The effects of trace metal exposure on agonistic encounters in juvenile rainbow trout, *Oncorhynchus mykiss*

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Abstract

The effects of five trace metals, copper, cadmium, nickel, zinc and lead (presented as soluble salts) on the ability of juvenile rainbow trout to form social relationships were investigated. Comparable concentrations of the five metals in relation to their acute 96 h LC50s (concentration at which population mortality = 50% at 96 h) were used (i.e. 15% of the 96 h LC50) and water quality parameters (hardness = 120 mg l\(^{-1}\) as CaCO\(_3\), pH 8; DOC = 3 mg l\(^{-1}\)) were kept constant throughout. In the first experiment, trout exposed to sublethal concentrations of cadmium for 24 h displayed significantly lower numbers of aggressive attacks during pair-wise agonistic encounters than fish paired in the copper, nickel, zinc, lead and control water. In a second experiment, fish were exposed to the same concentration of metal for 24 h, and then returned to normal water for 24 h. When these metal pre-exposed fish were paired with non-exposed fish only cadmium pre-exposure had a significant effect on social interaction. All of the cadmium pre-exposed fish became subordinate when paired with non-exposed fish, whereas the probability of a fish pre-exposed to copper, nickel, zinc or lead becoming subordinate did not significantly differ from random. Therefore, at around 15% of the 96 h LC50, different metals exert different effects on the social behaviour of fish, suggesting potential implications for social structure and population stability.

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1. Introduction

Pair-wise encounters of fish within natural salmonid populations lead to the establishment of dominance hierarchies, particularly among stream-dwelling species (Bachman, 1984). Fish compete with each other for finite resources such as food and shelter and as a result of these competitive encounters, one fish will become dominant over the other subordinate fish, resulting in a linear hierarchy. The most dominant fish will out-compete the fish below it in the hierarchy, which in turn will win agonistic encounters with the fish beneath it in the dominance hierarchy. Thus a linear ‘nip-order’ hierarchy is formed...
In general, dominant fish will obtain the most profitable positions in streams (Fausch, 1984). These social hierarchies, so characteristic of salmonid populations, are therefore, an expression of individual differences in competitive ability and have been shown to be responsible, in part, for the ecological stability of a population (Gurney and Nisbet, 1979). Contamination of a stream environment by pollutants impacting upon social behaviour, e.g. by influencing competitive ability, could therefore have serious consequences for population stability. The aim of the present study was to examine the effects of five trace metals, all environmentally-relevant aquatic contaminants, on the competitive ability of rainbow trout.

Five metals were used in the present study: cadmium, lead, copper, nickel and zinc, the latter three being actual (copper, zinc) or presumptive (nickel) essential requirements of vertebrates. Cadmium is a calcium antagonist, its mechanism of toxicity probably related to disruption of calcium homeostasis (Sorensen, 1991). Behavioural effects of cadmium are likely caused by neurological disturbances during chronic exposure. Lake whitefish, Coregonus clupeaformis, have been shown to avoid cadmium concentrations of 0.2 μg l⁻¹ (at hardness = 53 mg l⁻¹ as CaCO₃) (McNicol and Scherer, 1991) and cadmium has also been shown to affect established dominance hierarchies among bluegills, Lepomis macrochirus (Henry and Atchison, 1979). Chronic exposure (8–9 months) of lake trout, Salvelinus namaycush, to 0.5 μg l⁻¹ cadmium (at hardness = 90 mg l⁻¹ as CaCO₃) had significant effects on foraging behaviour (Scherer et al., 1997).

Lead is also a calcium antagonist and neurotoxin (Sorensen, 1991) that is known to have significant effects on behaviour. Impairment of goldfish avoidance behaviour in response to electric shock has been noted at concentrations of 70 μg l⁻¹ (at hardness = 50 mg l⁻¹ as CaCO₃; Weir and Hine, 1970). Copper is a sodium antagonist, and although a trace metal essential for vertebrate function, copper can be toxic in excess (Sorensen, 1991). Behavioural studies investigating the effects of copper on social status in rainbow trout have shown that 30 μg l⁻¹ copper (at hardness = 120 mg l⁻¹ as CaCO₃) does not disrupt previously established dominance hierarchies (Sloman et al., 2002), but the effects of copper during hierarchy establishment remain unknown.

Zinc, like cadmium, is a calcium antagonist and can cause hypocalcaemia by specifically disrupting calcium uptake at the gill (Hogstrand et al., 1995). Behavioural studies using zinc have demonstrated that rainbow trout show strong avoidance reactions to a sublethal concentration (5.6 μg l⁻¹; at hardness = 14 mg l⁻¹ as CaCO₃) of zinc sulphate (Sprague, 1968). Studies on the effects of zinc on social behaviour have illustrated that zinc may affect established dominance hierarchies (Henry and Atchison, 1979) and when paired fish are exposed to lethal concentrations of zinc, dominant fish show greater resistance than subordinates (Sparks et al., 1972). In comparison with the other four metals used in the present study, relatively little is understood about the toxic mechanisms of nickel in teleost fish. Although only a few studies have considered the effects of nickel on behaviour, nickel has been shown to induce a hypoxic response in goldfish, Carassius auratus, (Ellgaard et al., 1995) and rainbow trout will avoid concentrations of 23.9 μg l⁻¹ (at hardness = 25.2 mg l⁻¹ as CaCO₃) (Giattina et al., 1992).

Although more is known about the physiological effects of these five metal contaminants than their behavioural effects, literature describing the behavioural effects of all these metals is available. However, a confounding problem when comparing behavioural responses of fish to different aquatic contaminants, is the large variety of species, contaminant concentrations, experimental designs, and water quality, which makes valid comparisons almost impossible. Therefore, the aim of the present study was to compare the effects of five trace metal contaminants on the ability of fish to form dominance relations using the same experimental design, species and water quality and using comparable concentrations of the metals in terms of their known acute LC50s. Understanding the effects of toxicants on social behaviour in particular is of great importance as any contaminant impairing or preventing the formation of dominance hierarchies, so characteristic of salmonid populations, has the potential not
only to affect the individual fish but to threaten overall population structure.

2. Materials and methods

Juvenile rainbow trout were obtained from Rainbow Springs Hatchery (Thamesford, ON, Canada) and held in flow-through tanks (500 l) supplied with aerated, dechlorinated Hamilton city tap water (hardness = 120 mg l\(^{-1}\) as CaCO\(_3\), Na\(^+\) = 13.8 mg l\(^{-1}\); Cl\(^-\) = 24.8 mg l\(^{-1}\); Ca\(^{2+}\) = 40 mg l\(^{-1}\); 13 °C; pH 8.0; DOC = 3 mg l\(^{-1}\)). Natural background concentrations of the five metals in the water used in the present study were cadmium = 0.1 μg l\(^{-1}\); lead = 0.2 μg l\(^{-1}\); copper = 3 μg l\(^{-1}\); nickel = 0.5 μg l\(^{-1}\); zinc = 0.1 μg l\(^{-1}\). All experiments were carried out in this water quality. Fish were fed daily a 1% ration (dry food weight/wet body weight) of commercial trout pellets (Martin Mills Inc., Elmira, ON, Canada) until the start of experiments.

2.1. Experiment 1 — aggression between pairs of fish

Juvenile rainbow trout (weight = 0.58 ± 0.03 g; length = 3.83 ± 0.06 cm; \(n = 96\)) were anaesthetised in MS222 (0.08 g l\(^{-1}\)) and marked individually with alcian blue dye injected into their fins (Kelly, 1967). Initial fork lengths and weights were recorded. Fish were then placed in size-matched pairs in 2.25 l glass aquaria but separated from each other by an opaque plastic partition. Following a 24 h acclimation period, pairs of fish, still separated from each other, were allocated to an experimental treatment. Control pairs of fish continued to be held in dechlorinated Hamilton city tap water whilst experimental pairs of fish were exposed to one of the five metals in the same water. For each metal, a concentration equivalent to about 15% of the 96 h LC50 value was chosen, as outlined in Table 1.

Metal exposure was achieved by introducing stock solutions into mixing tanks (served with a flow of 100 ml min\(^{-1}\)) at a rate of 0.5 ml min\(^{-1}\) (stock solution concentrations: copper: 0.0118 g l\(^{-1}\) CuSO\(_4\) · 5H\(_2\)O; cadmium: 0.0018 g l\(^{-1}\) Cd(NO\(_3\))\(_2\) · 4H\(_2\)O; nickel: 1.823 g l\(^{-1}\) NiCl\(_2\) · 6H\(_2\)O; zinc: 0.132 g l\(^{-1}\) ZnSO\(_4\) · 7H\(_2\)O; lead: 0.048 g l\(^{-1}\) Pb(NO\(_3\))\(_2\); Fisher Scientific, Toronto, ON, Canada). Water of the chosen concentrations (nominally 15 μg l\(^{-1}\) copper; 3.3 μg l\(^{-1}\) cadmium; 2250 μg l\(^{-1}\) nickel; 130 μg l\(^{-1}\) zinc; 150 μg l\(^{-1}\) lead) then supplied the experimental tanks at a flow rate of 100 ml min\(^{-1}\), a 50% water exchange occurring within the experimental tanks every 16 min. Water samples were analysed by graphite furnace atomic absorption spectrophotometry (Varian AA-220, GTA 110, Varian Walnut Creek, CA, USA) for cadmium, lead, copper and nickel and by flame atomic absorption spectrophotometry (Varian AA-220, Varian Walnut Creek, CA, USA) for zinc to ensure that the chosen metal concentrations were achieved (see Table 1). Inorganic Ventures-certified standards and operating conditions as documented by the manufacturer were used. Pairs of fish, still separated from each other, were held in either control water or water containing one of the five metals for 24 h. Following the 24 h exposure, fish were introduced to each other by the removal of the partition. Behavioural observations were then made continuously for the next 15 min, during which time dominance was established (see below).

2.2. Experiment 2 — aggression between non-exposed and metal-exposed fish

As in experiment 1, rainbow trout (weight = 0.72 ± 0.03 g; length = 4.07 ± 0.06 cm; \(n = 80\)) were marked with alcian blue dye, initial weights and fork lengths recorded and allocated to size-matched pairs. Fish from each pair were then divided, one fish from each pair being placed in one of two 26 l plastic tanks supplied with control water. Fish were allowed 24 h to recover from the marking procedure before one of the two stock tanks was exposed to trace metal contaminants. Again the metals used were copper, cadmium, nickel, zinc or lead at the same concentrations as in experiment 1. Stock solutions were added to the experimental tanks via a mixing tank and supplied to the exposure tank at 1 l min\(^{-1}\), 50% being replaced every 18 min. Following a 24 h exposure period, one fish from each pair was drawn from
the control tank and the other from the metal-exposed tank. The fish were then placed in their size-matched pairs in 2.25 l glass aquaria, separated from each other by an opaque plastic partition. After 24 h acclimation to the aquaria, during which time the tanks were supplied with control dechlorinated Hamilton city tap water (no added metals), fish were introduced to each other by the removal of the plastic partition and behavioural observations were made for the following 15 min as dominance was established (see below). The identity of the fish that became dominant was noted.

2.3. Behavioural observations

For both experiments fish were observed continuously once the partition was removed and the fish introduced to each other. Social interactions were characteristic of those reported in other studies which observed pairs of salmonid fish (O’Connor et al., 1999; Sloman et al., 2000) with intense fighting generally lasting for 10 min. Once dominance was established, the subordinate fish would take a submissive position in the tank, either on the bottom of the tank or hovering at the water surface. Dominant fish would swim actively in the water column and continue to chase subordinate fish. Fish were scored on the intensity of their fighting. 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. Fish were observed until it was clear which fish was dominant within each pair, with resolution occurring within 15 min in all pairs.

2.4. Statistical analysis

Numbers of aggressive encounters between pairs of fish held in control water and water containing trace metals in experiments 1 and 2 were compared using analysis of variance (ANOVA) analyses followed by Scheffé’s tests for multiple compar-

<table>
<thead>
<tr>
<th>Metal</th>
<th>96 h LC50 (mg l⁻¹)</th>
<th>Measured concentration (mg l⁻¹)</th>
<th>Measured concentration (mg l⁻¹)</th>
<th>pH</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>22</td>
<td>3.17 ± 0.22</td>
<td>101.3 ± 5.32</td>
<td>8.0</td>
<td>Hollis et al. (1999)</td>
</tr>
<tr>
<td>Pb</td>
<td>1000</td>
<td>3.3</td>
<td>8.0</td>
<td>J.T. Rogers and C.M. Wood (unpublished data)</td>
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</tr>
<tr>
<td>Cu</td>
<td>100</td>
<td>120</td>
<td>120</td>
<td>8.0</td>
<td>Taylor et al. (2000)</td>
</tr>
<tr>
<td>Ni</td>
<td>15000</td>
<td>2250</td>
<td>2269 ± 5.32</td>
<td>8.0</td>
<td>E.F. Pane and C.M. Wood (unpublished data)</td>
</tr>
<tr>
<td>Zn</td>
<td>869</td>
<td>125.3 ± 26.3</td>
<td>125.3 ± 26.3</td>
<td>8.0</td>
<td>Alaab et al. (1999)</td>
</tr>
</tbody>
</table>

Values from our laboratory for water of the quality used in the present study are shown. The concentrations of metals selected for the present study are also given.
isons. Acquisition of dominance by non-exposed compared with metal-exposed fish in experiment 2 was compared using Wilcoxon Signed Ranks analyses. SPSS software was used for statistical analysis and the limit of significance in all analyses was 5%.

3. Results

3.1. Experiment 1 — aggression between pairs of fish

Behavioural observations compared aggression between pairs of fish exposed to each of the five metals for 24 h. Tests were run in the continual presence of the metal. The number of attacks attempted was significantly affected by the presence of trace metals (ANOVA: \( P < 0.001 \)). Pairs of fish exposed to cadmium displayed a lower number of attempted attacks during the establishment of dominance than those pairs held in copper, nickel, zinc, lead or control water (Fig. 1a). This pattern was also reflected in the number of attempts that were successful (Fig. 1b). A successful attack is defined as an attempted attack that involved actual body contact between individuals; those pairs of fish exposed to 3.3 \( \mu g \) l\(^{-1} \) cadmium demonstrated a significantly lower number of successful attacks than fish paired in the presence of lead (ANOVA: \( P < 0.003 \)). While numbers of successful attacks appeared low in those fish exposed to cadmium and elevated in those fish exposed to lead and were significantly different from each other, neither group was statistically significant from controls.

3.2. Experiment 2 — aggression between non-exposed and metal-exposed fish

In these tests, experimental fish were exposed to the metal for 24 h and then returned to normal water for 24 h prior to behavioural observations in normal water. Pairing of a metal-exposed fish with a non-exposed fish had varying outcomes depending upon the exposure metal. Pre-exposure of fish to 3.3 \( \mu g \) l\(^{-1} \) cadmium before pairing with a non-exposed fish had a significant effect on the outcome of the aggressive encounter (Wilcoxon Signed Ranks Test: \( Z = -2.824; P = 0.005 \); Fig. 2). Fish pre-exposed to cadmium had a decreased chance of becoming dominant than control fish, all of the dominant positions being scored by a control fish. The other four metals did not have an effect on the ability of a fish to become dominant with no significant differences noted between acquisition of dominant positions by non-exposed and pre-exposed fish (Wilcoxon Signed Ranks Test: Copper: \( Z = -0.378, P = 0.705 \); Nickel: \( Z = -0.378, P = 0.705 \); Zinc: \( Z = -1.414, P = 0.157 \); Lead: \( Z = -0.707, P = 0.480 \)).

To compare the competitive behaviour of fish in experiment 2 with those in experiment 1, the number of attempted and successful attacks for each fish was recorded. Although the same patterns are seen there are no statistical differences (ANOVA: attempted: \( P = 0.121 \); successful: \( P = 0.163 \)), most likely due to the different experimental conditions. The exposure conditions were not the same in experiment 2 because in each pair a non-exposed (control) was competing with a metal exposed fish and both fish were competing in control water. However, the number of attempted and successful attacks made by each group of fish reflected and confirmed those results seen in experiment 1 (Fig. 3). The exception to this is that the number of successful attacks completed by the lead exposed fish was not elevated. Cadmium still elicited a trend towards a decreased number of successful attacks.

4. Discussion

To allow for valid comparison between the five metals used in the present study, it was necessary to use concentrations of metals normalised to each other in relation to their acute 96 h LC50s for rainbow trout. One of the problems in determining 96 h LC50s for a population of fish is that the lethal concentrations will vary depending upon water hardness (Howarth and Sprague, 1978; Chakoumakos et al., 1979; Calamari et al., 1980; Bradley and Sprague, 1985), pH (Cusimano et al., 1986), species (Eisler, 1998), social status (personal observations) and age of fish (Davies et al., 1976;
Therefore, 96 h LC50 values were obtained from studies performed in our laboratory (Table 1) where experimental conditions were as similar to those used in the present study as possible. Fifteen percent of the 96 h LC50 was chosen as a sub-lethal concentration that would
elicited no mortality and the results suggest that this was a useful concentration for comparing behavioural effects of different metals.

In a preliminary study, performed at higher concentrations of cadmium, copper, nickel and zinc (i.e. approximately 30% of the 96 h LC50; Pb not tested), all four metals significantly reduced the number of attempted attacks in an experiment comparable to Experiment 1, indicating that this was not a suitable concentration for discerning differences in the behavioural effects of metals.

Social dominance hierarchies are an integral part of salmonid communities, with social structure being implicated in population stability (Gurney and Nisbet, 1979). Therefore, any contaminant that has the ability to influence the outcome of aggressive encounters provides a potential threat to salmonid populations. In the present study, only cadmium had a significant effect upon aggressive encounters at sublethal (15% of the acute 96 h LC50) concentrations. Furthermore, only cadmium had an impact upon behaviour when non-exposed fish were paired with metal-exposed fish, significantly affecting the outcome of contests in a negative manner (Fig. 2). Indeed, the results from experiment 2 suggest that the effects of cadmium on social behaviour persist for at least 24 h after transfer to control water, an effect not seen in any of the other metals. While in experiment 1 lead did appear to increase the number of successful attacks between pairs of fish, being statistically higher than that of cadmium, this result was not reflected in experiment 2. As the number of successful attacks in lead-exposed fish were not statistically higher than in control pairs it is unlikely that this is a true result of metal exposure. In a realistic environmental scenario, there are many situations where exposed fish could compete with non-exposed fish. Point sources of pollution result in some fish within a stream environment being exposed and migrations up and down stream could result in exposed fish interacting with non-exposed fish. Another scenario where this may occur includes restocking of streams where non-exposed fish may be introduced to streams where some fish have already been exposed to contaminants.

A difference in pattern of aggressive behaviour for fish exposed to trace metal contaminants is perhaps not surprising as previous studies have found changes in behaviour at low concentrations of each of these metals. Ellgaard et al. (1995) demonstrated a decreased locomotor activity of goldfish exposed to 25000 μg l⁻¹ nickel (at

![Fig. 2. Percentage of fish becoming dominant in contests between non-exposed fish and fish pre-exposed to 15% of the acute 96 h LC50 for 24 h, and then returned to clean water for 24 h before the contests (n = 8 pairs of fish per treatment). Asterisks denote significant differences between pre-exposed and non-exposed fish (Wilcoxon Signed Ranks: cadmium: Z = -2.824, P = 0.005).](image_url)
340 mg l$^{-1}$ as CaCO$_3$), followed by a general decrease in activity. Weir and Hine (1970) noted changes in conditioned behaviour of goldfish at lead concentrations of 70 µg l$^{-1}$ (at hardness = 50 mg l$^{-1}$ as CaCO$_3$). It is interesting to note that the difference in behavioural effects of trace metals seen in the present study may reflect the essentiality of the metal. Copper and zinc are both widely recognised as essential metals and undergo tightly controlled homeostasis (Sorensen, 1991). The essentiality of nickel is perhaps more debatable (Norseth and Piscator, 1979), but it is considered essential to animals and is homeostatically regulated (Eisler, 1998). As cadmium and lead are not essential metals, homeostatic mechanisms are lacking and therefore greater accumulation of these metals in internal organs is likely. It is possible that the internal regulation of cadmium in part explains its behavioural effects; however, no behavioural effects of lead were seen.

Cadmium and lead are both neurotoxins (Sorensen, 1991) and may exert toxic effects directly upon the central nervous system. The blood–brain barrier protects the central nervous system but many metal contaminants have the ability to reach the brain via the olfactory pathways. Cadmium is transported along the olfactory nerve and accumulates in the anterior part of the olfactory bulb of the brain (Gottfrey and Tjälve, 1991) but it cannot continue along secondary olfactory neurons and is in general restricted to one brain region. A recent study demonstrated the impairment of behavioural response to alarm pheromone due to uptake of cadmium into the olfactory apparatus during comparable waterborne exposures (G.R. Scott, K.A. Sloman, C. Rouleau, C.M. Wood, unpublished data). Transport of cadmium (at 5 µg l$^{-1}$) along the pituitary–olfactory neurons in pike is as a cadmium–metallothionein complex (Tallkvist et al., 2002). Nickel, however, can pass along primary, secondary and tertiary olfactory neurons to a larger area of the brain (Henriksson et al., 1997; Tallkvist et al., 1998) but at a slower rate than cadmium (Tjälve and Henriksson, 1999). Little appears to be known about transport of lead to the brain although low solubility of lead salts may impede transport across membranes (Baatrup, 1991). It is, therefore, possible that
differences in transport mechanisms of the two non-essential metals used in the present study, cadmium and lead, explain their differences in behavioural effect.

In conclusion, the behavioural effects of comparable sublethal concentrations (15% of the 96 h LC50) of five different metals, copper, cadmium, nickel, zinc and lead were considered in the present study on the same species and age of fish, at the same water hardness and pH. Only the non-essential metal, cadmium, exerted significant effects on social behaviour, severely confounding the ability of a fish to become dominant. Cadmium also appeared to have a persistent effect, social behaviour being impaired even after 24 h in control water. As cadmium appears to be so potent, it will be of interest in future studies to determine the threshold concentrations for behavioural effects of cadmium and how long these effects persist after return to clean water. In all cases the concentrations tested were above acute and chronic criteria levels given for most jurisdictions (e.g. Canadian Water Quality Guidelines, CCREM-CCME, 1987–1999), so implications for regulation should not be drawn. Nevertheless, the present data emphasise that the neurotoxic metal cadmium may influence the establishment of dominance and thereby have implications for population stability.

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