



# The effects of chronic cadmium exposure on repeat swimming performance and anaerobic metabolism in brown trout (*Salmo trutta*) and lake whitefish (*Coregonus clupeaformis*)



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## ABSTRACT

This study investigates the effect of chronic Cd exposure on the ability to perform repeat swim challenges in brown trout (*Salmo trutta*) and lake whitefish (*Coregonus clupeaformis*). Fish were exposed to waterborne Cd (18 nM) in moderately hard water (120 mg L<sup>-1</sup> CaCO<sub>3</sub>) for 30 days. This level of exposure has been shown to cause sublethal physiological disruption and acclimation responses but no impairment of sustained swimming capacity ( $U_{crit}$ ) in single swim challenges. Swim trials were done over the course of the exposure and each one consisted of an initial swim to 85% of the  $U_{crit}$  of control fish, a 30 min recovery period and finally a second swim challenge to determine  $U_{crit}$ . Plasma and tissue samples were collected before and after each of the swim periods. As expected from previous studies, Cd exposure resulted in significant accumulation of Cd in gills, liver and kidney but not in white muscle. Exposure also induced a loss of plasma Ca followed by subsequent recovery (in lake whitefish but not brown trout) with few mortalities (100% survival for lake whitefish and 93% for brown trout). Both control and exposed fish swam to 85% of the single swim  $U_{crit}$  and no differences in performance were seen. The  $U_{crit}$  of unexposed controls in the second swim challenges were not different from the single swim  $U_{crit}$ . However, second swim performance was significantly reduced in Cd exposed fish, particularly after a week of exposure where 31% and 38% reductions were observed for brown trout and lake whitefish respectively. Swimming to 85%  $U_{crit}$  resulted in metabolic expenditure with little recovery after 30 min. Few differences were observed between control and Cd exposed fish with the exception of a reduction in resting white muscle ATP stores of Cd exposed fish after 1 week of exposure. The results show that chronic sublethal Cd exposure results in an impairment of swimming ability in repeat swim challenges but this impairment is generally not related to metabolic processes in white muscle.

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## 1. Introduction

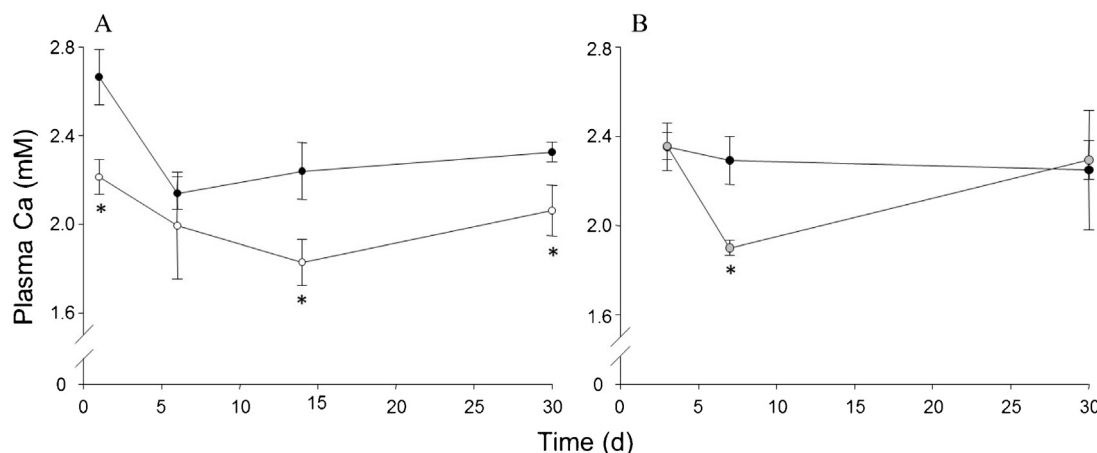
Swim performance is one of the most observable and relevant measures of the effects of stressors on fish (Beitinger and McCauley, 1990), with important ramifications on feeding, avoidance of unfavourable environmental conditions, reproductive behaviour and migration (Plaut, 2001). Salmonids provide a good model for evaluations of swimming performance as they are active predators and spend much of their life in motion. Exposure to waterborne metals has previously been shown to reduce swim performance in salmonids. Beaumont et al. (1995) showed that 96 h of copper exposure (0.08 µM) caused reductions in swimming performance

of brown trout (*Salmo trutta*). Similarly, Pane et al. (2004) found that swimming performance in rainbow trout (*Oncorhynchus mykiss*) was not affected by exposure to 6.5 µM Ni but exposure to 35 µM Ni resulted in a 42 and 35% reduction in the critical swimming speed ( $U_{crit}$ ) after 12 and 24 days of exposure, respectively. Some metals however, do not impair swim performance. For example, both Hollis et al. (1999) and McGeer et al. (2000a) found that chronic sublethal Cd exposure did not impact  $U_{crit}$  values despite other, obvious, impairments (e.g. mortalities, reduced feeding and significant fluctuations in plasma ion composition). Therefore, while chronic exposure to some metals (e.g. Cu or Ni) can impact swim performance, others such as Cd do not appear to impair sustained swimming ability even though fish clearly exhibit physiological impairment.

To date, swim performance testing for the assessment of the potential impacts of contaminant exposure has primarily employed

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**Fig. 1.** The effect of chronic exposure to waterborne Cd on plasma  $\text{Ca}^{2+}$  in brown trout (A) and on lake whitefish (B). Controls are shown as black points for both exposures while fish exposed to 18 nM Cd are shown as white points in brown trout and grey points in lake whitefish. Values are mean  $\pm$  1 SEM,  $n = 8$  for brown trout and  $n = 6$  for lake whitefish. A \* indicates a significant difference compared to controls at that time point ( $P < 0.05$ ).

single swim challenges. However, the study of repeated swim performance can provide a potentially more sensitive and ecologically relevant reflection of impacts of chronic sublethal exposure than the use of a single swim challenge alone. For example, Jain et al. (1998) found that the application of a repeat swimming protocol identified reduced performance in dehydroabietic acid (DHA) exposed fish that was not identified in a single swimming challenge test. Repeat swim performance has also been used to assess the effect of altering dietary lipid sources in manufactured feeds for Atlantic salmon, where reduced recovery ratios (calculated as second  $U_{\text{crit}}$ /initial  $U_{\text{crit}}$ ) were observed in fish fed a diet containing anchovy oil as the lipid source but not in those with plant-based lipid sources (Wagner et al., 2004). Temperature has also been shown to affect performance in subsequent bouts of swimming. Jain and Farrell (2003) showed a significant reduction in recovery ratio of fish acclimated to warm temperatures (17°C) compared with those acclimated to cold temperatures (5°C).

Several studies, completed with salmonids, have evaluated the amount of time required between swimming challenges in order to completely restore performance in the second swim trial relative to that of the first. Brauner et al. (1994) reported a return to full swimming capacity in juvenile coho salmon (*Oncorhynchus kisutch*) following 2 h of recovery. Shorter recovery times were identified as sufficient for the restoration of swimming ability. Randall et al. (1987) found that chinook salmon (*Oncorhynchus tshawytscha*) given a 60 min recovery period performed equally as well in successive  $U_{\text{crit}}$  tests, while a recovery period of only 45 mins was required to restore swim performance in successive swim challenges with mature sockeye salmon (*Oncorhynchus nerka*; Farrell et al., 1998). The responses of rainbow trout would appear to be similar to those of Pacific salmon. For example, Jain et al. (1998) found that hatchery reared rainbow trout fully recovered their swimming ability after a 70 min rest period. Even shorter recovery times of 40 (Jain and Farrell, 2003) and 45 mins (MacNutt et al., 2004) were observed as sufficient to completely restore swimming ability following exhaustive exercise in mature fish. The ability to recover quickly from exhaustive exercise can have significant ecological implications as organisms with impaired or extended recovery periods may suffer during migration and be more susceptible to predation. Performance in subsequent swim challenges is likely determined by the capacity to restore metabolic fuel reserves such as glycogen and to clear accumulated waste products such as lactate (Milligan, 1996) during recovery. Given that repeat swim performance may provide a sensitive measure of sublethal exposure (Jain et al., 1998) and that exposure to Cd does not impact

single swim performance in salmonids in spite of obvious physiological impairment (Hollis et al., 1999; McGeer et al., 2000a) our experiments were directed towards determining whether Cd influenced performance in swim challenges after a bout of exercise (i.e. second swim challenges).

In this study the effect of chronic waterborne Cd exposure on repeat swim performance was studied in brown trout (*S. trutta*) and lake whitefish (*Coregonus clupeaformis*). There is a paucity of data on the impacts of waterborne metals, particularly for lake whitefish and therefore this provided an opportunity to characterize the physiological effects of Cd on less studied fish. The effects of Cd exposure are not well studied in these fish, for example acute 96 h LC50 values are not available for our water chemistry. Therefore, we based our exposure concentration on the results of studies with rainbow trout where chronic sublethal exposure to 18 nM Cd (9% of the 96 h LC50 value) has been shown to induce physiological disruption and an acclimation response but did not impair swim performance (single  $U_{\text{crit}}$ ; Hollis et al., 1999; McGeer et al., 2000a). In the present study we selected an initial swim to 85% of the mean  $U_{\text{crit}}$  value of unexposed controls as a way to give a consistent, size adjusted, bout of exercise that would simulate naturally occurring conditions as during activities such as migration. We then assessed the effect of Cd exposure (18 nM) on recovery and performance in subsequent bout of swimming. A recovery period of 30 min was chosen to limit the potential for full metabolic recovery (Jain and Farrell, 2003) and determine the influence of Cd exposure. In addition to focusing on anaerobic aspects of repeated swim challenges we also studied ionoregulation and bioaccumulation as markers of exposure.

## 2. Materials and methods

### 2.1. Fish culture

Juvenile brown trout and lake whitefish were donated by the Ontario Ministry of Natural Resources (White Lake Hatchery; Sharbot Lake, ON). For both species of fish, eggs were collected from spawning adult fish and then incubated and reared in the hatchery for at least three months. In the case of lake whitefish, spawning adults were from Lake Simcoe Ontario while brown trout were from a variety of sources in southern Ontario. Fish were transported to the aquatics facility at Wilfrid Laurier University where they were held in 200 L polyethylene tanks with flowing fresh water that was a mixture of reverse osmosis processed water and dechlorinated Waterloo city tap water. Water chemistry for tests with brown trout

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