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Effects of acute and chronic waterborne lead exposure on the swimming performance and aerobic scope of fathead minnows (*Pimephales promelas*)

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ABSTRACT

Fathead minnows were subjected to an incremental velocity test using swim tunnel respirometry for the analysis of aerobic scope and swimming performance, as critical aerobic swim speed (U_{crit}), following chronic exposures (33–57 d) to 0.9 ± 0.4, 157 ± 18 or 689 ± 66 nmol L^{-1} Pb and an acute exposure (24 h) to 672 ± 35 nmol L^{-1} Pb (mean ± SEM). Assessment of Pb-induced anemia and neurological impairment were evaluated by blood hemoglobin (Hb) concentrations and a cost of transport (COT) analysis, respectively. Fish from the acute 672 ± 35 nmol L^{-1} Pb (24.4 ± 1.2 BL s^{-1}) and chronic 689 ± 66 nmol L^{-1} Pb (24.6 ± 0.9 BL s^{-1}) treatments exhibited reduced U_{crits} compared to control fish (27.6 ± 0.8 BL s^{-1}). Aerobic scope was reduced by acute Pb exposure (8.6 ± 2.6 μmol O_2 g^{-1} h^{-1} vs. 22.6 ± 3.8 μmol O_2 g^{-1} h^{-1} from controls) owing to a decrease in maximum oxygen consumption rate (38.8 ± 0.8 μmol O_2 g^{-1} h^{-1} vs. 54.0 ± 4.2 μmol O_2 g^{-1} h^{-1} from controls). However, no effect on aerobic scope was observed with fish chronically exposed to Pb. Significant differences were not observed for Hb concentrations or COT. These findings suggest that the impaired swimming performances arising from acute and chronic Pb exposures reflect different mechanisms of toxicity.

1. Introduction

Lead (Pb) is a non-essential heavy metal that has been used for a variety of applications throughout human history owing to its unique chemical and physical properties (e.g. low melting temperature, high malleability and resistance to corrosion). Today, most concern for Pb entering aquatic environments is from point-source discharges related to mining and smelting of Pb ores, largely for use in the production of storage batteries; however, natural sources such as erosion and atmospheric fallout from volcanoes and forest fires also contribute (Nriagu and Pacyna, 1988; World Health Organization, 1995). Lead has been shown to have toxic effects on a variety of freshwater organisms with sensitivity as low as 19 nmol L^{-1} (4 μg L^{-1}) (Grosell et al., 2006b). With respect to fish, the acute toxicity of Pb is putatively due to a mucus-induced respiratory asphyxiation under extreme conditions (Carpenter, 1927; Westfall, 1945) and the disruption of Ca^{2+} and Na^{+} homeostasis in more environmentally relevant Pb concentrations (Birceanu et al., 2008; Patel et al., 2006; Rogers et al., 2003). The chronic toxicity of Pb, on the other hand, is generally consistent between fish and mammals, involving primarily neurological (Davies et al., 1976; Holcombe et al., 1976) and hematological (Hodson et al., 1978) dysfunctions.

Such sublethal effects of Pb could lead to higher order effects, such as reduced swimming performance, with important ecological ramifications. For example, the neurological effects of Pb potentially involve the disruption of the coordinated sensory–motor responses required for capturing prey and eluding predation. Indeed, there are several lines of evidence to this effect. Lead has been shown to increase feeding duration (Mager et al., 2010; Weber et al., 1991; Weis and Weis, 1998), number of feeding miscues (Weber et al., 1991; Weis and Weis, 1998) as well as a reduced ability to avoid predation (Weis and Weis, 1998). Additionally, the Pb-induced developmental abnormality of lordoscoliosis commonly observed in salmonids is likely a direct result of neurological damage (Davies et al., 1976). The effect has significant implications for reproductive success as spawning mobility becomes severely limited as a result of the spinal curvature (Holcombe et al., 1976).

Impaired swimming performance could also arise from Pb-induced hematological effects that cause a reduction in oxygen carrying capacity (anemia). Lead-induced anemia is presumably the result of two separate but related effects, namely reduced heme synthesis and the destruction of mature erythrocytes (Goyer and Clarkson, 2001). The former is largely due to inhibition of the enzyme, delta-aminolevulinic acid dehydratase (ALAD, also known as porphobilinogen synthase), while the latter appears to occur as a result of reactive oxygen species- (ROS-) mediated hemolysis. Inhibition of ALAD not only impairs heme synthesis but also contributes to oxidative stress due to the accumulation of ALA which has been linked to the production of ROS via oxidative interactions with oxyHb

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(Monteiro et al., 1989) and ferritin (Oteiza et al., 1995; Rocha et al., 2003). Lead can also form ROS such as hydrogen peroxide and singlet oxygen via lipid peroxidation events (Sanders et al., 2009) thereby adding to the oxidative stress involved in hemolysis. Such ROS may also contribute to neuronal death (Sanders et al., 2009), thus providing another possible mechanism for Pb-induced sensory–motor and behavioral impairment.

Considering the above, there are multiple potential mechanisms of Pb toxicity that could adversely affect the ecological fitness of fish populations exposed to Pb by reducing swimming performance (e.g. by affecting migration, foraging and/or predator avoidance). Tests of critical aerobic swim speed (U_{crit}) utilizing swimming respirometry are aptly suited to assess subtle, yet ecologically-relevant, effects on swimming performance that might otherwise go unnoticed and may provide key insights on the underlying mechanisms of toxicity. While there remains some debate over the extent to which U_{crit} provides an accurate measure of fitness in the wild, and thus for its utility in ecological risk assessment, evidence continues to mount supporting the relevance of such tests in this regard (Plaut, 2001).

From our recent studies investigating toxicity to the fathead minnow (*Pimephales promelas*), a number of effects were observed supporting hematological and neurological dysfunctions as potential mechanisms of Pb toxicity. A time course toxicogenomic analysis of fathead minnows exposed to 170 nmol L^{-1} Pb (35 μg L^{-1} Pb) for 150 d revealed several genes indicating responses likely involved in ROS detoxification and anemia (Mager et al., 2008). In support of a neurological effect of Pb, a subsequent full life cycle (>300 d) investigation revealed an impaired prey capture ability in F1 larval fathead minnows exposed to 580 nmol L^{-1} Pb (120 μg L^{-1} Pb) (Mager et al., 2010). We therefore undertook the present study to investigate whether the potential hematological and neurological effects of chronic Pb exposure suggested by our previous studies translated into higher order effects on swimming performance (as U_{crit}) and aerobic scope as assessed using a swim tunnel respirometer and the incremental velocity test (Blazka et al., 1960). Hemoglobin (Hb) concentrations were measured to evaluate potential hematological responses to Pb, whereas oxygen consumption data were used to generate cost of transport (COT) values as an indirect assessment of motor function impairment by Pb. That is, we assumed that a Pb effect on motor function would lead to less efficient swimming and therefore greater COT. To facilitate a direct comparison with our previous studies (Mager et al., 2010; Mager et al., 2008), Pb exposures in the present study targeted the 170 and 580 nmol L^{-1} Pb (35 and 120 μg L^{-1} Pb) concentrations used previously.

2. Materials and methods

2.1. Experimental animals

Fathead minnows (*P. promelas*) were obtained from Aquatic Biosystems, Inc. (Fort Collins, CO). Upon arrival fish were gradually acclimated to a low-ionic strength tap water by receiving a constant flow of 2:1 dechlorinated Virginia Key tap water:deionized water for 48 h and then the reverse ratio for the remainder of the experiment using a gravity flow-through approach as previously described (Grosell et al., 2006a). For the chronic Pb exposures, fish were obtained at <24 h post-hatch and fed *ad libitum* a daily diet of *Artemia sp.* nauplii for the first 20 d, a mixture of *Artemia sp.* plus Tetramin flake food for the next 10 d and then flake food only thereafter. For the acute Pb exposures, fish were obtained at approximately 30 d of age and were fed flake food daily *ad libitum*. Prior to feeding, the bottoms of all exposure chambers were siphoned to remove feces and any leftover food from the previous day. Fish used for swim respirometry were starved for at least 24 h prior to initiation of the experiment.

2.2. Lead exposures and sampling protocol

Lead concentrations were maintained for all tests by the addition of concentrated $PbNO_3$ (Sigma-Aldrich, St. Louis, MO, USA) delivered at a constant rate of 1 mL min^{-1} to vigorously aerated mixing chambers using Mariotte bottles. Water was drained from the mixing chambers into 2 L exposure chambers at a rate of 15 mL min^{-1}. Addition of Pb was started 2–3 d prior to introducing fish to the exposure chambers to allow time for equilibration. Chronic Pb exposures targeting nominal Pb concentrations of either 0, 170 or 580 nmol L^{-1} Pb were initiated with 8 d old larvae and continued until time of collection for swim respirometry. Beginning at approximately 41 d of age (33 d of chronic Pb exposure), 2–3 fish were swum per day (1 at a time), alternating treatments with each day to avoid a significant time lag between treatments. To obtain a minimum n of 8 for each Pb treatment sampling was performed over a period of several weeks for the swimming experiments and therefore the durations of chronic Pb exposures ranged from 33–57 d with a mean of 41 d (mean 49 d of age). Following completion of the chronic Pb exposures, acute Pb exposures were performed with the fish obtained at 30 d of age using 2–3 fish per day (mean 46 d of age) by transferring from control water to 580 nmol L^{-1} Pb 24 h prior to swim respirometry. Control fish were swum for both acute and chronic exposures ($n=7$ from each; overall mean 53 d of age). As no significant differences in growth or swimming performance were observed between the acute and chronic control groups, data were pooled ($n=14$) for all comparisons with Pb treatments. A summary of the ages and measured growth parameters of fish used from each treatment is provided in Table 1.

2.3. Swim tunnel respirometry

Automated intermittent flow respirometry was performed using a miniature Blazka-type variable speed respirometer with a DAQ-1 control device and the AutoResp1 version 1.7 software (Loligo Systems, Denmark) (Blazka et al., 1960; Steffenson, 1989). Briefly, the system consists of a small swim tunnel (0.17 L) submerged inside an ~8 L reservoir of well-aerated water used for flushing the tunnel after each closed cycle. Water velocity was initially calibrated using stop-motion video and a ruler fastened above the swim tunnel to measure the velocity of a dye injected into the submerged swim tunnel at various speeds. Once placed in the swim tunnel, monitoring of the fish using a small video camera connected to a computer, as well as manual adjustments to swim speed, was performed remotely from an area partitioned off from the respirometer to avoid disturbing the fish. Oxygen concentrations were continuously measured and recorded by a computer using a Pt100 fiber-optic probe connected to the Fibox 3 minisensor oxygen meter (PreSens Precision Sensing GmbH, Germany). Temperature readings were also simultaneously collected via the oxygen meter using a separate probe. Each day prior to use the system was thoroughly cleaned and the oxygen sensor was calibrated using two partial pressures of O_2. The first (maximum O_2 saturation) was established by vigorous aeration with an air stone and the second (complete absence of O_2) was achieved using a solution of 10 g L^{-1} Na_2SO_3 (Sigma-Aldrich). Control experiments were periodically conducted using an empty swim tunnel to confirm that background microbial O_2 consumption was negligible.

Table 1
Mean ± SEM values for mass, total body length (BL) and age of fathead minnows used in this study. Measurements were collected the day on which fish were swum.

	Mass (mg)	BL (cm)	Age (d)	n
Control	99 ± 7	2.2 ± 0	53 ± 3	14
Acute high Pb	82 ± 7	2.1 ± 0	46 ± 0	8
Chronic high Pb	116 ± 4	2.3 ± 0	48 ± 3	8
Chronic low Pb	134 ± 9[a]	2.5 ± 0.1[a]	49 ± 3	8

[a] Significantly different from controls by one-way ANOVA.

Preliminary metabolic O_2 consumption (MO_2) tests were performed overnight to establish an acceptable duration for acclimation of the fish to the swim chamber as evaluated by the time necessary to achieve a stable routine MO_2 rate. A minimal water velocity of 0.7 cm s^{-1} was used during these tests to maintain mixing within the swim chamber without forcing the fish to exercise. From these initial experiments a stabilized routine metabolic rate was typically established by 1–1.5 h. Thus, prior to swimming, fish were initially acclimated at a flow rate of 0.7 cm s^{-1} for a minimum of 1–1.5 h or longer if necessary until 2 consecutive 30 min measurements of MO_2 were approximately the same.

To measure U_{crit}, fish were exercised at 30 min intervals beginning with a flow rate of approximately 20 cm s^{-1} with subsequent increments in flow of 10 cm s^{-1} every interval until the fish was exhausted. Exhaustion was designated as when the fish became pinned against the back screen of the tunnel and would not regain activity after briefly decreasing flow and then returning to the last speed achieved. The duration (T, in s) at the final swim speed (V_f, in cm s^{-1}) was recorded and the U_{crit} (in cm s^{-1}) calculated using the following equation originally described by Brett (1964):

$$U_{crit} = [V_f + (T / t)dV] / cm \quad (1)$$

where t is the time interval (30 min) and dV is the increment in swim speed (10 cm s^{-1}). Upon completion of the swimming experiment, the fish was removed and total body length measured to transform U_{crit} values to body lengths (BL) per second. As the cross-sectional areas of fish did not exceed 10% of the cross-sectional area of the swim tunnel, corrections for solid blocking effects were not made for measured swimming velocities (Smit et al., 1971; Webb, 1971). A regression equation was derived for each fish by plotting the logarithm of oxygen consumption versus swimming speed to estimate basal MO_2 (y intercept), maximum MO_2 (at U_{crit}) and aerobic scope (maximum − basal MO_2).

2.4. Hemoglobin concentration

Fish used for swim respirometry were subsequently sacrificed for the measurement of blood hemoglobin concentration. Following a blow to the head to stun the fish, the gill was lacerated with a scalpel and blood was collected using a 1–5 μL calibrated pipet (Drummond Scientific Co., Bromall, PA, USA) pre-filled with 0.5 μL 0.5 M EDTA, pH 8 (Ambion, Austin, TX, USA). The total volume within the pipet was then measured and the EDTA volume subtracted to obtain the volume of blood collected. Samples were then mixed with 20 μL Drabkin's Reagent with Brij 35 (Sigma-Aldrich, St. Louis, MO, USA), allowed to stand at room temperature for at least 15 min and then measured at 540 nM using a NanoDrop ND-1000 (Thermo Scientific, Wilmington, DE, USA). Hemoglobin concentrations were determined from a standard curve made with human hemoglobin (Sigma-Aldrich) spanning the range of measured experimental values.

2.5. Cost of transport

To calculate COT, the oxygen consumption rate (mg O_2 kg^{-1} h^{-1}) was converted to mg O_2 kg^{-1} s^{-1}, multiplied by the oxycaloric value of 14.1 J mg^{-1} O_2 and then divided by the corresponding swimming speed (in m s^{-1}) to obtain the final units of J kg^{-1} m^{-1} (Videler, 1993).

2.6. Water chemistry

For the chronic exposures, Pb concentrations were measured daily for the first 3 days of exposure and then once a week thereafter. All other water chemistry parameters were measured on a weekly basis. For the acute exposures, Pb concentrations were measured at the onset and completion (i.e. 24 h later) of exposure. For dissolved Pb, water samples were first passed through a 0.45 μm cellulose syringe filter (Acrodisc, Pall Life Sciences, MI), acidified to 1% HNO_3 (Fisher Scientific, trace metal grade) and concentrations measured via graphite furnace atomic absorption spectroscopy (Varian 200Z, Varian, Australia). Flame atomic absorption spectroscopy (Varian 220FS, Varian, Australia) and anion chromatography (DIONEX DX120, CA) were used to measure concentrations of major cations (Na^+, K^+, Ca^{2+}, and Mg^{2+}) and anions (Cl^- and SO_4^{2-}), respectively. Total CO_2 (total inorganic carbon) was measured using a Corning 962 carbon dioxide analyzer (UK) and pH was measured using a PHM201 meter (Radiometer, Copenhagen, Denmark) fitted with a combination glass electrode calibrated with IUPAC standards (Radiometer, Copenhagen, Denmark) prior to each use. Concentrations of DOC were determined by high temperature catalytic oxidations using a Shimadzu total organic carbon-VCSH analyzer (Kyoto, Japan) (Hansell and Carlson, 2001).

2.7. Statistical analysis

Data are presented as mean ± 1 standard error of the mean (SEM). Differences were tested for statistical significance by Student's t-test for comparisons involving controls and acute Pb treatments, or one-way analysis of variance (ANOVA) for comparisons involving controls and chronic Pb treatments using pairwise multi-sample comparison corrections (Fisher LSD Method) as appropriate. Cost of transport comparisons were analyzed separately for acute and chronic Pb exposures using two-way ANOVA. In all cases, differences were deemed significant at $P<0.05$.

3. Results

3.1. Lead exposures

Mean ± SEM values for measurements of dissolved Pb concentrations and general water chemistry parameters are provided in Table 2. For the high Pb treatments, measured dissolved Pb concentrations were higher than the targeted nominal value of 580 nmol L^{-1}, although mean values were similar between acute and chronic exposures. Conversely, for the chronic low Pb treatment, the measured dissolved Pb concentration was slightly lower than the targeted nominal value of 170 nmol L^{-1}.

Table 2
Measured concentrations for dissolved lead (in nmol L^{-1} and μg L^{-1}) and general water chemistry parameters (in μmol L^{-1} except where indicated otherwise). Values represent mean ± SEM.

Lead concentrations	nmol L^{-1} (μg L^{-1})
Control	0.9 ± 0.4 (0.2 ± 0.1)
Acute high Pb	672 ± 35 (139 ± 7)
Chronic high Pb	689 ± 66 (143 ± 14)
Chronic low Pb	157 ± 18 (33 ± 4)
General water chemistry parameters	
Na^+	569 ± 16
K^+	11 ± 0
Ca^{2+}	202 ± 29
Mg^{2+}	54 ± 1
Cl^-	698 ± 92
SO_4^{2-}	47 ± 2
Total CO_2	543 ± 69
DOC (μmol C L^{-1})	108 ± 4
Hardness (mg L^{-1})	26 ± 3
pH	7.50 ± 0.03
Temperature (°C)	21 ± 1

3.2. Swimming performance

Maximum aerobic swim speeds (U_{crit}) were extrapolated from data collected during swim respirometry experiments to evaluate whether acute and/or chronic Pb exposure impairs the swimming performance of fathead minnows. Fish from the acute 672 ± 35 nmol L^{-1} Pb treatment and chronic 689 ± 66 nmol L^{-1} Pb treatment exhibited reduced U_{crits} compared to control fish, whereas fish from the chronic 157 ± 18 nmol L^{-1} Pb treatment were not statistically different from controls (Fig. 1).

3.3. Oxygen consumption

Oxygen consumption measurements obtained during swim respirometry experiments revealed a significantly reduced aerobic scope and MO_{2max} for fish exposed acutely to 672 ± 35 nmol L^{-1} Pb compared to controls (Fig. 2). While the differences were not statistically significant, there was a trend toward higher basal and maximum oxygen consumption rates in fish chronically exposed to either 157 ± 18 or 689 ± 66 nmol L^{-1} Pb.

Mean ± SEM values for masses and total body lengths of fathead minnows measured just before and after swim respirometry, respectively, are provided in Table 1. Fish from the chronic low Pb treatment were significantly larger than control fish both in terms of mass and body length. An explanation for the increased growth following the chronic low Pb exposure was not immediately apparent and may simply reflect an uncontrollable natural variability in growth among the different treatments. This result is consistent, however, with previous reports of increased growth by fathead minnows following 30 d of Pb exposure (Grosell et al., 2006a; Mager et al., 2010).

To evaluate whether differences in body mass may have contributed to effects on oxygen consumption, one-way analyses of co-variance (ANCOVAs) were performed using the corresponding log-transformed data. A significant relationship was observed between body mass and MO_{2max} ($P<0.0258$) and aerobic scope ($P<0.0049$), but not between body mass and MO_{2basal} ($P<0.9918$). However, none of the relationships were significant when the data from the acute 672 ± 35 nmol L^{-1} Pb treatment were removed from the analyses, indicating that a Pb treatment effect was confounding the initial analyses of the influence of mass. Thus, although body mass was found to co-vary with MO_{2max} and aerobic scope in the acute 672 ± 35 nmol L^{-1} Pb treatment, the relationship (decrease in MO_{2max} and aerobic scope with a decrease in body mass) was opposite to expectations for a metabolic scaling effect (i.e. increase in MO_{2max} and aerobic scope with a decrease in body mass). Hence, any potential effect due to metabolic scaling should have resulted in increased MO_{2max} in the acute Pb-exposed fish which were the smallest. Therefore, the effect of body mass is a conservative error and the observed reductions in MO_{2max} and aerobic scope can be attributed to acute Pb exposure and not a difference in body mass.

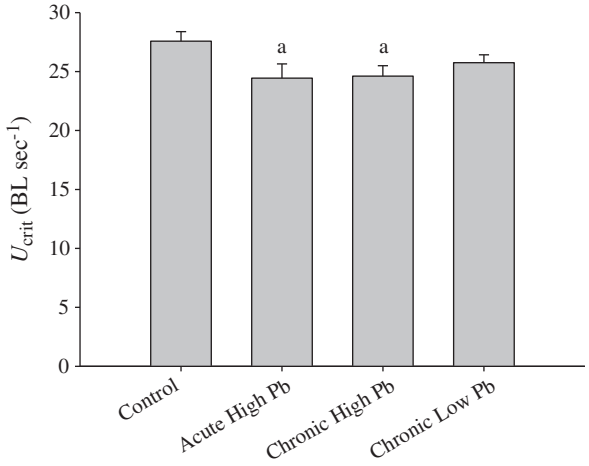

Fig. 1. Critical aerobic swimming speeds (U_{crit}) of juvenile fathead minnows exposed acutely to a high Pb concentration (672 nmol L^{-1}), chronically to either a high (689 nmol L^{-1}) or low (157 nmol L^{-1}) Pb concentration and control fish (mean ± SEM). [a]Significantly different from controls by Student's *t*-test (acute) or one-way ANOVA (chronic).

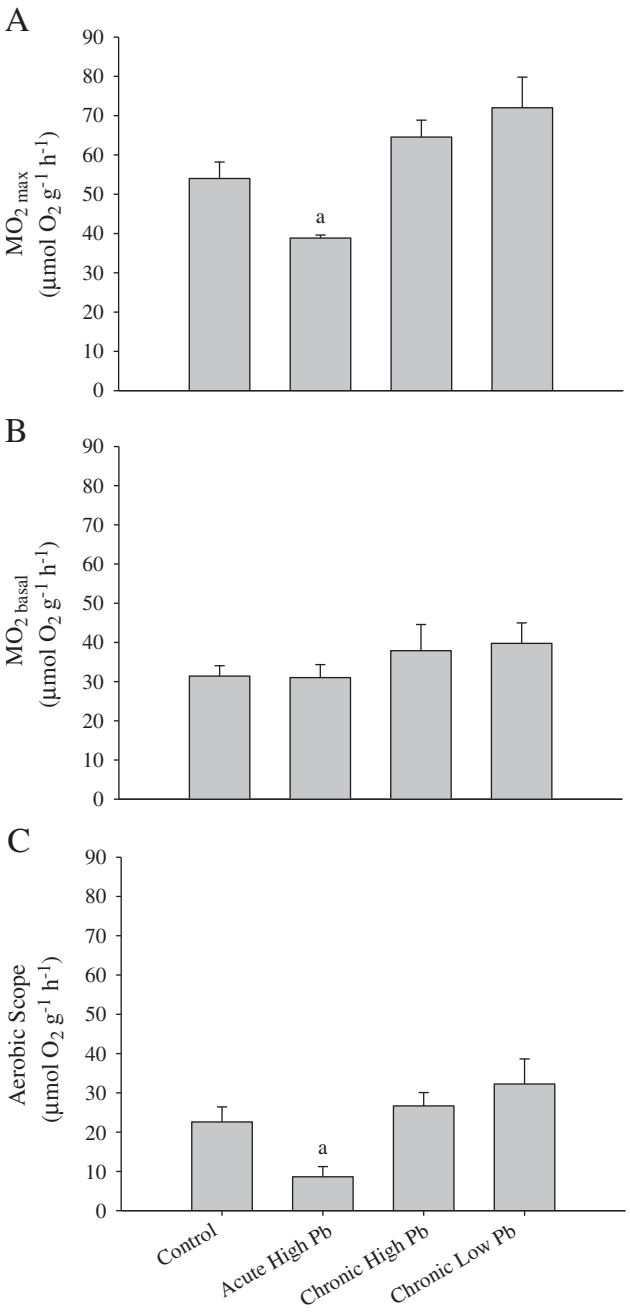

Fig. 2. Maximum (A) and basal (B) oxygen consumption rates of juvenile fathead minnows exposed acutely to a high Pb concentration (672 nmol L^{-1}), chronically to either a high (689 nmol L^{-1}) or low (157 nmol L^{-1}) Pb concentration and control fish. Aerobic scope (C) represents the difference of $MO_{2max} - MO_{2basal}$. Data are presented as mean ± SEM. [a]Significantly different from controls by Student's *t*-test.

3.4. Hemoglobin concentrations

Concentrations of Hb were measured from fish blood following swim respirometry experiments to test for an anemic response

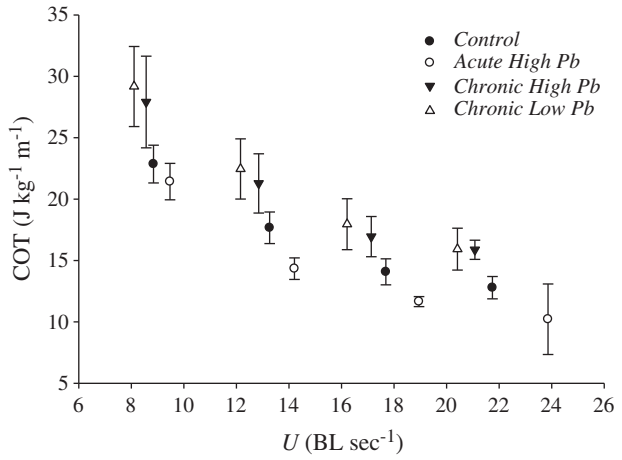

Fig. 3. Cost of transport (COT) as a function of swimming speed (U) for juvenile fathead minnows exposed acutely to a high Pb concentration (672 nmol L^{-1}), chronically to either a high (689 nmol L^{-1}) or low (157 nmol L^{-1}) Pb concentration and control fish (mean ± SEM).

induced by acute or chronic Pb exposure. Mean ± SEM (n) concentrations (mg mL^{-1}) of Hb measured from the control, acute 672 ± 35 nmol L^{-1} Pb, chronic 689 ± 66 nmol L^{-1} Pb and chronic 157 ± 18 nmol L^{-1} Pb treatments were 35 ± 2 (14), 34 ± 4 (8), 38 ± 3 (8) and 41 ± 4 (8), respectively. Although a trend toward an increase in Hb concentration appeared evident in fish chronically exposed to either 689 ± 66 or 157 ± 18 nmol L^{-1} Pb, no statistically significant differences among the treatments were determined.

3.5. Cost of transport

Metabolic rates and the corresponding swimming speeds obtained during the swim respirometry experiments were used to calculate COT as an indirect assessment of motor function impairment by Pb. Similar trends of decreasing COT with increasing swimming speed were observed regardless of treatment (Fig. 3). No statistically significant differences were found among any of the Pb treatments when compared to controls.

4. Discussion

Results from the present study revealed a Pb-induced impairment to the swimming performance (U_{crit}) of fathead minnows following acute and chronic exposures to 672 ± 35 and 689 ± 66 nmol L^{-1} Pb, respectively (Fig. 1). However, whereas the reduced U_{crit} from the acute Pb exposure was clearly associated with a reduction in aerobic scope, the chronic effect was not (Fig. 2). Two types of stress have been described by Brett (1958) that could account for the reduced aerobic scope exhibited by fish from the acute Pb exposure: (1) loading stresses that add to routine maintenance costs (MO_{2basal}) and (2) limiting stresses that reduce the maximum rate of O_2 consumption (MO_{2max}). Since MO_{2max} was significantly reduced, whereas MO_{2basal} was unchanged compared to controls (Fig. 2), the reduction in U_{crit} elicited by the acute Pb exposure appeared to reflect a limiting stress.

Possible limiting stresses include effects that reduce O_2 uptake from the water (e.g. filament clubbing and gill mucus secretion) and/or reduce O_2 delivery to the tissues (e.g. anemia and decreased cardiac output). Given that Hb concentrations from fish acutely exposed to Pb were similar to controls, a limiting stress due to anemia is not supported. However, considering the effects of other metals on swimming performance, a diffusional limitation in O_2 uptake appears plausible. Using a time course of swimming metabolism tests, Wilson et al. (1994) demonstrated that rainbow trout exposed to aluminum exhibited reductions in aerobic scope and MO_{2max} as early as 1 day post-exposure that had not recovered by the end of the 36 d exposure regime, and coincided with a similar trend for U_{crit}. These findings were consistent with the effects of nickel exposures to rainbow trout for similar durations (Pane et al., 2004). In both cases, effects were attributed to structural changes at the gill limiting oxygen uptake. A similar response could account for the observed effects on swimming performance and aerobic scope of fathead minnows following acute Pb exposure, although additional histological studies will be needed to examine this possibility.

An alternative type of limiting stress and mechanism for impaired swimming performance has been proposed for various species of salmonids and cyprinids following sublethal copper (Cu) exposures (Beaumont et al., 2000a; Beaumont et al., 2000b; De Boeck et al., 2006). Specifically, evidence indicates that Cu elicits reductions in swimming performance during chronic exposures that are due, not to an increased diffusion distance at the gill (Taylor et al. 1996; Waser et al. 2009), but rather to a state of hyperammonemia that causes a disruption of muscle membrane potentials (Beaumont et al., 2000a; Beaumont et al., 2000b). A reduction in aerobic scope therefore occurs due to a decrease in metabolic demand arising from impaired muscle fiber excitability (Beaumont et al., 2003). While it is possible that hyperammonemia might help explain the present results from the acute Pb exposures, it seems unlikely that a similar mechanism could account for the reduced U_{crits} following chronic Pb exposures given that no effect on MO_{2max} and aerobic scope was observed.

It is important to note, however, that neurological dysfunction could account for a reduction in swimming performance without a concurrent decrease in aerobic scope (such as that caused by hyperammonemia) through impairment of behavior and/or the optimal timing and coordination of muscle contractions necessary for efficient swimming. The COT analysis would seem to support this notion as mean values from both groups chronically exposed to Pb consistently trended higher than controls at similar swim speeds (Fig. 3), although again the differences were not statistically significant. However, there is additional evidence from previous studies in support of a possible neurological impairment in Pb-exposed fathead minnows that could explain a decrease in swimming performance. For example, although the reduction in β-globin mRNA expression from our earlier toxicogenomic study suggested an anemic response, at the same time, the apparent Pb-induced ROS production (also suggested by the microarray-identified genes) may have led to neurological damage (Mager et al., 2008). Furthermore, a follow-up study revealed behavioral impairment in larval offspring exposed to Pb as assessed by a prey capture assay (Mager et al., 2010). Aside from lordoscoliosis (Davies et al., 1976; Holcombe et al., 1976), other effects of Pb to the nervous system of fish have been reported including the disruption of various neurotransmitter systems (Rademacher et al., 2003; Sloman et al., 2005; Spieler et al., 1995), increased brain endocannabinoid levels (Rademacher et al., 2005) and injury to the hippocampus and optic tetum, regions of the brain controlling memory and visuomotor function (Giusi et al., 2008). Although the Pb concentration was higher than used in the present study (1450 nmol L^{-1}; 300 μg L^{-1}), Weber and Dingel (1997) found a 38% decrease in U_{crit} of rainbow trout (*Oncorhynchus mykiss*) following 1 week of Pb exposure that was attributed to neurobehavioral dysfunction as assessed by separate analyses of neurotransmitter levels and the startle response of fathead minnows. It therefore seems reasonable that neurological impairment could manifest in a decreased U_{crit} by fathead minnows during chronic Pb exposures in the absence of a corresponding reduction in aerobic scope, although additional work will be needed to explore this possibility.

Perhaps most surprising from this study was that the relatively large decreases in MO_{2max} and aerobic scope exhibited by fish from the acute Pb treatment translated into a rather small reduction in U_{crit}. It is difficult to explain the apparent discordance in magnitudes of

these two effects; however, results from the COT analysis may offer some potential insight. While the differences escaped statistical significance, it is interesting to note that values for the fish acutely exposed to Pb consistently trended lower than controls (Fig. 3) suggesting a possible increase in swimming efficiency. Such an increase in swimming efficiency could account for a partial maintenance of swimming performance in the face of reduced oxygen consumption capacity, although it is unclear as to how Pb might cause this to occur. In any event, an adverse effect of Pb on swimming performance due to neurological impairment of motor function appears unlikely during an acute exposure.

Finally, the present results indicate that fathead minnows may have an ability to recover from at least some of the acute effects of Pb given the restoration of aerobic scope (Fig. 2). In fact, both groups of fish chronically exposed to Pb revealed apparent elevations in MO_{2max} that overcame similar increases in MO_{2basal} (Fig. 2). Thus, while a Pb-induced elevation in MO_{2basal} indicates additional costs related to routine maintenance (loading stress), the cost, in terms of aerobic scope, appears compensated for by a similar increase in MO_{2max}. Although an increase in MO_{2basal} could reflect a variety of factors associated with increased costs of maintaining homeostasis during Pb exposure (e.g. detoxification and repair mechanisms, increased ventilation and/or ionoregulation), a greater MO_{2max} likely indicates increased hemopoiesis and Hb synthesis. Consistent with this notion, mean Hb concentrations from the fish chronically exposed to Pb trended toward higher levels than those of controls, although these differences were not statistically significant (see Section 3.4). It is interesting to note that the persistent decrease in β-globin mRNA expression observed up to 30 d of Pb exposure during our previous study was apparently recovered, and potentially reversed, at 150 d of Pb exposure (Mager et al., 2008). As the fish from the present study were exposed for 33–57 d (mean 43 d), this period may have coincided with a transition to recovery and reversal from the effects of Pb on Hb concentration. This possibility is supported by the results of Hodson et al. (1978) which demonstrated a recovery in hematocrits, likely owing to accelerated hemopoiesis, following an initial decline in hematocrits during the first 4 weeks of Pb exposures to rainbow trout. Thus, restoration of aerobic scope by fish chronically exposed to Pb may involve compensatory hematological responses in addition to recovery from acute Pb-induced effects (e.g. gill damage).

5. Conclusions

In summary, we have shown that the swimming performance of fathead minnows is impaired following acute and chronic exposures to Pb concentrations approximating 680 nmol L^{-1}. Given the reduction in aerobic scope (due to a reduced MO_{2max}), in addition to the lack of evidence for hematological or neurological dysfunction, the nature of the impaired swimming performance during acute Pb exposure remains unclear but may be due to reduced oxygen uptake at the gill, potentially owing to morphological alterations that cause an increase in diffusion distance, or possibly hyperammonemia. From the chronic Pb exposures, fathead minnows appear capable of recovering from the acute reduction in aerobic scope, potentially owing to acclimation and/or compensatory responses related to gill repair and/or increased hemopoiesis. Nevertheless, despite the recovery in aerobic scope, swimming performance remains impaired following chronic Pb exposure. These findings therefore suggest that the mechanisms of impaired swimming performance by fathead minnows arising from acute and chronic Pb exposure are different and may involve a transition from an acute limiting stress to a chronic effect on neuromotor function. Clearly, there is still much to be learned regarding the nature of the reduced aerobic scope and swimming performance in fathead minnows during acute and chronic Pb exposures. Additional experiments employing histological examinations of the gills, ammonia metabolism and alternative assessments of motor function (e.g. tail beat frequency) should help clarify the mechanisms of Pb impairment observed herein.

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