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Differences in Response to Hypoxia in the Three-Spined Stickleback from Lotic and Lentic Localities: Dominance and an Anaerobic Metabolite

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ABSTRACT

Dominance hierarchies of the three-spined stickleback Gasterosteus aculeatus from river and pond populations were subjected to hypoxia (20%, range$\pm$1%). Under hypoxia, the hierarchies were less stable in terms of rank position and tissue L-lactate was higher in river fish than pond fish under normoxia and hypoxia. Dominant fish gained mass under normoxia but lost mass under hypoxic conditions possibly due to them maintaining high levels of aggression.

Dominance hierarchies are a common feature of a variety of commercial fish species (Adams & Huntingford, 1996; Johnsson, 1997) and can be problematic in aquaculture (Brännäs et al., 2001). Dominant individuals tend to monopolize resources and as such show higher growth rates than subordinates who have limited access to food (Metcalfe, 1986). Altering environmental conditions is a possible route to ameliorating the undesirable effect of social hierarchies on individual growth. A few studies have shown that manipulating the environmental conditions can cause a breakdown of hierarchy structure in brown trout *Salmo trutta* L. (Sloman et al., 2001) and reduce aggression levels in brook charr *Salvelinus fontinalis* (McNicol & Noakes, 1984). Different species with different ecological preferences may respond in particular ways to environmental variation (e.g. oxygen tensions and fish distribution; Burleson et al., 2001). The purpose of this study was to investigate the impact of an environmental variable, hypoxia, on dominance behaviour and metabolic physiology. Hypoxia imposes metabolic constraints on energy production and should, therefore, affect behaviour (Sneddon et al., 1999). Three-spined sticklebacks *Gasterosteus aculeatus* L. are known to colonize various types of habitats and thus two populations were tested, one from a static pond area and the other from a fast flowing river to assess if they showed variable responses to the environmental manipulation.

Three-spined sticklebacks were caught by trapping and netting from a river [Westquarter Burn, Falkirk, central Scotland (56˚0' N; 3˚48' W)] and a pond [Balmaha pond, Loch Lomondside, Scotland (55˚55' N; 3˚11' W)] in January 2002. Water temperature was 8˚ C, range$\pm$1˚ C in both water bodies. The fish were transferred to an aquarium and placed into groups of four (n=8 for each habitat). The fish were likely to be of different genetic stock since they came from water bodies that are unlinked. The close proximity of the populations (c. 25 miles apart on the same latitude) probably ensures similar weather conditions including water temperature but, the fish would have experienced different water quality. Only non-parasitized fish

were used in the experiments and this was checked internally at the end of the experiment. Each tank (45 x 35 x 30 cm) was filled with dechlorinated water, pH 7·4, had a coarse gravel substratum and a 15 cm airstone connected to an airpump supplying air via airline tubing and an internal filter (Series 1, Interpet U.K.). The aquarium was kept at $12\pm1°$ C and a 10L : 4D photoperiod to maintain the nonbreeding condition of the fish. Tanks were screened from visual disturbance and left for 1 week to allow the fish to recover from the stress of transport and handling. The fish were individually tagged using plastic tubing placed over the dorsal spines and weighed (g) before the observations commenced. Observations were made for 15 min, morning and afternoon, and for each fish the number of attacks made and received were recorded as well as number of prey items eaten. Fish were fed daily in the morning observations using live chironomid larvae. The observations were recorded by speaking into a dictaphone and the data subsequently entered into a PC when the tapes were played back. Aggressive interactions allowed a daily dominance score for each fish to be calculated (number of attacks minus number of retreats) and a linear hierarchy could be determined for each group on each experimental day. After week 1, half of the tanks had nitrogen bubbled through the airstones and the oxygen concentrations were reduced to 20% of normal oxygen saturation ($PO_2 = 4\cdot4$ kPa). This level of hypoxia was chosen since pilot studies had demonstrated no observable change in behaviour or metabolism at PO_2 levels above this (C. Vance & L.U. Sneddon, unpubl. obs.) The flow of nitrogen was controlled by a solenoid valve connected to an oxygen controller and temperature compensated oxygen probe (Cole Parmer, U.S.A.) which was set at 20%, range $\pm$ 1% oxygen concentration. All tanks were measured for oxygen concentrations twice a day to ensure that the control tanks remained at c. 100%, range $\pm$1% normoxia and the treatment tanks were kept at 20 $\pm$1% normoxia. Behavioural observations were made as described before to assess the impact of hypoxia upon hierarchy structure for the following week and the results were compared to the remaining half of the groups that were kept under normoxia during this period. At the end of the 2 week experimental period, all fish were weighed, humanely killed by concussion and pithing and frozen in liquid nitrogen. Sample sizes were kept to a minimum for ethical reasons. Whole body L-lactate concentrations were measured by the method described for muscle tissue in Sneddon et al. (1999).

The stability of the hierarchies was calculated by correlating overall rank and dominance scores for each day over the normoxic and hypoxic periods giving r^2 values for weeks 1 and 2 for both pond and river fish in the control and treatment groups. (Mean r^2 values were 0·87 in week 1 and 0·89 in week 2 for the control group pond fish. For control river fish r^2 values were 0·83 in week 1 and 0·92 in week 2, whereas for the treatment group r^2 values were 0·80 in week 1 and 0·73 in week 2 for pond fish and 0·82 in week 1 and 0·53 in week 2 for river fish subject to hypoxia.) This gave a r^2 value between 0 and 1 with 0 meaning no stability and 1 meaning totally stable. Subsequently, the correlation data for each tank, treatment group and lotic v. lentic were transformed (ln x + 1) to make the data normal so that parametric comparisons could be made. Pond fish had more stable hierarchies than river fish ($F_{1,63}$=7·86, P=0·008). Groups that were not subject to hypoxia became more stable in week 2 whereas the groups enduring low oxygen became less stable in week 2 but this was only significant for river fish (Fig. 1; $F_{1,6}$=12·8, P=0·012). There was a difference in the levels of aggression between pond and river fish with river fish exhibiting higher levels of aggression than pond fish (mean frequency of aggression in river fish=0·77 min^{-1}; pond fish=0·08 min^{-1}; $F_{1,}$63=7·33, P=0·009). Reducing oxygen levels had a profound effect on the amount of aggression in each group with fish performing less aggressive acts under hypoxia (river fish=0·39 39 acts min^{-1}; pond fish=0·04 acts min^{-1}; $F_{1,63}$=6·92,P=0·011). The dominant individual, rank 1, was the most aggressive and the frequency of aggressive acts declined with rank position ($F_{1,63}$=13·15, P<0·001). Hypoxia did not effect the frequency of prey items consumed (mean number of larvae consumed per min per fish) so fish experiencing hypoxia did not alter their feeding rate ($F_{1,63}$=0·06, P=0·815).

FIG. 1. Mean ± s.d. Pearson's correlation coefficients, calculated from the dominance score and overall rank of each group of river (■) and pond (□) three-spined sticklebacks in week 1 and week 2. A value of 1 means a totally stable hierarchy and 0 means totally unstable. The value for week 1 was deducted from week 2 to give a change in stability for groups of river and pond fish held under normoxia and hypoxia (n=4 for each group). Pond fish had more stable hierarchies than river fish under both treatments (*, $P=0.008$).
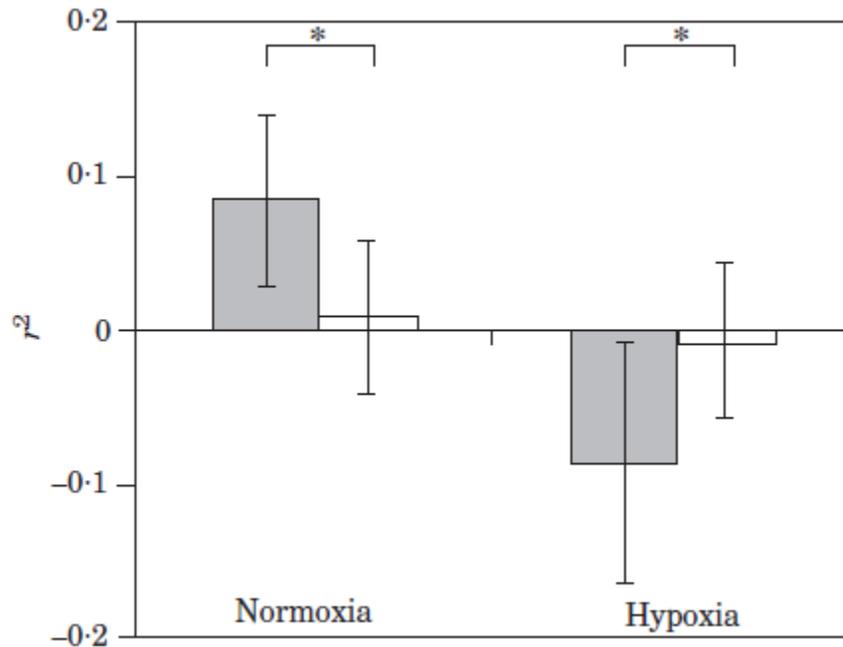
The final rank of the fish was taken from the average rank (using mean dominance score) over week 2. In both populations under normoxia dominant fish gained mass (g) whereas ranks 2 and 3 lost mass [Fig. 2(a); $F_{3,28}=6.40$, $P=0.44$]. Rank 4 in pond fish actually gained mass, however, rank 4 in river fish lost mass [Fig. 2(a)]. The mass change pattern was very different for fish in hypoxic conditions with the dominant fish losing mass and all other ranks gaining mass [Fig. 2(b)]. River fish had higher whole body L-lactate concentrations than pond fish for both treatments (Fig. 3; $F_{1,63}=13.65$, $P<0.001$). Fish in hypoxic conditions had elevated concentrations of L-lactate suggesting that anaerobic respiration may be greater under hypoxia (Fig. 3; river, $F_{1,30}=34.2$, $P<0.001$; pond, $F_{1,30}=23.7$, $P<0.001$). There was no relationship between rank and L-lactate concentrations ($F_{3,58}=1.01$, $P=0.394$).

The dominance hierarchies of the three-spined stickleback were disrupted under hypoxic conditions although this was only significant for river fish. River fish also had greatly elevated concentrations of L-lactate when compared with pond fish under hypoxia. In the turbulent environment of the river, it is less likely that fish will encounter low oxygen concentrations and so they may be less adapted to such conditions than pond fish. Static pond habitats can become hypoxic at night due to respiration of pond flora (Whorisky et al., 1985) and pond fish may have encountered low oxygen and therefore may be better physiologically to cope with hypoxia. Therefore environmental or physiological history may influence phenotypic responses with individuals from different habitats responding in specific ways to hypoxia.

FIG. 2. (a) Mean±s.d. mass change for each rank position within dominance hierarchies of river (■) and pond (□) three-spined sticklebacks held under (a) normoxia ($n=4$ fish per group; mass change for each rank was significantly different ($F_{3,28} = 6.40$, $P = 0.044$] and (b) hypoxia ($F_{3,28} = 4.46$, $P = 0.011$) ($n = 4$ fish per group).

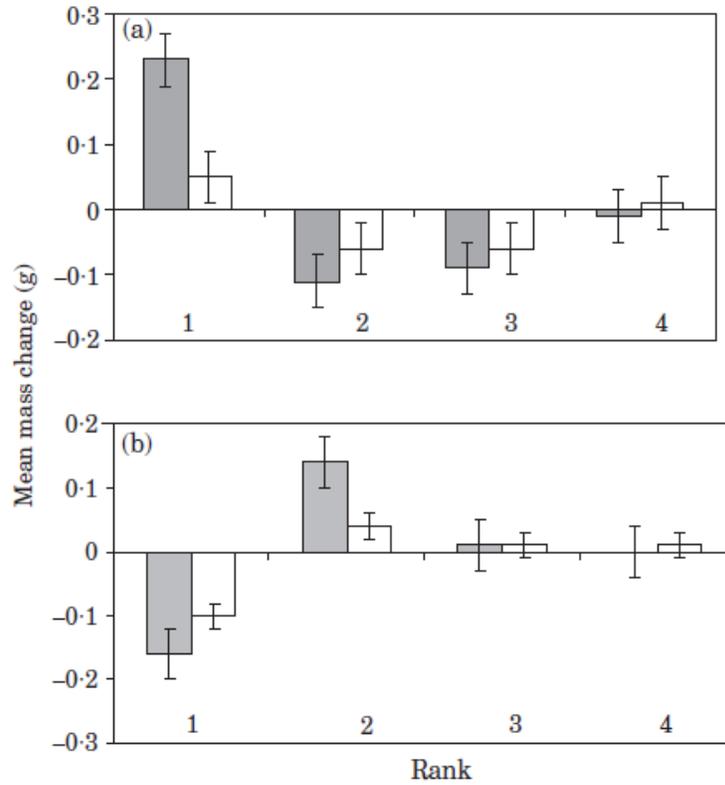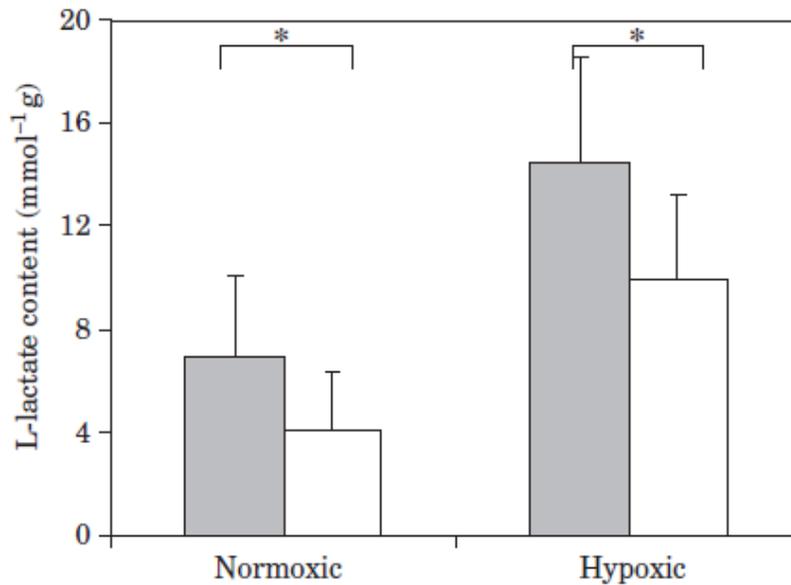FIG. 3. Mean±s.d. whole body L-lactate concentrations for three-spined sticklebacks from river (■) and pond (□) populations held under normoxia and hypoxia ($n = 16$ fish in each group). River fish had higher whole body L-lactate concentrations than pond fish for both treatments (*, $P<0.001$).

Fish under hypoxic conditions also performed fewer aggressive acts and this may be a strategy to conserve energy in the face of metabolic challenge, however, feeding rates remained unaffected. A variety of fish species reduce activity when oxygen concentrations drop in their environment (Kramer, 1987) and those that remain active suffer high mortality (van Raaij et al., 1996). Field studies on three-spined sticklebacks in salt marsh pools demonstrated that females were much less active under hypoxic conditions (Whorisky et al., 1985). A similar reduction in activity in terms of circadian activity rhythms, has also been observed in fishes exposed to toxicants (e.g. copper, aluminium and acidity) that cause internal hypoxia due to detrimental changes at the gills (Allin & Wilson, 1999, 2000; Campbell et al., 2002).

The benefits of being dominant in terms of gaining mass were lost under hypoxia. Ranks 2, 3 and 4 reduced the amount of aggression they performed when oxygen concentration declined, however, rank 1 continued to be highly aggressive. Maintaining such high levels of activity will require a greater amount of energy under hypoxia (Eckert, 1988) and so dominant individuals may have incurred greater energetic costs by not reducing their levels of aggression. The lower-ranked fish may have conserved energy by reducing aggressive activity and thus gained mass. In Atlantic salmon *Salmo salar* L., the dominant individuals have a higher basal metabolic rate and this may account for the maintenance of high aggression levels under hypoxia (Metcalfe et al., 1995). Therefore, the motivational drive for dominant three-spined sticklebacks to continue their high aggressiveness could be construed as maladaptive under non-ideal environmental conditions. Maintaining metabolic rates will only be more costly once oxygen levels fall below the critical PO_2 for a given species (i.e. once anaerobic respiration is the only option of delivering energy to the tissues). Fishes can often maintain their normal metabolic rates (and activity) aerobically during exposure to oxygen levels above this critical value. More research should be directed at whether these responses are genetic due to divergence of three-spined stickleback populations or whether these are acclimatory responses developed over the animal's lifetime rather than an adaptive response.

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