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Socially-induced changes in sodium regulation affect the uptake of water-borne copper and silver in the rainbow trout, *Oncorhynchus mykiss*

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Abstract

Subordinate fish take up more copper during water-borne exposure than dominant fish and consequently display higher tissue burdens. The present study demonstrated a similar effect of social status on water-borne silver uptake. We evaluated whether differences in copper and silver accumulation between individuals could be due to differences in metabolic rate, internal concentrations of cortisol or sodium uptake rates. In the absence of social interaction, experimentally increased metabolic rates (via moderate exercise) and elevated whole body cortisol concentrations (via feeding of a cortisol-spiked diet) did not result in increased metal uptake. However, elimination of the difference in sodium uptake rates between dominant and subordinate fish by exposing them to a saturating level of water-borne sodium (50 mM) resulted in an elimination of copper uptake differences. No significant differences in sodium and silver uptake rates were seen between dominant and subordinate fish exposed to elevated silver concentrations. Therefore, it appears that socially-mediated differences in copper and silver accumulation are a result of differences in sodium uptake rates as both silver and copper are known to cross the gill epithelia via sodium transport pathways.

Keywords: Apical sodium channel; Copper; Cortisol; Dominance; Gills; Silver; Sodium uptake rates; Respirometry

1. Introduction

Characteristic linear dominance hierarchies form among juvenile stream-dwelling salmonid fish, arising through competition for food and shelter (Bachman, 1984; Metcalfe et al., 1989; Nakano, 1995). These social interactions elicit individual differences in physiology. Relative to dominant fish, subordinate fish will generally have higher plasma cortisol concentrations (Laidley and Leatherland, 1988; Pottinger and Pickering, 1992; Øverli et al., 1999; Sloman et al., 2001a), higher metabolic rate (Wirtz, 1975; Sloman et al., 2000b), lower growth rates (Li and Brocksen, 1977; Abbott and Dill, 1989; Pottinger and Pickering, 1992), lower body condition (Sloman et al., 2000a), lower hepatic energy reserves (Ejike and Schreck, 1980; Sloman et al., 2001b), higher sodium uptake rates at the gills (Sloman et al., 2002), and a reduced resistance to disease (Peters et al., 1988).

The social status of a fish can also influence accumulation of trace metals from a contaminated environment (Sloman et al., 2002) and this is most likely a result of one or more of these socially-

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mediated variations in physiology. Sloman et al. (2002) demonstrated that uptake and accumulation of copper during water-borne exposure was dependent upon social status. Subordinate fish had a higher uptake of copper than dominant fish, both when confined in pairs in an artificial laboratory environment and when groups of fish were held in a simulated stream environment. However, the exact physiological reason why social status influences copper accumulation remains undetermined.

Copper and sodium transport across the gill are linked, as copper passes, at least in part, through the sodium channel on the apical membrane (Grosell and Wood, 2002). A similar mechanism has also been demonstrated for silver, which passes through the apical sodium channel (Bury and Wood, 1999) and is transported across the basolateral membrane by an ATP-dependent transporter (Bury et al., 1999). Since subordinate fish display higher rates of branchial sodium uptake than dominant fish (Sloman et al., 2002), one possible explanation for higher copper uptake in subordinates is greater transport via this sodium pathway. Whether dominant and subordinate fish also display differential uptake of silver during water-borne exposure is not known.

Another physiological parameter that varies with social status is metabolic rate. Subordinate fish can display elevated metabolic rates following social encounters due to the stress of received aggression from dominant fish (Sloman et al., 2000b) or simply the visual presence of a conspecific (Wirtz, 1975). Increased metabolic rate is associated with increased blood and water flow across the gills, both of which favour increased uptake of toxicants (Wood, 2001) and could explain increased copper uptake.

Subordinate fish also exhibit chronic elevation of plasma cortisol concentrations (Laidley and Leatherland, 1988; Pottinger and Pickering, 1992; Øverli et al., 1999; Sloman et al., 2001a). De Boeck et al. (2001) demonstrated a protective effect of cortisol during copper exposure and cortisol is known to protect against copper-induced necrosis in the gill epithelia (Bury et al., 1998). However, cortisol can alter gill ion transport mechanisms (Perry et al., 1992) and increased plasma cortisol, therefore, has the potential to affect toxicant uptake.

The first objective of the present study was to test whether silver uptake showed the same pattern of stimulation in subordinate fish during water-borne exposure as previously demonstrated for copper uptake (Sloman et al., 2002). The second objective, using copper uptake as a model, was to determine whether differences in (i) metabolic rate, (ii) plasma cortisol concentrations or (iii) sodium transport mechanisms could explain why social status influences the uptake of water-borne copper and silver.

2. Materials and methods

2.1. Experimental animals

Juvenile rainbow trout were obtained from Humber Springs Trout Hatchery (Thamesford, ON, Canada) and held in flow-through tanks (250 l) supplied with moderately hard dechlorinated Hamilton City tap water (hardness = 120 mg l^{-1} as $CaCO_3$, $Na^+ = 0.6$ mmol l^{-1}; $Cl^- = 0.7$ mmol l^{-1}; $Ca^{2+} = 1.0$ mmol l^{-1}; 12 °C; pH 7.99 ± 0.04; $Cu = 22 \pm 3$ nmol l^{-1}; Ag = < 0.46 nmol l^{-1}; dissolved organic carbon = 3 mg l^{-1}) for at least 2 weeks before experiments were performed. Fish were too small for gender to be reliably determined morphometrically and there is no evidence in the literature to suggest that sex of fish at this age has an effect on dominance. Fish were fed to satiation once daily on commercial trout food (Martin Mills, Inc., Elmira, Ontario).

2.2. Analytical techniques

2.2.1. Copper and silver concentrations

Copper and silver concentrations of water samples were determined by graphite furnace atomic absorption spectrophotometry (Varian AA-220, GTA 110) using Inorganic Ventures-certified standards. Water samples were analysed for sodium concentrations by flame atomic absorption spectrophotometry (Varian AA-220) using a Fisher Scientific-certified standard.

2.2.2. Copper, silver and sodium uptake rates

The short-lived radioisotope, ^{64}Cu ($CuSO_4$; 1 μCi l^{-1}; half life ~ 12.7 h; McMaster Nuclear Reactor, Hamilton), was used to measure copper uptake rates and the isotope ^{110m}Ag to measure silver uptake ($AgNO_3$; 1 μCi l^{-1}; half life ~ 250 days; Risø National Laboratory). Two isotopes were used to measure sodium, ^{24}Na, which has a short half life (NaCl; 5 μCi l^{-1}; half life ~ 15 h; McMaster Nuclear Reactor, Hamilton) and the

long-lived isotope ^{22}Na (NaCl; 1 μCi l^{-1}; half life ~ 950 days; NEN-Dupont). Where the uptake of two isotopes was measured simultaneously, a short-lived isotope was used in conjunction with a long-lived isotope (e.g. ^{64}Cu with ^{22}Na; ^{110m}Ag with ^{24}Na).

To measure uptake rates, water inflow to the tanks was stopped and the chosen isotopes were added to the water. A water sample was taken 15 min after the addition of radioisotope and analysed for gamma radioactivity and total copper, silver or sodium concentration to determine initial specific activity. Two hours after the addition of radioisotope, another reference water sample was taken for measurement of final specific activity. At the end of each experiment, fish were transferred, for a 2-min period, to a 'cold' displacement rinse containing a lethal dose of anaesthetic (0.5 mg ml^{-1} benzocaine) to remove any surface bound isotope, and then blotted dry. The displacement rinse contained a much higher concentration (at least 100-fold) of the non-radioactive salt of the specific isotope used (i.e. 0.5 mmol l^{-1} $CuSO_4$ $5H_2O$ for copper; 600 mM NaCl for sodium; 10 μmol l^{-1} $AgNO_3$ for silver). The fish were then placed in pre-weighed vials and re-weighed. The isotope activity was determined in the reference water samples and the whole fish using an 8 cm well NaI crystal gamma counter (Canberra Packard MINAXI Auto Gamma 5000). The measurement of ^{110m}Ag used the precautions of Hansen et al. (2002) for using ^{110m}Ag as a tracer of silver metabolism in ecotoxicology.

In dual-labelling experiments (where two isotopes were used) the samples were counted immediately after each experiment and then again after the short-lived isotope had decayed (1 week for ^{64}Cu ~ 13 half lives later; 2 weeks for ^{24}Na ~ 22 half lives later). Activities of the short-lived isotopes were corrected for decay either automatically by an on-board program or by manual calculation. The difference in counts was equal to the activity of the short-lived isotope and the remainder (the second set of counts) was equal to the activity of the long-lived isotope. Uptake rates of copper, sodium and silver were calculated separately using the equation:

$$\text{Uptake} = ab^{-1}c^{-1}d^{-1}$$

where a = counts per minute in the fish, b = mean specific activity of the water (activity per unit concentration), c = weight of fish (g) and d = duration of exposure (h) (Grosell et al., 1998).

2.2.3. Cortisol concentrations

Whole body cortisol concentrations were determined according to the method of A. Gravel, P.G.C. Campbell and A. Hontela (personel communication). In brief, fish were frozen in liquid nitrogen immediately after sampling and later homogenised in 0.01 M phosphate buffer (pH 7.5; 1:3 body weight to volume of buffer) on ice. Homogenates were centrifuged at 13 000 × g for 10 min and the supernatant was removed for analysis of cortisol by radioimmunoassay (ICN Pharmaceuticals).

2.3. Behavioural analyses

Social interactions between pairs of fish were observed to distinguish between dominant and subordinate fish. Fish were anaesthetised (benzocaine 0.05 mg ml^{-1}) and marked for individual identification with alcian blue dye injected into their fins (Kelly, 1967). Initial fork lengths and weights were recorded and fish were selected randomly and allocated to size-matched pairs. Each pair was placed in 4.5 l flow-through plastic aquaria; individual fish within each pair were separated from each other by an opaque partition for 48 h during acclimation to the tank and recovery from the marking procedure. The tanks were supplied with water of the same quality to which the fish were previously acclimated. After 48 h acclimation to the supply water and aquaria, the opaque partitions were removed to allow both fish to swim throughout the tank and socially interact with each other.

Behavioural observations of each pair of fish were made once daily throughout experiments at the same time each day (11.00–12.00 h). The following behavioural indicators were scored: position, response to food, and colouration (Sloman et al., 2000a,b, 2001a). Fish were scored according to their position in the tank: fish maintaining position in the water column scored three points, fish resting on the bottom of the tank scored two points and fish swimming at the water surface (indicative of subordinance) scored one point. One food pellet was introduced to the tank during each behavioural observation. The fish taking the food pellet scored one point, the other fish scored zero points. A fish that was light in colouration scored

one point, and a fish that was dark scored zero points. Darkening in colouration is believed to be associated with subordination (O'Connor et al., 1999). At the end of each experiment, the fish with the highest overall score within each pair was identified as the dominant fish.

2.4. Experimental series

2.4.1. Silver and sodium uptake rates in dominant and subordinate fish

The objective of this study was to test whether the same elevation in uptake rates of sodium and copper, previously seen in subordinates (Sloman et al., 2002) also occurred for sodium and silver. Rainbow trout (weight = 1.64 ± 0.09 g; length = 5.34 ± 0.09 cm; $n = 16$) were allocated to size-matched pairs (mean size difference = 0.088 ± 0.04 cm). Following 48 h of social interaction, silver and sodium uptake rates were measured by the addition of ^{110m}Ag and ^{24}Na. This created a measured silver concentration of 1.65 nmol l^{-1} (measured by dilution of the stock concentration). After 2 h exposure, fish were sampled for silver and sodium uptake.

2.4.2. Elevated metabolic rate and copper uptake rate

The goal here was to use moderate exercise (Lauff and Wood, 1996) to raise metabolic rate by about the same percentage as seen in subordinate trout (Sloman et al., 2000b), and to examine the consequences for copper uptake rate. Rainbow trout (weight = 6.20 ± 0.43 g; $n = 24$) were placed in individual 3.2 l Blazka-style respirometers (Lauff and Wood, 1996) that were continuously fed with aerated water from a header tank at a rate of 100 ml min^{-1}. Fish were placed in the respirometers and left at a current of less than 1 BL s^{-1} (where BL = body length) for 15–20 h before measurements were made. This allowed the fish to reach a post-absorptive state (Beamish, 1981) and to orientate to the direction of water flow. Fish were not fed whilst in the respirometers.

Following the settling period, the flow of new aerated water to the chambers was stopped, the respirometers were sealed but the current was maintained or increased (see below). The reduction in oxygen content caused by the respiring fish was determined within the sealed system by collecting water samples anaerobically with a gas-tight syringe and injecting them into a thermostatted cell containing a Cameron OM-200 oxygen electrode connected to a Cameron OM-200 oxygen meter. The oxygen electrode was calibrated using air-saturated water (100% saturation) and nitrogen-saturated water (0%).

As soon as the respirometers were sealed, ^{64}Cu (1 μCi l^{-1}) was added to the water yielding an overall copper concentration of 18.73 ± 3.78 nmol l^{-1}, i.e. not significantly elevated above background. In half of the respirometers the flow rate was then increased to 3 BL s^{-1} (high speed) and left unchanged in the remainder (low speed). Water samples (5 ml) were removed and analysed for oxygen content every hour over a 4 h period and replaced with the equivalent volume of air-saturated water to maintain the pressure within the sealed chambers. Preliminary studies showed that the addition of this small volume of air-saturated water did not measurably affect the overall oxygen content or specific activity of the water. On average, the P_{O_2} in the respirometers did not fall below 68% saturation. At the end of the experiment, fish were removed from the respirometers and gamma counted for measurement of whole body copper uptake.

2.4.3. Elevated cortisol and copper uptake rate

The goal here was to raise internal cortisol levels experimentally, as seen in subordinate fish (Sloman et al., 2000a, 2001a) and examine the consequences for copper uptake. Rainbow trout (weight: 1.36 ± 0.05 g; $n = 58$) were divided between two 20 l tanks supplied with flowing dechlorinated water. Fish were allowed 1 week to acclimate to the tanks and were maintained on a 1% food ration (commercial trout food). In one tank, during the experimental treatment, cortisol was added to the food by dissolving the desired amount of cortisol (Hydrocortisone; Sigma; 125 mg per kg of food) in a small volume (approx. 50 ml) of acetone. Food pellets were added to the cortisol solution and mixed continuously for 5 min to ensure equal coverage of the pellets. The mixture was then left at room temperature until the acetone evaporated. In the control treatment, food was mixed with acetone but without the addition of cortisol.

Fish were fed their respective diets for a period of 10 days. Water flow was then stopped and ^{64}Cu (1 μCi l^{-1}) added to the tanks to yield an overall concentration of 30.06 ± 1.89 nmol l^{-1} ($n = 4$). After 2 h exposure, a lethal dose of anaesthetic

(benzocaine; 0.5 mg ml^{-1}) was introduced to the tanks. Ten fish from each tank were placed in pre-weighed vials for gamma counting for the determination of copper uptake rates and the remainder of the fish (19 from each tank) were frozen immediately in liquid nitrogen, placed in pre-weighed vials and stored at −80 °C for later analysis of whole body cortisol. Fish were not fed 48 h prior to sampling to ensure that all food had been evacuated from the gut. The fish that were sampled for whole body cortisol concentration were stored at −80 °C for a 1-week period to ensure sufficient decay of ^{64}Cu so that there would be no interference with the radioimmunoassay. Whole body cortisol concentrations were measured in the present study due to the small size of the fish. Another group of fish was held in size-matched pairs as described above and sampled after 48 h of social interaction for whole body cortisol concentrations in dominant and subordinate fish.

2.4.4. Copper uptake rates of dominants and subordinates during exposure to elevated sodium

The objective of this experiment was to use an elevated environmental sodium concentration to saturate the branchial sodium uptake mechanism, and to examine the consequences for copper uptake. Rainbow trout (weight: 2.33±0.23 g; length: 5.98±0.18 cm; $n=16$) were acclimated to Hamilton City tap water spiked with 50 mM NaCl (nominal) for a period of 7 days (measured Na^+ = 55.89±0.36 mM; Cl^- = 53.37±3.28 mM; pH 7.77±0.02; $n=7$). All other water parameters were left unchanged during this period. Water of this consistency was created by pumping a concentrated NaCl solution (250.5 g l^{-1}) at 7 ml min^{-1} into a mixing tank supplied with dechlorinated water at a flow rate of 600 ml min^{-1}. The mixing tank supplied the exposure tank at a flow rate of 400 ml min^{-1}; a 50% water exchange occurred every 45 min.

After 7 days acclimation, fish were allocated to size-matched pairs (mean size difference = 0.23±0.06 cm; $n=8$). Following 48 h of social interaction, dominant and subordinate fish were identified as described above. Copper and sodium uptake rates were then measured by the addition of ^{64}Cu (1 μCi l^{-1}) and ^{22}Na (1 μCi l^{-1}) yielding a measured concentration of 236.36±46.11 nmol l^{-1} copper. This was a slightly higher copper concentration than background due to the lower specific activity of this batch of ^{64}Cu (i.e. a greater amount of cold copper present) but it was not high enough to affect the differences in sodium or copper uptake between dominants and subordinates (Sloman et al., 2002). After 2 h exposure, fish were sampled for determination of copper and sodium uptake by gamma counting.

2.4.5. Uptake rate of silver and sodium during exposure to a high silver concentration

The goal here was to produce a partial inhibition of sodium uptake (Morgan et al., 1997; Webb and Wood, 1998) and examine the consequences for silver uptake. Here, experiment 1 (Section 2.4.1) was repeated (rainbow trout: weight = 3.09±0.15 g; length = 6.44±0.11 cm) to examine the uptake of silver and sodium between pairs of size-matched fish (mean size difference = 0.075±0.04 cm) but a cold silver spike (measured silver = 24.47±2.41 nmol l^{-1}; $n=16$) was added at the same time.

2.5. Statistical analysis

Data are given as means±S.E.M. Physiological measurements were compared between pairs of fish using paired samples Student's *t*-test analyses and between treatment groups using independent samples Student's *t*-tests. Physiological parameters were also compared using linear regression analyses. SPSS® software was used for statistical analyses and the limit of significance in all analyses was $P<0.05$.

3. Results

3.1. Silver and sodium uptake rates in dominant and subordinate fish

As shown in previous studies, subordinate fish demonstrated a three-fold increase in sodium uptake rates ($P=0.015$; Fig. 1a) as a result of the chronic stress associated with subordination. At the same time, silver uptake rate (background levels of silver as ^{110m}Ag = 1.65 nmol l^{-1}; measured by dilution of stock) in subordinate fish was two-fold higher when compared with their dominant counterparts ($P=0.033$; Fig. 1b; $n=8$ pairs of fish). Previously, a 50-fold higher copper uptake rate has been shown in subordinates compared to dominant fish (Sloman et al., 2002; Fig. 1).

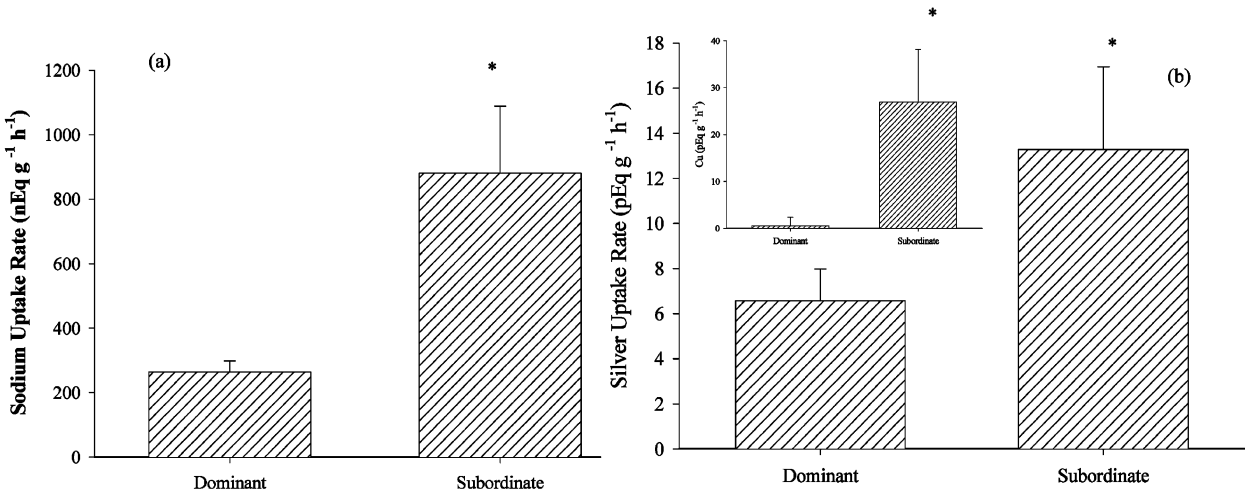

Fig. 1. (a) Sodium and (b) silver uptake rates of dominant and subordinate fish where asterisks denote statistical differences (sodium: $t=-3.213$; $P=0.015$; silver: $t=-2.644$; $P=0.033$; $n=8$ pairs of fish). The insert shows the significant difference in copper uptake demonstrated by Sloman et al. (2002). Data are given as means ± S.E.M.

3.2. Elevated metabolic rate and copper uptake rate

Fish that were swum at a higher speed had a 33% higher oxygen consumption when compared with those fish at rest ($P=0.034$; Fig. 2a), similar to the increase in metabolic rate demonstrated previously between dominant and subordinate fish (Sloman et al., 2000b). This difference in metabolic rate between the two experimental groups did not result in a significant difference in copper uptake rate ($P=0.486$; Fig. 2b). When individuals were compared by correlation analysis, there was no significant correlation between metabolic rate and copper uptake rate ($r^2=0.019$; $P=0.526$).

3.3. Elevated cortisol and copper uptake rate

Fish that were fed a diet containing cortisol for the 1-week period exhibited whole body cortisol concentrations seven-fold higher (22.56 ng g^{-1} vs. 3.28 ng g^{-1}) than those of fish fed on a control diet ($P<0.001$; Fig. 3a). Elevation of whole body cortisol concentrations had no effect on copper uptake from the water ($P=0.562$; Fig. 3b). A significant two-fold difference (4.63 ng g^{-1} vs. 9.10 ng g^{-1}) in whole body cortisol concentration between dominants and subordinates was shown in the present study ($P=0.024$; Fig. 3c).

3.4. Copper uptake rate of dominants and subordinates during exposure to elevated sodium

When pairs of fish were acclimated to 50 mM sodium before and during social interaction, the differences in sodium uptake between dominant and subordinate fish were eliminated ($P=0.365$; Table 1). Note that sodium uptake rates at these high environmental sodium concentrations were much higher than in Fig. 1. Acclimation to 50 mM sodium caused a 10-fold increase in sodium uptake, hence saturating sodium uptake pathways. There were no longer any significant differences in copper uptake rates between dominants and subordinates ($P=0.544$; Table 1). Copper uptake rates of both dominant and subordinate fish were similar to those found in subordinate fish by Sloman et al. (2002).

3.5. Uptake rates of silver and sodium in the presence of a high silver concentration

In the presence of 24.47 nmol l^{-1} silver, no significant difference in sodium uptake between dominant and subordinate fish ($P=0.292$; Table 2) was noted. Neither was there a significant effect of social status on silver uptake ($P=0.655$; Table 2). This level of silver did not appear to inhibit sodium uptake in dominant fish but the sodium uptake rate of subordinate fish was significantly

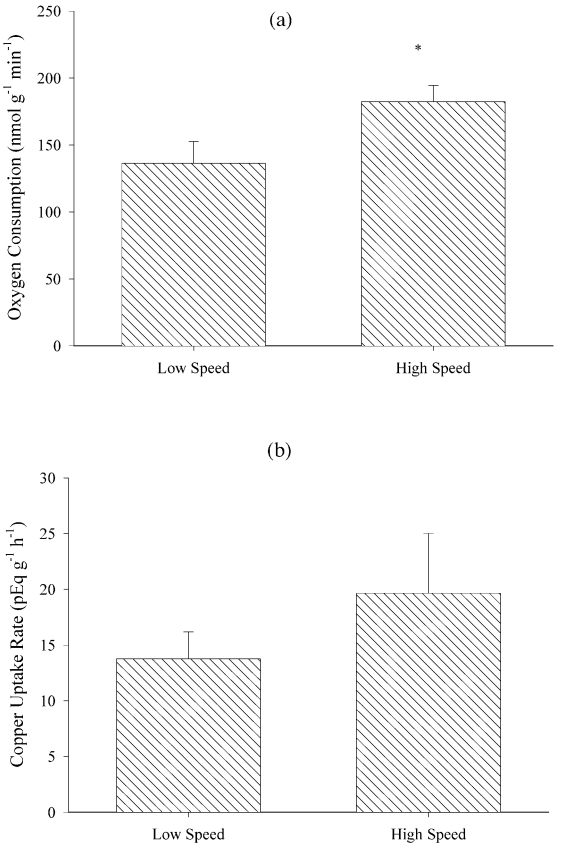

Fig. 2. (a) Oxygen consumption rate and (b) copper uptake in fish swum at control (1 BL s^{-1}; low) and experimental (3 BL s^{-1}; high) speeds. Data are presented as means ± S.E.M. Asterisk denotes a significant difference (O_2 consumption: $t=2.258$; $P=0.034$; copper uptake: $t=-0.709$; $P=0.486$; $n=12$).

reduced ($P=0.019$; $n=16$) compared with subordinates exposed to background silver concentrations in the present study.

4. Discussion

Many differences in the physiology of subordinate and dominant fish have been documented (see review by Sloman and Armstrong, 2002). These individual variations in physiology mean that within a population of fish, individuals may display different responses to toxicants. It has been shown recently, and also in the present study, that the social status of a fish affects the uptake rate and internal accumulation of copper and silver during water-borne exposure (Sloman et al., 2002). The aim of the present study was then to elucidate which of the socially-mediated changes in physiology are responsible for differences in copper and silver uptake. Three explanations were proposed for differential copper uptake by Sloman et al. (2002): differences in (i) metabolic rate, (ii) circulating plasma cortisol concentrations or (iii) ionoregulatory physiology. These explanations could also explain socially-induced differences in silver uptake.

Increased metabolic rate can be associated with an increased uptake of toxicants due to elevated blood and water flow across the gills (Wood, 2001). In the present study, a 33% elevation in metabolic rate compared with controls was induced through forced activity of the fish. This elevation is comparable to previous studies investigating the effects of social status on metabolic rate that have demonstrated a 28% elevation in metabolic rate in subordinates of paired brown trout (Sloman et al., 2000b). However, this relatively small elevation of oxygen consumption induced in the absence of social interaction, did not result in a significant increase in uptake of copper, nor was there any correlation between copper uptake rate and oxygen consumption rate in individual fish.

These data support rejection of the hypothesis that metabolic rate is responsible for socially-mediated differences in copper uptake. If differences in metabolic rate between dominant and subordinate fish resulted in differential copper uptake, this relationship should hold true for other water-borne trace metals such as cadmium. Recently, we have found exactly the opposite in the presence of cadmium, dominant fish accumulate the most metal from the water (Sloman et al., in press). Furthermore, cadmium accumulation among social hierarchies does not reflect the pattern of copper accumulation.

Whole body cortisol concentrations were increased in the present study in fish fed a cortisol-supplemented diet. Subordinate fish also displayed elevated whole body cortisol concentrations but to a lesser extent than those fish fed a cortisol-spiked diet. Cortisol is synthesised and released during the primary response to stress and has both metabolic (Pickering and Pottinger, 1995) and ionoregulatory effects. Cortisol affects the morphology of the gill epithelia (Perry et al., 1992) and, therefore, chronically elevated plasma cortisol concentrations seen in subordinate fish (Laidley and Leatherland, 1988; Pottinger and Pickering, 1992; Øverli et al., 1999; Sloman et al., 2001a) have the potential to alter toxicant uptake. However, it

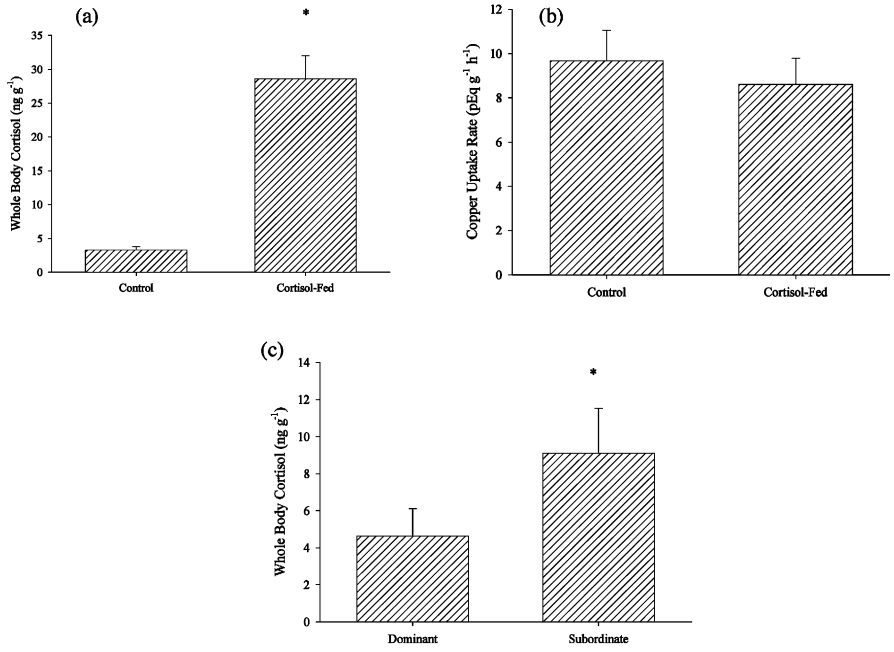

Fig. 3. (a) Whole body cortisol concentrations and (b) copper uptake rates in fish fed a control and cortisol-supplemented diet. (c) Whole body cortisol concentrations of dominant and subordinate pairs of fish. Data are presented as means ± S.E.M. Asterisks denote significant differences (whole body cortisol: $t = 5.546$; $P < 0.001$; $n = 10$; copper uptake: $t = 0.591$; $P = 0.562$; $n = 19$; pairs of fish: $t = -2.518$; $P = 0.024$; $n = 16$ pairs).

Table 1
Sodium and copper uptake rates for dominant and subordinate fish acclimated to 50 mM sodium. No significant differences were demonstrated (sodium: $t = -0.969$; $P = 0.365$; copper: $t = -0.638$; $P = 0.544$; $n = 8$ pairs of fish). Data are presented as means ± S.E.M.

	Dominant	Subordinate
Sodium uptake rate (nEq g^{-1} h^{-1})	2482 ± 323	2955 ± 505
Copper uptake rate (pEq g^{-1} h^{-1})	39 ± 3.9	42 ± 6.3

appears that the higher uptake of copper in subordinate fish is not due to elevated cortisol concentrations. An increase in whole body cortisol concentration in the absence of social interaction did not elicit a significant increase in copper uptake. Copper uptakes rates measured in this experiment were approximately 9 pEq g^{-1} h^{-1}, between those values found in subordinate and dominant fish by Sloman et al. (2002). The hypothesis that elevated cortisol concentrations are responsible for socially-mediated differences in metal uptake is, therefore, rejected. Again, supporting this conclusion is the fact that among social hierarchies the same effects on cadmium uptake are not seen as for silver and copper uptake rates.

Sloman et al. (2002) noted higher sodium uptake rates in subordinate fish following social interaction. In freshwater teleosts, sodium crosses the apical membrane of the gill epithelia via a sodium channel driven by proton exchange (Avella and Bornancin, 1989; Bury and Wood, 1999;

Table 2
Sodium and silver uptake rates for dominant and subordinate fish exposed to 24.27 nmol l^{-1} silver as $AgNO_3$. No significant differences were demonstrated (sodium: $t = -1.139$; $P = 0.292$; silver: $t = -0.466$; $P = 0.655$; $n = 8$ pairs of fish). Data are presented as means ± S.E.M.

	Dominant	Subordinate
Sodium uptake rate (nEq g^{-1} h^{-1})	255.89 ± 49.36	307.94 ± 57.63
Silver uptake rate (pEq g^{-1} h^{-1})	60 ± 10	60 ± 10

Fenwick et al., 1999). Once inside the cell, sodium is transported across the basolateral membrane by Na^+K^+ATPase. The pathways via which copper crosses the gill epithelia into the blood plasma are less certain. Current theories suggest that copper (Grosell and Wood, 2002), at least in part, and silver (Bury and Wood, 1999) cross the gill by a similar mechanism, that is through the sodium channel on the apical membrane. Approximately 50% of copper and silver transport can be eliminated by pharmacological blockade of the sodium channel, by pharmacological blockade of the H^+ ATPase, and by high external sodium levels (Bury and Wood, 1999; Grosell and Wood, 2002). At high concentrations both copper (Laurén and McDonald, 1985, 1986) and silver (Morgan et al., 1997; Webb and Wood, 1998) inhibit branchial sodium transport, supporting the concept of shared common transport pathways of sodium with silver and copper.

Therefore, the explanation of why subordinate fish accumulate more copper and silver could be their increased sodium uptake rates. Subordinate fish have higher uptake rates of sodium when compared with dominant fish (Sloman et al., 2002), probably as a consequence of higher branchial sodium efflux rates and stress-induced diuresis (Sloman et al., submitted). Sodium uptake in the present study was measured at approximately 900 nEq g^{-1} h^{-1} in subordinates compared with approximately 200 nEq g^{-1} h^{-1} in dominant fish. The average sodium uptake rate for fish of similar size held in groups of eight is approximately 550 nEq g^{-1} h^{-1} (Grosell and Wood, 2002). If subordinate fish have a higher number of sodium channels open in the apical membrane, and/or greater Na^+K^+ATPase activity in the basolateral membranes of the gill cells, in order to counteract their increased loss of sodium, then this could in consequence lead to higher uptake rates of copper and silver.

In order to test this hypothesis, the sodium uptake pathways of dominant and subordinate fish were saturated with a very high external sodium concentration so they were no longer different. In this water quality, previous evidence suggests that sodium uptake rates would be saturated at 50 mM sodium (Wood and Goss, 1990; Goss and Wood, 1990). This resulted in the elimination of socially-mediated differences in sodium uptake rates, allowing for consideration of the subsequent effects on copper uptake. In the absence of a difference in sodium uptake rates between dominants and subordinates, the difference in copper uptake rates was also eliminated suggesting that differences in copper uptake are indeed mediated by changes in sodium transport. It is likely that cations, which are known to cross the gill via sodium pathways (i.e. copper and silver) follow this pattern but that cations transported by different ion pathways (e.g. cadmium via calcium pathways; Verbost et al., 1987; Wickland Glynn et al., 1994) may not. This is in agreement with the finding that dominant fish accumulate more cadmium from the water than subordinates (Sloman et al., in press).

As an alternative test of this hypothesis for silver, pairs of fish were exposed to a concentration of water-borne silver (24.47 nmol l^{-1}), that would cause a partial inhibition of sodium uptake in this water quality (Morgan et al., 1997; Webb and Wood, 1998). This higher concentration of silver eliminated the differences in sodium uptake between fish of different social status by competing with sodium uptake and resulted in the elimination of differences in silver uptake between dominant and subordinate fish. In larger fish, exposure to 31 nmol l^{-1} of silver in water of similar hardness has been shown to cause approximately 50% inhibition of sodium uptake (Morgan et al., 1997). However, in the present study inhibition of sodium uptake appeared to occur to a lesser extent.

In conclusion, the present study highlights the toxicological implications of the wide range in physiological differences that exist between social ranks of fish. Water-borne exposure to a variety of trace metals that move through different gill pathways may have strikingly different effects. While dominance hierarchies that form in the natural environment are more complex than the pairs of fish in the present study (Sloman and Armstrong, 2002), there is a clear need to see whether metal accumulation correlates with social status in the wild. Moreover, the present study reinforces that, due to socially-mediated differences in physiology among ranks of fish, it can never be assumed that all individuals within a population will be equally affected by the presence of water-borne contaminants. Subordinate fish may be more susceptible to the presence of water-borne copper and silver because of their increased uptake rates of sodium.

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