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The alkaline tide and ammonia excretion after voluntary feeding in freshwater rainbow trout

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SUMMARY

We investigated the potential acid–base and nitrogenous waste excretion challenges created by voluntary feeding in freshwater rainbow trout, with particular focus on the possible occurrence of an alkaline tide (a metabolic alkalosis created by gastric HCl secretion during digestion). Plasma metabolites (glucose, urea and ammonia) were measured at various time points before and after voluntary feeding to satiation (approximately 5% body mass meal of dry commercial pellets), as was the net flux of ammonia and titratable alkalinity to the water from unfed and fed fish. Arterial blood, sampled by indwelling catheter, was examined for post-prandial effects on pH, plasma bicarbonate and plasma CO_2 tension. There was no significant change in plasma glucose or urea concentrations following feeding, whereas plasma ammonia transiently increased, peaking at threefold above resting values at 12 h after the meal and remaining elevated for 24 h. The increased plasma ammonia was correlated with an increase in net ammonia excretion to the water, with fed fish significantly elevating their net ammonia excretion two- to threefold between 12 and 48 h post feeding. These parameters did not change in unfed control fish. Fed fish likewise increased the net titratable base flux to the water by approximately threefold, which resulted in a transition from a small net acid flux seen in unfed fish to a large net base flux in fed fish. Over 48 h, this resulted in a net excretion of 13 867 μmol kg^{-1} more base to the external water than in unfed fish. The arterial blood exhibited a corresponding rise in pH (between 6 and 12 h) and plasma bicarbonate (between 3 and 12 h) following feeding; however, no respiratory compensation was observed, as Pa_{CO_2} remained constant. Overall, there was evidence of numerous challenges created by feeding in a freshwater teleost fish, including the occurrence of an alkaline tide, and its compensation by excretion of base to the external water. The possible influence of feeding ecology and environmental salinity on these challenges, as well as discrepancies in the literature, are discussed.

Key words: acid–base regulation, base excretion, digestion, glucose, *Oncorhynchus mykiss*, plasma, urea.

INTRODUCTION

The gut is indispensable for multicellular life, and is responsible for meeting the nutritional and energy demands of an organism. However, while digestion can affect numerous other physiological systems through associated processes, some of the most basic consequences of feeding and assimilation in fish have only just begun to be discovered. For example, recently, the effects of digestion on overall osmotic and ionic balance in freshwater rainbow trout (*Oncorhynchus mykiss*) have been addressed (Bucking and Wood, 2006a; Bucking and Wood, 2006b; Bucking and Wood, 2007), revealing several beneficial consequences to digestion in these teleosts in addition to those offered by nutrition itself. However, digestion could also potentially create challenges for fish, especially carnivorous fish such as rainbow trout, because of the formation of excess ammonia during the catabolism of dietary proteins (Handy and Poxton, 1993), as well as excess base during the formation of HCl by the stomach (reviewed by Hersey and Sachs, 1995; Niv and Fraser, 2002). In this regard, a marked systemic alkaline tide during digestion has recently been described in a carnivorous marine elasmobranch, the dogfish shark (Wood et al., 2005; Wood et al., 2007a; Wood et al., 2007b).

As the amino acid surplus from protein-rich diets cannot be directly stored in fishes, it is deaminated and converted into energetic compounds (Ballantyne, 2001; Stone et al., 2003), resulting in post-prandial increases in plasma total ammonia levels (Kaushik and Teles, 1985) and ammonia excretion rates (van Weerd et al., 1995; Dosdat et al., 1996; Gelineau et al., 1998; Leung et al., 1999). More than 80% of this metabolic ammonia production is excreted across the gills, a portion of which may be in direct (NH_4^+) or indirect ($H^+ + NH_3$) exchange with Na^+ uptake (reviewed by Evans et al., 2005). Indeed a direct relationship between protein intake and ammonia excretion has been found in fish (Li and Lovell, 1992; Jayaram and Beamish, 1992; Ballestrazzi et al., 1998; Medale et al., 1995; Cai et al., 1996; Chakraborty and Chakraborty, 1998). The amino acid surplus is created through the hydrolysis of dietary proteins, first initiated in the stomach by pepsin and completed by the combined action of trypsin and chymotrypsin in the intestine. Pepsin is the proteolytically active form of the enzyme pepsinogen, which is secreted by gastric cells and autocatalytically activated in acidic environments. This is a conserved mechanism across species from fish (e.g. Bomgren et al., 1998; Hernandez et al., 2001; Lo and Weng, 2006) to mammals (reviewed by Kageyama, 2002), although the cells responsible for the production of pepsinogen vary, with mammals possessing two distinct acid secreting cells (chief cells) and pepsinogen secreting cells (parietal cells), whereas lower vertebrates such as the rainbow trout posses only one secreting cell, the oxynticopeptic cell (Bomgren et al., 1998). Although HCl secretion is essential for protein digestion through the aforementioned pepsinogen activation as well as by direct acid

hydrolysis, it can also add to the challenges created by digestion by generating an alkaline tide.

Historically defined as the alkalinization of the blood and urine during the digestion of a meal (Rune, 1965; Rune, 1966), the term alkaline tide in essence refers to the increase in blood HCO_3^- concentration that occurs as a consequence of increased secretion of HCl at this time. It is believed that gastric cells use a basolateral Cl^-/HCO_3^- exchanger to import extracellular Cl^- needed for HCl formation, and simultaneously export intracellular HCO_3^- that is formed *via* the hydration of CO_2 by intracellular carbonic anhydrase [which forms a proton and a HCO_3^- ion (reviewed by Hersey and Sachs, 1995; Niv and Fraser, 2002)]. This ultimately results in the equimolar secretion of H^+ into the lumen for HCl formation, and HCO_3^- into the blood that is responsible for the alkaline tide (Rune 1965; Rune, 1966; Niv et al., 1993). To date, the phenomenon has been documented in mammals, birds, reptiles and elasmobranchs (Wang et al., 2001b; Niv and Fraser, 2002; Wood et al., 2005; Wood et al., 2007a; Wood et al., 2007b) but there is, as yet, no evidence that it occurs in teleost fish. Indeed, neither Taylor and Grosell (Taylor and Grosell, 2006) using the marine toadfish, *Opsanus beta*, nor Taylor et al. (Taylor et al., 2007) using the euryhaline European flounder, *Platichthys flesus*, could detect a post-prandial alkaline tide in the blood of teleosts. Furthermore, in frogs there is a tight correlation between the reduction in plasma Cl^- concentrations and the increase in plasma HCO_3^- concentrations following feeding (Busk et al., 2000), but Bucking and Wood (Bucking and Wood, 2006a) found no such post-prandial reduction in plasma Cl^- levels in rainbow trout.

Gill function may be one reason why it has not been possible to see the symptoms of the alkaline tide in teleost fish. In addition to Na^+ uptake and NH_3/NH_4^+ and H^+ excretion, the gills are the main site of base (in the form of HCO_3^-) excretion and concurrent Cl^- uptake (reviewed by Evans et al., 2005), believed to be facilitated by an apical Cl^-/HCO_3^- exchanger, either belonging to the SLC4 anion exchanger (AE) family (Claiborne et al., 1997; Wilson et al., 2002), or the SLC26 AE family (Piermarini et al., 2002). In a recent review, Tresguerres et al. (Tresguerres et al., 2006) proposed a model of Cl^- uptake by freshwater fish through an apical Cl^-/HCO_3^- anion exchanger, cytoplasmic carbonic anhydrase and a basolateral V-type H^+-ATPase. If Cl^-/HCO_3^- excretion across the gills were fast enough to keep up with the HCl secretion and associated Cl^-/HCO_3^- exchange at the stomach, then alkalotic disturbances of blood pH, HCO_3^- and Cl might be avoided. However, a net base excretion into the water should still be detected; this was seen in the elasmobranch *Squalus acanthias* (Tresguerres et al., 2007; Wood et al., 2007b), but not in the euryhaline European flounder *Platichthys flesus* (Taylor et al., 2007). Potentially, the large ammonia excretion after feeding could make it difficult to detect net metabolic base efflux, a problem which would not occur in the ureotelic elasmobranch.

With this background in mind, we examined the effect of feeding on acid–base exchange with the environment using the original single end-point titration methodology of McDonald and Wood (McDonald and Wood, 1981) to separate ammonia and metabolic base fluxes, together with measurements of systemic acid–base status and plasma metabolites (glucose, urea and ammonia) in freshwater rainbow trout. The overall hypothesis behind this study was that digestion of a meal would create numerous physiological challenges to freshwater rainbow trout, including increases in plasma ammonia, increases in plasma pH and HCO_3^- concentration (an alkaline tide), and excretion of both the excess ammonia and excess base to the water *via* the gills.

MATERIALS AND METHODS

Rainbow trout (*Oncorhynchus mykiss* Walbaum) were obtained from a commercial supplier (Humber Springs Trout Hatchery; Orangeville, ON, Canada) and acclimated to laboratory conditions for a 2-week period before experimentation. The animals (ranging in body mass from 300 to 400 g) 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 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]. Except during experimentation, the animals were fed a 2% body ration [crude protein 41%; carbohydrates 30%; crude fat 11%; Martin Mills; Elmira, ON, Canada] every 48 h. All experiments were carried out at 12°C.

Post-prandial changes in plasma ammonia, urea and glucose

Sampling occurred immediately prior to (0 h), and at several time points following (2, 4, 8, 12, 24, 48 h) a single feeding to satiation of the trout (amounting to 5% of body mass) in the 500 l holding tanks. In a parallel study, these satiation feeding events resulted in the majority of fish (95%) consuming between 80 and 110% of the offered ration (C.P.B. and C.M.W., unpublished data). The meal consisted of commercial trout pellets with a measured ionic composition of Na^+ 215±15, Cl^- 188±16, K^+ 97±2, Ca^{2+} 194±3, Mg^{2+} 109±1 μmol g^{-1} original food mass. The fish were netted and sampled individually to reduce processing time (typically <60 s) and any resultant stress. Each trout was randomly netted from the holding tank and lightly anaesthetized using MS-222 (tricaine methane sulphonate; 0.03 g l^{-1}; Sigma, St Louis, MO, USA) before a blood sample was obtained *via* a caudal puncture using a no. 22 needle attached to an ice-cold heparinized syringe. The whole blood was immediately centrifuged at 13 000 ***g***, and the resultant plasma was removed, placed into liquid nitrogen, and stored at –80°C for future analyses. The fish were allowed to recover in fresh water and returned to a separate holding tank to avoid repeated sampling.

Plasma total ammonia (T_{amm}) was measured enzymatically (based on the glutamate dehydrogenase/NAD method) using a commercial kit (Raichem; San Diego, CA, USA) and plasma total urea ($[urea]_p$) was measured using a colorimetric urea assay modified from Rahmatullah and Boyde (Rahmatullah and Boyde, 1980). The plasma was then deproteinized and neutralized before analyzing for plasma total glucose ($[glucose]_p$) by the hexokinase, glucose-6-phosphate dehydrogenase method (Sigma, 301A). All samples were read on a microplate reader (SpectraMax 340PC; Sunnyvale, CA, USA).

Fluxes to the water

Individual trout were removed from the 500 l holding tanks immediately before the scheduled feeding time to serve as unfed controls (N=6). The remaining trout were then fed to satiation (>5% body mass ration) and then more individual fish (fed fish, N=6) were removed from the holding tank. The removed fish were placed in individual darkened flux boxes supplied with flow-through Hamilton city tap water and vigorous aeration. Flux measurements were then performed over successive 6 h intervals for the next 48 h. For each flux measurement, the water level was set to 4 l (excluding the mass of the animal) and the water flow suspended. An initial water sample was then taken followed by another water sample 6 h later, serving as starting and final flux samples, respectively. At the end of each 6 h flux period, following the final water sample, the box was thoroughly flushed with fresh water by repeatedly lowering

and raising the water level, before the volume was reset to 4 l. This procedure was repeated every 6 h for 48 h. The fish were then returned to the holding tanks.

The initial and final water samples were taken in triplicate and measured for total ammonia and titratable alkalinity, the latter by the single end-point technique of McDonald and Wood (McDonald and Wood, 1981) 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. HCl was added to each water sample until the pH was brought below pH 5.0. The sample was then aerated for 15 min to remove excess CO_2, and then more HCl was slowly added to determine the total quantity of acid needed to lower the pH of the water sample to a final end-point pH of 3.8. Continual aeration ensured mixing and CO_2 removal. A standardized acid (0.02 mol l^{-1} HCl; Sigma) was used to lower the pH and was accurately delivered by a Gilmont (Barrington, IL, USA) microburette. The amount of acid titrant, factored by the volume of the sample required to reach pH 3.8, represented the concentration of titrable alkalinity in basic equivalents. Water total ammonia concentration ($[NH_3/NH_4^+]_w$) was measured using the salicylate–hypochlorite method (Verdouw and Dekkers, 1978). Fluxes were calculated from changes in concentration (i.e. from initial to final samples), factored by volume, time and trout mass, and expressed as μmol kg^{-1} h^{-1}. The net acid–base flux was calculated as the difference between the flux of titratable alkalinity (J_{TAlk}) and the flux of total ammonia (J_{Tamm}) to the external water (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).

The $[NH_3/NH_4^+]_w$ in the chambers of the unfed fish did not exceed 150 μmol l^{-1} by the end of the 6 h flux period; however, some of the fed fish experienced a $[NH_3/NH_4^+]_w$ close to 300 μmol l^{-1}. To ensure that this had no influence on the outcome of the present experiment, a validation experiment was conducted wherein the present flux study with fed fish was repeated but J_{Tamm} was measured over 3 h flux periods within intervening flushes, so that the $[NH_3/NH_4^+]_w$ did not exceed 150 μmol l^{-1} in any of the fish chambers. The J_{Tamm} over the 3 h periods were then combined and compared with the 6 h fluxes in the present study. The results demonstrated that the high ammonia levels had no significant effect on the overall net flux of ammonia or acid–base equivalents.

At the same time, an additional validation experiment was performed to address concerns that the single end-point titration method used to measure titratable alkalinity fluxes may have incurred error if the buffer capacity of the water changed over the flux period. Theoretically, this could occur as a result of regurgitation of food or defecation, although such events were never observed. In this parallel trial, fluxes were measured at 0–6, 6–12 and 42–48 h post feeding (0 h). The water samples were titrated as in the single end-point technique to below 3.8 with 0.02 mol l^{-1} HCl, and the moles of acid added to reach this single end-point was calculated. However, the samples were then titrated back up to pH 7 (a second end-point) with 0.02 mol l^{-1} NaOH (which was verified against the 0.0 mol l^{-1} HCl). The difference between the number of moles of acid and base added was used to calculate the total titratable alkalinity of each sample, which was then used to determine the change from initial to final water samples to calculate the titratable alkalinity flux (i.e. a double end-point titration). The titratable alkalinity fluxes that were measured, either with the single titration or the double titration method, were essentially identical (i.e. no significant differences), although the latter were more variable (50% larger standard error of the means), as would expected for measurements based on the difference between double end-points and single end-points. We therefore conclude that the single end-point titration method used in this study is more accurate and appropriate for this type of investigation.

Systemic acid–base status

Additionally, following the acclimation to laboratory conditions, 18 fish were transferred from the holding tank to individual 25 l tanks supplied with flow-through dechlorinated Hamilton city tap water and individual aeration. These fish were fed daily at a set time point to entrain feeding in the individual tanks and ensure a synchronization of any feeding-associated activities. The fish were cleaned daily of any waste accumulation several hours before feeding. Training continued for several weeks until all the fish ate readily when food was supplied. Following training, the fish were starved for 1 week to clear the gastrointestinal tract.

After 1 week of starvation, the trout were anaesthetized with MS-222 (0.07 g l^{-1}) and artificially ventilated on an operating table. Dorsal aortic catheters (Clay-Adams PE-50; Sparks, MD, USA) were then implanted according to the method of Soivio et al. (Soivio et al., 1972) and filled with 0.3 ml of Cortland saline (NaCl 120, KCl 5, $CaCl_2 \cdot 2H_2O$ 2, $MgSO_4 \cdot 7H_2O$ 1, $NaH_2PO_4 \cdot H_2O$ 3, glucose 5 mmol l^{-1}; adjusted to pH 7.8 with $NaHCO_3$) (Wolf, 1963) containing 50 i.u. ml^{-1} of lithium heparin (Sigma) and sealed. Each trout was then returned to its individual 25 l tank and allowed to recover for 1 day. Following this recovery period, nine of the fish were then fed to satiation (again, typically a 5% body mass meal). The other nine were used as unfed control animals.

Blood samples (250 μl) were taken from the dorsal aorta catheter, using an ice-cold pre-heparinised, gas-tight Hamilton syringe, before and after feeding at various time points (–6, –3, 0, 3, 6, 9, 12, 24, 48 h). Approximately 70 μl of the whole blood was immediately used to measure arterial blood pH (pHa) using a Radiometer GK2401C glass combination electrode inserted into a tightly sealed chamber which was thermostatted to 12°C. The remaining whole blood was centrifuged at 13 000 ***g*** for 30 s to separate plasma and red blood cells. Plasma samples were then immediately measured for total CO_2 ($T_{a_{CO_2}}$; Corning 965 Total CO_2 Analyser; Lowell, MA, USA). Plasma CO_2 tension ($P_{a_{CO_2}}$) and bicarbonate concentration ($[HCO_3^-]_a$) were calculated using a rearrangement of the Henderson–Hasselbalch equation with values of plasma pK′ and CO_2 solubility coefficients for trout blood at 12°C (Boutilier et al., 1984).

Statistics

All data passed normality and homogeneity tests prior to statistical investigation, and are reported as mean ± s.e.m. (*N*=number of animals) unless otherwise specified. Temporal changes in T_{amm}, $[glucose]_p$, and $[urea]_p$ were examined with a one-way ANOVA followed by a *post-hoc* HSD (Tukey's honest significant difference) test. The temporal changes in J_{Tamm}, J_{TAlk}, $J_{net}OH^-$, and $J_{net}H^+$, pHa, $[HCO_3^-]_a$ and $P_{a_{CO_2}}$ were examined with a repeated measures two-way ANOVA followed by a *post-hoc* HSD test. Values were considered significantly different at $P<0.05$.

RESULTS

Post-prandial changes in plasma ammonia, urea and glucose

Feeding had no significant effect on $[glucose]_p$, which was maintained at an average of 6.84±1.60 mmol l^{-1} (*N*=49) for the

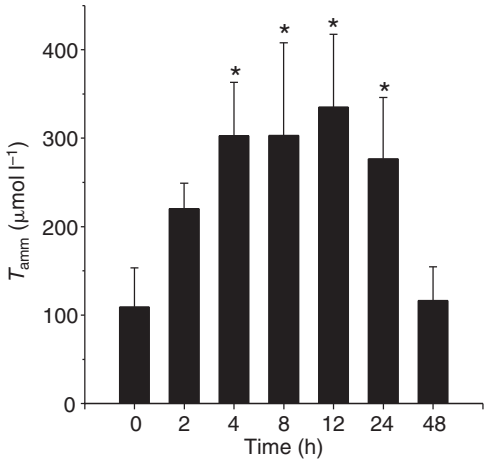

Fig. 1. Changes in total plasma ammonia concentration (T_{amm}; μmol l^{-1}) following feeding to satiation. Feeding occurred immediately following the time 0 h sampling, which was used as the control. Values are means ± s.e.m.; N=7. Each value was from a separate fish. *Significant difference (P<0.05) from the control.

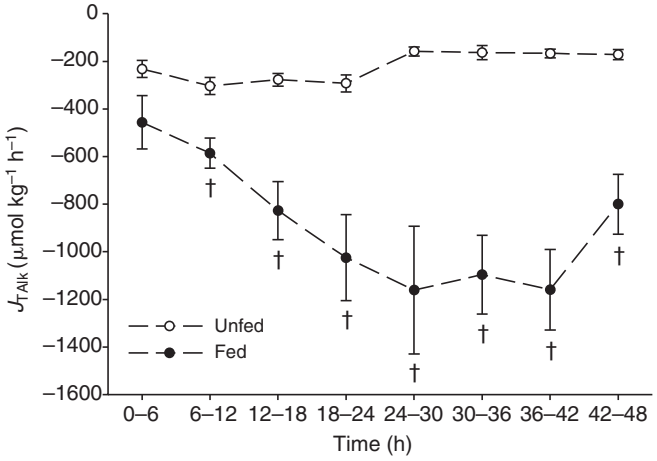

Fig. 3. Flux of total titratable alkalinity to the water (J_{TAlk}; μmol kg^{-1} h^{-1}) from fed and unfed fish. Open symbols indicate unfed fish, filled symbols indicate fish that were fed immediately before the experiment began. Data were simultaneously obtained from the fish used in Fig. 2. Values are means ± s.e.m.; N=6 for each treatment. †Significant difference (P<0.05) between fed and unfed fish.

duration of digestion. Likewise, $[urea]_p$ concentrations remained unchanged following feeding, averaging 1.39±0.11 mmol l^{-1} (N=49) over the 48 h of experimentation. By contrast, feeding had a dramatic effect on T_{amm}, which significantly increased more than threefold from pre-prandial values (109±44 μmol l^{-1}, N=7; 0 h), to peak 12 h following ingestion (335±82 μmol l^{-1}, N=7), before subsequently returning to resting values by 48 h (116±38 μmol l^{-1}, N=7; Fig. 1).

Fluxes to the water from fed and unfed fish

Confined unfed fish (i.e. control fish) showed a J_{Tamm} that remained unchanged over the course of experiment, averaging 320±8 μmol kg^{-1} h^{-1} (N=48; Fig. 2). The control fish showed a likewise unaffected J_{TAlk}, which averaged 220±19 μmol kg^{-1} h^{-1} (N=48; Fig. 3), slightly lower than the J_{Tamm}, resulting in a steady $J_{net}H^+$ of –100±14 μmol kg^{-1} h^{-1} (N=48; Fig. 4).

By contrast, while the confined pre-fed trout initially showed a J_{Tamm} similar to that of the unfed fish (between 0 and 6 h; Fig. 2), the J_{Tamm} increased more than twofold, eventually peaking between 36 and 42 h post-feeding, at a rate of –817±133 μmol kg^{-1} h^{-1} (N=6; Fig. 2). J_{Tamm} then decreased to –760±156 μmol kg^{-1} h^{-1} (N=6) at 48 h post-feeding; however, it was still significantly elevated when compared with the J_{Tamm} of unfed fish (Fig. 2). Similarly, the J_{TAlk} of fed fish increased when compared to that of unfed fish from initially comparable values (–456±112 μmol kg^{-1} h^{-1} at 0–6 h; Fig. 3)

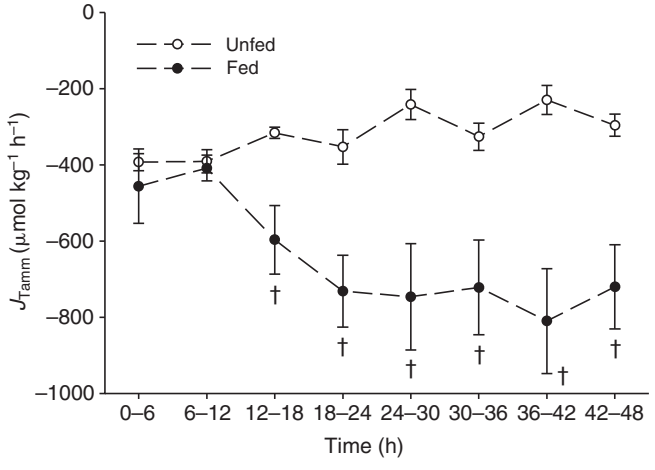

Fig. 2. Flux of total ammonia to the water (J_{Tamm}; μmol kg^{-1} h^{-1}) from fed and unfed fish. Open symbols indicate unfed fish, filled symbols indicate fish that were fed immediately before the experiment began. Values are means ± s.e.m.; N=6 for each treatment. The same six fish were measured at each interval. †Significant difference (P<0.05) between fed and unfed fish.

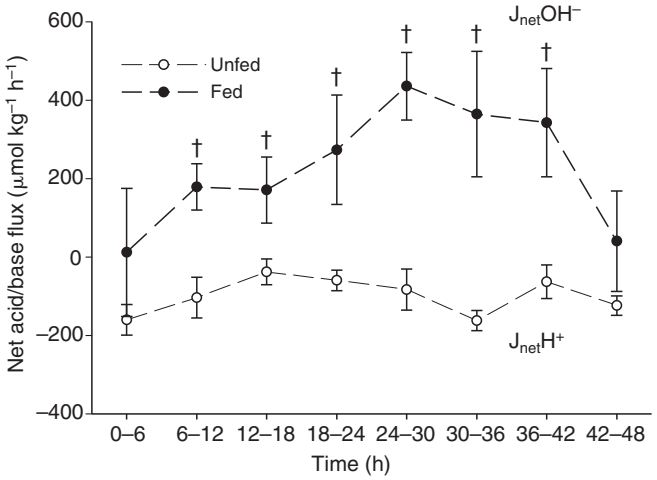

Fig. 4. The overall net acid or base flux to the water from fed and unfed fish. Positive values indicate a net base flux ($J_{net}OH^-$; μmol kg^{-1} h^{-1}), whereas negative values indicate a net acid flux ($J_{net}H^+$; μmol kg^{-1} h^{-1}). Open symbols indicate unfed fish, filled symbols indicate fish that were fed immediately before the experiment began. Data were calculated from the difference between values shown in Fig. 2 and Fig. 3 on an individual fish basis (see Materials and methods for further explanation). Values are means ± s.e.m.; N=6 for each treatment. †Significant difference (P<0.05) between fed and unfed fish.

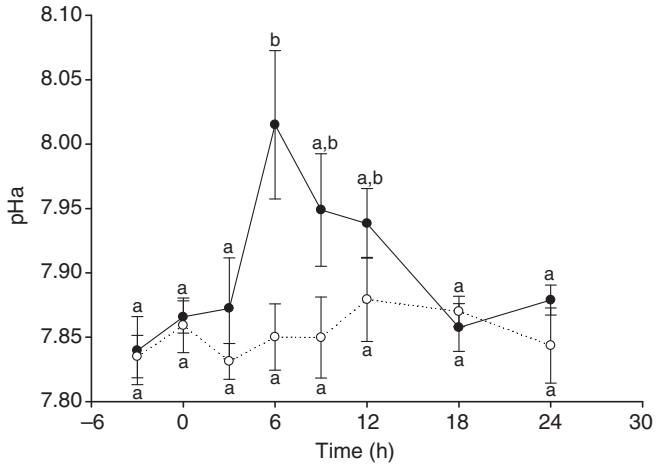

Fig. 5. Arterial blood pH (pHa) in unfed and fed fish. One set of fish (indicated by the filled symbols) were fed immediately following the time 0 sample, while another set of fish (open symbols) remained unfed. Values are means ± s.e.m.; *N*=8 for each treatment. Time points with the same letters are not significantly different (*P*>0.05).

to peak threefold higher at 30 h post-feeding at a rate of –1161±189 μmol kg^{-1} h^{-1}, before decreasing to –800±88 μmol kg^{-1} h^{-1} at 48 h (Fig. 3). Hence, feeding altered the net acid–base flux of the fed fish from an initial $J_{net}H^+$ at 0–6 h (–11±163 μmol kg^{-1} h^{-1}) that was similar to that of unfed fish (Fig. 4) to a $J_{net}OH^-$ that was significantly different from control values until 42 h post-feeding. The $J_{net}OH^-$ peaked between 24 and 30 h after the ingestion of the meal at 435±87 μmol kg^{-1} h^{-1} (*N*=6; Fig. 4).

Post-prandial arterial blood gases and acid–base status

Unfed cannulated fish maintained their pHa and $[HCO_3^-]_a$ unchanged over the course of the experiment, at an average of 7.85±0.01 mmol l^{-1} (*N*=64; Fig. 5) and 8.52±0.11 mmol l^{-1} (*N*=64; Fig. 6B), respectively. In the experimental group, pHa was maintained at an average of 7.86±0.01 (*N*=24; Fig. 5) before and for 3 h after the ingestion of the meal, which was not significantly different from unfed fish. However, thereafter, pHa significantly increased by over 0.15 pH units at 6 h (Fig. 5). This transient increase was slowly dissipated over the next 12 h, falling back to resting pHa levels 18 h after feeding (Fig. 5). This feeding-induced increase in pHa was mirrored by a post-prandial increase in $[HCO_3^-]_a$, which also significantly increased from pre-prandial values of 8.41±0.27 mmol l^{-1} (*N*=16; –3 to 0 h) as well as from unfed controls, to similarly peak 6 h following feeding at 12.19±0.43 mmol l^{-1} (*N*=8); a 1.4-fold increase (Fig. 6A). Meanwhile, there was no apparent difference in the Pa_{CO_2} (2.48±0.08, *N*=64) between fed and unfed controls, as well as no temporal change (Fig. 6B), indicating that no respiratory compensation was made during the metabolic alkalosis.

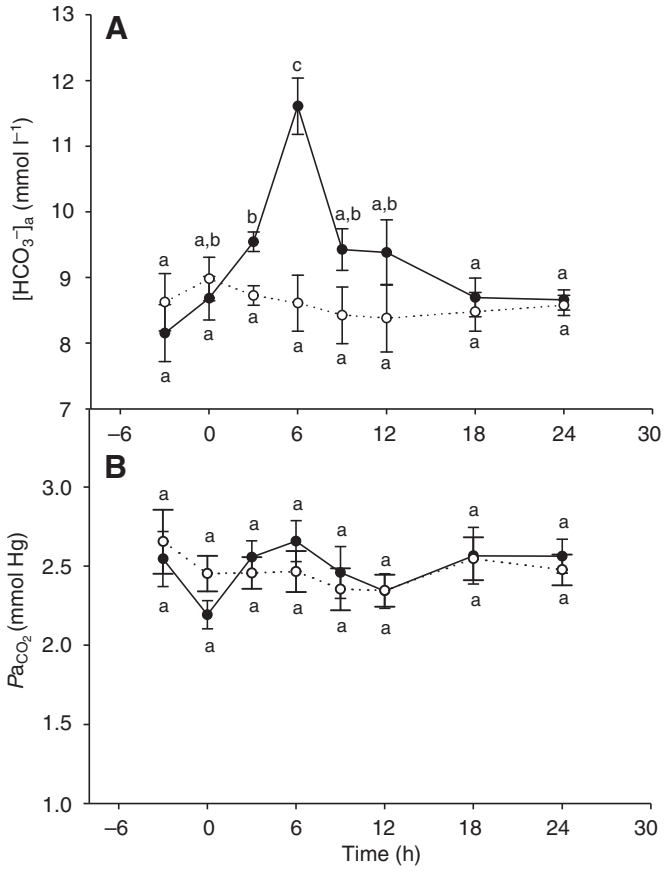

Fig. 6. (A) Arterial blood plasma bicarbonate concentration ($[HCO_3^-]_a$; mmol l^{-1}) and (B) CO_2 tension (Pa_{CO_2}; mmol Hg). One set of fish (indicated by the filled symbols) were fed immediately following the time 0 sample, while another set of fish (open symbols) remained unfed. Values are means ± s.e.m.; *N*=8 for each treatment. Time points with the same letters are not significantly different (*P*>0.05).

DISCUSSION

The majority of amino acids absorbed after the ingestion of protein, in excess of requirements for protein synthesis, are catabolized in the liver (Campbell, 1991), resulting in an increase in plasma ammonia levels in fish (Fig. 1) (Kaushik and Teles, 1985; Wicks and Randall, 2002). In association, there are changes in ammonia excretion in fish during the postprandial period (Fig. 2) (van Weerd et al., 1995; Dosdat et al., 1996; Alsop and Wood, 1997; Gelineau et al., 1998; Taylor et al., 2007) and plasma ammonia concentrations typically peak 12 h following feeding in trout (Fig. 1) (Wicks and Randall, 2002). Therefore, it is not surprising that an increase in dietary protein results in an increase in ammonia excretion in fish (Li and Lowell, 1992; Jayaram and Beamish, 1992; Ballestrazzi et al., 1998; Medale et al., 1995; Cai et al., 1996; Chakraborty and Chakraborty, 1998). An early study by Beamish and Thomas (Beamish and Thomas, 1984) on trout fitted with urinary catheters and trained to feed in small flux boxes attributed the majority of the post-prandial increase in ammonia-N excretion to the gills (>96%), with the remainder excreted by the kidneys, emphasizing the role of the gills in ammonia regulation. In contrast to the present study on ammoniotelic rainbow trout, feeding and digestion in the ureotelic dogfish resulted in only a very small rise in ammonia-N excretion, amounting to less than 3% of the total-N in the meal, which was also accompanied by only a modest increase in plasma ammonia-N concentration (Wood et al., 2005; Wood et al., 2007b).

This present study, together with the simultaneous investigation of Cooper and Wilson (Cooper and Wilson, 2008), both on rainbow trout, present the first evidence for an alkaline tide in any teleost fish. Notably, the fish in our study were feeding voluntarily, so there was no confounding effect of disturbance. Cooper and Wilson (Cooper and Wilson, 2008), working with a smaller ration (1% *vs* the 5% used in the present study) compared voluntary and forced-feeding, and found that the latter resulted in larger, longer-lasting

acid–base disturbance than voluntary feeding. In our study, voluntary feeding clearly induced an alkaline tide in the arterial blood of rainbow trout, evidenced by marked increases in pHa (Fig. 5) and plasma [HCO_3^-]a (Fig. 6A) from 3 to 12 h after the meal, without change in $P_{a_{CO_2}}$ (Fig. 6B) – i.e. a classical metabolic alkalosis. This disturbance was larger than seen in the voluntarily feeding fish of Cooper and Wilson (Cooper and Wilson, 2008), probably reflecting the difference in meal size between the two studies. As mentioned in the Introduction, the response almost certainly reflects the addition of metabolic base to the blood by oxynticopeptic cells of the gastric mucosa (reviewed by Hersey and Sachs, 1995) (Niv and Fraser, 2002). Upon stimulation, these cells secrete HCl into the stomach lumen to facilitate digestion, as well as HCO_3^- into the extracellular fluid compartment in order to maintain intracellular pH. A K^+-stimulated, H^+-ATPase is responsible for the apical H^+ secretion, and has been identified in oxynticopeptic cells of elasmobranchs (Smolka et al., 1994) as well as those of the rainbow trout (Sugiura et al., 2006). Although there is a vigorous secretion of gastric acid at this time, the pH of the stomach fluid actually increases substantially due to the buffering action of the ingested food (Sugiura et al., 2006; Bucking and Wood, 2008). As a Cl^-/HCO_3^- exchanger is believed to be responsible for the basolateral HCO_3^- export and Cl^- entry, the net transfer of HCl to the stomach can lead to a reduction in plasma [Cl^-] that has been correlated with an alkaline tide in toads (Busk et al., 2000).

Interestingly, a post-prandial drop in plasma [Cl^-] was not seen by either Bucking and Wood (Bucking and Wood, 2006a) or Cooper and Wilson (Cooper and Wilson, 2008) in rainbow trout that had fed voluntarily, but was reported by the latter authors in rainbow trout that had been force-fed. Based on the results of the present study, a probable explanation is that during the removal of the excess base to the water by the gills, the branchial Cl^-/HCO_3^- exchanger is able to compensate for the loss of Cl^- to the stomach lumen by uptake of Cl^- from the dilute external environment. Interestingly, increased activity of this branchial exchanger may also explain why our study revealed a net excretion of base to the water to relieve the alkaline tide, whereas Cooper and Wilson (Cooper and Wilson, 2008) found no such clearance of base to the water (in either of their feeding treatments) despite observing clear alkaline tides in the bloodstream. The lower Cl^- levels in the water in the Cooper and Wilson study may have limited the exchange of Cl^- for HCO_3^- at the gills and prevented the clearance of the metabolic alkalosis to the water, a theory that is further corroborated by the reduction in plasma [Cl^-] at least in the force-fed fish of the Cooper and Wilson study (Cooper and Wilson, 2008), because of the lack of environmental Cl^- available for replacement.

However, the extent to which the differences in water chemistry can contribute to the differences observed between the two studies is unknown. It has been shown that water Cl^- concentrations had only a modest effect on branchial Cl^-/HCO_3^- exchange rates in the flounder (Taylor et al., 2007). However, the higher affinity and capacity of branchial uptake kinetics of Cl^- in the rainbow trout [K_m 150–300 μmol l^{-1}, J_{max} ~360 μmol kg^{-1} h^{-1} (e.g. Goss and Wood, 1990; Wilkie et al., 1999)] *vs* those in the flounder [K_m ~650 μmol l^{-1}, J_{max} ~198 μmol kg^{-1} h^{-1} (Taylor et al., 2007)] suggest that water Cl^- concentrations may play a larger role in determining the rate of exchange in the trout. The potential limitations of low water Cl^- concentrations in relieving a metabolic alkalosis requires further investigation. If this massive base excretion (Fig. 4) had not occurred, at least 13 867 μmol kg^{-1} of base (i.e. HCO_3^- equivalents) would have to have been buffered in the body fluids over 48 h. It is not possible to precisely calculate the effect on blood pH without knowledge of how this 13.9 mmol kg^{-1} HCO_3^- load might distribute between intra- and extra-cellular compartments. However, applying the traditional technique pioneered by Rune (Rune, 1965; Rune, 1966) and now widely used in humans (Niv and Fraser, 2002), the assumption is made that the excess base of the alkaline tide is distributed in a 'blood buffer space', equivalent to 0.3 body mass. Using a blood non-HCO_3^- buffer capacity of 10.8 slykes for rainbow trout (Wood et al., 1982), the Henderson–Hasselbalch equation, the pK^1 and αCO_2 constants tabulated for trout blood plasma by Boutilier et al. (Boutilier et al., 1984) and a simple Davenport (Davenport, 1974) diagram analysis, the blood pH would have risen to about 8.55, an increase of about 0.7 units, in contrast to the 0.2 pH unit increase measured here (Fig. 5). Thus the excretion of excess base to the environment 'prevented' about 70% of the anticipated rise in blood plasma pH, an increase that very likely would have been fatal.

Although the alkaline tide appeared to be relieved by 18 h post-feeding (Fig. 5), this does not necessarily mean that gastric acid secretion has subsided. Fasted fish exhibited a small net acid flux at all time points, or an overall negative base excretion of –4344 μmol kg^{-1} over the 48 h of experimentation (Fig. 4). By contrast, fed fish transitioned from a net acid flux to a net base flux as the alkaline tide progressed (Fig. 4), which remained significant relative to non-fed animals during the 6–42 h post-feeding period (Fig. 4) and resulted in the excretion of 13 867 μmol kg^{-1} more base than by the unfed fish. In comparison, unfed dogfish exhibited approximately one half the net acid flux [–2160 μmol kg^{-1} (Wood et al., 2007b)], which is most likely due to the inherent differences in nitrogen metabolism between the two species (i.e. ammoniotelism *vs* ureotelism). However, following feeding, the dogfish showed a net flux of base to the water that was similar, although quantitatively smaller [10 470 μmol kg^{-1} (Wood et al., 2007b)], to that seen in the current study, suggesting that the rainbow trout had a larger alkaline tide than the dogfish. These measurements suggest a substantial role for branchial Cl^-/HCO_3^- exchange in alleviating the alkaline tide through increased base excretion to the water.

The ability of freshwater teleosts to utilize branchial ion transport mechanisms to correct acid–base disturbances is well established (e.g. Perry et al., 2003; Evans et al., 2005; Tresguerres et al., 2006) and the restoration of resting blood acid–base chemistry likely reflects the ability of branchial base excretion mechanisms to adequately compensate for the metabolic alkalosis created during digestion, as in the elasmobranch *Squalus acanthias* (Wood et al., 2005; Wood et al., 2007a; Wood et al., 2007b; Tresguerres et al., 2007). The alkaline tide, at least for mammals, is also accompanied by excretion of alkaline urine (Rune, 1965; Rune 1966; Niv and Fraser, 2002) resulting from a reduction in the metabolic acid load normally excreted in the urine (Brunton, 1933). In fact, Finke and Litsenberger (Finke and Litsenberger, 1992) determined that post-prandial pH of urine produced by cats was linearly correlated with meal size. In humans, Johnson et al. (Johnson et al., 1990) observed a correlation between changes in postprandial urine acid output and titratable gastric acid output. While branchial excretion in freshwater fish of acid–base equivalents generally accounts for the majority of the total exchange, the urine can play an important supplementary role in the compensation of metabolic acid–base disturbances (Wood et al., 1999). The net fluxes of ammonia and HCO_3^- to the water measured in this experiment combined contributions from both branchial and urinary sources, and the urinary contribution to both the excretion of base to the water and the relief of the alkaline tide can only be speculated.

The alkaline tide observed in reptiles and amphibians (e.g. Coulson et al., 1950; Wang et al., 2001a; Andrade et al., 2004) results in only very modest increases in pHa, as a result of respiratory compensation [i.e. an increase in $P_{a_{CO_2}}$ (Wang et al., 1995; Wang et al., 2001a; Overgaard et al., 1999; Busk et al., 2000a; Busk et al., 2000b; Andersen and Wang, 2003)] that appears to be caused by hypoventilation (Hicks et al., 2000; Secor et al., 2000; Wang et al., 2001b). This phenomenon has also been observed in humans although to a lesser degree (Higgins, 1914; Erdt, 1915; Van Slyke et al., 1917; Ou and Tenney, 1974). However, neither the freshwater rainbow trout of the present study, those studied by Cooper and Wilson (Cooper and Wilson, 2008) nor the marine dogfish shark (Wood et al., 2005) exhibited any increase in $P_{a_{CO_2}}$ during the post-prandial period. Fish appear to have no ability for respiratory compensation of the metabolic alkalosis created by the alkaline tide. In essence, the gills are believed to be hyperventilated with respect to CO_2 excretion because of the much lower solubility of O_2 relative to CO_2 in water. This results in minimal adjustments of blood $P_{a_{CO_2}}$ even if ventilatory changes occur (Perry and Wood, 1989).

Although the alkaline tide phenomenon is commonly reported in amphibians and reptiles (Wang et al., 2001a), it appears to be more controversial in humans and fish. Several studies in humans have failed to see alkaline urine and respiratory compensation following feeding (e.g. Brunton, 1933; Johnson et al., 1995). These authors have even suggested that any respiratory or urinary compensation for gastric acid secretion is too small to be of physiological or clinical significance. When considering fish species, studies on the gulf toadfish (Taylor and Grosell, 2006) and European flounder (Taylor et al., 2007) likewise reported no evidence for a classic alkaline tide. By contrast, the present study and that of Cooper and Wilson (Cooper and Wilson, 2008) on the rainbow trout, as well as several investigations on the dogfish shark (Wood et al., 2005; Wood et al., 2007a; Wood et al., 2007b; Tresguerres et al., 2007) clearly demonstrate evidence for its existence. Differences in methodology may contribute to these discrepancies; for example, the study of Cooper and Wilson (Cooper and Wilson, 2008) demonstrates that the nature of feeding (voluntary *vs* forced) will alter the extent of the alkaline tide. It is also possible that the differences are related to differences in environmental salinity (discussed subsequently) or feeding ecology among species. For example, many reptiles and amphibians feed at irregular intervals, but are able to ingest meals that are very large relative to their own body mass (e.g. Greene, 1997; Shine et al., 1998). Digestion of these large meals is associated with considerable increments in oxygen uptake that lasts for several days (Benedict, 1932; Secor and Diamond, 1998; Wang et al., 2001b). By contrast, humans and some fish ingest relatively smaller meals more frequently, indicating a possible role for meal size in the occurrence of an alkaline tide. Fish that have exhibited an alkaline tide large enough to elicit compensation by excretion of base to the environment appear to exhibit either a sporadic feeding ecology more similar to that of a reptile than of a mammal [dogfish (e.g. Jones and Green, 1977; Hanchet, 1991; Tanasichuk et al., 1991)], or were starved for more than a week and then consumed a ration of food >5% of body mass (rainbow trout, this study).

Differences within species with similar feeding ecology also probably exist. The rainbow trout used in this study do not normally fast for more than one week, and while their natural feeding patterns are probably more similar to the toadfish and flounder than the dogfish, our results suggest a difference in acid–base disturbances between the species as pointed out above. The reasons are unclear as of yet, but perhaps it is related to the diet itself. Indeed, as mentioned earlier the net flux of base equivalents from fed trout in the current study were greater than those seen from fed dogfish (Wood et al., 2007b), and although both studies estimated feeding at >5% body mass, the food used in the current study was a commercial diet that was approximately 10–20% water whereas the natural diet fed to the dogfish was ~80% water. Commercial diets may in fact be digested in a very different manner than natural diets, as aside from differing water contents, commercial diets may possess a higher buffering capacity and hence require greater acid secretion to reach the low pH required for protein digestion. In fact, the titration of a commercial fish meal down to pH 3 (Cooper and Wilson, 2008) required 10-fold more acid than that of a natural ragworm diet (Taylor et al., 2007). It is unlikely that titration *in vitro* exactly duplicates the real titration that occurs as chyme is progressively digested and diluted *in vivo* (Bucking and Wood, 2006a; Bucking and Wood, 2008). Nevertheless, greater acid secretion with a commercial diet may reflect the greater acid–base disturbances observed in the present study when compared with studies using natural diets (Wood et al., 2007b; Taylor et al., 2007). In fact, this may lead to a variety of acid–base challenges in the wild, where fish that feed primarily on invertebrates may secrete less gastric acid, than fish that eat mainly vertebrates. The cause(s) behind the incongruities between the base excretion observed in this study and the lack of base excretion observed by Cooper and Wilson (Cooper and Wilson, 2008) and Taylor et al. (Taylor et al., 2007) may be a result of either different meal sizes or the availability of environmental Cl^- to relieve the alkaline tide through branchial Cl^-/HCO_3^- exchange, as suggested earlier. Additionally, the current study was conducted on freshwater rainbow trout, however, marine teleosts such as the flounder studied by Taylor et al. (Taylor et al., 2007) may very well react differently to feeding because of altered gastrointestinal and branchial transporter expression, as well as essentially opposing physiological needs. Marine teleosts secrete large quantities of HCO_3^- into the intestine for purposes associated with osmoregulation [water absorption and Ca^{2+} precipitation, as reviewed by Grosell (Grosell, 2006)], so it is possible that 'recycling' of HCO_3^- in this manner will attenuate or prevent the systemic alkaline tide and/or base excretion to the water. Finally, the diet itself, in its composition and size, may play a strong role in determining the extent, duration and mechanism of compensation for this metabolic disturbance.

Unlike plasma ammonia, plasma glucose was not significantly affected by feeding, which is symptomatic of poor utilization of carbohydrates by rainbow trout. A rapid and transient increase in plasma glucose concentrations has been reported in rainbow trout 1 h after feeding (Wicks and Randall, 2002); however, this could have been reflective of a stress response to the experimental procedure. Overall, carnivorous fish (such as rainbow trout) are recognized for their inefficiency in utilizing dietary carbohydrates (Moon, 2001; Wilson, 1994). Carnivorous fish express a lower abundance of intestinal glucose transporters relative to omnivorous and herbivorous fish (Buddington et al., 1997). The current study also revealed no post-prandial changes in plasma urea concentrations, as a result of either a lack of increase in urea production or a matching increase in urea excretion to maintain plasma levels. This is not the case with glucose, as glucose is highly reabsorbed by the kidney, the primary site of glucose 'excretion' (Bucking and Wood, 2004). Previous studies (e.g. Brett and Zala, 1975; Wiggs et al., 1989) revealed no significant increase in urea excretion following feeding in several fish species; however, these findings have been contradicted in other species (e.g. Alsop and Wood, 1997; Wright, 1993). Reasons for this discrepancy may reflect differences in metabolic pathways

among species and/or diet composition. Notably, Alsop and Wood (Alsop and Wood, 1997), working on juvenile rainbow trout, reported that steady feeding to satiation increased urea excretion rate about fourfold relative to that of fasted fish, though urea-N excretion remained only about 10% of the similarly elevated ammonia-N excretion.

Future areas of interest generated by this study include identifying the urinary contribution to the total increased ammonia and base excretion to the water, as well as the details of the branchial base excretion mechanism, as mentioned earlier. Additionally, it may be possible to pharmacologically manipulate gastric acid secretion using inhibitors, and thereby evaluate whether HCl production is the direct cause of the alkaline tide. In humans, Odera et al. (Odera et al., 2002) observed an increase in mean gastric pH after the administration of proton pump inhibitors, and urinary acid output significantly decreased when compared with control fed subjects. Holstein (Holstein, 1975) reported that teleostean fish possess the histamine H_2 receptor in the stomach, which is believed to be responsible for stimulating gastric acid secretion, and Trischitta et al. (Trischitta et al., 1998) demonstrated *in vitro* evidence for histamine stimulation of gastric acid secretion by the eel stomach as well as inhibition by carbachol (a histamine H_2 receptor antagonist). Finally, the effect of water chemistry and diet composition should be evaluated.

In summary, feeding and digestion created numerous physiological challenges in the freshwater rainbow trout, including increased plasma ammonia levels, increased ammonia and base excretion to the water, as well as an overall systemic metabolic alkalosis. Although the metabolic alkalosis can be thought of as a challenge to fish created by digestion, it may serve to maintain plasma ion concentrations, especially Cl^- through branchial transport mechanisms. It has long been known that freshwater fish have a high capacity for branchial base excretion, as usually demonstrated by $NaHCO_3$ infusion (Perry et al., 2003; Evans et al., 2005; Tresguerres et al., 2006). The present demonstration of the alkaline tide and associated base efflux provides a natural purpose (i.e. acid–base homeostasis following feeding) for the existence of this mechanism.

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