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Osmoregulation, ionoregulation and acid–base regulation by the gastrointestinal tract after feeding in the elasmobranch (*Squalus acanthias*)

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Summary

In order to study the physiological consequences of voluntary feeding in the gastrointestinal tract of a ureotelic marine elasmobranch, dogfish (fasted for 96 h) were sampled at various times up to 360 h after consuming a 5–6% ration of teleost fish (hake) under natural feeding conditions. Digestion and absorption were completed between 120 and 360 h post-feeding. The tissue masses of different segments of the gastrointestinal tract increased and decreased markedly as the chyme moved through, mainly because of fluid engorgement rather than hyperplasia. In fasted dogfish, the cardiac and pyloric stomachs contained only small volumes of highly acidic fluid (pH 1.77±1.12, 2.05±0.08) similar in composition to seawater. Feeding resulted in gastric pHs of 3.20±0.31 and 3.95±0.40 at 6 h, followed by slow declines through 60 h. An alkaline tide in the blood also occurred at 6 h. In the face of large changing masses of highly acidic chyme in the stomachs, the pH (6.50±0.10), ionic composition and volume of chyme in the intestine (spiral valve) were precisely regulated from 6 to 60 h post-feeding at very different values from those in the stomachs, and intestinal HCO_3^- remained low (5.12±0.83 mmol l^{-1}). The colon was usually empty and its pH constant at 7.20±0.16 at all times. Despite the ingestion of strongly hypo-osmotic teleost tissue, the osmolality of the chyme remained in equilibrium with that of the blood plasma in all segments at all times after feeding. Much of the osmotic equilibration was because of the secretion of urea into the chyme, particularly in the intestine. After feeding, gastric fluid concentrations of Na^+ and Mg^{2+} declined, K^+ and Ca^{2+} increased, whereas Cl^- exhibited little change, indicating that additional drinking of seawater was minimal. Na^+, K^+, water and especially Cl^- were absorbed in the intestine, whereas Mg^{2+} and Ca^{2+} were largely excluded. Our results illustrate the complex integration of digestive and ionoregulatory function in the elasmobranch digestive tract, and marked differences from the teleost pattern.

Key words: gastric acid secretion, chyme composition, alkaline tide, urea, osmolality, dogfish, shark.

Introduction

The feeding ecology of marine sharks has been widely investigated (Cortes, 1997; Cortes, 1999; Wetherbee and Cortes, 2004), and there is an abundance of studies on the spiny dogfish *Squalus acanthias* (Bonham, 1954; Holden, 1966; Jones and Geen, 1977; Tanasichuk et al., 1991; Hanchet, 1991; Laptikhovsky et al., 2001; Alonso et al., 2002). In general, the dogfish appears to be an opportunistic predator with catholic tastes, feeding at irregular intervals and exploiting a wide variety of prey according to their availability. Many dogfish are caught with empty stomachs, but a single meal may be massive (up to 10% of body mass). Averaged on a daily basis, consumption appears to be in the range of 0.5–2.5% (Jones and Geen, 1977) or 0.8–4.1% of body mass (Tanasichuk et al., 1991) in *Squalus acanthias*, with substantial year-to-year and seasonal variation. Gut passage time is calculated to be more than 5 days in *Squalus acanthias* (Jones and Geen, 1977) and 8 days in *Scyliorhinus canicula* (Sims et al., 1996), with estimates of 3–4 days in other elasmobranch species at warmer temperatures (Menon and Kewalramani, 1959; Wetherbee et al., 1987; Wetherbee and Gruber, 1990; Cortes and Gruber, 1990; Cortes, 1999). All authors except Menon and Kewalramani concur that the digestive process is much slower in sharks than in similarly sized teleost carnivores at comparable temperature (Menon and Kewalramani, 1959).

In contrast to this abundance of trophic information, there are relatively few data on the physiological events associated with feeding (reviewed by Holmgren and Nilsson, 1999), so

recently, we have begun to address this deficit with studies on *Squalus acanthias* (Wood et al., 2005; Kajimura et al., 2006; Walsh et al., 2006). These investigations have revealed pronounced systemic disturbances including a marked alkaline tide in the bloodstream peaking 6 h after feeding, presumably reflective of HCl secretion into the stomach (Wood et al., 2005), and a slower activation of the ornithine urea cycle in both liver and skeletal muscle associated with a rise in plasma urea and osmolality levels (Kajimura et al., 2006). In both studies, postprandial ammonia-N and urea-N excretion was minimal, indicative of strong N-conservation. There was also an activation of aerobic enzymes, particularly those of ketone body metabolism, with the most pronounced effects seen in the rectal gland, the organ thought to deal with any excess NaCl load accompanying a meal (Walsh et al., 2006).

In the current investigation, our focus has turned to the events occurring in the gastrointestinal tract itself during the processing of a meal. Previous studies are sparse, and have concentrated on the pH of the stomach, with two patterns described in various species. In one, the pH of the gastric fluids is close to neutrality when food is not present in the stomach, and decreases greatly upon feeding (Sullivan, 1905; Rebolledo and Vial, 1979; Caira and Jolitz, 1989; Papastamiatiou and Lowe, 2005). In the other, the gastric pH is always low, but increases because of the buffering action of the food once a meal is taken in (Babkin et al., 1935; Menon and Kewalramani, 1959; Papastamiatiou and Lowe, 2004). The only report on *Squalus acanthias* (Sullivan, 1905) suggested that it follows the first pattern (i.e. acid secretion occurs only when food is present), although no actual pH data were recorded. There is negligible information on pH levels in the remainder of the tract, or on ion concentrations, osmolality or water content of the chyme in any part of the gastrointestinal system during the processing of a meal. There is also no information on whether the mass of the gastrointestinal tract itself changes in response to feeding, as has been documented in another intermittent eater, the python (Secor and Diamond, 1995; Secor and Diamond, 1998).

With this background in mind, we addressed the following issues, using a natural 'feeding frenzy' protocol in which dogfish were fed a meal of the teleost hake (*Merluccius productus*), and then terminally sampled at various times up to 15 days. First, we wished to describe the processing of the food through the tract over time after natural feeding in a quantitative fashion, and to establish whether there were any 'trophic' changes in the mass of the gastrointestinal tissue. Second, we sought to establish that the alkaline tide (build-up of plasma HCO_3^-), which had previously been seen in animals fed by stomach tube (Wood et al., 2005), also occurred after natural feeding, because the stomach tube feeding protocol represented involuntary food intake. Third, we recorded pH and HCO_3^- levels in the digestive fluids and chyme throughout the tract in starved and post-feeding dogfish. Our goal here was to validate the conclusions of Sullivan (Sullivan, 1905), and to understand whether neutralization occurs in the intestine (spiral valve) in light of recent reports of high HCO_3^- levels in this region in starved specimens of the bamboo shark *Chiloscyllium plagiosum* (Taylor and Grosell, 2006a; Anderson et al., 2006). A fourth goal was to document levels of osmolality, major electrolytes and water content in the digestive fluids and chyme during the processing of the meal. We suspected that ingestion of osmoregulating teleost tissue by an osmoconforming elasmobranch would create large osmotic and ionic gradients between the chyme and the blood plasma. The acid–base, ionic and osmotic status of the gall bladder bile was also followed to assess its contribution to such events. Finally, in light of our recent finding that the enzymes of the ornithine urea cycle occur in the intestine of *Squalus acanthias* (Kajimura et al., 2006), we determined urea levels in the various fluids to understand whether this major osmolyte of the systemic fluids was used to adjust the osmolality of the chyme.

Materials and methods

Feeding

Dogfish sharks (*Squalus acanthias*, 0.9–3.7 kg) of both sexes were collected by trawl or by angling in Barkley Sound, British Columbia in July and August 2005. Prior to experimentation, the fish were held as a large group (~120 animals) for several weeks in a 200 000 l circular indoor tank served with running seawater at the experimental temperature (11±1°C), salinity (32±1 p.p.t.) and pH (7.90±0.15). *Squalus acanthias* will not readily feed when held in small tanks, but in the large group tank a few fish started feeding after 1 week in captivity, and thereafter the others quickly learned to do so, as reported by Kajimura et al. (Kajimura et al., 2006). During holding, the dogfish were fed every fourth day (i.e. at approximately 96-h intervals) with freshly thawed whole hake (*Merluccius productus*) from which the heads had been removed. This is one of the most common natural prey of dogfish in British Columbia coastal waters (Jones and Geen, 1977). A feeding frenzy ensued, and all the food was consumed within 30 min. The ration supplied at each feeding was approximately 3% of body mass, based on the estimated mass of all the dogfish in the group tank. With practice, it was possible to discern which dogfish had fed based on the bulging profile of the abdomen, and at 1 h after feeding, some of these animals were caught by dip-net and removed to isolation enclosures. The enclosures were individual 40 l polyurethane-coated wooden boxes (seawater flow=1 l min^{-1}), as described by Wood et al. (Wood et al., 1995; Wood et al., 2005). In this manner, a group of animals (N=6–8) was set aside for sampling at each of the post-feeding times (6 h, 20 h, 30 h, 60 h, 120 h and 360 h).

Sampling

At each sample time, fish were terminally anaesthetized in their isolation boxes by stopping the water flow, lowering the water level to 6 l and adding an overdose of tricaine methanesulphonate (MS-222) (0.2 g l^{-1}). The fish were then quickly removed, weighed and blood sampled by caudal puncture with a #22 needle attached to a lithium-heparinized

10 ml syringe. Blood samples were centrifuged at 10 000 ***g*** for 2 min, the plasma was removed, a subsample was divided into aliquots for total CO_2 analysis, and the remainder was frozen in liquid N_2 for later analyses. The body cavity was opened by a long mid-ventral incision. The bile duct was ligated with silk suture, the gall bladder removed intact, and then cut and drained so as to collect the entire bile volume into a tared weighboat. The bile was weighed and then decanted into a sealed centrifuge tube, from which aliquots were removed for pH and total CO_2 analysis, before the remainder was similarly frozen in liquid N_2. Double ligatures were placed around the junctions of the esophagus with the cardiac stomach, the cardiac stomach with the pyloric stomach, at the level of the pyloric sphincter, at the posterior end of the intestine (spiral valve), and at the end of the colon (rectum). This delineated four closed sacs: cardiac stomach (stomach-1), pyloric stomach (stomach-2), intestine and colon. The very short duodenum was thereby taken as part of the intestinal sac, which was dominated by the spiral valve [see Holmgren and Nilsson for anatomy (Holmgren and Nilsson, 1999)]. Cuts were made between the double ligatures, and the sacs removed to tared weighboats. The entire procedure took approximately 10 min, after which additional tissues were harvested for measurements not reported in the current paper.

Each sac was weighed and then cut open for the collection of its contents of digestive juices and chyme into a separate weighboat. The empty sac was reweighed, so as to yield the mass of the tissue and the mass of the contents. The pH of the fluid portion of the contents was then measured, or if this was not possible because of insufficient volume, a pH measurement was taken from the mucosal wall of the tissue. Tests demonstrated that the measurements from the fluid phase and mucosal wall were virtually identical. The fluid phase was then manually stirred and a sample (up to 1.5 ml) was collected. If the sample was cloudy, it was centrifuged to yield a clear supernatant, from which a subsample was divided into aliquots for total CO_2 analysis and the remainder was frozen in liquid N_2 for later analyses. The rest of the chyme and the tissue of the empty sac (except the colon) were then dried to a constant mass so as to yield their respective water contents.

Samples of the food and the ambient seawater were also frozen for later analysis.

Analyses

The pH of the bile and the fluid phase of the chyme samples were measured using a Radiometer GK2401C (Copenhagen, Denmark) glass combination electrode fitted into a custom-built chamber thermostatted to the experimental temperature of 11±1°C. The electrode was calibrated with Radiometer precision buffers and its output displayed on a Radiometer pHM 72 acid–base analyzer. For very low-volume samples or when it was necessary to take measurements from the mucosal wall, an esophageal electrode set (MicroElectrodes Inc., Bedford, NH, USA) calibrated with the same thermostatted buffers was used. Total CO_2 concentrations of the various fluids were determined by the method of Cameron (Cameron, 1971). When both total CO_2 and pH were measured, HCO_3^- concentrations were calculated from rearrangement of the Henderson-Hasselbalch equation using appropriate constants from Boutilier et al. (Boutilier et al., 1984).

Samples of the food were homogenized in a blender, then digested in 5 volumes of 1N HNO_3 at 65°C prior to assay. The remainder of the measurements were made on fluid samples, which had been frozen and then later thawed and thoroughly mixed. Na^+, K^+, Ca^{2+} and Mg^{2+} concentrations were determined by atomic absorption spectroscopy (SpectrAA-220FS; Varian) and Cl^- by the colorimetric assay of Zall et al. (Zall et al., 1956). Osmolality was measured by vapour pressure osmometry (Wescor 5100C, Logan, UT, USA). Urea was measured by the diacetyl monoxime method (Rahmatullah and Boyde, 1980). Water contents of chyme, gut tissues and food samples were determined by drying to a constant mass at 65°C for 72–96 h.

Data have been expressed as means ± 1 s.e.m. (*N*). Masses of the gastrointestinal contents and the gastrointestinal tissue segments themselves were normalized to total body mass prior to analysis. Data were tested for normality and homogeneity of variances, and in some instances data were log-transformed prior to further analysis to pass Bartlett's χ^2 test for homogeneity of variances. Percentage data were arc-sin transformed. Data were then analyzed by one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference test to detect specific differences (Statistix for Windows). In those few instances in which the data still did not pass Bartlett's test, the non-parametric Kruskal–Wallis signed-ranks test was used in place of the ANOVA and Tukey's test. In the current study, data were collected from animals that had been kept on a 4-day feeding cycle and then sacrificed at various times after their last meal. Thus, in the figures a dotted line at 96 h post-feeding indicates the probable pre-feeding condition (i.e. 96 h fasting), and significant differences among points at different times post-feeding are indicated in the figure legends. Comparisons between compartments were made using Student's paired *t*-test (two-tailed) with the Bonferroni correction for multiple comparisons, and appropriate log or arc-sin transformations where necessary. A significance level of $P<0.05$ was used throughout.

Results

Masses and movements of chyme

Based on the mass (fluid plus solid) of the contents of stomach-1 plus stomach-2 at 6 h and 20 h post-feeding (Table 1), and taking into account water content differences, the average mass of food consumed in the single meal was 5–6% of body mass. This was greater than the estimated ration (3%) fed to the entire group, but an effort was made to select those dogfish that we knew had eaten, based on bulging abdomens. The mass of ingested food was significantly greater in stomach-1 than in stomach-2 at 6 h, but this difference was reversed by 20 h (Table 1). At 30 h and 60 h, this difference persisted, although the total masses in each section were greatly

Table 1. *Qualitative and quantitative tabulation of the time course of digestion from sampling of four sections of the gastrointestinal tract of the dogfish shark at various times after feeding*

Time after meal (h)	*N*	Stomach-1	Stomach-2	Intestine	Colon
6	7	Large mass of solid, some liquid chyme	Large mass of solid, some liquid chyme	Yellow-brown fluid	Empty
		36.6±5.2 (a,A)	17.3±7.0 (a,B)	6.2±1.1 (a,C)	0.0±0.0 (a,D)
20	6	Smaller mass of solid, more liquid chyme	Greater mass of solid, more liquid chyme	Similar amount of yellow-brown fluid, but pastier	Empty
		22.0±12.8 (a,b,A)	48.5±11.5 (a,B)	7.3±1.3 (a,A)	0.0±0.0 (a,C)
30	7	Smaller mass of solid, smaller amount of liquid chyme	Smaller mass of solid, similar amount of liquid chyme	Similar amount of yellow-brown fluid-paste	Empty or small amount of dark paste
		4.2±2.5 (b,c,A)	17.9±5.8 (a,B)	6.2±2.2 (a,A)	0.2±0.1 (a,A)
60	6	Little solid (mainly bone), similar amount of chyme or clear fluid	Similar mass of solid (mainly bone), similar amount of liquid chyme	Similar amount of yellow-brown fluid-paste, small pieces of bone	Empty or small amount of dark paste
		5.5±2.9 (b,A)	18.6±7.2 (a,B)	5.6±1.3 (a,A)	0.1±0.1 (a,A)
120	8	Very small volume of clear fluid only	Very small volume of clear fluid only	Very small volume of watery yellow-brown fluid, tiny pieces of bone	Empty
		0.9±0.4 (c,A)	0.4±0.1 (b,A)	0.5±0.1 (b,A)	0.0±0.0 (a,A)
360	8	Very small volume of clear fluid only	Very small volume of clear fluid only	Very small volume of watery yellow-brown fluid	Empty
		0.4±0.1 (c,A)	0.3±0.1 (b,A)	0.2±0.1 (b,A)	0.0±0.0 (a,A)

Values (means ± 1 s.e.m.) are the relative mass (g kg^{-1} body weight) of the contents in each segment.
Note: qualitative comparisons are with respect to conditions in the same compartment at the preceding sample time.
Within the same compartment, values sharing the same lower-case letter are not significantly different (P>0.05).
Within the same time, values sharing the same upper-case letter are not significantly different (P>0.05).

reduced. The digestion of bone clearly lagged behind the digestion of soft tissue. By 120 h there was no food or chyme remaining in either stomach compartment, only a small amount of clear fluid, and this situation persisted at 360 h. Right from 6 h through 60 h post-feeding there was a constant volume (approximately 6 ml kg^{-1}) of yellow-brown fluid-paste in the intestine, although its consistency changed somewhat over time. By 120 h this intestinal fluid was reduced approximately 10-fold, and only tiny pieces of bones remained. Similar small amounts of intestinal fluid were present at 360 h. The colon was empty at most times, and only at 30 h and 60 h post-feeding was any material found in this small compartment, and only in a few animals, indicating that faecal material was not held here for any prolonged period. These observations indicate that digestion and absorption were largely completed by 120 h post-feeding, and finished before 360 h post-feeding.

Changes in mass of the gastrointestinal tract tissues

The relative masses of the tissues of the gastrointestinal tract changed during the processing of the meal (Fig. 1). Thus, the mass of the tissue of stomach-1 was significantly lower at 30 h and 60 h post-feeding than at some other time points, a difference of 18–24% (Fig. 1A). By contrast, the mass of stomach-2 was greatest at these times, a difference of approximately 31–42% relative to the 6 h value, and 68–83% relative to the 360 h value, where stomach-2 mass was at its lowest (Fig. 1B). The intestine underwent a similar pattern of increase followed by decrease in mass after feeding, with the lowest relative mass again occurring at 360 h. Relative to the 6 h value, the peak at 20 h was a 28% rise, and relative to the 360 h value it was a 74% rise.

To a very large extent, these changes were driven by alterations in the water contents of the tissues, with only small and non-significant changes in the dry masses (Fig. 1A-C). For example, when the relative mass of stomach-2 tissue increased from 9.13±0.53 (N=8) to 12.36±0.62 (N=7) g kg^{-1} body mass at

30 h, the percentage of water increased from 80.43±0.62% (*N*=8) to 81.82±0.44% (*N*=7), so the water content increased from 7.34±0.42 (*N*=8) to 10.19±0.51 (*N*=7) g kg^{-1} body mass. Therefore, these changes were largely associated with alterations in the extent to which the tissues were engorged with fluid. Indeed, we noted that at the peak of the increases, the excised tissue continued to secrete fluid into the weighboat for approximately 30 min after the original chyme had been removed and the original tissue mass taken. This did not happen with tissues from animals at 120 or 360 h post-feeding.

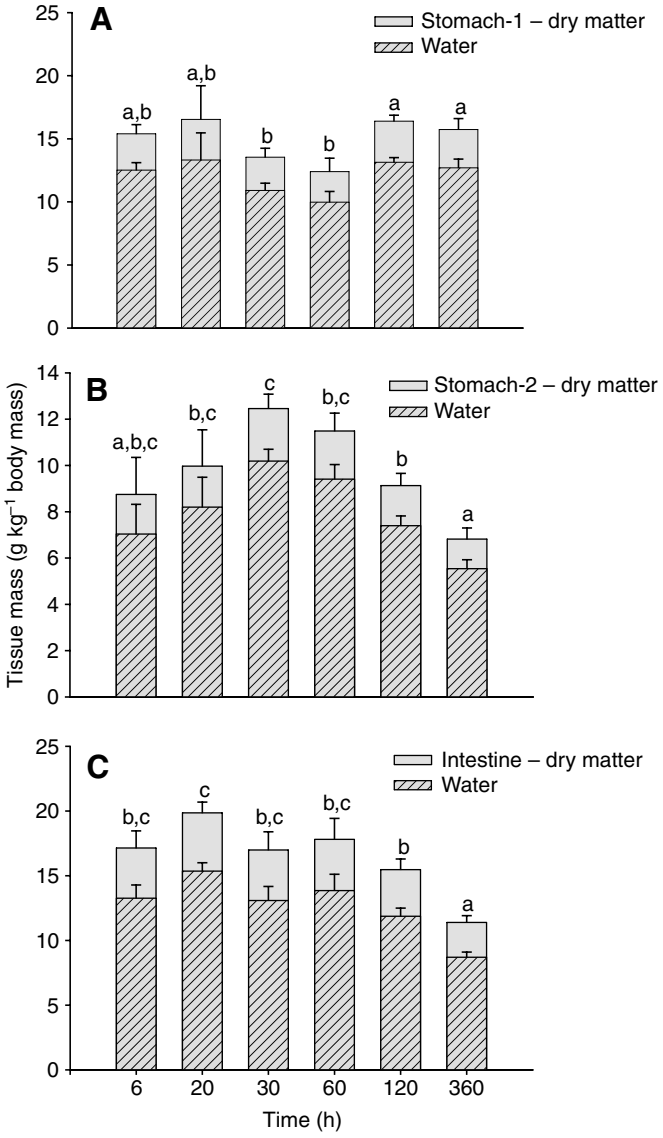

Fig. 1. The influence of feeding on the relative mass of the tissue (expressed as g kg^{-1} body mass) of various segments of the gastrointestinal tract in the dogfish shark – (A) stomach-1 (cardiac stomach), (B) stomach-2 (pyloric stomach) and (C) intestine. The inset bars indicate the mass contributed by the tissue water content, and the differences represent the mass contributed by the tissue dry-matter content. Means ± 1 s.e.m. (*N*=6–8 at each time point). Means not sharing the same letter are significantly different ($P<0.05$); the same symbols apply to the mass contributed by tissue water content and so have not been repeated.

Acid–base changes

On the systemic side, total CO_2 measurements on blood plasma (Fig. 2) revealed clear evidence of a postprandial alkaline tide in the systemic bloodstream of these naturally feeding sharks, with a rise of approximately 4.5 mmol l^{-1} at 6 h post-feeding relative to points at 30 h onwards. As plasma pH was not measured, P_{CO_2} and HCO_3^- could not be calculated, but HCO_3^- represented more than 90% of this increase, based on any reasonable estimate of P_{CO_2}.

On the luminal side, feeding caused marked changes in the acid–base status of the gastric fluids (Fig. 3). In fasted fish (i.e. 120 h), pHs in the fluids of both stomach-1 and stomach-2 were low, averaging 1.77 and 2.05, respectively. However, these values reached 3.20 and 3.95 at 6 h post-feeding, indicating a substantial reduction of H^+ concentration by the buffering action of the food, despite the probable increase in H^+ secretion rate at this time. Thereafter, pH gradually fell in both parts of the stomach as digestion proceeded until minima were reached at 120 h. At all times, pH was lower in stomach-1 than in stomach-2. At no time was there any detectable HCO_3^- in the gastric fluids.

In contrast to these substantial changes in the stomach compartments, pHs in the intestine (~6.5) and colon (~7.2) remained high and remarkably stable, with no significant changes at any times throughout the regime (Fig. 3). Similarly, the pH of gall bladder bile remained unchanged (data not shown), with an overall mean of 6.42±0.08 (*N*=42). HCO_3^- concentrations in the intestinal fluid were rather variable, ranging from 0 to 19.6 mmol l^{-1} in individual animals, but there were no significant differences over time (data not shown). The overall mean was 5.12±0.83 (*N*=36) mmol l^{-1}. HCO_3^- concentrations in gall bladder bile were low (0–4.21 mmol l^{-1}) with an overall mean of 0.61±0.12 (*N*=41) mmol l^{-1}. There was

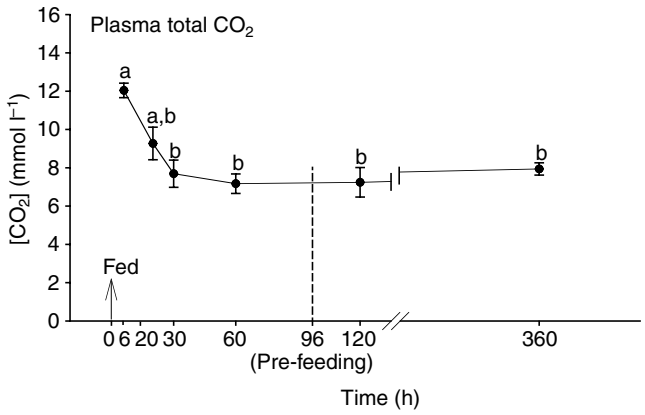

Fig. 2. The influence of feeding on plasma total CO_2 concentration in blood samples drawn by caudal puncture in the dogfish shark. Means ± 1 s.e.m. (*N*=6–8 at each time point). Means not sharing the same letter are significantly different ($P<0.05$).

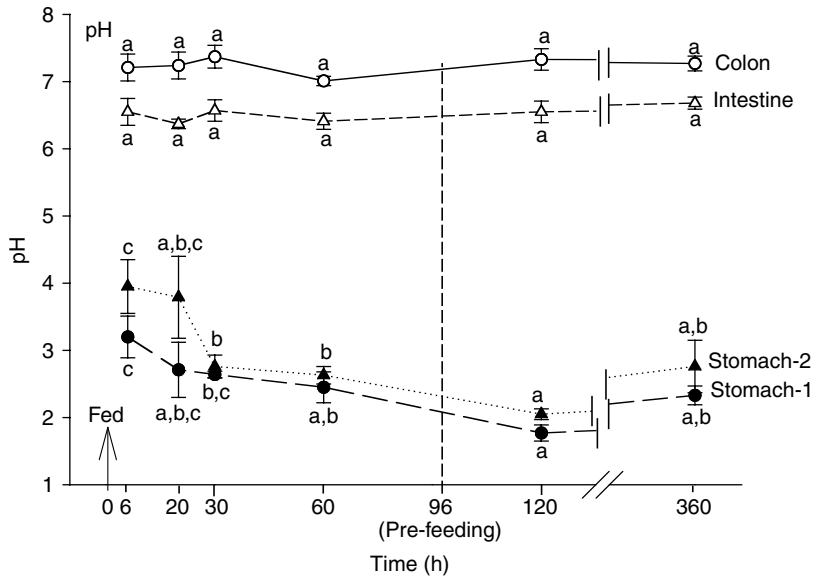

Fig. 3. The influence of feeding on the pH of the fluid phase of the gastrointestinal contents in stomach-1 (cardiac stomach), stomach-2 (pyloric stomach), intestine and colon in the dogfish shark. Means ± 1 s.e.m. (N=6–8 at each time point). Within a compartment, means not sharing the same letter are significantly different (P<0.05).

never sufficient fluid in the colon to obtain a measurement of HCO_3^- or of any other ions or osmolality.

Ionic and osmotic changes

In the first sample taken after eating (6 h), the percentage of water in the contents of stomach-1 (78.0%, Table 2) was approximately the same as in the original food (80.2%, Table 3), and this remained more or less unchanged when the bolus passed into stomach-2 at 20 h (81.9%, Table 2). This suggests that very little seawater was ingested with the original meal. However, as digestion progressed, the percentage of water in the chyme increased in stomach-1, stomach-2 and the intestine (Table 2).

When expressed on a percentage basis as in Table 2, the changes in water content are deceptively small. Fig. 4 illustrates the large changes in total water volumes in the chyme as it passed down the tract. Water volumes peaked at approximately 30 ml kg^{-1} body mass at 6 h in stomach-1 and 38 ml kg^{-1} body mass at 20 h in stomach-2, and remained significantly elevated through 60 h. Of course, much of this water was contained within the ingested food, and at least initially remained within the solid phase. Water volume in the intestinal chyme remained at approximately 5–6 ml kg^{-1} body mass throughout the 6 h to 60 h post-feeding period.

A comparison of the composition of the seawater, the teleost food, the blood plasma and the small amount of fluid in stomach-1 [0.86±0.42 (N=8) ml kg^{-1} body mass] in fasting fish (120 h post-feeding) illustrates several points (Table 3). First, the osmolality of seawater, plasma and stomach-1 fluid were statistically identical. Second, in other aspects, stomach-1 fluid was much closer to seawater than to blood plasma. These similarities include higher and identical K^+ and Ca^{2+} concentrations, much higher Na^+, Cl^- and Mg^{2+} levels, and much lower urea levels in both stomach-1 fluid and seawater than in blood plasma. Finally, the food was very different in composition from any of the fluids, and would clearly offer an initial dilution of the osmolality, Na^+ and Cl^- levels of the body fluids (stomach-1 or plasma), unless it was ingested together with considerable amounts of seawater. Similarly, urea concentrations in food were only a fraction of those in these body fluids. At the same time, however, K^+ and Ca^{2+} concentrations were considerably higher in the food than in either plasma or stomach-1 fluid, whereas Mg^{2+} concentration was intermediate.

The intake of the teleost food had a profound impact on the composition of the fluid phase in the gastrointestinal contents, but surprisingly, osmolality remained at approximately plasma and seawater levels (~950 mOsm kg^{-1}) in all compartments at all times (Fig. 5A). This occurred despite the fact that the

Table 2. *Changes over time in the percentage of water of the contents in various compartments of the gastrointestinal tract in the dogfish shark*

Time after meal (h)	N	Stomach-1	Stomach-2	Intestine
6	7	78.00±0.95 (a,A)	84.71±3.05 (a,A,B)	88.51±2.00 (a,B)
20	6	90.05±2.73 (b,A)	81.95±3.01 (a,B)	87.91±1.18 (a,A,B)
30	7	87.48±3.45 (b,A)	86.09±2.96 (a,A)	91.64±1.25 (a,A)
60	6	87.58±3.53 (b,A)	81.22±0.98 (a,B)	90.88±1.32 (a,A)
120	8	97.25±0.49 (c,A)	96.81±1.71 (b,A)	93.71±1.79 (a,b,A)
360	8	97.75±0.15 (c,A)	97.77±0.14 (b,A)	96.13±0.51 (b,A)

Gastrointestinal compartments studied were stomach-1 (cardiac stomach), stomach-2 (pyloric stomach) and the intestine. Values are the means ± 1 s.e.m.

Within the same compartment, values sharing the same lower-case letter are not significantly different (P>0.05).

Within the same time, values sharing the same upper-case letter are not significantly different (P>0.05).

Table 3. *A comparison of osmolality and the concentrations of major osmolytes and water in seawater, food (deheaded hake, Merluccius productus), blood plasma and the fluid of stomach-1 (cardiac stomach) prior to feeding (120 h fasting) in the dogfish shark*

	Seawater (*N*=4)	Food† (*N*=5)	Blood plasma (*N*=8)	Stomach-1 fluid (*N*=8)
Osmolality (mosmol kg^{-1})	967±3 (a)	400‡	973±6 (b)	949±25 (a)
Ion concentrations (mmol l^{-1})				
Na^+	452±3 (a)	55.6±2.6 (b)	254±3 (c)	400±9 (d)
Cl^-	515±3 (a)	46.4±2.4 (b)	249±2 (c)	440±14 (d)
K^+	9.8±0.5 (a)	112.9±2.3 (b)	3.5±0.9 (c)	11.6±0.6 (a)
Ca^{2+}	9.5±0.1 (a)	73.0±3.2 (b)	4.3±0.1 (c)	8.7±0.4 (a)
Mg^{2+}	52.0±0.4 (a)	15.9±0.2 (b)	1.4±0.1 (c)	35.3±1.2 (d)
Urea	0.0±0.0 (a)	4.2±1.0 (b)	438±22 (c)	145±15 (d)
Water (%)	(96.8)*	80.18±0.23 (a)	96.15±0.39 (b)	97.25±0.49 (b)

Values are the means ± 1 s.e.m.
*Calculated from salinity.
†Units for food are on a per kg rather than a per l basis.
‡Estimated value (see Holmes and Donaldson, 1969).
Measured values sharing the same letter for a parameter are not significantly different (P>0.05).

original osmotic concentration of the food was less than 50% of that of these fluids. The only consistent difference between compartments was a slightly higher osmolality (by approximately 50 mOsm kg^{-1}) in the fluid of the intestine, which was significant at some sample times. Plasma osmolality exhibited minor fluctuations.

Urea levels in the fluids of stomach-1 and stomach-2 were approximately 200 mmol l^{-1} lower than in intestinal fluid, whereas intestinal fluid urea was only approximately 100 mmol l^{-1} below that in plasma (Fig. 5B). These differences were maintained at all times. Thus, a considerable addition of urea must occur when the chyme moves from stomach-2 to the intestine. The impact of the meal, which contained negligible amounts of urea (~4 mmol kg^{-1}), appeared to further lower urea levels in stomach-1 and stomach-2 up to 30–60 h post-feeding. Notably, plasma urea peaked at 20 h post-feeding.

In fasted fish (120–360 h post-feeding), Na^+ concentrations in the fluid of stomach-1 (~400 mmol l^{-1}) were higher than in stomach-2 (~325 mmol l^{-1}), blood plasma (~255 mmol l^{-1}) or intestinal fluid (~140 mmol l^{-1}), but lower than in seawater (~450 mmol l^{-1}) (Fig. 6A). All these differences were significant. The impact of the meal, which contained a much lower concentration of Na^+ (~55 mmol kg^{-1}), was to cause lower Na^+ levels in stomach-1 and stomach-2 by 30–50% at all times through 60 h. By contrast, Na^+ levels in the intestinal fluid tended to be greater at these times. Plasma Na^+ level was maintained constant at all times.

Cl^- concentrations in the gastrointestinal fluids (Fig. 6B) were generally higher than those of Na^+, particularly in the intestine (c.f. Fig. 6A), and underwent less marked changes. In fasted fish (120–360 h post-feeding), Cl^- concentrations were similar in the fluids of stomach-1 and stomach-2 (~430 mmol l^{-1}) and much higher than in the intestine or blood plasma (both ~240 mmol l^{-1}), although lower than in seawater (~515 mmol l^{-1}). Despite the intake of a meal that contained much lower levels of Cl^- (~45 mmol kg^{-1}), these substantial differences were maintained after feeding, and there were only small decreases in the Cl^- concentrations in the gastric fluid. As with Na^+, intestinal fluid Cl^- tended to be greater through 60 h post-feeding. Plasma Cl^- remained unchanged.

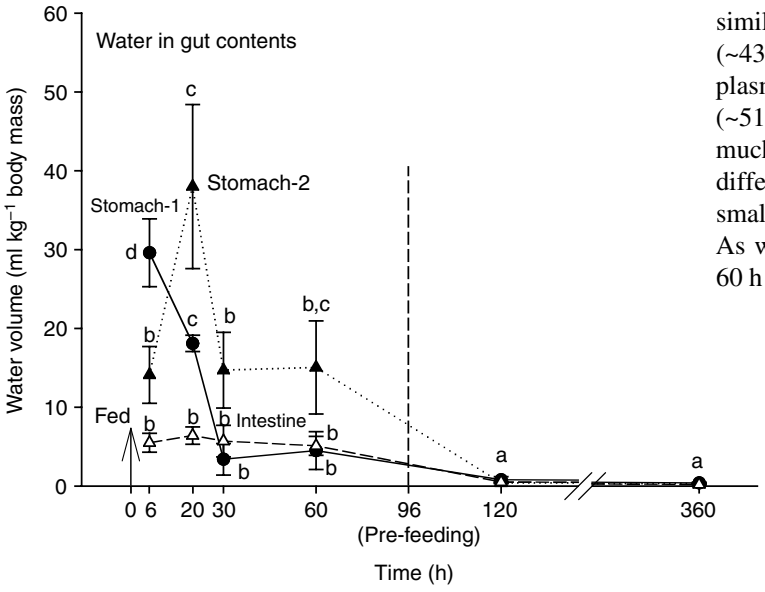

Fig. 4. The influence of feeding on total water volumes (expressed as ml kg^{-1} body mass) of the gastrointestinal contents in stomach-1 (cardiac stomach), stomach-2 (pyloric stomach) and intestine in the dogfish shark. This measurement includes water in both the fluid and solid phases. Means ± 1 s.e.m. (N=6–8 at each time point). Within a compartment, means not sharing the same letter are significantly different (P<0.05).

In contrast to Na^+ and Cl^-, K^+ concentration in the food (~115 mmol kg^{-1}) was more than 10-fold higher than in seawater, blood plasma or any of the gastrointestinal fluids of fasted fish (120–360 h post-feeding; Fig. 6C). Seawater, stomach-1 and stomach-2 fluids were all approximately 10 mmol l^{-1}, whereas plasma and intestinal fluid were substantially lower at 3–5 mmol l^{-1}. The impact of the K^+-rich meal was also to triple the K^+ concentration in the fluids of both stomach-1 and stomach-2, and these elevations remained significant through 30 h. K^+ levels in the intestinal fluid were also tripled and remained elevated through 60 h, although the absolute changes were smaller than in the gastric fluids, and K^+ concentrations were lower in the intestinal fluids than in the gastric fluids at all times. There were no significant changes in plasma K^+.

Like K^+, Ca^{2+} was in substantially higher concentration in the food (~73 mmol kg^{-1}) than in any of the fluids sampled (Fig. 7A). Plasma Ca^{2+} levels were approximately 4 mmol l^{-1}, seawater, stomach-1 and stomach-2 fluids were all approximately 10 mmol l^{-1}, whereas intestinal fluids were significantly higher at approximately 20 mmol l^{-1} in fasted fish (120–360 h post-feeding). In response to the Ca^{2+}-rich meal, concentrations increased significantly by 15–20 mmol l^{-1} in all three compartments of the gastrointestinal tract from 6 h through 60 h. Notably, Ca^{2+} levels remained higher in the intestinal fluid than in the gastric fluids throughout the entire period. Plasma Ca^{2+} did not change.

Mg^{2+} was in lower concentration in the food (~16 mmol kg^{-1}) than in seawater (~52 mmol l^{-1}), but both of these values were far greater than the low levels (1.0–1.5 mmol l^{-1}) in the blood plasma (Fig. 7B). At 120 h post-feeding, concentrations of Mg^{2+} were all very similar (~35 mmol l^{-1}) in the fluids of stomach-1, stomach-2 and the intestine. These values were significantly lower than in seawater but higher than in food. However, after longer-term fasting (360 h), intestinal fluid Mg^{2+} concentrations were threefold greater (~120 mmol l^{-1}), although highly variable, whereas gastric fluid values remained the same. After ingestion of the meal, Mg^{2+} concentrations were lowered by approximately 10 mmol l^{-1} in the fluids of stomach-1 and stomach-2 from 6 h through 30 h. By contrast, Mg^{2+} concentrations in the intestinal fluid tended to rise after the meal, with an initial doubling at 6 h. Mg^{2+} concentrations in the plasma exhibited minor fluctuations but remained less than 1.5 mmol l^{-1} at all times.

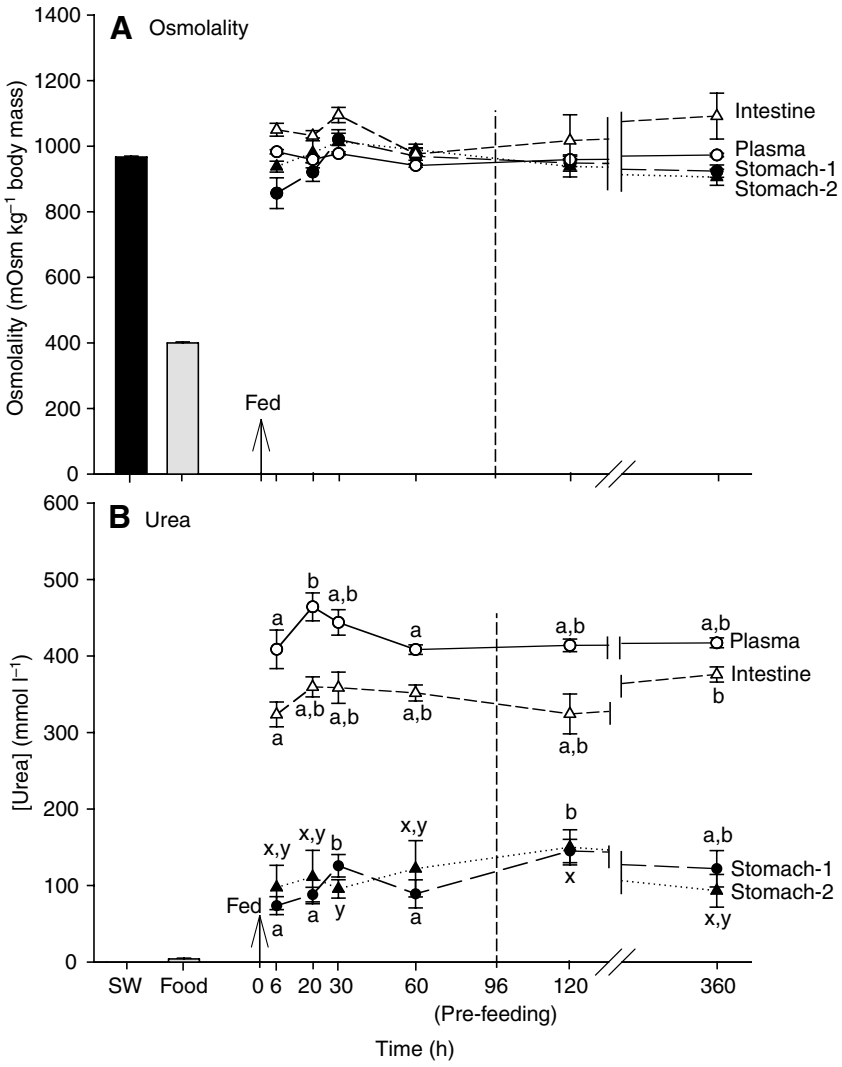

Fig. 5. The influence of feeding on (A) the osmolality and (B) the urea concentration of the fluid phases of the gastrointestinal contents in stomach-1 (cardiac stomach), stomach-2 (pyloric stomach) and intestine in the dogfish shark. Simultaneously measured values in the blood plasma are also shown. Means ± 1 s.e.m. (N=6–8 at each time point). As points of reference, the dark bars represent the measured values for ambient seawater (SW) (N=4), and the light bars represent the estimated value for the ingested food [N=5; value for osmolality from Holmes and Donaldson (Holmes and Donaldson, 1969)]. Significant differences are not marked in A so as to avoid clutter; the only significant differences (P<0.05) within compartments are in plasma osmolality, as indicated by means not sharing the same letter in the following series: a=6 h, 30 h, 360 h; b=20 h, 120 h, 360 h; c=20 h, 60 h, 120 h. In B, within a compartment, means not sharing the same letter are significantly different (P<0.05). For clarity, x,y refers to stomach-2 and a,b to stomach-1.

Gall bladder bile

The gall bladder clearly contracted after the meal. The volume of bile in the gall bladder was only 0.38±0.09 (N=7), 0.38±0.12 (N=6) and 0.45±0.06 (N=7) ml kg^{-1} body mass at 6 h, 20 h and 30 h after the meal, respectively, but then rose to significantly higher levels at 60 h [0.62±0.13 (N=6)], 120 h [0.82±0.06 (N=8)] and 360 h [0.71±0.18 (N=8)] ml kg^{-1} body mass.

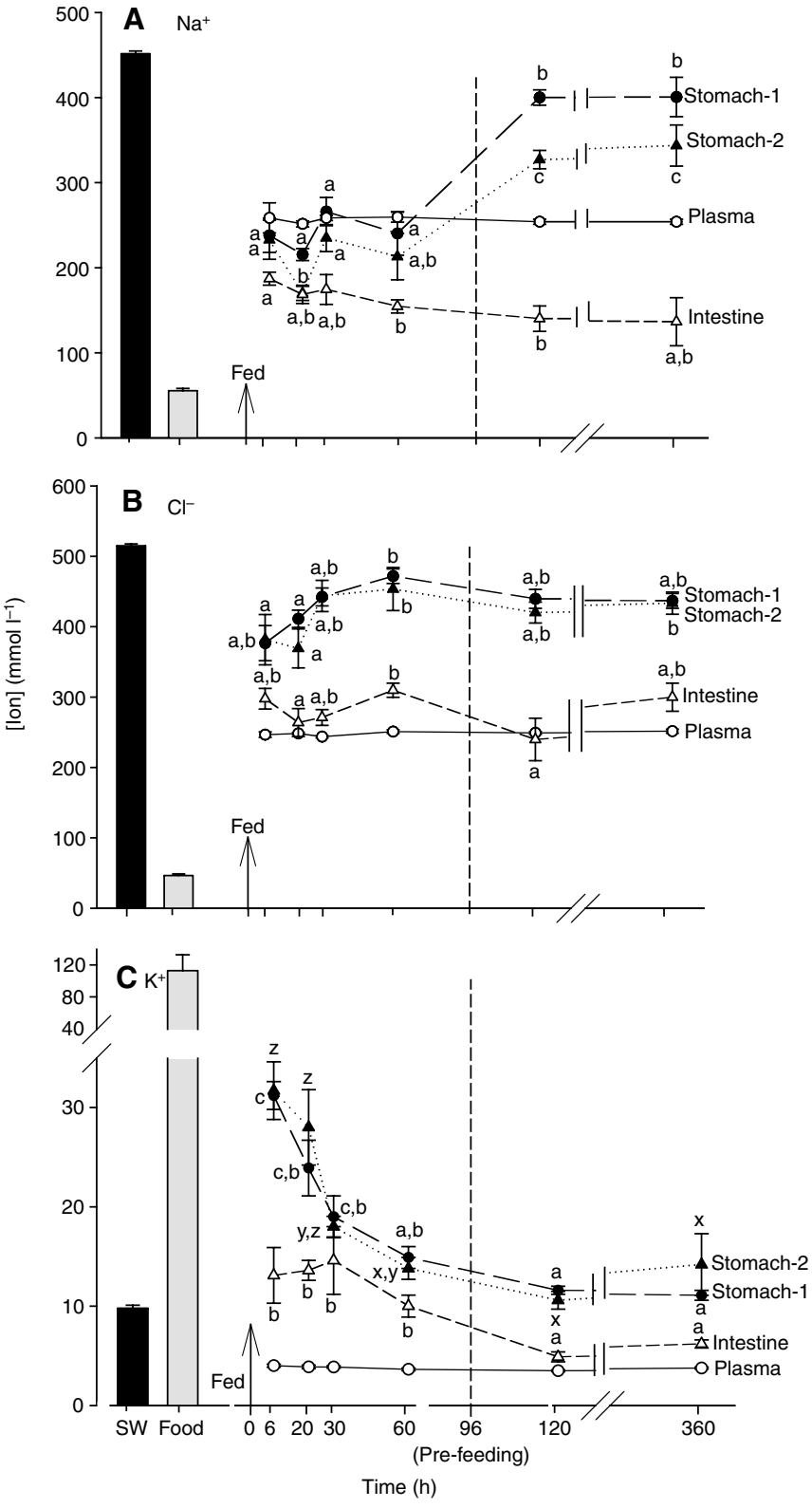

Fig. 6. The influence of feeding on (A) sodium, (B) chloride and (C) potassium concentrations of the fluid phases of the gastrointestinal contents in stomach-1 (cardiac stomach), stomach-2 (pyloric stomach) and intestine in the dogfish shark. Simultaneously measured values in the blood plasma are also shown. Means ± 1 s.e.m. (N=6–8 at each time point). As points of reference, the dark bars represent the measured values for ambient seawater (SW) (N=4), and the light bars represent the measured values for the ingested food (N=5). Within a compartment, means not sharing the same letter are significantly different (P<0.05). There were no significant differences in the plasma compartment in A–C. In C, for clarity, x,y,z refers to stomach-2 and a,b,c to stomach-1.

Bile was in osmotic equilibrium with blood plasma (~950 mOsm kg^{-1}) and exhibited similar Na^+ concentrations (~270 mmol l^{-1}), but other aspects of its composition were rather different (Fig. 8). Thus, K^+ (~7 mmol l^{-1}), Mg^{2+} (~12 mmol l^{-1}) and Ca^{2+} (~30 mmol l^{-1}) concentrations were all substantially higher than in blood plasma, whereas urea levels (~310 mmol l^{-1}) were lower (Fig. 8 *versus* Table 3). Particularly notable were the very low levels of Cl^- in bile (~60 mmol l^{-1}), only approximately 25% of those in plasma, indicating that much of the negative charge was carried by an unmeasured anion, presumably bile acid anions.

There were only modest changes in the composition of gall bladder bile after feeding (Fig. 8). These included slightly lower levels of osmolality and Ca^{2+} at 20 h and 60 h, and of Cl^- at 6 h, and a higher level of urea at 20 h. Biliary Mg^{2+}, Na^+ and Ca^{2+} concentrations were also slightly higher after 360 h of starvation than at other times.

Discussion

The pattern of feeding and digestion

Contrary to the early observations of Sullivan (Sullivan, 1905), the dogfish shark clearly falls into that group of elasmobranchs that maintain a highly acidic pH in the gastric fluids during fasting, and exhibit a rise in pH once the bolus of food is ingested. The pH changes in the stomach of *Squalus acanthias* (from approximately 1.8 to 3.9 at 6 h after the meal; Fig. 3) were very similar to those in the leopard shark *Triakis semifasciata* (1.5 to 3.1) (Papastamiatiou and Lowe, 2004) but much more marked than in several other species exhibiting this same pattern (3.5 to 5.0) (Babkin et al., 1935; Menon and Kewalramani, 1959). Papastamiatiou and Lowe speculated that maintaining stomach pH continuously low may be advantageous for an opportunistic predator (Papastamiatiou and Lowe, 2004), allowing it to be physiologically prepared to digest a meal soon after a feeding event. The digestion and gut passage time observed in the current study (largely complete

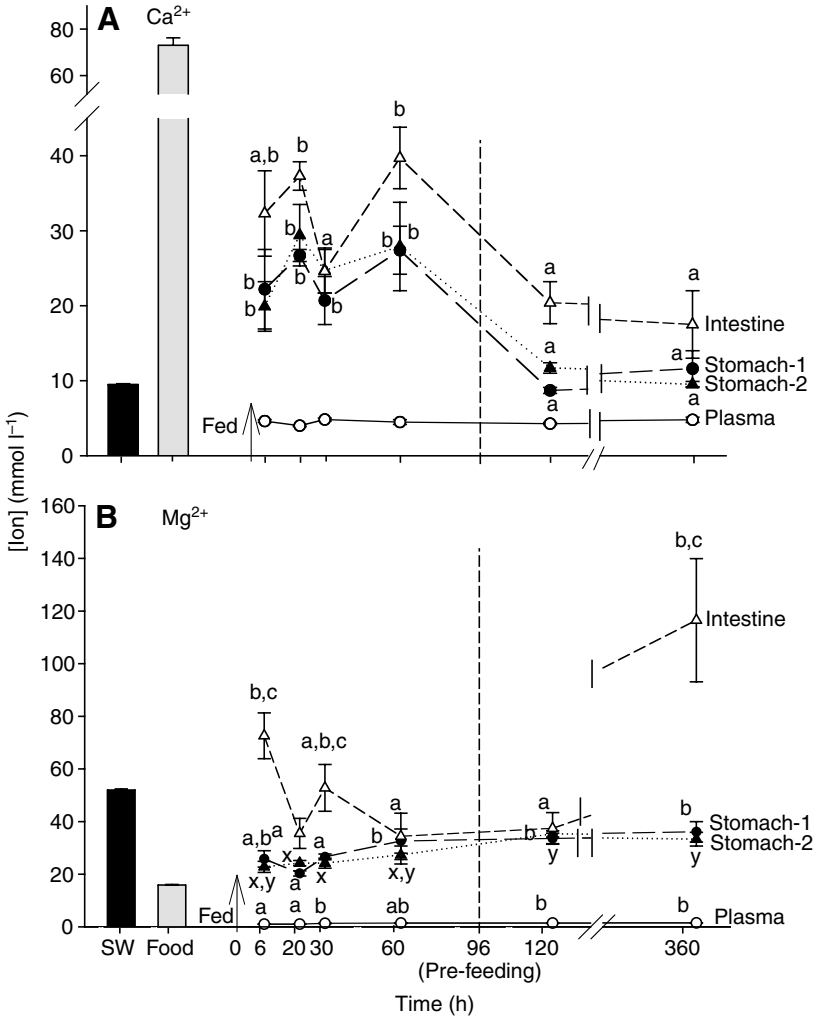

Fig. 7. The influence of feeding on (A) Ca and (B) Mg concentrations of the fluid phases of the gastrointestinal contents in stomach-1 (cardiac stomach), stomach-2 (pyloric stomach) and intestine in the dogfish shark. Simultaneously measured values in the blood plasma are also shown. Means ± 1 s.e.m. (N=6–8 at each time point). As points of reference, the dark bars represent the measured values for ambient seawater (SW) (N=4), and the light bars represent the measured values for the ingested food (N=5). Within a compartment, means not sharing the same letter are significantly different (P<0.05). There were no significant differences in the plasma compartment in A. In B, for clarity, x,y refers to stomach-2 and a,b to stomach-1.

by 5 days, and finished by 15 days; Table 1) was comparable to that reported by Jones and Geen for the same species force-fed comparable food items (Jones and Geen, 1977). Our recent measurements of N-budget in naturally feeding *Squalus acanthias* (Kajimura et al., 2006) indicate that the animals must have to feed every 5–6 days just to maintain enough N for urea-based osmoregulation, let alone grow.

In accord with these ideas, stomach-1 did not appear to increase its mass after initial ingestion of the meal (Fig. 1A), suggesting that it is maintained in a state of readiness during starvation so as to be able to accept a meal at any time. The fall in stomach-1 mass at 30 h and 60 h post-feeding was associated with a fall in tissue water content of unknown etiology. We speculate that contractile activity at these times resulted in the mechanical displacement of fluid from the muscle tissue. By contrast, stomach-2 did increase its mass substantially, as did the intestine, indicating that these segments can be activated fairly quickly when required (Fig. 1B,C). Although these changes were relatively large, they were mainly because of fluid engorgement rather than actual proliferation of the tissue. We attribute these changes to increased blood flow and secretory activity for digestion and absorption. By 360 h after the last meal, these were probably greatly reduced, resulting in the observed falls in tissue masses at this time. By way of comparison, in the 30-day starved Burmese python, which is perhaps the champion in terms of upregulation of the digestive tract (Secor and Diamond, 1995), stomach mass increased by 26%, mainly because of increased hydration only at 6 h post-feeding, whereas intestinal mass increased more slowly, reaching 300% by 3 days, but the relative roles of hydration *versus* proliferation were not separated in that study. However, more recently, Holmberg et al. have reported that dry as well as wet intestinal mass increases in the python at 48 h after feeding, although the percentage of water decreases (Holmberg et al., 2003). By analogy to teleosts (e.g. Axelson et al., 1989; Axelson and Fritsche, 1991), overall increases in regional blood flow are expected after feeding, but these have not yet been measured in elasmobranchs (Holmgren and Nilsson, 1999).

The volume of chyme in the intestine (approximately 6 ml kg^{-1} body mass; Table 1) and its ionic and osmotic characteristics (Figs 5-7) remained approximately constant from 6 h through 60 h post-feeding, despite large changes in these parameters in stomach-1 and stomach-2. The composition of the chyme was also very different from that in the stomachs, with much higher pH (Fig. 3) and urea levels (Fig. 5B), higher Ca^{2+} (Fig. 7A) and Mg^{2+} (Fig. 7B) concentrations, and much lower Na^+ (Fig. 6A), Cl^- (Fig. 6B) and K^+ (Fig. 6C) concentrations. This suggests that the pyloric sphincter acts as an efficient meter, letting through only as much material from stomach-2 as the neutralization, transport and absorptive processes in the intestine can deal with at any one time. As in higher vertebrates, this pyloric transit is probably under complex neural and hormonal control (Holmgren and Nilsson, 1999).

In most vertebrates, the final section of the tract, the colon or rectum, serves for water absorption, thereby drying the faeces (Holmgren and Nilsson, 1999). In the dogfish, there was

rarely much material in the colon, and when present, it was a thick paste (Table 1), in accord with this idea. Our impression is that overall absorptive efficiency is very high in *Squalus acanthias*, and that the amount of faecal material discharged is small.

Acid–base responses associated with feeding and digestion

The increase in plasma HCO_3^- concentration at 6 h after the natural meal (5–6% ration; Table 1) was more than 4 mmol l^{-1} (Fig. 2), substantially greater than the 1.5–2.5 mmol l^{-1} seen earlier in dogfish fed a 2% ration by stomach tube (Wood et al., 2005). Thus, the original observation of a postprandial alkaline tide was not an artifact of involuntary force-feeding, and these data suggest that the magnitude of the response is proportional to the ration. *Squalus acanthias* has a remarkable capacity to resist alkalosis during experimental HCO_3^- loading, accomplished by excretion of base (HCO_3^- equivalents) at a high rate through the gills (Wood et al., 1995). This appears to occur *via* a mechanism driven by a basolateral V-H^+-ATPase in specialized branchial cells (Tresguerres et al., 2005; Tresguerres et al., 2006). Therefore, the 'true' extent of metabolic base generation during digestion may actually have been much greater than indicated by the observed alkalosis in the systemic bloodstream. Measurements of possible base flux to the water after feeding will be required to address this possibility.

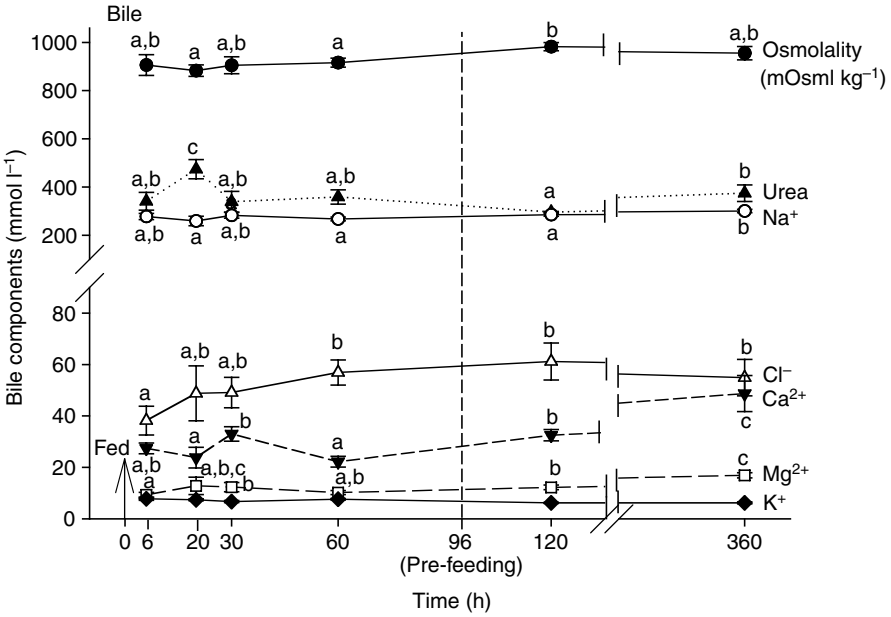

Fig. 8. The influence of feeding on the composition of gall bladder bile in the dogfish shark. Means ± 1 s.e.m. (N=6–8 at each time point). For each parameter, means not sharing the same letter are significantly different (P<0.05). There were no significant differences for K^+.

The cause of the alkaline tide is the secretion of acidic equivalents by the gastric mucosa to digest the food bolus: basolateral HCO_3^- efflux into the extracellular compartment matches the rate of apical H^+ secretion. In higher vertebrates (reviewed by Sachs et al., 1995; Hersey and Sachs, 1995; Niv and Fraser, 2002; Wang et al., 2001), a K^+-stimulated, H^+-translocating ATPase is responsible for the apical H^+ secretion, and a Cl^-/HCO_3^- exchanger for the basolateral HCO_3^- export and Cl^- entry. Cl^- is believed to exit apically *via* a Cl^- channel, such that there is a net secretion of HCl into the stomach. At present, it is uncertain as to whether the mechanism is the same in elasmobranchs where the HCl and pepsinogen secretion functions appear to be combined into a single gastric gland cell type, the oxynticopeptic cell (Hogben, 1967; Rebolledo and Vial, 1979). Certainly, a K^+-stimulated, H^+-ATPase very similar to that of mammals has been localized in gastric gland cells of the elasmobranch *Dasyatis sabina* (Smolka et al., 1994), but HCl secretion occurs with the generation of a negligible transepithelial potential, in contrast to higher vertebrates (Hogben, 1959; Hogben, 1967; Rehm, 1962; Kidder, 1976; Kidder, 1991).

The chyme entering the intestine from stomach-2 during the period from 6 h to 60 h post-feeding had a pH in the range 2.6–3.95, yet pH in the intestinal chyme was precisely regulated at 6.5 (Fig. 3). This indicates a very accurate and effective neutralization mechanism in the intestine. Although the volume of the gall bladder was depressed at 6–30 h, gall bladder bile is unlikely to have played much of a role in neutralization because it contained only very low HCO_3^- concentrations (mean=0.61 mmol l^{-1}). However, in starved *Squalus acanthias*, Boyer et al. reported HCO_3^- concentrations in hepatic bile to be 10-fold higher (5.8 mmol l^{-1}), with a substantial flow rate (74 μl kg^{-1} h^{-1}) (Boyer et al., 1976). Potentially, these values could be even higher in fed animals, so HCO_3^- originating from the bile duct epithelia could play a significant role, as in higher vertebrates.

The other, more important source of HCO_3^- in higher vertebrates is the acinar duct epithelia of the pancreas, and the flow rate and HCO_3^- concentration of pancreatic juice increase after a meal (Guyton and Hall, 2006). The dogfish shark has a large discrete pancreas (Holmgren and Nilsson, 1999), so the role of pancreatic secretion in neutralization could be substantial. However, we are not aware of any reports on this topic in elasmobranchs.

A third possible source is HCO_3^- secretion by the intestinal epithelium itself. In marine teleost fish, this is known to occur at a very high rate in exchange for Cl^- uptake, and to be involved in the precipitation of divalent cations (principally Ca^{2+}) and CO_3^- in the intestine, thereby allowing the absorption of more free water from the imbibed seawater (Wilson et al., 1996; Wilson et al., 2002; Grosell et al., 2005). Recent work has reported very high HCO_3^- concentrations (45–75 mmol l^{-1}) in the intestinal fluid of starved specimens of the bamboo shark,

Chiloscyllium plagiosum, experiencing a hypertonic shock and resulting in substantial drinking (Taylor and Grosell, 2006a; Anderson et al., 2006). These HCO_3^- concentrations were comparable to those in marine teleosts that drink regularly. However, HCO_3^- secretion rates were very low in isolated intestinal sac preparations from these hypertonically exposed elasmobranchs, as well as from control specimens of *Chiloscyllium plagiosum* (Anderson et al., 2006), so the source of that HCO_3^- in the shark is uncertain. In the current study on *Squalus acanthias*, HCO_3^- concentrations in intestinal fluid were much lower (mean=5.1 mmol l^{-1}) than in *Chiloscyllium plagiosum* and did not vary between starved and fed animals. We conclude that the source of the base used for precise pH control of the intestinal chyme is unclear and deserves future investigation.

Iono- and osmoregulatory responses associated with feeding and digestion

Contrary to our initial hypothesis, ingestion of a large meal of teleost tissue (approximately 600 mOsm kg^{-1} below shark plasma values) never resulted in osmotic gradients between the gastrointestinal contents and the body fluids (Fig. 5A). Within 6 h, the gastrointestinal fluids were brought into osmotic equilibrium (Fig. 5A), although large ionic gradients persisted throughout the digestive process (Figs 6, 7). One possible explanation would be a large osmotic water flux across the stomach wall. Based on a 5.5% ration, an 80% water content in the food (Table 3) and the 600 mOsm kg^{-1} osmotic difference, this would necessitate a flux of approximately 17.6 ml of water per kg of dogfish mass across the stomach wall from food to plasma, reducing the water content of the ingested material to approximately 61.5%. However, water content of the intestinal contents stayed more or less unchanged initially and later increased (Table 2), so it is unlikely that this large absorptive flux of water occurred. Another possible explanation for the rapid adjustment of chyme osmolality would be the ingestion of appreciable amounts of seawater with the food. The whole question of drinking in elasmobranchs has been controversial since the original conclusion of Smith that these animals do not need to drink as part of their osmoregulatory strategy (reviewed by Anderson et al., 2006; Smith, 1936). However, earlier we documented drinking at a very low rate (~0.16 ml kg^{-1} h^{-1}) in starved specimens of *Squalus acanthias* (Webb and Wood, 2000), and the composition of the small amount of fluid in stomach-1 in the current study suggests that it was derived from seawater (Table 3). The particularly high, although variable Mg^{2+} concentration in intestinal fluid at 360 h (Fig. 7B) could reflect a greater drinking rate in longer-term starvation – i.e. intestinal fluid absorption with Mg^{2+} exclusion, as discussed subsequently. To our knowledge, drinking has never been measured in elasmobranchs during or soon after feeding. However, the facts that the percentage of water of stomach-1 contents at 6 h after the meal (Table 2) was virtually identical to that of the food (Table 3), and that the Na^+ and, to a lesser extent, Mg^{2+} concentrations in the fluid phase both decreased at this time through 30–60 h (Fig. 6A,C) suggest that drinking remained low following feeding. Osmolality appeared to be sustained by the addition of ions, urea and unmeasured substances to the gastrointestinal fluids. Although some of these unmeasured substances were undoubtedly the organic anions normally present in the prey, additional unmeasured osmolytes may have been created by the subsequent digestion of proteins to polypeptides and amino acids, and of triglycerides to free fatty acids, etc. The degree to which they contributed to chyme osmolality would depend on the balance between the rate at which they were created by digestion *versus* the rate at which they were removed by absorption.

By comparison of concentrations of measured osmolytes, the urea must have originated from the dogfish systemic fluids rather than the food (Fig. 5B); indeed, MacIntosh first documented urea secretion in the stomachs of two ray species (MacIntosh, 1936). However, Na^+ (Fig. 6A) and Cl^- (Fig. 6B) must have originated from the systemic fluids and/or imbibed seawater rather than the food, whereas K^+ (Fig. 6C) and Ca^{2+} (Fig. 7A) clearly came from the food. Mg^{2+} probably originated mainly from seawater (Fig. 7B).

Fig. 9 compares total measured osmolyte concentrations with measured osmolality in the fluid phases of the gastrointestinal tract and the blood plasma. No correction has been made for osmotic activity coefficients. These coefficients for inorganic ions and urea are in the range of 0.90–0.96 in dogfish plasma (Robertson, 1975; Robertson, 1989), but are unknown for inorganic and organic osmolytes in dogfish chyme. It is likely that there is some degree of overestimation in the summated osmolyte concentrations, and therefore underestimation of unmeasured osmolytes (mainly organic osmolytes) in this analysis, but it serves to illustrate important trends.

In starved dogfish, measured osmolytes satisfactorily accounted for total osmolality in all compartments except the intestinal fluid (Fig. 9A). In fed animals (20 h), there were discrepancies of 200–300 mOsm kg^{-1} in all three gastrointestinal compartments (Fig. 9B), probably because of organic osmolytes from the digested food, with perhaps small contributions from bile acids (Boyer et al., 1976) and SO_4^- (Taylor and Grosell, 2006a; Anderson et al., 2006). In the fluids of stomach-1 and stomach-2 in both starved and fed dogfish, the largest measured component was Cl^-, and the other major components were Na^+ and urea. The fact that Cl^- concentration was well-maintained (Fig. 6B) relative to Na^+ (Fig. 6A), urea (Fig. 5B) and Mg^{2+} (Fig. 7B) in the fluids of stomach-1 and stomach-2 after feeding was indicative of HCl secretion by the gastric mucosa.

When chyme moved from stomach-2 into the intestine, Ca^{2+} and Mg^{2+} levels both increased, despite strong concentration gradients favouring their uptake into the plasma after feeding (Fig. 7A,B). This suggests that these potentially toxic divalents were poorly taken up or perhaps even secreted into the lumen in this part of the tract. In quantitative terms, much more important was a two- to threefold increase in its urea concentration, which was particularly marked in the fed

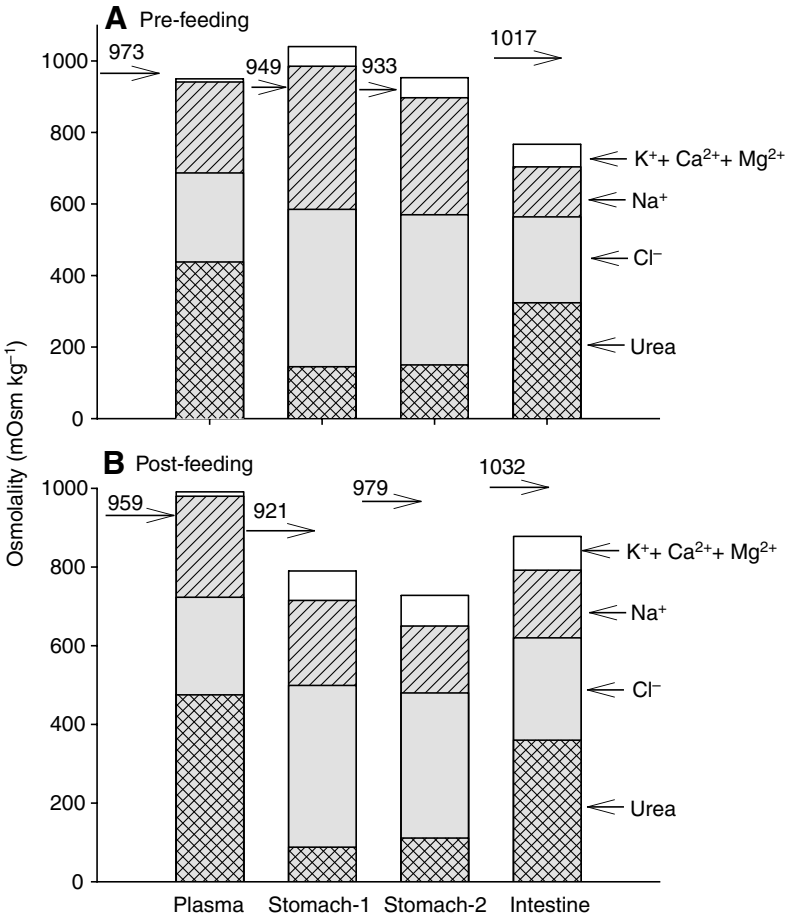

Fig. 9. A comparison of the summation of total measured osmolytes in the blood plasma and the fluid phases of stomach-1 (cardiac stomach), stomach-2 (pyloric stomach) and intestine prior to feeding (A) and at 20 h post-feeding (B). Arrows indicate measured osmolalities. (A) Prior to feeding, note the reasonable agreement between total measured osmolytes and measured osmolality in blood plasma, stomach-1 and stomach-2, but not in intestine. (B) After feeding, note continued agreement in blood plasma, but the discrepancies in all three gastrointestinal fluids. Note also the different contributions of urea *versus* Na^++Cl^- in the various compartments.

animals (Fig. 5B, Fig. 8), together with a corresponding fall in its Cl^- concentration (Fig. 6B, Fig. 8). K^+ also fell (Fig. 6C). Probable explanations are that urea was secreted, whereas Cl^- and K^+ were absorbed in the intestine, all three movements being in accord with concentration gradients from plasma to gastrointestinal fluids (Fig. 5B, Fig. 6B,C). Cl^- may have been absorbed at least in part in effective exchange for the HCO_3^- needed to neutralize the acidic chyme entering from the stomach, as discussed earlier. However, as Na^+ also fell to a lesser degree (Fig. 6A), absorptive cotransport of Na^+ and Cl^- or Na^+, K^+ and 2 Cl^- may also have occurred against the Na^+ concentration gradient from plasma to chyme, thereby promoting water absorption. Kajimura et al. recently documented the presence of a urea-synthetic capacity (full ornithine urea cycle, OUC) in the tissues of the intestine of *Squalus acanthias* and suggested it served for direct N-trapping from the diet (Kajimura et al., 2006). To this explanation, we may now add the possibility that the intestinal OUC also serves as a source of urea secretion for osmolality adjustment in the intestinal chyme. The above movements must be considered speculative in the absence of a non-absorbed marker (e.g. Bucking and Wood, 2006a; Bucking and Wood, 2006b). However, at least in terms of direction, they agree with ion concentration changes from stomach to intestine reported *in vivo* (Taylor and Grosell, 2006a), and with intestinal sac fluxes (except for K^+) measured *in vitro* by Anderson et al. in starved specimens of the bamboo shark *Chiloscyllium plagiosum* (Anderson et al., 2006).

Since the bile duct drains into the duodenum (Holmgren and Nilsson, 1999), the addition of bile could also have influenced the composition of intestinal chyme. The present analyses on gall bladder bile (Fig. 9) were within the ranges reported for most substances by Boyer et al. for starved *Squalus acanthias* (Boyer et al., 1976), although these workers did not record urea concentrations. Biliary urea was surprisingly high in the current study (Fig. 9), although less than plasma values (c.f. Fig. 5B). Boyer et al. noted that free-flowing hepatic bile exhibited rather lower Na^+ and much higher Ca^{2+} and Cl^- concentrations than gall bladder bile (Boyer et al., 1976). However, by comparing biliary concentrations to the present intestinal data (Figs 5-7), urea and Ca^{2+} were the only substances (together with HCO_3^-, as discussed earlier) for which biliary secretion might have exerted an appreciable influence in terms of intestinal chyme composition.

Comparison with teleosts

Although there have been many measurements of gastrointestinal fluid composition in starved marine teleosts (e.g. Shehadeh and Gordon, 1969; Wilson et al., 1996; Wilson et al., 2002; Grosell et al., 2005), the recent investigation of Taylor and Grosell on gulf toadfish (*Opsanus beta*) is the only one to address the consequences of feeding (Taylor and Grosell, 2006b). The picture that emerges from that study suggests profound differences between the teleost and the elasmobranch, some of which reflect their different iono- and osmoregulatory strategies. Principal amongst these are a pronounced osmotic disequilibrium between chyme and plasma in the teleost after the meal, which probably constrains a flux of water into the lumen, in contrast to the rapid equilibration seen in the elasmobranch (Fig. 5A), where urea movements play a large role in this adjustment. Interestingly, the same disequilibrium has been reported in another teleost, the freshwater trout (*Oncorhynchus mykiss*), fed commercial pellets, where seawater drinking is not an exacerbating factor (Bucking and Wood, 2006a; Bucking and

Wood, 2006b). Stomach fluid pH falls after feeding in the toadfish, rather than rising as in the dogfish (Fig. 3), but never reaches the very acidic values seen in the latter. The alkaline tide (Fig. 2) appears to be negligible in the marine teleost. In addition, the toadfish maintains its intestine at a much higher pH (>8.0 *versus* 6.5 in the dogfish, Fig. 3) and exhibits clear evidence of activation of intestinal Cl^-/HCO_3^- exchange. This intestinal HCO_3^- secretion in the teleost serves for postprandial neutralization of the chyme, as well as to facilitate Ca^{2+} precipitation, together with Cl^- and water absorption, and may prevent an alkaline tide in the bloodstream. However, there are some similarities: both the teleost and elasmobranch appear to absorb Na^+, Cl^-, K^+ and water in the intestine, and to exclude the potentially toxic divalents Mg^{2+} and Ca^{2+} (Figs 5-7).

Given the dual function of the fish gastrointestinal tract in feeding and ionoregulation, it is surprising that only the few studies discussed above have addressed how the two processes are integrated. Already, these studies have revealed remarkable differences between fed and starved states, and between teleosts and elasmobranchs. This is a rich area for future experimental investigation.

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