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Renal responses to acute lead waterborne exposure in the freshwater rainbow trout (*Oncorhynchus mykiss*)

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Abstract

The possible nephrotoxic effects of waterborne lead exposure (as $Pb(NO_3)_2$) were investigated in the freshwater rainbow trout (*Oncorhynchus mykiss*). Kidney lead accumulation was time-dependent, increasing upon exposure to 0.57 ± 0.01 mg dissolved Pb L^{-1} for up to 96 h with a significantly higher burden occurring in the posterior kidney compared to the anterior segment. Urine analyses in trout exposed to 1.20 ± 0.09 mg dissolved Pb L^{-1} revealed a significant increase in urinary lead excretion rate throughout 96 h of exposure. Urine flow rate and glomerular filtration rate (GFR) were not impacted with the exception of a significant decrease in GFR from 84 to 96 h in lead-exposed trout. Urine pH decreased significantly over time in lead-exposed fish. Correspondingly, urine ammonia excretion rate showed a marked increase from 48 h onwards. In experimental fish, urine glucose excretion was significantly greater by 96 h while urine lactate, urea and protein excretion were not significantly altered by lead exposure. The urine excretion rate of Ca^{2+} increased significantly by approximately 43% after only 24 h of lead exposure, and was maintained at a higher rate than controls for up to 96 h. Magnesium excretion increased in a time-dependent fashion, reaching a two- to three-fold rise by 96 h. In contrast, rates of Na^+ and Cl^- excretion were decreased in experimental fish by approximately 30% by 48 h, this trend continuing for the duration of lead-exposure. There were no changes in any of these parameters in similarly treated control fish. Clearance ratio analyses indicated progressive decreases in the net reabsorption efficiencies of the renal system for Ca^{2+}, Mg^{2+}, Pb, and glucose, suggesting that the active tubular transport mechanisms for these substances were inhibited by lead exposure, while Na^+, K^+, Cl^-, lactate, and protein reabsorptions were unaffected. Net ammonia secretion increased. We conclude that changes in renal function both reflect and help to minimize some of the associated disturbances in systemic physiology. Lead-induced ionoregulatory toxicity in rainbow trout, particularly the disturbance of Ca^{2+} homeostasis, is not exclusively a branchial phenomenon, but is in part a result of disruption of ionoregulatory mechanisms at the kidney. This action of lead outside the gills is critical to consider when developing guidelines for water quality.
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Keywords: Waterborne lead; Acute toxicity; Ionoregulation; Kidney function; Calcium reabsorption; Rainbow trout

1. Introduction

As a common contaminant in industrially impacted waters, waterborne lead (Pb) exerts toxic effects in fish through disturbance of ionoregulatory mechanisms, evident in the disruption of Ca^{2+} balance and the interference with Na^+ and Cl^- regulation (Sorensen, 1991; Rogers et al., 2003, 2005; Rogers and Wood, 2004). This places the toxic mechanism of lead midway between metals that disrupt Ca^{2+} homeostasis such as cadmium and zinc (Verbost et al., 1987, 1989; Spry and Wood, 1989; Hogstrand et al., 1995, 1996) and those that disrupt Na^+ and Cl^- balance such as silver (Morgan et al., 1997; Bury and Wood, 1999). A number of studies have shown that significant gill lead burden occurs after both acute exposure (MacDonald et al., 2002; Rogers et al., 2003) and chronic exposure to sublethal concentrations of waterborne lead (Davies et al., 1976; Hodson et al., 1978). Therefore, it has been assumed that the gill is the major site of lead-induced toxicity. Once it has crossed the gill, however, lead is distributed throughout other soft tissues within the organism, including the kidney (Demayo et al., 1982; Sorensen, 1991). Rogers et al. (2003) demonstrated significant renal accumulation in juvenile rainbow trout exposed to waterborne lead concentrations approaching the 96-h LC_{50} of 1 mg Pb L^{-1}, determined in moderately hard Lake Ontario water. Reichert et al. (1979) have also noted significant lead accumulation in the salmonid kidney. Similarly, Alves et al. (2006) and Alves and Wood (2006) reported

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that the highest internal lead concentrations (except for the gastro-intestinal tract) occurred in the kidney when trout were chronically exposed to dietary lead. Both Reichert et al. (1979) and Alves and Wood (2006) reported that the greatest concentrations occurred in the posterior kidney, the portion which contains the great majority of functional renal tubules (Wood, 1995). This suggests that the kidney may serve as a route of lead excretion, but it is presently unknown whether the processing of lead within the renal system creates similar disturbances to those noted at the gill, and whether these effects contribute to the ionoregulatory disruption previously observed (Rogers et al., 2003, 2005; Rogers and Wood, 2004) in freshwater teleosts.

Studies investigating the nephrotoxic effects of lead in amphibians have shown that prominent histological damage occurs during acute and chronic lead exposure (Loumbourdis, 2003). This damage is manifested in vacuolization of the proximal convoluted tubules and the formation of inclusion bodies, which are essentially aggregates of protein-bound lead absorbed by tubular epithelium and sequestered within the apical cell. Mammalian systems show similar indications of lead poisoning with a loss of proximal tubule integrity, and associated deficiencies in renal homeostatic mechanisms (Goyer, 1985).

Given the tendency of lead to bind protein sulfhydryl groups with high affinity (Sorensen, 1991), it is likely that ion transport enzymes such as Ca^{2+} ATPase, Na^+/K^+ ATPase, and carbonic anhydrase that are inhibited by lead at the gill (Rogers et al., 2003; Rogers and Wood, 2004; Rogers et al., 2005) are also disabled at the kidney. For example, renal Ca^{2+} reabsorption relies upon the action of a brush-border high-affinity Ca^{2+} ATPase (Flik et al., 1993), a transport enzyme inhibited at the baso-lateral membrane of branchial cells by lead (Rogers and Wood, 2004). Nephrotoxic effects of lead in mammals also include disruption of renal production of 1,25-dihydroxy Vitamin D (Goyer, 1985), a hormone involved in the regulation of Ca^{2+} uptake from the diet and Ca^{2+} reabsorption at the kidney.

The objective of this study was to investigate the effect of lead on the renal system of the freshwater rainbow trout (*Oncorhynchus mykiss*: Walbaum) using basic physiological techniques for analysis of renal function (Wood and Patrick, 1994). The study was performed in parallel to that of Rogers et al. (2003), and as far as possible the exposure regimes (water quality, temperature, lead concentrations) were the same to allow comparison of the results. Specifically, we evaluate whether or not disturbance of kidney function could contribute to the systemic physiological responses associated with acute lead toxicity reported in that study. This is of importance to the development of suitable mechanism-based models of toxicity, such as the biotic ligand model (BLM) (Paquin et al., 2000; Niyogi and Wood, 2004) that can be used to establish effective water-quality guidelines. Though a gill-binding model for lead does exist (MacDonald et al., 2002), further understanding of key binding sites involved in lead toxicity is required to evaluate whether internal binding of lead (e.g. at the kidney) and resulting disturbances of physiology (e.g. renal functions) can contribute to the acute toxic effects of waterborne lead.

2. Materials and methods

2.1. Experimental animals

This study was performed in parallel to that of Rogers et al. (2003), and an identical stock of fish, holding conditions, and temperature regime were used. Adult rainbow trout (180–230 g) for use in tissue bioaccumulation analysis and catheterization studies were obtained from Humber Springs Trout Farm in Orangeville, Ontario. Fish were held in a vigorously aerated flow-through system receiving a continuous flow of de-chlorinated City of Hamilton tap water (from Lake Ontario) at seasonal temperatures of 7–12 °C, and fed commercial trout feed at a ration of 1% total body weight per day. Water composition was in mM: $Ca^{2+} = 1.0$, $Mg^{2+} = 0.2$, $Na^+ = 0.6$, $Cl^- = 0.8$, $K^+ = 0.05$, total Pb = 0.68 μg L^{-1} (0.003 μM), dissolved organic carbon (DOC) = 3 mg L^{-1}, hardness (as $CaCO_3$) approximately 140 mg L^{-1} and pH 7.9–8.0. Experiments were conducted at a temperature of 9–12 °C and experimental animals were starved 72 h prior to and throughout all experiments.

2.2. Kidney lead accumulation

Methods used for lead bioaccumulation analysis were similar to those outlined in Rogers et al. (2003). The nominal waterborne lead concentration used was 0.5 mg L^{-1}, half of the Pb 96 h LC_{50} (Rogers et al., 2003) in Hamilton tap-water. This concentration was selected to avoid mortality over the course of the 96 h exposure. Following transfer to experimental chambers, adult rainbow trout were allowed a 'settling' period of 24 h. At $t = 0$ h, a control sampling took place ($n = 6$) after which stock solutions prepared from $Pb(NO_3)_2$ were used to spike the exposure chamber to achieve the appropriate lead concentration. Subsequent terminal sampling was done daily up to 96 h of lead exposure ($n = 6$ fish per sampling) for analysis of total kidney lead accumulation. In a separate exposure to 0.5 mg Pb L^{-1}, comparable samples were taken at 3, 8, 24, and 96 h for comparison of anterior and posterior kidney lead burden. In both exposures, water samples were taken daily, both filtered (0.45 μm) and unfiltered, for measurement of dissolved and total lead concentrations, respectively. These water samples were immediately acidified to 1% HNO_3 and refrigerated until the time of analysis.

Immediately following kidney dissection, tissues were blotted dry, weighed, and digested in 5:1 (v/w) ratio of 1N HNO_3 at 55 °C for 48 h. Samples were then homogenized by vortexing, centrifuged at 13,000 × g for 10 min, and the supernatant analyzed for total lead concentration as outlined below.

2.3. Renal responses to acute lead exposure

Adult rainbow trout were anaesthetized with MS-222 and fitted with both dorsal aorta catheters for blood collection (PE 50; Soivio et al., 1972) and urinary catheters (PE 60) for urine sampling using the technique outlined by Wood and Patrick (1994) for internal urinary catheterization. Ureteral urine is continually drained from the urinary bladder by this method, and thus is not subject to modification by the bladder, allowing observation of kidney function exclusively. Following surgery, fish

were transferred to darkened plexiglass boxes (3 L) which were continually aerated and received approximately 150 mL min^{-1} de-chlorinated Hamilton tap-water. A recovery period of 48 h was allowed after which urine flow rates were monitored every 12 h for a further 48 h to ensure that urinary catheters were functional and that urine flow rates (UFR) were normal. The final 24 h of this second 48 h period served as the pre-exposure control period. At 48 h post-surgery, control and experimental fish (to be exposed to 1.2 mg L^{-1} of lead for 96 h, see below) were each injected, via the implanted dorsal aorta catheters, with 17 μCi [^{3}H] polyethylene glycol ([^{3}H]-PEG 4000; New England Nuclear) in 0.66 mL Cortland saline (Wolf, 1963) for measurement of glomerular filtration rate (GFR), again using methods similar to those documented in Wood and Patrick (1994) and McDonald and Wood (1998).

2.4. Waterborne lead exposure

Methods used for waterborne lead exposures were identical to those outlined in Rogers et al. (2003). Water samples, both filtered and unfiltered, were taken for lead analysis by methods identical to those outlined above. Briefly, water from a head-reservoir tank was diverted to a separate, heavily aerated, mixing chamber, into which a stock solution of $Pb(NO_3)_2$ was dripped at a rate of 1 mL min^{-1} to achieve a nominal waterborne lead concentration of 1 mg L^{-1}. This nominal exposure concentration, which approximates the 96 h LC_{50} determined in Hamilton tap-water (Rogers et al., 2003), was chosen to maintain consistency with our previous studies on blood homeostasis during acute waterborne lead exposure (Rogers et al., 2003). In the present investigation, we wished to avoid taking large blood samples needed for plasma ion analyses as this sampling might disturb measurements of kidney function. Therefore, we used the plasma ion data of Rogers et al. (2003), a study performed under identical exposure conditions in the same water quality, for the renal clearance ratio analyses of the present study.

Urine collection took place over successive 12 h periods during which time urine volume was measured for determination of urine flow rate (UFR) and an aliquot of urine removed for determination of [^{3}H] PEG 4000 radioactivity for the purpose of calculating glomerular filtration rate (GFR). Urine pH was measured every 24 h. The remaining urine was immediately frozen in liquid nitrogen and stored at −80 °C until further analysis for ions and metabolites could be conducted. Small plasma samples (25 μL) were collected simultaneous to urine sampling every 12 h for determination of [^{3}H] PEG 4000 radioactivity. A final large blood plasma sample (1 mL) was taken at the end of the experiment (96 h) to confirm that the plasma ion changes in the present study were similar to those reported by Rogers et al. (2003). These samples were similarly frozen in liquid nitrogen immediately after collection and stored at −80 °C until further analysis.

2.5. Analytical techniques

Urine protein was measured using the method of Bradford (1976) and bovine serum albumin standards (Sigma–Aldrich). Urine lactate was measured enzymatically (L-lactate dehydrogenase/NADH; Sigma–Aldrich) on samples de-proteinized with ice cold 8% perchloric acid. Urine glucose was measured enzymatically using the hexokinase/glucose-6-phosphate dehydrogenase method (Sigma–Aldrich). Total plasma and urine ammonia concentration was measured enzymatically (glutamate dehydrogenase/NADP; Sigma–Aldrich). Urine urea was measured by the colorimetric assay of Rahmatullah and Boyde (1980). Urine and plasma Na^+, K^+, Ca^{2+}, and Mg^{2+} concentrations were determined by flame atomic absorption spectroscopy (FAAS) using a 220FS SpectrAA (Varian, Australia). Urine and blood plasma were diluted with 0.2% lanthanum for plasma Ca^{2+} determination. Urine and plasma Cl^- was analyzed by the mercuric thiocyanate spectrophotometric method (Zall et al., 1956). Lead concentrations in water samples, tissue digests, plasma, and urine were measured using graphite furnace atomic absorption spectroscopy (GFAAS) on a 220FS SpectrAA (Varian, Australia) against certified atomic absorption standards (Fisher Scientific) employing appropriate blank and reference standards. The lead standard used was within 3% of the certified reference material (National Water Research Institute). The detection limit was 0.06 μg Pb L^{-1}. The mean spike recovery for tissue samples spiked with a known amount of Pb was 98 ± 2.4%. The data were not corrected for total lead recovery.

2.6. Calculations

Urine flow rate was calculated from the cumulative collected volume of urine ($\sum V_{total}$):

$$\mathrm{UFR} = \frac{\sum V_{\mathrm{total}}}{\mathrm{mass} \times \mathrm{time}}$$

Measurement of [^{3}H]-PEG 4000 radioactivity in both urine (cpm_u) and plasma (cpm_p) samples was done by scintillation counting (Rackbeta 1217; LKB Wallac, Turka, Finland) with appropriate quench correction. GFR was then calculated as

$$\mathrm{GFR} = \frac{\mathrm{UFR} \times \mathrm{cpm_u}}{\mathrm{cpm_p}}$$

Urine excretion rate (U) of any substance (X) was given by$U_x = [X]_u$(UFR)and corresponding clearance ratio (CR_x) were calculated using measurements of substance X in the plasma ($[X_p]$) at the same time points from Rogers et al. (2003) and the formula below

$$\mathrm{CR}_x = \frac{[X]_u(\mathrm{UFR})}{[X]_p(\mathrm{GFR})}$$

This analysis relates the clearance rate of a substance (X) to the clearance rate of the non-reabsorbed, non-secreted marker [^{3}H]-PEG 4000 (i.e. GFR). Values of CR_x greater than 1.0 indicate that X is secreted on a net basis by the renal system, while values of CR_x less than 1.0 indicate that X is reabsorbed on a net basis (see Wood and Patrick, 1994; Wood, 1995). For example, a CR_x value of 0.1 would indicate 90% net reabsorption of the filtered load of X, while a CR_x value of 2.0 would indicate that the rate of excretion of X was twice as large as that attributable

to glomerular filtration alone—i.e. net secretion of *X* must have occurred.

2.7. Statistical analysis

Data are expressed as mean ± 1 S.E.M. (N = number of fish). Experimental means were compared with corresponding control mean values at the same time point by an unpaired two-tailed Student's *t*-test. Time-dependent responses in both control and experimental groups were tested against initial 0 h measurements using a one-way ANOVA with a two-sided Dunnett's post hoc multiple comparison test. All statistical significance are calculated at $p < 0.05$.

3. Results

Total and dissolved lead concentrations in the exposure waters did not differ significantly, thus only the latter are reported.

3.1. Kidney lead accumulation

Total renal lead burden increased significantly upon exposure to 0.57 ± 0.01 mg dissolved Pb L^{-1} ($n = 6$ at every time point, no significant variation over time; Fig. 1A). Accumulation was rapid with significant elevation after 24 h of waterborne lead exposure, compared to $t = 0$ h controls. Burden increased significantly in a time-dependent fashion through to 96 h of exposure.

A second series focused on early time points and a comparison of burden measured in the anterior versus posterior sections of the kidney (Fig. 1B). Accumulation of lead was significant after only 3 h in the posterior section, whereas it took 24 h for significant elevation in the anterior section. Anterior accumulation was consistently lower than that at the posterior end (57% lower at 24 h and 35% lower at 96 h).

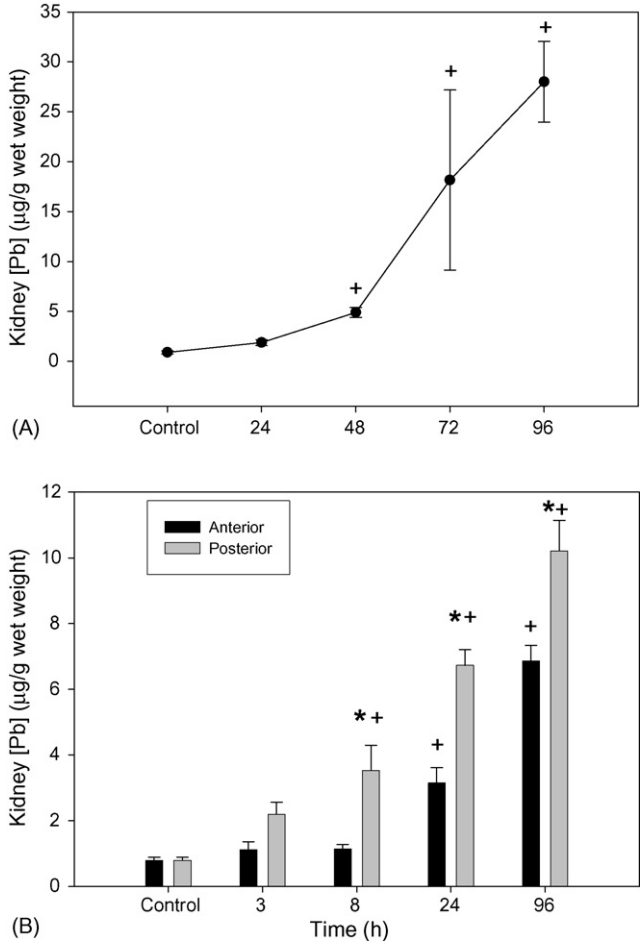

Fig. 1. Lead accumulation in the adult rainbow trout (*Oncorhynchus mykiss*) exposed to control conditions ($t = 0$ h) or elevated waterborne lead. (A) Total kidney lead accumulation at 0, 24, 48, 72, and 96 h, and (B) ($N = 6$ at each time) lead accumulation in the posterior kidney (white bars) and in the anterior kidney (black bars) at 0, 3, 8, 24, and 96 h ($N = 6$ at each time). Data are expressed as mean ± 1 S.E.M. Asterisk "*" indicates significant difference ($p < 0.05$, two-tailed Student's *t*-test) between sample groups; "+" indicates a significant difference ($p < 0.05$; Dunnett's one-way ANOVA) from 0 h control mean.

3.2. Renal responses to acute lead exposure

Adult rainbow trout implanted with dorsal aortic and urinary catheters were exposed to 1.20 ± 0.09 mg ($n = 11$) dissolved Pb L^{-1}. Dissolved lead in control water was 0.040 ± 0.006 mg L^{-1} ($n = 8$). Neither of these values varied significantly over time. Changes in measured plasma constituents (Na^+, Cl^-, Ca^{2+}, Mg^{2+}, K^+, and Pb) by 96 h were virtually identical to those reported by Rogers et al. (2003) in response to a similar lead exposure, and so have not been repeated here. Notably, plasma Na^+, Cl^-, and Ca^{2+} levels all decreased significantly, the latter by the greatest relative extent, while plasma Mg^{2+} increased slightly.

Corresponding with the trends of lead accumulation (Fig. 1), a significant amount of lead was excreted in the urine of catheterized adult rainbow trout (Fig. 2A). At $t = 0$ h, urine lead was not detectable, however by 24–96 h of lead exposure, the concentration had reached 60–95 µg Pb L^{-1}, yielding an excretion rate of about 0.08–0.12 µg Pb kg^{-1} h^{-1}. Clearance ratio analysis indicated that lead was about 80–90% reabsorbed by the renal system, though this reabsorption tended to decrease slightly over time (Table 1).

Urine flow rate (UFR; Fig. 2B) and glomerular filtration rate (GFR; Fig. 2C), both strong indicators of renal function, were unaltered in control and experimental groups for up to 72 h. At 96 h, UFR remained stable, however, a significant decrease in GFR occurred in lead-exposed trout compared to corresponding 96 h controls (Fig. 2C). Clearance ratio analysis indicated that about 70% of the filtered water load was reabsorbed on a net basis, but this decreased to 55% at 96 h (Table 1).

Fig. 3A illustrates urine pH measurements taken in control and lead-exposed trout. Overall, significant decreases in urine pH were evident in the lead treatment group at 72 and 96 h. A significant decline apparent at 96 h compared to corresponding control measurements was observed in lead-exposed fish.

Rates of urinary ammonia excretion (Fig. 3B) were very much in-line with pH changes shown in Fig. 3A. Stable excretion occurred from 0 to 48 h in control and experimental groups,

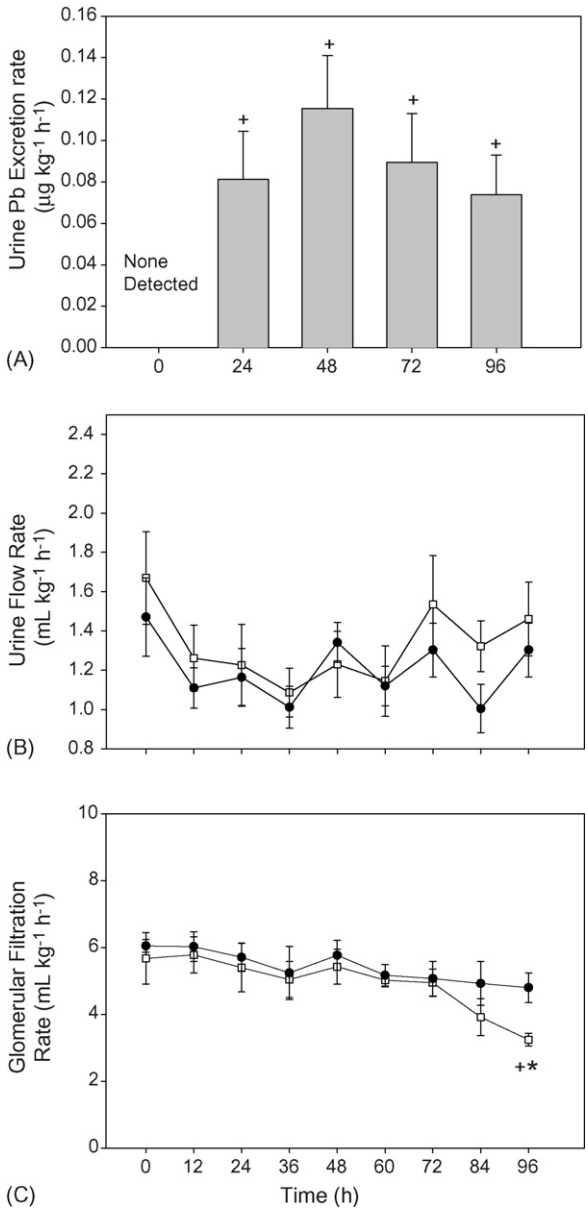

Fig. 2. Measurements of (A) urine lead concentration in trout (*O. mykiss*) exposed to elevated waterborne lead for up to 96 h, (B) urine flow rate (UFR), and (C) glomerular filtration rate (GFR) in rainbow trout exposed to control conditions (black circles; $N = 8$) or 1.2 ± 0.09 mg dissolved Pb L^{-1} (white squares; $N = 11$). Data and symbols as per Fig. 1.

however, with prolonged lead-exposure, ammonia excretion increased significantly by two-fold compared to both 0 h pre-exposure values and 72 h control values. This trend continued at 96 h of lead exposure. Changes in ammonia handling were also reflected in clearance ratios which were always greater than 1.0 indicating net secretion, and increased in a time-dependent fashion from 2.80 at 0 h to 5.90 at 96 h (Table 1), indicating a marked increase in net ammonia secretion. Urea excretion rates remained approximately stable in both treatment groups (Fig. 3C). Plasma urea data were not available for clearance ratio analysis.

Rates of urinary glucose excretion (Fig. 4A) showed a time-dependent increase in lead-exposed fish. At 96 h, this culminated

Table 1
Clearance ratios of various substances under $t = 0$ h control conditions and upon exposure to 1.20 mg dissolved Pb L^{-1} for up to 96 h

Substance	Clearance ratio				
	Control	0–24 h	24–48 h	48–72 h	72–96 h
Ca^{2+}	0.15	0.24	0.26	0.34	0.48
Mg^{2+}	0.39	0.61	0.56	0.9	1.3
Na^{+}	0.02	0.01	0.01	0.01	0.02
Cl^{-}	0.02	0.01	0.01	0.01	0.02
K^{+}	0.41	0.2	0.49	0.53	0.38
Pb	0	0.08	0.14	0.1	0.21
Ammonia	2.8	3.12	3.41	4.69	5.9
Glucose	0.07	0.06	0.08	0.18	0.43
Lactate	0.04	0.02	0.01	0.01	0.03
Protein	0.0012	0.0006	0.001	0.001	0.0012
Water	0.29	0.22	0.23	0.31	0.45

Data are calculated from mean urinary values of the present study and mean plasma values of Rogers et al. (2003) for trout exposed under very similar conditions.

in a significant 2.5-fold increase in rates of urine glucose excretion in experimental fish while control measurements were stable throughout the experiment. Glucose clearance ratios (Table 1) also showed a decline in net reabsorption as they increased in

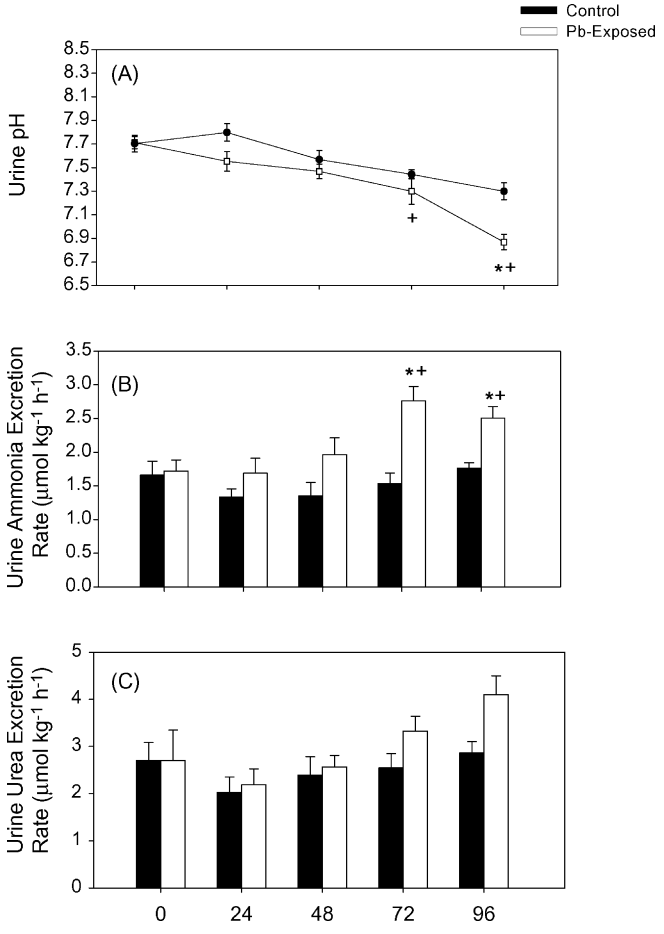

Fig. 3. Measurements of (A) urinary pH, (B) urine ammonia excretion rate, and (C) urine urea excretion rate in adult rainbow trout (*O. mykiss*) exposed to control conditions (black circles and bars; $N = 8$) or to 1.2 ± 0.09 mg dissolved Pb L^{-1} (white squares and bars; $N = 11$). Data and symbols as per Fig. 1.

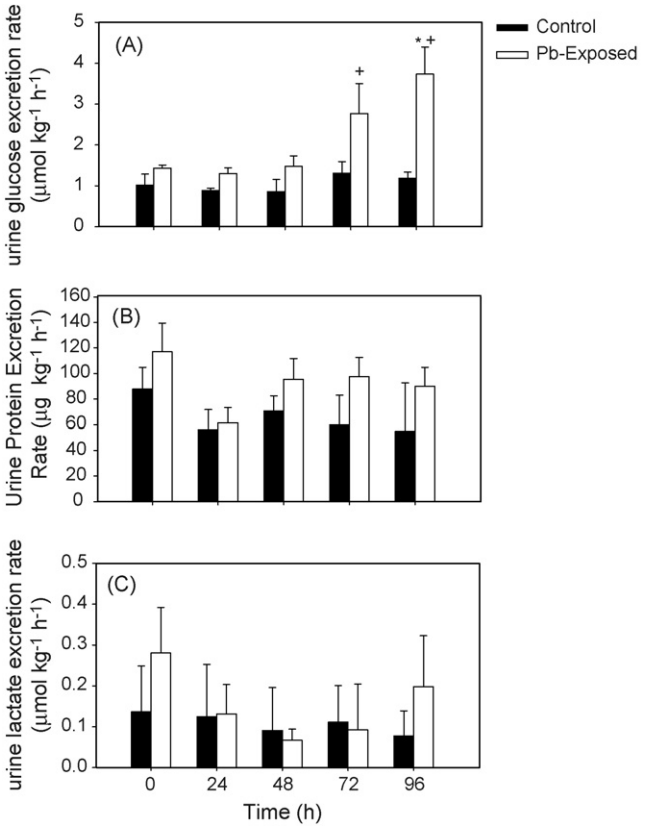

Fig. 4. Urinary metabolite parameters obtained from rainbow trout (*O. mykiss*) exposed to control conditions (black circles and bars; $N = 8$) or 1.2 ± 0.09 mg dissolved Pb L^{-1} (white circles and bars; $N = 11$). (A) Urine glucose, (B) urine protein, and (C) urine lactate. Data and symbols as per Fig. 1.

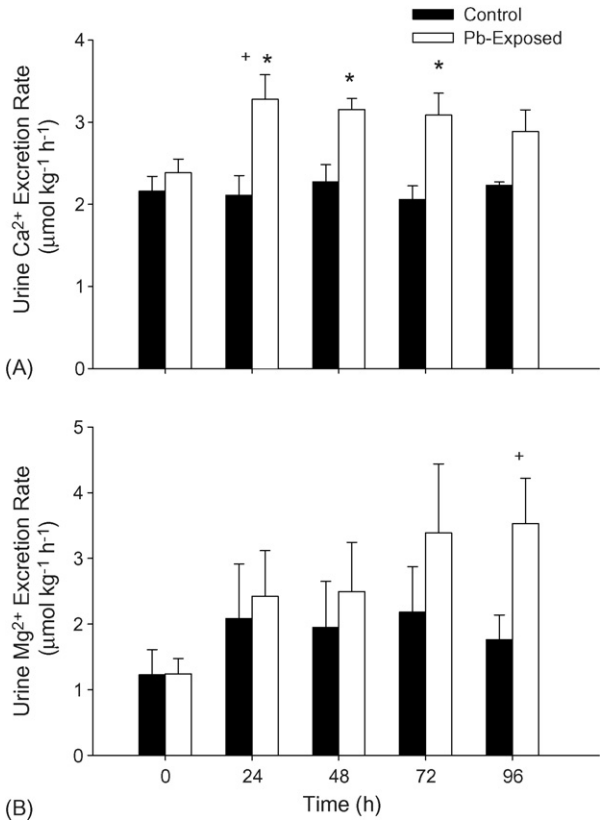

Fig. 5. Urine ion excretion rates in adult rainbow trout (*O. mykiss*) exposed to control conditions (black bars; $N = 8$) or 1.2 ± 0.09 mg dissolved Pb L^{-1} (white bars; $N = 11$). (A) Urine Ca^{2+} excretion rate and (B) urine Mg^{2+} excretion rate. Data and symbols as per Fig. 1.

an accelerating fashion over time. Unlike glucose, rates of urine protein excretion (Fig. 4B) and lactate excretion (Fig. 4C) were not significantly altered by waterborne lead exposure. Excretion rates of both protein and lactate were extremely low, and corresponding clearance ratio analysis (Table 1) indicated that both substances were very strongly reabsorbed at constant levels (>96% for lactate, >99.8% for protein) by the renal system.

Fig. 5A and B illustrates the urinary excretion rates of Ca^{2+} and Mg^{2+}, respectively, in control and experimental rainbow trout. In contrast to comparable values measured under 0 h control conditions, exposure to 1.20 ± 0.09 mg dissolved Pb L^{-1} resulted in a significant 43% increase in urinary Ca^{2+} excretion compared to 0 h and corresponding 24 h controls (Fig. 5A). Calcium excretion remained significantly higher at 24–48 and 48–72 h of lead exposure. Corresponding clearance ratio analysis illustrated that the net reabsorption of Ca^{2+}, which was initially high (85%), was progressively reduced (Table 1).

Magnesium excretion rates (Fig. 5B) were variable, however, a time-dependent increase in Mg^{2+} excretion was evident in lead-exposed fish, with a significant two- to three-fold higher rate measured at 96 h, compared to 0 h controls. In control fish, Mg^{2+} excretion was relatively stable from 72 to 96 h of experimentation. Clearance ratio analysis for Mg^{2+} (Table 1) indicated a less efficient net reabsorption (61%) than for Ca^{2+} (85%) under control conditions, but thereafter trends were qualitatively similar to Ca^{2+}, indicating a progressive reduction in reabsorption during lead exposure. At 72–96 h, Mg^{2+} handling changed to net secretion.

Unlike those observed for Ca^{2+} and Mg^{2+}, excretion rates for Na^+ and Cl^- (Fig. 6A and B, respectively) decreased significantly over time with exposure to waterborne lead. Decreases were approximately 30%. Urinary Na^+ excretion rate in lead-exposed fish, first fell at 48 h and the effect persisted thereafter (Fig. 6A). Urinary Cl^- excretion rates showed a similar trend in lead-exposed fish with significantly lowered Cl^- excretion by 72 h of lead exposure. Control excretion rates for Na^+ and Cl^- remained constant for the duration of the 96 h experiment. Excretion rates for K^+ (Fig. 6C) were not significantly altered from 0 to 96 h of exposure to control conditions or to lead exposure.

Correspondingly, clearance ratios (Table 1) for these ions were stable over the course of lead exposure despite the decrease in excretion rates, showing that functional reabsorption was unaffected for Na^+, Cl^-, and K^+. Na^+ and Cl^- reabsorption efficiencies were about 98%, while the values for K^+ were only about 60%.

4. Discussion

The concentrations of lead used in the present study were relatively high, close to the LC_{50} range, and chosen to parallel the study of Rogers et al. (2003) so that the physiological changes seen would be diagnostic of mechanisms of toxicity. Neverthe-

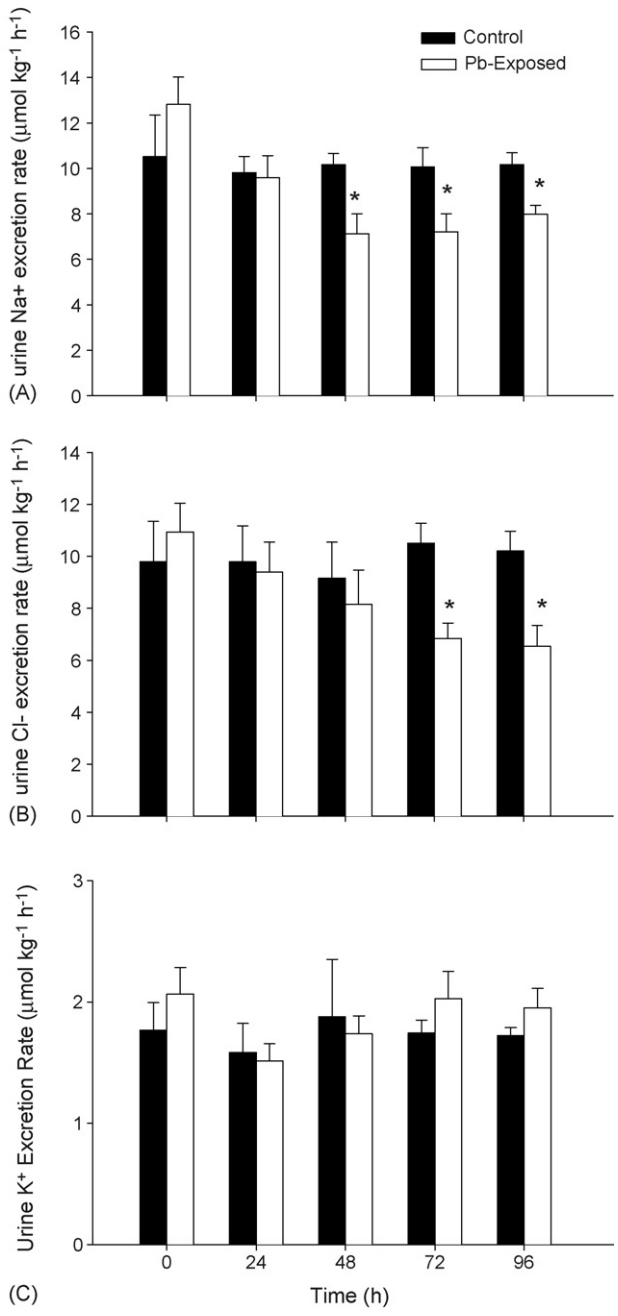

Fig. 6. Urine ion excretion rates in adult rainbow trout (*O. mykiss*) exposed to control conditions (black bars; $N = 8$) or 1.2 ± 0.09 mg dissolved Pb L^{-1} (white bars; $N = 11$). (A) Urine Na^+ excretion rate, (B) urine Cl^- excretion rate, and (C) urine K^+ excretion rate. Data and symbols as per Fig. 1.

less, waterborne lead concentrations in lead-impacted natural waters as high as 0.89 mg L^{-1} have been reported (Research Triangle Institute, 1999). Lead has been previously characterized as an ionoregulatory toxicant in the rainbow trout, affecting not only Ca^{2+} influx and homeostasis, but also the balance of Na^+ and Cl^- (MacDonald et al., 2002; Rogers et al., 2003, 2005; Rogers and Wood, 2004). The interaction of lead at the teleost gill is becoming increasingly well understood, however, this study has presented evidence that lead-induced ionoregulatory toxicity cannot be characterized exclusively as a branchial phenomenon, but may also be the product of disruption at the kidney.

Consistent with results reported in Hodson et al. (1978), Reichert et al. (1979), Rogers et al. (2003), Alves and Wood (2006) and Alves et al. (2006), lead readily accumulated in the trout kidney (Fig. 1A and B). Under relatively short term acute exposures, it appears that the kidney acts as a high capacity 'sink' whereby lead accumulates without reaching an obvious steady state. This high capacity for lead binding has also been demonstrated in the mammalian renal system (Goyer, 1985) and has previously been associated with the formation of inclusion bodies, essentially precipitates of protein-bound lead, observable in the apical cells of the proximal renal tubules, and is recognized as a detoxification mechanism (Goyer, 1985; Ceruti et al., 2002; Loumbourdis, 2003).

The renal accumulation of lead observed in the present study (Fig. 1B) followed a similar pattern to that reported in Reichert et al. (1979), whereby lead accumulation in chronically exposed coho salmon (*Oncorhyncus kisutch*) was significantly higher in the posterior kidney when compared to the anterior, or head portion. A similar pattern was seen in the dietary lead study of Alves and Wood (2006). Given that the posterior aspect contains the functional nephrons and associated reabsorptive and excretory mechanisms (Wood, 1995), it is apparent that the kidney plays a large role in the metabolism and excretion of lead.

The excretory role of the kidney during lead exposure is further supported by the significant presence of lead in urine collected from lead-exposed catheterized trout (Fig. 2A). Interestingly, UFR was not impacted by lead from 0 to 96 h of exposure despite the increased concentration of urine lead (Fig. 2B), and there was no significant change in urinary protein loss (Fig. 4B). The latter is a classical indicator of damage to the glomerular filtration barrier, and in this respect the action of waterborne lead appears to differ from that of waterborne nickel which causes a large increase in urinary protein excretion rate (Pane et al., 2005). However, a significant decrease in glomerular filtration rate did occur from 72 to 96 h of lead exposure. This change in GFR without a corresponding impact on urine flow was also reported during waterborne nickel exposure (Pane et al., 2005) and may reflect subtle damage resulting from lead accumulation, causing a loss of function in some nephrons. A partially compensating increase in single-nephron GFR would likely occur in those nephrons which are still filtering so as to maintain osmotic balance. This scenario is consistent with the observation that the overall GFR fell (Fig. 2C) yet UFR was maintained (Fig. 2B) so the efficiency of water reabsorption was reduced (Table 1). A loss in filtering nephrons could also indicate a compensatory response to the significant decreases in plasma Ca^{2+}, Na^+ and Cl^- after 96 h of acute lead exposure (Rogers et al., 2003). Hormonal regulation may be an additional factor in lead-induced changes in GFR. In response to reduced concentrations of plasma Na^+ and Cl^-, it is possible that a compensatory release of an anti-diuretic hormone such as arginine vasotocin (AVT) (Brown and Balment, 1997) may occur resulting in systemic changes in blood vessel constriction (Larsen and Perkins, 2001) and subsequently lowered rates of filtration at the kidney.

Urine pH fell in response to waterborne lead exposure (Fig. 3A). Since the plasma pH and other acid–base parameters remained unaltered in the lead-exposed trout (Rogers et al., 2003), this suggests that the urinary buffering system was disturbed. An example of such a disturbance would be the role of inorganic phosphate (P_i) in excreting protons as H_2PO_4; in future studies it will be of interest to measure the full-acid base status of the urine and its buffer constituents (e.g. Wood et al., 1999; Pane et al., 2005) in lead-exposed fish. Urinary ammonia excretion rates increased after 72 h in lead-exposed fish (Fig. 3B). This was mirrored by elevated plasma ammonia concentrations after 72 h of Pb exposure (Rogers et al., 2003). However, as ammonia was clearly secreted into the urine on a net basis (i.e. clearance ratio > 1.0) and this secretion increased during lead exposure (Table 1), the increase in urinary ammonia excretion was not solely attributable to increased plasma levels and filtered loads. Rather, the increase in secretion was likely consistent with greater diffusion-trapping of NH_3 as NH_4^+ by the more acidic urine pH (Wood, 1995; Wood et al., 1999). Notably, urea excretion did not increase with lead exposure, so the effect was specific to ammonia excretion and not a general effect on nitrogenous waste metabolism.

There was no effect of lead exposure on renal lactate excretion, which was in accordance with the similar lack of effect on plasma lactate (Rogers et al., 2003). However, lead-exposed trout had significantly higher glucose excretion rates than their control counterparts (Fig. 4A). Since plasma glucose concentration increased only slightly and non-significantly despite a substantial rise in cortisol (Rogers et al., 2003), remaining well below the threshold concentration (22.5 mmol L^{-1}) where reabsorption is normally saturated in this species (Bucking and Wood, 2005), the increased glucose excretion rates was probably not caused by an increase in filtered load. However, the clearance ratio analysis indicated that the efficiency of net glucose reabsorption was clearly reduced at 48–96 h (Table 1). Therefore, increased urinary glucose excretion could be due to a more specific interaction of lead with the Na^+-glucose co-transport mechanism involved in renal glucose reabsorption at the teleost brush-border membrane (Freire et al., 1995). Normally, glucose is freely filtered at the glomerulus and almost completely reabsorbed, however, interference with key transporters involved in this uptake process would result in glucose excretion via the urine (Bucking and Wood, 2005).

Lead excretion had a significant and highly specific impact on renal handling and reabsorption of Ca^{2+} (Fig. 5A). After only 24 h of exposure, Ca^{2+} excretion rates were increased significantly, and this effect was associated with a progressive decrease in the efficiency of net Ca^{2+} reabsorption (Table 1). By analogy to observations reported for gill tissue by Rogers and Wood (2004), lead inhibition of Ca^{2+} transport could occur at a number of steps in the reabsorption process. Firstly, the increased presence of lead in the lumen of the proximal tubule could have a competitive effect at key Ca^{2+} uptake sites along the brush border. Once entering the apical membrane of cells lining the proximal tubule, lead would accumulate in the form of inclusion bodies, resulting in increased tubular cell nuclei size which would damage the proximal tubule cell plasma membrane and cause a loss of microvilli and vasolateral invaginations (Goyer, 1985; Tanimoto et al., 1993; Ceruti et al., 2002; Loumbourdis, 2003) which could disrupt the capacity for Ca^{2+} reabsorption. Similar to inhibition of high-affinity Ca^{2+}-ATPase by lead at the gill (Rogers and Wood, 2004), inhibition at the kidney resulting in disrupted transport of Ca^{2+} across the basolateral membrane of proximal tubule cells could be the cause of increased urinary Ca^{2+} loss.

Accumulation of lead at the kidney may also have profound effects on hormonal regulation of Ca^{2+} homeostasis. In mammals, re-absorption of Ca^{2+} at the proximal tubule in response to decreasing plasma Ca^{2+} concentrations can be stimulated by Vitamin D. Thus, the production of 1,25-dihydroxyVitamin D (metabolically active form of Vitamin D; Boyle et al., 1971) at the kidney is inhibited by lead (World Health Organization, 1995; Fullmer, 1997), therefore suggesting an additional point of lead-induced Ca^{2+} disruption in fish. The progressive lead-induced interference with the reabsorptive transport of Ca^{2+} may be linked to the pattern of lead accumulation at the kidney (Fig. 1A and B). As shown in Rogers and Wood (2004), lead burden at the gill was strongly correlated with reduced high-affinity Ca^{2+} ATPase activity and with reduced rates of Ca^{2+} influx. The disturbances at the kidney may be mechanistically similar, however, further study is required. For example, studies employing *in vitro* procedures for investigating brush-border ion transport (e.g. Freire et al., 1995) would be useful in characterizing the effect of lead on Ca^{2+} transport at the trout kidney. This would include investigating the effect of lead on vesicular Ca^{2+} uptake and on the activity of brush-border bound high-affinity Ca^{2+} ATPase.

In contrast to plasma Ca^{2+}, plasma Mg^{2+} exhibited a modest increase in response to waterborne lead exposure (Rogers et al., 2003). However, urinary Mg^{2+} excretion increased, though not quite in parallel to urinary Ca^{2+} excretion (Fig. 5A and B), so decreased renal Mg^{2+} loss was not the explanation, and the etiology of the phenomenon remains unknown. At the kidney, the clearance ratio analysis indicated that the reduction in net reabsorption efficiency was greater for Mg^{2+} than for Ca^{2+}, and by 72–96 h, there was clear evidence of net Mg^{2+} secretion as the clearance ratio surpassed 1.0 (Table 1). It is well established that the teleost kidney is capable of both tubular reabsorption and tubular secretion of Mg^{2+}, and the two processes likely operate simultaneously in different parts of the tubule (Oikari and Rankin, 1985; Bijvelds et al., 1998; Beyenbach, 2000). Therefore, it seems likely that lead interfered with tubular Mg^{2+} reabsorption, perhaps in a similar manner to its interference with tubular Ca^{2+} reabsorption, such that the influence of secretory processes became greater over time. Indeed, Bijvelds et al. (1998) characterized Mg^{2+} homeostasis and reabsorption at the kidney as a Ca^{2+}-sensitive process. Additionally, the rise in plasma Mg^{2+} concentrations may have triggered an increase in the Mg^{2+} secretory processes (Oikari and Rankin, 1985; Beyenbach, 2000). Interestingly, clearance ratio values for Pb, Ca^{2+}, and Mg^{2+} all increased in a similar manner during waterborne lead exposure (Table 1), suggesting that inhibition of a common reabsorptive mechanism may have occurred. There is a clear need for further mechanistic analysis, perhaps using the

brush-order membrane vesicle techniques pioneered for trout by Freire et al. (1995).

There were significant declines in the plasma concentrations of Na^+ and Cl^- after 72 h of exposure to waterborne lead (Rogers et al., 2005). From measurements of urine Na^+ and Cl^- excretion rates (Fig. 6A and B), it appears that the kidney was responsive in compensating for ion and osmoregulatory imbalance resulting from waterborne lead exposure from 48 to 72 h onward. Since the clearance ratios for Na^+ and Cl^- did not change and remained indicative of very strong net reabsorption (Table 1), the decrease in Na^+ and Cl^- excretion rates was simply a function of the decrease in the filtered loads, reflecting decreased plasma Na^+ and Cl^- concentrations, with an additional contribution from the late reduction in GFR (Fig. 2C). Thus, renal function did not contribute to dysfunction of Na^+ and Cl^- regulation, but rather helped to minimize it.

Given the capacity of the teleost kidney to absorb Na^+ and Cl^- at not only the proximal tubule I, but at other sites along the nephron such the proximal tubule II (Wood, 1995) and the distal segment (Nishimura et al., 1983), it can only be speculated in the present study that lead does not have an impact on renal Na^+ and Cl^- transport processes, in contrast to its documented inhibitory effects on active Na^+ and Cl^- uptake at the gills (Rogers et al., 2003, 2005). Based on the increasing urinary excretion of glucose upon acute lead exposure (Fig. 4A), there is a possibility that interference with Na^+ transport sites still occurs. For example, phlorizin inhibition of the Na^+-dependent transport of glucose (Kleinzeller et al., 1977; Freire et al., 1995) at the proximal tubule resulted in increased glucose excretion in the urine of freshwater rainbow trout without detectable effect on net Na^+ reabsorption (Bucking and Wood, 2005). Further studies investigating the interaction of lead with Na^+ transport sites are required.

5. Conclusions

Based on the evidence presented in this study, the ionoregulatory and osmoregulatory impacts of exposure to acute concentrations of waterborne lead occur, at least in part, by disruption of reabsorption processes at the kidney (for Ca^{2+}, Mg^{2+}, glucose, and water), in addition to previously documented interference with active ion uptake at the gill (Rogers and Wood, 2004; Rogers et al., 2003, 2005). This dual action, most obvious when considering measurements of increased urinary Ca^{2+} excretion coupled with reduced branchial Ca^{2+} influx, explains the hypocalcemic effects associated with lead poisoning (Sorensen, 1991).

Application of predictive models such as the biotic ligand model (BLM: Paquin et al., 2000) to the development of water quality guidelines requires a firm understanding of the acute toxic mechanism of a metal, which involves using diagnostic concentrations of the toxicant close to the LC_{50}. Data presented here using this approach suggest that the acute toxic mechanism for lead in the rainbow trout involves disrupted ionoregulation at the kidney in addition to previously considered gill effects used in modeling (MacDonald et al., 2002; Niyogi and Wood, 2004). Clearly, future studies should address the impact of lower more environmentally relevant waterborne lead concentrations, and should examine the renal system as an important site of lead processing and lead-induced physiological impact.

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