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Post-prandial metabolic alkalosis in the seawater-acclimated trout: the alkaline tide comes in

Carol Bucking*, John L. Fitzpatrick, Sunita R. Nadella and Chris M. Wood

McMaster University, 1280 Main Street West, Hamilton, Ontario, Canada L8S 4K1 and Bamfield Marine Sciences Center, 100 Pachena Road, Bamfield, British Columbia, Canada V0R 1B0

*Author for correspondence (e-mail: bucking@zoology.ubc.ca)

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SUMMARY

The consequences of feeding and digestion on acid–base balance and regulation in a marine teleost (seawater-acclimated steelhead trout; *Oncorhynchus mykiss*) were investigated by tracking changes in blood pH and [HCO_3^-], as well as alterations in net acid or base excretion to the water following feeding. Additionally the role of the intestine in the regulation of acid–base balance during feeding was investigated with an *in vitro* gut sac technique. Feeding did not affect plasma glucose or urea concentrations, however, total plasma ammonia rose during feeding, peaking between 3 and 24 h following the ingestion of a meal, three-fold above resting control values (~300 μmol ml^{-1}). This increase in plasma ammonia was accompanied by an increase in net ammonia flux to the water (~twofold higher in fed fish *versus* unfed fish). The arterial blood also became alkaline with increases in pH and plasma [HCO_3^-] between 3 and 12 h following feeding, representing the first measurement of an alkaline tide in a marine teleost. There was no evidence of respiratory compensation for the measured metabolic alkalosis, as $P_{a_{CO_2}}$ remained unchanged throughout the post-feeding period. However, in contrast to an earlier study on freshwater-acclimated trout, fed fish did not exhibit a compensating increase in net base excretion, but rather took in additional base from the external seawater, amounting to ~8490 μmol kg^{-1} over 48 h. *In vitro* experiments suggest that at least a portion of the alkaline tide was eliminated through increased HCO_3^- secretion coupled to Cl^- absorption in the intestinal tract. This did not occur in the intestine of freshwater-acclimated trout. The marked effects of the external salinity (seawater *versus* freshwater) on different post-feeding patterns of acid–base balance are discussed.

Key words: acid–base balance, digestion, fish, gastric acid secretion, gill, intestine, teleost.

INTRODUCTION

The role of the marine teleost intestine in salt and water homeostasis is well established. In brief, to survive in the hyperosmotic environment, fish must drink and absorb water along the intestine to replace water that is lost to the environment. The mechanisms behind water absorption along the gastrointestinal (GI) tract are suspected to involve both NaCl absorption and HCO_3^- secretion (reviewed by Grosell, 2006). The secretion of HCO_3^- is believed to be coupled to the absorption of Cl^- from the intestinal tract as well as the precipitation of Ca^{2+} ions, which removes the osmotically active ions from the intestinal milieu and hence reduces the osmotic pressure of the intestinal fluid in favour of water absorption (Grosell, 2006; Wilson et al., 2002). However, the intestinal tract of marine teleosts is not only important for osmoregulation, it is also the locus for feeding and digestion, a fact largely ignored in osmoregulatory studies of this tissue, though there are several notable exceptions (Taylor and Grosell, 2006; Taylor et al., 2007).

In most teleost fish, digestion begins in the stomach with a secretion of HCl, which facilitates the breakdown of proteins and begins the process of nutrient assimilation. In fact the parietal cells have been shown to secrete HCl with a pH of 0.8 (Demarest et al., 1989). In freshwater rainbow trout (Bucking and Wood, 2009) and seawater steelhead trout (C.B. and C.M.W., unpublished data), the pH of the stomach chyme during digestion has been shown to decrease to pH ~2–4.

The production of the protons involved with HCl formation is associated with animal-wide metabolic changes, particularly in acid–base balance. Specifically, the ‘alkaline tide’ phenomenon refers to the metabolic base load that is generated in the plasma as a result of the mechanisms of gastric acid secretion. Essentially, the rate of apical H^+ extrusion is matched by basolateral HCO_3^- excretion into the blood plasma by the Cl^-/HCO_3^- exchanger. This process is catalyzed by intracellular carbonic anhydrase, which converts H_2O and CO_2 to HCO_3^- and H^+, and also provides H^+ for apical H^+ secretion in parallel with Cl^- secretion (reviewed by Niv and Fraser, 2002).

The presence of a post-prandial alkaline tide in marine fish has been recorded recently in elasmobranchs (Wood et al., 2005; Wood et al., 2007), whereas evidence of an alkaline tide in marine teleosts [Gulf toadfish (Taylor and Grosell 2006); European flounder (Taylor et al., 2007)] has been elusive. However, there is now evidence that at least one teleost species does experience an alkaline tide, as freshwater rainbow trout have been shown to exhibit a post-prandial metabolic alkalosis (Bucking and Wood, 2008; Cooper and Wilson, 2008).

The confirmation of an alkaline tide in both freshwater rainbow trout and seawater elasmobranchs suggests that seawater-acclimated trout should encounter a similar disturbance in acid–base regulation following feeding. However, how this acid–base disturbance is dealt with may vary with both phylogeny and environment. There is evidence that like the marine dogfish (Wood et al., 2007), freshwater trout relieve the alkaline tide through branchial base excretion to the water (Bucking and Wood, 2008). However, considering the important role of intestinal HCO_3^- secretion in marine

osmoregulation (Wilson et al., 2002; Grosell, 2006), seawater-acclimated trout may have an additional route for the excretion of the excess metabolic base load (Wilson et al., 1996). This could in fact result in a shorter, smaller alkaline tide when compared to freshwater fish.

In the present study the effects of feeding on acid–base status and net acid–base balance were investigated in seawater steelhead trout following the force-feeding of a single commercial meal. Based on the revelation of an alkaline tide in freshwater rainbow trout that is corrected through branchial base excretion (Bucking and Wood, 2008), the overall hypothesis was that feeding would result in a systemic metabolic alkalosis that would increase both net branchial base excretion and intestinal HCO_3^- secretion in seawater trout, resulting in a smaller and shorter alkaline tide when compared to freshwater fish, with less excretion of base to the external water.

MATERIALS AND METHODS

Animals

Freshwater steelhead trout (*Oncorhynchus mykiss* Walbaum; 50–150 g body mass) were obtained from Robertson Creek Hatchery (Port Alberni, BC, Canada) and housed in outdoor tanks (~200 l), which were supplied with air and both fresh- and seawater sources at Bamfield Marine Science Station. Through periodic increases in the salinity of the water in the housing tanks (~10‰ every 2 weeks), the trout were gradually acclimated to full strength seawater [~35‰; Bamfield Marine Station seawater (Na^+ 489±5, Cl^- 539±8, K^+ 9.4±1.2, Ca^{2+} 10.8±1.1, Mg^{2+} 52±3 mmol l^{-1})] over the course of 2 months. Housing and all experiments were conducted at ambient water temperatures (~12°C) under natural photoperiods (April to July). The animals were fed every second day with a 2% body mass ration of commercial fish food excluding 3 days after each incremental increase in the salinity of the housing tanks, during which feeding was suspended. Over the course of all experiments, the trout were fed a commercial fish feed (3 point floating pellets, EWOS Pacific [Surrey, BC, Canada; (Na^+ 234±6, Cl^- 197±4, K^+ 99.2±3, Ca^{2+} 186±6, Mg^{2+} 108±5 µmol g^{-1} wet food mass; *N*=7)].

Additionally, freshwater rainbow trout (*Oncorhynchus mykiss*, 200–250 g body mass) were obtained from a commercial supplier (August; Humber Springs Trout Hatchery; Orangeville, ON, Canada) and acclimated to McMaster University laboratory conditions for a 2-week period before experimentation. The animals were held in 500 l holding tanks at a density of approximately 40 fish per tank and supplied with flow-through dechlorinated Hamilton (ON, Canada) city tap water (Na^+ 0.6, Cl^- 0.7, K^+ 0.05, Ca^{2+} 1.0, Mg^{2+} 0.1 mg l^{-1}; total hardness 140 mg l^{-1} as $CaCO_3$; pH 8.0). During the holding period, the animals were fed a 2% body mass ration of commercial floating (5 point) pellets [Martin Mills, ON, Canada (Na^+ 215±15, Cl^- 188±16, K^+ 97±2, Ca^{2+} 194±3, Mg^{2+} 109±1 µmol g^{-1} wet food mass; *N*=7)]; every 48 h. The animals were housed with a 12 h:12 h light:dark cycle. All experiments were carried out at 12°C. These freshwater rainbow trout were used during Series 3 only.

Series 1: acid–base flux to the water in fed and unfed seawater-acclimated fish

Following acclimation to full strength seawater, several trout were removed from the holding tanks immediately before (*N*=6 unfed) and shortly after (*N*=6 fed) the scheduled feeding. However, instead of the usual 2% body ration, the trout were fed to satiation (>3% body mass ration). The fish were individually netted, placed in a darkened transfer container, and transferred to individual darkened flux boxes supplied with flow-through Bamfield Marine Station seawater and perimeter aeration. Chemical anaesthesia was not utilized as it induced vomiting in previous experiments; however, the transfer from the holding tanks to the flux boxes was rapid (<45 s) to reduce stress. Subsequently, the water level was set to approximately 4 l (excluding the mass of the animal), the water flow suspended (while aeration was maintained) and an initial water sample was then taken, representing the starting flux water sample. Another water sample was then taken after 6 h of housing the fish in static water and represented the final flux sample. Following the collection of the final water sample, the box was thoroughly flushed with clean seawater by repeatedly lowering and raising the water level. This was accomplished by first raising the water level with a running seawater supply, then lowering the water level by the removal of a bung on the outside lower quadrant of the flux box. This process was repeated several times with care taken to minimize stress to the fish, before the volume was reset to 4 l. This procedure was repeated every 6 h for 42 h before the fish were returned to the holding tanks.

The starting and final water samples of each 6 h flux period were measured for titratable alkalinity and total ammonia. Titratable alkalinity was determined by the titration of 10 ml water samples past pH=3.8, using a Radiometer GK2401C glass combination electrode that was connected to a Radiometer PHM 82 standard pH meter. A Gilmont microburette was used to accurately deliver standardized acid (0.04 N HCl) until the pH of the water sample fell below pH 5.0. The water samples were then aerated for 15 min to remove CO_2 and the titration was continued until a pH of 3.8 was reached. Water total ammonia concentrations ($[NH_3]_w$+$NH_4^+]_w$) were measured by the indophenol blue method (Ivancic and Degobbis, 1984). The fluxes were then calculated from the changes in concentration from initial to final samples, factored by volume, time and trout mass. The fluxes were then expressed as either the net flux of ammonia (J_{amm}; µmol kg^{-1} h^{-1}) or the net flux of titratable alkalinity (J_{TAlk}; µmol kg^{-1} h^{-1}). Following this, the net acid–base flux was calculated as the difference between J_{TAlk} and J_{amm}, signs considered (e.g. McDonald and Wood, 1981). A net base flux to the environment (i.e. a net flux of HCO_3^- equivalents; $J_{net}OH^-$) is shown by a positive difference, while a net acid flux to the environment (i.e. a net flux of H^+ equivalents; $J_{net}H^+$) is shown by a negative difference. A previous study conducted on freshwater rainbow trout showed that the ammonia concentrations encountered during the static fluxes had no significant effect on the outcome of the experiment (Bucking and Wood, 2008).

Series 2: the alkaline tide in seawater-acclimated fish

In a separate study, after a 1 week starvation, trout (*N*=14) were anaesthetized with MS-222 (tricaine methane sulphonate; 0.07 g l^{-1}; Sigma Chemical Co., St Louis, MO, USA) and artificially ventilated on an operating table. Dorsal aortic catheters (Clay-Adams PE-50, Sparks, MA, USA) were then implanted according to the method of Soivio et al. (Soivio et al., 1972) and filled with 0.3 ml of modified Cortland saline (Na^+ 160; Cl^- 150; K^+ 5; Ca^{2+} 1, Mg^{2+} 2, glucose 5.5 mmol l^{-1}; pH 7.8) (Wolf, 1963) containing 50 i.u. ml^{-1} of lithium heparin (Sigma) and sealed. The trout were then returned to individual 15 l tanks and allowed to recover for 1 day.

Following this recovery period, the fish were force-fed a 3% body mass ration of a slurry of ground commercial trout food and seawater (*N*=7; 50:50 ratio; total dry mass ration 1.5%). The composition of the slurry was based on observations of the amount of seawater that was imbibed during a voluntary ingestion of the commercial diet by seawater steelhead trout (C.B. and C.M.W., unpublished data). Force-feeding achieved by gently placing a long catheter into the

stomach that was connected to a 20 ml syringe containing the food slurry, and injecting ~6 ml, depending on the body mass of the trout. Fish were not anaesthetized because of poor recovery in initial trials. Control fish (N=7) were sham fed a 3% body mass ration of seawater only. Blood samples (200 µl) were then taken *via* the arterial catheter into an iced and pre-heparinized gas-tight Hamilton syringe before and after (–3, 0, 3, 6, 9, 12, 18, 24 h) the force-feeding (or sham-feeding), which occurred immediately following the 0 h time point. Approximately 100 µl of the whole blood was immediately used to measure arterial blood pH (pH_a) inside a tightly sealed and thermostatted chamber using a Radiometer GK2401C glass combination electrode connected to a Radiometer PHM 82 standard pH meter. Plasma samples were then obtained from the remaining whole blood through centrifugation (13,000 ***g*** for 30 s) and were immediately assayed for total CO_2 (Ta_{CO_2}; Corning 965 Total CO_2 analyzer; Lowell, MA, USA); the remainder was placed in liquid nitrogen and stored at –80°C for later analysis of blood glucose, urea and ammonia. Through rearrangement of the Henderson–Hasselbalch equation, and using values of plasma pK' and CO_2 solubility coefficients for trout blood at 12°C (Boutilier et al., 1984), arterial plasma CO_2 tension (Pa_{CO_2}) and bicarbonate concentration ($[HCO_3^-]_a$) were calculated.

Blood plasma was also analyzed for changes in ammonia, urea and glucose. Both plasma total ammonia (T_{amm}) and plasma total glucose ($[glucose]_p$ were measured enzymatically. T_{amm} measurement was based on the glutamate dehydrogenase/NAD method using a commercial kit (Raichem; San Diego, CA, USA), and $[glucose]_p$ was quantified through the hexokinase/glucose-6-phosphate dehydrogenase assay (Sigma, 301A). A colorimetric assay modified from Rahmatullah and Boyde (Rahmatullah and Boyde, 1980) was used to measure plasma total urea ($[urea]_p$). All samples were read on a microplate reader (SpectraMax 340PC; Sunnyvale, CA, USA) at appropriate wavelengths.

Series 3: *in vitro* intestinal gut sacs from seawater- and freshwater-acclimated fish

In a separate experiment, the intestinal tract was removed from unfed (fasted for 1 week to clear the GI tract of undigested food; N=7) or fed seawater-acclimated fish (12 h following meal ingestion, commercial fish feed, 3% body ration; N=7) and then flushed with saline to remove the contents (food, faeces, etc.). Each intestinal section (anterior, mid and posterior excluding the rectum) was identified based on morphological characteristics. The posterior end of each section was then ligated with double silk ligatures and each section was separated. The intestinal sections were then rinsed with saline, and a subsample of the rinse solution was taken for analysis. A short piece of heat flared PE 50 tubing was inserted into the anterior end of each section, which was then ligated at the anterior end with double silk sutures, forming a 'sac'. The PE 50 tube served to fill each sac preparation with a physiological saline ('gut' saline), and was later used to drain each section as well. The intestinal gut sacs were then blotted dry externally, weighed and incubated in saline equilibrated to 99.7% O_2, 0.3% CO_2 (12 ml) for 2 h. Following incubation, the gut sacs were removed from the saline, blotted dry and reweighed. The internal saline was then drained from the sacs, which were subsequently cut open by a longitudinal incision and the gross surface area was obtained by tracing the outline onto graph paper (Grosell and Jensen, 1999). The internal saline was measured for changes in pH (Radiometer GK2401C glass combination electrode connected to a Radiometer PHM 82 standard pH meter), Cl^- (chloridometer; CMT 10 Chloride Titrator, Radiometer, Copenhagen, Denmark) and total CO_2 (Corning 965 Total CO_2 analyzer). The $[HCO_3^-]+[2CO_3^{2-}]$ was calculated according to rearrangement of the Henderson–Hasselbalch equation and the carbonic acid pK' values at one-third the strength of seawater at 12°C. A previous study (Grosell et al., 1999) found that this method yielded identical results to those determined using the double-end point titration method (Hills, 1973).

The standard mucosal saline used for gut sac incubations ('gut saline') consisted of the following (in mmol l^{-1}): 100 NaCl, 50 $MgSO_4$, 35 $MgCl_2$, 5 $CaCl_2$, 5 KCl, 1 $KHCO_3$ (pH 7.4) (Wilson et al., 1996); the serosal saline was modified Cortland saline (143 NaCl, 5 KCl, 1 $CaCl_2{\cdot}2H_2O$, 1.9 $MgSO_4\,7H_2O$, 11.9 $NaHCO_3$, 2.9 $NaH_2PO_4{\cdot}H_2O$, glucose 5.5 mmol l^{-1}; pH 7.8) (Wolf, 1963).

Fluid transport rates (FTR; µl cm^{-2} h^{-1}) were calculated as:

$$\text{Fluid transport rate (FTR)} = \frac{(M_1 - M_2)}{ISA \times \text{T}}\ , \qquad (1)$$

where M_1 was the initial mass of the gut sac (mg), M_2 was the final mass of the gut sac (mg), ISA was the intestinal surface area (cm^2) as estimated by tracing the outline of the GI tract section onto graph paper (Grosell and Jensen, 1999), and T was the time (h). This calculation provided the amount of fluid that was transported from the gut saline to the serosal saline.

The Cl^- absorption rate (µmol cm^{-2} h^{-1}) was calculated as:

$$Cl^- \text{ absorption rate} = \frac{(V_1 \times G_{Cl1}) - (V_2 \times G_{Cl2})}{ISA \times \text{T}}\ , \qquad (2)$$

where V_1 was the initial volume of injected fluid and G_{Cl1} was the concentration of Cl^- (µmol ml^{-1}) in the gut saline at the start of the flux. V_2 was the final volume of the gut fluid and G_{Cl2} was the concentration of Cl^- (µmol ml^{-1}) in the final flux sample (ISA and T were defined above). This calculation gave the absorption of Cl^- from the mucosal saline as a function of intestinal area over the course of the gut sac incubation, taking into account the changing volume of the system.

HCO_3^- ($+2CO_3^{2-}$) secretion rates were calculated by first calculating the concentration of HCO_3^- and $2CO_3^{2-}$ in the starting gut saline and final mucosal saline collected from the gut sacs by rearranging the Henderson–Hasselbalch equation. The HCO_3^- ($+2CO_3^{2-}$) secretion rate (µmol cm^{-2} h^{-1}) was then calculated as:

$$HCO_3^- (+2CO_3^{2-}) \text{ secretion rate} = \frac{(V_2 \times G_{HCO_3^-}\text{f}) - (V_1 \times G_{HCO_3^-}\text{i})}{ISA \times \text{T}}\ , \qquad (3)$$

where $G_{HCO_3^-}$f is the concentration of HCO_3^- ($+2CO_3^{2-}$; µmol ml^{-1}) in the gut saline at the end of the flux and $G_{HCO_3^-}$i is the concentration of HCO_3^- ($+2CO_3^{2-}$; µmol ml^{-1}) in the initial flux sample (V_1, V_2, ISA, T were defined above). This provided the amount of HCO_3^- ($+2CO_3^{2-}$) that was secreted into the mucosal saline as a function of intestinal area over the course of the gut sac incubation, taking into account the changing volume of the system.

Series 3 was repeated on freshwater-acclimated rainbow trout at McMaster University.

Statistics

Data are means ± s.e.m. (N is the number of fish), unless otherwise stated. All data passed normality and homogeneity tests before statistical analyses were performed using SigmaStat (version 3.1). Temporal changes in plasma ionic composition, T_{amm}, $[glucose]_p$, and $[urea]_p$ were examined with a one-way ANOVA, whereas the temporal changes in J_{Tamm}, J_{TAlk}, $J_{net}OH^-$ and $J_{net}H^+$, pH_a, $[HCO_3^-]_a$

and Pa_{CO_2}, were examined with a repeated measures two-way ANOVA. All of the statistical tests were followed by a HSD *post-hoc* test (Tukey's honest significant difference) or a Bonferroni's correction as appropriate. Effects of feeding on *in vitro* pH, fluid transport rates, Cl^- absorption rates and HCO_3^- secretion rates were determined using a paired Student's *t*-test. Values were considered significantly different at $P<0.05$.

RESULTS

Post-prandial plasma concentrations

Feeding had little effect on $[urea]_p$ (3.5±1.2 μmol ml^{-1}; N=49) and $[glucose]_p$ (4.8±1.1 μmol ml^{-1}; N=49) in seawater-acclimated trout and both were maintained throughout the experimental period. Feeding did, however, have a significant effect on plasma ammonia levels. Prior to feeding, control levels of T_{amm} were 121±44 μmol l^{-1}. A prolonged, approximately threefold increase in T_{amm} was observed following the meal, which remained significantly elevated between 4 and 24 h following feeding, before returning to control values at 48 h (Fig. 1).

Acid–base regulation during feeding

Confined unfed fish exhibited an average net J_{amm} to the water of –375±12 μmol kg^{-1} h^{-1} (N=42), that remained unchanged over the course of confinement (Fig. 2A). In addition, unfed fish showed a net J_{TAlk} to the water of –135±19 μmol kg^{-1} h^{-1} (N=42) that remained consistent over time (Fig. 2B). By contrast, significantly elevated J_{amm} was observed between 0 and 36 h after fish feeding, peaking between 18 and 24 h post-feed at –901±154 μmol kg^{-1} h^{-1} (N=6; Fig. 2A). However, the J_{TAlk} of fed fish was variable, and was elevated only for two brief periods (6–12 h and 30–36 h^{-1}; Fig. 2B). Otherwise, the J_{TAlk} of fed fish was not significantly different from unfed fish. When the net acid–base flux was examined, unfed fish exhibited a net acid flux to the water (–240±18 μmol kg^{-1} h^{-1}; N=42; Fig. 2C). Feeding significantly increased the net acid excretion to the water over the first 24 h after feeding (Fig. 2C) before net acid flux returned to control values. Integrating the area between the fed and fasted curves in Fig. 2C, the excess net efflux of acidic equivalents in the fed trout was ~8490 μmol kg^{-1} over the course of digestion, equivalent to the uptake of 8490 μmol kg^{-1} of basic equivalents from the external seawater.

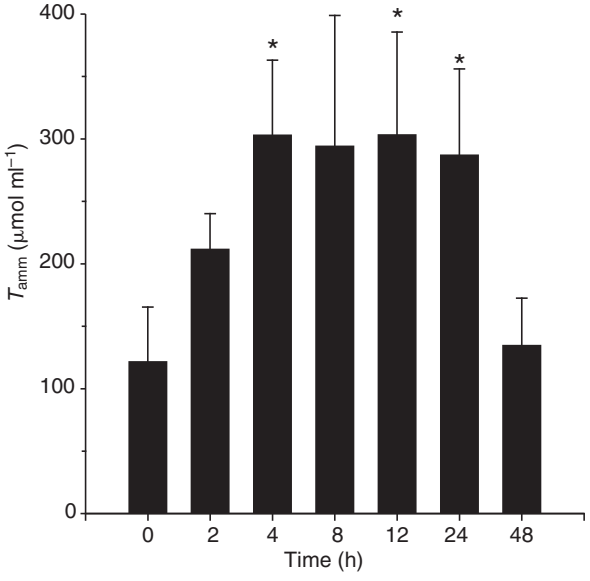

Fig. 1. Effect of feeding on plasma total ammonia concentration (T_{amm}; μmol ml^{-1}). Feeding occurred immediately after 0 h. Values are means ± s.e.m.; N=7. *A significant difference from the control value (0 h).

pH_a was not significantly affected by sham feeding and was maintained at an average of 7.85±0.02 (N=56; Fig. 3A). However, force-feeding did significantly increase pH_a between 3 and 12 h after manipulation, peaking at 8.01±0.04 (N=7; Fig. 3A), before returning to control values. This increase in pH_a was accompanied by an increase in $[HCO_3^-]_a$ (Fig. 3B), which was elevated by 2–3 mmol kg^{-1} between 3 and 12 h. $[HCO_3^-]_a$ in unfed fish was maintained unchanged at an average of 7.5±0.2 mmol (N=56; Fig. 3B). By contrast, Pa_{CO_2} was unaffected by feeding and no difference existed between fed and unfed fish (2.36±0.06 mmol Hg, N=98; data not shown).

In vitro effect of feeding on pH, Cl^- and HCO_3^- transport by the intestine of seawater- and freshwater-acclimated fish

In seawater-acclimated trout, feeding greatly increased HCO_3^- secretion in gut sacs from all three intestinal sections (the anterior from 0.21 to 0.99 μmol cm^{-2} h^{-1}, the mid from 0.11 to 0.75 μmol cm^{-2} h^{-1}, and the posterior from 0.12 to 0.49 μmol cm^{-2} h^{-1}, respectively) (Fig. 4), By contrast, in freshwater-acclimated trout, $[HCO_3^-]$ secretion remained unchanged in all three sections (Fig. 5). This increase in HCO_3^- secretion by seawater-acclimated fish was accompanied by a significant increase in Cl^- absorption in the anterior and posterior intestine only (from 5.3 to 6.1 μmol cm^{-2} h^{-1}, and 2.9 to 4.2 μmol cm^{-2} h^{-1}, 15–45% increases; Fig. 4). Interestingly, in seawater fish feeding significantly stimulated fluid absorption rates with about 60–130% increases in all three intestinal sections (Fig. 4). by contrast, fluid absorption was generally higher in unfed freshwater fish, a trend that was significant in the mid intestine (Fig. 5). In general, rates of HCO_3^-, Cl^-, and fluid transport in the gut sacs of fed and unfed freshwater fish were comparable to unfed rates in seawater fish.

DISCUSSION

In contrast to two previous studies on Gulf toadfish (Taylor and Grosell, 2006) and European flounder (Taylor et al., 2007), the present study on seawater-acclimated steelhead trout provides the first evidence of a post-prandial alkaline tide in a seawater teleost. Reasons for the difference may be phylogenetic or methodological (e.g. differences in food quality, amount, or administration, differences in blood sampling techniques), and are worthy of future investigation. Our original hypotheses were that feeding would increase both net branchial base excretion and intestinal HCO_3^- secretion in seawater trout, resulting in a smaller and shorter alkaline tide when compared with freshwater trout, with less excretion of base to the external water. With the exception of increased intestinal HCO_3^- secretion following feeding, these hypotheses were not confirmed, as discussed below.

In the seawater trout plasma T_{amm}, pH_a and $[HCO_3^-]_a$ were disturbed by feeding (Fig. 1; Fig. 3A,B), in a similar manner as seen in freshwater-acclimated specimens of the same species (Bucking and Wood, 2008). The mechanism of HCl acid secretion would presumably not change during acclimation to seawater, hence the presence of an alkaline tide in the bloodstream of seawater fish is not surprising. However, an elevation of net H^+ excretion at the gills following feeding (i.e. substantial net base uptake from the water) was certainly unexpected (Fig. 2C). In freshwater, the alkaline tide was excreted to the environment, a response that was postulated to prevent a blood alkalosis that would have produced blood pH

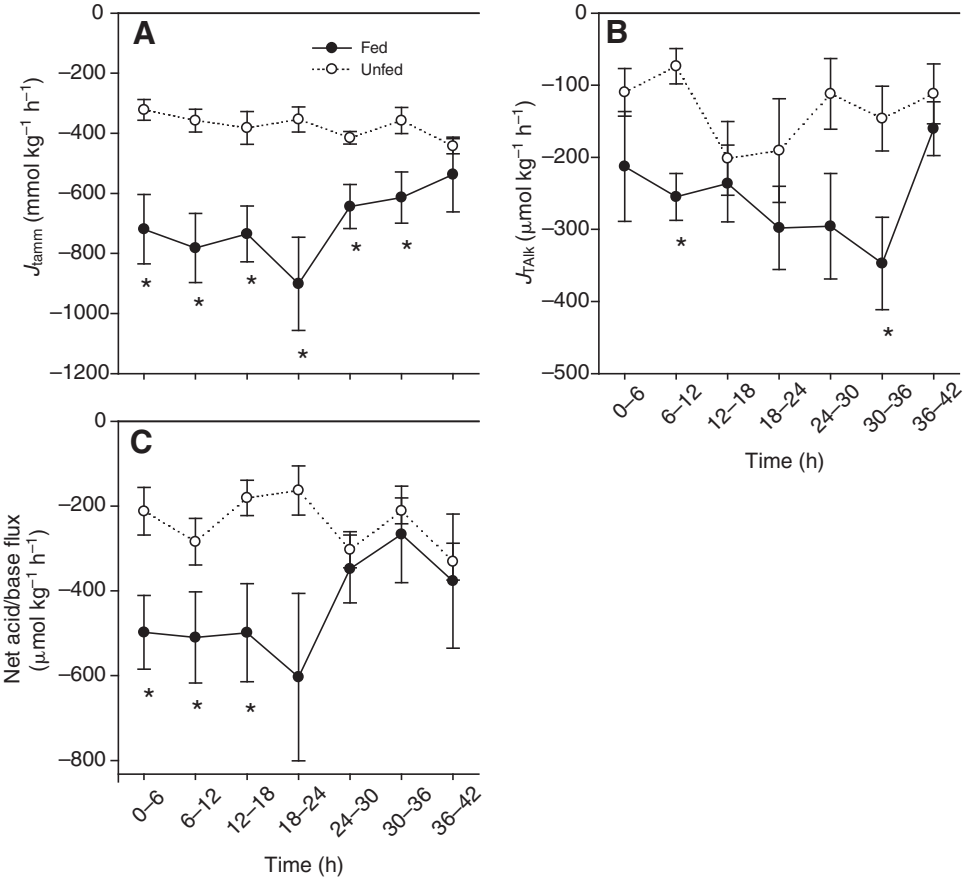

Fig. 2. (A) Net flux of ammonia to the water (J_{amm}; μmol kg^{-1} h^{-1}) from unfed (open circles) and fed (closed circles) seawater-acclimated steelhead trout. Feeding occurred immediately after 0 h sampling. Values are means ± s.e.m.; *N*=7. *Significant difference between fed and unfed fish at the same time point. (B) Net flux of titratable alkalinity to the water (J_{TAlk}; μmol kg^{-1} h^{-1}) from unfed (open circles) and fed (closed circles) seawater-acclimated steelhead trout. Feeding occurred immediately after 0 h sampling. Values are means ± s.e.m.; *N*=7. *Significant difference between fed and unfed fish at the same time point. (C) The overall net acid or base flux to the water from unfed (open circles) and fed (closed circles) seawater-acclimated steelhead trout. Data were calculated from the difference between the values in A and B. Values are means ± s.e.m.; *N*=7. *Significant difference between fed and unfed fish at the same time point. Negative values indicate a net acid flux ($J_{net}H^+$).

values close to 8.55 (Bucking and Wood, 2008). By contrast, fed seawater fish excreted ~8490 μmol kg^{-1} more acid to the external water (Fig. 2C) resulting in an additional uptake of ~8490 μmol kg^{-1} base units. This could account for the prolongation of the metabolic alkalosis in the bloodstream of seawater fish for up to 18 h (Fig. 3B) when compared with freshwater fish (Bucking and Wood, 2008). The reduction (but not full correction) in pH_a and $[HCO_3^-]_a$ by 12 h post-feeding in seawater trout, suggests that the base load was being removed from the blood, most likely by the intestine and certainly not by excretion into the water *via* the gills or kidney. Although the role of the kidney was not directly examined, its contribution to acid–base flux was probably very small in seawater trout because of the low urine flow rate in seawater teleosts (McDonald et al., 1982). The base excreted into the intestine would probably precipitate with Ca^{2+} to form $CaCO_3$, mixed into or coating the faecal material. When this solid material is excreted to the environment, it would be undetectable as a dissolved base, explaining the absence of an increased base efflux detected in the water. Wilson et al. (Wilson et al., 1996) succeeded in collecting the rectal output from trout in seawater, and were able to demonstrate that net base excretion through the rectum quantitatively matched net acid excretion through the gills, though they were able to do this in unfed fish only.

We speculate that the HCO_3^- added to the blood plasma across the basolateral surface of the gastric cells (associated with the apical secretion of HCl into the chyme) becomes fuel for the apical exchange for Cl^- in the enterocytes of the intestine. For HCO_3^- to enter the enterocyte it would either have to be transported across the basolateral membrane *via* a transporter (e.g. the sodium bicarbonate co-transporter; NBC) (Grosell and Genz, 2006) or become dehydrated to CO_2 in the blood, enter the enterocyte diffusively and become rehydrated intracellularly with a compensating net extrusion of H^+ across the basolateral membranes. Recent studies indicate that the majority of HCO_3^- excreted into the intestinal lumen is in fact from the hydration of intracellular CO_2 (Grosell and Genz, 2006), but this conclusion is based on unfed marine teleosts. The source of the HCO_3^- generated during active digestion is unknown. The present data on the *in vitro* net secretion of HCO_3^- into each intestinal section indicate that the rates are greatly increased at this time (Fig. 4). This probably relates to increased need for neutralization of HCl coming down the tract from the stomach, as well as perhaps the need for increased Cl^- and fluid absorption through the intestinal wall after feeding. Using an estimate of the intestinal surface area of each section for a 250 g rainbow trout (Nadella et al., 2006), intestinal base secretion could have accounted for approximately 186 μmol HCO_3 kg^{-1} h^{-1} or 7810 μmol HCO_3 kg^{-1} over the 42 h of digestion in this study. This would be approximately sufficient to relieve the additional base load (8490 μmol HCO_3 kg^{-1}) taken up from the water at the gills during this period. However, the gut sac technique utilized may have over- or underestimated the net transport of HCO_3^- occurring in the post-prandial period.

The 'gut sac' technique used allowed for relatively easy measurements of intestinal Cl^-, HCO_3^- and water transport and eliminated complications encountered *in vivo*. It has been well documented (e.g. Barthe et al., 1999; Grosell and Jensen, 1999; Handy et al., 2000; Bury et al., 2001; Grosell et al., 2005) that gut sacs maintain tissue viability for prolonged periods and give reliable data in spite of being a closed system. However, the technique provides only an estimate of transport processes occurring. The

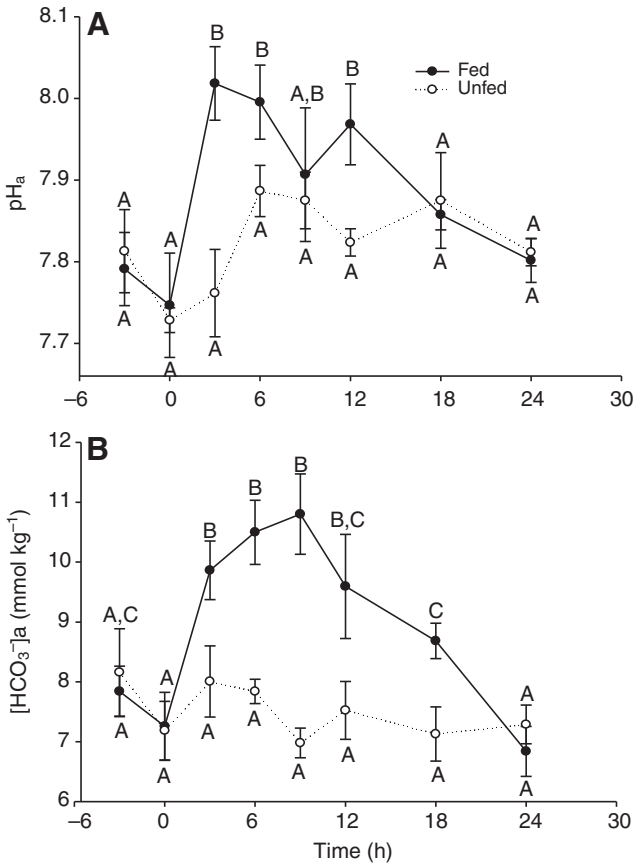

Fig. 3. (A) Arterial blood pH (pH_a) in unfed (open circles) and fed (filled circles) seawater-acclimated steelhead trout. Feeding occurred immediately after 0 h sampling. Values are means ± s.e.m.; *N*=7. Time points with the same letter are not significantly different. (B) Arterial plasma bicarbonate concentration ($[HCO_3^-]_a$) in unfed (open circles) and fed (filled circles) seawater-acclimated steelhead trout. Feeding occurred immediately after 0 h sampling. Values are means ± s.e.m.; *N*=7. Time points with the same letter are not significantly different.

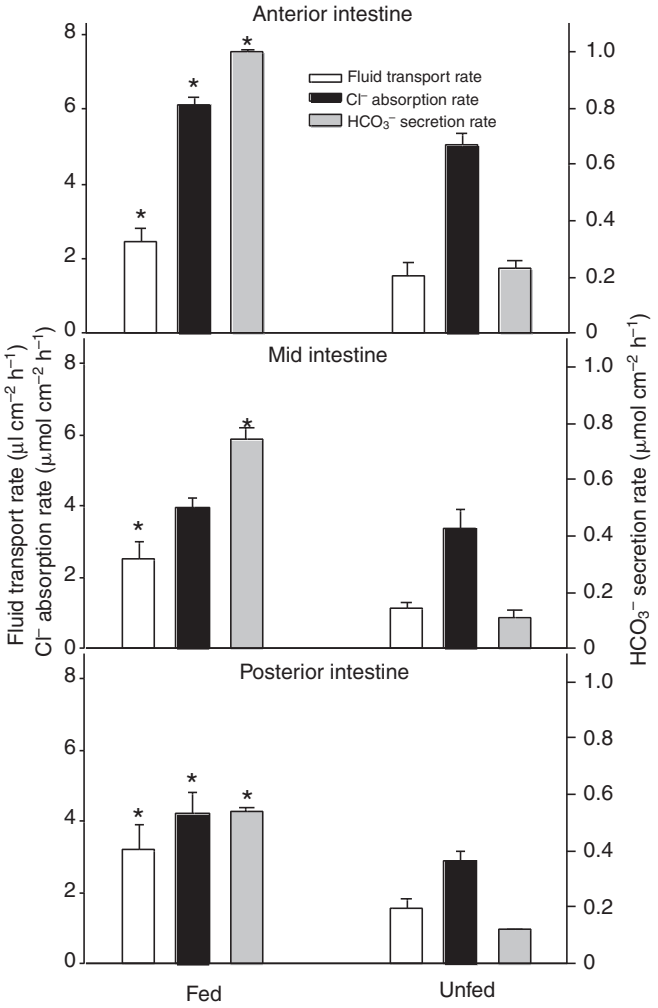

Fig. 4. *In vitro* fluid transport rate (µl cm^{-2} h^{-1}) and Cl^- absorption rate (µmol cm^{-2} h^{-1}) from the mucosal saline, and the HCO_3^- secretion rate (µmol cm^{-2} h^{-1}) into the mucosal saline of intestinal gut sacs from fed and unfed seawater-acclimated steelhead trout. Values are means ± s.e.m.; *N*=7. *Significant difference between fed and unfed samples.

removal of the interactions with dietary components, hormones associated with digestion, neural inputs, and blood supply to the tissue, all of which create an easier system to study, may at the same time affect the comparability of the data obtained *in vitro* and *in vivo*. Nevertheless, we were able to detect a marked effect of feeding, and a marked difference between seawater and freshwater fish with this approach.

Apart from the marked acid–base disturbance, only one perturbation in plasma composition was observed throughout the experiment (T_{amm}; Fig. 1). Additionally, ion composition of plasma appears to be tightly regulated during feeding in both seawater (C.B. and C.M.W., unpublished data; Taylor and Grosell, 2006) and freshwater, where only brief transient changes occurred in plasma Na^+, Ca^{2+} and Mg^{2+} concentrations (Bucking and Wood, 2006a; Bucking and Wood, 2006b; Bucking and Wood, 2007) despite large-scale absorption of the majority of ions, and large concentration gradients from chyme to blood plasma. This suggests that branchial, renal and other homeostatic systems tightly regulate the plasma composition during feeding. The complicated interactions between the GI tract and numerous other organs, suggests organism-wide alterations in homeostatic regulation during feeding. These interactions could be controlled by hormonal signals. For example, feeding has been associated with increases in blood cortisol when compared to fasted freshwater rainbow trout (Polakof et al., 2007) and channel catfish (Small, 2005). Additionally, Wood et al. (Wood et al., 2008) argued that the alkaline tide in elasmobranchs could provide a systemic signal to activate post-prandial changes in metabolism and ion and acid–base transport processes. Clearly, feeding and digestion in fish affects numerous physiological processes, creating large and repetitive alterations in homeostasis that fish must endure and/or exploit.

Comparisons with freshwater rainbow trout

It is of interest to directly compare the current data set on seawater steelhead trout with earlier studies on the freshwater rainbow trout.

The ingestion of a somewhat larger meal in freshwater trout (5% ration *versus* 3% ration in the present study) resulted in a transient increase in T_{amm}, that was similar in magnitude and duration (Bucking and Wood, 2008) to the results of the current study. This resulted in, on average, a 1.5- to 3-fold post-prandial increase in

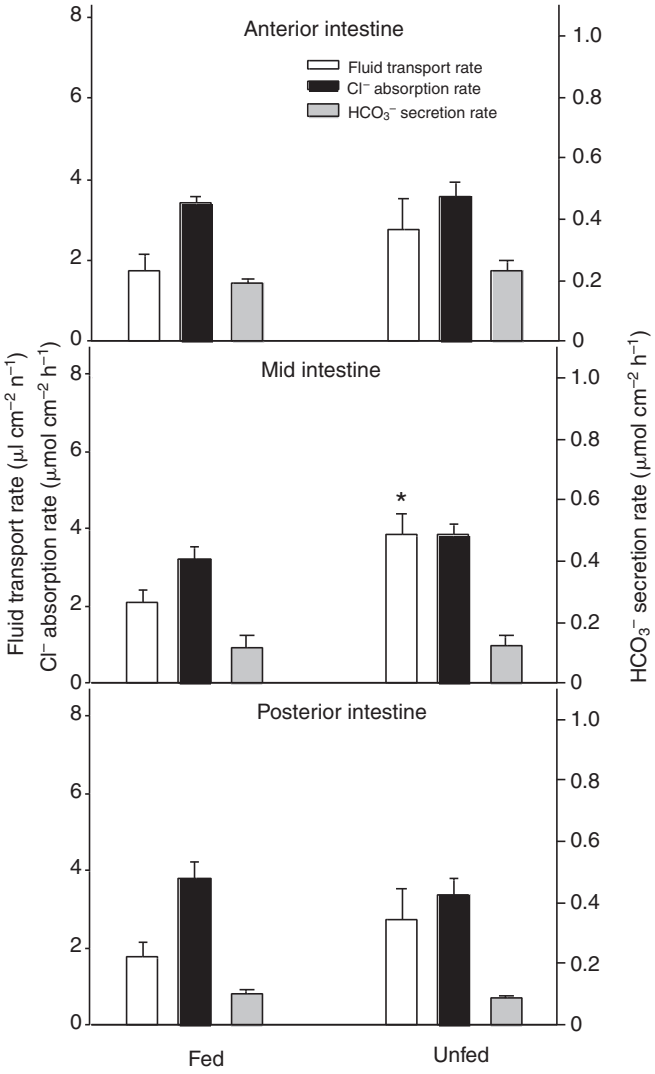

Fig. 5. *In vitro* fluid transport rate (µl cm^{-2} h^{-1}) and Cl^- absorption rate (µmol cm^{-2} h^{-1}) from the mucosal saline, and the HCO_3^- secretion rate (µmol cm^{-2} h^{-1}) into the mucosal saline of intestinal gut sacs from fed and unfed freshwater-acclimated rainbow trout. Values are means ± s.e.m.; *N*=7. *Significant difference between fed and unfed samples.

the net branchial excretion of ammonia to the external environment in both freshwater and seawater. Additionally, freshwater rainbow trout experienced an increase in pH_a and $[HCO_3^-]_a$, resulting in an increase in titratable base excretion to the water. The increase in titratable base excretion exceeded the increase in the net ammonia secretion, resulting in an overall net branchial base flux to the water (Table 1) (Bucking and Wood, 2008); however, this may depend on environmental Cl^- levels (Cooper and Wilson, 2008). The route of excretion is believed to be primarily branchial, though the kidney can make a significant contribution to the regulation of metabolic alkalosis in freshwater trout (Wheatley et al., 1984; Wood et al., 1999). The role of the kidney in the alleviation of the alkaline tide, and urinary acid and/or base excretion following feeding remain to be investigated.

By contrast, seawater teleosts exhibited a more prolonged metabolic alkalosis. Like freshwater trout, they also showed an increase in net titratable base excretion to the water; however, it was of a lower magnitude than the net ammonia secretion,

Table 1. Contributions of various routes to net acid or base excretion during digestion in freshwater and seawater trout

	Freshwater	Seawater
Gill	–13,890	+8490
Intestine	0	–7810
Total	–13,890	+680

Values are µmol kg^{-1} h^{-1}. Negative values indicate net base excretion; positive values indicate net acid excretion.

resulting in a net acid flux to the water (i.e. base uptake; Table 1). The route of base uptake is undoubtedly branchial, as seawater fish exhibit a reduced glomerular filtration rate, which minimizes renal water loss (Nishimura and Imai, 1982) and the capacity for urinary acid–base excretion (McDonald et al., 1982). This indicates that although a fed seawater fish excreted ~8490 µmol kg^{-1} more acid than an unfed fish, it took up ~8490 µmol kg^{-1} of base at the gill during digestion, a surprising result considering the existing metabolic base load in the blood. The *in vitro* study suggested that the intestine is largely responsible for the removal of the metabolic base load in seawater-acclimated fish whereas the role of the intestine in freshwater fish appears to be minimal (Table 1).

Plasma alkalinization of freshwater fish was shorter in duration and smaller in magnitude than that of seawater fish (pH_a increased between 6 and 12 h in freshwater and between 3 and 12 h in seawater, whereas $[HCO_3^-]$ increased between 3 and 12 h in freshwater and between 3 and 18 h in seawater), in direct opposition to the original hypothesis. This may reflect the fact that the post-prandial net base uptake from the water in the seawater trout, in contrast to the increased base excretion to the water in the freshwater trout, augments the systemic alkaline tide. This assumes that there is a temporal and/or quantitative mismatch between the rates of base uptake at the gills and stomach *versus* the rates of secretion at the intestine.

However, there is a confounding methodological issue. The fish in the present study were force fed, a technique that has been shown to increase the size (by 2-fold) and duration (by 48 h) of the alkaline tide in freshwater rainbow trout, possibly because of the short circuiting of the natural feeding process (Cooper and Wilson, 2008). This would suggest that force-fed seawater-acclimated trout should have experienced an even greater alkaline tide than observed, and suggests that intestinal base secretion is able to greatly reduce the systemic base load, be it either gastric or branchial in origin. Additionally, the rate and quantity of gastric acid production in both studies is unknown. A comparison of the gastric pH profiles of freshwater rainbow trout (Bucking and Wood, 2008) with those of seawater steelhead trout (C.B. and C.M.W., unpublished data), fed similar rations of similar commercial meals, indicates that the rate and amount of acid produced are circumstantially similar, if pH is used as a proxy measure of protons secreted.

Additionally, rainbow trout and steelhead trout are the freshwater and anadromous forms, respectively, of *Oncorhynchus mykiss* and most evidence does not support a taxonomic distinction between steelhead and rainbow trout at either the species or subspecies level (McCusker et al., 2000; Docker and Heath, 2003). However, it can be argued that different rearing environments of the two fish populations used in the above studies, as well as time separation of the populations, may have resulted in genetic differences (McCusker et al., 2000; Docker and Heath, 2003). Although lineage differences may have had confounding effects on the results seen, the extent of these genetic variations, as well as their contributions to the differing

responses to the alkaline tide seen in rainbow and steelhead trout, is unknown.

Conclusions

Feeding in seawater resulted in a metabolic alkalosis in seawater-acclimated steelhead trout that was slightly larger and longer in duration than in freshwater rainbow trout. This could reflect the differing mechanisms of compensation, as freshwater fish rapidly increase net base excretion to the water at the gills, whereas the mechanism in seawater trout appears to involve base uptake at the gills and secretion by the intestine.

All experiments conformed to CACC guidelines and were conducted under approved animal use protocols at both McMaster University and Bamfield Marine Sciences Center. C.M.W. is supported by an NSERC Discovery Grant as well as the Canada Research Chair Program. C.B. is supported by an NSERC CG Scholarship.

REFERENCES

Barthe, L., Woodley, J. and Houin, G. (1999). Gastrointestinal absorption of drugs: methods and studies. *Fundam. Clin. Pharmacol.* **13**, 154-168.

Boutilier, R. G., Heming, T. A. and Iwama, G. K. (1984). Appendix-physicochemical parameters for use in fish respiratory physiology. *Fish Physiol. Biochem.* **10**, 403-430.

Bucking, C. and Wood, C. M. (2006a). Water dynamics in the digestive tract of the freshwater rainbow trout during the processing of a single meal. *J. Exp. Biol.* **209**, 1883-1893.

Bucking, C. and Wood, C. M. (2006b). Gastrointestinal processing of Na^+, Cl^-, and K^+ during digestion: implications for homeostatic balance in freshwater rainbow trout. *Am. J. Physiol.* **291**, R1764-R1772.

Bucking, C. and Wood, C. M. (2007). Gastrointestinal transport of Ca^{2+} and Mg^{2+} during the digestion of a single meal in the freshwater rainbow trout. *J. Comp. Physiol. B* **177**, 349-360.

Bucking, C. and Wood, C. W. (2008). The alkaline tide and ammonia excretion after voluntary feeding in freshwater rainbow trout. *J. Exp. Biol.* **211**, 2533-2541.

Bucking, C. and Wood, C. W. (2009). The effect of postprandial changes in pH along the gastrointestinal tract on the distribution of ions between the solid and fluid phases of chyme in rainbow trout. *Aquacult. Nut.* **15**(3), 282-296.

Bury, N. R., Grosell, M., Wood, C. M., Hogstrand, C., Wilson, R. W., Rankin, J. C., Busk, M., Lecklin, T. and Jensen, F. B. (2001). Intestinal iron uptake in the European flounder (*Platichthys flesus*). *J. Exp. Biol.* **204**, 3779-3787.

Cooper, C. A. and Wilson, R. W. (2008). Post-prandial alkaline tide in freshwater rainbow trout: effects of meal anticipation on recovery from acid-base and ion regulatory disturbances. *J. Exp. Biol.* **211**, 2542-2550.

Demarest, J. R., Loo, D. D. F. and Sachs, G. (1989). Activation of apical chloride channels in the gastric oxyntic cell. *Science* **245**, 402-404.

Docker, M. F. and Heath, D. D. (2003). Genetic comparison between sympatric anadromous steelhead and freshwater resident rainbow trout in British Columbia, Canada. *Conserv. Genet.* **4**, 227-231.

Grosell, M. (2006). Intestinal anion exchange in marine fish osmoregulation. *J. Exp. Biol.* **209**, 2813-2827.

Grosell, M. and Genz, J. (2006). Ouabain-sensitive bicarbonate secretion and acid absorption by the marine teleost fish intestine play a role in osmoregulation. *Am. J. Physiol.* **291,** R1145-R1156.

Grosell, M. and Jensen, F. B. (1999). NO_2^- uptake and HCO_3^- excretion in the intestine of the European flounder (*Platichthys flesus*). *J. Exp. Biol.* **202**, 2103-2110.

Grosell, M., DeBoeck, G., Johannsson, O. and Wood, C. M. (1999). The effects of silver on intestinal ion and acid-base regulation in the marine teleost fish *Parophrys vetulus*. *Comp. Biochem. Physiol. C* **124**, 259-270.

Grosell, M., Wood, C. M., Wilson, R. W., Bury, N. R., Hogstrand, C., Rankin, C. and Jensen, F. B. (2005). Bicarbonate secretion plays a role in chloride and water absorption in the European flounder intestine. *Am. J. Physiol.* **288,** R936-R946.

Handy, R. D., Musonda, M. M., Philips, C. and Fella, S. J. (2000). Mechanisms of gastrointestinal copper absorption in the African walking catfish: copper dose-effects and a novel anion-dependent pathway in the intestine. *J. Exp. Biol.* **203**, 2365-2377**.**

Hills, H. G. (1973). *Acid-Base Balance: Chemistry, Physiology, Pathophysiology*. Baltimore, MD: Williams and Wilkins.

Ivancic, I. and Degobbis, D. (1984). An optimal manual procedure for ammonia analysis in natural waters by the indophenol blue method. *Water Res.* **18**, 1143-1147.

McCusker, M. R., Parkinson, E. and Taylor, E. B. (2000). Mitochondrial DNA variation in rainbow trout (*Oncorhynchus mykiss*) across its native range: testing biogeographical hypotheses and their relevance to conservation. *Mol. Ecol.* **99**, 2089-2108.

McDonald, D. G. and Wood, C. M. (1981). Branchial and renal acid and ion fluxes in the rainbow trout, Sa*lmo gairdneri*, at low environmental pH. *J. Exp. Biol.* **93**, 101-118.

McDonald, D. G., Walker, R. L., Wilkes, P. R. H. and Wood, C. M. (1982). H^+ excretion in the marine teleost, *Parophrys vetulus*. *J. Exp. Biol.* **98**, 403-414.

Nadella, S. R., Grosell, M. and Wood, C. M. (2006). Physical characterization of high-affinity gastrointestinal Cu transport *in vitro* in freshwater rainbow trout *Oncorhynchus mykiss*. *J. Comp. Physiol. B* **176**, 793-806.

Nishimura, H. and Imai, M. (1982). Control of renal function in fresh water and marine teleosts. *Fed. Proc.* **41**, 2355-2360.

Niv, Y. and Fraser, G. M. (2002). Esophageal and gastric diseases; the alkaline tide phenomenon. *J. Clin. Gastroenterol.* **35**, 5-8.

Polakof, S., Ceinos, R. M., Fernandez-Duran, B., Miguez, J. M. and Soengas, J. L. (2007). Daily changes in parameters of energy metabolism in brain of rainbow trout: dependence on feeding**.** *Comp. Biochem. Phyisol. A* **146**, 265-273.

Rahmatullah, M. and Boyde, T. R. C. (1980). Improvements in the determination of urea using diacetyl monoxime: methods with and without deproteinization. *Clin. Chim. Acta* **107**, 3-9.

Small, B. C. (2005). Effect of fasting on nychthemeral concentrations of plasma growth hormone (GH), insulin-like growth factor I (IGF-I), and cortisol in channel catfish (*Ictalurus punctatus*). *Comp. Biochem. Physiol.* **142B**, 217-223.

Soivio, A., Westman, K. and Nyholm, K. (1972). Improved method of dorsal aorta catheterization: haematological effects followed for 3 weeks in rainbow trout (*Salmo gairdneri*). *Finn. Fish Res.* **1**, 11-21.

Taylor, J. R. and Grosell, M. (2006). Feeding and osmoregulation: dual function of the marine teleost intestine. *J. Exp. Biol.* **209**, 2939-2951.

Taylor, J. R., Whittamore, J. M., Wilson, R. W. and Grosell, M. (2007). Postprandial acid-base balance and ion regulation in freshwater and seawater-acclimated European flounder, *Platichthys flesus*. *J. Comp. Physiol. B* **177**, 597-608.

Wheatly, M. G., Hobe, H. and Wood, C. M. (1984). The mechanisms of acid-base and ionoregulation in the freshwater ranbow trout during environmental hyperoxia and subsequent normoxia II. The role of the kidney. *Respir. Physiol.* **55**, 155-173.

Wilson, R. W., Gilmour, K. M., Henry, R. P. and Wood, C. M. (1996). Intestinal base excretion in the seawater-adapted rainbow trout: a role in acid-base balance? *J. Exp. Biol.* **199**, 2331-2343.

Wilson, R. W., Wilson, J. M. and Grosell, M. (2002). Intestinal bicarbonate secretion by marine teleost fish – why and how? *Biochim. Biophys. Acta* **1566**, 182-193.

Wolf, K. (1963). Physiological salines for freshwater teleosts. *Prog. Fish Cult.* **25**, 135-140.

Wood, C. M., Kajimura, M., Mommsen, T. P. and Walsh, P. J. (2005). Alkaline tide and nitrogen conservation after feeding in an elasmobranch (*Scualus acanthias*). *J. Exp. Biol.* **208**, 2693-2705.

Wood, C. M., Milligan, L. M. and Walsh, P. J. (1999). Renal responses of trout to chronic respiratory and metabolic acidosis and metabolic alkalosis. *Am. J. Physiol.* **277,** R482-R492.

Wood, C. M., Bucking, C. P., Fitzpatrick, J. and Nadella, S. R. (2007). The alkaline tide goes out and the nitrogen stays in after feeding in the dogfish shark, *Squalus acanthias*. *Respir. Physiol. Neurobiol.* **159**, 163-170.

Wood, C. M., Kajimura, M., Mommsen, T. P. and Walsh, P. J. (2008). Is the alkaline tide a signal to activate metabolic or ionoregulatory enzymes in the dogfish shark (*Squalus acanthias*)? *Physiol. Biochem. Zool.* **81**, 278-287.