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Hydromineral balance in the marine gulf toadfish (*Opsanus beta*) exposed to waterborne or infused nickel

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Abstract

The effects of acute Ni exposure on the marine gulf toadfish (*Opsanus beta*) were investigated via separate exposures to waterborne nickel (Ni) and arterially infused Ni. Of the plasma electrolytes measured after 72 h of waterborne exposure (215.3 and 606.1 μM Ni in SW (salinity of 34)), only plasma [Ca^{2+}] was significantly impacted (∼55% decrease at both exposure concentrations). At both exposure concentrations, plasma [Ni] was regulated for 24 h, after which a linear accumulation over time occurred. Accumulation of Ni in the plasma, and in tissues in direct contact with seawater (gill, stomach, and intestine), was roughly proportional to the Ni concentration of the exposure water. Hydromineral balance in the intestinal fluid (IF) was markedly impacted, with Na^+, Cl^-, SO_4^{2-}, K^+, and Mg^{2+} concentrations elevated after 72 h of exposure to waterborne Ni.

Following arterial Ni infusion (0.40 μmol Ni kg^{-1} h^{-1}), perturbation of hydromineral balance of the intestinal fluid was specific only to Na^+ (significantly elevated by Ni infusion) and Mg^{2+} (significantly decreased by Ni infusion). Nitrogen excretion was not significantly impacted by Ni infusion. In all tissues save the kidney, Ni accumulation via infusion was only a fraction of that observed during waterborne exposures. Remarkably, the kidney Ni burden following infusion was almost identical to that resulting from both waterborne exposures, suggesting homeostatic control. Ni excretion, dominated at 24 h by extrarenal routes, was primarily a function of renal excretion by 72 h of infusion. The sum excretion from infused toadfish was relatively efficient, accounting for over 40% of the infused dose by 72 h.

Mechanistic knowledge of the mechanisms of toxicity of waterborne Ni in marine systems is a critical component to the development of physiologically based modeling approaches to accurately predict Ni toxicity in marine and estuarine ecosystems.

Keywords: Nickel; Gulf toadfish; Marine toxicity; Mechanistic physiology; Ion balance; Waterborne; Infusion; Excretion

1. Introduction

As a general rule, mechanistic investigations of metal toxicity in marine species lag far behind those in freshwater species. This discrepancy is well explained by the freshwater roots of the physiologically based biotic ligand model (BLM; Di Toro et al., 2001), for which many mechanistic studies have been conducted over the last decade. The biotic ligand model is a practical modeling approach designed to reliably predict variations in the toxicity of metals based on site-specific variations in water chemistry (Di Toro et al., 2001). The approach aims to supplement non-biological freshwater parameters such as water hardness, traditionally used to derive water quality guidelines and risk assessments (USEPA, 1986), with geochemically relevant parameters (specific ions) and true biological parameters (De Schamphelaere et al., 2002). Inclusion of these additional parameters directly integrates the physiological impact of elevated concentrations of metal contaminants into the creation of water quality guidelines.

As such, one of the key elements to creating a useful BLM for a certain metal in a certain species is knowledge of the physiological mechanism of toxicity of that metal to the species in question. Over the past 15 years, this knowledge has been gained for a multitude of metals, including silver (Ag), copper (Cu), zinc (Zn), cadmium (Cd), aluminum (Al), lead (Pb), and nickel (Ni), in the most studied of freshwater fish, the rainbow trout, *Oncorhynchus mykiss* (see Wood, 2001 for a review).

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If the history of the BLM lies in freshwater toxicology, its future, to some extent, will include marine toxicology. To date, the most advanced freshwater BLMs, those for Ag and Cu, have been extensively developed, calibrated, and are being incorporated into current freshwater regulatory policy (Di Toro et al., 2001; Bury et al., 2002; De Schamphelaere and Janssen, 2002; De Schamphelaere et al., 2002; Paquin et al., 2002; Niyogi and Wood, 2004). Not surprisingly, mechanistic investigations of the modes of toxicity of these metals in marine species is underway (e.g. Wood et al., 2004; Grosell et al., 2004a,b; Bianchini et al., 2005) in anticipation of development of eventual marine BLMs for these two metals.

In freshwater, Ni^{2+} is typically considered the most relevant toxic species, comprising about 10–40% of total Ni at pH below about 8 (Pyle et al., 2002; WERF, 2002). In the freshwater rainbow trout the acute toxic mechanism of Ni is respiratory in nature, as Ni (presumably as Ni^{2+}) binds extensively to the freshwater trout gill, causing ultrastructural damage to the fish gill and subsequent impairment of respiratory gas exchange (Pane et al., 2003a, 2004a).

To our knowledge, the toxic mechanisms of nickel (Ni) have not been investigated in any marine species. At the higher pH of full seawater (~8), $NiCO_3$ becomes more prominent, though the toxicity of this species is unknown (Hoang et al., 2004). Additionally, in full seawater (SW), increased anionic complexation of Ni (USEPA, 1986; Sadiq, 1989), and cationic competition with Ni (Eisler, 1998), will reduce to some degree the availability of free Ni^{2+} ions to bind to the gill. The ionic differences between FW and SW notwithstanding, the critical importance of gill Ni accumulation (Meyer et al., 1999) and subsequent Ni-induced blockade of branchial gas exchange (Pane et al., 2003a, 2004a) should remain key mechanisms of acute Ni toxicity in marine fish and worthy subjects of investigation. This report, however, focuses solely on the ionic and osmotic implications of Ni exposure in the gulf toadfish.

Ni acts as an ionoregulatory toxicant in the freshwater invertebrate, *Daphnia magna* (Pane et al., 2003b), as well as in the freshwater rainbow trout under certain conditions (Pane et al., 2005). Accordingly, our first objective in the present report was to investigate the impact of Ni on the overall ionoregulatory health of a marine fish, the gulf toadfish (*Opsanus beta*). This was accomplished by exposing toadfish to Ni via two routes, waterborne exposure and arterial infusion, and measuring the ionic and osmotic balance of major body fluids. In conjunction, the potential effects of Ni exposure on the nitrogen balance of the facultatively ureotelic toadfish were assessed.

Our second objective was more specifically related to our previous findings that Ni-induced ionoregulatory disruption in certain aquatic species appears specific to magnesium (Mg) (Pane et al., 2003b, 2005). Marine fish face a unique dilemma with respect to Mg homeostasis, related to the regulation of plasma Mg^{2+} in these animals at concentrations typically 50-fold lower than the Mg^{2+} concentration of seawater (Beyenbach, 2004). This steep water to blood gradient is exacerbated across the intestinal epithelium by drinking of the seawater for osmoregulation (Smith, 1930), and preferential absorption of water over Mg^{2+}, resulting in a 3- or 4-fold concentration of Mg^{2+} in the intestinal fluids. A similar gradient must also be maintained across the renal epithelium as excess Mg^{2+} absorbed across the intestinal epithelium is secreted from the blood to the urine against a 150-fold gradient (Beyenbach, 2004).

Given the specific antagonistic interactions between Ni and Mg in a whole host of species (Adiga et al., 1962; Kaltwasser and Frings, 1980; Enyedi et al., 1982; Kasprzak and Poirier, 1985; Kasprzak et al., 1986; Brommundt and Kavaler, 1987; Miki et al., 1987; Sunderman, 1989; Costa, 1991; Costa et al., 1993; Ross, 1995; Smith et al., 1995; Pane et al., 2003b, 2005, 2006), and given that marine fish face the greatest uphill Mg^{2+} transport challenge known to date (Beyenbach, 2004), we were particularly interested to know whether Ni could specifically perturb Mg homeostasis in the marine gulf toadfish. Putative Ni-induced disruption of the powerful magnesium excretory processes of marine teleosts would result in hypermagnesia.

Befitting the first investigations into the mechanisms of Ni toxicity in a marine teleost, and the necessity of determining modes of toxicity for regulatory modeling purposes, the waterborne Ni concentrations employed in this study were diagnostic and therefore outside the range of environmental relevance. The lower concentration used (~200 μM) is similar to that used in our mechanistic studies with freshwater rainbow trout (Pane et al., 2003a, 2004a) and was chosen to facilitate comparison of freshwater and seawater modes of toxicity. The higher concentration (~600 μM) is near the freshwater LC_{50} value for adult rainbow trout (Segner et al., 1994), though below the range of LC_{50} values (1–2 mM) for adult marine fish typically highly tolerant of waterborne Ni exposure (Eisler, 1998). The value of using diagnostic metal concentrations as a first step to highlight metal-sensitive physiological processes for subsequent investigation of chronic, sublethal toxicity at metal concentrations within the range of environmental concern has been well documented both for Ni in freshwater aquatic organisms (Pane et al., 2003a,b, 2004a), and for the marine gulf toadfish exposed to Cu (Grosell et al., 2004a,b).

The ionoregulatory and osmoregulatory investigations reported in the current study, in combination with future investigations into putative acute Ni-induced respiratory toxicity, will provide a necessary introduction to the mechanisms of Ni toxicity in marine fish.

2. Materials and methods

2.1. Experimental animals

Gulf toadfish (*Opsanus beta*) weighing 50–200 g were caught by commercial shrimpers in Biscayne Bay, Florida. Toadfish were kept in running seawater in outdoor tanks at Jimbo's on Virginia Key for <24 h before transfer to the laboratory. Collection from Jimbo's was limited by availability, necessitating the use of fish with a 4-fold range of weight. Within experimental treatments, however, variation in any given parameter was independent of fish weight.

On the day of transfer, fish were immersed for several hours in Biscayne Bay seawater treated with malachite green (final concentration 0.05 mg L^{-1}) in formalin (15 mg L^{-1}) (McDonald et

al., 2003) to prevent infection by the ciliate, *Cryptocaryon irritans* (Stoskopf, 1993). Following treatment, fish were held in 45 L glass aquaria in well aerated, flowing Biscayne Bay seawater (salinity = 33–35, temperature = 24–25 °C) at a density of less than 1 kg per tank. An excess of short lengths of PVC tubes were provided for shelter. Fish were held for four or five days before experimentation, and were starved throughout the holding and experimental periods.

2.2. Waterborne Ni exposures

2.2.1. Exposure conditions

Following a holding period, fish were transferred randomly to one of three 45 L glass aquaria ($n = 9$ per aquarium) under conditions identical to those of the holding period. The relatively high loading density was offset by high-rate flow through conditions, an excess of shelter (in the form of PVC tubes) and the tolerance of the gulf toadfish to crowding (Walsh et al., 1994). After a 12 h settling period, two of the aquaria were spiked with a concentrated stock of $NiSO_4 \cdot 6H_2O$ to bring tanks up to nominal concentrations of 200 μM (measured = 215.3) or 600 μM (measured = 606.1) Ni. Immediately following, Ni was delivered by gravity flow from a concentrated stock to maintain two aquaria at their respective Ni concentrations under flow-through conditions (~1 L min^{-1}) for 72 h. The third aquarium was used as a nominally Ni-free control, as Ni concentrations in uncontaminated seawater are typically very low (5–15 nM; Eisler, 1998). Duplicate water samples were taken from each tank every 18 h and one set filtered through a 0.45 μm filter. [Ni] was measured by graphite furnace atomic absorption spectrophotometry (GFAAS; Varian 220Z) against certified atomic absorption standards (Fisher Scientific). As with freshwater Ni samples (Pane et al., 2003a), filtered and unfiltered seawater samples differed by less than 5% with no predictable pattern. Therefore, seawater concentrations reported in the following are for 0.45 μm filtered samples, and are considered dissolved Ni concentrations.

2.2.2. Blood and tissue sampling

At 24 and 48 h of exposure, fish from each tank were quickly netted, immediately sampled for blood (~30 μL) by caudal puncture in heparin-rinsed syringes, and returned to the tank. Plasma was separated by centrifugation (30 s at 2100 × *g*), and aliquoted for Ni analysis. Additionally, at 72 h, fish were euthanized by an overdose of MS222 (10 g L^{-1}), and a larger (~100 μL) terminal blood sample was drawn by caudal puncture.

Following the terminal blood sampling, gill, trunk kidney, white muscle (epaxial and posterior to dorsal fin), liver, heart, stomach, and intestine were excised for Ni analysis. Additionally, intestinal fluid was collected and the $CaCO_3$ precipitate (see Walsh et al., 1991; Wilson et al., 2002) separated from the intestinal fluid by centrifugation (20 min at 16,000 × *g*).

2.2.3. Analyses

The Ni concentration of plasma and other tissues was analyzed by graphite furnace atomic absorption spectrophotometry (GFAAS) as described above after digestion in 5–10 volumes of 1N HNO_3 (overnight at 70 °C) and appropriate dilution in 1% HNO_3. Plasma and intestinal fluid were analyzed for Na^+ and K^+ by flame atomic absorption spectrophotometry (FAAS; Varian 220FS) after dilution with 1% HNO_3, while Ca^{2+} and Mg^{2+} were analyzed by FAAS after dilution with 1% HNO_3 and 1% $LaCl_3$. Concentrations of Cl^- and SO_4^{2-} (intestinal fluid only) were analyzed by anion chromatography (Dionex DX120) after dilution with double distilled water using 0.5 mL sample vials in a AS40 autosampler, 25 μL injection volumes, AS14A analytical and AG14A guard columns. Data were collected on a PC using PeakNet 6.0 2000 DIONEX corporation software and compared to values obtained using a certified 7 anion standard (Lot 23-172AS). The osmolality of plasma and intestinal fluid was measured on 10 μL samples by vapor pressure osmometry (Vapro 5520; Wescor, Inc.; Logan, UT). Additionally, the total carbon dioxide concentration (C_{CO_2}) of intestinal fluids was measured with a Corning 965 total CO_2 analyzer. All plasma samples were sonicated on ice for 5 s at 5 W prior to dilution.

2.3. Ni infusion experiments

2.3.1. Surgery

Prior to surgery, toadfish were anaesthetized in MS222 (1 g L^{-1} in seawater), blotted dry, weighed to the nearest 0.1 g, and wrapped in paper towel soaked with anaesthetic. Indwelling arterial and ureteral catheters were implanted following identically the procedures described in McDonald et al. (2003). Briefly, arterial catheterization was accomplished by inserting a piece of Clay-Adams PE-50 tubing into the caudal artery exposed by surgical incision. A sleeve of PE160 was fitted over the catheter, glued into place, and then sutured into the body wall for support. The wound was treated with powdered oxytetracycline and sutured. The catheter was filled with sodium-heparinized (60 IU mL^{-1}) Hank's saline (Walsh, 1987), and sealed.

Ureteral catheterization was accomplished via a modification (McDonald et al., 2000, 2003) of a technique originally developed by Howe and Gutknecht (1978) and Lahlou et al. (1969). Briefly, a piece of PE 10 tubing was filled with distilled water and inserted through the urogenital papilla and into the urinary sinus. A sleeve of PE60 was fitted over the catheter, glued into place with Vetbond and then sutured to the body wall. The urinary bladders were then exposed by surgical incision, gently voided of urine, and ligated at the intersection with the ureter. The wound was dusted with oxytetracylcline and sutured.

Fish were then returned to individual, shielded, containers of 2–3 L of vigorously aerated seawater. During a 24 h recovery period, and throughout 96 h of infusion, urine was continuously collected by gravity into vials set 3 cm below the water line (Wood and Patrick, 1994). Cannulation of the urinary sinus, ligation of the urinary bladders, and constant siphoning result in collection of ureteral urine unmodified by the physiological processes of the bladder (Wood and Patrick, 1994).

2.3.2. Infusion protocol

Following the 24 h recovery period, all fish were infused with a solution of 150 mM NaCl for 24 h by connection of

arterial catheters to a peristaltic pump. After 24 h of saline infusion, fish were randomly assigned to two groups ($n = 8$ for each group) and infused for 72 h with individual solutions of either 150 mM NaCl (control fish), or 150 mM plus $NiCl_2$ added from a stock of 50 mM to give individual infusion rates of 0.40 μmol Ni kg^{-1} h^{-1}. The infusion rate was chosen to match that used with freshwater rainbow trout in a previous study (Pane et al., 2004a) to facilitate comparison of Ni handling between freshwater and marine fish. The rate of fluid infusion for both groups of fish ranged from 1.4 to 2.5 mL kg^{-1} h^{-1}, representing the lowest rates possible, given the size of the fish and the delivery rates of the peristaltic pump. Average weight gain over the infusion period was 7.7 ± 1.4%, and was not statistically different between control and Ni-infused fish.

Both groups of fish were held under static renewal conditions of vigorously aerated, Ni-free seawater with 95% replacement every 24 h. A static renewal protocol was chosen to facilitate analysis of excretion rates of various compounds during Ni infusion.

2.3.3. Seawater, blood, urine, and tissue sampling

To determine excretion rates of Ni, total ammonia (T_{amm}), and urea, seawater samples (5 mL) were taken at the beginning and end of each 24 h static renewal period.

Blood samples (~80 μL) were collected via the arterial catheter into a heparin-rinsed syringe at −12, 0, 24, 48, and 72 h of exposure. Plasma was separated by centrifugation and aliquoted as described above, and blood was replaced with an appropriate volume of Hank's saline. Urine was collected in preweighed vials over 12 h periods ending at 0, 24, 36, 48, 60, and 72 h of exposure. Vials were post-weighed to determine urine flow rate (UFR) and urine was frozen for later analysis of total ammonia, urea, Ni, and osmolality.

At 72 h of exposure, infusion was stopped, and fish were euthanized by an overdose of MS222. Fish were blotted dry and post-weighed to the nearest 0.1 g. Gill, kidney, white muscle, liver, heart, stomach, and intestine were all excised for determination of Ni concentration as described above. Additionally, intestinal fluid was collected as described above.

2.3.4. Analyses

Ni in SW was analyzed by GFAAS following a double extraction procedure. In brief, Ni was extracted from seawater using a solvent extraction procedure described in Kinrade and Van Loon (1974) with subsequent back extraction as described by Danielsson et al. (1978). Standards made from commercially available certified solutions in the appropriate medium were subjected to the solvent extraction procedure to assess extraction efficiency. Measured values were corrected according to extraction efficiency, which in all cases was >90%.

The T_{amm} and urea concentrations of seawater were determined by the salicylate/hypocholite method (Verdouw et al., 1978) and the diacetyl monoxime method (Rahmatullah and Boyde, 1980), respectively.

Plasma and intestinal fluid were analyzed for Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+}, Ni, and osmolality. Intestinal fluid was additionally analyzed for SO_4^{2-} and total CO_2. Ni concentrations of the gill, kidney, liver, heart, stomach, intestine, and urine were determined as described above. The osmolality of urine was measured on 10 μL samples by vapor pressure osmometry (see above), and the T_{amm} and urea concentrations of urine were determined as described above.

2.3.5. Excretion rates

Continuous sampling of urine via an indwelling ureteral catheter, in combination with periodic seawater sampling under static renewal conditions, allows for simultaneous determination of renal (or urinary) excretion rates and extrarenal excretion rates, respectively. The sum of the two is taken to be the excretion rate of the whole animal. Concentrations of Ni, T_{amm}, and urea in the urine were converted to urinary excretion rates (μmol kg^{-1} h^{-1}) according to the formula:

$$\text{Urinary excretion rate} = [X]_u\,(\text{UFR}) \quad (1)$$

where $[X]_u$ is the concentration of the relevant analyte in the urine collected over a 12 h period, and UFR is the urine flow rate over that same period. Extrarenal excretion rates (μmol kg^{-1} h^{-1}) of Ni, T_{amm}, and urea were calculated by the formula:

$$\text{Extrarenal excretion rate} = \frac{[X]_f - [X]_i}{WVt} \quad (2)$$

where $[X]_f$ and $[X]_i$ are the final and initial concentrations of analyte in the seawater, W the mass of the animal, V the volume of water in the chamber, and t is the time period over which the samples were taken.

2.4. Statistics

All measured values are presented as mean ± 1 standard error of the mean (S.E.M.; n = number of fish). At 72 h of waterborne exposure, the 606.1 μM Ni treatment yielded only five functional blood samples despite a starting n number of 9 and only one mortality during the 72 h exposure period. In three fish from this treatment, blood sampled by caudal puncture was badly lysed and was therefore not used for analysis of plasma ions.

In the infusion experiments, data appear for only four control fish and six Ni-infused fish (from a starting n of 8) that retained functional arterial and ureteral catheters across the whole infusion and exposure periods. There were no mortalities in the control group and only one in the Ni-infused group during the course of the experiment.

Data were tested for normality (Shapiro–Wilk test) and homogeneity of variance (Levene test) prior to statistical treatment. Data meeting these assumptions were analyzed for significant differences using either an unpaired two-tailed Student's t-test, or a one-way ANOVA followed by a two-sided Dunnett's post hoc multiple comparison test. Data not meeting parametric assumptions were compared using either a Mann–Whitney U-test, or a Kruskal–Wallis test followed by multiple comparison testing according to the method of Dunn (1964), as described in Zar (1984). Statistical significance in all cases was accepted at the $P < 0.05$ level.

3. Results

3.1. Waterborne Ni exposures

3.1.1. Plasma hydromineral balance

Ni accumulated in the plasma of toadfish exposed to waterborne Ni in a time- and concentration-dependent manner (Fig. 1). In both Ni treatments (215.3 and 606.1 μM Ni), plasma Ni accumulation was nearly linear with time from 24 h onward. This trend was particularly evident in the lower treatment, where plasma Ni concentration was very well regulated for the first 24 h of exposure. At 72 h of exposure, the plasma Ni concentration of fish exposed to the higher waterborne Ni concentration was about 2.3-fold greater than that of fish exposed to the lower Ni concentration. This ratio of plasma Ni concentrations was similar to the ratio of the two exposure concentrations (606.1:215.3 or 2.8:1).

In toadfish exposed to both waterborne Ni concentrations for 72 h, plasma Ca^{2+} dropped by approximately 50% from control concentrations (Table 1). No other plasma ion measured, nor the osmolality of the plasma, was significantly perturbed by waterborne Ni exposure (Table 1).

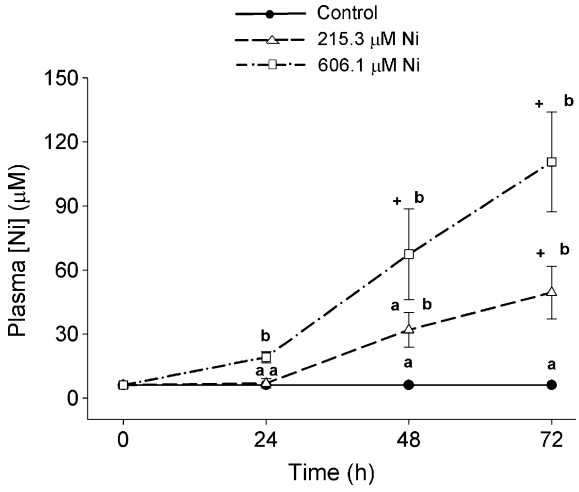

Fig. 1. Time course of plasma Ni accumulation over 72 h of exposure to three concentrations of waterborne Ni, control (nominally zero), 215.3, and 606.1 μM. Data are presented as mean ± 1 S.E.M. (n = 5–8). Within treatments, symbol "+" indicates a mean significantly different from the time 0 value. Among treatments, simultaneous means not sharing the same letter are significantly different.

Table 1
Plasma ion concentrations and osmolality following 72 h of exposure to three concentrations of waterborne Ni, control (nominally zero), 215.3, and 606.1 μM

	Control	215.3 μM	606.1 μM
Na^+ (mM)	152.69 ± 8.32	143.91 ± 1.65	139.47 ± 2.36
Cl^- (mM)	144.12 ± 7.37	142.00 ± 2.68	145.04 ± 2.21
K^+ (mM)	4.38 ± 0.33	4.24 ± 0.48	4.08 ± 0.16
Ca^{2+} (mM)	1.95 ± 0.24[a]	0.90 ± 0.06[b]	0.84 ± 0.08[b]
Mg^{2+} (mM)	1.38 ± 0.25	0.84 ± 0.07	1.18 ± 0.26
Osmolality (mOsm)	342.9 ± 12.9	332.5 ± 3.9	343.5 ± 8.1

Data are presented as mean ± 1 S.E.M. (n = 5–9). Among treatments, means not sharing the same letter are significantly different.

Table 2
Intestinal fluid ion concentrations, osmolality, and total CO_2 concentration following 72 h of exposure to three concentrations of waterborne Ni, control (nominally zero), 215.3, and 606.1 μM

	Control	215.3 μM	606.1 μM
Na^+ (mM)	21.5 ± 2.2[a]	29.7 ± 2.9[a,b]	42.4 ± 5.7[b]
Cl^- (mM)	85.1 ± 8.7[a]	118.3 ± 7.4[b]	116.5 ± 9.1[b]
SO_4^{2-} (mM)	89.9 ± 6.8[a]	148.2 ± 15.4[b]	203.7 ± 23.7[b]
K^+ (mM)	1.19 ± 0.22[a]	3.04 ± 0.66[b]	2.76 ± 0.51[b]
Ca^{2+} (mM)	6.06 ± 1.10	5.34 ± 1.43	6.06 ± 0.75
Mg^{2+} (mM)	176.2 ± 9.9[a,b]	146.6 ± 4.8[a]	224.6 ± 24.6[b]
Ni (μM)	2.9 ± 0.3[a]	894.9 ± 153.1[b]	3694.4 ± 1315.2[b]
Osmolality (mOsm)	349.9 ± 10.9	367.4 ± 9.6	394.9 ± 23.9
Total CO_2 (mM)	82.3 ± 2.6	74.5 ± 6.2	103.1 ± 10.4

Data are presented as mean ± 1 S.E.M. (n = 7–10). Among treatments, means not sharing the same letter are significantly different.

3.1.2. Intestinal fluid hydromineral balance

Waterborne Ni exposure had a marked impact on the ionic composition of the toadfish intestinal fluid sampled at 72 h of exposure (Table 2). Na^+, Cl^-, K^+, and SO_4^{2-} concentrations were all significantly elevated by Ni exposure. The Ca^{2+} concentration of the intestinal fluid was well conserved in both treatments, and neither the osmolality nor the total CO_2 concentration of intestinal fluid was significantly impacted by waterborne Ni exposure (Table 2).

The Ni concentration of intestinal fluid increased markedly with both waterborne treatments (Table 2). The final concentrations of Ni in the intestinal fluid of toadfish exposed to the two concentrations of waterborne Ni were approximately 4–6-fold higher than the respective water exposure concentrations. In the higher exposure of 606.1 μM, the resultant Ni concentration of the intestinal fluid was almost 4000 μM, or three orders of magnitude higher than the typical Ni concentration found in the intestinal fluid and plasma of naïve fish.

3.1.3. Tissue Ni accumulation

In addition to concentration-dependent plasma accumulation, Ni accumulation was also concentration-dependent in the urine, and in tissues such as gill, liver, heart, stomach, and intestine (Fig. 2). The kidney accumulated an almost identical amount of Ni in the two waterborne treatments. The ratio of tissue Ni accumulation between fish exposed to the two waterborne concentrations was similar in the gill (2.6:1), stomach (3.1:1), and intestine (2.4:1) to that of the water (2.8:1) and plasma (2.3:1) (see above). Additionally, intestinal Ni accumulation in the two waterborne exposures matched very closely the water Ni concentrations (Fig. 2). White muscle did not significantly accumulate Ni at either exposure concentration.

3.2. Ni infusion experiments

3.2.1. Plasma hydromineral balance

Plasma ion balance in the gulf toadfish was tracked for 12 h of arterial infusion of a 150 mM NaCl solution, followed by either 72 h of continued infusion of 150 mM NaCl (control),

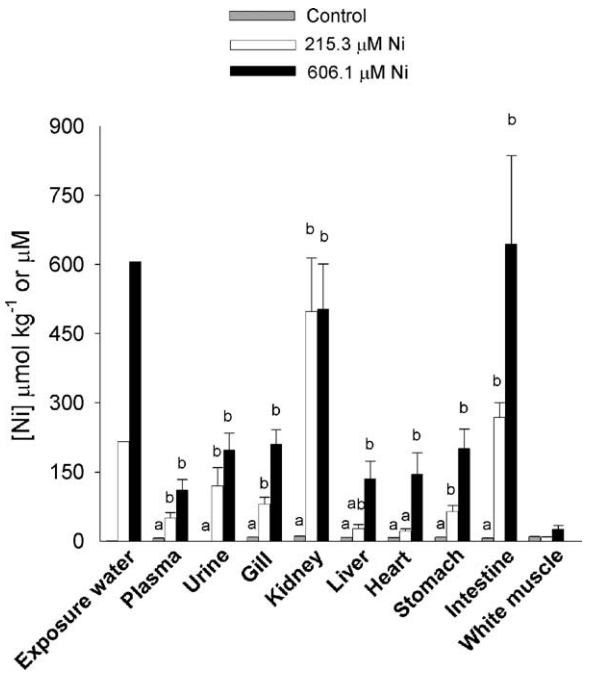

Fig. 2. Tissue Ni burdens following 72 h of exposure to three concentrations of waterborne Ni, control (nominally zero), 215.3, and 606.1 μM. Data are presented as mean ± 1 S.E.M. (n = 7–8). For each tissue, bars not sharing the same letter are significantly different.

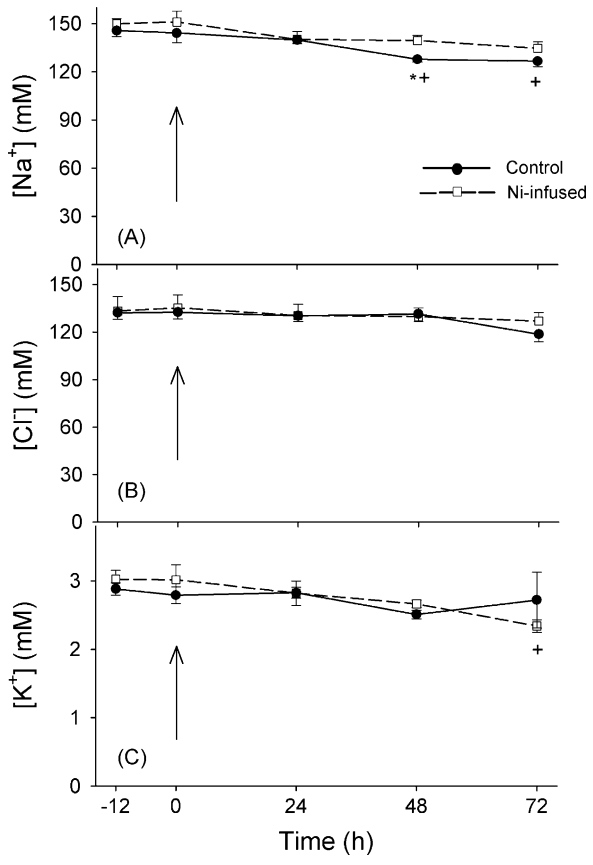

Fig. 3. Plasma ions measured over 72 h of infusion of either 150 mM NaCl (control), or 0.40 μmol Ni kg^{-1} h^{-1} delivered in a 150 mM NaCl solution (Ni-infused). Both groups of fish were control infused (150 mM NaCl) from −12 to 0 h. Arrow represents start of differential (control or Ni) infusion. Data are presented as mean ± 1 S.E.M. (n = 4–6). (A) Na^+; (B) Cl^-; (C) K^+. Within treatments, symbol "+" indicates a mean significantly different from the time 0 value. Between treatments, symbol "*" indicates simultaneous means that are significantly different.

or 72 h of infusion of 0.40 μmol Ni kg^{-1} h^{-1} delivered in a solution of 150 mM NaCl (Ni-infused). While unchanged in Ni-infused fish, the plasma Na^+ concentration of control fish decreased in a time-dependent manner, being significantly reduced from 48 h onward (Fig. 3A). As no other measured ion was impacted by control infusion (Figs. 3 and 4), the statistically significant time-dependent decrease in the plasma (Fig. 5), urine (Fig. 6B), and intestinal fluid (Table 3) osmolality of control-infused fish was presumably driven by reduced plasma [Na^+]. Plasma Na^+ loss in control fish over the course of infusion was approximately 12% (Fig. 3A), closely matching the 13% decrease in plasma osmolality in these fish (Fig. 5).

Table 3
Intestinal fluid ion concentrations, osmolality, and total CO_2 concentration following 72 h of infusion of either 150 mM NaCl (control), or 0.40 μmol Ni kg^{-1} h^{-1} delivered in a 150 mM NaCl solution (Ni-infused)

	Control	Ni-infused
Na^+ (mM)	36.4 ± 9.2	106.6 ± 15.8[a]
Cl^- (mM)	65.7 ± 4.8	81.0 ± 5.3
SO_4^{2-} (mM)	101.7 ± 22.2	64.1 ± 17.8
K^+ (mM)	2.49 ± 0.83	3.48 ± 0.50
Ca^{2+} (mM)	1.67 ± 0.57	1.02 ± 0.28
Mg^{2+} (mM)	117.6 ± 14.2	40.6 ± 18.5[a]
Ni (μM)	2.90 ± 0.27	3.04 ± 0.66
Osmolality (mOsm)	294.7 ± 7.0	330.0 ± 18.2[a]
Total CO_2 (mM)	65.0 ± 5.8	76.5 ± 5.4

Data are presented as mean ± 1 S.E.M. (n = 4–6).
[a] Significant difference between treatments.

Ionic and osmotic balance of the plasma of Ni-infused toadfish was unchanged over 72 h of Ni-infusion (Figs. 3–5), save for a slight time-dependent decrease in plasma K^+ at 72 h of infusion (Fig. 3C).

3.2.2. Urinary hydromineral balance

There were no significant differences in urine flow rate (UFR) either between infusion treatments, or across time within a specific treatment (Fig. 6A). Typically, UFR was variable (Hickman and Trump, 1969; Cameron, 1980), decreasing over time from a rate (0.7–0.9 mL kg^{-1} h^{-1}) indicative of stress-induced diuresis (Evans, 1967), to a 72 h rate (~0.3 mL kg^{-1} h^{-1}) more consistent with the balance between the limited urine flow of an aglomerular, seawater-adapted teleost, and the slight volume loading associated with the infusion protocol (Fig. 6A).

3.2.3. Intestinal fluid hydromineral balance

Seventy-two hours of Ni infusion resulted in an increased Na^+ concentration and a decreased Mg^{2+} concentration of intestinal fluids (Table 3). The approximately 70 mM increase in the Na^+ concentration of intestinal fluid of Ni-infused toadfish was

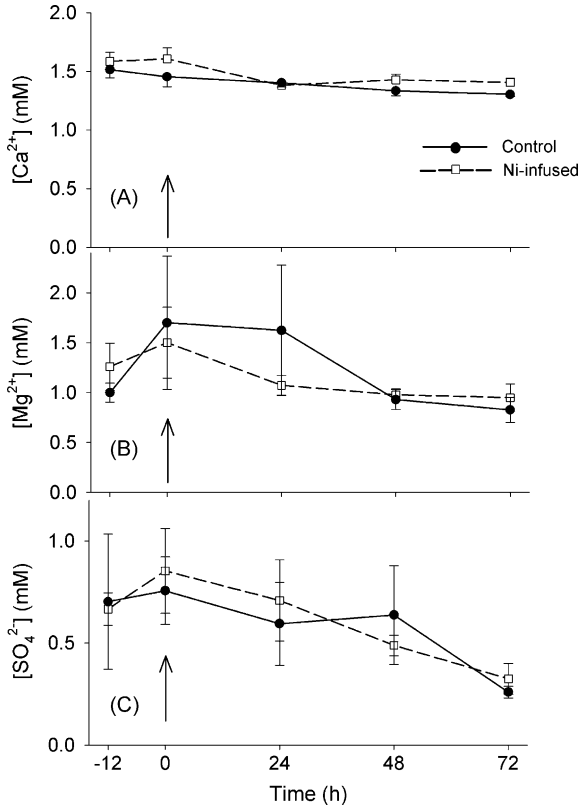

Fig. 4. Plasma ions measured over 72 h of infusion of either 150 mM NaCl (control), or 0.40 μmol Ni kg^{-1} h^{-1} delivered in a 150 mM NaCl solution (Ni-infused). Both groups of fish were control infused (150 mM NaCl) from −12 to 0 h. Arrow represents start of differential (control or Ni) infusion. Data are presented as mean ± 1 S.E.M. (n = 4–6). (A) Ca^{2+}; (B) Mg^{2+}; (C) $SO_4{}^{2-}$.

closely matched by a 77 mM decrease in Mg^{2+} concentration. That these Ni-induced effects on intestinal fluid are not a function of overall ionic perturbance is evidenced by the lack of significant change in the intestinal fluid concentration of Cl^-, $SO_4{}^{2-}$, K^+, and Ca^{2+} (Table 3). Additionally, the total CO_2 concentration was unaffected by Ni infusion. Intestinal fluid osmolality, however, was significantly increased by Ni infusion (Table 3).

During infusion, nickel was not secreted from the blood into the intestinal fluid, as the Ni concentration of intestinal fluids was essentially identical between the two infusion treatments (Table 3; Fig. 7).

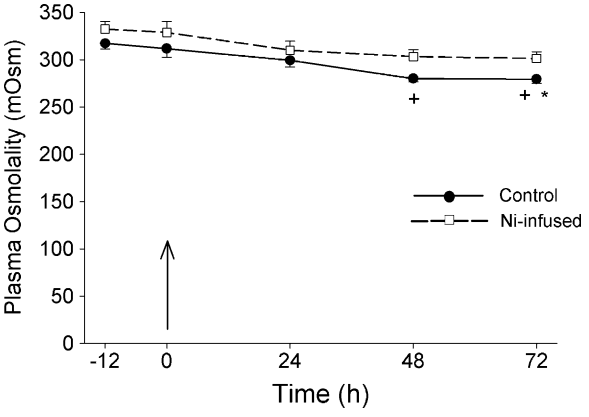

Fig. 5. Plasma osmolality measured over 72 h of infusion of either 150 mM NaCl (control), or 0.40 μmol Ni kg^{-1} h^{-1} delivered in a 150 mM NaCl solution (Ni-infused). Both groups of fish were control infused (150 mM NaCl) from −12 to 0 h. Arrow represents start of differential (control or Ni) infusion. Data are presented as mean ± 1 S.E.M. (n = 4–6). Within treatments, symbol "+" indicates a mean significantly different from the time 0 value. Between treatments, symbol "*" indicates simultaneous means that are significantly different.

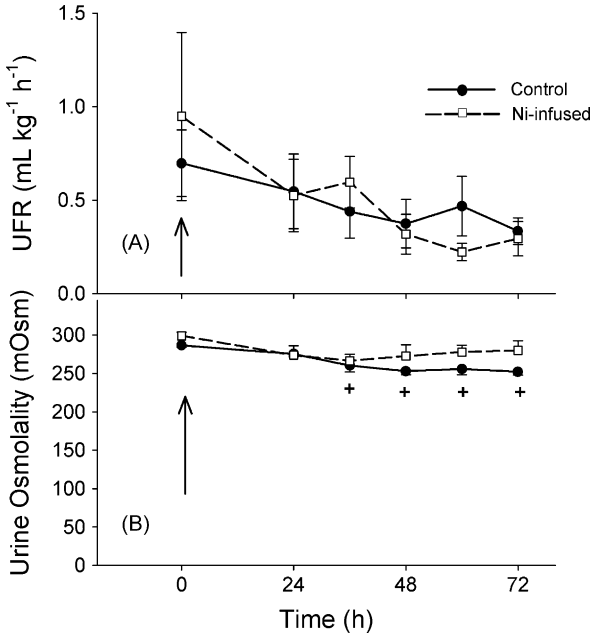

Fig. 6. Urine flow rate (UFR) and osmolaltiy of urine continuously collected over 72 h of infusion of either 150 mM NaCl (control), or 0.40 μmol Ni kg^{-1} h^{-1} delivered in a 150 mM NaCl solution (Ni-infused). Both groups of fish were control infused (150 mM NaCl) from −12 to 0 h. Arrow represents start of differential (control or Ni) infusion. Data are presented as mean ± 1 S.E.M. (n = 4–6). Urine was collected over 12 h periods ending at the time points indicated. (A) UFR; (B) urine osmolaltiy. Within treatments, symbol "+" indicates a mean significantly different from the time 0 value.

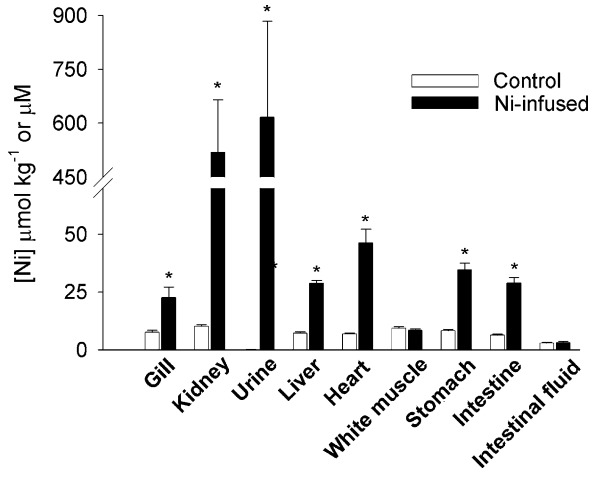

Fig. 7. Tissue Ni burdens following 72 h of infusion of either 150 mM NaCl (control), or 0.40 μmol Ni kg^{-1} h^{-1} delivered in a 150 mM NaCl solution (Ni-infused). Data are presented as mean ± 1 S.E.M. (n = 6–8). Symbol "*" indicates significant difference between treatments. Note the break in the Y-axis.

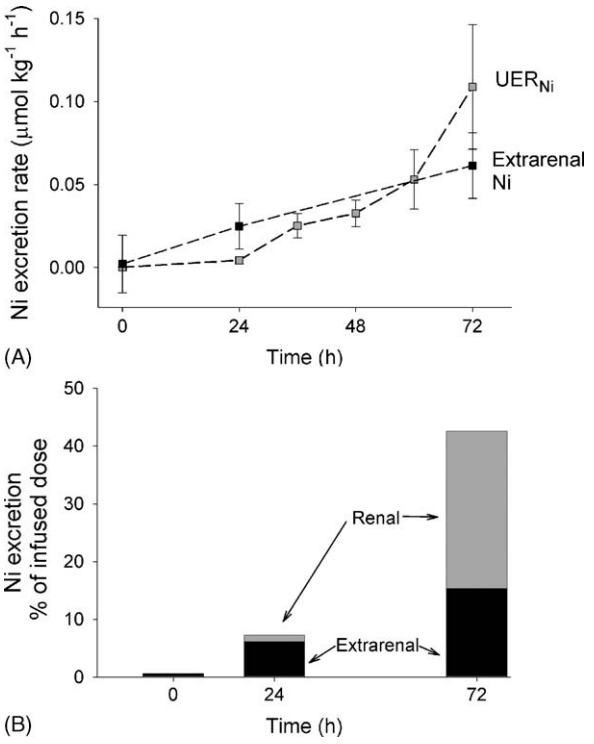

Fig. 8. Ni excretion in gulf toadfish infused for 72 h with either 150 mM NaCl (control), or 0.40 μmol Ni kg^{-1} h^{-1} delivered in a 150 mM NaCl solution (Ni-infused). (A) Renal and extrarenal excretion rates. Data are presented as mean ± 1 S.E.M. (n = 4–6). (B) Excretion rates expressed as percentages of infused dose.

3.2.4. Tissue Ni accumulation and Ni excretion

Ni infusion resulted in significantly elevated terminal Ni burdens in the gill, kidney, liver, heart, stomach, and intestine (Fig. 7). With the exception of the kidney, these increases in Ni burden were moderate, relative to the accumulation of Ni during waterborne exposure to 606.1 μM. Tissue burdens following 72 h of Ni infusion ranged from only 4% (intestine) to a maximum of 33% (heart) of tissue burdens following 72 h of waterborne exposure to 606.1 μM (Fig. 7; compare Fig. 2).

Remarkably, though, the kidney accumulated a similar amount of Ni across the three Ni treatments including the two waterborne exposures and an infusion treatment (Fig. 7; compare Fig. 2). Kidney Ni burden was approximately 500 μmol kg^{-1}, varying by less than 4% across the three quite distinct treatments.

Fig. 8 plots the sum excretion of Ni (combination of urinary and extrarenal excretion) both in absolute values (Fig. 8A) and as a percentage of the infused Ni dose (Fig. 8B). At 24 h of Ni infusion, extrarenal Ni excretion dominated total Ni excretion, while at 72 h, urinary excretion of Ni accounted for the majority of excreted Ni.

3.2.5. Ni and nitrogen excretion

Arterial infusion of Ni had no significant effect on extrarenal nitrogen excretion, as extrarenal excretion rates of ammonia and urea were not significantly different between the two infusion treatments (data not shown). Similarly, urinary excretion rates (UER) of ammonia and urea were not significantly different between treatments (data not shown).

4. Discussion

4.1. Waterborne Ni exposures

4.1.1. Plasma hydromineral balance

The nearly linear time-dependent increase in the plasma Ni concentration of gulf toadfish during waterborne exposure (Fig. 1) is similar to that seen in the freshwater rainbow trout and apparently unique to Ni (Pane et al., 2003a, 2004a). The primary difference, however, between the plasma Ni profiles in the freshwater and marine species is the rate of Ni accumulation over the first 24 h. Freshwater rainbow trout accumulate Ni in the plasma at a very constant rate over the course of a 96 h exposure (Pane et al., 2003a, 2004a), whereas the marine gulf toadfish was more resistant to Ni accumulation in the first 24 h. This was particularly evident in the lower Ni treatment of 215.3 μM, where Ni was actually excluded from the plasma for the first 24 h (Fig. 1).

This difference may reflect the increased anionic complexation of Ni in seawater versus freshwater (USEPA, 1986; Sadiq, 1989), or the greatly increased seawater concentrations of cations such as Ca^{2+} or Mg^{2+} competing for uptake pathways in marine fish (Eisler, 1998). Both processes would theoretically limit the amount of free Ni ion taken up by transporting epithelia of marine teleosts. This point is supported by the fact that after 72 h of exposure to 215.3 μM Ni in seawater, the plasma Ni concentration in the gulf toadfish was only half of that observed in the freshwater rainbow trout exposed for 72 h to a very similar Ni concentration (Fig. 1; cf. Pane et al., 2003a, 2004a). In the gulf toadfish, the relative resistance to plasma Ni accumulation for the first 24 h of waterborne exposure suggests an ability to curb excessive accumulation either via a reduced influx pathway, or a stimulated efflux capacity.

The significant decrease in the plasma concentration of only Ca^{2+} following 72 h of waterborne Ni exposure (Table 1) is surprising for several reasons. Firstly, after 72 of Ni exposure, Ca^{2+} was the only ion in the intestinal fluid not significantly altered (Table 2). Additionally, we have previously found no evidence of calcium antagonism by Ni in freshwater exposure scenarios involving different water hardness, exposure concentrations, exposure times, and species (Pane et al., 2003a,b, 2004b, 2005). It should be noted, though, that Ni accumulation in the intestinal fluid of the gulf toadfish during exposure to the highest waterborne Ni concentration reached almost 4 mM (Table 2). This concentration is an order of magnitude higher than the Ni concentration of any fluid we have measured in the rainbow trout, and easily enters the range of normally supraphysiological concentrations at which Ni blocks voltage-gated calcium channels (typically 1–2 mM; Pane et al., 2003a). While the conservation of intestinal fluid calcium levels during both waterborne Ni exposures suggests that 4 mM Ni in the intestinal fluid had no direct negative impact on intestinal fluid calcium balance, the possibility remains that Ni may have accumulated to similar, typically calcium-antagonistic concentrations in other tissues of the gulf toadfish, with direct bearing on the observed decrease in plasma Ca^{2+} concentration.

4.1.2. Intestinal fluid hydromineral balance

The ionic imbalance in the intestinal fluid of the gulf toadfish following 72 h of waterborne Ni exposure was extensive and not specific to any one particular ion (Table 2). A possible partial explanation for this phenomenon is not a direct result of specific Ni-induced alterations to intestinal ion transport, rather a secondary effect of a profound Ni-induced increase in drinking rate. The intestine of the marine teleost is charged with the task of water absorption to replace the osmotically driven loss of body water to the strongly hypertonic seawater medium (Smith, 1930). To accomplish this task, marine fish drink the seawater medium, absorbing water, and salts. Monovalent ions such as Na^+ and Cl^- are typically excreted at the gill (Shehadeh and Gordon, 1969; Skadhauge, 1974), while divalent cations such as Mg^{2+} and SO_4^{2-} are typically excreted by the kidney (Hickman, 1968; Marshall and Grosell, 2005).

An increased drinking rate in the gulf toadfish, as observed during exposure to waterborne Cu (Grosell et al., 2004b), would bring an increased load of water and ions to the intestinal epithelium. The data of Table 2 are consistent with the hypothesis of increased drinking in combination with moderate increases in the absorption of excess monovalent and divalent ions.

In contrast to the intestinal epithelium of higher vertebrates, the epithelium of marine teleosts absorbs a fluid (~650 mOsm) that is highly hypertonic to the blood plasma (Grosell et al., 2005; Grosell and Genz, 2006; Grosell, 2006). This normally results in strongly reduced NaCl concentrations in intestinal fluids and highly elevated $MgSO_4$ concentrations. The general increase in ionic concentrations of the intestinal fluids from Ni-exposed fish likely represents a combination of increased salt intake (drinking) and a reduction in the osmolality of the fluids absorbed by the intestine. Continued absorption of water would explain elevated $MgSO_4$ concentrations, but NaCl must be elevated as a result of reduced NaCl concentrations in the fluids absorbed by the intestine of Ni-exposed fish. It should be noted that intestinal water absorption in marine teleosts occurs from the intestinal fluid to the plasma, both of which have osmotic pressures of approximately 320–330 mOsm. The strongly hypertonic absorbate thus easily explains how water absorption can occur despite a lack of net osmotic pressure change between the intestinal fluid and the plasma.

Building on the data of Table 2, further in vivo or in vitro experiments would be of great interest detailing the specific effects of intestinal fluid Ni loading on the absorptive capacity of the gulf toadfish intestine. Different regions of the intestine, including the rectum, handle water, and salts differently and respond to another metal (Cu) differently (Grosell et al., 2004a). Accordingly, the Ni-induced perturbation of intestinal hydromineral balance, as observed in Table 2 for fluid collected from the entire intestinal tract, may be specific to one or more regions of the intestine or could represent the sum of differential effects along the gastrointestinal tract. Additionally, a detailed time course of drinking rate during waterborne Ni exposure would shed light on the possible causes of intestinal fluid ion imbalance.

The lack of a statistically significant change in the osmolality of intestinal fluids, despite the increase in the concentration of most ions measured (Table 2), suggests that the osmotic coefficient is lower in intestinal fluids of Ni-exposed fish compared to control fish. It is worth noting that $MgSO_4$ displays a much lower osmotic coefficient than NaCl (Taylor and Grosell, submitted for publication). The compensatory response in marine teleosts to control the osmotic pressure of the intestinal fluid is typically accomplished by secretion of bicarbonate ions into the intestinal lumen to precipitate accumulated Ca^{2+} ions (see Wilson et al., 2002 for a review of this process). The unchanged Ca^{2+} concentration of the intestinal fluid following waterborne Ni exposure, where all other electrolytes are increased (Table 2) supports this idea.

4.1.3. Tissue Ni accumulation

The higher waterborne Ni concentration of 606.1 μM was about 2.8-fold higher than the lower exposure concentration of 215.3 μM. It is interesting that plasma, gill, stomach, and intestine exhibited a very similar pattern of Ni accumulation resulting in ratios of Ni accumulation between about 2.3 and 3.1 to 1 when comparing accumulation at the higher waterborne concentration to accumulation at the lower concentration (Fig. 2). The gill, stomach, and intestine are the tissues in most intimate contact with the seawater medium, suggesting that Ni accumulation in these epithelial tissues of the gulf toadfish is a relatively unregulated process. A pattern of unregulated accumulation of Ni has been reported in both FW and SW invertebrates where whole-body Ni burdens increase proportionally with water Ni concentrations (Hardy and Roesijadi, 1982; Darmono, 1990; Keithly et al., 2004).

Ni moving through the epithelia of the gill, stomach, and intestine enters the plasma, another compartment exhibiting a similar ratio of Ni accumulation. The tight coupling of Ni burden in these tissues with water Ni concentration will benefit the development of a marine BLM, in that short-term (i.e. 96 h) Ni accumulation in such tissues, frequently the targets of metal toxicity, would be predictably related to the water Ni concentration.

In contrast, the kidney accumulated a remarkably consistent amount of Ni following both waterborne treatments and the Ni infusion treatment (Fig. 2; cf. Fig. 7). This consistency of renal Ni burden is even more remarkable given a greater than 5-fold difference in urine Ni concentrations among the three treatments (Figs. 2 and 7). Further studies are necessary to determine how and why this similar renal burden is a consistently regulated level in the face of very different Ni challenges.

4.2. Ni infusion experiments

4.2.1. Hydromineral balance

The effects of Ni infusion on the hydromineral balance of the gulf toadfish were slightly complicated by the unavoidable volume loading associated with the infusion protocol in both control and treated fish. Weight gain for both groups of fish over the infusion period was about 8%. In the control group, however, this volume loading was associated with significant time-dependent reductions in plasma Na^+ concentration (Fig. 3A) as

well as the osmolality of the plasma (Fig. 5), intestinal fluid (Table 3), and urine (Fig. 6B). The time-dependent loss of Na^+ closely paralleled the losses in fluid osmolality, suggesting that plasma osmotic losses in saline-infused control fish, and possibly osmotic losses in intestinal fluid and urine also, were driven by plasma sodium loss. Such a compensatory response, perhaps in combination with a reduction in drinking rate, would be expected to offset the volume loading associated with infusion.

Despite similar trends, however, time-dependent sodium and osmotic losses in Ni-infused toadfish were not statistically significant. These data suggest that Ni interfered with a putative natriuretic response observed in toadfish infused with a Ni-free 150 mM NaCl solution. At the level of the intestine, any possible effect of luminal Ni interfering with a mechanism for sodium loss can be ruled out, as Ni infusion, unlike waterborne exposure (Table 2), did not cause any Ni loading of the intestinal fluid (Table 3). Additionally, intestinal accumulation of Ni following infusion was only moderate (~4-fold; Fig. 7), as compared to the striking 100-fold increase in intestinal Ni burden following waterborne Ni exposure (Fig. 2).

Notwithstanding the lack of effect on the osmotic and ionic balance of toadfish plasma (Figs. 3–5), Ni infusion had a marked effect on the specific intestinal transport of Na^+ and Mg^{2+} (Table 3). Na^+ concentrations in the intestinal fluid increased markedly and in concert with reduced Mg^{2+} concentrations. In the absence of luminal Ni, this effect was achieved either serosally or cellularly. The effect was also specific to only these two ions and did not extend systemically, as plasma concentrations of Na^+ (Fig. 3A) and Mg^{2+} (Fig. 4B) were not significantly altered by Ni infusion.

An ATP-dependent Na^+/Mg^{2+} antiport, using inwardly directed Na^+ gradients to drive Mg^{2+} extrusion from cells, has been described in many systems, including squid axons (DiPolo and Beauge, 1988) and human erythrocytes (Feray and Garay, 1986). In freshwater fish, such a putative system would occur in the enterocytic basolateral membrane (e.g. Flik et al., 1993), finalizing transcellular mucosal to serosal uptake of dietary Mg^{2+}. In marine fish, the constant battle against hypermagnesia requires transport of Mg^{2+} in the opposite direction, against a 100-fold serosal to mucosal gradient. Intracellular Mg^{2+} concentrations kept well below equilibrium concentrations (Flik et al., 1993), coupled with mucosally directed transepithelial Mg^{2+} transport, dictate that Mg^{2+} be actively pumped across the apical membrane of the enterocyte of marine fish. The mechanisms underlying this process are poorly understood, and the role of Na^+ in mucosally driven Mg^{2+} transport in unclear, although it is interesting to note that the intestinal fluid data of Table 3 suggest an inhibition of a putative Na/Mg exchange process. This is an important area for future investigation by both in vivo and in vitro approaches (e.g. Wilson et al., 2002).

Regardless of the mechanisms responsible, plasma hypermagnesia did not occur despite the sharply reduced Mg^{2+} concentration of the intestinal fluid. Though not measured in the present study, the Mg^{2+} concentration of the urine may be increased in Ni-infused toadfish to augment urinary Mg^{2+} excretion, the predominant excretory route in marine teleosts (Hickman, 1968; Marshall and Grosell, 2005).

Specific effects of both serosal and mucosal Ni on intestinal transport pathways of Mg^{2+} (and other ions) in marine teleosts would be well investigated by in vitro techniques such as gut sac preparations (e.g. Grosell et al., 2005), Ussing chamber experiments (Grosell and Genz, in press), or in situ intestinal perfusions (e.g. Grosell et al., 1999).

4.2.2. Tissue Ni accumulation

The relatively low Ni burdens in all tissues except the kidney, following Ni infusion (Fig. 7), suggest, as was observed during Ni infusion in the freshwater rainbow trout (Pane et al., 2004a), that Ni loading of toadfish tissues from the plasma is far less efficient than via the exposure water. In the case of the freshwater rainbow trout, trapped plasma analysis determined that Ni accumulated in tissues during infusion included a substantial percentage of bloodborne Ni perfusing vascularized tissues such as the heart, stomach, and intestine (Pane et al., 2004a). Under the conditions in the present experiment, we would expect similar results for the gulf toadfish infused with Ni.

4.2.3. Ni excretion

Under control conditions, the freshwater trout kidney reabsorbs about 98% of the Ni filtered by the glomeruli (Pane et al., 2005). Even under conditions of extensive Ni loading, both acutely and chronically, renal reabsorption of Ni dominates over renal secretion in the freshwater rainbow trout, making renal excretion a relatively poor means of offloading accumulated Ni in this species (Pane et al., 2005). Under such conditions, it is hypothesized that Ni is inappropriately reabsorbed via highly efficient renal reabsorptive Mg^{2+} transport pathways designed to prevent hypomagnesia in freshwaters containing very low concentrations of Mg^{2+}.

The gulf toadfish, on the other hand, appears to be fairly proficient at Ni excretion, particularly renal Ni excretion. By 72 h of Ni infusion, over 40% of the infused dose was excreted by the toadfish, with almost 30% of the excreted load accomplished renally (Fig. 8b). This efficient Ni excretion observed in the gulf toadfish is consistent with our hypothesis (Pane et al., 2006) that a portion of Ni movement across the renal epithelium is via Mg^{2+} transport pathways. In marine teleosts, these Mg^{2+} transport routes comprise a powerful renal excretory system (Beyenbach, 2004).

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