

# Cadmium affects the social behaviour of rainbow trout, *Oncorhynchus mykiss*

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## Abstract

The present study investigated both the effects of cadmium on the social interactions of rainbow trout and the differential accumulation of waterborne cadmium among social ranks of fish. Fish exposed to waterborne cadmium concentrations of  $2 \mu\text{g l}^{-1}$  for 24 h, followed by a 1, 2 or 3 day depuration period in clean water, had a decreased ability to compete with non-exposed fish. However, the competitive ability of exposed fish given a 5 day depuration period was not significantly impaired. Cadmium accumulated in the olfactory apparatus of fish exposed to waterborne cadmium for 24 h and decreased significantly only after 5 days depuration in clean water. Among groups of ten fish held in stream tanks, where all fish were exposed to cadmium, there were significant effects on social behaviour and growth rate. Dominance hierarchies formed faster among fish exposed to cadmium than among control fish, and overall growth rates were higher in the cadmium treatment. In groups of ten fish, social status also affected tissue accumulation of cadmium during waterborne exposure, with dominant fish accumulating more cadmium at the gill. In conclusion, exposure to low levels of cadmium affects the social behaviour of fish, in part due to accumulation in the olfactory apparatus, and dominant fish accumulate more gill cadmium than subordinates during chronic waterborne exposure.  
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**Keywords:** Cadmium; Behaviour; Dominance; Olfaction; Calcium

## 1. Introduction

Cadmium is known to accumulate in the tissues of freshwater fish during waterborne exposure, in particular the gills, liver and kidney (Roberts et al., 1978; Reichert et al., 1979; Hollis et al., 2000). Little is known about the possible mechanisms of

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sublethal toxicity of accumulated cadmium in fish. Hypocalcemia is a common hypothesis for the acute toxic action of cadmium (Roch and Maly, 1979; Pratap et al., 1989). Indeed it is now clear that cadmium may cross the gill epithelia, at least in part via calcium transport pathways in the chloride cells (Wicklund-Glynn et al., 1994). Cadmium entry into branchial epithelial cells via  $\text{La}^{3+}$ -sensitive apical  $\text{Ca}^{2+}$  channels has been demonstrated in vitro (Verbost et al., 1987). It is suggested that the primary mechanisms of cadmium toxicity may be cadmium-induced inhibition of  $\text{Ca}^{2+}$  transport proteins (Wood, 2001).

Cadmium directly affects the behaviour of fish. Scherer et al. (1997) demonstrated dose-dependent decreases in foraging rates of lake trout, *Salvelinus namaycush*, exposed to 0.5–5  $\mu\text{g l}^{-1}$  cadmium (at hardness = 90  $\text{mg l}^{-1}$  as  $\text{CaCO}_3$ ) for 8–9 months. Sloman et al. (2003) found that social interaction was severely affected by exposure to 3.3  $\mu\text{g l}^{-1}$  cadmium (at hardness = 120  $\text{mg l}^{-1}$   $\text{CaCO}_3$ ) for 24 h. Fish exposed to cadmium displayed less aggression and were less able to compete with non-exposed fish. A possible mechanism for the behavioural effects of cadmium is disruption of the olfactory system which is known to play an important role in many behaviours including foraging (Hara, 1986) and social interactions (Griffiths and Armstrong, 2000). Cadmium accumulates in the olfactory system during waterborne exposure and alters the ability of fish to respond to natural pheromones (Tjälve et al., 1986; Scott et al., 2003).

Within populations of stream-dwelling salmonid fish, dominance hierarchies are known to form as a result of competition for limited resources such as food and shelter (Keenleyside and Yamamoto, 1962; Fausch, 1984; Sloman and Armstrong, 2002). These characteristic dominance hierarchies have been documented in the field (Bachman, 1984; Nakano, 1995) and can be recreated in the laboratory environment (Noakes and Leatherland, 1977; Sloman et al., 2001b). Dominance hierarchies may be constant over long periods of time (Bachman, 1984) and add stability to population structure (Gurney and Nisbet, 1979). Therefore, alteration of social behaviour by aquatic toxicants and disruption of the formation of social hierar-

chies could have implications for population structure and stability.

Recently, Sloman et al. (2002) demonstrated that the social behaviour of individual fish may influence accumulation of trace metals. Within a dominance hierarchy of rainbow trout, social status was found to affect the uptake of waterborne copper. Subordinate fish had a greater tendency to take up copper from the water and consequently displayed higher tissue burdens. Therefore, we hypothesised that cadmium would alter the social behaviour of rainbow trout, in part by inhibiting olfaction, and that the social position of a fish may affect tissue cadmium accumulation.

The concentrations of cadmium used in the present study fall between the concentrations in the Canadian water quality guidelines intended to protect fish against chronic toxicity (0.039  $\mu\text{g l}^{-1}$  at a hardness of 120  $\text{mg l}^{-1}$   $\text{CaCO}_3$ ; CCREM-CCME, 1987–1999) and those in the USEPA freshwater criteria for aquatic life of 4.3 and 2.2  $\mu\text{g l}^{-1}$  (at hardness = 100  $\text{mg l}^{-1}$   $\text{CaCO}_3$ ) for acute and chronic cadmium exposures, respectively (USEPA, 1999). The acute 96 h LC50 for cadmium in the water quality used in the present study is 22  $\mu\text{g l}^{-1}$  (Hollis et al., 1999). The concentrations of cadmium used in the present study were chosen in light of results from previous behavioural studies, and represent levels that may be realistically encountered by fish as a result of anthropogenic pollution of their natural environment. The objectives of the present study were (a) to investigate the effects of cadmium on the social interactions of rainbow trout, (b) to study the accumulation and depuration of cadmium in the olfactory apparatus and (c) to consider differential accumulation of cadmium among social ranks of fish.

## 2. Materials and methods

### 2.1. Experimental animals

Rainbow trout were obtained from Humber Springs Trout Hatchery (Thamesford, Ont., Canada) and held in flow-through tanks (250 l) supplied with dechlorinated Hamilton City tap

water (hardness  $\sim 120 \text{ mg l}^{-1}$  as  $\text{CaCO}_3$ ,  $\text{Na}^+ \sim 14 \text{ mg l}^{-1}$ ;  $\text{Cl}^- \sim 25 \text{ mg l}^{-1}$ ;  $\text{Ca}^{2+} \sim 40 \text{ mg l}^{-1}$ ; pH  $8.0 \pm 0.04$ ; natural background cadmium concentration of  $0.016 \pm 0.001 \text{ } \mu\text{g l}^{-1}$  ( $n = 32$ ); dissolved organic carbon  $\sim 3 \text{ mg l}^{-1}$ ) for at least 2 weeks before experiments were performed. Temperature was controlled to  $12 \pm 0.5 \text{ }^\circ\text{C}$ .

During all experiments, lights remained on in the aquaria and the tanks were sheltered from the observers by thin black plastic sheeting, thus producing continuous, shaded light conditions. While this served to simplify the experimental conditions, there was no apparent effect on social behaviours. Specifically, social competition and dominance hierarchies occurred as documented in previous studies (O'Connor et al., 1999; Sloman et al., 2001a).

## 2.2. Cadmium effects on social interactions between paired fish

Rainbow trout (weight:  $0.8 \pm 0.03 \text{ g}$ ; fork length:  $4.3 \pm 0.04 \text{ cm}$ ;  $n = 206$ ) were anaesthetised using a solution of benzocaine ( $0.05 \text{ mg ml}^{-1}$ ; Sigma-Aldrich Co. Canada Ltd, Oakville, Ont.) and individually marked by the injection of alcian blue dye into their fins (Kelly, 1967). Initial fork lengths and weights were recorded and fish were allocated to size-matched pairs, as in many behavioural analyses of dominance (Abbott and Dill, 1989; O'Connor et al., 1999; Øverli et al., 1999), to ensure that the physiological condition of each fish within the pair was as similar as possible at the start of the experiment. The pairs of fish were then divided, each fish from the pair being placed in one of two 26 l plastic tanks supplied with control water. Fish were left for 24 h to recover from the marking procedure and then one of the two stock tanks was exposed to cadmium at a nominal concentration of  $2 \text{ } \mu\text{g l}^{-1}$  (measured  $2.26 \pm 0.05 \text{ } \mu\text{g l}^{-1}$ ;  $n = 70$ ). To produce the exposure concentration, a mixing tank was fed with control water at a rate of  $1.14 \text{ l min}^{-1}$ . A concentrated cadmium stock solution ( $0.0126 \text{ g l}^{-1}$  of  $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ; Fisher Scientific, Toronto, Ont.) was added to the mixing tank at a drip rate of  $0.5 \text{ ml l}^{-1}$  and the exposure tank was then supplied with water at a flow rate of  $250 \text{ ml min}^{-1}$ , 50% being replaced

every 72 min. Following a 24 h exposure period, the exposure tank was switched back to control water.

Fish were allocated to size-matched pair tests at different time points post exposure. Some pairs of fish were moved to observation tanks immediately after the 24 h cadmium exposure period. Some fish were removed 48 h later and the remainder of the fish were paired 96 h after exposure to cadmium was stopped. For each pair, one fish was taken from the control tank and the other from the metal-exposed tank and placed in 4.5 l glass aquaria, separated from each other by an opaque plastic partition. After 24 h acclimation to the 4.5 l aquaria, during which time the tanks were supplied with control water (no added cadmium), the plastic partition was removed and behavioural observations were made for the following 15 min as dominance was established. In summary, when interaction between the pairs of fish was initiated, each pair consisted of one non-exposed fish and one fish exposed to  $2.26 \text{ } \mu\text{g l}^{-1}$  cadmium for 24 h followed by a 24, 48, 72 or 120 h depuration period in control water (including acclimation time to 4.5 l tanks).

Fish were observed continuously once the partition was removed. Intense social interactions occurred between the trout for approximately 10 min as seen in other salmonid studies (O'Connor et al., 1999; Sloman et al., 2001a). In all pairs, dominance was established within 15 min and then aggression diminished. At the end of the contests, submissive fish were located either on the bottom of the tank or hovering at the water surface. Dominant fish swam actively in the water column and continued to chase subordinate fish. During the contest, the number of attacks attempted by each fish was recorded, where an attempted attack was counted as a chasing, lunging, biting or nipping behaviour. The number of successful attacks was also recorded; a successful attack was defined as any attempted attack that resulted in physical contact with the other fish, i.e. when one fish bit the other fish, and was counted as a successful attack rather than an attempted attack. The position and colour of each fish was noted. Darkening in coloration is believed to be associated with subordination (O'Connor et al., 1999)

and so at the end of the observation period the lightest fish in the tank scored one point and the darkest fish zero points. Position in the tank was scored at the end of the observation period. Fish swimming in the water column scored two points, fish resting on the bottom of the tank scored one point and fish swimming at the water surface scored zero points. Fish, therefore, scored points for attempted and successful attacks, position in the tank and coloration. At the end of the observation period, the fish with the highest overall score within each pair was identified as the dominant fish.

The experiment was then repeated using a nominal concentration of  $0.8 \mu\text{g l}^{-1}$  cadmium (measured =  $0.80 \pm 0.04 \mu\text{g l}^{-1}$ ;  $n = 8$ ) followed by a 24 h acclimation period only. The exposure concentration was achieved using the same system as before with a stock concentration of  $0.0050 \text{ g l}^{-1}$  of  $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ . Fish were not fed during the experimental period.

Water samples were taken twice daily and acidified to 0.1% with concentrated nitric acid (Fisher Scientific, Trace Metal Grade). Cadmium concentration was analysed by graphite furnace atomic absorption spectrophotometry (AA-220, GTA 110, Varian, Walnut Creek, CA) using Inorganic Ventures (Lakewood, NJ) certified standards. Acquisition of dominance by non-exposed compared with metal-exposed fish was compared using Wilcoxon Signed Ranks analyses. Comparison of attempted attacks performed was carried out using an ANOVA followed by Scheffé's test for multiple comparisons. Data are given as mean  $\pm$  standard error of the mean (S.E.M.). SPSS software (SPSS, Chicago, IL) was used for statistical analysis and the limit of significance in all analyses was  $P < 0.05$ .

### 2.3. Accumulation of cadmium in the olfactory apparatus

Rainbow trout (weight:  $17.2 \pm 1.0 \text{ g}$ ;  $n = 25$ ) were weighed and placed into a 26 l tank containing aerated, control water. Fish were then statically exposed to a nominal cadmium concentration of  $5 \mu\text{g l}^{-1}$  (measured  $5.30 \pm 0.28 \mu\text{g l}^{-1}$ ;  $n = 10$ ; added as  $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ) containing  $0.05 \mu\text{Ci}$

$\text{l}^{-1} \text{ }^{109}\text{Cd}$  (measured =  $0.046 \pm 0.002 \mu\text{Ci l}^{-1}$ ;  $n = 10$ ;  $^{109}\text{CdCl}_2$  Perkin–Elmer, Boston, MA) for 24 h. Water samples were taken, acidified and analysed by graphite furnace atomic absorption spectrophotometry for cadmium content. Water samples were also counted in a Minaxi 8 cm well NaI crystal gamma counter (Canberra Packard Instrument Company, Meriden, CT) to determine  $^{109}\text{Cd}$  activity. Following the 24 h exposure period, five fish were quickly removed from the tank, killed, and immediately frozen in liquid nitrogen (time = 0 h). The remainder of the fish were transferred to a 26 l tank supplied with control water. Five fish were then sampled at 24, 48, 72 and 120 h depuration. Fish were not fed throughout the experiment.

Once sampled, the fish were stored at  $-20^\circ\text{C}$  for later analysis of cadmium uptake by autoradiography. Fish were embedded in carboxymethyl-cellulose gel and frozen in hexane–dry ice slurry. The blocks were then sectioned sagittally (whole body, vertical plane) on tape using a cryomicrotome (Leica CM3600, Nussloch, Germany) to a thickness of  $20 \mu\text{m}$ . At least ten sections of the olfactory system of each fish were taken and the sections then freeze-dried. Freeze-dried sections were mounted on phosphor screens (Canberra–Packard, Mississauga, Ont.) for whole body autoradiography. The screens were then exposed for a 2 week period and the  $^{109}\text{Cd}$  activities in selected tissues and organs quantified using a Cyclone Storage Phosphor Imager and OPTIQUANT© software (Canberra–Packard). Activity was expressed in digital light units per  $\text{mm}^2$  ( $\text{DLU mm}^{-2}$ ). Depuration of cadmium from the olfactory apparatus over time was analysed with an ANOVA.

### 2.4. Cadmium effects on social interactions among groups of fish

Rainbow trout (weight:  $3.3 \pm 0.2 \text{ g}$ ; fork length:  $6.3 \pm 0.1 \text{ cm}$ ;  $n = 100$ ) were anaesthetised with benzocaine ( $0.05 \text{ mg ml}^{-1}$ ) and each individually marked by implantation of a 12-mm long passive integrated transponder (PIT) tag (TX1400L-125 kHz, Destron Fearing, South St Paul, MN) into the peritoneal cavity. After a 1-week recovery period, fish were re-anaesthetised ( $0.05 \text{ mg ml}^{-1}$

benzocaine), and fork lengths and weights recorded. The trout were then placed in size-matched groups of ten in 10 l stream tanks (width of channel = 8 cm; depth = 0.05 m; Fig. 1). Water was pumped around the tank creating a unidirectional flow of water at  $5 \text{ cm s}^{-1}$ . A standpipe was located in the pump area and the cadmium was delivered at the opposite end of the tank. A belt feeder (Aquatic Ecosystems, Apopka, FL) introduced food at the upstream end of the tank and a 1% ration was supplied daily at a continual rate over a 12 h period. The water current was not strong enough to carry food far downstream so to access the food fish had to swim upstream and pass through a PIT tag transceiver (FS2001, Destron Fearing).

The PIT tag transceiver continually recorded the entry of fish into the feeding area through a pocket reader (HS 6100L, Destron Fearing) connected to a computer. The transceiver was set to record any fish that was either directly underneath the transceiver, in the feeding area, or slightly outside the area. The transceiver was also set to detect any fish that sat permanently under the transceiver

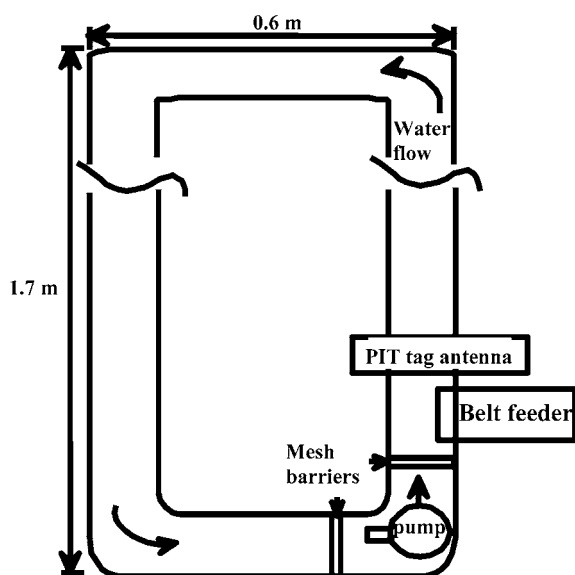


Fig. 1. Plan of the experimental stream tank. The direction of water flow is indicated. Water was driven by a pump with an outflow also in the pump area. Fish could not cross the pump area. Food was delivered by a belt feeder to the upstream end of the tank.

although throughout the experiment this did not occur. The software program SOFTWARE WEDGE for WINDOWS 1.2v (TAL Technologies, Philadelphia, PA) collected the PIT tag data in an EXCEL (Microsoft, Redmond, WA) spreadsheet. Previous studies (Sloman et al., 2002) have shown that the PIT tag method is an effective method of identifying dominance hierarchies among groups of fish in stream tanks.

The stream tanks were allocated to either a control or experimental treatment where control stream tanks were supplied with control (no added cadmium) water and the experimental stream tanks with water containing  $2 \mu\text{g l}^{-1}$  cadmium (measured =  $1.78 \pm 0.071 \mu\text{g l}^{-1}$ ;  $n = 42$ ). To produce the exposure concentration, a head tank was fed with control water at a rate of  $2.28 \text{ l min}^{-1}$ . A concentrated cadmium stock solution ( $0.025 \text{ g l}^{-1}$  of  $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ) was added to the head tank at a drip rate of  $0.5 \text{ ml l}^{-1}$  and the stream tanks were then supplied with water at a flow rate of  $250 \text{ ml min}^{-1}$ . A 50% water exchange occurred every 28 min. Water samples were taken daily, acidified and analysed by graphite furnace atomic absorption spectrophotometry as before. Water samples were taken randomly from different sections of the stream tank to ensure an even distribution of cadmium. The groups of ten rainbow trout were kept in the stream tank environment for 1 week and then the experiment was terminated.

The PIT tag recordings (i.e. the number of times a tag was detected by the reader) were used to determine the time taken for a dominance hierarchy to form. The dominant fish was characterised as the fish with the highest total number of PIT tag readings over the whole week period. Dominant fish are generally more active and feed more than subordinate fish. A positive correlation between activity and dominance has been demonstrated in many previous studies (Winberg et al., 1993; Øverli et al., 1998). The percentage of the total number of PIT tag records per day that were acquired by the dominant fish was calculated for the control and experimental groups of fish. Rates of increase in the percentage of total behaviour score acquired by the dominant fish in the stream tank experiments were compared with an ANCOVA following arcsine transformation of the

data. A log probit transformation was used to calculate the time at which the dominant fish achieved 50% of the total behavioural scores.

### 2.5. Accumulation of cadmium by different social ranks

Stream tanks were used to generate social dominance hierarchies among groups of ten rainbow trout as in the previous experiment. For the first week of the experiment, rainbow trout (weight:  $3.2 \pm 0.2$  g; fork length:  $5.8 \pm 0.2$  cm;  $n = 80$ ) were left undisturbed in the stream tanks supplied with control (no added cadmium) water to allow dominance hierarchies to establish. After 1 week, fish were either left in control water or exposed to a nominal  $2 \mu\text{g l}^{-1}$  cadmium concentration (measured =  $1.86 \pm 0.079 \mu\text{g l}^{-1}$ ;  $n = 42$ ) for a further week (cadmium treatment) in the stream tank. Exposure conditions were achieved as before. Daily water samples were acidified and analysed for cadmium content by graphite furnace atomic absorption spectrophotometry. Four replicates of both control and cadmium treatment were carried out and the results combined for statistical analysis.

At the end of the 2 week experimental period, fish were killed by a sharp blow to the head and final weights and fork lengths recorded. Gill (branchial basket), liver and kidney tissues were dissected from the fish and placed separately in pre-weighed 1.5 ml microtubes. The remaining carcass was also placed in pre-weighed 50 ml Falcon<sup>®</sup> tubes. Tissue weights were recorded and all tissues were then digested in five volumes of 1 N HNO<sub>3</sub> at 55 °C for 48 h. Tissue digests were analysed for cadmium concentration by graphite furnace atomic absorption spectrophotometry. Fish were ranked according to the number of PIT tag entries. Dominant fish (rank 1) had the highest total number of PIT tag readings, the fish with the second highest number of readings was ranked second (rank 2), and so forth. Fish ranked five and above were not easy to differentiate due to the infrequency of entry to the feeding area and so were grouped together for statistical analyses.

The equation  $[(\ln y_2 - \ln y_1)/(t_1 - t_2)] \times 100$  where  $y_2$  = final weight,  $y_1$  = initial weight,  $(t_2 - t_1)$  =

duration of experiment (days)] was used to calculate specific growth rate of individual fish as percent change in weight per day. Physiological and behavioural values among ranks of fish from the stream tank experiments were compared with a two-way ANOVA followed by Scheffé's tests for multiple comparisons and by linear regression analyses. Linear regression analyses were carried out to relate original with final behaviour scores and also overall behaviour with tissue cadmium burdens.

## 3. Results

### 3.1. Cadmium affects social interactions between paired fish

Exposure to  $2 \mu\text{g l}^{-1}$  waterborne cadmium for 24 h had a significant negative effect on the ability of fish to socially compete with a non-exposed fish even after 24, 48 and 72 h depuration in clean water ( $P = 0.034, 0.039$  and  $0.007$ ; Fig. 2). Competitive ability is defined here as the ability to achieve a higher behavioural score, and thus become dominant, based upon levels of aggression, position in the tank and coloration. However, exposed fish that had experienced 120 h depuration in clean water were able to compete effectively with non-exposed fish ( $P = 0.513$ ). Exposure to  $0.8 \mu\text{g l}^{-1}$  cadmium for 24 h followed by 24 h depuration in clean water did not appear to have any significant effect on the competitive ability of the fish ( $P = 0.275$ ; Fig. 2a). Fish becoming dominant (both exposed and non-exposed) were more aggressive than fish becoming subordinate ( $P < 0.001$ ; Fig. 2b). Social encounters occurring between fish exposed to  $2 \mu\text{g l}^{-1}$  cadmium followed by 72 h in clean water, and those fish exposed to  $0.8 \mu\text{g l}^{-1}$  cadmium followed by 24 h in clean water were generally less aggressive than those fish exposed to  $2 \mu\text{g l}^{-1}$  followed by a 24 h depuration period ( $P < 0.001$ ; Fig. 2b).

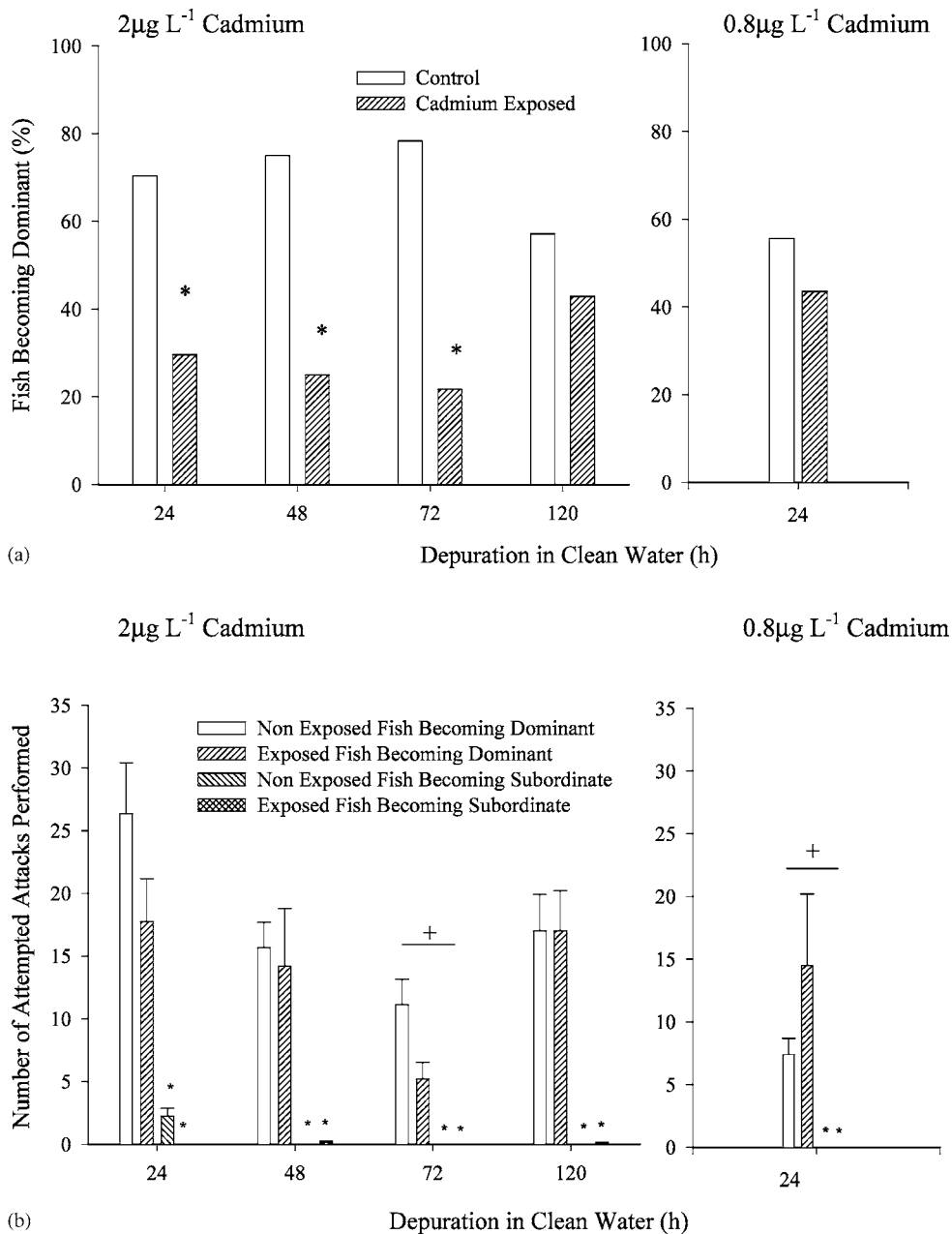


Fig. 2. (a) The percentage of fish becoming dominant in contests between non-exposed fish and fish pre-exposed to 2 and 0.8  $\mu\text{g l}^{-1}$  cadmium for 24 h, and then returned to clean water for a defined depuration period before the contests ( $n = 23$  pairs per treatment). Asterisks denote significant differences between pre-exposed and non-exposed fish (Wilcoxon Signed Ranks: 2  $\mu\text{g l}^{-1}$  cadmium: 24 h depuration:  $Z = -2.117$ ,  $P = 0.034$ ; 48 h depuration:  $Z = -2.065$ ,  $P = 0.039$ ; 72 h depuration:  $Z = -2.711$ ,  $P = 0.007$ ). (b) Number of attempted attacks performed by non-exposed and exposed fish becoming dominant and non-exposed and exposed fish becoming subordinate. Asterisks denote significant differences between ranks ( $F_{1,3} = 59.618$ ,  $P < 0.001$ ) and crosses denote groups that are significantly different from the 24 h depuration following exposure to 2  $\mu\text{g l}^{-1}$  cadmium ( $F_{1,4} = 5.042$ ,  $P < 0.001$ ). Data are presented as mean  $\pm$  S.E.M.

### 3.2. Accumulation of cadmium in the olfactory apparatus

Exposure to  $5 \mu\text{g l}^{-1}$  waterborne cadmium for 24 h resulted in accumulation in the olfactory rosette ( $6882 \text{ DLU mm}^{-2}$  at 0 h depuration). A significant decrease in cadmium in the rosette occurred with increasing depuration time in control water. Cadmium accumulation was not significantly decreased in fish after 24 h depuration ( $4588 \text{ DLU mm}^{-2}$ ) but was significantly lower in fish with a 120 h depuration time ( $4119 \text{ DLU mm}^{-2}$ ;  $P = 0.009$ ; Fig. 3).

### 3.3. Cadmium affects social interactions among groups of fish

The time taken for dominance hierarchies to form in the presence and absence of cadmium was calculated using PIT tag recordings for groups of ten fish held in stream tanks. Fig. 4 shows the increasing percentage of the total number of daily PIT tag recordings acquired by the dominant fish.

In the presence of cadmium, dominant fish acquired 50% of the total daily number of PIT tag records at  $t = 3.02$  days, significantly faster than dominant fish in control treatments which acquired 50% at  $t = 5.64$  days (time:  $P < 0.001$ ; treatment (covariate):  $P < 0.001$ ).

### 3.4. Accumulation of cadmium by different social ranks

In the 2-week stream tank experiments with groups of ten fish, stable dominance hierarchies formed in the control treatments. There was a significant positive correlation between initial and final PIT tag scores, i.e. the number of times the fish entered the feeding area in the first and second week of the experiment, ( $r^2 = 0.559$ ,  $P < 0.001$ ). A positive correlation is indicative of the formation of a stable dominance hierarchy as the social ranking of the fish remains the same throughout the experiment (Sloman et al., 2001b, 2002). Fish exposed to  $2 \mu\text{g l}^{-1}$  cadmium during the second week of the experiment (experimental treatments)

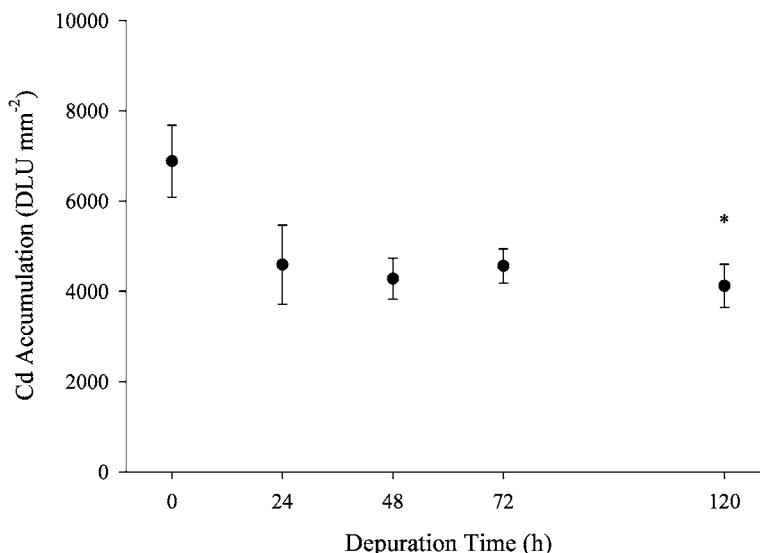


Fig. 3. Accumulation of cadmium in olfactory rosette after 24 h exposure to waterborne cadmium ( $5 \mu\text{g l}^{-1}$ ) followed by a depuration period in clean water of 0, 24, 48, 72 and 120 h. Asterisks denote statistical differences from 0 h depuration. At least three fish and six sections were used for each data point. Data are presented as mean  $\pm$  S.E.M.



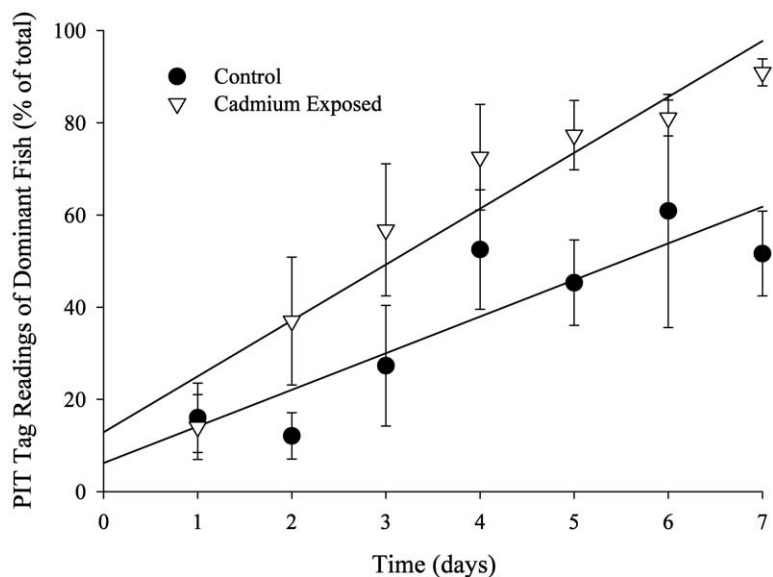


Fig. 4. Log-Probit transformation showing the increase over time of the percentage of readings acquired by dominant fish in control (dark circles) and cadmium-exposed ( $2 \mu\text{g l}^{-1}$ ) (open triangles) treatments. Dominant fish acquired 50% of the total daily number of PIT tag readings at  $t = 5.64$  within control treatments (95% confidence limits: +4.45, -8.54), and at  $t = 3.02$  in the presence of cadmium (+2.26, -3.62). Statistical analysis was carried out after arcsine transformation of the raw data. (time:  $P < 0.001$ ; treatment (covariate):  $P < 0.001$ ). Data are presented as mean  $\pm$  S.E.M.

also displayed a significant positive correlation between initial and final PIT tag scores ( $r^2 = 0.420$ ,  $P < 0.001$ ) indicating that hierarchies formed in

the first week of the experiment in control water and remained stable during exposure to cadmium. The activity patterns of different ranks of fish are

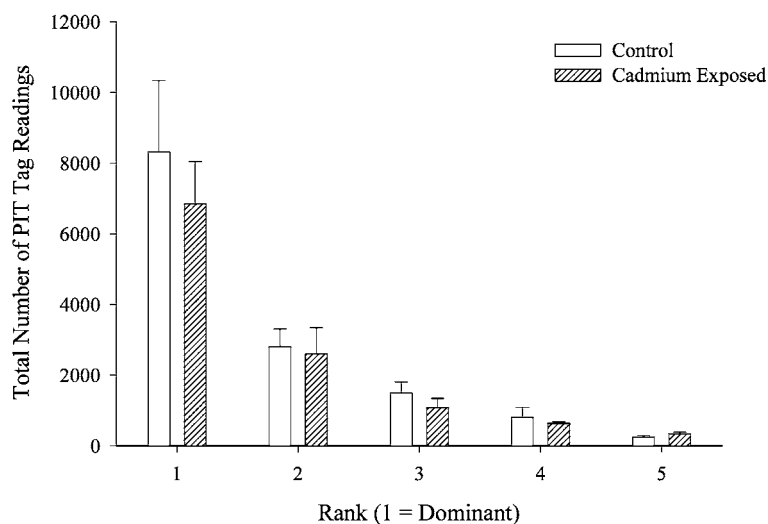


Fig. 5. Total number of PIT tag readings acquired over the 2-week period for tanks of fish (where 1 = dominant) in the control (open bars) and cadmium treatments ( $2 \mu\text{g l}^{-1}$ ) (hatched bars) ( $n = 4$ ). Data are presented as mean  $\pm$  S.E.M. (rank:  $P < 0.001$ ; treatment:  $P = 0.415$ ).

shown as the total acquired number of PIT tag readings throughout the experiment in Fig. 5. Higher ranks of fish acquired higher numbers of PIT tag readings ( $P < 0.001$ ) and there was no significant effect of treatment ( $P = 0.415$ ).

In both treatments dominant fish had significantly higher growth rates than subordinates (Fig. 6; rank:  $P = 0.001$ ; treatment:  $P = 0.022$ ; rank  $\times$  treatment interaction:  $P = 0.545$ ). However, there was also a significant effect of treatment. Fish that were exposed to cadmium for the second week of the experiment had higher growth rates over the whole 2 week period than control fish.

Within the experimental treatments, dominant fish had higher gill cadmium ( $P = 0.029$ ; Table 1). Gill cadmium accumulation during the experimental treatments also increased with specific growth rate ( $r^2 = 0.275$ ;  $P = 0.001$ ; Fig. 7). There were no significant differences in liver, kidney or carcass cadmium burdens among ranks. For all tissues cadmium concentrations were higher in the cadmium-exposed treatments (gill:  $P < 0.001$ ; liver:  $P < 0.001$ ; kidney:  $P < 0.001$ ; carcass:  $P < 0.001$ ; Table 1).

#### 4. Discussion

Fish exposed to  $2 \mu\text{g l}^{-1}$  waterborne cadmium had an impaired ability to compete with non-exposed fish that persisted after 3 days depuration in control water, but not after 5 days depuration. It is likely that this disruption in social behaviour is linked in part to accumulation of cadmium in the olfactory system. Olfaction plays an important role in social interaction in fish. Salmonid fish can differentiate between kin and non-kin by chemical cues (Brown and Brown, 1993) and there is evidence to suggest that odour detection can alter levels of aggression in Atlantic salmon, *Salmo salar* (Griffiths and Armstrong, 2000).

Cadmium accumulates in the olfactory rosette as seen in the present study, and during longer periods of exposure is transported along the olfactory nerve and accumulates in the olfactory bulb (Scott et al., 2003). Unlike manganese and mercury, cadmium does not leave the pre-synaptic neurons of the olfactory bulb and cannot cross the blood-brain barrier (Tjälve and Henriksson, 1999). Concentrations of cadmium similar to those used

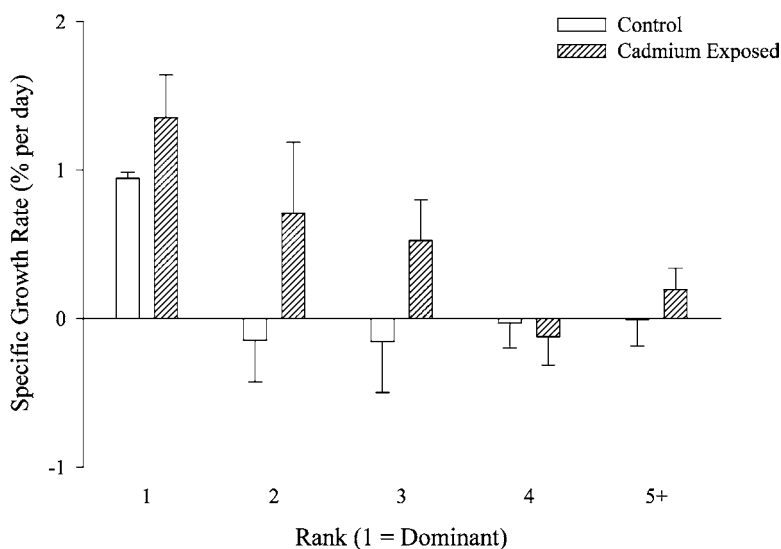


Fig. 6. Specific growth rates (percent increase in weight per day) for the 2-week period for ranks of fish (where 1 = dominant) in the control (open bars) and cadmium treatments ( $2 \mu\text{g l}^{-1}$ ) (hatched bars) ( $n = 4$ ). Data are presented as mean  $\pm$  S.E.M. (rank:  $P = 0.001$ ; treatment:  $P = 0.022$ ).

Table 1

Tissue cadmium concentrations for control (not exposed to cadmium) and experimental (exposed to  $\sim 2 \mu\text{g l}^{-1}$  cadmium for 1 week) ranks of fish held in groups of ten in stream tanks (1 = dominant)

Rank of fish	Tissue cadmium concentrations ( $\text{ng g}^{-1}$ wet tissue)			
	Gill	Liver	Kidney	Carcass
<i>Control treatments</i>				
1	$6 \pm 1$	$10 \pm 4$	$26 \pm 9$	$4 \pm 2$
2	$4 \pm 1$	$14 \pm 4$	$61 \pm 23$	$4 \pm 2$
3	$21 \pm 13$	$9 \pm 4$	$123 \pm 73$	$2 \pm 1$
4	$10 \pm 2$	$13 \pm 5$	$73 \pm 33$	$6 \pm 2$
5–10	$7 \pm 1$	$10 \pm 3$	$49 \pm 9$	$7 \pm 2$
<i>Experimental treatments</i>				
1	$832 \pm 155$	$85 \pm 10$	$229 \pm 20$	$134 \pm 52$
2	$831 \pm 161$	$65 \pm 12$	$198 \pm 29$	$176 \pm 74$
3	$819 \pm 106$	$89 \pm 12$	$289 \pm 73$	$225 \pm 78$
4	$371 \pm 52$	$90 \pm 8$	$458 \pm 277$	$177 \pm 51$
5–10	$627 \pm 44$	$84 \pm 9$	$248 \pm 36$	$163 \pm 29$
<i>P</i> value for experimental rank differences	0.029	0.832	0.401	0.934

Data are expressed as mean  $\pm$  S.E.M. ( $n = 4$ ).

in the present study affect other olfaction-mediated behaviours in fish including migration (Baker and Montgomery, 2001), foraging (Scherer et al., 1997) and response to alarm substance (Scott et al., 2003).

The inability of cadmium-exposed fish to successfully compete with non-exposed fish could be explained by olfactory impairment. There appeared to be no difference in competitive ability between non-exposed fish and fish exposed to

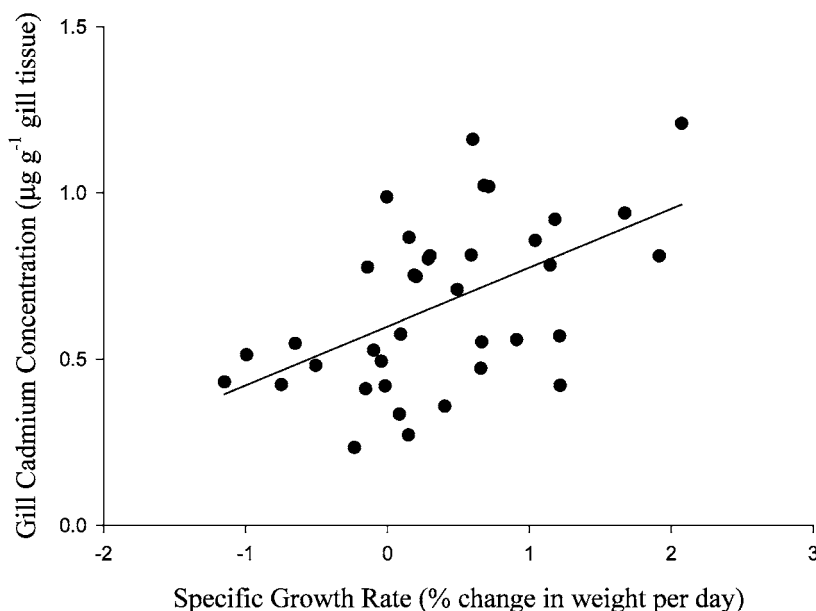


Fig. 7. Relationship between gill cadmium and specific growth rate ( $r^2 = 0.275$ ;  $P = 0.001$ ;  $n = 36$ ) for fish held in groups of ten in stream tanks. Fish were held for 1 week in control water and then exposed to  $2 \mu\text{g l}^{-1}$  cadmium for a further week.

cadmium followed by a 5 day recovery period in control water. The significant reduction in the amount of cadmium accumulated in the olfactory rosette after 5 days depuration in control water may explain this result. Olfactory neurons are capable of regeneration so it is also possible that the 5 day depuration period allowed for recovery of the olfactory system. The results of the present study are consistent with the hypothesis that cadmium disrupts social behaviour by inhibiting olfaction but this is likely to be only part of the explanation. Other physiological mechanisms that are closely linked to social behaviour include neurotransmitters (Winberg and Nilsson, 1993) and hormone concentrations (Sloman et al., 2001a). Interference with both of these systems during cadmium exposure could also alter competitive ability.

In the natural environment, non-exposed fish may compete with exposed fish. For example, a point source of pollution may affect only fish downstream of the source and any future migration of these fish upstream would result in competition with non-exposed fish. Introduction of fish to polluted streams would cause competition between non-exposed and exposed fish as well. However, it is also important to consider the competitive interactions between fish where all fish have been exposed.

Sloman et al. (2003) demonstrated an effect of  $3.3 \mu\text{g l}^{-1}$  waterborne cadmium exposure on the interaction between pairs of fish, where both fish were exposed to cadmium. In the present study, an effect of waterborne cadmium on the competitive interactions among groups of ten fish was seen but perhaps surprisingly, the rate at which exposed fish formed dominance hierarchies was faster than that of control fish.

Dominance hierarchies formed more quickly in the presence of cadmium with the dominant fish becoming established at an earlier time point than in control treatments. Sloman et al. (2003) found that competition between pairs of fish exposed to cadmium was less aggressive than between control fish and that dominance was not easily determined. However, among groups of fish it appears that dominance hierarchies do still form, and that cadmium does not inhibit social formation.

Disruption of the olfactory system could explain this difference in rate of hierarchy formation. Increased re-circulation of water flow can alter aggression patterns among Atlantic salmon, (Griffiths and Armstrong, 2000), and therefore, the inability to detect odours in the water may reduce aggression. The aggressive behaviour of the American lobster, *Homarus americanus*, is also influenced by chemical cues (Breithaupt et al., 1999) and in contrast to the Atlantic salmon, aggression is seen to increase in lobsters when olfactory cues are decreased (by removal of olfactory appendages), resulting in continuous fighting (Karavanich and Atema, 1998). In a relatively artificial environment where only two fish are competing with each other, this may result in a breakdown of social structure but in a more complex environment, hierarchies may actually form faster. The natural environment is, of course, more complex than the stream tanks used in the present study and it is likely that dominance hierarchies will form at different rates under different environmental conditions.

The final part of the present study aimed to determine whether differential accumulation of cadmium during waterborne exposure occurred among ranks of fish as demonstrated for copper (Sloman et al., 2002). Among those fish exposed to waterborne cadmium dominant fish accumulated significantly higher gill cadmium concentrations than subordinate fish but this relationship was not seen in liver, kidney or carcass tissues. The results of the present study are in direct contrast to the results of Sloman et al. (2002) where subordinates accumulated more copper both in gills and internal tissues than dominant fish. Copper uptake is believed to be higher in subordinate fish due to their higher sodium turnover rates (Sloman et al., *in press*). Copper crosses the gill epithelia via sodium transport pathways (Grosell and Wood, 2002), and therefore, increased sodium uptake can result in concomitant copper uptake. Cadmium crosses the gill epithelia, at least in part, via calcium transport pathways in the chloride cells (Verbost et al., 1989; Wicklund-Glynn et al., 1994) and it is possible that dominant fish display higher cadmium uptake due to higher calcium uptake rates.

Wagner et al. (1985) have demonstrated a cyclic uptake of waterborne calcium in concert with a cyclic pattern of growth in rainbow trout (Wagner and McKeown 1985). Therefore, dominant fish may take up more calcium than subordinate fish to meet the calcium demands created by a higher specific growth rate, and, in consequence, take up more cadmium from the water. In mammals, calcium for skeletal growth is obtained solely from the diet across the intestine, but fish can also absorb calcium from their external environment. Water calcium concentrations may affect growth of brook trout (Rodgers, 1984) with lower calcium concentrations decreasing growth rate.

We speculate that subordinate fish more readily accumulate waterborne trace metals (e.g. copper and silver) that cross the gill epithelia by sodium transport pathways and interfere with sodium regulation (Bury and Wood, 1999; Grosell and Wood, 2002). This is because the chronic stress associated with subordination increases sodium uptake across the gill (Sloman et al., 2002). In contrast, dominant fish would more readily accumulate metals (e.g. cadmium and zinc) that cross the gill epithelia by calcium transport pathways (Verbost et al., 1988; Wicklund-Glynn et al., 1994; Hogstrand et al., 1995). This is most likely due to increased uptake of calcium across the gill by faster growing, dominant fish.

Within the 2 week stream tank study dominant fish grew faster, similar to many previous studies (Li and Brocksen, 1977; Pottinger and Pickering, 1992; Sloman et al., 2001b). Although cadmium-exposed fish exhibited a higher growth rate than control fish, the growth rate of the fish in the experimental treatments represents 1 week of growth in control water and 1 week of growth during cadmium exposure. Various studies have shown no effect of low levels of waterborne cadmium on growth (Giles, 1988; Hollis et al., 2000) so it is difficult to draw any strong conclusions on the effects of cadmium on growth from the results of the present study. However, an interesting possibility for the lack of growth in the subordinates of the control treatments compared with subordinates in the cadmium-exposed treatments is the potential difference in aggression levels. If cadmium decreases aggression due to

olfactory impairment, the dominant fish in the control treatments could be monopolising the food source more aggressively than in experimental treatments, resulting in lower growth in the subordinates. It could also be possible that the faster establishment of hierarchies among the cadmium-exposed fish allows a reduction of metabolic costs, and therefore, higher growth rates.

In conclusion, 24 h exposure to  $2 \mu\text{g l}^{-1}$  of cadmium impairs the ability of exposed fish to compete with non-exposed fish. Five days depuration in clean water eliminates the behavioural effects of this cadmium exposure. Cadmium accumulation in the olfactory rosette decreases following 5 days of depuration. A concentration of  $2 \mu\text{g l}^{-1}$  cadmium altered the rate of formation of hierarchies among groups of fish when compared with non-exposed fish, with hierarchies forming faster among exposed fish. Dominant fish accumulate more cadmium in the gill tissue than subordinate fish during waterborne exposure. A correlation between dominance and accumulation of gill cadmium may occur as a consequence of higher growth rates among dominant fish and a greater uptake of calcium with a concomitant increase in cadmium. Cadmium appears to have a subtle yet significant effect on the social behaviour of fish most likely mediated by disruption of the olfactory system. The present study reinforces the fact that it can never be assumed that all individuals within a population will be affected equally by the presence of aquatic toxicants. Moreover, the present study highlights how the wide variety of differences in physiology mediated by competition may elicit different results when social hierarchies are exposed to different trace metals.

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