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Post-prandial alkaline tide in freshwater rainbow trout: effects of meal anticipation on recovery from acid–base and ion regulatory disturbances

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SUMMARY

The post-feeding alkaline tide (elevated blood pH and HCO_3^-) has been well characterised in air-breathing animals, but to date this phenomenon has only been demonstrated in one piscine species, a marine elasmobranch. We have investigated the acid–base and ion regulatory responses of a freshwater teleost to voluntary feeding as well as to involuntary filling of the stomach *via* an indwelling gastric intubation tube. One group of rainbow trout (*Oncorhynchus mykiss*) were fed a 1% body mass ration of homogenised food *via* the gastric intubation tube. Another group fed voluntarily on a 1% body mass ration. Blood samples were taken *via* dorsal aortic catheters from fish in both groups before feeding and over the subsequent 72 h. Trout fed *via* the gastric intubation tube exhibited post-prandial metabolic alkalosis of the blood (pH and plasma HCO_3^- increases of up to ~0.2 pH units and 3 mmol l^{-1}, respectively), that was more than twofold greater than the voluntary feeding fish, and took three times as long to recover (72 *versus* 24 h). Arterial P_{CO_2} was unchanged in both groups indicating that freshwater trout do not retain CO_2 to compensate for a post-prandial alkaline tide. Although excretion of HCO_3^- to the water increased post-prandially, NH_4^+ excretion followed a similar pattern, such that net acid equivalent fluxes were unaffected. Thus, sites other than the gills or kidney must be responsible for recovery of blood acid–base status, with intestinal HCO_3^- secretion being a likely candidate. In addition, fish fed *via* the gastric intubation tube experienced a large (17 mmol l^{-1}) but acute (6 h) drop in plasma chloride and a very large (53%) and long lasting decline in plasma magnesium concentration, that were absent in voluntarily feeding fish. These results further indicate a potentially important role for neuro-endocrine mediated mechanisms when fish feed voluntarily, in promoting the earlier initiation of compensatory responses that regulate blood ion levels and acid–base status. This aspect should also be considered when interpreting studies on other aspects of post-prandial physiology, where force feeding by gavage is commonly used in preference to voluntary feeding.

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

INTRODUCTION

Gastric acid secretion following a meal results in an equal amount of bicarbonate base (HCO_3^-) being transported into the blood which causes a metabolic alkalosis known as the post-prandial ‘alkaline tide’ in both mammals and ectothermic terrestrial vertebrates (Wang et al., 2001; Niv and Fraser, 2002). During feeding, isotonic hydrochloric acid (HCl) is secreted into the stomach lumen by parietal acid secreting oxyntic cells (mammals) or oxyntopeptic cells (non-mammalian vertebrates) (Niv and Fraser, 2002; Taylor and Grosell, 2006a). The protons required for gastric acid secretions are produced when carbonic anhydrase catalyses the hydration of CO_2 that also produces HCO_3^- (Perry and Gilmour, 2006). Protons (H^+) and Cl^- ions are then extruded from the parietal cells into the lumen *via* apically bound K^+/H^+ ATPase proteins and Cl^- channels, respectively (Niv and Fraser, 2002). To prevent intracellular alkalinisation an equivalent concentration of HCO_3^- is excreted basolaterally into the blood in exchange for Cl^-, fuelling the net transcellular movement of Cl^- required for gastric HCl secretion (for a review, see Niv and Fraser, 2002). Mammals and ectothermic terrestrial vertebrates compensate for a post-prandial alkaline tide by the hypoventilatory retention of respiratory CO_2, raising the partial pressure of CO_2 (P_{CO_2}) in the blood, and partially restoring blood pHi (Wang et al., 1995; Wang et al., 2001; Overgaard et al., 1999; Andersen et al., 2003).

The rate of apical gastric acid secretion will ultimately affect the extent of basolateral HCO_3^- excretion into the blood, and so is therefore a key component behind the post-prandial alkaline tide. Ivan Pavlov won the Nobel Prize in 1904 for his work on the concept of ‘nervism’ or the entire neural control of gastric acid secretion. He demonstrated that gastric acid secretion in fasted dogs started almost immediately following exposure to appetising food even without the entrance of this food into the stomach. It was later shown by James Black (who also won a Nobel Prize in 1972) that neural control was only part of the gastric acid secretion process, with hormonal regulation involving the gastrin-histamine pathway also being major components (Konturek et al., 2004; Konturek et al., 2005). The regulation of post-prandial gastric acid secretion in mammalian systems is now classically divided into three overlapping phases: cephalic (or neural), gastric and intestinal, with each phase including neural and hormonal components (Konturek et al., 2004). Information for the neural or cephalic phase of gastric acid secretion is communicated *via* the vagal nerve, which links the medulla oblongata to the oesophagus, stomach and most of the abdominal viscera (Fox, 2006). Gastric acid secretion regulation *via* the vagal neural phase overlaps and interacts with the gastric and intestinal phases (Katschinski, 2000), thus highlighting the complex and interdependent mechanisms that contribute to post-prandial acid secretory responses.

There are a number of studies on the vagal neural system in fish most of which have either examined the link with cardio-respiratory activity (Schwerte et al., 2006; Campbell and Eggington, 2007) or its role in relaying sensory information between the taste palette and the brain (Morita and Finger, 1985; Lamb and Finger, 1994; Finger, 1997). There is also a relatively good understanding of brain regulation of food intake by fish. For example, studies on goldfish have shown that, as with mammals, the hypothalamic area is associated with the regulation of food intake and the monitoring of long term energy expenditure/intake balance (Lin et al., 2000). How these neural processes and feedback mechanisms in fish are affected during short term post-prandial activity (e.g. in response to an alkaline tide) has yet to be studied.

Campbell (Campbell, 1920) was one of the first to report and discuss the alkaline tide phenomenon in humans and link the rise in blood pH with the secretion of HCl into the stomach. There are now many examples in the literature of post-prandial alkaline tides occurring in reptiles and mammals (Regev et al., 2001; Ozaki et al., 2000; Andersen et al., 2003; Arvedsen et al., 2005; Hartzler et al., 2006; Weber and White, 1986). Comparative information of a post-prandial alkaline tide in fish (i.e. water-breathing vertebrates) has been limited to only one species, a marine elasmobranch, the Pacific spiny dogfish (*Squalus acanthias*) (Wood et al., 2005). Wood et al. (Wood et al., 2005) concluded that the post-prandial alkaline tide in the spiny dogfish was not compensated by respiratory acidosis, which raises an interesting question: why not and what alternate mechanisms do fish use? To further complicate matters, other studies on marine and euryhaline teleost fish have shown no evidence of a post-prandial alkaline tide (Taylor and Grosell, 2006a; Taylor et al., 2007). Finally, although there are some studies on the vagal neural system and the hypothalamic brain regulation of food intake in fish, little is known about the control of gastric acid secretion in fish, so it is of interest to ascertain whether the anticipation of a meal can influence any post-prandial regulatory networks and feedback systems by examining differences between voluntarily feeding fish and fish fed directly *via* an oesophageal catheter. The aim of the present study was to therefore use a commercially important freshwater teleost fish species, rainbow trout (*Oncorhynchus mykiss*) to (1) further elucidate the effects of feeding on blood acid–base and ionic regulation and (2) to determine the effects of meal anticipation on such regulatory responses.

MATERIALS AND METHODS

Fish husbandry

Immature (mixed sex) rainbow trout (*Oncorhynchus mykiss* Walbaum) were obtained from Spring Fish Farm (Houghton, Dorset, UK) and housed at Exeter University. Prior to experimentation, the fish were maintained in 300–400 l tanks with flow-through, aerated, dechlorinated freshwater [Na^+ 390, K^+ 47, Ca^2 598, Mg^{2+} 152, Cl^- 400 μmol l^{-1}; titratable alkalinity (to pH 4.0) ~0.85 mmol l^{-1}; pH 7.5; temperature 10.4±1.5°C]. Fish used in experiments were 211.4±35.3 g and were fed daily with a 1% (w/w) ration of commercial trout pellets (Aqualife, from Biomar A/S, Brande, Denmark; 42.0% protein, 22.0% fat, 3.3% fibre, 8.0% ash; Na^+ 236±8; K^+ 157±8; Ca^{2+} 319±24; Mg^{2+} 74±2; Cl^- 164±6 μmol g^{-1}), but were not fed for 7 days prior to experimentation. All experiments were conducted with the approval of the University of Exeter Ethics Committee and under a UK Home Office license (PPL 30/2217).

Feeding experiments

To allow repetitive blood sampling without disturbance, all fish were surgically fitted with dorsal aortic catheters (i.d. 0.58 mm, o.d. 0.96 mm; Portex, Scientific Laboratory Supplies Ltd, Nottingham, UK) under anaesthesia with buffered MS-222 (60 mg l^{-1}; Pharmaq Ltd, Fordingbridge, Hants, UK) using the 'guided wire' technique described by Soivio et al. (Soivio et al., 1972), with the exception that catheters were exited from the mouth *via* a pin hole made in the thin membrane between the maxillary and the preorbital. This was in preference to punching a hole through the snout (which can potentially damage sensitive neural/olfactory tissues near the nares). Trout fed *via* a gastric intubation tube (which from now on will be referred to as 'catheter-fed'; N=11) were additionally surgically fitted with rectal catheters (Wilson et al., 2002) and gastric catheters (i.d. 1.19 mm, o.d. 1.70 mm; Portex) entering *via* the oesophagus and exiting *via* a small hole in the corner of the mouth (as above for dorsal aorta catheters). Following surgery all fish were allowed a 72 h recovery period, and were maintained in individual fixed-volume aerated chambers (5 and 40 l for catheter-fed and voluntary feeding fish, respectively).

Catheter-fed fish were fed a 1% body mass ration, *via* the oesophageal catheter, of commercial trout pellets that had been freshly homogenised (IKA, Laboratory Analysis Ltd, Exeter, Devon, UK) in two volumes of water. This food:water ratio was based approximately on the findings of Bucking and Wood (Bucking and Wood, 2006), who showed that freshwater rainbow trout imbibed just over 1.6 ml of water for every 1 g of food eaten. We added slightly more water to ensure a consistency of homogenate that would allow easy infusion *via* the catheter. The homogenised meal was gradually injected into the stomach over a period of 10 min, a similar period for the voluntary feeding fish to consume an equivalent sized meal. Voluntarily fed rainbow trout (N=8) were left to feed freely on a 1% body mass ration of whole pellets introduced into the water. Although voluntarily feeding fish were without rectal catheters, there was no visible faecal contamination of the tanks throughout the duration of the experiment, and prior to fluxes any excess food was removed and the tank flushed thoroughly.

Analysis of blood and plasma

Arterial blood samples (~800 μl) were taken using a gas-tight 1 ml Hamilton syringe before feeding and at 6, 12, 24, 48 and 72 h post-feeding and various parameters were measured. Blood pH was measured on whole blood (~300 μl) in a system thermostatted to the experimental temperature [Cameron E301 glass and E351 electrodes (Cameron Instrument Company, Port Aransas, TX, USA) connected to an Alpha 600 metre; Oxford Laboratories, High Wycombe, Bucks, UK]. P_{O_2} was measured on whole blood in a system thermostatted to the experimental temperature (Strathkelvin 1302 electrode and 781 meter; StrathKelvin Instruments Ltd, Glasgow, UK). Whole blood oxygen content (T_{O_2}) was measured using the method of Tucker (Tucker, 1967) with a Cameron E101 electrode and BGS200 chamber at 38°C, connected to Strathkelvin 781 meter. Plasma was isolated by centrifuging the remaining blood, and it was then kept on ice. The remaining blood (~300 μl of total whole blood taken) was returned to the animal, along with ~500 μl 0.9% NaCl to replace the volume taken. Plasma ions (Pye SP9 series AAS/FES and Corning chloride analyser 925, Pye Unicam Ltd, Cambridge, UK and Ciba Corning Diagnostics, Halstead, Essex, UK), osmolality (Wescor Vapro 5520 vapour pressure osmometer; Chemlab Scientific Products, Laindon, Essex, UK) and T_{CO_2} (Mettler Toledo 965 carbon dioxide analyzer; Ciba Corning Diagnostics) were measured. Plasma P_{CO_2} and [HCO_3^-] were calculated from

plasma T_{O_2} and blood pH measurements using a rearrangement of the Henderson–Hasselbalch equation and values for solubility (α_{CO_2}=0.064 mmol l^{-1} mmHg^{-1}) and pK_{app} (6.11–6.17, temperature and pH dependent), based on Boutilier et al. (Boutilier et al., 1984).

Net acid–base fluxes and the analysis of food

Initial and final water samples were taken for each flux period for the measurement of net fluxes of acid–base relevant ions between the animal and its external medium. Catheter-fed fish were held in static water for up to 12 h in the 5 l chambers and voluntarily fed fish were held for 24 h in the 40 l chambers, conditions in which average final water total ammonia concentrations did not exceed 132 μmol l^{-1}. At the end of each flux period chambers were flushed with fresh water to ensure restoration of normal levels of these ions. Total ammonia was measured on 2 ml water samples using the salicylate method [modified from Verdouw et al. (Verdouw et al., 1978)] and the titratable alkalinity measured on 20 ml water samples using an auto-titrator (TIM845 titration manager and SAC80 automated sample changer radiometer) performing single titrations with 0.02 mol l^{-1} HCl [as described by Wilson et al. (Wilson et al., 2002)]. Single titrations were deemed sufficient for the analysis of water HCO_3^- excretion rates (J_{TAlk} – see below), as a comparison with double titrations (i.e. using 0.02 mol l^{-1} NaOH to titrate back starting pH) only revealed a significant decrease at 24 h post-feed (–30.6±7.4 μmol kg^{-1} h^{-1}; P=0.003). When taking into account all data from all time points, J_{TAlk} when measured using double titrations was only 19.6±10.4 μmol kg^{-1} h^{-1} lower than when using single titrations, and this difference was not significantly different from zero. A decrease of 30.6 μmol kg^{-1} h^{-1} at 24 h is relatively small when considering J_{TAlk} rates at this time point are >300 μmol kg^{-1} h^{-1} (see Results). Furthermore, none of our conclusions would be altered by the different absolute rates produced by using the single and double titration methods (see Discussion). All net flux data from the catheter-fed fish (i.e. 12 h fluxes) were subsequently compiled into 24 h groups to enable direct comparisons to be made with voluntarily fed fish.

To estimate stomach acid secretion, the homogenised food was titrated by the auto-titrator using 0.02 mol l^{-1} HCl to pH 5.0 and 3.0 (the approximate pH range of rainbow trout stomach chyme during feeding; C. Bucking and C. M. Wood, personal communication).

The net fluxes of acid–base relevant ions between the fish and external water were calculated using the following equation:

$$J_{net}X = [([X]_i - [X]_f) \times V] / (M \times t) ,$$

where V is the volume of water (l) in the chamber (after the initial sample was taken), M is the mass of the fish (kg), t is the duration of the flux period (h), and $[X]_i$ and $[X]_f$ are the ion concentrations in the chamber water (μmol l^{-1}) at the beginning and end of the flux period, respectively. Titratable alkalinity flux rates (J_{TAlk}) were calculated from the above equation using titratable alkalinity measurements. 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). It should be noted that $J_{net}H^+$ can result from the movement of any of the following: H^+, NH_4^+, HCO_3^- or OH^-. Although it is not possible to distinguish between these forms, H^+ and NH_4^+ excretion, and HCO_3^- and OH^- uptake are all equivalent in terms of the acid–base status of the fish.

To estimate the range of predicted total load of base (HCO_3^-) introduced into the bloodstream of fish post-prandially, it was assumed that it would be equivalent to the quantity of acid required to titrate the mass of food in the stomach to either pH 3.0 (putative maximum blood base load) or pH 5.0 (putative minimum blood base load). This range was based on measurements of stomach chyme pH from voluntary feeding freshwater rainbow trout (C. Bucking and C. M. Wood, personal communication). To give an indication of how the predicted post-prandial base load might compare with the potential amount of HCO_3^- excreted to the water, the 'excess' HCO_3^- excretion was calculated for each fish (i.e. pre-feed J_{TAlk} was subtracted from each of the post-feed J_{TAlk} flux values, and then multiplied by the duration of each flux). The sum of these provided a cumulative 'excess' base excretion (i.e. in excess of the control rate) over the whole 72 h post-prandial period. Changes in J_{TAlk} fluxes on their own are difficult to interpret as an increase in J_{TAlk} can result from an increased efflux of NH_3 gas or HCO_3^- ions, or a combination of the two. However, the above calculation was considered to be a useful theoretical exercise if only to rule in (or out) the potential for detecting clearance of a HCO_3^- load from the blood. The calculation of cumulative 'excess' base excretion therefore assumes such a change in J_{TAlk} would be entirely due to increased HCO_3^- excretion.

We wished to compare the post-prandial responses of voluntary feeding and catheter-fed fish. Therefore a relative change (compared with its own pre-feed control) was calculated for each fish, for each variable of interest, at each time point (e.g. ΔpH_{24h}, $\Delta[HCO_3^-]_{48h}$ etc.). This allowed direct statistical comparison between the two treatments of each Δ value at each time point.

Statistical analysis

All data are presented as means ± s.e.m. Normality was checked with the Kologorov–Smirnov test and those data that were not normally distributed were log transformed. Where appropriate a Student's t-test or a one-way repeated measures analysis of variance (RM-ANOVA), followed by a multiple pairwise control (pre-feed) comparison *versus* post-feed groups using the Bonferroni t-test method was used to test the normal and lognormal data. Means were considered significantly different based on the adjusted P<0.05 (SigmaStat 3.1 statistical program).

RESULTS

Catheter-fed trout – acid–base and ion data

Fish fed a 1% body mass ration *via* the oesophageal catheter exhibited a post-prandial alkaline tide (Fig. 1A). On average, blood pH reached a maximum of pH 8.10 12 h post-feed (a rise of ~0.2 pH units when compared with the pre-feed control value) and remained significantly elevated until 48 h post-feed (Fig. 1A). Concomitant with this rise in blood pH were increased levels of plasma HCO_3^- (maximum elevation of ~3 mmol l^{-1} at 6 h post-feed; Fig. 1A) but blood P_{CO_2} was unaffected (Fig. 1B).

Plasma Cl^- levels significantly dropped sharply by 17 mmol l^{-1} at 6 h post-feed, but recovered by 12 h and were significantly higher than pre-feed levels after 72 h (Fig. 2A). Conversely, plasma Na^+ levels were unaffected by feeding (Fig. 2A). There were no changes in plasma concentrations of K^+ or Ca^{2+} (Fig. 2B) or osmolality (data not shown) post-feeding when compared with the pre-fed values. However, plasma Mg^{2+} levels dropped significantly and remained low (almost half the control level) between 12 and 72 h post-feeding (Fig. 2B).

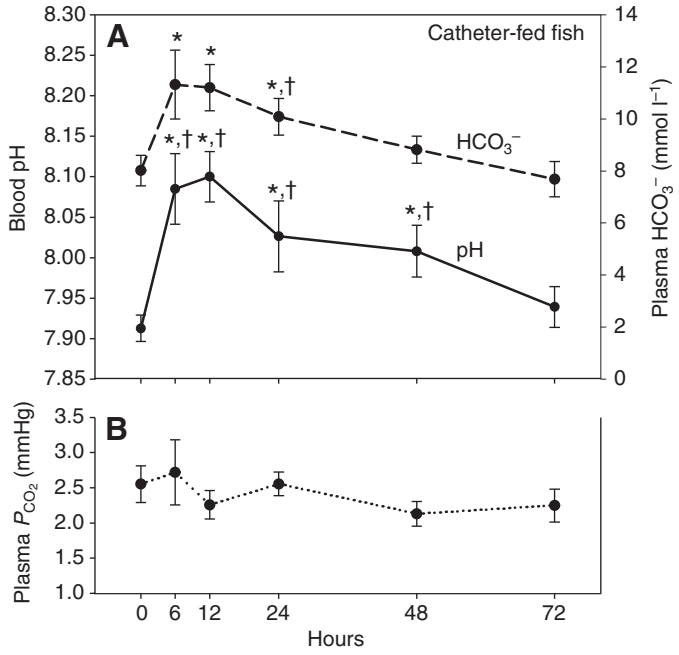

Fig. 1. The change in (A) blood pH (solid line), plasma HCO_3^- (mmol l^{-1}; dashed line), and (B) the partial pressure of CO_2 (P_{CO_2}) over the duration of the experiment in which fish were fed a 1% of body mass ration *via* an oesophageal catheter. Values are means ± s.e.m. (N=11). An asterisk denotes a significant difference when compared with pre-feed levels [adjusted P<0.05; RM-ANOVA followed by a multiple pairwise control (pre-feed) comparisons *versus* post-feed groups using the Bonferroni t-test method], whereas a dagger indicates a significant difference of ΔpH when compared with voluntarily fed fish (P<0.05; paired t-test).

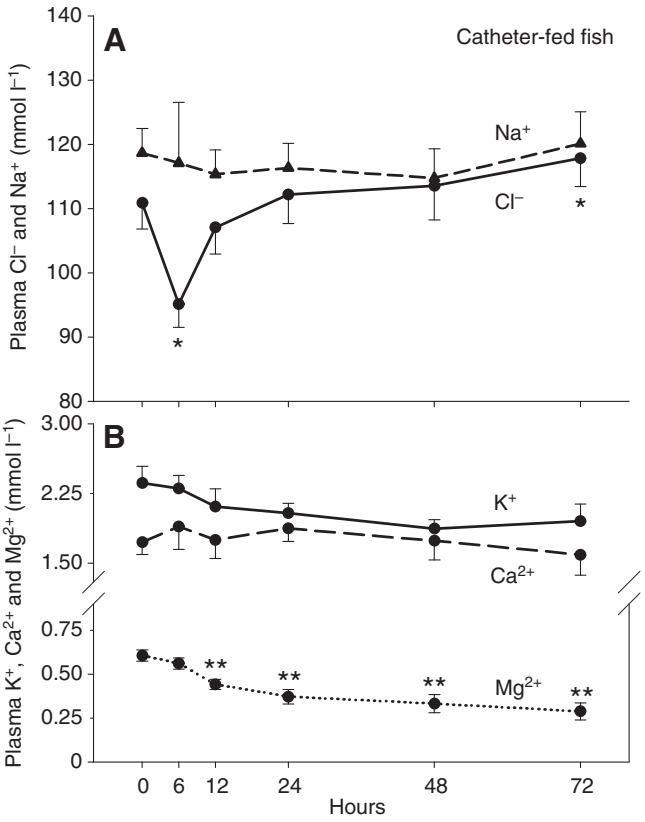

Fig. 2. Plasma concentrations of (A) chloride (Cl^-, dashed line), sodium (Na^+, solid line), (B) potassium (K^+, solid line), calcium (Ca^{2+}, dashed line) and magnesium (Mg^{2+}, dotted line), over the duration of the experiment in which fish were fed a 1% of body mass ration *via* an oesophageal catheter. Values are means ± s.e.m. (N=11). A single asterisk denotes P<0.05, whereas a double asterisk indicates P<0.01 [adjusted P value; RM-ANOVA followed by a multiple pairwise control (pre-feed) comparisons *versus* post-feed groups using the Bonferroni t-test method].

Titratable alkalinity fluxes were all positive (equivalent to efflux of HCO_3^- into the water) and were significantly increased 0–48 h post-feed when compared with pre-feed levels, before recovering by the 48–72 h period (Fig. 3). Total ammonia efflux into the water was also significantly higher than pre-feed levels from 0–48 h post-feed, which again was recovered by 72 h (Fig. 3). As total ammonia and titratable alkalinity fluxes (i.e. NH_4^+ and HCO_3^- excretion rates) followed a very similar pattern the net acid–base flux did not vary significantly over the duration of the feeding experiment and was effectively not different to zero throughout the experiment (Fig. 3).

Voluntarily fed trout – acid–base and ion data

Fish that fed voluntarily on a 1% body mass ration of whole pellets also exhibited a post-prandial alkaline tide (Fig. 4A), although it was much less pronounced (only half the rise in pH and [HCO_3^-]) when compared with catheter-fed fish (Fig. 1A), and both these variables were significantly elevated for only 6 h post-feed, before returning to pre-feed levels by 24 h (Fig. 4A). Blood P_{CO_2} did not significantly change over the duration of the feeding experiment (Fig. 4B). Levels of plasma Cl^-, Na^+ (Fig. 5A), K^+, Mg^{2+}, Ca^{2+} (Fig. 5B) and osmolality (data not shown) remained unchanged over the duration of the experiment in voluntary fed fish.

Titratable alkalinity fluxes were positive (i.e. equivalent to HCO_3^- efflux into the water) and significantly increased 0–24 h post-feed, before returning to pre-feed levels during the 24–48 h post-feed period, whereas net total ammonia flux remained significantly elevated up to 48 h, recovering during the 48–72 h period (Fig. 6). As with the catheter-fed trout (Fig. 3), both titratable alkalinity and total ammonia fluxes (i.e. HCO_3^- and NH_4^+ excretion rates) were similar, so net acid–base flux remained unchanged and effectively not different from zero over the whole experiment (Fig. 6).

Catheter-fed fish versus voluntarily fed fish – acid–base and ion data

The relative change, compared with their own pre-feed control (i.e. Δ), of plasma pH, HCO_3^- and water acid–base fluxes were calculated and statistically tested. The increase in plasma pH was significantly greater in catheter-fed from 6 to 48 h post-prandially, when compared with voluntarily fed fish ($\Delta pH_{6\text{-}48h}$ P<0.05; compare Fig. 1A and Fig. 4A). Similarly, the rise in plasma HCO_3^- was significantly greater in catheter-fed fish 24 h post-prandially ($\Delta HCO_3^-{}_{24h}$ P<0.05; compare Fig. 1A and Fig. 4A). Conversely, the increases in both titratable alkalinity and total ammonia fluxes were significantly greater 24 h post-prandially in voluntarily fed fish ($\Delta J_{TAlk0\text{-}24h}$ and $\Delta T_{amm0\text{-}24h}$, P<0.05; compare Fig. 3 and Fig. 6).

Titrating food to either pH 5 or 3 in the stomach (a range of stomach chyme pH values which have been observed in freshwater rainbow trout; C. Bucking and C. M. Wood, personal communication) required 680±0.15 or 2280±1.14 μmoles HCl per g of food, respectively. Therefore, to titrate a 1% ration of food to pH 5 or 3 would require 6800±15 or 22 800±114 mmoles HCl per kg of fish, respectively.

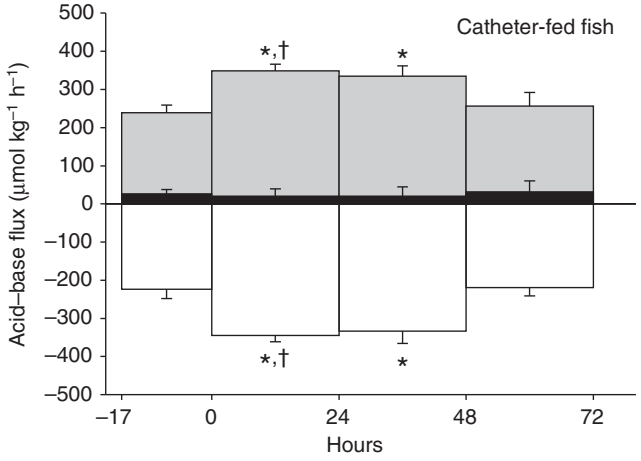

Fig. 3. Fluxes of titratable alkalinity (J_{TAlk}, grey bars), total ammonia (J_{Tamm}, white bars) and net acidic equivalents ($J_{net}H^+$ black bars) over a 72 h period after fish were fed a 1% of body mass ration *via* an oesophageal catheter. Positive values indicate base excretion and negative values indicate acid excretion. Values are means ± s.e.m. (N=8–11). An asterisk denotes a significant difference when compared with pre-feed levels [adjusted P<0.05; RM-ANOVA followed by a multiple pairwise control (pre-feed) comparisons versus post-feed groups using the Bonferroni t-test method], whereas a dagger indicates a significant difference of ΔJ_{TAlk} or J_{Tamm} when compared with voluntarily fed fish (P<0.05; paired t-test).

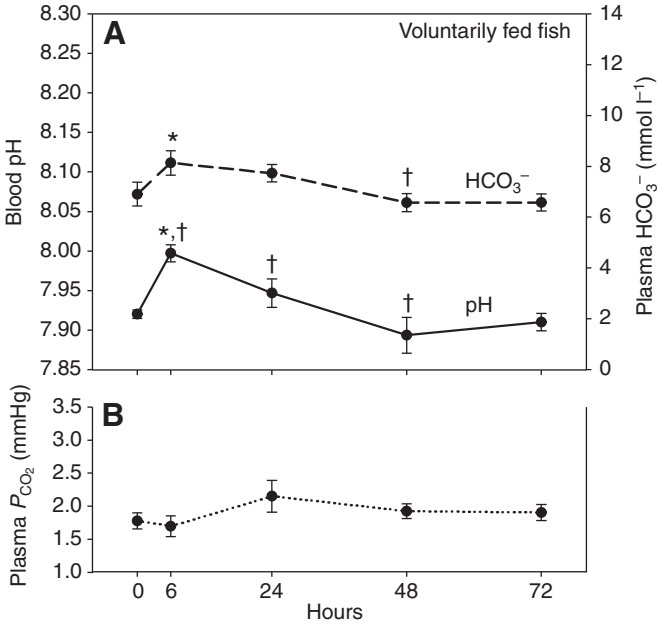

Fig. 4. The change in (A) blood pH (solid line), plasma HCO_3^- (mmol l^{-1}, dashed line) and (B) the partial pressure of CO_2 (P_{CO_2}), over the duration of the experiment when fish fed voluntarily on a 1% of body mass ration. Values are means ± s.e.m. (N=8). An asterisk denotes a significant difference when compared with pre-feed levels [adjusted P<0.05; RM-ANOVA followed by a multiple pairwise control (pre-feed) comparisons *versus* post-feed groups using the Bonferroni t-test method], whereas a dagger indicates a significant difference in ΔpH when compared with catheter-fed fish (P<0.05; paired t-test).

Blood oxygen measurements for catheter and voluntarily fed fish

Data for blood oxygen variables (P_{O_2} and total O_2 content) are not shown, but P_{O_2} did not vary with time or between treatments (catheter-fed *versus* voluntary feeding). P_{O_2} remained unchanged over time in both treatment groups, with an average value of 119±1.8 mmHg. By contrast, the total blood O_2 content declined over time, with no differences between the two groups. This is typical of previous studies in trout using repetitive blood sampling and the consequent sequential removal of a small proportion of the circulating blood cells (e.g. Wilson and Taylor, 1993).

DISCUSSION

The present study and the simultaneous work of Bucking and Wood (Bucking and Wood, 2008) on the same species are the first records of a post-prandial alkaline tide in teleost fish. This alkalosis, induced by an elevation of HCO_3^- base in the blood, occurred in fish from both feeding regimes in the present study (voluntary feeding and catheter-fed fish), and in both groups recovery of blood acid–base status was complete within 72 h. It is worth noting that the least amount of HCO_3^- we predict would be added to the blood following the meal (assuming gastric pH of only 5 – see below) would have raised [HCO_3^-] in the extracellular fluid space [assuming 37% of body mass (Milligan and Wood, 1982)] by 18 mmol l^{-1}. Given that this is 6 and 12 times higher than the average increase in plasma [HCO_3^-] observed post-prandially in catheter-fed and voluntary fed fish, respectively, it is clear that they must have been actively compensating for this significant HCO_3^- base load. There were also important differences in the degree and timing of the acid–base and ionic disturbances and recovery between the two feeding treatments, which will form the basis of further discussion on the mechanisms and sites of recovery together with the potential control processes involved.

Role of the gills and kidney in recovery from the alkaline tide

In fish from both groups (voluntarily fed and catheter-fed), and as also shown by Bucking and Wood (Bucking and Wood, 2008), blood P_{CO_2} did not change following feeding, indicating that rainbow trout, like spiny dogfish (Wood et al., 2005), do not retain CO_2 to compensate for a post-prandial alkaline tide. The primary mechanism of post-prandial pH regulation must therefore be removal of the excess HCO_3^- from the blood. Wood et al. (Wood et al., 2005) highlighted the potential ability of the gill in elasmobranchs to deal with the excess blood HCO_3^- load, and Wood et al. (Wood et al., 2007) demonstrated that there is indeed a large efflux of base to the external water after voluntary feeding in the dogfish shark (*Squalus acanthias*).

In the present study on freshwater rainbow trout, rates of HCO_3^- excretion to the water (i.e. positive J_{TAlk} values) did increase post-prandially in both treatments; in some cases these flux rates increased up to fourfold within the first 24 h. Normally the vast majority of such net acid–base fluxes to the water occur at the gills, with the minority *via* the kidney (Wood, 1992). Thus the gills of trout would appear to respond quite dynamically to the post-prandial acid–base disturbance which would fit with the branchial base excretion mechanism induced by blood alkalosis as described by Tresguerres et al. (Tresguerres et al., 2007). Indeed, by making some simple assumptions about the degree of gastric acid secretion during digestion we can estimate whether the stimulation of J_{TAlk} (presumably reflecting branchial HCO_3^- secretion) was sufficient to remove the post-prandial blood HCO_3^- load. Titrating their 1% ration to either pH 5 or 3 in the stomach (a range of stomach chyme pH values which have been observed in freshwater rainbow trout;

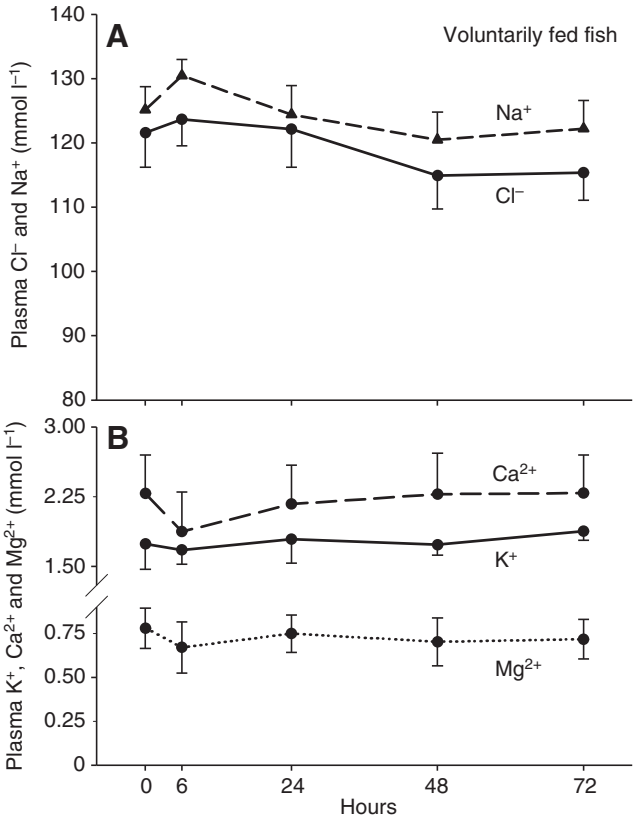

Fig. 5. Plasma concentrations of (A) chloride (Cl^-, solid line), sodium (Na^+, dashed line), (B) potassium (K^+, solid line), calcium (Ca^{2+}, dashed line) and magnesium (Mg^{2+}, dotted line), over the duration of the experiment when fish were allowed to feed voluntarily on a 1% of body mass ration. Values are means ± s.e.m. (*N*=8).

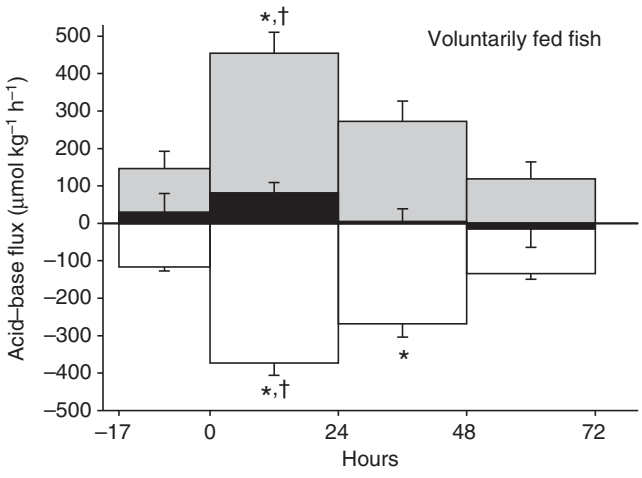

Fig. 6. Fluxes of titratable alkalinity (J_{TAlk}, grey bars), total ammonia (J_{Tamm}, white bars) and net acidic equivalents ($J_{net}H^+$ black bars) over a 72 h period after fish fed voluntarily on a 1% of body mass ration. Positive values indicate base excretion and negative values indicate acid excretion. Values are means ± s.e.m. (*N*=8). An asterisk denotes a significant difference when compared with pre-feed levels (adjusted $P<0.05$; RM-ANOVA followed by a multiple pairwise control (pre-feed) comparisons *versus* post-feed groups using the Bonferroni *t*-test method), whereas a dagger indicates a significant difference of ΔJ_{TAlk} and J_{Tamm} when compared with voluntarily fed fish ($P<0.05$; paired *t*-test).

C. Bucking and C. M. Wood, personal communication) would require either 6800 or 22 800 μmoles HCl per kg of fish, respectively, and create equivalent HCO_3^- base loads in the blood. Based on the J_{TAlk} flux data, we calculated that on average, the theoretical cumulative 'excess' amount of HCO_3^- excreted to the water (i.e. above control pre-feed levels) over the post-prandial period was 7205±1447 μmoles kg^{-1} in catheter-fed fish, and 17 803±4174 μmoles kg^{-1} in voluntary feeding fish. Both these values fall within a range that could potentially account for a reasonable proportion of the predicted base load in the blood for the typical stomach pH range found in fed rainbow trout.

However, because in the present study ammonia excretion rates followed a similar pattern to the J_{TAlk} fluxes, the net acidic equivalent flux to the water was actually negligible during the entire experimental period in both treatment groups. So despite relatively dynamic responses in the fluxes of acid–base relevant ions to the water, the gill cannot be considered as an important site of recovery from the alkaline tide following the 1% ration meal used. In effect, the increased HCO_3^- excretion to the water was sufficient to match the increased NH_4^+ excretion that resulted from post-prandial deamination of excess amino acids (Ballantyne, 2001), but not enough to additionally account for the blood HCO_3^- load resulting from gastric acid secretion.

By contrast, the companion paper by Bucking and Wood (Bucking and Wood, 2008) did report an increase in net base excretion into the water by rainbow trout, with post-prandial HCO_3^- excretion rates being up to 400 μmol kg^{-1} h^{-1} higher than ammonia excretion rates in the same fish, and three times higher than the maximum J_{TAlk} rates in the present study. This suggests that the gills, either instead of or in addition to the intestine, can play a role in compensating for the alkaline tide in freshwater trout. A likely explanation for the different results between the present study and that of Bucking and Wood is the fivefold difference in ration size (1% *versus* 5%, respectively). With a much greater digestive load (and presumably gastric acid secretion rate) created by the larger ration, regulation of blood acid–base status may simply require both gill and intestinal processes to work in tandem to maintain a functional blood acid–base status. One further interesting difference between these two studies is the water chemistry. In the study of Bucking and Wood (Bucking and Wood, 2008) the freshwater Cl^- concentration was almost twofold higher than in the present study (i.e. hard *versus* soft water, respectively), increasing the potential for maximising HCO_3^- excretion *via* apical Cl^-/HCO_3^- exchange at the gill.

It has been shown that Cl^- uptake kinetics *via* the gill of rainbow trout has a K_m of ~150–300 μmol l^{-1} Cl^- in a variety of freshwater chemistries (Kerstetter and Kirschner, 1972; Wilkie et al., 1999; Williams and Eddy, 1986), which is intermediate for K_m values in freshwater zebrafish (Boisen et al., 2003) and flounder (Taylor et al., 2007). Taylor et al. (Taylor et al., 2007) showed that flounder in seawater (compared with freshwater) had significantly elevated titratable alkalinity flux rates at 6 h after a meal, which might be attributed to the 1000-fold higher concentration of Cl^-. However, the gill ionoregulatory apparatus is very different in seawater and freshwater acclimated fish, therefore interpretation of their result is not straightforward. It remains to be seen whether more subtle changes in freshwater Cl^- concentration, including environments with almost zero chloride, might influence the post-prandial recovery from the alkaline tide, and specifically the involvement of branchial Cl^-/HCO_3^- exchange.

The teleost kidney plays an important role in reabsorbing the majority of filtered HCO_3^- [*via* an equivalent rate of renal acid secretion (Perry and Fryer, 1997; Perry and Gilmour, 2006)] which consumes substantial metabolic energy. Thus small changes in these renal transport processes could result in considerable net acid or base excretion *via* the urine. Indeed, it is possible (though not likely) that the post-prandial increases in J_{TAlk} fluxes (i.e. HCO_3^- excretion to the water) were at least partly the result of increased net removal of filtered HCO_3^- *via* the urine, rather than the gill. However, even if this were the case, the net excretion of acid–base relevant ions to the external water remained essentially zero in these fish, indicating that the post-prandial blood HCO_3^- load was dealt with 'internally' rather than excreted to the external medium *via* the gills and/or kidney. The most likely candidate for this removal of the HCO_3^- load from the blood is the intestine, and this possibility will be discussed below.

The role of the intestine in recovery from a post-prandial alkaline tide

In contrast to the present findings with freshwater rainbow trout, two previous feeding studies on teleost fish found no evidence for a post-prandial alkaline tide (Taylor and Grosell, 2006a; Taylor et al., 2007). In these cases, the intestine was put forward as playing a pivotal role in the recovery from this metabolic alkalosis. The teleost species used in these previous experiments were the Gulf toadfish (*Opsanus beta*) (Taylor and Grosell, 2006a) and the European flounder (*Platichythus flesus*) (Taylor et al., 2007). The authors concluded that as these fish were adapted to, or able to adapt to living in seawater, intestinal mechanisms were in place that could circumvent a post-prandial alkaline tide (Taylor and Grosell, 2006a; Taylor et al., 2007). It was hypothesised in the these studies that any blood load of HCO_3^- during gastric acid secretion was simultaneously matched by transport of HCO_3^- from the blood into the intestinal lumen, which was supported by the fact that in both toadfish and flounder post-prandial intestinal HCO_3^- concentrations were significantly elevated (Taylor and Grosell, 2006a; Taylor et al., 2007). By contrast, although the freshwater rainbow trout used in the current study are euryhaline, they require a considerable acclimation period (usually days) to fully express the appropriate ion transport mechanisms typical of marine fish (including intestinal bicarbonate secretion). Thus a delayed expression of transporters required for intestinal HCO_3^- secretion may explain the observation of a significant alkaline tide in these freshwater trout, and the rather prolonged delay in recovery from this blood alkalosis.

Potentially, limited expression of transporters required for intestinal HCO_3^- secretion may also explain the occurrence of the post-prandial alkaline tide and subsequent recovery *via* net base efflux to the water observed in Pacific spiny dogfish (*Squalus acanthias*) (Wood et al., 2007). Marine elasmobranchs are osmoconformers and so have extremely low drinking rates in sea water compared with their teleost counterparts and as such do not require constitutive expression of intestinal HCO_3^- secretion (Taylor and Grosell, 2006b). This would mean little potential to use intestinal HCO_3^- secretion as a way to compensate for the alkaline tide (much like the freshwater trout).

A further explanation for the absence or presence of an alkaline tide in different studies could be the food type itself, i.e. commercial fish food pellets *versus* a natural diet. Taylor et al. (Taylor et al., 2007) utilised similar techniques and calculations as in the present study to determine gastric acid secretion in flounder after a meal of ragworm (*Nereis diversicolor*). The amount of acid required to titrate our commercial fish food pellets down to pH 3.0 was 13 times greater than that required for the ragworm [2280 *versus* 175 μmol $H^+ g^{-1}$, respectively (Taylor et al., 2007)]. This difference is partly due to water content being higher in the ragworm, but also because commercial fish food has more buffering capacity than ragworm because of the high calcium phosphate content from skeletal material (i.e. in fish meal), whereas ragworm are soft-bodied invertebrates with no such skeletal material. This highlights the potential for dietary variations to affect the post-prandial alkaline tide, something that will have significance especially for comparison of species with different feeding habits in the wild e.g. carnivores that eat mainly invertebrates or vertebrates, or a mixture, and also when comparing wild with farm-reared fish.

Effects of meal anticipation on the post-prandial alkaline tide, subsequent recovery and ion regulation

To our knowledge, there have been no previous studies that explored the effects of bypassing the initial neural stage of gastric acid secretion and how this subsequently affects post-prandial acid–base regulation in teleost fish. In the present study, when comparing the change in blood pH between the two groups of fish, the alkaline tide in catheter-fed fish was significantly higher 6 to 48 h post feed when compared with voluntarily fed fish ($P<0.05$) and the pH disturbance was recovered much earlier (compare Fig. 1A and Fig. 4A). The change in plasma HCO_3^- was also significantly higher in catheter-fed fish after 24 h post feed, when compared with voluntarily fed fish ($P<0.05$; compare Fig. 1A and Fig. 4A). Furthermore, although net acid excretion into the water was unchanged in both groups of fish before and after feeding, both HCO_3^- and ammonia excretion into the water 24 h post-prandially was significantly elevated in voluntarily fed fish ($P<0.05$; compare Fig. 3 and Fig. 6).

We cannot dismiss that a possible cause for the observed differences in acid–base disturbances between catheter and voluntarily fed fish could be the state of the food when it was delivered to the stomach. In catheter-fed fish the food had already been ground into powder and then added to water prior to being injected into the stomach. In contrast, voluntarily fed fish were fed on whole pellets. However, we speculate that the state of the food in the stomachs of the fish from the two groups after ~2 h of digestion will be comparable. This is because rainbow trout start drinking at least within the first 2 h following feeding (Bucking and Wood, 2006) and possibly sooner, and they imbibe a similar food:water ratio as used for catheter-fed fish.

In the parallel study of Bucking and Wood (Bucking and Wood, 2008) that used trout voluntarily feeding on a 5% ration, the observed alkaline tide was more comparable to that of the catheter-fed fish in the present study (receiving only a 1% ration). By comparing these two studies, it is apparent that bypassing the initial neural/cephalic stage of gastric acid secretion with a 1% ration delivered involuntarily to the stomach, produces a post-prandial blood pH and HCO_3^- disturbance equivalent to voluntary feeding on a five times larger ration. This underlines the functional importance of meal anticipation in the physiological responses associated with an alkaline tide.

In catheter-fed fish the first neural stage (i.e. anticipation of the food) of gastric acid secretion would be bypassed. So gastric acid secretion would presumably be initiated by vagal reflexes derived from the distention of the stomach by food, chemical irritation of gastric mucosal receptors (Konturek et al., 2004), and potentially by satiation signalling molecules (Lin et al., 2000). In voluntarily feeding fish the food is sensed by either visual or olfactory cues prior to ingestion and it are these that commence the first neural

phase of gastric acid secretion. This difference in recovery from acid–base disturbance in the two feeding treatments indicates a potentially important role for neuro-endocrine-mediated mechanisms when fish anticipate feeding, in promoting the earlier initiation of compensatory responses (e.g. in the gills, intestine or kidney) that regulate blood acid–base status during digestive processes in the gut.

Fish from both feeding regimes were able to maintain relatively consistent levels of plasma Na^+, Ca^{2+}, K^+ (Fig. 2A,B and Fig. 5A,B) and osmolality (data not shown) throughout the duration of the experiment. However, catheter-fed fish experienced a significant drop in plasma Cl^- 6 h post-prandially before returning to normal (Fig. 2A). A number of studies have shown that plasma Cl^- levels drop following a meal in some ectotherms (Busk et al., 2000a; Andersen and Wang, 2003; Hartzler et al., 2006), whereas it remains stable in others (Overgaard et al., 1999; Busk et al., 2000b). Studies have also shown that HCO_3^-/Cl^- exchange is 1:1 (for a review, see Grosell, 2006), which would not explain why there is such a substantial difference between the large drop in plasma Cl^- (17 mmol l^{-1}) and the moderate rise in plasma HCO_3^- (4 mmol l^{-1}) in catheter-fed fish after 6 h (Fig. 2A and Fig. 1A, respectively). Furthermore, Mg^{2+} levels in catheter-fed fish significantly dropped by 12 h and remained at almost half the pre-feed level throughout (Fig. 2B). The perfect regulation of Mg^{2+} and Cl^- in the voluntary feeding fish, compared with the dramatic reduction (and complete lack of recovery) of Mg^{2+} and the un-proportional drop in Cl^- in catheter-fed fish, suggests an intriguing role for the neural phase in the homeostasis of these ions following a meal.

Conclusions

The present and the companion study by Bucking and Wood (Bucking and Wood, 2008) are the first reports of a post-prandial alkaline tide in a teleost fish species. From these data, rainbow trout do not compensate for a post-prandial alkaline tide using the same mechanisms as mammals and ectothermic vertebrates, i.e. the retention of CO_2. There are considerable differences between fish that are able to anticipate feeding (voluntarily fed fish) and those that presumably have the initial neural or cephalic phase of gastric acid secretion bypassed by filling the stomach *via* a catheter. The former group were able to recover faster from a less pronounced deviation in blood acid–base balance and maintain ionic balance following a meal. We hypothesise that the reason for this difference is that the initial anticipatory phase of gastric acid secretion has been circumvented. It would therefore be of interest to ascertain how this phase initiates the appropriate processes, and explore the feedback pathways and regulatory transport mechanisms that enable a rapid recovery response to any post-prandial acid–base and ionic disturbances. This should also be borne in mind when interpreting the results of studies on other aspects of post-prandial physiology, where force feeding by gavage is commonly used in preference to voluntary feeding.

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