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The intestinal response to feeding in seawater gulf toadfish, *Opsanus beta*, includes elevated base secretion and increased epithelial oxygen consumption

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SUMMARY

Intestinal HCO_3^- secretion is essential to marine teleost fish osmoregulation and comprises a considerable source of base efflux attributable to both serosal HCO_3^- and endogenous CO_2 hydration. The role of intestinal HCO_3^- secretion in dynamic acid–base balance regulation appears negligible in studies of unfed fish, but evidence of high intestinal fluid [HCO_3^-] in fed marine teleosts led us to investigate the source of this HCO_3^- and its potential role in offsetting the postprandial 'alkaline tide' commonly associated with digestion. Specifically, we hypothesized that elevated metabolic rate and thus endogenous CO_2 production by intestinal tissue as well as increased transepithelial intestinal HCO_3^- secretion occur post-feeding and offset a postprandial alkaline tide. To test these hypotheses changes in HCO_3^- secretion and O_2 consumption by gulf toadfish (*Opsanus beta*) isolated intestine were quantified 0, 3, 6, 12, 24 and 48 h post-feeding. Intestinal tissue of unfed fish in general showed high rates of HCO_3^- secretion (15.5 μmol g^{-1} h^{-1}) and O_2 consumption (8.9 μmol g^{-1} h^{-1}). Furthermore, postprandial increases in both intestinal HCO_3^- secretion and O_2 consumption (1.6- and 1.9-fold peak increases, respectively) were observed. Elevated intestinal HCO_3^- secretion rates preceded and outlasted those of O_2 consumption, and occurred at a magnitude and duration sufficient to account for the lack of alkaline tide. The dependence of these high rates of postprandial intestinal base secretion on serosal HCO_3^- indicates transepithelial HCO_3^- transport increases disproportionately more than endogenous CO_2 production. The magnitude of postprandial intestinal HCO_3^- secretion indicates the intestine certainly is capable of postprandial acid–base balance regulation.

Key words: postprandial, anion exchange, Cl^-/HCO_3^-, NBC, Na^+/HCO_3^- cotransport, acid–base balance regulation, alkaline tide.

INTRODUCTION

Intestinal HCO_3^- secretion has become widely recognized as an essential component of marine fish osmoregulation (Wilson et al., 2002; Grosell et al., 2005; Grosell, 2007; Grosell et al., 2009b), and the excretion of the resulting carbonates occurs at rates high enough to impact on the inorganic oceanic carbon cycle (Wilson et al., 2009). Studies have shown intestinal base excretion to be countered by branchial acid efflux in unfed marine teleost fish (Wilson et al., 1996; Wilson and Grosell, 2003; Genz et al., 2008), suggesting it is unlikely to play a role in the dynamic regulation of acid–base balance. However, the possibility that intestinal HCO_3^- secretion plays a role in acid–base balance regulation during digestion has been a topic of recent interest (Taylor and Grosell, 2006; Taylor et al., 2007; Wood et al., 2007b; Bucking and Wood, 2008; Cooper and Wilson, 2008; Bucking et al., 2009). Stomach acidification in fish proceeds *via* a mechanism of HCl secretion parallel to that of mammals, resulting in an equimolar secretion of base, as HCO_3^-, to the systemic circulation by gastric parietal cells (Hersey and Sachs, 1995; Niv and Fraser, 2002). The ensuing metabolic alkalosis is commonly referred to as 'alkaline tide' and ranges from a modest response in most mammals (Rune, 1965), to a more extreme alkalosis of 0.3 pH units with a 2.5-fold increase in plasma [HCO_3^-], lasting upwards of 6 days after meal consumption in opportunistically feeding reptiles (Secor and Diamond, 1995; Wang et al., 2001). The occurrence of alkaline tide in fish has received increasing attention in recent years, but has thus far, to our knowledge, been ascertained in only two fish species including freshwater and seawater-acclimated rainbow trout (Bucking and Wood, 2008; Cooper and Wilson, 2008; Bucking et al., 2009) and a marine elasmobranch (Wood et al., 2005; Wood et al., 2007a). These studies show increases in pH (0.15–0.25 units) with approximately 1.5-fold increases in plasma [HCO_3^-] between 3 and 9 h after feeding (Wood et al., 2005; Bucking and Wood, 2008; Cooper and Wilson, 2008; Bucking et al., 2009), with no evidence of the respiratory compensation often observed in the postprandial metabolic response in air-breathing animals (Wang et al., 2001; Andrade et al., 2004). Conversely, investigations of the postprandial metabolic response in other seawater teleosts including gulf toadfish, *Opsanus beta* (Taylor and Grosell, 2006), and European flounder, *Platichthys flesus* (Taylor et al., 2007), have shown no evidence of either alkaline tide or respiratory compensation, and indicate that increases in base excretion are insufficient to compensate for the systemic base gain resulting from gastric acid secretion (Taylor et al., 2007). Furthermore, the composition of postprandial intestinal fluids in gulf toadfish (Taylor and Grosell, 2006) indicates a substantial increase in anion exchange during digestion, and led us to examine the intestine more closely as a potential site of dynamic postprandial acid–base balance regulation.

Intestinal anion exchange in marine teleosts occurs at the apical membrane and draws from both serosal HCO_3^- and endogenous CO_2, the latter undergoing hydration facilitated by carbonic anhydrase (CA), yielding HCO_3^- and H^+ (Wilson et al., 2002; Grosell, 2006). As HCO_3^- arising from the CO_2 hydration reaction is exchanged across the apical membrane for Cl^-, equimolar extrusion of H^+ maintains cellular acid–base balance and has been shown to occur *via* several mechanisms in marine teleosts (Wilson et al., 2002; Grosell, 2007; Grosell et al., 2009a). An additional source of HCO_3^- for anion exchange stems from serosal HCO_3^-

taken up across the basolateral membrane *via* Na^+/HCO_3^- cotransport (NBC), driven by the electrochemical gradient for Na^+ created by basolateral Na^+/K^+-ATPase (NKA). Basolateral NBC has recently been characterized in seawater teleosts (Kurita et al., 2008) including toadfish (Taylor et al., 2010) and provides a route for transepithelial HCO_3^- secretion ideally suited for dynamic regulation of systemic acid–base balance. While the serosal source of HCO_3^- for intestinal HCO_3^- secretion by marine teleosts has direct implications for alkaline tide compensation, endogenous CO_2 production is also likely to be affected by feeding. Any changes in endogenous CO_2 production by the intestinal epithelium may result in proportional changes in basolateral H^+ extrusion to the serosa, a process also countering the development of an alkaline tide. In addition to the alkaline tide, the postprandial metabolic response includes a well-characterized increase in metabolic rate referred to as specific dynamic action (SDA) (Jobling, 1981; McCue, 2006; Secor, 2009). SDA has been shown to comprise an average maximum metabolic rate increase of 25% in humans, persisting for 4–6 h after feeding (Secor, 2009). Animals with more opportunistic feeding strategies, such as certain snakes, have been shown to experience postprandial metabolic rate increases lasting 6 days following feeding, with an average maximum increase of 687% (Secor, 2009). The SDA response of most fishes falls between the modest response seen in humans and the extreme response of snakes to feeding; postprandial maximum metabolic rate increases average 136% in fish, with elevated metabolic rates persisting 20 h after feeding (Jobling, 1981; Secor, 2009). The variability associated with feeding lends an exceptional complexity to these investigations; the SDA response in all animals is dependent on a suite of variables including meal mass and composition, body temperature and body size (Jobling, 1981; McCue, 2006; Secor, 2009). One consequence of this variability both within and among species is that SDA has been well characterized in a variety of animals, but the exact source of the metabolic rate increase associated with feeding is difficult to discern and remains relatively speculative.

In mammals, the gastrointestinal (GI) tract is a site of high O_2 uptake that amounts to 20–25% of the whole-body O_2 consumption, even in the post-absorptive or fasting state (Britton and Krehbiel, 1993; Duee et al., 1995). This energy consumption is disproportionate for the size of the tissue (about 6% of body mass in these studies). Accordingly, the coupling of active transport to oxidative metabolism in epithelial tissues (Zerahn, 1956; Mandel and Balaban, 1981) suggests that the GI tract likely elicits an even greater SDA response, relative to its mass, than that of the whole animal, although investigations of postprandial oxygen consumption by isolated intestinal epithelia are scant. The importance of the marine fish intestine in osmoregulation suggests the baseline (i.e. fasting) oxygen demand by this organ may be higher than that in other animals. The intestinal tissue oxygen demand is a particular point of interest due to the reliance of intestinal HCO_3^- secretion on endogenous CO_2 production.

We hypothesized that elevated metabolic rate and thus endogenous CO_2 production by intestinal tissue as well as increased transepithelial intestinal HCO_3^- secretion occur post-feeding and offset a postprandial alkaline tide. The objectives of the present study were therefore to quantify the postprandial changes in HCO_3^- secretion and oxygen consumption by the toadfish intestine. Monitoring these parameters in a parallel postprandial time course allowed for determination of the source of high postprandial intestinal fluid [HCO_3^-] and its possible contribution to the dynamic regulation of acid–base balance.

MATERIALS AND METHODS

Gulf toadfish (*Opsanus beta* Goode and Bean 1880), were obtained by trawl from Biscayne Bay as by-catch by shrimp fishermen local to Miami, FL, USA, during January and February 2008. Within hours of capture fish were transported to the wetlab facility at the Rosenstiel School of Marine and Atmospheric Science at the University of Miami, FL, USA, and subjected to an ecto-parasite treatment (McDonald et al., 2003). Prior to experimentation, fish were maintained in the laboratory for a minimum of 2 weeks in aquaria with a constant flow of filtered, aerated seawater (32±1‰) at 21±2°C from Bear Cut, FL, USA. These aquaria were equipped with PVC shelters to reduce stress and aggression among fish. Toadfish were fed chopped whole squid, *Loligo* sp., to satiation twice weekly until experimentation, during which time food was offered as outlined below.

A natural feeding protocol was employed in which toadfish in each 80 l tank (<10 toadfish per tank, ranging from 15 to 30 g unless otherwise noted) were presented with chopped squid and allowed to feed to satiation for 1 h before excess food was vigilantly removed from the tank. For all isolated tissue work described below toadfish intestinal tissue was collected before feeding and 3, 6, 12, 24 and 48 h after feeding as follows. Toadfish showing gastric distension were selected for all postprandial time points, and all fish were killed by a lethal dose (5 g l^{-1}) of tricaine methanesulfonate (MS-222) after which the spine was severed. Anterior intestinal tissue was excised from just posterior to the bile duct, cut open lengthwise, and mounted on the appropriate tissue holder for pH-stat or O_2 consumption measurements as described below. Stomach contents were recovered from fish sampled at 3, 6, 12 and 24 h postprandially and used to estimate meal size with respect to body mass. All experimental procedures were approved by the University of Miami Animal Care and Use Committee under protocol 08–017.

Quantification of GI tract contribution to whole-animal mass

In order to more accurately quantify the contribution of intestinal tissue HCO_3^- secretion and O_2 consumption to whole-animal acid–base balance and metabolism, the percentage body mass accounted for by this base-secreting, likely metabolically demanding tissue was determined under fasted and postprandial conditions. Toadfish (mass=46.7±1.6 g) were sampled before feeding (N=12), at which time these and all remaining experimental animals were weighed to obtain pre-feeding mass. Postprandial sampling was done 3 days (N=18) and 6 days (N=6) after feeding. Fish were killed as described above, their mass recorded, and the entire GI tract excised. The GI tract was separated into stomach and intestinal (including rectal) tissue at the pyloric sphincter. The wet mass of each tissue was measured in pre-weighed plastic weigh trays, and tissues were desiccated overnight to a constant mass at 50°C. Dry mass was then recorded and compared with wet mass to estimate tissue water content in control and postprandial fish.

Isolated intestine HCO_3^- secretion and electrophysiology

To simultaneously investigate electrophysiological parameters and HCO_3^- secretion rates of isolated intestinal epithelia, Ussing chambers (Physiological Instruments, San Diego, CA, USA) were set up in combination with an automated pH-stat titration system (model TIM 854 or 856, Radiometer, Copenhagen, Denmark) as described previously (Grosell and Genz, 2006). A segment of anterior intestine was excised as described above and mounted onto a tissue holder (model P2413, Physiological Instruments) exposing 0.71 cm^2 of the isolated tissue, and positioned between two half-chambers (model P2400, Physiological Instruments) containing

Table 1. Composition of serosal and mucosal salines used in isolated intestinal tissue experiments

	Serosal		
	0 mmol l^{-1} HCO_3^-	10 mmol l^{-1} HCO_3^-	Mucosal
NaCl (mmol l^{-1})	151	151	69
KCl (mmol l^{-1})	3	3	5
$MgCl_2$ (mmol l^{-1})			22.5
$MgSO_4$ (mmol l^{-1})	0.88	0.88	77.5
Na_2HPO_4 (mmol l^{-1})	0.5	0.5	
KH_2PO_4 (mmol l^{-1})	0.5	0.5	
$CaCl_2$ (mmol l^{-1})	1	1	5
$NaHCO_3$ (mmol l^{-1})	0	10	
Hepes			
Free acid (mmol l^{-1})	11	11	
Sodium salt (mmol l^{-1})	11	11	
Urea (mmol l^{-1})	4.5	4.5	
Glucose (mmol l^{-1})	5	5	
Osmolality (mosmol l^{-1})	320*	320	320*
pH	7.800‡	7.800‡	7.800†
Gas	O_2	0.3% CO_2 in O_2	O_2

*Adjusted with mannitol to ensure transepithelial isosmotic conditions in all experiments.
†pH 7.800 was maintained by pH-stat titration.
‡pH adjusted to 7.800 by NaOH addition.

1.6 ml of the appropriate, pre-gassed, mucosal or serosal saline (Table 1). The saline in each half-chamber was continually mixed by airlift gassing with either O_2 or 0.3% CO_2 in O_2 (Table 1), and a constant temperature of 25°C was maintained by a thermostatic water bath. Current and voltage electrodes connected to amplifiers (model VCC600, Physiological Instruments) recorded the transepithelial potential (TEP) differences under current-clamp conditions at 0 μA, with 3 s, 50 μA pulses from the mucosal to the serosal side at 60 s intervals. These current-clamped conditions were maintained across all treatments. TEP measurements were logged on a personal computer using Biopac systems interface hardware and Acqknowledge software (version 3.8.1; Biopac, Goleta, CA, USA). TEP values are reported with a luminal reference of 0 mV. A pH electrode (model PHC4000.8, Radiometer) and microburette tip were submersed in the luminal half-chamber and were connected to the automated pH-stat titration system which was grounded to the amplifier to allow for pH readings during current pulsing. The pH-stat titrations were performed on luminal salines at a physiological pH of 7.800 throughout all experiments (creating symmetrical pH conditions on either side of the epithelium), with pH values and rate of acid titrant (0.005 mol l^{-1} HCl) addition logged to a PC using Titramaster software (version 5.1, Radiometer). Titratable alkalinity, representing HCO_3^- secretion (Grosell and Genz, 2006), was calculated from the rate of titrant addition and its concentration. Intestinal preparations from gulf toadfish are viable and stable for at least 5 h under these experimental conditions (Grosell and Genz, 2006). The titration curves and electrophysiological measurements of each preparation were allowed to stabilize for 60 min after introduction to the chamber before HCO_3^- secretion rates and electrophysiological parameters were averaged and reported for the following 60 min of exposure.

Postprandial HCO_3^- secretion and electrophysiology

The combined pH-stat titration/electrophysiology approach described above was employed to quantify changes in HCO_3^- secretion rates and electrophysiology of the isolated intestine after feeding. For these experiments, tissue measurements were made from fish sampled in the postprandial time course (N=8 at each time point) described above.

Dependence on serosal HCO_3^-

To quantify any changes in the contribution of endogenous CO_2 to intestinal HCO_3^- secretion under fed *vs* control conditions, additional experiments were completed in unfed and 3 h postprandial fish (N=6). For each fish, whether control or fed, the intestine was dissected out as described above, and then split lengthwise into two halves which were mounted onto tissue holders (model P2404, Physiological Instruments) exposing 0.25 cm^2 tissue to the chamber. The two tissue preparations taken from the same length of intestine were run simultaneously in two parallel systems. One of the preparations was exposed to HCO_3^--free serosal saline for 120 min (60 min stabilizing period and 60 min measurement period, as described above), while the other was exposed to serosal saline containing 10 mmol l^{-1} HCO_3^- (Table 1). These parallel measurements of HCO_3^- secretion in the presence of serosal HCO_3^- and under HCO_3^--free conditions by epithelia taken from the same animal and same length of intestine allowed us to calculate the contribution of endogenous *vs* serosal substrate to HCO_3^- secretion before and after feeding.

O_2 consumption by isolated intestinal tissue

Newly designed, custom-made glass Ussing chambers (Loligo Systems, Tjele, Denmark, model CH10500) were used to measure O_2 consumption (reported in μmol h^{-1} relative to intestinal tissue surface area and/or mass) by the isolated toadfish anterior intestine, while simultaneously monitoring tissue electrophysiological parameters to ensure tissue viability. Glass–Teflon-connected current and voltage electrode ports allowed for electrophysiology measurements to be collected by a hardware and software combination identical to that used during pH-stat measurements (described above). These glass Ussing chambers are designed to have a small volume (2.95 ml in each half-chamber) to epithelia surface area (0.87 cm^2) ratio, and the salines contained therein (Table 2) were continually mixed *via* micromagnetic stirrers rather

Table 2. Dependence of HCO_3^- secretion rates, TEP and conductance on serosal HCO_3^- in control and 3 h postprandial isolated epithelia

	HCO_3^- secretion (μmol cm^{-2} h^{-1})	TEP (mV)	Conductance (mS cm^{-2})
Control			
HCO_3^- free	0.41±0.03^A	−18.93±2.42	17.28±0.99
10 mmol l^{-1} serosal HCO_3^-	0.82±0.08^B	−16.17±3.87	16.14±1.10
3 h postprandial			
HCO_3^- free	0.53±0.08^A	−16.39±1.16	16.66±1.58
10 mmol l^{-1} serosal HCO_3^-	1.14±0.05^b	−19.84±2.42	19.01±0.35

TEP, transepithelial potential.
N=6 for each set (control *vs* 3 h postprandial) of paired (HCO_3^- free *vs* 10 mmol l^{-1} serosal HCO_3^-) measurements, with statistically significant differences indicated by upper and lower case letters. Data are displayed as means ± s.e.m.

than airlift gassing. Teflon tissue holders ensure a gas-tight connection between the epithelium and glass chambers. Oxygen measurements were made using a fiber-optic oxygen sensor connected to Fibox 3 single-channel oxygen meters (Loligo), with data recorded by Oxy-View software (www.oxyview.com) allowing for parallel measurement of O_2 consumption in each (mucosal and serosal) half-chamber. The fiber-optic light source is secured on the outside of the glass chambers while O_2 sensors are glued to the inside of the chamber, reflecting light through the glass chamber wall. This approach minimizes the number of ports in the half chambers and thereby the source of error compared with measurements made using traditional O_2 electrodes. Calibration of O_2 sensors was done daily by gassing mucosal and serosal salines (Table 1) with N_2 for their respective zero, and gassing with air for calibration to 100% air. In all experiments, O_2 consumption measurements are performed using intermittent-flow respirometry with flushing controlled manually using a gravity-fed saline replacement system. Initial experiments using this novel system quantified O_2 consumption during exposure of control epithelia sequentially to (1) mucosal salines at 100% air saturation and serosal salines at 0% air (gassed with N_2), and (2) mucosal salines at 0% air saturation and serosal salines at 100% air. These experiments served to quantify any unidirectional or bidirectional diffusion of O_2 across the isolated toadfish intestine.

Intestinal tissue substrate utilization

In order to ensure fasted toadfish intestinal tissue O_2 consumption was not limited by substrate availability, a series of experiments was undertaken using varying combinations of mucosal and HCO_3^--free serosal salines (Table 1). While salines used in isolated teleost intestine experiments typically include approximately 5 mmol l^{-1} glucose in the serosal saline (Grosell et al., 2005; Grosell and Genz, 2006; Grosell et al., 2009a), literature on substrate utilization in isolated mammalian intestinal epithelia (Duee et al., 1995) led us to investigate the effects of adding 5 mmol l^{-1} glutamate to the 'standard' (Table 1) HCO_3^--free serosal saline and also the addition of these two substrates to the 'standard' (Table 1) mucosal saline. Furthermore, the impact of a mucosal saline containing a more complete mixture of amino acids, intended to more closely mimic the composition of chyme, was investigated on fasted intestinal tissue using L-15 cell culture media (Sigma, St Louis, MO, USA). The O_2 consumption was quantified for isolated toadfish intestine exposed to the following four saline combinations: (1) mucosal (no metabolic substrate) + serosal (with glucose), (2) mucosal (no metabolic substrate) + serosal (with glucose and glutamate), and (3) mucosal (with glucose and glutamate) + serosal (with glucose and glutamate), applied sequentially in randomized order to single preparations (N=8 fasted), and (4) mucosal (L-15) + serosal (with glucose), applied to separate preparations (N=5 fasted). Oxygen depletion from mucosal and serosal salines was measured in parallel and O_2 consumption was quantified between 90% and 95% air saturation in each half-chamber. These measurements from mucosal and serosal half-chambers were combined to give O_2 consumption of the isolated tissue.

Dependence of O_2 consumption on O_2 availability

To investigate the influence of P_{O_2} on O_2 consumption by isolated toadfish anterior intestine, a series of experiments was completed on tissue from fasted toadfish (N=8) in which oxygen tension was allowed to drop 50% from air saturation as a consequence of tissue O_2 consumption. Standard mucosal and HCO_3^--free serosal (with 5 mmol l^{-1} glucose) salines (Table 1) were used in these experiments. O_2 consumption was calculated in parallel for mucosal and serosal half-chambers for every 5% drop in O_2 from air saturation and combined to give total tissue O_2 consumption.

Specific dynamic action (SDA)

The presence and magnitude of SDA by the isolated toadfish intestine was investigated by measuring O_2 consumption of tissues sampled in a time course following feeding as described above. Eight fish were sampled at each time point, with mean tissue O_2 consumption analyzed as an indicator of tissue metabolic rate. Standard mucosal and HCO_3^--free serosal (with 5 mmol l^{-1} glucose) salines (Table 1) were used in these experiments. Furthermore, mucosal L-15 media was applied to 3 h post-fed intestinal tissue (N=5) to investigate the effects of a saline more closely resembling chyme on postprandial epithelia. Standard serosal saline (Table 1) was used during mucosal L-15 treatment. In all control and postprandial intestinal preparations, O_2 consumption was calculated in parallel for mucosal and serosal half-chambers during 90–95% air saturation and combined to give total tissue O_2 consumption.

Statistics

Data are presented as means ± s.e.m. Statistical comparisons were made using one-way ANOVA followed by the Student–Newman–Keuls test for multiple comparisons as appropriate. Paired t-tests were used to determine the dependence of HCO_3^- secretion and electrophysiology on serosal HCO_3^- within each group (control *vs* fed). Statistical significance was determined at $P<0.05$ in all cases.

RESULTS

Meal size in naturally feeding toadfish

Stomach contents were recovered from 3, 6, 12 and 24 h postprandial toadfish at 9.1(±0.7), 7.8(±1.0), 6.3(±0.9) and 5.2(±1.3)% body mass, respectively (Fig. 1). Reciprocal transformation (Jobling, 1981) of these data yielded the best-fitting curve (R=0.55), indicating non-linear rates of gastric emptying. The y-intercept of the linear equation fitted to these data indicates 9.5% body mass to be the maximal meal size, suggesting relatively little gastric emptying occurs in the first 3 h after feeding.

Quantification of GI tract contribution to whole-animal mass

The GI tract of fasted toadfish was found to contribute 2.84±0.08% of body mass, with 81.3% water content (Fig. 2). Intestinal tissue was responsible for 64.0% of GI tract mass, and was 81.5% water by mass. Feeding made no statistically significant changes to GI mass (Fig. 2A), but a trend of increased wet and dry GI mass 3 days post-feeding appears to be attributable to increases in intestinal, rather than stomach, mass. Indeed, the intestinal contribution to body mass was elevated by 14.6% at this time, an increase that cannot be accounted for by its water content which was 81.1±0.33% (Fig. 2B).

Isolated intestine HCO_3^- secretion and electrophysiology

Postprandial HCO_3^- secretion and electrophysiology

Isolated toadfish intestine exhibited a significant postprandial increase in HCO_3^- secretion that persisted 48 h after feeding, with an apparent peak at 3 h (Fig. 3A). Corresponding electrophysiological data (Fig. 3B,C) show no statistically significant changes in either TEP or epithelial conductance at any point after feeding.

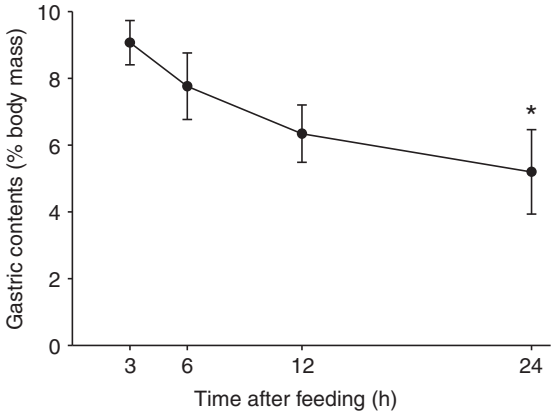

Fig. 1. A time course of stomach contents relative to body mass allowed for estimation of meal size and gastric emptying time as described in the text. Data are best fitted (R=0.55) to the equation $1/y=0.0065x+0.1056$. Stomach contents were collected 3 h (N=21), 6 h (N=8), 12 h (N=8) and 24 h (N=7) postprandially and are displayed as means ± s.e.m., with asterisks indicative of a statistically significant decline from 3 h values.

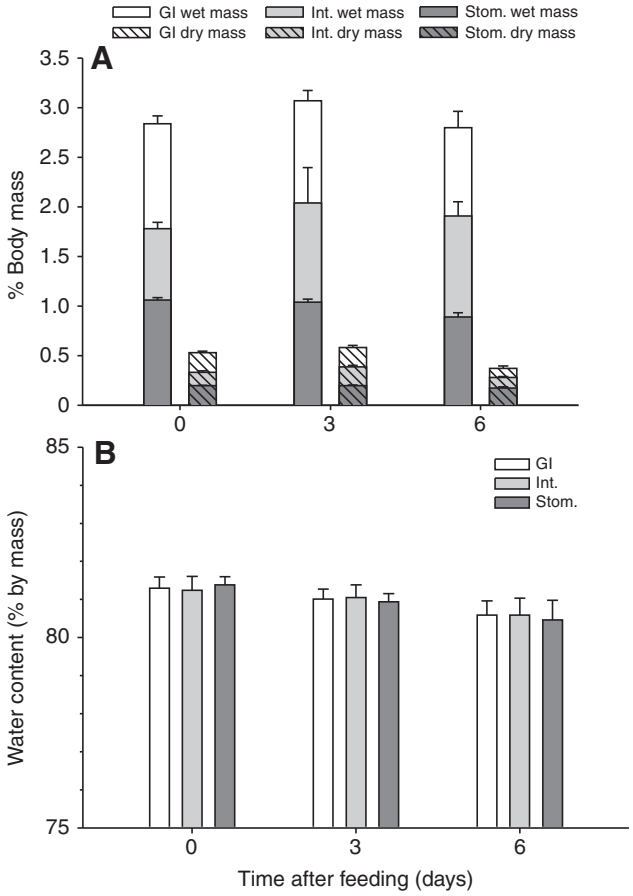

Fig. 2. (A) The contribution of the gastrointestinal (GI) tract to body mass was quantified under control (N=12), and 3 day (N=18) and 6 day (N=6) postprandial toadfish. Stomach (Stom.) and intestinal (Int.) tissue samples were analyzed for wet *vs* dry mass in order to estimate changes in (B) organ water content resulting from feeding. Data are displayed as means + s.e.m.

Dependence on serosal HCO_3^-

Bicarbonate secretion by the isolated toadfish intestine showed a strong dependence on serosal HCO_3^- availability, with the contribution of serosal HCO_3^- increasing following feeding (Fig. 4). Total postprandial HCO_3^- secretion rates were 39% greater than control rates (Fig. 4); however, the fraction attributable to serosal HCO_3^- was 49% higher in postprandial epithelia than in unfed controls. Under HCO_3^--free conditions, rates of HCO_3^- secretion were not significantly different between control and postprandial intestinal epithelia (Fig. 4). While electrophysiological parameters showed no statistically significant dependence on serosal HCO_3^-, we noted a trend towards increased epithelial conductance in the presence of serosal HCO_3^- in 3 h postprandial epithelia, but not in control preparations (Table 2).

O_2 consumption by isolated intestinal tissue

Initial experiments (N=3) using the newly designed Ussing chamber tissue respirometer showed a significant decrease in total tissue O_2 consumption when one half-chamber was deprived of O_2. During exposure to 0% air saturation, the mucosal and serosal salines had very minimal rates of O_2 appearance (0.03±0.02 and 0.01±0.01 μmol cm^{-2} h^{-1}, respectively). When exposed to 100% air saturation, the mucosal and serosal salines showed O_2 depletion at rates (0.36±0.14 and 0.19±0.02 μmol cm^{-2} h^{-1}, respectively) not significantly different from those measured during tissue exposure to symmetrical 100% air saturation. A representative trace of O_2 content in each half-chamber during these experiments is shown in Fig. 5A.

Substrate utilization

No significant differences were observed between total fasted tissue O_2 consumption for the different substrate combinations (Fig. 5B), suggesting that the 'standard' asymmetrical salines (Table 1) do not impose substrate limitations on fasted, isolated toadfish intestine. Furthermore, no significant differences in rates of O_2 depletion between the mucosal and serosal half-chambers were observed in any treatment when measurements in the serosal and luminal half-chambers were made in parallel at 90–95% air saturation (Fig. 5B). Tissue metabolic rate was constant over the approximately 4 h experimental period used in the substrate utilization experiments. Based on these results, the 'standard' asymmetrical mucosal and HCO_3^--free serosal (with 5 mmol l^{-1} glucose) salines were used for all subsequent measurements of tissue O_2 consumption which were confined to last for less than 4 h.

Dependence of O_2 consumption on O_2 availability

Our results show that O_2 consumption by the isolated toadfish intestine occurs at significantly lower rates between 50% and 75% air saturation compared with the highest rates which occur between 75% and 100% air saturation (Fig. 5C). No significant differences were observed among O_2 consumption rates recorded between 75% and 100% air saturation (Fig. 5C). To ensure that measurements of O_2 consumption were not limited by P_{O_2}, all subsequent measurements of O_2 consumption were performed at 90–95% air saturation.

Specific dynamic action (SDA)

Isolated toadfish intestine is subject to a significant postprandial increase in O_2 consumption indicative of SDA in this tissue. O_2 consumption was significantly elevated over control rates (0.47±0.02 μmol cm^{-2} h^{-1}) between 3 h (0.67±0.04 μmol cm^{-2} h^{-1}) and 6 h (0.88±0.04 μmol cm^{-2} h^{-1}) following feeding, with peak values at 6 h significantly greater than at all other time points (Fig. 6). The

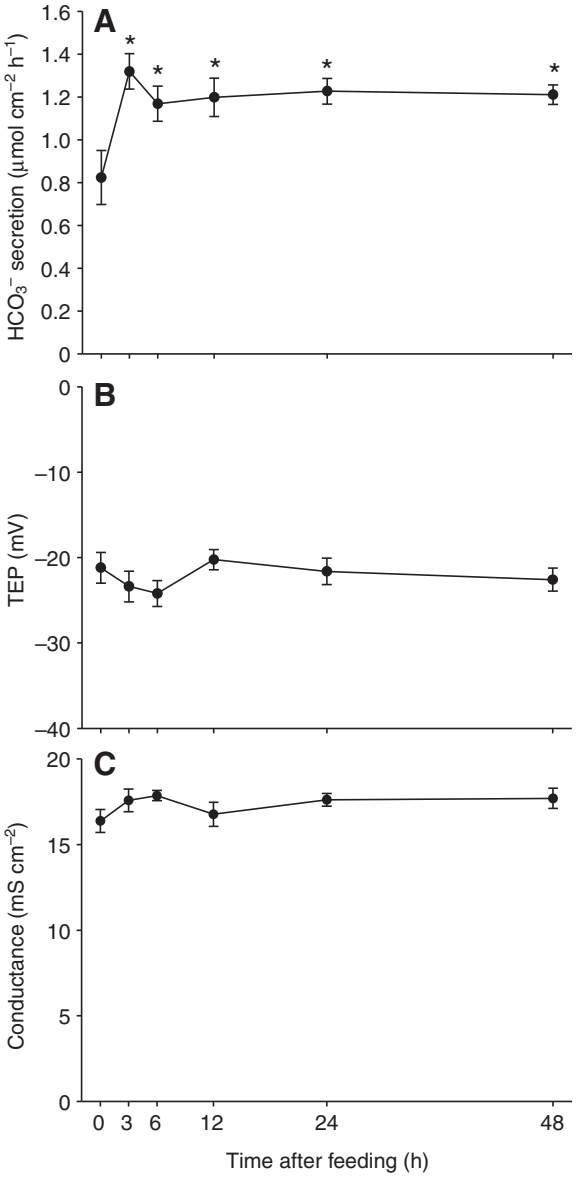

Fig. 3. Postprandial (A) HCO_3^- secretion, (B) transepithelial potential (TEP) and (C) conductance by isolated toadfish intestine. *N*=8 for each time point, with statistically significant differences from control (0 h) indicated by asterisks. Data are displayed as means ± s.e.m.

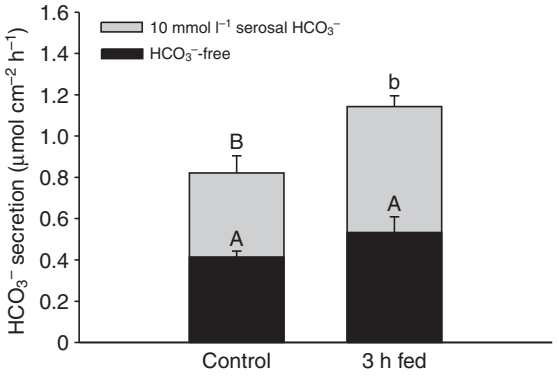

Fig. 4. Dependence of HCO_3^- secretion rates on serosal HCO_3^- in control and 3 h postprandial isolated epithelia. *N*=6 for each set (control *vs* 3 h postprandial) of paired (HCO_3^- free *vs* 10 mmol l^{-1} serosal HCO_3^-) measurements, with statistically significant differences indicated by upper and lower case letters. Data are displayed as means + s.e.m.

application of L-15 media to the mucosa of 3 h postprandial epithelia did not increase the SDA response by the isolated tissue. Mucosal application of L-15 caused no significant changes in 3 h postprandial O_2 consumption (0.51±0.07 μmol cm^{-2} h^{-1}) compared with glucose-containing mucosal saline, although these rates are significantly higher than those measured in epithelia from unfed fish exposed to mucosal L-15 (0.43±0.06 μmol cm^{-2} h^{-1}) as described above.

DISCUSSION

The present study suggests that the postprandial increases in intestinal fluid [HCO_3^-] measured in marine teleosts (Taylor and Grosell, 2006; Taylor et al., 2007) are attributable largely to increases in transepithelial HCO_3^- secretion. Measurements of epithelial HCO_3^- secretion suggest that adjustments in the contribution of endogenous CO_2 production by isolated toadfish intestine are modest 3 h after feeding (Fig. 4); however, epithelial O_2 consumption (Fig. 6) indicates a clear rise in tissue metabolic rate post-feeding. This discrepancy can be explained in part by the peak postprandial HCO_3^- secretion preceding the peak postprandial metabolic response by the intestinal tissue. This temporal disparity has noteworthy implications for postprandial marine fish physiology. Specifically, with alkaline tide peaking 6–12 h after feeding in the teleost fish species showing this postprandial response (Bucking and Wood, 2008; Cooper and Wilson, 2008; Bucking et al., 2009), an appropriately preceding peak in transepithelial intestinal HCO_3^- secretion may eliminate any measurable accumulation of blood HCO_3^-. The following estimates have provided insight into the feasibility of this hypothesis. Based on measurements of mass and surface area of isolated intestinal tissue used in pH-stat and tissue respirometry experiments (0.053 g tissue cm^{-2}), whole-animal intestinal tract surface area was estimated to be 7.2 cm^2 [1.9% body mass (as pooled from fasted and fed toadfish, Fig. 2)×20 g fish=0.38 g intestinal tract tissue; 0.38 g/0.053 g tissue cm^{-2}=7.2 cm^2 intestinal tract tissue] in a 20 g fish. Assuming constant HCO_3^- secretion rates along the intestinal tract [as is shown in gulf toadfish (Grosell, 2006)], the calculated intestinal tract surface area of 7.2 cm^2 and the difference in total HCO_3^- secretion between postprandial and control epithelia (1.14–0.82=0.32 μmol cm^{-2} h^{-1}) indicate that whole-animal postprandial base efflux to the intestinal fluids exceeds control base efflux by approximately 2.3 μmol h^{-1}. Extracellular fluid volume in marine teleosts has been estimated to comprise approximately 20% body mass (Thorson, 1961; Takei, 2000), giving an extracellular fluid volume of approximately 4 ml in a 20 g toadfish. Considering equilibrium of HCO_3^- between the plasma and extracellular fluids, an alkaline tide of 2.5 mmol l^{-1} HCO_3^- [in line with that reported in dogfish (Wood et al., 2005; Wood et al., 2007a) and trout (Bucking and Wood, 2008; Cooper and Wilson, 2008; Bucking et al., 2009)] in 4 ml of extracellular fluid could be offset by a base efflux of 10 μmol. Simple division of this total by the observed increase in intestinal HCO_3^- secretion following feeding suggests that the 39% increase in base secretion by postprandial intestinal tissue would be sufficient to counteract an alkaline tide of this magnitude within 4–5 h. Based on our results of significantly elevated postprandial intestinal HCO_3^- secretion peaking 3 h post-feeding but persisting over the entire 48 h postprandial period, the toadfish intestine certainly is capable of postprandial regulation of acid–base balance. Furthermore, while

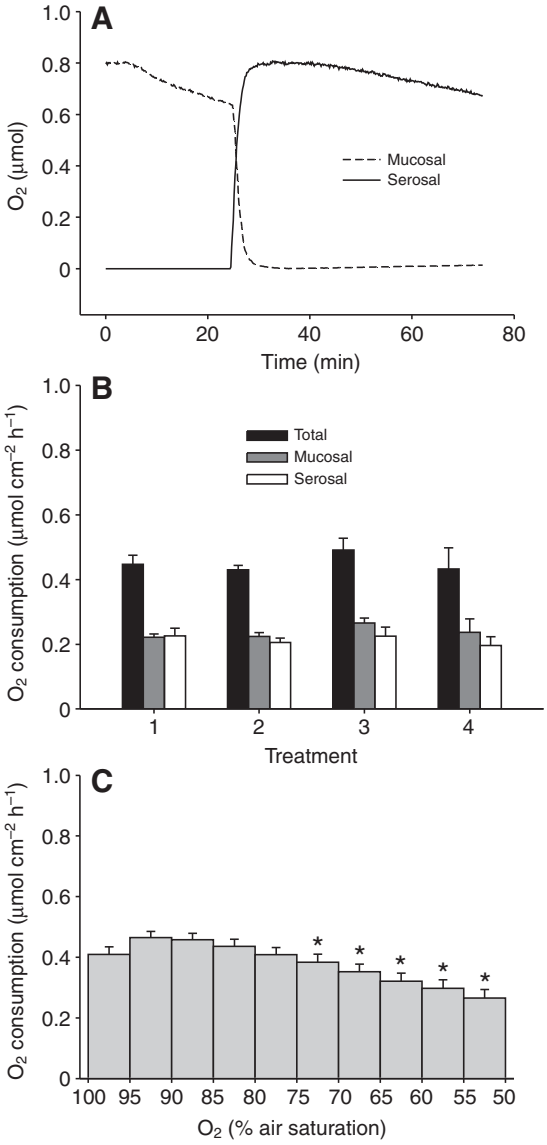

Fig. 5. Influence of backflux/diffusion (A), substrate (B) and O_2 availability (C) on experimental determination of epithelial oxygen consumption. (A) A representative trace of O_2 depletion from mucosal and serosal half-chambers during exposure of control epithelia sequentially to (between 0 and 45 min) mucosal salines at 100% air saturation and serosal salines at 0% air (gassed with N_2), and (between 45 and 100 min) mucosal salines at 0% air saturation and serosal salines at 100% air. (B) Fasted, isolated toadfish intestinal epithelia O_2 consumption shows no significant change between the sequential, randomized application of (1) mucosal (no metabolic substrate) + serosal (with 5 mmol l^{-1} glucose), (2) mucosal (no metabolic substrate) + serosal (5 mmol l^{-1} glucose + 5 mmol l^{-1} glutamate) and (3) mucosal (5 mmol l^{-1} glucose + 5 mmol l^{-1} glutamate) + serosal (5 mmol l^{-1} glucose + 5 mmol l^{-1} glutamate) salines (N=8). Separate fasted epithelia (N=5) show no significant difference in O_2 consumption on exposure to mucosal L-15 + serosal (5 mmol l^{-1} glucose) salines (treatment 4). Furthermore, O_2 consumption is consistent between the mucosal and serosal half-chambers in all experimental treatments. Data are displayed as means + s.e.m. (C) Isolated toadfish intestinal epithelia show a marked decrease in total (mucosal + serosal) O_2 consumption with percentage air saturation. Asterisks indicate statistical significance of this trend (N=8) with data displayed as means + s.e.m.

transepithelial HCO_3^- secretion contributes direct adjustments to plasma [HCO_3^-], any secretion of endogenously produced CO_2 as HCO_3^- into the intestinal fluids will also serve to acidify the plasma

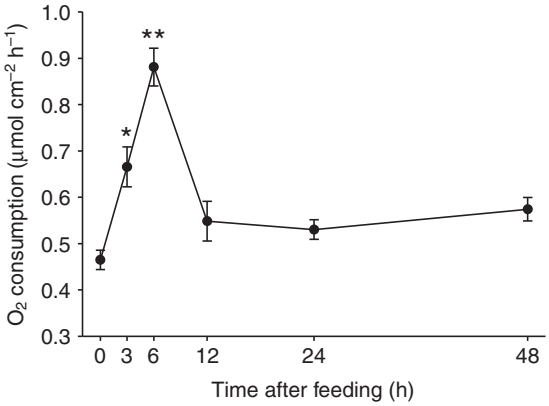

Fig. 6. Isolated toadfish intestinal epithelia show significant (*) elevation in total (mucosal + serosal) O_2 consumption between 3 and 6 h after feeding, with a peak at 6 h that is significantly greater than at all other time points (**). N=8 at each time point, with data displayed as means ± s.e.m.

via basolateral H^+ extrusion (Fig. 7) serving cellular acid–base balance regulation (Grosell, 2006).

The focus of this study was to quantify any postprandial elevation in intestinal HCO_3^- secretion and deduce the source of postprandial high intestinal fluid [HCO_3^-] and thereby its possible contribution to dynamic regulation of acid–base balance after feeding. Our measurements of postprandial O_2 consumption (Fig. 6) used a novel technique to investigate largely unexplored intestinal tissue-specific metabolic rates post-feeding. Initial experiments showed very low rates of O_2 accumulation during O_2-free conditions, indicating a highly gas-tight system with minimal rates of back-flux of atmospheric O_2. The rate of O_2 consumption exceeded the rate of O_2 appearance under O_2-free conditions by more than 100-fold demonstrating that O_2 back-flux was an insignificant source of error in these experiments. Rates of O_2 depletion during exposure of one half-chamber to 100% air saturation, with anoxic conditions in the opposing half-chamber, suggest that diffusion limitations occur at both the mucosal and serosal membranes of the epithelium and prevent O_2 consumption from one half-chamber from maintaining the whole-tissue O_2 consumption. Diffusion limitations were expected at the serosal membrane due to the lack of perfusion of the often substantial muscle layer during these *in vitro* measurements.

The lack of constancy in O_2 consumption measured at levels of air saturation below 75% was unexpected in these experiments, largely because teleost plasma is far less saturated with O_2 than air (Krogh and Leitch, 1919; Evans, 1997), and thus should not be O_2 limited even in the 50–75% air saturation range tested. We have applied several of our results to further investigate this apparent limitation to metabolic rate. Considering that glucose appears to be a sufficient substrate for unfed toadfish intestinal tissue, the possibility of glucose depletion during the relatively prolonged experiments examining the influence of O_2 availability on metabolic rate must be considered. A relationship between O_2 consumption and glucose metabolism of 6 mol O_2 mol^{-1} glucose suggests the isolated toadfish intestine depletes glucose from the tissue respirometer at maximal rates of 0.066 μmol h^{-1}. Thus the 5 mmol l^{-1} serosal glucose (14.75 μmol in the half-chamber volume of 2.95 ml) used in all experiments is sufficient to sustain the carbohydrate metabolism of the epithelium for well past the experimental period (>200 h).

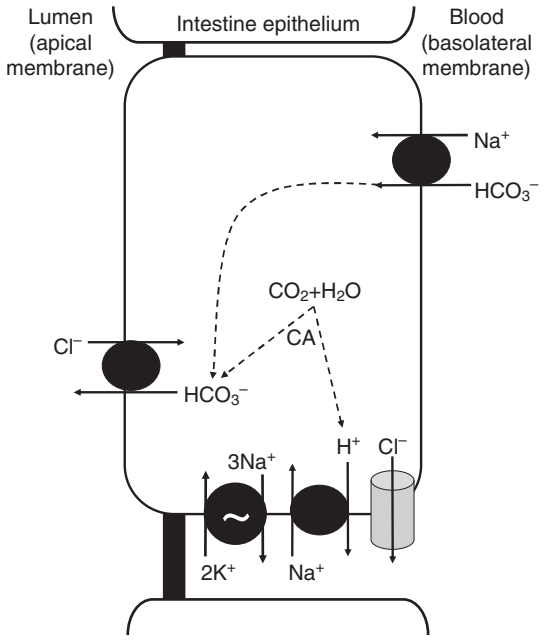

Fig. 7. Schematic cellular model of the mechanism of HCO_3^- secretion by the marine teleost intestine. Hydration of endogenous CO_2 is facilitated by carbonic anhydrase (CA) and the resulting HCO_3^- is exchanged across the apical membrane in exchange for Cl^-, while H^+ is extruded across the basolateral mechanism in exchange for Na^+, driven by the electrochemical gradient for Na^+ created by basolateral Na^+/K^+-ATPase (NKA). Alternatively, serosal HCO_3^- is taken up across the basolateral membrane *via* Na^+/HCO_3^- cotransport (NBC), also driven by the Na^+ electrochemical gradient. The Cl^- taken up from the luminal fluids leaves the cell through basolateral Cl^- channels. Note that this figure is limited to ion transport mechanisms pertaining to intestinal HCO_3^- secretion and is not inclusive of other important transport processes occurring across marine teleost intestinal epithelia.

By comparing tissue O_2 consumption with HCO_3^- secretion under HCO_3^--free conditions as a proxy for endogenous CO_2 production, we have calculated the respiratory quotient (moles of CO_2 produced/moles of O_2 consumed; RQ) of control (RQ=0.89) and 3 h postprandial (RQ=0.80) epithelia. The high RQ in control tissue suggests the utilization of a carbohydrate-rich (carbohydrate RQ=1.0) substrate. This result is in accordance with studies suggesting the intestine metabolizes predominantly carbohydrate and protein (RQ=0.83) substrates (Duee et al., 1995) and that these macromolecules are also the predominant fuels for osmoregulation (Tseng and Hwang, 2008), a process in which the marine teleost intestine plays a critical role (Grosell, 2007). Furthermore, the high RQ under control conditions is in agreement with our observations of glucose alone being a sufficient metabolic substrate for toadfish intestinal tissue. The drop in RQ seen in postprandial epithelia can be explained by at least two theories, which are not mutually exclusive. First, the postprandial intestine may indeed metabolize a higher fraction of protein and/or lipid (RQ as high as 0.71) than that of an unfed fish. Second, as a caveat in both control and postprandial RQ calculations, CO_2 production may exceed that accounted for by measured HCO_3^- secretion. This could be attributable to simple diffusion of endogenously produced CO_2 out of the tissue, and/or the activity of an apical H^+-ATPase which could lead to titration of secreted HCO_3^- beyond that accounted for by our pH-stat methodology (i.e. an underestimation of intestinal HCO_3^- secretion rates) as illustrated in two recent studies (Grosell et al., 2009a; Grosell et al., 2009b). Furthermore, the possibility of endogenous amino acid metabolism by the isolated tissue has been considered. However, during substrate preference experiments, we saw no significant decrease in O_2 consumption when glucose (without glutamate) was applied as the final treatment as opposed to the first treatment. This result suggests that an experimental time of 4 h is not sufficient to deplete the tissue of any internal amino acid stores it may be metabolizing. It furthermore supports the former explanation of alternative CO_2 fates leading to a reduction in apparent RQ. In addition to the aforementioned implications, calculated RQ values validate the decision to investigate both glucose and glutamate as potentially limiting metabolic substrates. The high RQ suggests carbohydrate is an important metabolic substrate in isolated toadfish intestine, a result supported by substrate utilization experiments including those showing a similar SDA response with L-15 application compared with glucose alone. With respect to P_{O_2} dependence of O_2 consumption below 75% air saturation, our results suggest that neither O_2 back-flux nor metabolic substrate limitation is likely to account for this unexpected outcome. Regardless of the reasons for significantly reduced O_2 consumption below 75% air saturation, this experiment validates the use of 75–100% air saturation in these experiments, and also suggests that tissues subjected to pH-stat experiments conducted at 100% O_2 likely display the same metabolic rates as tissues exposed to air, since 75% air saturation is sufficient for the observed O_2 consumption rates. Factors influencing the dependence of isolated tissue O_2 consumption on P_{O_2} are certainly a source of interest for future investigations.

Measurements of O_2 consumption by the isolated toadfish intestine also allow predictions about the intestinal contribution to whole-animal O_2 consumption and SDA. Our results indicate unfed intestinal tissue consumes O_2 at rates approximately 3-fold higher than those measured in whole toadfish (3.0 mmol kg^{-1} h^{-1}) (Gilmour et al., 1998). In a 20 g toadfish, intestinal O_2 consumption accounts for 3.4 μmol h^{-1}, or 5.6% of whole-animal O_2 consumption. This is a surprisingly low fraction considering studies suggesting the non-fed, non-osmoregulating mammalian GI tract accounts for 20–25% of whole-animal O_2 consumption (Duee et al., 1995). Peak postprandial intestinal O_2 consumption equates to 6.3 μmol h^{-1} for a 20 g toadfish, and this intestinal SDA response of a 2.9 μmol h^{-1} increase in O_2 consumption can account for approximately 4.8% of an assumed 60 μmol h^{-1} increase in metabolic rate of a 20 g toadfish as suggested by typical increases in fish O_2 consumption associated with SDA (Jobling, 1981; McCue, 2006; Secor, 2009). These calculations suggest that the intestinal contribution to whole-toadfish SDA is just over 2-fold higher than that suggested by its mass. This contribution of the intestinal tract to SDA is rather modest considering its location at 'ground zero' with respect to nutrient absorption (requiring ATP-driven maintenance of a sodium gradient), but corresponds with the view that SDA is attributable predominantly to post-absorptive processes including protein synthesis, rather than pre-absorptive and absorptive processes (Secor, 2009). Furthermore, our L-15 data suggest that the presence of chyme in the lumen is not likely to further stimulate the intestinal SDA response. However, considering the remarkable variation in magnitude between studies of fish SDA (Secor, 2009), comparison of tissue-specific and whole-animal postprandial O_2 consumption is an important area for future study and would shed further light on the intestinal contribution to SDA.

The duration of whole-animal SDA in fishes also varies greatly among studies, ranging from 1.3 to 390 h, and is dependent largely on meal mass and temperature (Jobling, 1981; McCue, 2006; Secor, 2009). The duration of the intestinal tissue SDA response in toadfish

appears markedly shorter than that shown for whole-fish SDA during acclimation to similar temperatures (Jobling, 1981; Secor, 2009). An estimated meal size of 9.5% body mass in our experiments suggests an approximate meal energy of 6.3 kJ, based on measurements of mean energy content in squid (Croxall and Prince, 1982). For a meal of this energetic value, the gastric evacuation time of 24–48 h seen in our experimental animals is in line with that shown for other benthic fishes acclimated to similar temperatures (Jobling, 1981). The meal size in our experimental animals was considerably larger than that used in other studies of postprandial fish physiology (Bucking and Wood, 2008; Cooper and Wilson, 2008; Secor, 2009). Despite this relatively large meal size, our data on postprandial GI tract morphology showed little evidence of the intestinal hyperplasia (Lignot et al., 2005) or fluid engorgement (Wood et al., 2007b) that has been shown in digestive tissues of opportunistically feeding animals consuming similar rations. However, we did see a trend of increased contribution of the intestinal (rather than gastric) tissue to body mass following feeding. This result is not surprising considering the prominent role of the intestinal tract in nutrient and water absorption, but the source of the postprandial intestinal mass increase deserves further attention as it appears to be more complicated than a simple increase in tissue perfusion.

Our data suggest that elevated postprandial intestinal HCO_3^- secretion occurs predominantly by transepithelial transport rather than increased endogenous CO_2 production (i.e. SDA), although both are occurring, and that it is capable of offsetting alkaline tide. Postprandial increases in basolateral NKA activity associated with SDA to fuel Na^+-driven nutrient uptake may also act to strengthen the electrochemical gradient favoring transport by basolateral NBC (Fig. 7). The serosal HCO_3^- substrate for NBC would be simultaneously increased during alkaline tide (Hersey and Sachs, 1995; Niv and Fraser, 2002), adding further driving force for transepithelial HCO_3^- secretion owing to the high affinity of basolateral NBC for serosal HCO_3^- (Taylor et al., 2010). Because intestinal HCO_3^- secretion has been shown to be limited to the basolateral membrane rather than by apical Cl^-/HCO_3^- exchange (Taylor et al., 2010), postprandial elevation of the driving force(s) for basolateral NBC would facilitate intestinal base efflux and thereby form the basis of this mechanism for dynamic regulation of postprandial acid–base balance.

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REFERENCES

Andrade, D. V., De Toledo, L. F., Abe, A. S. and Wang, T. (2004). Ventilatory compensation of the alkaline tide during digestion in the snake *Boa constrictor*. *J. Exp. Biol.* **207**, 1379-1385.

Britton, R. and Krehbiel, C. (1993). Nutrient metabolism by gut tissues. *J. Dairy Sci.* **76**, 2125-2131.

Bucking, C., Fitzpatrick, J. L., Nadella, S. R. and Wood, C. M. (2009). Post-prandial metabolic alkalosis in the seawater-acclimated trout: the alkaline tide comes in. *J. Exp. Biol.* **212**, 2159-2166.

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

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

Croxall, J. P. and Prince, P. A. (1982). Calorific content of squid (Mollusca: Cephalopoda). *Br. Antarct. Surv. Bull.* **55**, 27-31.

Duee, P. H., Darcyvrillon, B., Blachier, F. and Morel, M. T. (1995). Fuel selection in intestinal-cells. *Proc. Nutr. Soc.* **54**, 83-94.

Evans, D. H. (1997). *The Physiology Of Fishes*, 2nd edn, pp. 1-544. Boca Raton, FL; CRC Press.

Genz, J., Taylor, J. R. and Grosell, M. (2008). Effects of salinity on intestinal bicarbonate secretion and compensatory regulation of acid–base balance in *Opsanus beta*. *J. Exp. Biol.* **211**, 2327-2335.

Gilmour, K., Perry, S. F., Wood, C. M., Henry, R. P., Laurent, P., Pärt, P. and Walsh, P. J. (1998). Nitrogen excretion and the cardiorespiratory physiology of the gulf toadfish, *Opsanus beta*. *Physiol. Zool.* **71**, 492-505.

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

Grosell, M. (2007). Intestinal transport processes in marine fish osmoregulation. In *Fish Osmoregulation* (ed. B. Baldisserotto, J. M. Mancera and B. G. Kapoor), pp. 332-357. Enfield, NH: Science Publishers.

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

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

Grosell, M., Genz, J., Taylor, J. R., Perry, S. F. and Gilmour, K. M. (2009a). The involvement of H^+-ATPase and carbonic anhydrase in intestinal HCO_3^- secretion in seawater acclimated rainbow trout. *J. Exp. Biol.* **212**, 1940-1948.

Grosell, M., Mager, E. M., Williams, C. and Taylor, J. R. (2009b). High rates of HCO_3^- secretion and Cl^- absorption against adverse gradients in the marine teleost intestine: the involvement of an electrogenic anion exchanger and H^+-pump metabolon? *J. Exp. Biol.* **212**, 1684-1696.

Hersey, S. J. and Sachs, G. (1995). Gastric acid secretion. *Physiol. Rev.* **75**, 155-189.

Jobling, M. (1981). The influences of feeding on the metabolic-rate of fishes – a short review. *J. Fish Biol.* **18**, 385-400.

Krogh, A. and Leitch, I. (1919). The respiratory function of the blood in fishes. *J. Physiol.* **52**, 288-300.

Kurita, Y., Nakada, T., Kato, A., Doi, H., Mistry, A. C., Chang, M. H., Romero, M. F. and Hirose, S. (2008). Identification of intestinal bicarbonate transporters involved in formation of carbonate precipitates to stimulate water absorption in marine teleost fish. *Am. J. Physiol. Regul. Intgr. Comp. Physiol.* **294**, R1402-R1412.

Lignot, J. H., Helmstetter, C. and Secor, S. M. (2005). Postprandial morphological response of the intestinal epithelium of the Burmese python (Python, molurus). *Comp. Biochem. Physiol. A* **141**, 280-291.

Mandel, L. J. and Balaban, R. S. (1981). Stoichiometry and coupling of active transport to oxidative metabolism in epithelial tissues. *Am J. Physiol. Renal Physiol.* **240**, F357-F371.

McCue, M. D. (2006). Specific dynamic action: A century of investigation. *Comp. Biochem. Physio. A Mol. Integr. Physiol.* **144**, 381-394.

McDonald, M. D., Grosell, M., Wood, C. M. and Walsh, P. J. (2003). Branchial and renal handling of urea in the gulf toadfish, Opsanus beta: the effect of exogenous urea loading. *Comp. Biochem. Physio. A Mol. Integr. Physiol.* **134**, 763-776.

Niv, Y. and Fraser, G. M. (2002). The alkaline tide phenomenon. *J. Clin. Gastroenterol.* **35**, 5-8.

Rune, S. J. (1965). The metabolic alkalosis following aspiration of gastric acid secretion. *Scand. J. Clin. Lab. Invest.* **17**, 305-310.

Secor, S. (2009). Specific dynamic action: a review of the postprandial metabolic response. *J. Comp. Physiol. B. Biochem. Syst. Environ. Physiol.* **179**, 1-56.

Secor, S. M. and Diamond, J. (1995). Adaptive responses to feeding in Burmese pythons: pay before pumping. *J. Exp. Biol.* **198**, 1313-1325.

Takei, Y. (2000). Comparative physiology of body fluid regulation in vertebrates with special reference to thirst regulation. *Jpn. J. Physiol.* **50**, 171-186.

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

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

Taylor, J. R., Mager, E. M. and Grosell, M. (2010). Basolateral NBCe1 plays a rate-limiting role in transepithelial intestinal HCO_3^- secretion serving marine fish osmoregulation. *J. Exp. Biol.* **213** (in press).

Thorson, T. B. (1961). The partitioning of body water in Osteichthyes: phylogenetic and ecological implications in aquatic vertebrates. *Biol. Bull.* **120**, 238-254.

Tseng, Y. C. and Hwang, P. P. (2008). Some insights into energy metabolism for osmoregulation in fish. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **148**, 419-429.

Wang, T., Busk, H. and Overgaard, J. (2001). The respiratory consequences of feeding in amphibians and reptiles. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **128**, 535-549.

Wilson, R. W. and Grosell, M. (2003). Intestinal bicarbonate secretion in marine teleost fish-source of bicarbonate, pH sensitivity, and consequence for whole animal acid–base and divalent cation homeostasis. *Biochim. Biophys. Acta* **1618**, 163-193.

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

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

Wilson, R. W., Millero, F. J., Taylor, J. R., Walsh, P. J., Christensen, V., Jennings, S. and Grosell, M. (2009). Contribution of fish to the marine inorganic carbon cycle. *Science* **323**, 359-362.

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

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

Wood, C. M., Kajimura, M., Bucking, C. and Walsh, P. J. (2007b). Osmoregulation, ionoregulation and acid-base regulation by the gastrointestinal tract after feeding in the elasmobranch (*Squalus acanthias*). *J. Exp. Biol.* **210**, 1335-1349.

Zerahn, K. (1956). Oxygen consumption and active sodium transport in the isolated and short-circuited frog skin. *Acta Physiol. Scand.* **36**, 300-318.