, increases in turbidity as a result of anthropogenic activities such as logging, agriculture, construction and mining (Bash et al. 2001; Bilotta & Brazier 2008) represent a serious problem. The detectability of a signal is dependent on (1) the type of information being conveyed, (2) the environment through which it is transmitted, (3) the functioning of sensory organs and (4) the psychology of the signal receiver (Guilford & Dawkins 1991; Dawkins & Guilford 1994). Therefore, changes in the environment through which a signal is transmitted can alter signalling interactions (Endler 1993). In fish, interference with visual signals associated with mate choice (Engstrom-Öst & Candolin 2006; Heubel & Schlupp 2006) can result in sexual selection and reproductive isolation (Seehausen et al. 1997). Turbidity may also cause more direct physiological costs to a fish by clogging gills and compromising respiration (Newcomb & Flagg 1983; Bash et al. 2001).

While there are numerous studies considering the effects of turbidity on a suite of fish behaviours (Meager & Batty 2007; Salonen

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et al. 2009; Salonen & Engström-Öst 2010; Sundin et al. 2010), to our knowledge, the effects of turbidity on signals used during competitive encounters have yet to be examined. There is evidence that in turbid environments, fish may increase vigilance for predators (Martel & Dill 1993; Shingles et al. 2005) and direct attention away from aggressive interactions. However, if turbidity masks signals of subordination used by juvenile salmonids then fights may escalate more than in clear environments. In the present study, we investigated the behaviour and physiology of pairs of juvenile brown trout competing in control, low- and high-turbidity environments. Our first hypothesis was that subordinate individuals would be able to alter the intensity of their signalling in response to turbidity and, therefore, aggression levels would not be affected by turbid conditions. Indeed, a decrease in aggression might be predicted if more attention was diverted away from aggression to increased predator vigilance associated with turbid environments. The alternative to this hypothesis was that a decreased ability of subordinates to signal defeat in a turbid environment would increase levels of aggression. Our second hypothesis was that alteration of intensity of social signalling would be related to changes in physiology caused by the stress of turbidity exposure; to address this hypothesis we measured a suite of physiological indexes of stress along with gill morphology to determine any effects of turbidity on the gills. The alternative hypothesis was that changes in social signalling were mediated without changes in stress physiology.

METHODS

Juvenile brown trout (fork length: 7.7 ± 0.1 cm; mass: 5.2 ± 0.2 g) used in this experiment were from an existing stock reared at the University of Plymouth. Before the experiment, fish were housed in 25-litre stock tanks connected to an 800-litre recirculating system (16.2 ± 0.1 °C; pH 6.6 ± 0.05; > 95% dissolved oxygen; 12:12 h light:dark). Fish were held at a stocking density of approximately 5 g/litre and were fed twice daily to satiation on a commercial trout feed. At the start of the experiment, fish were lightly anaesthetized (MS222, 0.08 g/litre according to Sloman et al. 2003) and individually marked on the caudal fin using Alcian blue dye (Kelly 1967). No adverse effects of anaesthetization or marking were seen, with all fish quickly resuming normal behaviours. Length measurements of the fish were accurate to the nearest 0.1 cm and pairs of fish were size matched before allocation to treatment. The average size difference between fish of the same pair was 0.1 ± 0.03 cm (mean ± SEM). Pairs were allocated to either a control treatment ($N = 10$ pairs; mean size difference between fish of the same pair: 0.2 ± 0.06 cm), or a low-turbidity ($N = 8$; mean size difference between fish of the same pair: 0.1 ± 0.05 cm) or high-turbidity ($N = 7$; mean size difference between fish of the same pair: 0.1 ± 0.02 cm) treatment. Differences in size between fish within the same pair were, therefore, extremely small and there was no significant difference in the size difference of matched pairs between treatments (one-way ANOVA: $F_{2,24} = 3.157$, $P = 0.062$). Each pair was placed into a 25-litre glass tank with the two individuals separated from each other by an opaque partition. Each treatment was held on the same recirculating system as the stock tanks. For the low- and high-turbidity treatments, we added Polsperse 10 Kaolin to the water to increase turbidity (Meager et al. 2005; Shingles et al. 2005). There are no known chemical effects of Polsperse 10 Kaolin at the concentrations used in the present study and we noticed no adverse reactions (e.g. coughing, agitation) when the Polsperse was added to the water. The fish were given 72 h acclimation to the tanks before the Polsperse 10 Kaolin was delivered to the tanks via a peristaltic pump. Polsperse 10 Kaolin was removed from the system as water passed through the filtration system so it was continuously added from a stock solution for the duration of the experiment. Turbidity measurements were taken daily (in nephelometric turbidity units [NTU]) using a YSI hydrodata 6600 multiparameter water quality monitor. Turbidity measured 0.2 ± 0.1 NTU in control treatments, 27.2 ± 0.94 NTU in low-turbidity treatments and 46.9 ± 1.04 NTU in high-turbidity treatments.

Partitions were removed 96 h after the fish were placed into the tanks to allow social contests to take place. Once the partitions were removed, we observed social interactions between fish daily for 30 min for 3 days. During each observation period, fish were scored for aggression, position in the tank and acquisition of food (see Behavioural Observations) based on scoring systems used in previous studies (Sloman et al. 2002). At the start of the first observation period and at the end of the last observation period, fish were also scored for body and sclera coloration. After the 3-day observation period, pairs of competing fish were captured within 2 min of each other and terminally anaesthetized (MS222, 0.8 g/litre). Mass and fork length of each fish were recorded and a blood sample taken by caudal severance. Blood was centrifuged at 13 000*g* and the plasma snap frozen in liquid nitrogen and stored at −80 °C for later analysis of cortisol. Livers were removed, weighed to allow calculation of hepatosomatic index, snap frozen in liquid nitrogen and stored at −80 °C for later analysis of glycogen content. The first two left gill arches of each fish were removed and placed in 10% formalin for later histological analysis of gill structure.

Behavioural Observations

Each aggressive act performed within the 30 min period (biting, lunging, chasing and gill flaring) scored one point. One score for position was given for each 30 min period. A fish holding a mid-tank position scored three points, a fish resting on the tank bottom scored two points and a fish swimming at the surface scored one point. At the end of the 30 min period, food was added to the tank one pellet at a time. If a fish acquired food it scored one point, while fish that did not acquire food scored zero. Food was not given at any other time. The body and sclera coloration of each fish was scored both at the beginning and end of the behavioural observations. Colour was scored between one and three according to the methods of O'Connor et al. (1999); a very light coloration scored three points, an intermediate coloration scored two points and a very dark coloration scored one point. All colour scoring was done by L.E. The use of visual observation for quantifying body and eye coloration in juvenile salmonids has been validated by O'Connor et al. (1999). However, to ensure objectivity of the colour grading, we used a similar method as O'Connor et al. (1999) where, separate to the main experiment, five independent fish biologists were asked to grade five fish, which gave an 80% agreement with L.E. There was no overall difference in the colour scoring of the fish by different observers (Friedman's rank test: $\chi^2_4 = 1.913$, $P = 0.752$) for body and sclera colour combined. The dominant fish of each pair was the fish with the highest behavioural score after the 3-day observation period. In addition to the absolute colour scores, for each fish, we calculated the change in colour between the start and end of the behavioural observations to generate one colour score per fish. Then, to remove any observational bias between treatments, we calculated the difference in body and sclera colour combined between the individuals of each pair according to the following equation:

$$\left[\left(\mathrm{BD_i} - \mathrm{BD_f}\right) - \left(\mathrm{BS_i} - \mathrm{BS_f}\right)\right] + \left[\left(\mathrm{ScD_i} - \mathrm{ScD_f}\right) - \left(\mathrm{ScS_i} - \mathrm{ScS_f}\right)\right]$$

where BD is the initial (i) and final (f) body colour of the dominant individual and BS that of its subordinate counterpart, ScD is the sclera colour of the dominant and ScS that of its subordinate counterpart.