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The role of the kidney in compensating the alkaline tide, electrolyte load, and fluid balance disturbance associated with feeding in the freshwater rainbow trout, *Oncorhynchus mykiss*

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ABSTRACT

The effect in freshwater rainbow trout of digesting a commercial pellet meal on the renal handling of water, ions and acid–base equivalents was investigated through urine collection over a 48 h period following meal ingestion. The glomerular filtration rate (GFR) and urine flow rate (UFR) were reduced in fed fish between 12 and 24 h following the meal, likely reflecting a loss of endogenous water across the gastric epithelium as a result of ingesting dry, ion-rich food pellets. The kidney was also responsible for the excretion of some excess dietary Ca^{2+}, and, to a much lesser extent, Na^{+} and Cl^{-}, while the urinary excretion of K^{+} was unaffected. The most dramatic effect of feeding was the elevation of renal Mg^{2+} excretion, with the kidney transitioning from net Mg^{2+} reabsorption to net Mg^{2+} secretion during digestion. The renal handling of dietary ions accounted for 3–27% of the total ions absorbed from the diet, indicating that a majority of the ions are excreted extra-renally or incorporated into growth. However this does highlight the underestimation of renal ion handling when using unfed fish models. The metabolic alkalosis created by digestion (the alkaline tide) resulted in an increase in urine pH as well as a transition from net acidic equivalent excretion in the urine to net basic equivalent excretion. This was due to a decrease in the titratable acidity minus bicarbonate component of urine as well as a decrease in ammonia secretion. Additionally, the experimental separation of the urinary component of acid–base excretion from that of the gills highlighted the substantially larger contribution of the latter. During the alkaline tide, renal excretion accounted for ~5% of the total basic equivalent excretion to the external water.

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1. Introduction

Numerous physiological studies have shown that unfed freshwater fish are hyperosmotic to their environment and face excessive water gain and ion loss. To compensate, freshwater teleosts exhibit active branchial ion uptake and produce copious volumes of very dilute urine (reviewed by Marshall and Grosell, 2006; Evans et al., 2005; Evans, 2008). Additionally, acid–base balance is maintained through regulated branchial and renal excretion of basic or acidic equivalents to the external environment. However, digestion may affect a myriad of physiological processes and the subsequent effects on these overall homeostatic mechanisms are not well known. Recently, the alkaline tide, a metabolic alkalosis generated by the mechanisms associated with gastric HCl secretion (reviewed by Niv and Fraser, 2002) has been shown to accompany the digestion of a meal in the freshwater rainbow trout (Bucking and Wood, 2008; Cooper and Wilson, 2008). The diet can also act as a source of water and ions. Excess water could potentially be counter-productive in freshwater fish, while electrolytes could be beneficial. In fact, dietary uptake of ions has been found to be especially important in maintaining internal electrolyte homeostasis when branchial ion transport processes are inhibited (Bucking and Wood, 2007; D'Cruz et al., 1998).

During digestion, freshwater fish have several potential compensatory mechanisms to prevent disturbances in ion and water balance, as well as to relieve the alkaline tide. The plasma composition is relatively well maintained during digestion despite the absorption of large ion loads (Bucking and Wood, 2006a,b). The gills appear to be of great importance. By altering branchial transport rates of ions when faced with ion loads (Na^{+}; Smith et al., 1995), fish may avoid large plasma ion disturbances (Bucking and Wood, 2006b). Is the kidney also involved? In unfed fish, renal ion excretion generally accounts for only about 10% of total ion efflux when compared to the gill (Perry et al., 2003), but potentially this could become more important in the face of dietary ion loading. For example, approximately 100% of an excess infused Mg^{2+} load was excreted renally in freshwater rainbow

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trout although the mechanism was unclear (Oikari and Rankin, 1985). In contrast, less than 5% of an infused Na^+ and Cl^- load was excreted renally (Curtis and Wood, 1992).

With respect to the alkaline tide, alterations in the transport of acid–base equivalents to the environment may attenuate the extent of the internal metabolic alkalosis (Goss and Wood, 1990; Goss and Perry, 1994; Bucking and Wood, 2008). However, none of these studies separated the contribution of the gills from that of the kidney. In contrast to other vertebrates (Wang et al., 2001), there is no evidence that fish, either elasmobranchs or teleosts, utilize respiratory compensation (i.e. respiratory acidosis due to restriction of ventilation) to mitigate the alkaline tide (Wood et al., 2007; Bucking and Wood, 2008, 2009). Is the kidney involved in compensating the postprandial alkaline tide in fish? In mammals, this is the other important mechanism besides respiratory compensation (e.g. Johnson et al., 1990; Oderda et al., 2002) and, in fact, the alkaline tide was first identified in the urine of hospitalized patients due to the renal regulation of the metabolic alkalosis (Jones, 1845). In freshwater teleosts, the kidney plays an important role in normal acid–base balance by reabsorbing most of the filtered bicarbonate, and produces urine of variable pH, titrable acidity, and bicarbonate content. However, as for mineral ion excretion, the kidney in fish is again thought to account for less than 10% of the total acid or base excretion to the environment (Perry et al., 2003; Perry and Gilmour, 2006).

With this background in mind, we examined the effect of feeding on the renal handling of various electrolytes, acid–base equivalents, and various metabolites in freshwater rainbow trout. Specifically we hypothesized that the kidney would play a larger role in regulating plasma divalent ions when compared to monovalent ions. Renal regulation of water balance during digestion was also investigated as several recent studies have suggested that endogenous water is lost across the GI tract during the digestion of a commercial trout chow meal (Bucking and Wood, 2006a). As such, we hypothesized that the kidney would produce less urine volume during digestion when recently fed fish were contrasted with unfed fish. Finally, recent evidence for acid/base disturbances during feeding in freshwater fish have highlighted the need to determine the route of compensation (i.e. branchial vs. renal; Bucking and Wood, 2008; Cooper and Wilson, 2008). We hypothesized the urine would be an avenue to help alleviate the alkaline tide seen in freshwater rainbow, through decreased H^+ excretion (i.e. decreased HCO_3^- reabsorption); however altered branchial acid/base handling would account for a majority of the base excretion.

2. Materials and methods

2.1. Experimental animals

All experiments were conducted at McMaster University, in accordance with Canadian Council on Animal Care guidelines and within approved animal use protocols.

Adult rainbow trout (*Oncorhynchus mykiss*, Salmonidae; mixed sexes; 232 ± 29 g) were obtained from Humber Springs Trout Farm (Orangeville, Ontario, Canada) and acclimated to laboratory conditions prior to experimentation. Fish were held in 500 L tanks and supplied with flow-through dechlorinated Hamilton City tap water (Ca^{2+} = 1.8 mmol L^{-1}; Cl^- = 0.8 mmol L^{-1}; Na^+ = 0.6 mmol L^{-1}; Mg^{2+} = 0.5 mmol L^{-1}; K^+ = 0.04 mmol L^{-1}; titration alkalinity (to pH 4.0) = 1.9 mequiv. L^{-1}; total hardness = 140 mg L^{-1} as $CaCO_3$; pH 8.0) and aeration. During laboratory acclimation, fish were fed every 48 h with a 2 % body mass ration of a commercial trout pellet diet (5 point floating trout chow, Martin Mills, Ontario, Canada; Na^+ = 215 ± 15, Cl^- = 188 ± 16, K^+ = 97 ± 2, Ca^{2+} = 194 ± 3, Mg^{2+} = 109 ± 1 µmol g^{-1} original food mass), and were subjected to a 12:12 light:dark cycle.

2.2. Experimental protocols

Following acclimation, feeding was suspended for 5 days and internal bladder catheters (Clay-Adams PE60) were surgically implanted (described by Curtis and Wood, 1992; Wood and Patrick, 1994) while the fish was under MS-222 anesthesia (0.07 g L^{-1}; Sigma, St Louis, MO, USA) and artificially ventilated on an operating table. Subsequently, fish were injected, via a caudal puncture with a gas-tight Hamilton syringe, with 17 µCi (0.629 MBq) of [1,2-^{3}H] polyethylene glycol-4000 (PEG-4000; New England Nuclear, Boston, MA, USA) in 0.64 mL of Cortland saline (Na^+ = 140; Cl^- = 130; K^+ = 5; Ca^{2+} = 1, Mg^{2+} = 2 mmol L^{-1}; pH 7.8; Wolf, 1963). Care was taken to ensure the entirety of the [^{3}H]PEG-4000 load was placed into the bloodstream by periodically withdrawing blood during the injection procedure. The fish were then placed in individual darkened flux boxes (10 L) for a 24 h recovery period which also allowed the [^{3}H]PEG-4000 to equilibrate within the extracellular space. The internal urinary catheters drained by gravity into glass vials placed 3 cm below the water level of the flux boxes, and collections were made over successive 12 h periods. By placement of the catheter inside the bladder, any reabsorptive/secretory role of the bladder was prevented and therefore the function of the kidney was solely examined (see Curtis and Wood, 1992; Wood and Patrick, 1994). The functionality of the catheters was monitored, and following the 24 h period for recovery and [^{3}H] PEG-4000 equilibration, fish with working catheters were either sham-fed (1.5% body mass ration of water, referred to as unfed fish hereafter) or fed a slurry of ground commercial fish feed at approximately a 3% body weight ration (referred to as fed fish). The slurry consisted of 50% commercial fish feed (composition as above) ground to a fine powder in a blender (PowerMax; Braun, USA) and combined with 50% dechlorinated Hamilton city tap water. The slurry composition was based on previous gastric water content observations following the voluntary ingestion of a similar commercial trout diet (Bucking and Wood, 2006b). The slurry presented the GI tract with large ions loads (Na^+ = 3225, Cl^- = 2825, K^+ = 1450, Ca^{2+} = 2900, Mg^{2+} = 1625 µmol kg^{-1} fish body mass) which were estimated based on the ion composition of the food, the ration size, and fish body mass. The feeding (and sham-feeding) was administered under light anesthesia (0.03 g L^{-1} MS-222), through a large tube inserted into the stomach through the buccal cavity and occurred 24 h following the initial surgery. The time of feeding was designated as 0 h.

Subsequently, the water flow to the tanks was suspended for a series of 12-h periods. At the beginning of each flux period the water level within the flux boxes was set to a predetermined volume, a fresh urine collection was started and a water sample taken (triplicates of 10 mL each). At the end of the 12-h period, another water sample was taken (10 mL in triplicate) and the flux boxes were flushed thoroughly with fresh water. The water levels within the boxes were then reset to the previous volume, water flow re-suspended, a new urine collection was started, and a starting water sample was taken. All water samples were stored at 4 °C for measurements of titratable alkalinity and ammonia concentrations, which were conducted within 24 h of collection. The urine was weighed to determine urine volume, a sub-sample collected to determine pH, and the remainder stored at −20 °C for later analysis.

At 48 h following feeding, the fish were terminally anesthetized, and a blood sample was taken via caudal puncture with an ice-cold, pre-heparinized gas-tight Hamilton syringe. The sample was then immediately centrifuged to obtain the plasma fraction which was placed in liquid nitrogen for later analysis.

2.3. Water analysis

The initial and final water samples were measured for total ammonia and titratable alkalinity. Water total ammonia (sum of $NH_3 + NH_4^+$

concentrations = $[Amm]_w$) was measured using the salicylate–hypochlorite method (Verdouw et al., 1978). Titratable alkalinity was determined by the single end-point technique (McDonald and Wood, 1981), which was earlier validated against the double end-point method in a similar study by comparing the results obtained with both techniques (Bucking and Wood 2008). Briefly, titratable alkalinity was determined by the titration of 10 mL water samples to pH 3.8 using a Radiometer (Copenhagen, Denmark) GK2401C glass combination electrode coupled to a Radiometer PHM 82 standard pH meter through the addition of a standardized acid (0.02 mol L^{-1} HCl; Sigma) delivered using a Gilmont (Barrington, IL, USA) microburette. The amount of acid titrant required to reach pH 3.8, factored by the volume of the sample, represented the concentration of titratable alkalinity in basic equivalents.

Additionally, 10 mL of ACS scintillation fluor (Amersham, U.K.) was added to 5 mL of water obtained from the flux box. The water samples were then counted for [^{3}H]PEG-4000 loss to the water over each 12 h flux period (LKB Rackbeta 1217 Counter (Turku, Finland), using internal standardization to check that quench was constant.

2.4. Urine analysis

The [^{3}H]PEG-4000 radioactivities of the urine (1 mL) were measured by diluting with water to a total volume of 5 mL, then adding 10 mL of ACS scintillation fluor. The samples were then counted as above. Urine samples were also diluted with 1% HNO_3^- and measured for Na^+, K^+, Ca^{2+} and Mg^{2+} concentrations using a Varian 1275 Atomic Absorption Spectrophotometer. La^{3+} was added to the Ca^{2+} and Mg^{2+} dilutions, while Cs^{2+} was added to the K^+ urine dilution, to prevent interferences. Urine Cl^- was determined according to a spectrophotometric assay (Zall et al., 1956). Total urine ammonia (sum of $NH_3 + NH_4^+$ concentrations = $[Amm]_u$) was determined by the salicylate hypochlorite method (Verdouw et al., 1978). Urine glucose was measured by the hexokinase, glucose-6-phosphate dehydrogenase method (Sigma, 301A). Urine urea was measured according to the diacetyl monoxime method of Rahmatullah and Boyde (1980).

Urine titratable acidity minus bicarbonate (titratable acidity–bicarbonate or [TA–HCO_3^-]) was measured as a single value by means of the double titration procedure (Hills, 1973), detailed elsewhere (Wheatly et al., 1984; Curtis and Wood, 1992). Briefly, the urine is titrated with acid (HCl) to neutralize the bicarbonate portion of the urine and then titrated with base (NaOH) to reveal the titratable acid components. The endpoint of the titration was pH = 7.8, representing an average control arterial pH (pH_a) measured in freshwater rainbow trout (e.g. Bucking and Wood, 2008). A Radiometer (Copenhagen, Denmark) GK2401C glass combination electrode coupled to a Radiometer PHM 82 standard pH meter was employed. Titrants used were standardized 0.02 N HCl (Sigma) and 0.02 N NaOH (standardized by titration with 0.02 N HCl) delivered by Gilmont microburettes. Total renal output of acidic equivalents was taken as the sum of the [TA–HCO_3^-] and NH_4^+ components (Hills, 1973).

2.5. Plasma analysis

The [^{3}H]PEG-4000 radioactivities of the plasma samples (25 µl) were also measured by diluting with water to a total volume of 5 mL, then adding 10 mL of ACS scintillation fluor, and counting as above.

2.6. Calculations

The branchial flux of total ammonia (J_{Tamm}), as well as the branchial flux of total titratable alkalinity (J_{Talk}), to the external water in these bladder-catheterized trout was calculated as:

$$J_X = \frac{([X]_{wi} - [X]_{wf})}{V^*T^*M} \quad (1)$$

where X was either the concentration of ammonia in the water (µmol L^{-1}), or the titratable alkalinity of the water (i.e. the amount of acid required to lower the pH of the water to pH 3.8; µmol L^{-1}), in either initial samples ($[X]_{wi}$) or final samples ($[X]_{wf}$). The change from initial to final concentration was factored by volume of the flux box (V; l), time (T; h) and trout mass (M; kg), and expressed as µmol kg^{-1} h^{-1}.

The net acid–base flux was calculated as the difference between J_{Talk} and J_{Tamm} (McDonald and Wood, 1981). An overall net base flux (i.e. HCO_3^- equivalent flux; $J_{net}OH^-$) is shown by a positive difference and is plotted as a negative value (i.e. net base loss from the animal), while a net acid flux (i.e. H^+ equivalent flux; $J_{net}H^+$) is shown by a negative difference and is plotted as a positive value (i.e. net base uptake = net acid loss).

Urinary excretion rates (U_x) of any substance (X) were calculated as:

$$U_x = [X]_u{}^*UFR \quad (2)$$

using measured values of urine flow rates (UFR; mL kg^{-1} h^{-1}) and urine concentrations of the substance of interest ($[X]_u$; µmol mL^{-1}).

Glomerular filtration rates (clearance rates of [^{3}H]PEG-4000) were calculated as the excretion rate of [^{3}H]PEG-4000 radioactivity in the urine (cpm_u*UFR) divided by its concentration in the blood plasma (cpm_p):

$$GFR = \frac{cpm_u{}^*UFR}{cpm_p} \quad (3)$$

While the cpm_u was measured directly from the urine, to avoid stress associated with repeated caudal blood sampling, the cpm_p was back calculated from the plasma [^{3}H]PEG-4000 radioactivity that was measured at the end of the experiment. Back-calculation consisted of taking the final cpm_p and accounting for the assumed extracellular fluid volume (250 mL kg^{-1}) to calculate the total [^{3}H]PEG-4000 remaining in the fish at the end of the experiment. The total amount of [^{3}H]PEG-4000 lost to the urine was calculated and added to the total [^{3}H]PEG-4000 remaining in the fish. This was subtracted from the total amount injected to yield the amount of [^{3}H]PEG-4000 lost across the gills. Assuming a linear loss across the gills with time, the cpm_p was calculated as the amount of [^{3}H]PEG-4000 remaining in the fish from the original amount injected after accounting for losses to the water and to the urine. Then, as the concentration of [^{3}H]PEG-4000 in the plasma declined over time, the concentration of [^{3}H]PEG-4000 in blood plasma was taken as the mean of measurements at the start and end of each 12 h period.

All of the above rates were related to fish body mass, i.e. mL kg^{-1} h^{-1}.

The clearance ratios of a variety of parameters were calculated based on the present measurements of urinary parameters and our previously published values for plasma constituents in freshwater rainbow trout following the ingestion of a similar meal (Bucking and Wood, 2006a,b, 2007; Bucking et al., 2009). The clearance ratio (relative clearance) of a substance (CR_x; Wood, 1995) relates the excretion rate of a substance (U_x; equation 2) to the rate that it is filtered at the glomerulus:

$$CR_x = \frac{[X]_u{}^*UFR}{[X]_p{}^*GFR} \quad (4)$$

A CR_x of more than 1 indicates that secretion of X has occurred on a net basis; less than 1 indicates that net reabsorption of X has occurred (see Wood and Patrick, 1994 for detailed description).

2.7. Statistics

All data have been examined for normality prior to statistical testing. The effect of time and feeding on the various parameters was

determined using a two-way ANOVA, followed by a post-hoc test (Tukey's; SigmaStat 3.0), with $P<0.05$ as the level of significance.

3. Results

Feeding occurred at 0 h, up until which time the two groups , “fed” and “unfed” (= sham-fed), were treated identically.

3.1. Renal responses

The GFR of the unfed fish did not change significantly over time between 12 and 48 h (6.1 ± 0.6 mL kg^{-1} h^{-1} ($N=21$); Fig. 1A), after falling from initial values (0–12 h; 10.4 ± 1.2 mL kg^{-1} h^{-1}). The UFR of the unfed fish displayed a similar trend as the GFR, initially falling and thereafter remaining unchanged over 12–48 h (4.5 ± 0.5 mL kg^{-1} h^{-1} ($N=21$); Fig. 1B). Feeding resulted in a significant decrease in both GFR (5.2 ± 0.6 mL kg^{-1} h^{-1} ($N=7$); Fig. 1A) and UFR (3.4 ± 0.4 mL kg^{-1} h^{-1} ($N=7$); Fig. 1B) by approximately 20%, when compared to values from unfed fish between 12 and 24 h. There were no significant differences between the GFR and UFR of fed and unfed fish at the remaining time points (Fig. 1A, B).

Feeding had variable effects on the urinary excretion rates of the ions measured (Table 1, Fig. 2). There were no significant effects of feeding or time on the excretion rate of K^+ which was maintained at an average of 2.6 ± 0.1 μmol kg^{-1} h^{-1} ($N=56$) across all treatments and time points (Table 1). In contrast, the excretion rate of Mg^{2+} in fed fish increased significantly within the fist 12 h, and was further elevated at 24–48 by 5–10-fold relative to rates observed in unfed fish

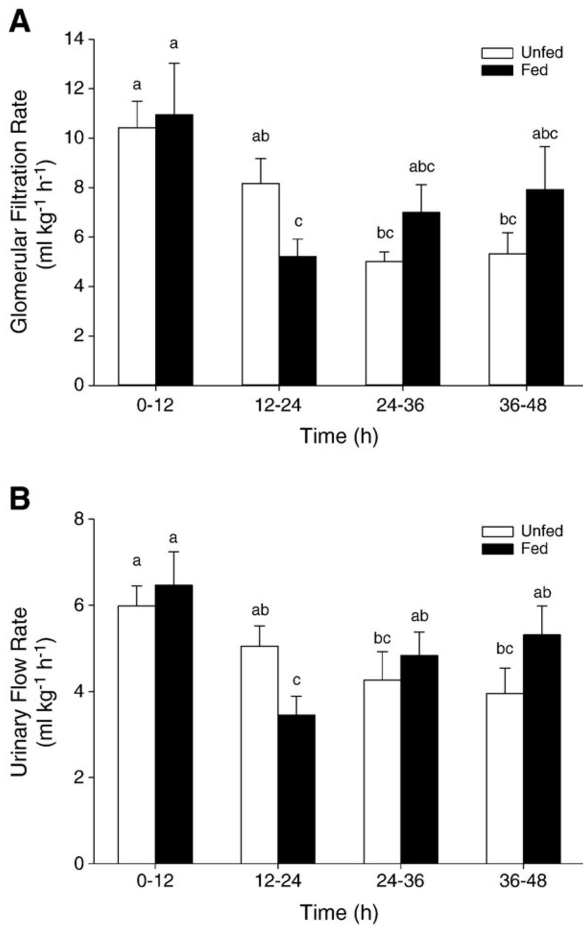

Fig. 1. A) The glomerular filtration rate (GFR; mL kg^{-1} h^{-1}) and B) the urinary flow rate (UFR; mL kg^{-1} h^{-1}) in unfed (clear bars) and fed (black bars) freshwater rainbow trout. Feeding or sham-feeding (unfed group) occurred at 0 h. Means ± 1 SEM ($N=7$). Bars that share letters are not significantly different.

(Fig. 2D). The excretion rates of Na^+, Cl^- and Ca^{2+} showed variable patterns with respect to time and feeding. All three ions exhibited significantly higher urinary excretion rates in fed fish when compared to unfed fish between 0 and 12 h, however this difference disappeared at 12–24 h due to a decrease in excretion rates in fed fish (Fig. 2 A,B,C). The excretion rate of Na^+ then increased again in fed fish between 24–48 h (Fig. 2A), while the excretion rate of Ca^{2+} in fed fish increased between 36–48 h (Fig. 2C), and that of Cl^- remained stable (Fig. 2B). Despite variations in UFR and GFR, the urinary excretion rates of all ions measured did not vary significantly over time in unfed fish (Table 1, Fig. 2). There were also no significant effects of feeding or time on the urinary excretion rates of urea (3.1 ± 0.4 μmol kg^{-1} h^{-1} ($N=56$)) and glucose (0.7 ± 0.1 μmol kg^{-1} h^{-1} ($N=56$); Table 1).

Unfed fish maintained a positive and very constant excretion rate of [TA–HCO_3^-] (i.e. TA>HCO_3^-, 1.5 ± 0.2 μequiv kg^{-1} h^{-1}; Fig. 3B) throughout the experiment. When combined with the similarly constant total ammonia excretion rate (3.8 ± 0.5 μequiv kg^{-1} h^{-1} ($N=56$); Fig. 3A), unfed fish had a positive net H^+ excretion rate (5.3 ± 0.4 μequiv kg^{-1} h^{-1} ($N=56$); Fig. 3C). In contrast, feeding resulted in a negative net H^+ excretion (i.e. net basic equivalent excretion, −3.3 ± 0.6 μequiv kg^{-1} h^{-1}($N=56$) averaged over the entire 48 h (Fig. 3C) due to a negative [TA–HCO_3^-] (i.e. HCO_3^->TA) urinary excretion rate (Fig. 3B). The tendency for net H^+ excretion rates to become less negative over time in fed fish was not significant (Fig. 3C), however there was a significant decrease in the negative [TA–HCO_3^-] excretion rate in fed fish after 12 h (Fig. 3B). The urinary excretion rate of total ammonia was initially higher in fed fish, but this difference was no longer significant between 12–48 h (Fig. 3A). Overall, these changes were reflected in an increase in urine pH (7.78 ± 0.04 ($N=21$) 0–36 h; Fig. 3D) in fed fish compared to unfed fish (7.38 ± 0.02 ($N=28$) 0–48 h; Fig. 3D). Relative to the unfed control group, fed fish excreted about 415 μmol kg^{-1} of basic equivalents via the urine during the 48-h post-feeding period.

3.2. Branchial responses

Unfed fish exhibited a net branchial H^+ excretion to the water ($J_{net}H^+ = 115 \pm 36$ μmol kg^{-1} h^{-1} ($N=56$), Fig. 4C) due to larger J_{Tamm} (342 ± 24 μmol kg^{-1} h^{-1} ($N=56$), Fig. 4A) when compared to J_{Talk} (227 ± 41 μmol kg^{-1} h^{-1} ($N=56$), Fig. 4B). None of these parameters changed significantly between 0–48 h. In contrast, J_{Tamm} increased over time in fed fish (1.5 fold from 12–48 h; Fig. 4A) along with J_{Talk} (2.8 fold from 12–48 h; Fig. 4B). This resulted in a net branchial OH^- excretion to the water ($J_{net}OH^-$) by the fed fish which averaged −104 ± 16 μmol kg^{-1} h^{-1} ($N=21$) over 12–48 h (Fig. 4C). Compared to the unfed control group (which displayed a net flux of acid equivalents), fed fish excreted about 8155 μmol kg^{-1} of basic equivalents across the gills during the 48-h post-feeding period.

4. Discussion

4.1. Overview

The experimental design used in the current study was necessarily invasive, and likely resulted in artifacts associated with stress (from confinement and/or anesthesia) that are not normally seen in naturally feeding fish. For example, UFR and GFR measurements in the first 12 hours (for both groups) indicate a diuresis is occurring when compared to other studies (e.g. McDonald and Wood, 1981; Curtis and Wood, 1991, 1992). However, the conclusions based on comparing the fed and unfed groups take these into account, as both were subjected to similar handling stresses. Hence, the present study presents the first quantitative assessment of the responses of the kidney to the ingestion of a single meal in a freshwater fish, in terms of fluid balance, ion excretion, and acid–base regulation. With respect to our initial hypotheses, these findings confirm that fed trout produce a

Table 1
Ion and metabolite urinary excretion rates (μmol kg^{-1} h^{-1}) in unfed and fed fish. Means ± 1 SEM (*N* = 7).

	K^+		Urea		Glucose	
	Unfed	Unfed	Unfed	Unfed	Unfed	Fed
0–12 h	2.9 ± 0.3 [a]	3.3 ± 0.5 [a]	3.5 ± 0.9 [a]	3.5 ± 0.9 [a]	0.71 ± 0.23 [a]	0.89 ± 0.18 [a]
12–24 h	2.6 ± 0.3 [a]	1.8 ± 0.4 [a]	3.8 ± 1.1 [a]	1.9 ± 1.1 [a]	0.69 ± 0.13 [a]	0.69 ± 0.12 [a]
24–36 h	2.5 ± 0.5 [a]	2.3 ± 0.2 [a]	2.7 ± 0.8 [a]	3.1 ± 1.0 [a]	0.55 ± 0.16 [a]	0.79 ± 0.17 [a]
36–48 h	2.5 ± 0.5 [a]	2.8 ± 0.4 [a]	2.8 ± 1.0 [a]	3.1 ± 1.1 [a]	0.70 ± 0.23 [a]	0.72 ± 0.20 [a]

Numbers within columns that share letters are not significantly different.
Feeding or sham-feeding (unfed group) occurred at 0 h.

lower urine volume, that the kidney plays a significant role in divalent cation excretion after feeding, and that the kidney contributes to compensation of the post-prandial alkaline tide, though its contribution is much less than that of the gills in this regard.

4.2. Fluid balance

By tracking changes in the water content of the chyme relative to an inert marker, Bucking and Wood (2006a) estimated that the ingestion of a meal of commercial trout feed comparable to that used in the present study resulted in a net water loss from the animal into the GI tract and out the rectum of about 17 mL kg^{-1} over 72 h, thereby decreasing the osmotic water load. The present observations provide strong support for this conclusion, inasmuch as fed fish excreted about 18 mL kg^{-1} less water through the kidney at 12–24 h after the meal (Fig. 1B), the only time period during which their UFR was significantly different than unfed fish. This was most likely caused by the creation of an additional water loss pathway through the gastric epithelium due to both the secretion of HCl and bile into the tract, as well as osmotically driven water loss to the ion-rich commercial diet. Indeed Bucking and Wood (2006a) measured an osmotic gradient from chyme supernatant to plasma of 100 – 500 mosm kg^{-1} at various times after feeding and in various sections of the gut, with the gradient always favoring fluid secretion into the tract. The resulting reductions in UFR (Fig. 1B) and GFR (Fig. 1A) will likely result in economies for both osmotic and mechanical (i.e. glomerular filtration) energy expenditure in freshwater fish fed standard commercial diets. Interestingly, in a somewhat different experimental design, Wood (1995) reported a similar finding: trout fed a commercial diet up until the time of cannulation exhibited UFRs

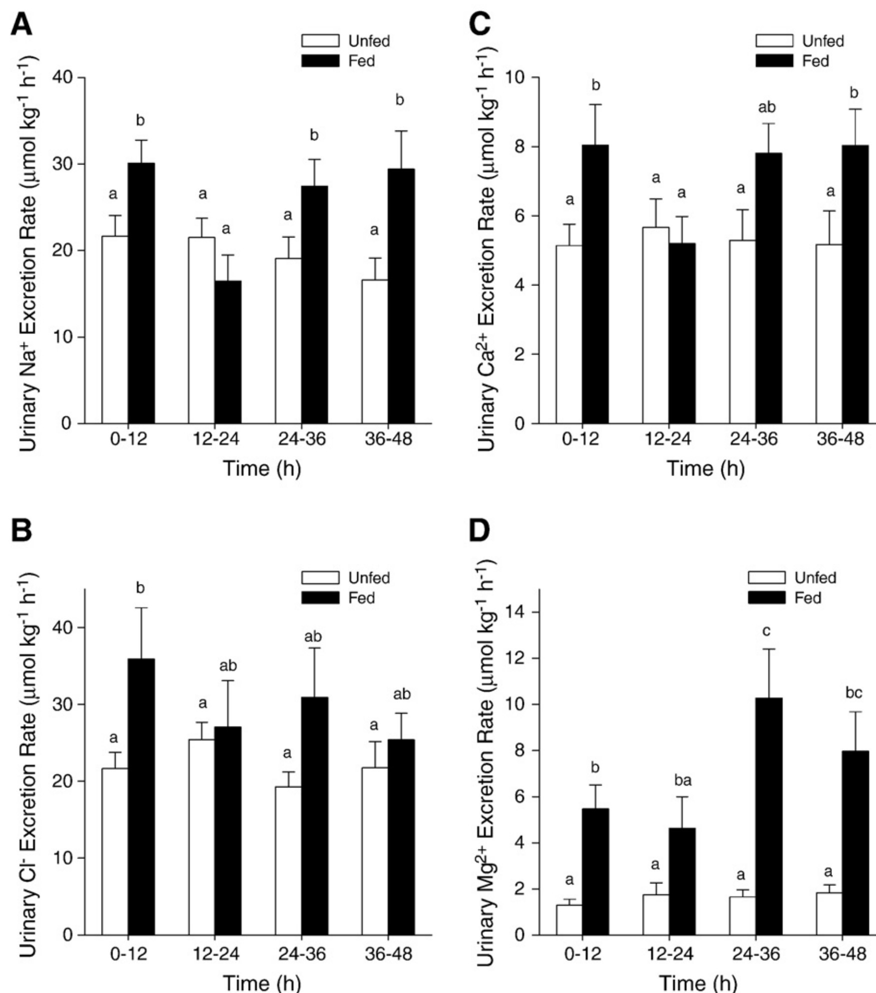

Fig. 2. The urinary excretion rates (U_x; μmol kg^{-1} h^{-1}) of A) Na^+, B) Cl^-, C) Ca^{2+} and D) Mg^{2+} in unfed (clear bars) and fed (black bars) freshwater rainbow trout. Feeding or sham-feeding (unfed group) occurred at 0 h. Means ± 1 SEM (*N* = 7). Bars that share letters within a panel are not significantly different.

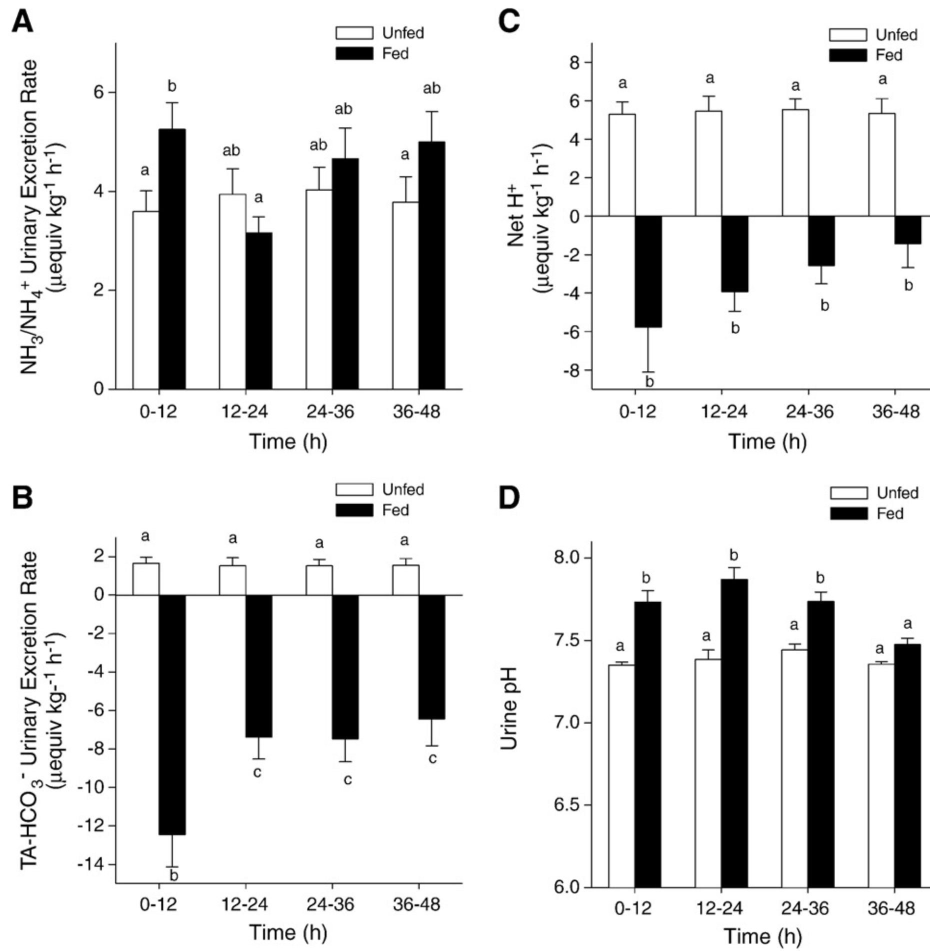

Fig. 3. Urinary acid–base balance in unfed (clear bars) and fed (black bars) freshwater rainbow trout. A) Urinary ammonia excretion rate ($U_{NH3+NH4+}$; μmol kg^{-1} h^{-1}). B) Urinary titratable acidity–bicarbonate excretion rate ($U_{TA-HCO3-}$; μequiv kg^{-1} h^{-1}). C) Urinary net acid or base excretion rate (μequiv kg^{-1} h^{-1}). D) Urine pH. Feeding or sham-feeding (unfed group) occurred at 0 h. Means ± 1 SEM ($N=7$). Bars that share letters within a panel are not significantly different.

which were about 20% lower for the next 72 h than those of fish fasted for 7–10 d prior to test.

These findings go against some earlier predictions. Ingestion of a commercial diet appears to require an additional liquefaction step that natural diets do not need, so as to facilitate digestive and absorptive processes (Ruohonen et al., 1997). While a portion of this extra liquid is supplied through increased drinking rates, the remainder is supplied through secretion of endogenous water across the gastric epithelium (Ruohonen et al., 1997; Kristiansen and Rankin, 2001). It was proposed that the elevated drinking rate would constitute an additional water load (Eddy, 2007) that would result in elevated GFR and UFR (Salman and Eddy, 1988). Indeed Salman and Eddy (1988) reported that trout fed a diet containing a much higher NaCl content than in the present study (12% vs. 1.5%) exhibited elevated UFR. However, such an elevated dietary NaCl concentration is abnormal and has been shown to result in elevated arterial blood pressure (Chen et al., 2007) and blood volume (Olson and Hoagland, 2008). In contrast, the present study using commercial feed with standard ion content demonstrates that the net secretion of water into the tract may actually result in the opposite effect, a decrease in GFR and UFR (Fig. 1A and B). In future studies, it will be of interest to evaluate kidney function in fish fed natural diets (i.e. aquatic prey items). Such diets would have higher water contents and lower ion concentrations, and so should not require additional water secretion, nor should they create the large chyme-to-plasma osmotic gradients seen with a standard commercial diet.

It remains unknown how the gastric water secretion results in a decreased UFR and GFR. It may simply be a case of decreased blood volume, plasma osmolality slightly increased approximately 8 h following the ingestion of a similar meal (Bucking and Wood, 2006a). However, digestion is also associated with gastrointestinal hyperaemia (i.e. increased blood flow to the gastrointestinal tract; e.g. Altimiras et al., 2008; Eliason et al., 2008) caused by an increased cardiac output (e.g. Axelsson and Fritsche, 1991; Axelsson et al., 2000, 2002; Altimiras et al., 2008) as well as preferential distribution of blood to the digestive system from the systemic circulation (Seth and Axelsson, 2009; Seth et al., 2009). Whether this results in a decrease in blood flow to the kidney and hence a decrease in GFR and UFR is unknown.

4.3. Ionoregulation

Renal clearance ratios (CR_X) for each of the major ions measured were calculated based on previously published plasma ion concentrations (Bucking and Wood, 2006a,b, 2007; Bucking et al., 2009), and are presented in Fig. 5. The most dramatic results were seen with Mg^{2+}. In the first 12-h measurement period, CR_{Mg2+} was already elevated about 3-fold above the value seen in unfed trout (0.25) and thereafter increased from 0.8 to about 1.5 at 34–48 h post-feeding (Fig. 5D). This means that Mg^{2+} handling by the kidney changed over from net reabsorption to net secretion, as previously seen in trout (Oikari and Rankin, 1985) and freshwater lamprey (Rankin et al., 1983) experimentally infused with Mg^{2+} loads. These workers could detect no evidence of branchial Mg^{2+} excretion. Furthermore, Oikari and Rankin (1985) reported that a single freshwater trout force-fed a Mg^{2+} load of ~5000 μmol kg^{-1} (commercial feed soaked in a $MgCl_2$ solution, a 3-

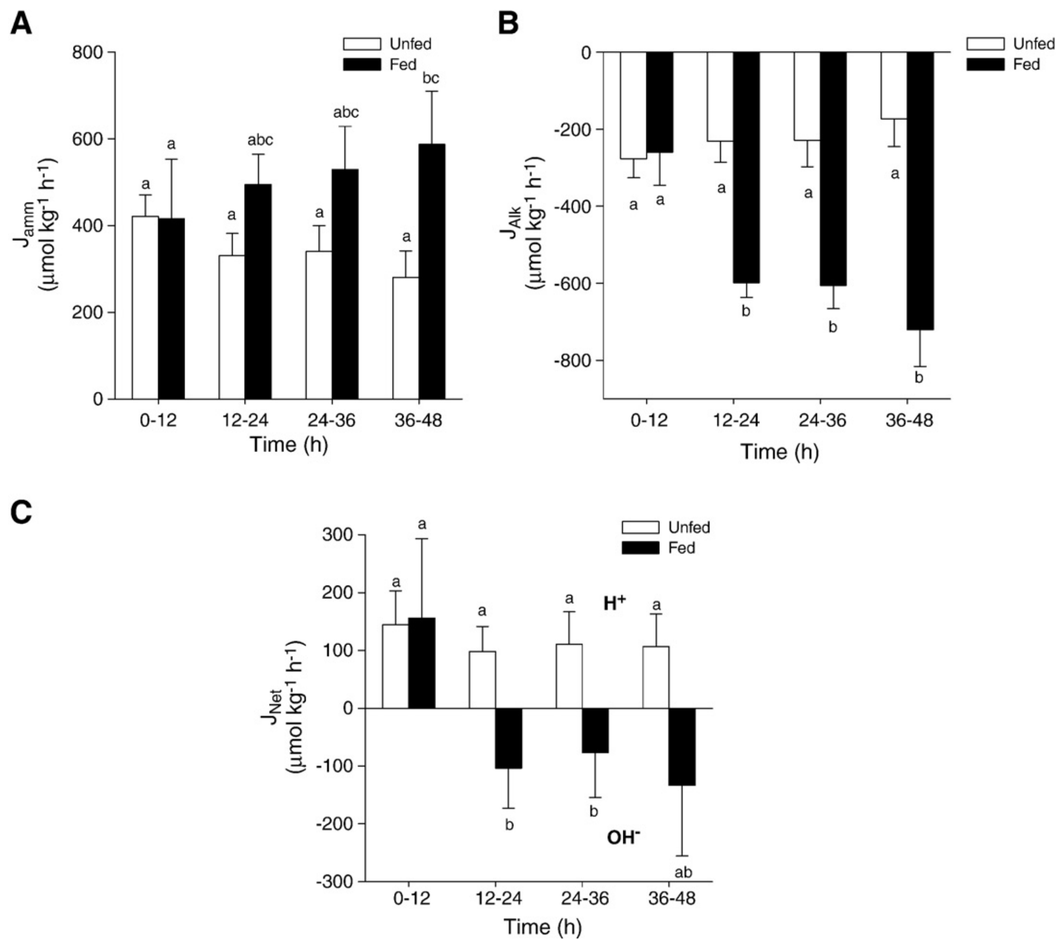

Fig. 4. Branchial acid–base balance in unfed (clear bars) and fed (black bars) freshwater rainbow trout. A) Net branchial ammonia excretion (J_{Tamm}; µmol kg^{-1} h^{-1}). B) Net branchial titratable alkalinity excretion (J_{Talk}; µmol kg^{-1} h^{-1}). C). Net branchial acid base excretion (J_{net}; µmol kg^{-1} h^{-1}). Feeding or sham-feeding (unfed group) occurred at 0 h. Means ± 1 SEM (N = 7). Bars that share letters within a panel are not significantly different.

fold greater load than that used in the present study) eliminated ~100% of the load to the urine. In the present study, about 16% of the ingested Mg^{2+} load (~1625 µmol kg^{-1} fish mass) in the meal, was excreted by the kidney (0.265 mmol kg^{-1}). However, considering that previous studies have demonstrated that only 60% of the ingested Mg^{2+} load was actually absorbed (Bucking and Wood, 2007), the kidney then becomes responsible for excreting more than 27% of the "absorbed" Mg^{2+} load over 48 h. Presumably, the remainder of the excess Mg^{2+} load is excreted at a later time through the kidney, or stored for growth.

Freshwater fish must obtain Mg^{2+} from either the water or the diet, however there is only limited evidence for active branchial Mg^{2+} uptake (Flik et al. 1993; Bijvelds et al. 1996); absorption from the alimentary canal appears to be the normally dominant route of uptake (Cowey et al., 1977; Bucking and Wood, 2007; reviewed by Bijvelds et al., 1998). The kidneys of freshwater trout seem to function to maintain optimal extracellular fluid magnesium concentrations by switching from net reabsorption to net secretion and vice versa in varying nutritional situations. The renal response for Mg^{2+} is in stark contrast to K^+, where the dietary load was similar (1450 µmol kg^{-1} fish mass) but there was no elevation of excretion in the urine (compared to unfed fish), despite the fact that 90% of the ingested load was absorbed through the GI tract (Bucking and Wood, 2006b). There was also no effect on the CR_{K+} (0.1 = 90% of filtered K reabsorbed) with the kidney maintaining K^+ reabsorption throughout digestion (Fig. 5C).

The dietary Na^+ and Cl^- loads (3225 and 2825 µmol kg^{-1} fish mass, respectively) were larger than for Mg^{2+} or K^+. Many studies suggest complete Na^+ absorption from the diet within a few hours of ingestion (Smith et al., 1989, 1995; Phillips, 1944). Bucking and Wood (2006b) have shown that this occurred mainly in the stomach in freshwater rainbow trout; however on a net basis, overall absorption of Na^+ from the meal was negligible because of Na^+ secretory processes lower in the tract. In contrast, 85% of the ingested Cl^- load was absorbed on a net basis (Bucking and Wood, 2006b). Despite this difference in net absorption, the kidney appeared to handle these two ions in a similar fashion after the meal. For both, there was no observable effect on Na^+ or Cl^- clearance ratios (Fig. 5A and B), which remained below 0.04 (i.e. >96% reabsorption for both ions) despite significant increases in excretion rates. These increased excretion rates amounted to only about 10% of the ingested Na^+ and Cl^- loads over 48 h, in agreement with earlier NaCl loading studies by infusion (Curtis and Wood, 1992) or by salt-enriched diets (Salman and Eddy, 1988). The gills appear to be the main sites for excretion of excess NaCl loads (Salman and Eddy, 1988; Smith et al., 1995; Pyle et al., 2003).

CR_{Ca2+} values of 0.2–0.4 in unfed fish indicated that 60–80% of the Ca^{2+} filtered at the glomeruli was reabsorbed in the tubules (Fig. 5C). Following feeding, the CR_{Ca2+} were significantly elevated over unfed values over the first 24 h, and reached about 0.6 at 24–48 h, indicating that only 40% of the filtered Ca^{2+} was being reabsorbed. However the kidney did not transition to active secretion as it did for Mg^{2+}. Between 3% (based on total ingestion) and 11% (based on the assimilation efficiency of 28% reported by Bucking and Wood, 2007) of the dietary Ca^{2+} load (2900 µmol kg^{-1} fish mass) was excreted by the kidney.

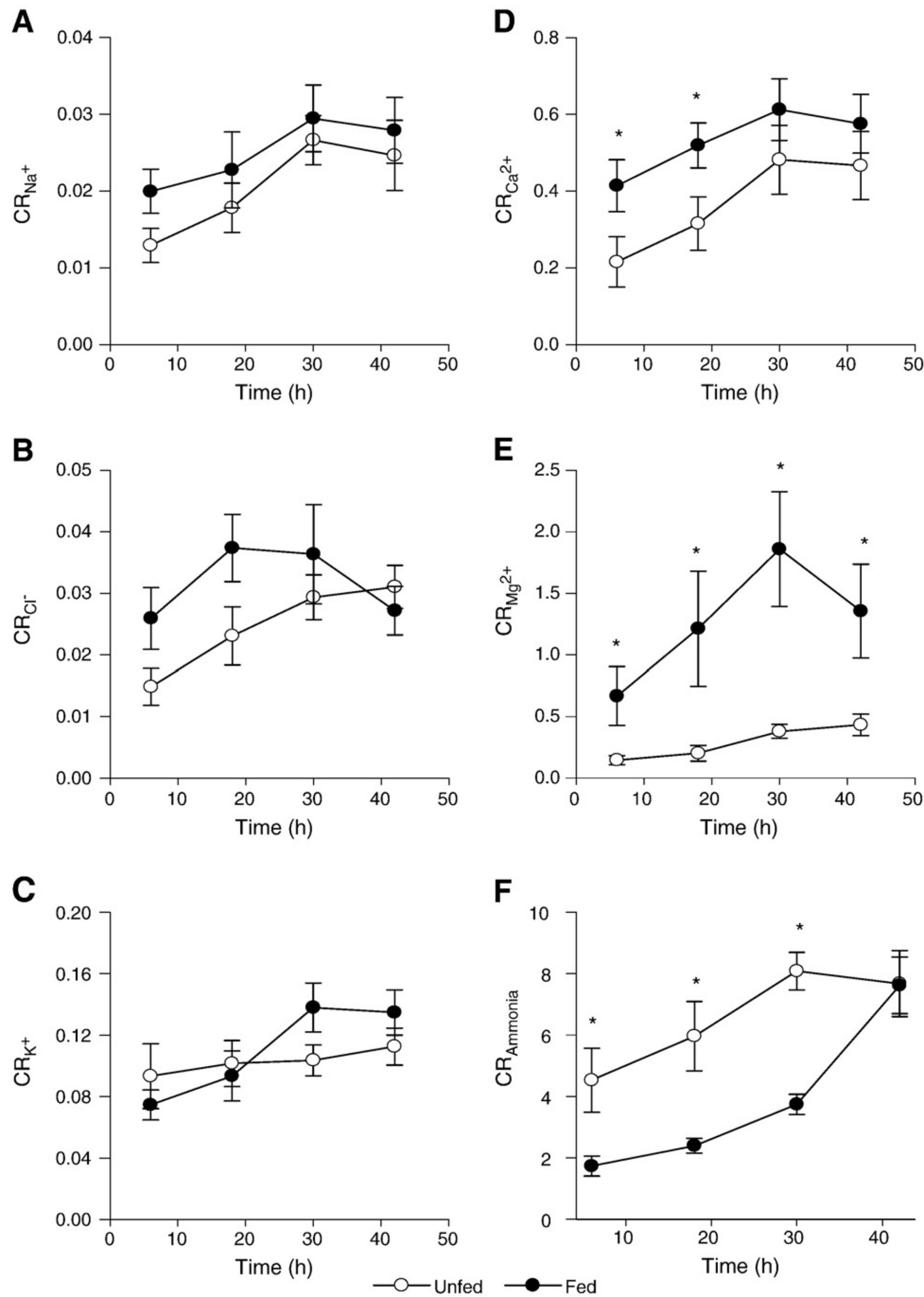

Fig. 5. Clearance ratios (CR_X; plasma values obtained from Bucking and Wood, 2006a, b, 2007; Bucking et al., 2009; see Materials and methods section for calculation details) in unfed (clear circles) or fed (black circles) freshwater rainbow trout. A) Na^+ clearance ratio. B) Cl^- clearance ratio. C) K^+ clearance ratio. D) Ca^{2+} clearance ratio. E) Mg^{2+}clearance ratio. F) Total ammonia clearance ratio. Feeding or sham-feeding (unfed group) occurred at 0 h. Means ± 1 SEM (N = 7). Estimated clearance ratios were plotted at mid time points (6, 18, 30 and 42 h). *Indicate significant differences compared to unfed values at the same time point.

As the kidney handled between 3–26% of the absorbed loads, it appears that a majority of the ions absorbed from the diet (Bucking and Wood, 2006b, 2007) are either excreted extrarenally in order to maintain relatively stable plasma concentrations, or incorporated into growth of the animal. Almost all previous studies conducted on renal regulation of ion balance have been conducted on unfed fish. This study suggests that overall we are underestimating the renal ion excretion rates in fish which are feeding, at least for those fed an aquacultural diet.

4.4. Acid base status

Previous work demonstrated a post-prandial alkaline tide in freshwater rainbow trout (Bucking and Wood, 2008; Cooper and Wilson, 2008), although the pervasiveness throughout the class of ray-finned fishes (Actinopterygii) is unclear inasmuch as other fishes have not shown an alkaline tide (Taylor and Grosell, 2006; Taylor et al., 2007). The mechanisms behind the alkaline tide phenomenon in higher vertebrates have been reviewed elsewhere (Wang et al., 2001; Niv and Fraser, 2002). Essentially it is caused by increased HCl secretion by H^+-ATPase in the gastric mucosa for the purposes of gastric digestion. A basolateral Cl^-/HCO_3^- exchanger transports Cl^- from the plasma into the gastric cell that is then used for HCl secretion across the apical surface of the cell. The HCO_3^- that is in turn secreted into the plasma is produced intracellularily by carbonic anhydrase, which catalyses the conversion of water and CO_2 into H^+ and HCO_3^-. This proton is also used for HCl secretion, while the HCO_3^- crosses the basolateral surface and accumulates in the plasma resulting in the

alkaline tide. Recently, Wood et al. (2009) demonstrated that partially blocking the gastric H^+-ATPase using omeprazole attenuated the extents of post-prandial chyme acidification, systemic alkalosis, and net basic equivalent excretion to the water in an elasmobranch fish, the seawater dogfish shark.

The magnitude of the alkaline tide appears to be directly related to the size of the meal in mammals, and the same appears to be true in the rainbow trout, perhaps not a surprising outcome considering that the alkaline tide is created by a 1:1 H^+: HCO_3^- secretion rate in the stomach. The larger the meal, the larger the amount of H^+ needed to be secreted in order to digest the meal. A meal of ~2-fold the size of that used in the current study produced greater increases in branchial J_{Tamm} and J_{Talk}, and net basic equivalent to the external water over the same time period was approximately −13,800 μmol kg^{-1}; Bucking and Wood, 2008). This figure is about 70% greater than that seen in the present study.

However, while the previous studies demonstrated the presence of an alkaline tide in freshwater rainbow trout, and that the metabolic alkalosis was relieved through excretion of basic units to the environment (Bucking and Wood, 2008; Cooper and Wilson, 2008), the specific contribution of the gills versus the kidney to the process remained to be investigated. Unlike fish, mammals have only one major excretory system, the kidneys, and the presence of an alkaline tide was first detected in the urine of hospitalized humans who were digesting meals, indicating the important role of the kidney.

The present results indicate that the urine is a vehicle for the excretion of basic equivalents during the post-prandial alkaline tide in fish as well (Fig. 3), but its contribution is quantitatively small (approximately 5%; Fig. 3C vs 4C) relative to that of the gills. In this regard, the present results are in accord with studies on the role of the freshwater teleost kidney in compensating other types of acid–base perturbations, where its contribution was generally less than 10% relative to that of the gills (Wheatly et al., 1984; Perry et al., 2003; Perry and Gilmour, 2006).

In terms of mechanism, the changeover from net renal acidic equivalent excretion to net basic equivalent excretion (Fig. 3C) after feeding was mainly due to the changeover of the TA–HCO_3^- component from positive to negative values (Fig. 3B) . The NH_4^+ component exhibited little change (Fig. 3A), though on a relative basis, renal ammonia secretion was reduced (Fig. 5F). The kidney of the rainbow trout is believed to function in a similar manner as the mammalian kidney to help control systemic acid–base status (Wood et al., 1999). Briefly, HCO_3^- may be reclaimed from the filtrate or regenerated by the renal cells. Reclamation involves converting filtered HCO_3^- into CO_2 through a membrane bound carbonic anhydrase (Georgalis et al., 2006). The CO_2 then enters the renal cell and is subsequently converted back into HCO_3^- via intracellular carbonic anhydrase and exported back to the blood via a basolateral Na^+/ HCO_3^- co-transporter (potentially NBC1; Perry et al., 2003). HCO_3^- may also be regenerated through the removal of intracellular H^+ via an apical V-type H^+-ATPase (Perry and Fryer, 1997; Perry et al., 2000) or a Na^+/H^+ antiporter (NHE3). Removal of H^+ drives the intracellular carbonic anhydrase to produce more HCO_3^- through the hydration of CO_2 obtained across the basolateral membrane. All but the NHE3 and the NBC have been specifically localized to the expected cellular domains within rainbow trout renal cells, and the expression of many of the transporters and enzymes have been found to be responsive to disturbances in acid–base status (e.g. NBC, the H^+-ATPase and both carbonic anhydrases; Perry et al., 2003; Georgalis et al., 2006). Whether the decrease in HCO_3^- reabsorption after feeding is due to a decrease in reclamation and/or regeneration remains to be seen.

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