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Feeding and osmoregulation: dual function of the marine teleost intestine

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Summary

Experiments on Gulf toadfish *Opsanus beta* demonstrate how feeding impacts osmoregulation in the marine teleost intestine. A high Ca^{2+} diet of pilchards *Sardina pilchardus* ($[Ca^{2+}]$=404.2 mmol kg^{-1}) was compared to a low Ca^{2+} diet of common squid *Loligo forbesi* ($[Ca^{2+}]$=1.3 mmol kg^{-1}), as high $[Ca^{2+}]$ has been shown to stimulate intestinal anion exchange. Gastrointestinal fluids and blood plasma were collected over a time course from pre-feeding to 216 h post feeding. Following food intake, monovalent ions were largely absorbed across the intestinal epithelium, leaving a fluid rich in divalent ions, which have a lower osmotic coefficient and effectively reduce osmotic pressure in the lumen to allow for enhanced fluid absorption. Concentrations of Cl^- and HCO_3^- in fluid along the gastrointestinal tract of fish fed both diets, particularly 1 and 2 days post-feeding, demonstrate that apical Cl^-/HCO_3^- exchange plays a vital role in postprandial Cl^- and water absorption. Postprandial acid–base balance disturbance as indicated by plasma alkalinization was limited or absent, indicating compensation for gastric acid secretion in this teleost fish. Plasma osmolality peaked 12 h post-feeding in toadfish fed squid, but was not accompanied by a significant increase in inorganic ion concentrations. Transient fluid secretion by the gastrointestinal tract was evident from reduced luminal Mg^{2+} and SO_4^{2-} concentrations for 24–48 h post feeding. Discrepancy between the sum of inorganic osmolytes and measured osmotic pressure was attributed to organic osmolytes, which occurred at high concentrations in the stomach and anterior intestine for up to 24 h post feeding.

Key words: acid–base balance, alkaline tide, Cl^-/HCO_3^- exchange, Gulf toadfish, *Opsanus beta.*

Introduction

Feeding and subsequent nutrient absorption provide life-sustaining energy to all animals, and are inevitably accompanied by physiological consequences. Most comparative physiology studies of fishes in the last century have been done on animals starved at least 72 h to control for physiological variability resulting from a meal. Although significant work has been done regarding the effects of feeding on physiology in other vertebrate groups, similar studies of fishes have been less comprehensive or are specifically designed to meet aquaculture goals. Dabrowski et al. (Dabrowski et al., 1986) found that feeding in seawater and freshwater drastically changes the ionic balance in the euryhaline fish intestine, even though the salt intake that accompanies drinking in seawater fish largely surpassed dietary intake (Shehadeh and Gordon, 1969; Dabrowski et al., 1986). The acute nature of dietary salt intake may in itself induce an osmoregulatory challenge for the gastrointestinal tract. Marshall and Grosell note that a large meal for a piscivorous fish, for example, will provide a substantial K^+ and Ca^{2+} load (from prey intracellular K^+ and bone phosphate, respectively) (Marshall and Grosell, 2006), the consequences of which remain to be examined. Additional intestinal salt load and systemic fluid loss might also result from bile, pancreatic and gastric secretions. Biliary secretions can carry Na^+ concentrations of over 300 mmol l^{-1} (Grosell et al., 2000), pancreatic secretions in many species contain HCO_3^- concentrations up to 140 mmol l^{-1} (Novak, 2000) (for a review, see Steward et al., 2005), and gastric parietal cells are capable of secreting Cl^- into the mammalian gastric lumen at rates up to 70 mmol l^{-1} min^{-1} (Thomas and Machen, 1991). In addition to potential osmoregulatory consequences of salt intake and gastrointestinal secretions, postprandial disruption to acid–base balance as a result of gastric acid secretion is possible, as occurs in many higher vertebrates. The ‘alkaline tide’ phenomenon recognized in amphibians, reptiles and mammals (for a review, see Wang et al., 2001) was recently demonstrated in an elasmobranch (Wood et al., 2005), representing the first instance of alkaline tide reported in fishes. The term alkaline tide refers to a significant alkalinization of the blood following gastric acid secretion. The rate of gastric apical H^+ secretion into the lumen is countered by basolateral efflux of HCO_3^- (metabolic base) *via* Cl^-/HCO_3^- exchange, which prevents alkalosis of the acid-secreting oxyntic

(mammals) or oxyntopeptic (non-mammalian vertebrates) cell. Basolateral Cl^-/HCO_3^- exchange provides Cl^- for gastric HCl secretion; H^+ and HCO_3^- are created in these gastric epithelial cells by CO_2 hydration, catalyzed by intracellular carbonic anhydrase (for a review, see Niv and Fraser, 2002).

In addition to digestion, the gastrointestinal tract of marine fish plays an important role in osmo- and iono-regulation, as hypo-osmoregulating fish have long been known to drink seawater to replace water lost diffusively to their environment. Ingested seawater passes through the gastrointestinal tract and ions must be differentially absorbed across the intestinal epithelium to facilitate water absorption. A large portion of Cl^- and water absorption in the intestine is accomplished *via* apical Cl^-/HCO_3^- exchange (Grosell et al., 2005), making intestinal anion exchange an important addition to long-recognized cotransporters Na^+-Cl^- and Na^+-K^+-$2Cl^-$ in the marine teleost intestine. The HCO_3^- that is consequently secreted into the intestinal lumen is present at levels approximately five- to tenfold plasma concentrations (Wilson et al., 2002; Taylor and Grosell, 2006) in starved fishes, indicating an active transport mechanism (for a review, see Grosell, 2006).

Bicarbonate secretion also seems to play a role in calcium homeostasis, inhibiting intestinal Ca^{2+} absorption by precipitating $CaCO_3$, which is subsequently excreted (Wilson et al., 2002; Wilson and Grosell, 2003). This carbonate precipitation in itself also promotes water absorption, lowering osmolality by removing Ca^{2+} and CO_3^{2-} from solution (Wilson et al., 1996; Wilson et al., 2002; Marshall and Grosell, 2006). High Ca^{2+} concentrations alone (Wilson et al., 2002) and elevated ambient salinity (Walsh et al., 1991; McDonald and Grosell, 2006; Taylor and Grosell, 2006) have been shown to stimulate HCO_3^- secretion, which is not surprising considering the role of intestinal anion exchange in Cl^- and water absorption (Grosell et al., 2005) (for a review, see Grosell, 2006).

In designing our experiments we considered the potential of high salinity in general, and high Ca^{2+} concentrations specifically, to stimulate Cl^-/HCO_3^- exchange, along with the great osmoregulatory challenge we imagine must be associated with ingesting a large meal and associated salts. We aimed to explore the effects of a high Ca^{2+} and a low Ca^{2+} diet (and feeding in general) on intestinal HCO_3^- secretion and osmoregulation in Gulf toadfish *Opsanus beta*. By investigating the effects of feeding on gastrointestinal fluid chemistry, and thereby gaining more information about intestinal anion exchange, we aim to better understand the function, regulation, and mechanism(s) of apical Cl^-/HCO_3^- exchange. Gastrointestinal fluids and blood plasma were sampled in a detailed time course post-feeding to reveal the timeline for organic and inorganic nutrient absorption across the intestine for differing diet composition. In addition, a detailed time course of blood plasma chemistry allowed for investigation of the possibility of postprandially disturbed acid–base balance and osmoregulatory compromise.

Materials and methods

Animal care

Gulf toadfish *Opsanus beta* (Goode and Bean 1880) were obtained as bycatch from local shrimp fishermen based on Virginia Key, FL, USA. Following transport to the University of Miami (RSMAS), fish were subject to a prophylactic treatment against ectoparasites as described previously (McDonald et al., 2003). Toadfish were held in aerated, 75 liter tanks receiving a flow-through of natural seawater at ambient temperature and salinity (26±2.0°C, 35±2.0‰, mean and range) and fed their respective experimental diet (see below) to satiation once weekly, for approximately 3 weeks prior to experimentation.

General experimental procedures

Two natural experimental diets were determined based on our knowledge of Ca^{2+}-induced HCO_3^- secretion in European flounder. A squid diet (*Loligo forbesi*, see Table 1 for inorganic ion composition) represented a low Ca^{2+} meal, while a fish diet (*Sardina pilchardus*, see Table 1 for inorganic ion composition) corresponded to a relatively high Ca^{2+} meal. Both experimental diets represent realistic meals for *O. beta*. Gulf toadfish were starved for 12 days prior to feeding to ensure resting conditions. In the initial experiment, 60 fish (mass 64±3.5 g, mean ± s.e.m.) were fed one of the two experimental diets to satiation (all uneaten food was removed after 30 min) and terminally sampled in groups of five fish pre-feeding (12 days following their last meal), 1, 2, 3, 6 and 9 days after feeding. Based on the results of this first experiment, follow-up sampling was done on another 50 toadfish (mass 80±3.5 g) fed the same two diets and sampled in groups of five fish pre-feeding (again 12 days following their last meal), 3, 6, 12 and 24 h after feeding. The final data set comprises timepoints 0, 3, 6, 12, 24, 48, 72, 144 and 216 h post-feeding. Data reported in the 24 h timepoint were taken exclusively from the second

Table 1. *Mean composition of experimental diets (per kg wet mass) for pilchards Sardina pilchardus and common squid Loligo forbesi*

	Concentration (mmol kg^{-1})					
	$[Mg^{2+}]$	$[Ca^{2+}]$	$[Na^+]$	$[K^+]$	$[Cl^-]$	$[SO_4^{2-}]$
Loligo forbesi	21.0±0.73	1.3±0.21	121.1±5.54	61.0±3.33	141.2±2.87	4.9±0.39
Sardina pilchardus	30.2±2.33	404.2±24.8	123.3±4.44	70.2±3.89	116.6±7.54	4.6±0.22

Values are means ± s.e.m. (*N*=3).

experiment as sample time was monitored more precisely in this more detailed trial. Experimental fish were euthanized by a tricaine methane sulfonate overdose (0.5 g l^{-1} MS-222, pH 8.0), after which a blood sample (200–400 μl) was obtained by caudal puncture into a heparinized syringe fitted with a gauge 22 needle, and placed on ice. The entire gastrointestinal tract was then carefully removed and ligated with silk ligatures into stomach, anterior, mid, posterior and rectal segments as previously described (Grosell et al., 2004). Each section was removed and contents were drained into 2 ml plastic microcentrifuge tubes (15 ml Falcon tubes for stomach contents) for analysis. At the 3 h timepoint, while most of the ingested meal remained in the stomach, contents were weighed to estimate meal size relative to body mass. Our experimental design was such that fish sampled at each timepoint (N=5) were taken exclusively from one 75 liter tank containing only these fish. Thus, any effects of dominance/hierarchy on food intake would have revealed themselves amongst the five fish from each diet sacrificed at 3 h post-feeding and used to estimate meal size.

Sample analysis

All blood samples were promptly analyzed for hematocrit and then centrifuged (2 min at 15 700 ***g***). Plasma was retained in addition to gastrointestinal fluid samples (only the supernatant of gastrointestinal fluid samples was retained after centrifugation to eliminate reaction with solid meal remains) for immediate analysis of pH (Accumet pH electrode connected to a Radiometer PHM220 pH meter, Copenhagen, Denmark). Total CO_2 was analyzed using a Corning 965 Carbon Dioxide Analyzer (Corning, NY, USA) and osmolality was measured using a vapor pressure osmometer (Wescor Vapro 5520, Logan, UT, USA). Samples were stored at –20°C for subsequent analysis of inorganic anions (Dionex DX-120, Sunnyvale, CA, USA) and cations (Fast Sequencial atomic absorption spectrophotometry, Varian FS220, Palo Alto, CA, USA) after appropriate dilution.

Osmotic coefficient calculation

Osmotic coefficients of representative monovalent (NaCl) and divalent ($MgSO_4$) solutions were calculated by making, in triplicate, 150 mmol l^{-1} solutions of each and measuring osmolality using a vapor pressure osmometer (Wescor Vapro 5520). The osmotic coefficient was calculated by dividing the measured osmotic pressure of each solution by 300 mOsm (the expected osmotic pressure assuming an osmotic coefficient=1).

Statistical analyses

Due to variable amounts of fluid present in each gastrointestinal segment at different stages after a meal, means were generally from five samples (except for the control timepoint, in which up to ten samples were collected for each parameter), but at times, some samples were too small to be analyzed for every parameter. Of a total 720 means, 8% (59) contained 10 samples; 3% (19) contained 9 samples; <1% (2) contained 8 samples; 63% (455) contained 5 samples; 17% (119) contained 4 samples; 5% (36) contained 3 samples, 3% (21) contained 2 samples, 1% (8) contained 1 sample, and <1% (1) means were omitted because no samples contained sufficient fluid for analysis. Data are presented as means ± 1 s.e.m. In analyses of organic nutrient absorption, total inorganic ion concentration was calculated from the inorganic ion concentrations measured in each sample for each time point (including only samples in which sufficient fluid was collected to measure every ion). This provides a conservative estimate of osmotic pressure exerted by inorganic ions by assuming an osmotic coefficient of one. Because data were not normally distributed, Kruskal–Wallis one-way analysis of variance (ANOVA) on ranks was used to evaluate all data, followed, where applicable, by comparisons of each parameter at each time point to control values by Dunn's method. When only two groups were compared, a Mann–Whitney Rank Sum test was used to determine statistically significant differences. Means were considered significantly different at P<0.05. For the sake of clarity, significant differences are not noted in figures or tables but rather are described in detail in the Results section.

Results

The meal sizes of toadfish fed our two experimental diets were not significantly different when fed to satiation. Toadfish fed sardines ingested an estimated 9±2.4% of their body mass (N=5), and toadfish fed squid consumed an estimated 9±1.3% of their body mass (N=5; based on the mass of stomach contents in fish sampled 3 h after feeding). Feeding to satiation ensured that adequate food was available to all fish, and we did not observe signs of hierarchy during feeding events. We found the ingested meal to be cleared completely from the stomach between 24 and 48 h after feeding for both diets.

Postprandial intestinal Cl^-/HCO_3^- exchange

In both high and low Ca^{2+} diets, pH (Fig. 1) and total CO_2 (HCO_3^- equivalents, Fig. 2) levels in gastrointestinal fluids indicated that postprandially increased HCO_3^- secretion acted as a buffer for H^+ secreted and subsequently released from the stomach with chyme. Between 3 and 24 h post-feeding, intestinal fluid HCO_3^- equivalents were reduced to as little as 50% of control levels (Fig. 2, Table 2), a decrease that was statistically significant only in the mid intestinal fluid 3 h after feeding a squid diet. Luminal pH was also slightly depressed at these timepoints, especially in the anterior and mid intestinal fluid (Fig. 1, Table 2), where pH was significantly reduced from control levels 3 h after feeding sardines. Correspondingly, 48 h after feeding (presumably once stomach secretions were neutralized), a dramatic increase in HCO_3^- equivalents was measured relative to control levels. Bicarbonate equivalents on average were present at 231% and 204% of control levels in the anterior intestinal fluid 48 h after feeding fish and squid diets, respectively (Fig. 2, Table 2), although limited sample sizes prohibited statistical significance. This rise over control HCO_3^- concentrations was

Table 2. *Chemistry and inorganic ion composition of intestinal fluid from O. beta fed (A) squid and (B) sardines*

		Concentration (mmol l^{-1})						
	pH	Total CO_2	$[Cl^-]$	$[Na^+]$	$[Ca^{2+}]$	$[K^+]$	$[Mg^{2+}]$	$[SO_4^{2-}]$
(A) Squid diet								
Pre-feeding (0 h)								
Anterior	8.51±0.155	32.7±11.38	93.0±19.47	62.3±16.74	2.5±0.52	6.2±2.99	95.3±23.38	63.7±11.71
Mid	8.50±0.120	62.4±4.03	51.9±4.82	29.8±6.07	1.8±0.65	1.7±0.43	158.5±11.69	97.1±6.44
Posterior	8.70*	72.9±3.72	49.4±4.83	19.8±2.78	2.3±0.86	5.2±1.99	133.9±10.41	104.7±3.19
Rectal	8.78±0.013	68.3±7.71	30.7±4.22	10.4±0.79	2.1±0.69	1.1±0.04	135.8±14.04	93.8±8.96
3 h post-feeding								
Anterior	8.14±0.096	20.6±3.63	36.0±8.28	71.9±9.12	4.0±0.48	7.0±1.02	34.4±5.31	26.8±1.40
Mid	8.33±0.042	29.2±4.47	35.3±8.72	83.0±17.77	3.6±1.25	3.9±0.56	62.0±18.03	41.1±11.34
Posterior	8.47±0.081	54.9±3.73	38.0±5.76	48.2±8.44	3.4±0.49	3.1±0.52	144.2±16.15	83.9±8.59
Rectal	8.80±0.032	59.0±9.26	28.7±6.64	17.7±6.12	3.1±0.99	1.8±0.22	127.5±18.39	78.7±11.94
6 h post-feeding								
Anterior	8.08±0.119	50.5±10.05	23.7±8.34	64.7±14.02	10.3±3.56	3.6±0.37	66.0±16.87	48.3±8.18
Mid	8.27±0.112	55.2±14.72	13.5±3.77	71.0±19.40	6.9±2.84	2.7±0.26	80.7±30.47	56.4±15.01
Posterior	8.37±0.025	65.2±18.11	19.5±6.54	56.6±18.96	3.4±0.79	2.9±0.32	73.2±14.34	77.2±19.24
Rectal	8.74±0.141	55.1±11.27	15.5±6.97	16.2±5.90	5.5±0.36	1.4±0.14	106.0±22.94	78.5±16.07
12 h post-feeding								
Anterior	7.99±0.021	34.4±2.11	37.3±5.07	85.3±14.23	9.6±0.37	4.5±0.42	77.1±7.32	36.1±2.79
Mid	8.39±0.024	49.0±3.86	13.7±1.47	39.2±6.62	9.7±1.56	2.0±0.15	111.4±13.46	55.6±4.27
Posterior	8.44±0.036	48.6±4.56	15.6±2.00	21.5±3.70	8.5±1.81	1.9±0.05	108.8±13.65	56.5±6.47
Rectal	8.16±0.575	62.0±36.60	24.5±9.36	9.6±4.17	3.9*	1.0±0.10	78.6±32.74	60.7±26.26
24 h post-feeding								
Anterior	7.81±0.084	42.1±5.63	29.9±3.41	54.3±22.00	13.0±1.55	5.0±0.26	100.4±18.64	67.1±8.21
Mid	8.06±0.035	41.7±3.66	14.4±2.18	64.6±16.76	15.6±2.09	3.6±0.26	91.5±16.50	84.9±4.62
Posterior	8.24±0.101	44.4±5.44	18.5±2.18	40.4±11.86	11.9±2.16	2.8±0.24	115.7±12.75	76.0±5.81
Rectal	8.56±0.113	50.4±5.99	13.6±2.33	8.5±2.97	6.7±1.87	1.9±0.35	81.5±22.49	56.4±8.07
48 h post-feeding								
Anterior	8.20±0.162	66.5±5.22	53.6±10.56	37.6±4.54	7.7±0.80	2.1±0.36	140.6±3.02	109.1±6.39
Mid	8.14±0.204	69.1±5.68	41.9±10.95	37.1±4.78	7.8±1.68	1.8±0.19	167.4±5.29	137.2±10.16
Posterior	8.36±0.122	74.1±7.19	37.5±8.63	38.7±10.14	7.6±1.37	2.9±0.57	166.5±10.33	129.4±10.17
Rectal	8.41±0.093	63.0±6.77	21.7±1.67	20.7±7.95	8.1±0.76	1.8±0.34	167.2±10.23	131.5±7.67
72 h post-feeding								
Anterior	8.55*	52.1±9.82	101.6±14.66	60.3±18.21	4.5±1.05	4.2±1.28	113.9±17.75	73.1±17.80
Mid	8.66±0.020	71.3±6.75	76.0±10.20	27.5±7.43	5.9±1.08	3.1±0.45	141.5±12.96	79.3±9.39
Posterior	8.73*	79.1±4.07	82.2±8.67	33.9±7.76	4.0±1.43	4.4±0.98	159.5±14.06	98.0±10.42
Rectal	8.80±0.035	78.4±5.26	49.8±8.25	13.9±1.24	4.1±0.93	1.2±1.05	133.1±15.50	87.8±10.50
144 h post-feeding								
Anterior	8.61±0.046	60.0±3.18	81.6±4.10	31.4±3.44	4.1±0.69	3.9±1.09	119.3±7.70	69.6±7.86
Mid	8.64±0.045	81.4±5.71	57.6±5.53	22.3±4.28	2.6±0.61	2.5±0.83	147.6±19.78	72.9±15.93
Posterior	8.62*	84.8±4.45	71.1±10.80	23.3±2.48	3.3±1.32	1.5±0.47	173.0±8.27	90.9±5.42
Rectal	8.76±0.055	83.7±8.13	41.4±5.38	12.7±1.65	1.3±0.37	0.3±0.09	158.0±19.43	81.4±11.04
216 h post-feeding								
Anterior	8.68±0.035	78.5±8.12	78.5±7.32	41.9±6.35	3.4±0.70	5.1±1.00	132.5±12.93	38.1±22.66
Mid	8.68±0.027	77.5±12.36	63.8±11.49	28.8±4.18	2.7±0.87	2.6±0.85	158.6±14.51	75.2±9.93
Posterior	8.69±0.027	81.4±10.47	60.1±7.66	22.4±2.46	1.9±0.73	1.7±0.47	153.7±9.92	46.6±15.97
Rectal	8.72±0.018	89.0±3.92	53.2±3.00	18.4±2.17	2.9±0.11	0.9±0.19	170.9±5.42	58.8±11.89

Table 2. *Continued*

	pH	Concentration (mmol l^{-1})						
		Total CO_2	$[Cl^-]$	$[Na^+]$	$[Ca^{2+}]$	$[K^+]$	$[Mg^{2+}]$	$[SO_4^{2-}]$
(B) Sardine diet								
Pre-feeding (0 h)								
Anterior	8.47±0.047	34.0±13.00	101.2±8.19	91.0±13.84	2.4±0.63	6.5±1.08	52.2±22.33	45.4±13.33
Mid	8.86±0.125	48.3±6.38	84.4±5.48	76.5±21.61	2.3±0.52	4.1±0.71	81.7±26.66	44.5±14.88
Posterior	†	66.5±17.50	84.2±9.61	77.5±32.29	2.4±1.31	4.7±0.98	115.5±34.43	62.3±18.81
Rectal	8.86±0.079	54.2±9.06	43.2±11.10	15.1±3.31	1.0±0.23	1.7±0.07	133.3±37.83	65.2±16.46
3 h post-feeding								
Anterior	7.19±0.202	18.2±2.75	72.3±7.80	88.9±17.21	17.3±5.94	10.1±1.40	56.9±10.56	41.7±4.89
Mid	8.05±0.083	40.3±5.03	38.6±6.36	73.9±17.53	12.1±4.39	3.6±0.46	78.1±16.75	54.0±6.04
Posterior	8.27±0.108	46.9±8.02	31.7±5.91	66.2±17.06	8.5±3.07	3.2±0.39	91.0±21.87	64.8±7.92
Rectal	8.87±0.128	49.6±3.53	19.6±4.69	18.5±9.61	1.6±0.85	1.5±0.20	86.6±23.46	61.7±8.45
6 h post-feeding								
Anterior	7.50±0.105	23.2±5.26	64.7±7.84	75.6±8.23	21.3±4.48	4.7±0.45	63.9±2.58	49.9±4.33
Mid	8.29±0.047	41.6±3.01	30.4±4.57	58.5±6.14	14.6±1.20	2.8±0.17	94.5±3.66	67.7±3.26
Posterior	8.54±0.061	49.7±4.63	25.9±4.47	44.8±6.66	10.9±1.72	2.6±0.23	127.1±15.13	76.0±7.34
Rectal	8.82±0.054	68.7±10.02	24.3±5.32	17.2±5.10	2.9±1.44	1.6±0.24	125.5±25.18	70.7±13.57
12 h post-feeding								
Anterior	7.79±0.146	32.8±4.44	40.1±6.11	92.2±12.26	19.3±5.27	5.2±0.64	68.0±14.24	22.6±8.77
Mid	8.18±0.077	42.9±5.86	16.9±3.06	82.4±12.91	14.4±2.06	3.4±0.40	93.1±16.66	47.4±10.68
Posterior	8.41±0.120	51.3±7.32	14.9±3.46	41.9±4.64	11.1±1.82	2.2±0.20	134.7±15.19	67.8±9.59
Rectal	8.58±0.057	48.5±15.52	16.1±8.05	17.8±3.80	5.9±2.04	1.5±0.14	115.1±26.29	41.6±12.41
24 h post-feeding								
Anterior	8.06±0.262	47.2±9.73	41.5±8.63	77.1±24.12	7.9±3.05	3.7±0.83	110.0±26.17	45.8±13.55
Mid	8.24±0.151	51.1±9.09	15.6±7.17	64.5±17.36	11.4±2.07	2.8±0.44	140.0±19.30	83.2±6.35
Posterior	8.49*	47.0±8.29	19.9±3.25	73.0±22.63	10.1±3.62	2.9±0.48	131.3±26.75	76.6±10.33
Rectal	8.37±0.048	32.7±7.82	8.3±5.37	26.7±7.81	12.2±2.65	2.0±0.09	129.6±18.20	72.8±12.03
48 h post-feeding								
Anterior	8.59±0.138	78.6±15.15	76.7±16.06	57.2±21.84	2.8±0.06	2.3±0.82	112.5±30.06	72.4±14.26
Mid	8.67±0.086	100.2±13.18	58.8±11.57	72.8±25.71	5.0±1.65	2.8±0.54	128.6±24.09	83.6±11.21
Posterior	8.73±0.120	105.6±14.58	43.8±7.64	71.4±26.13	2.8±0.33	3.4±0.86	125.3±29.48	82.4±13.45
Rectal	7.93±0.260	68.5±7.27	43.0±10.47	41.4±16.72	7.0±2.28	4.5±1.56	165.5±40.27	121.9±19.35
72 h post-feeding								
Anterior	8.33*	43.0±11.78	100.1±11.37	62.5±14.74	3.1±0.21	4.1±1.06	104.3±17.66	58.6±11.00
Mid	8.66±0.114	68.9±5.53	74.1±3.17	32.5±1.77	2.7±0.39	4.5±1.84	145.7±4.40	83.7±2.85
Posterior	8.90±0.087	82.8±4.34	62.9±2.00	26.2±1.84	2.5±0.37	2.5±1.06	149.3±4.50	86.8±2.79
Rectal	8.77±0.064	84.3±8.13	45.5±4.42	19.1±1.35	3.6±1.08	0.7±0.10	197.5±1.11	123.6±7.87
144 h post-feeding								
Anterior	8.69±0.030	52.6±9.61	100.6±5.94	63.7±14.71	4.6±0.41	7.1±1.36	98.3±17.44	52.3±9.36
Mid	8.57±0.050	73.5±10.08	81.9±4.57	31.5±4.24	3.5±0.43	3.3±0.66	139.6±6.35	76.9±4.46
Posterior	8.66*	69.4±6.48	88.6±6.75	29.2±3.67	5.0±1.30	4.4±1.17	151.4±9.66	82.4±5.88
Rectal	8.75±0.031	69.7±5.73	73.5±6.96	23.2±3.70	2.5±0.14	0.8±0.22	170.8±9.46	93.6±6.87
216 h post-feeding								
Anterior	8.52±0.025	55.8±9.01	80.2±8.63	41.6±2.92	3.2±0.80	2.3±0.44	98.9±15.89	54.0±8.65
Mid	8.69±0.040	77.4±5.41	63.9±5.39	29.3±4.57	3.1±0.63	1.8±0.48	156.7±11.03	86.3±6.43
Posterior	8.56±0.020	78.5±6.95	62.9±3.44	22.1±1.26	1.9±0.54	1.8±0.35	162.4±6.58	90.7±3.20
Rectal	8.63±0.050	65.3±13.96	59.7±10.33	15.1±1.30	2.7±1.25	0.9±0.34	163.7±14.63	84.9±6.69

Values are means ± s.e.m. (*N* varies, see text).
*N=1.
†N=0.

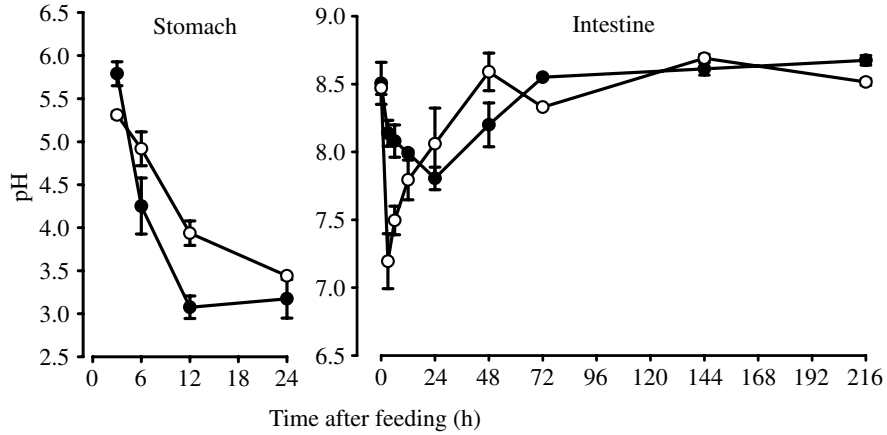


Fig. 1. pH in the stomach and anterior intestinal fluids of *Opsanus beta* fed squid (filled circles) and fish (open circles). Values are means ± 1 s.e.m., *N*=5 for most samples, as described in text.

most dramatic in the anterior intestine of both diets 48 h post feeding, and was sequentially less pronounced in the more posterior sections, indicating that postprandial HCO_3^- secretion might be prevalent in the anterior intestine. Notably, these HCO_3^- measurements are based exclusively on intestinal fluid concentrations, and neglect to account for any additional increases in HCO_3^- concentration accounted for by $CaCO_3$ precipitation. Overall, HCO_3^- concentrations of intestinal fluids ranged from 4- to 20-fold control plasma levels (Tables 2 and 3, respectively).

Postprandial luminal Cl^- concentrations (Fig. 3, Table 2) in both diets support an increase in intestinal Cl^-/HCO_3^- exchange post-feeding. Intestinal fluid Cl^- concentrations were reduced from control levels between 3 and 48 h post-feeding in both squid and sardine diets. This difference from control conditions was statistically significant in the anterior, mid and posterior intestinal fluid between 6 and 24 h post-feeding in toadfish fed a squid diet, and at 12 h and 24 h timepoints in toadfish fed sardines. Additionally, a slight (though not statistically significant using Kruskal–Wallis one-way ANOVA on ranks) increase in plasma Cl^- concentration over control levels was measured between 12 and 48 h post feeding in toadfish fed squid (Table 3).

While luminal Cl^- concentrations were reduced post-feeding, no such trend was observed for Na^+ concentrations (Fig. 4, Table 2), yielding additional evidence for enhanced postprandial apical Cl^-/HCO_3^- exchange as opposed to Na^+-Cl^- cotransport.

Consequences of high dietary Ca^{2+} and K^+ loads

While unfed (control) toadfish maintained low intestinal Ca^{2+} concentrations, fed toadfish experienced up to a tenfold postprandial increase in intestinal Ca^{2+} concentrations (Fig. 5, Table 2). In toadfish fed a squid diet, Ca^{2+} concentrations were significantly elevated over control conditions at 24 h post-feeding in the anterior intestine fluid, 12 and 24 h post-feeding in mid and posterior intestine fluid, and 48 h post-feeding in rectal fluid. In toadfish fed sardines, however, Ca^{2+} seemed to be liberated into the intestinal fluid sooner, as concentrations were significantly elevated at 6 and 12 h post-feeding in the anterior and mid intestine fluid, and 24 h post-feeding in the rectal fluid. A notable difference in diet composition was evident in stomach Ca^{2+} concentrations, which were significantly higher in fish fed sardines and maximal 12 h post-feeding (77.4±6.52 mmol l^{-1}) in these fish (Fig. 5B). In toadfish fed a squid diet, stomach Ca^{2+} concentrations were maximal 3 h post-feeding (6.8±0.32 mmol l^{-1}; Fig. 5A). Despite the intense Ca^{2+} load

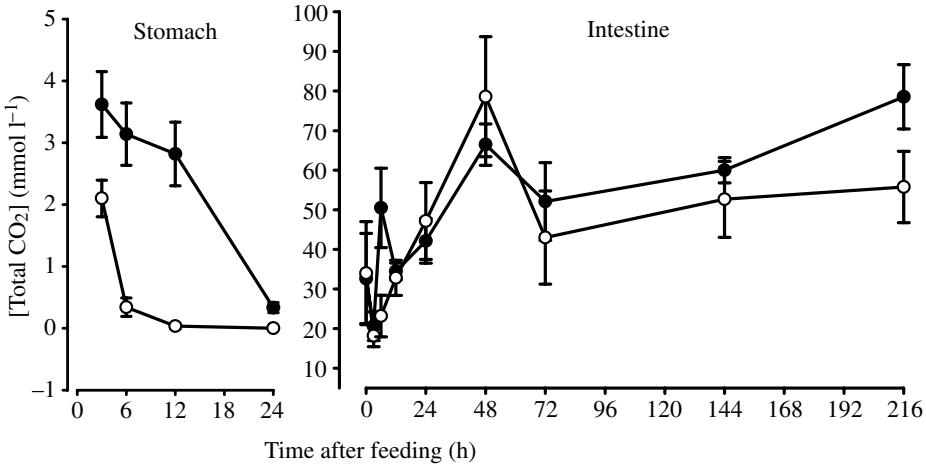


Fig. 2. Total CO_2 (HCO_3^- equivalents) in the stomach and anterior intestinal fluids of *Opsanus beta* fed squid (filled circles) and fish (open circles). Values are means ± 1 s.e.m., *N*=5 for most samples, as described in text.

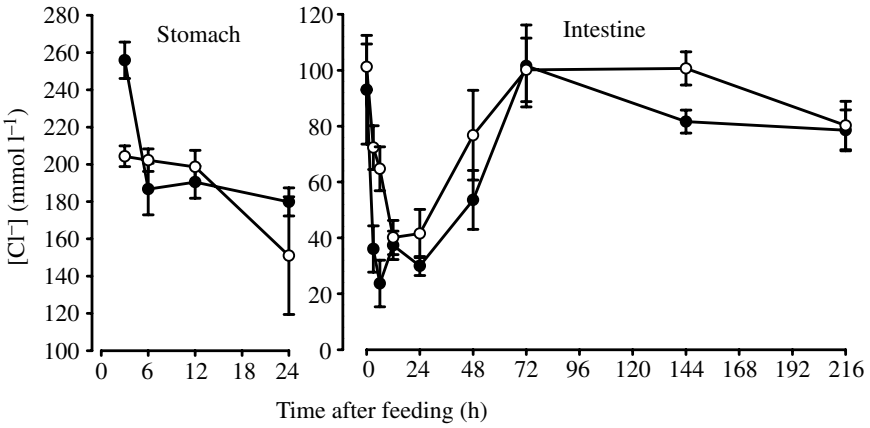


Fig. 3. Cl^- concentrations in the stomach and anterior intestinal fluids of *Opsanus beta* fed squid (filled circles) and fish (open circles). Values are means ± 1 s.e.m., *N*=5 for most samples, as described in text.

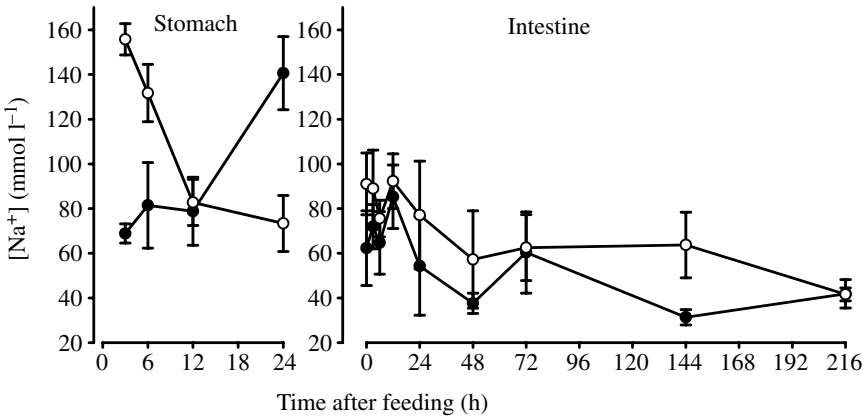


Fig. 4. Na^+ concentrations in the stomach and anterior intestinal fluids of *Opsanus beta* fed squid (filled circles) and fish (open circles). Values are means ± 1 s.e.m., *N*=5 for most samples, as described in text.

Table 3. *Chemistry and inorganic ion composition of blood plasma from O. beta fed (A) squid and (B) sardines*

Time after feeding (h)	pH	Osmolality (mOsm)	Hematocrit (%)	Concentration (mmol l^{-1})				
				Total CO_2	$[Cl^-]$	$[Na^+]$	$[K^+]$	$[Ca^{2+}]$
(A) Squid diet								
0 (Control)	7.74±0.038	310±2.4	34±2.3	5.2±0.59	118.5±1.44	143.6±2.95	2.5±0.15	2.0±0.14
3	7.96±0.038	319±1.7	23±1.6	3.3±0.51	116.2±0.70	134.9±3.72	2.6±0.25	1.5±0.11
6	7.75±0.041	318±1.9	27±2.6	4.7±0.37	118.1±2.76	137.5±4.47	3.2±0.59	2.3±0.15
12	7.86±0.018	326±2.4	31±3.0	5.5±0.39	126.4±1.99	141.7±4.50	3.8±0.23	2.4±0.34
24	7.84±0.055	315±11.9	31±4.3	4.7±0.43	124.1±6.58	140.6±12.62	4.0±0.32	2.5±0.70
48	7.87±0.062	292±3.3	28±2.7	4.6±0.30	132.1±5.93	143.7±6.70	2.8±0.14	1.5±0.06
72	7.94±0.033	287±3.1	33±3.2	5.0±0.10	115.2±1.22	131.9±1.57	2.7±0.15	1.7±0.11
144	8.03±0.013	267±6.7	24±1.3	5.2±0.23	109.0±2.93	123.7±4.25	3.8±0.38	1.7±0.18
216	7.91±0.041	286±4.6	23±2.4	4.6±0.64	111.2±4.10	133.7±4.86	2.4±0.12	1.8±0.16
(B) Sardine diet								
0 (Control)	8.21±0.022	321±5.5	21±0.5	2.7±0.21	129.5±4.14	158.3±5.45	4.5±0.13	2.5±0.28
3	8.31±0.029	322±4.0	20±2.4	2.4±0.19	120.0±1.64	149.9±3.25	6.4±0.14	1.9±0.20
6	8.34±0.040	323±2.9	21±1.0	3.8±0.23	124.3±1.42	145.1±1.61	4.4±0.34	2.3±0.06
12	8.30±0.032	315±2.5	23±1.3	4.2±0.46	127.5±1.52	145.8±1.81	4.1±0.15	2.6±0.09
24	7.95±0.033	312±3.9	17±1.9	2.2±0.50	114.2±2.46	139.1±3.60	4.7±0.30	2.8±0.58
48	7.65±0.015	320±2.1	28±2.8	5.3±0.25	130.0±2.18	147.1±1.75	2.8±0.19	1.6±0.12
72	8.01±0.047	319±1.2	35±1.2	5.0±0.24	130.3±1.39	149.5±1.68	3.7±0.18	1.3±0.03
144	7.74±0.063	337±4.2	35±1.6	3.4±0.68	130.0±2.06	154.1±2.20	6.8±1.41	1.4±0.08
216	7.86±0.043	321±3.6	23±3.1	5.3±0.47	127.3±2.45	155.6±3.66	4.2±0.09	1.6±0.13

Values are means ± s.e.m. (*N*=10 for control samples, *N*=5 for all others).

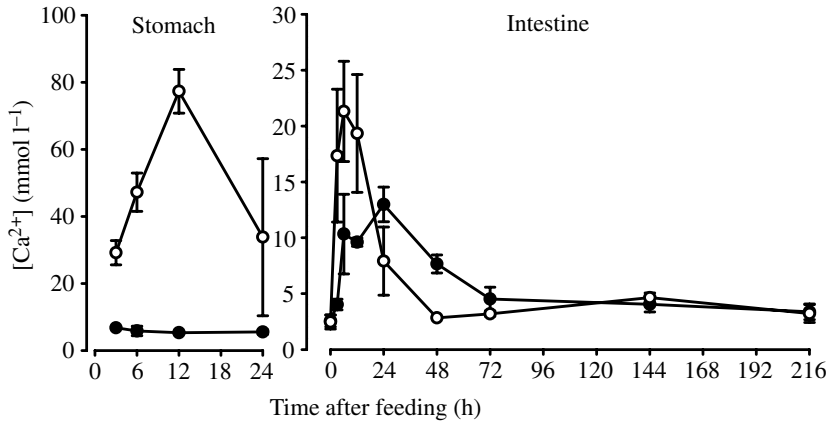


Fig. 5. Ca^{2+} concentrations in the stomach and anterior intestinal fluids of *Opsanus beta* fed squid (filled circles) and fish (open circles). Values are means ± 1 s.e.m., *N*=5 for most samples, as described in text.

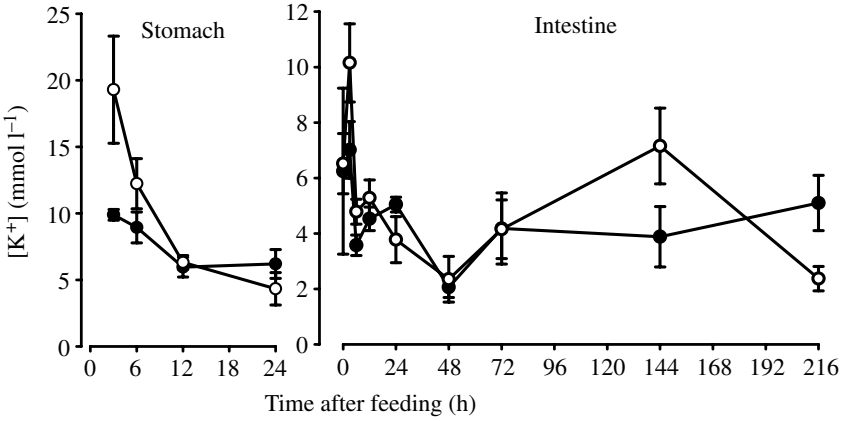


Fig. 6. K^+ concentrations in the stomach and anterior intestinal fluids of *Opsanus beta* fed squid (filled circles) and fish (open circles). Values are means ± 1 s.e.m., *N*=5 for most samples, as described in text.

to the gastrointestinal tract of toadfish fed sardines, no increase in plasma Ca^{2+} concentration was observed in fish fed either diet (Table 3).

Another difference in diets was suggested by the K^+ loads carried to the gastrointestinal tract. While K^+ levels of the two diets were similar (Table 1), mean K^+ concentrations in the stomach and anterior intestine of toadfish fed sardines were approximately 2 and 1.5 times those in squid-fed toadfish, respectively (Fig. 6). Additionally, we saw a nearly 50% increase in mean plasma K^+ concentrations 3 h post-feeding in toadfish fed sardines (Table 3B), although sample sizes were too small to gain statistical significance.

Intestinal water absorption and divalent ion concentration

Immediately following feeding, mean intestinal Mg^{2+} and SO_4^{2-} concentrations were reduced to as little as 36% and 42% of control concentrations, respectively, in toadfish fed a squid diet (Fig. 7A and Fig. 8A, respectively, and Table 2). This decline in both Mg^{2+} and SO_4^{2-} concentrations was statistically significant only in the mid intestinal fluid 3 h post-feeding. A

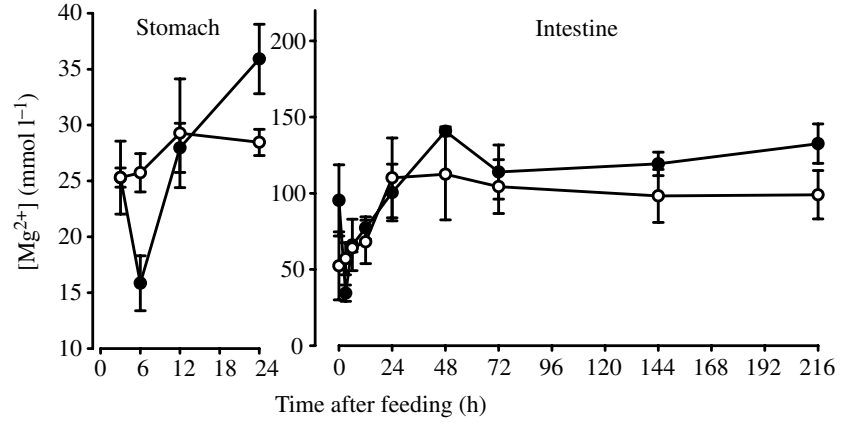


Fig. 7. Mg^{2+} concentrations in the stomach and anterior intestinal fluids of *Opsanus beta* fed squid (filled circles) and fish (open circles). Values are means ± 1 s.e.m., *N*=5 for most samples, as described in text.

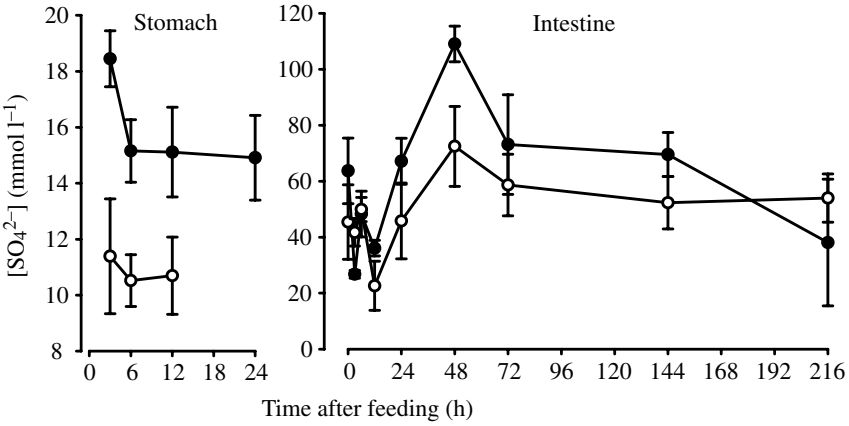


Fig. 8. SO_4^{2-} concentrations in the stomach and anterior intestinal fluids of *Opsanus beta* fed squid (filled circles) and fish (open circles). Values are means ± 1 s.e.m., *N*=5 for most samples, as described in text.

postprandial reduction in intestinal Mg^{2+} and SO_4^{2-} concentrations was also noted in toadfish fed sardines (Fig. 7B and Fig. 8B, respectively, and Table 2), though these ions were only reduced to as little as 65% and 50% control conditions, respectively, and exhibited no statistically significant differences from control concentrations. By 48 h post feeding, however, water absorption rather than secretion appears to have resumed in full force as intestinal Mg^{2+} and SO_4^{2-} concentrations return to and even exceed their high levels in control fish.

Osmotic coefficients in monovalent and divalent solutions

Our experimental determination of divalent and monovalent solution osmotic coefficients yielded an osmotic coefficient of 0.91 (±0.002) for the monovalent solution NaCl, and an osmotic coefficient of 0.56 (±0.004) for the divalent solution $MgSO_4$. Thus a replacement along the gastrointestinal tract of monovalent ions with divalent ions will facilitate water absorption by lowering osmotic pressure in the lumen.

Organic nutrient absorption

By calculating the difference between measured osmolality and the sum of inorganic ion concentrations in a given sample, we were able to conservatively predict the concentration (mEqv) of organic solutes present in the sample. Measured plasma osmolality is consistently significantly higher than the sum of inorganic ions (Fig. 9A) of fish fed both diets. In toadfish fed a squid diet (Fig. 9Ai), there is a statistically significant rise in plasma osmolality 12 h post-feeding, but not a significant increase in the sum of inorganic ion concentration.

The very high stomach osmolality 3 h post-feeding in toadfish fed a squid diet comprises less than 45% inorganic ions (Fig. 9Bi), while the stomach osmolality in toadfish fed sardines was significantly lower and composed of approximately 75% inorganic ions (Fig. 9Bii). The stomach of toadfish fed sardines also held a significantly greater concentration of inorganic ions than did the squid diet. The gap between stomach osmolality and inorganic ion sum became progressively narrower as time after feeding increased, until most organic solutes in the stomach were absorbed or passed on to the intestine by 24 h post feeding in toadfish fed squid, and 12 h in toadfish fed fish. In the stomach, this gap (thus the estimated concentration of organic solutes) was statistically significant between 3 and 12 h after feeding in toadfish fed a squid diet, and between 3 and 6 h post-feeding in toadfish fed sardines. Along the intestine, osmolality exceeds total ion sum until 24–48 h post-feeding, when osmolality drops and total inorganic ion sum increases above the corresponding osmotic pressure as gastrointestinal conditions return to baseline levels.

Disturbed acid–base balance?

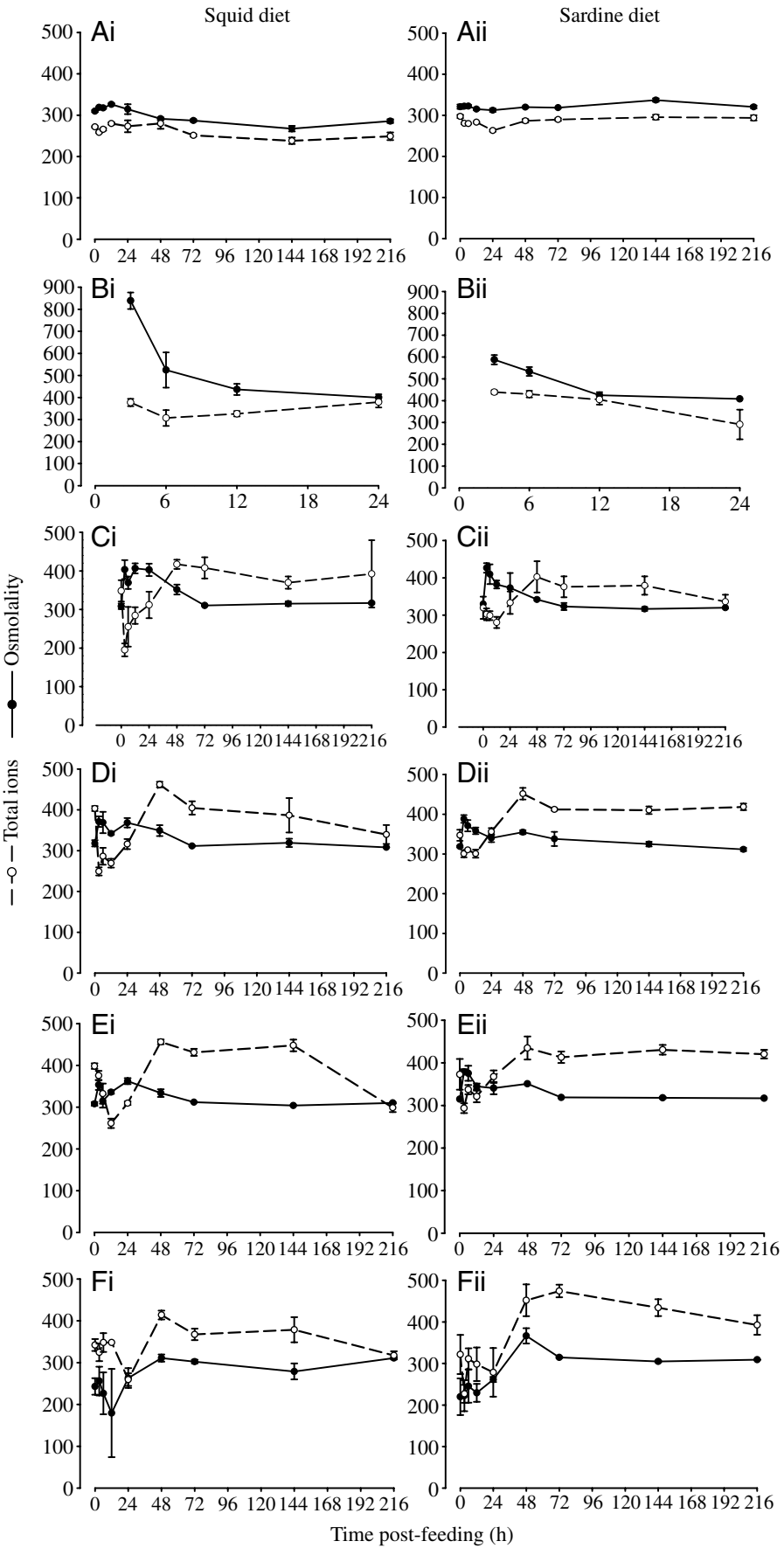
Plasma pH (Table 3) did not exhibit strong trends in toadfish fed either diet, although it was significantly increased over control conditions at 3 h post-feeding in toadfish fed a squid diet. No statistically significant changes were seen in plasma pH of toadfish fed sardines. Also, no significant changes in plasma HCO_3^- (Table 3) were measured postprandially in either diet.

Discussion

Feeding results in substantial changes in the ionic composition of intestinal fluids; notably, an acute influx of dietary salts prompts apparent transient changes in fluid absorption/secretion and stimulation of intestinal HCO_3^- secretion. The mechanisms of these intestinal transport properties (and changes therein) may be unique to digestive physiology or may be identical to mechanisms utilized to osmoregulate in a marine environment.

Postprandial intestinal Cl^-/HCO_3^- exchange

While we anticipated an influx of Cl^- to the anterior intestine due to the passage of gastric HCl secretions, we measured intestinal Cl^- concentrations that markedly decreased immediately (by 3 h) following feeding and remained depressed for at least 48 h. Based on these reduced luminal Cl^- concentrations and on measurements of unchanged Na^+ concentrations between 3 and 48 h post-feeding, we presume that elevated levels of intestinal HCO_3^- at 48 h post-feeding cannot be accounted for by pancreatic-like secretion. Pancreatic secretions at least in higher vertebrates consist of a $NaHCO_3$-rich solution (Steward et al., 2005). Buffering of



highly acidic chyme entering the intestine from the stomach to circumneutral and alkaline pH likely consumes much of the HCO_3^- secreted into the intestine. Increased apical Cl^-/HCO_3^- exchange may be stimulated postprandially either by a signal directly related to the act of feeding, by reduced luminal pH as has been shown in European flounder (Wilson and Grosell, 2003), or by osmoregulatory challenges to the gastrointestinal tract that result from ingesting a large meal. Regardless of the mechanism of stimulation, intestinal Cl^-/HCO_3^- exchange appears beneficial both in neutralizing luminal pH and in serving Cl^- and water absorption across the intestinal epithelium between 3 and 48 h after feeding. Immediately (3 h) post-feeding, the anterior intestine of toadfish fed sardines becomes more acidified (Fig. 1) and has a higher Cl^- concentration (Fig. 3) than that of toadfish fed a squid diet. These observations together seem to indicate elevated gastric HCl secretion in fish fed sardines. Correspondingly, toadfish fed sardines experienced the highest intestinal HCO_3^- levels we measured during these experiments at 48 h post-feeding (Fig. 2B, Table 2). While stomach pH was not significantly different between fish fed the two diets at any time point, we imagine that a larger volume of acid would be required to acidify chopped fish (buffered by carbonate and phosphate salts characteristic of cycloid scales and bone) to a similar pH as chopped squid. Common sense attests that increased gastric acid secretion and/or a prolonged gastric holding time should increase assimilation efficiency for a more difficult to digest meal (i.e. a fish diet containing scales and bone). This topic has so far only been discussed with reference to the potential of diet

Fig. 9. Osmolality (solid line) and ion sum (broken line) of plasma (A), stomach (B), anterior intestine (C), mid intestine (D), posterior intestine (E), and rectal (F) fluid of *Opsanus beta* fed squid (Ai–Fi) and fish (Aii–Fii). Values are means ± 1 s.e.m., *N*=5 for most samples, as described in text.

acidification to increase phosphorus (as bone phosphate in fish meal) assimilation in aquaculture as a means of reducing eutrophication by undigested P in these systems (Vielma and Lall, 1997; Vielma et al., 1999; Sugiura and Hardy, 2000). A review of the pylorus (Ramkumar and Schulze, 2005) indicates that, at least in mammals tested to date, the pylorus adjusts gastric outflow resistance to meet physiological needs as a function of chemical (acidification) and physical (mechanical) action by the stomach. We assume that the release of chyme is controlled by a similar mechanism in toadfish which, like most teleosts, possess a true stomach and pyloric sphincter. Notably, because our fish were fed to satiation, we observed upon dissection that the stomach of nearly all fish was so distended as to indicate the possibility that small amounts of chyme had entered the anterior intestine regardless of the state of contraction of the pyloric sphincter – a likely explanation for the immediate decrease in anterior intestine pH following feeding. Many ectothermic vertebrates also experience very large meals (often containing bone) at infrequent intervals; an exceptionally high volume of gastric acid secretion and long holding time are likely reasons for an especially pronounced alkaline tide response in these animals (for a review, see Wang et al., 2001).

Intestinal HCO_3^- secretion may also act in part to regulate acute dietary Ca^{2+} influx. In addition to a larger influx of HCl to the intestine of toadfish fed sardines, maximal Ca^{2+} concentrations (reached 6 h post-feeding) in the anterior intestine were over twofold those in fish fed a squid diet, yielding another possible reason for a larger peak of postprandial HCO_3^- levels in toadfish fed sardines. This may suggest an additional role of postprandial intestinal HCO_3^- secretion in leading to increased $CaCO_3$ precipitation to serve Ca^{2+} excretion during digestion of high calcium meals, as plasma Ca^{2+} concentrations were not increased postprandially in either diet (Table 3). Notably, Ca^{2+} concentrations in the posterior intestinal and rectal fluids never reached levels as high as those measured in the anterior and mid intestine fluid (Table 2). This difference could be accounted for by increased $CaCO_3$ precipitation, which was unaccounted for by our measurements, and likely is a main route of Ca^{2+} excretion in the absence of major Ca^{2+} absorption to the extracellular fluid.

Disturbed acid–base balance?

The statistically significant yet transient increase in plasma pH we measured 3 h post-feeding in toadfish fed a squid diet (Table 3A) indicates the possibility of a postprandial alkaline tide; however, the inconsistency in both control and treatment plasma pH values is a cause for reservation. The absence of plasma alkalinization in toadfish fed sardines (Table 3B), and unchanged plasma HCO_3^- concentrations in fish fed both diets (Table 3), suggest that feeding-induced acid–base balance disturbance is lacking in Gulf toadfish even when fed a 9% (of body mass) meal. Notably, due to the nature of the caudal puncture technique, blood samples may have contained a variable mixture of arterial and venous blood and may also have been influenced by tissue lactic acid release following anesthetic overdose. While our measurements indicated the absence of acid–base balance disturbance, plasma pH and HCO_3^- concentrations were increased between 3 and 9 h after feeding in Pacific spiny dogfish (Wood et al., 2005), with virtually no evidence of respiratory compensation that is common among other vertebrate classes (Andrade et al., 2004).

It has been shown in fishes that ventilatory adjustments have only a small effect on blood P_{CO_2} levels (Perry and Wood, 1989; Wood et al., 2005) and thus we would expect an alkaline tide to reveal itself exclusively in plasma pH and HCO_3^- concentrations as in the spiny dogfish (Wood et al., 2005). We suggest additional experiments employing a more detailed time course, along with cannulation to provide for continual sampling, on a larger number of fish, before concluding the presence or absence of a postprandial alkaline tide in Gulf toadfish. It is possible that plasma alkalinization might be absent or reduced by branchial base efflux, although this was not measured in our experiments. Metabolic HCO_3^- is secreted by the gastric oxyntopeptic cell (gastric epithelial cells in non-mammalian vertebrates, secreting both HCl and pepsinogen) basolaterally into the extracellular fluid as a mechanism of alleviating cellular alkalosis. This HCO_3^- may be immediately transported across the gill, assumedly *via* either a Cl^-/HCO_3^- exchanger or Na^+-HCO_3^- cotransport [NBC; see Evans et al. (Evans et al., 2005)], thus no plasma alkalinization would be measured. If this is indeed the case, one may expect a more pronounced alkaline tide in freshwater fish, in which branchial anion exchange would presumably be limited by low environmental Cl^- concentrations.

Another possible explanation for the lack of alkaline tide response lies in intestinal HCO_3^- secretion. As apical acid secretion by the gastric oxyntopeptic cell prompts basolateral secretion of metabolic HCO_3^- into the extracellular fluid, it is possible that this HCO_3^- provides additional substrate for intestinal Cl^-/HCO_3^- exchange, which appears to be enhanced during digestion. This hypothesis, however, has several caveats. One involves the uncertainty of possible mechanisms responsible for transporting HCO_3^- to the intestine from the serosal side of the gastric oxyntopeptic cell. In fact, the mechanism transporting HCO_3^- to the systemic blood circulation thus creating an alkaline tide has not yet been described (for a review, see Niv and Fraser, 2002). A second caveat lies in the mechanism of HCO_3^- entry into the intestinal epithelium cell. Recent reports show that the majority of HCO_3^- secreted into the intestinal lumen to serve osmoregulation arises from hydration of endogenous CO_2 in the epithelial cells of the intestine (Wilson et al., 2002; Grosell and Genz, 2006) (reviewed by Grosell, 2006). Therefore, we suppose that HCO_3^- secreted basolaterally by the gastric parietal cells would have to either be dehydrated to CO_2 to enter the intestinal epithelium cell diffusively, or an additional transport pathway, such as basolateral NBC, must be supplying the intestinal epithelial cells with excess HCO_3^- during the period of postprandially stimulated HCO_3^- secretion. Clearly, postprandial acid–base balance in seawater and freshwater fishes deserves additional attention.

Gastrointestinal water and monovalent ion absorption and divalent ion concentration

Intestinal Mg^{2+} and SO_4^{2-} concentrations are good markers for water absorption (Smith, 1930), as these divalent ions see only very modest absorption across the intestinal epithelium, and are not known to precipitate to the extent that Ca^{2+} does in intestinal carbonate pellets (Walsh et al., 1991). A drop in luminal Mg^{2+} and SO_4^{2-} concentrations between 3 and 48 h after feeding presumably indicates an influx of gastric, biliary, intestinal and perhaps pancreatic secretions in addition to transient diffusive water gain. The intestinal lumen and certainly the stomach contents are hyperosmotic to the extracellular fluids, which would facilitate diffusive water movement into the gastrointestinal tract. Additionally, seawater ingestion has been shown both prandially *via* incidental intake and postprandially *via* drinking (Kristiansen and Rankin, 2001). Together, it appears that these factors act to temporarily dilute the divalent ions that normally persist in marine teleost intestinal fluids (Marshall and Grosell, 2006). An increase in Mg^{2+} and especially SO_4^{2-} concentrations over control levels at 48 h post-feeding, though not statistically significant, indicates the possibility of a period of highly increased intestinal water absorption. This is conveniently the same time point at which intestinal HCO_3^- levels are maximal, luminal Cl^- concentrations are still reduced, and organic nutrients have been largely absorbed. Anterior intestinal fluid in both starved and fed fish is composed predominantly of monovalent ions Cl^- and Na^+, while a notable shift towards divalents Mg^{2+} and SO_4^{2-} occurs posteriorly. Based on our calculated osmotic coefficients for monovalent and divalent solutions, an intestinal fluid rich in divalent ions will act to facilitate water absorption in the posterior intestine by enhancing the transepithelial osmotic gradient by reducing the effective osmolality on the luminal side.

Organic nutrient absorption

Osmolality and inorganic ion sum measurements of gastrointestinal fluids allow us to predict a timeline for organic nutrient absorption. Organic nutrient absorption seems to occur in the greatest magnitude in the stomach of toadfish fed a squid diet (Fig. 9Bi), and be completed in all segments of the intestine by 48 h post feeding in both diets (Fig. 9C–F). In addition to diminishing over time, the amount of organic solutes in the fluids of each intestinal segment at any given time is slightly reduced in progressively posterior intestinal sections. This not only indicates a consistent ability of the entire length of the intestinal epithelium to absorb organic nutrients, but also indicates that organic nutrients are truly being absorbed and not just passed along to posterior sections of the gastrointestinal tract. In addition, we saw a clear shift in intestinal fluid composition between 24 and 48 h post-feeding in which osmolality decreases markedly and is exceeded by the sum of inorganic ions. This indicates a trend towards divalent ions, which have a lower osmotic coefficient than monovalents and thus account for the difference between inorganic ion sum (which assumes an osmotic coefficient of one) and actual osmolality. This difference between inorganic ion sum and actual osmolality is elevated progressively in posterior sections, indicating that in addition to organic nutrient absorption, water absorption also is consistent and cumulative along the intestine.

A transient but significant increase in plasma osmolality 12 h post-feeding in toadfish fed a squid diet in the absence of a significant increase in inorganic ion sum might be attributed to a high amino acid content in squid, although organic solute concentrations were not measured directly in these experiments.

Conclusions

Above all, we have shown with these experiments that feeding transiently and acutely alters the gastrointestinal physiology of Gulf toadfish, and that these physiological changes are dependent upon diet composition. In addition to dramatically changing both the inorganic and organic ionic composition along the gastrointestinal tract, we also saw evidence for transient changes in water and solute secretion and absorption across the gastric and intestinal epithelia as time passed after feeding. By demonstrating a limited or lack of postprandial alkaline tide, we have raised questions about postprandial disruption to acid–base balance in seawater and freshwater fish, and the fate of metabolic HCO_3^- secreted basolaterally by gastric oxyntopeptic cells. The next question begging an answer is the regulation of intestinal HCO_3^- secretion. Since we have shown strong evidence that apical Cl^-/HCO_3^- exchange is stimulated in the intestine by feeding, regardless of the diet, it is possible that intestinal anion exchange is stimulated by parameters relating to ingestion of a meal itself and/or by the osmoregulatory and acid–base challenges that result. Conjecture might tie these questions of acid–base balance and anion exchange together by explaining the lack of an alkaline tide with increased intestinal HCO_3^- secretion, although this remains to be documented.

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