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Physiological adaptations of the gut in the Lake Magadi tilapia, *Alcolapia grahami*, an alkaline- and saline-adapted teleost fish

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Abstract

We describe the gut physiology of the Lake Magadi tilapia (*Alcolapia grahami*), specifically those aspects associated with feeding and drinking while living in water of unusually high carbonate alkalinity (titratable base = 245 mequiv l^{-1}) and pH (9.85). Drinking of this highly alkaline lake water occurs at rates comparable to or higher than those seen in marine teleosts. Eating and drinking take place throughout the day, although drinking predominates during hours of darkness. The intestine directly intersects the esophagus at the anterior end of the stomach forming a 'T', and the pyloric sphincter, which comprises both smooth and striated muscle, is open when the stomach is empty and closed when the stomach is full. This unique configuration (a functional trifurcation) allows imbibed alkaline water to bypass the empty stomach, thereby avoiding a reactive mixing with acidic gastric fluids, and minimizes interference with a full stomach. No titratable base was present in the stomach, where the mean pH was 3.55, but the intestine was progressively more alkaline (foregut 6.96, midgut 7.74, hindgut 8.12, rectum 8.42); base levels in the intestinal fluid were comparable to those in lake water. The gut was highly efficient at absorbing water (76.6%), which accompanied the absorption of Na^+ (78.5%), titratable base (80.8%), and Cl^- (71.8%). The majority of Na^+, base and water absorption occurred in the foregut by an apparent Na^+ plus base co-transport system. Overall, more than 70% of the intestinal flux occurred via Na^+ plus base co-transport, and less than 30% by Na^+ plus Cl^- co-transport, a very different situation from the processes in the intestine of a typical marine teleost.

Keywords: Acid–base regulation; Alkaline environment; Base transport; Digestive function; Drinking; Gut anatomy; Ionoregulation

1. Introduction

Alcolapia grahami, the Lake Magadi tilapia, is faced with a concert of extreme environmental conditions that would prove lethal to other teleosts. The most outstanding of these are a high, stable pH of approximately 10, dissolved oxygen levels fluctuating diurnally from near hypoxia at night to supersaturation at mid-day, a corresponding diel fluctuation in temperature from less than 25 °C at night to near 40 °C mid-day, an osmolality of 525

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mOsmol kg^{-1} (about half-strength seawater), and a total carbon dioxide concentration of approximately 180 mmol l^{-1}, reflecting an extremely high level of HCO_3^- and $CO_3^=$ (Coe, 1966; Narahara et al., 1996). Formerly classified as *Oreochromis alcalicus grahami*, it is the sole fish species living in Lake Magadi in the Rift Valley of Kenya (Coe, 1966; Seegers and Tichy, 1999). This animal has been shown to produce urea as its primary nitrogenous waste, via the ornithine–urea cycle (Randall et al., 1989; Walsh et al., 1993), a rare metabolic activity in teleosts with potential acid–base implications because of the consumption of HCO_3^- in the process of urea production (Wood et al., 1994). Based on what is already known of the physiology of its gills and chemistry of its plasma, *A. grahami* appears to behave physiologically like a fish in seawater. Its general strategy appears to be the maintenance of a hypo-osmotic state (Wright et al., 1990) by drinking the alkaline lake water at a relatively high rate (Wood et al., 2002a) and excreting excess ions at the gill (Eddy et al., 1981; Eddy and Malloiy, 1984; Wright et al., 1990) via a typical marine chloride cell system on its branchial epithelium (Laurent et al., 1995).

In addition to drinking the alkaline lake water for osmoregulation, this fish must also process large quantities of food via the intestine to support exceptionally high metabolic demands (Franklin et al., 1995; Narahara et al., 1996; Wood et al., 2002b) for survival, growth and activity at high temperatures. Coe (1966) found 90% of the stomach contents of *A. grahami* were algal material (the primary species of cyanobacteria was *Arthrospira platensis*) with the remainder being crustacean (Copepods) and dipteran larvae. Very likely the mechanism for cell lysis is via acid digestion in the stomach as in *O. niloticus*, *O. mossambicus*, *Tilapia guineesis*, and *Saratherodon melanotheron* (Fish, 1960; Moriarty, 1973; Payne, 1978; Gargiulo et al., 1997). A paradox then emerges. How is the Magadi tilapia able to process the highly alkaline lake water while maintaining stomach pH sufficiently low for the initial digestion of cell walls? Apart from titrating the necessarily low pH towards a more neutral pH, the mixing of large quantities of highly alkaline lake water with very low pH stomach fluids would evolve CO_2 as a gas. The reaction in the stomach would be:

$$(Na)_2CO_3 + 2HCl \leftrightarrow CO_2 + 2NaCl + H_2O.$$

This would potentially disturb acid–base homeostasis and make it difficult for the fish to maintain neutral buoyancy in the water.

Smith (1982), in speculating about the (much lesser) problem that marine teleosts have in drinking slightly alkaline seawater, insightfully mentioned several possible untested solutions to this paradox. Anatomically, the esophagus and pyloric sphincter could be located very close together so that water ingested by drinking might bypass the stomach. A behavioral adaptation of not drinking during gastric digestion may also occur. Physiologically, additional stomach acid could be produced to offset the neutralizing effect of drinking alkaline water, or food could be digested within a layer of mucus, pepsin, and acid on the surface of the food. Given the potential size of the alkaline load in the Magadi tilapia (lake water is >100 times more alkaline than seawater), we paid particular attention to the first two possibilities, as they might circumvent the problem entirely.

An additional issue is whether a fish that drinks highly alkaline lake water actually secretes base into the intestine. Recent studies of seawater-adapted teleosts (Wilson et al., 1996, 2003b; Wilson, 1999; Grosell et al., 2001) show the gastrointestinal tract to be a major site of base secretion. It remains an open question whether the Magadi tilapia gut plays this role.

Therefore, the goal of this field study was to examine the anatomical and physiological role of the gut in osmoregulatory, ionic and acid–base balance, relative to feeding in Magadi tilapia freshly collected from the wild. Specifically, we wished to test whether the standard saltwater teleost model of drinking and intestinal water absorption applies to this species, and if so, how it might have been modified to cope with the competing demands of digestion and osmoregulation, given the unusual extreme alkalinity of the environmental lake water.

2. Materials and methods

2.1. Experimental animals

Between 12 January 1997 and 18 February 1997, *A. grahami* were captured with a 45-m seine from the Fish Springs Lagoon (FSL) site at Lake Magadi. This site was described in detail by Coe (1966), Narahara et al. (1996), and Wilson et al. (2003a). The largest fish caught, typically 10–20 g, were selected for the gut content and physiolog-

ical measurements of this study, while the smaller ones were used in studies of ionoregulation and urea metabolism reported elsewhere (Lindley et al., 1999; Walsh et al., 2001; Wood et al., 2002a,b; Wilson et al., 2003a). We found that the fish were able to survive capture and transport better when they were seined early in the morning (lower air and water temperatures), and this regime was adhered to whenever possible. However, for the purposes of surveying possible diurnal rhythms in feeding, fish were collected from FSL at all times from 06.00 to 22.00 h. We did not make net hauls in the middle of the night because of safety concerns.

All fish were transported in FSL water to an outdoor lab (set up on a balcony kindly supplied by Magadi Soda PLC) in the nearby town of Magadi. Most fish were killed within a few hours of capture, but fish held for experiments were kept in aerated FSL water in round 20- or 100-l plastic containers where water temperature fluctuated diurnally from approximately 30–36 °C and photoperiod was natural. Water in these holding tanks was changed daily and the fish were fed commercial Tetramin cichlid diet at approximately 3% wet body weight per day.

2.2. Dissection and sampling of the gastrointestinal tract

Prior to all dissections, fish were anaesthetized in metomidate HCl (5 mg l^{-1} in FSL water). Total length and weight of each fish were measured. Upon dissection, sex was determined, and total gut length was measured. The stomach, easily recognizable as a separate pouch, plus four discrete sections of the intestine were readily identified (foregut, midgut, hindgut, rectum). For these studies, foregut was defined as the first section of intestine from the pyloric sphincter until it narrows. It was yellow, with large, longitudinal ridges obvious on the luminal surface. In some dissections it was sampled in two halves: anterior foregut and posterior foregut. The narrow middle section was defined as the midgut, and the larger-diameter section following this to just prior to the anus was defined as hindgut. Rectal tissue refers to the final centimeter of dark-colored intestine immediately before the vent.

The functional relationship between the esophagus, stomach, pyloric sphincter, and foregut was examined by perfusing isotonic saline (200 mM NaCl) between the various compartments using a small catheter (Clay–Adams PE50 tubing). In some cases, tissues were examined under a dissecting scope in the field and then fixed for light microscopy. In particular, we focused on the trifurcated connection between the esophagus, the duodenum, and the stomach pouch in *A. grahami* to compare it to those from related species. The region was gently inflated with 5% formalin solution, severed from the digestive tract, and kept in 70% ethanol for binocular examination and macrophotography. In addition, we determined the distribution of muscle (smooth and striated) within the wall of the different compartments. Fixative solutions for classic histology (Bouin's fluid or 4% buffered glutaraldehyde solutions for semi-thin sections to allow better light microscope definition) were injected in an anterograde fashion via the esophagus or a retrograde fashion via the intestine. Tissue samples were excised from various regions of the trifurcation, embedded in paraffin, serial-sectioned (5 μm) for histology, and sections stained according to the Masson's trichromic procedure (Langeron, 1949) for light microscopy.

For sampling of gut contents, the stomach was ligated with suture at its junction with the esophagus and pyloric sphincter, and additional ligatures were placed around each of the intestinal sections to create sacs. A small incision was made at the posterior end of each sac and the contents, including both solids and fluids, were gently squeezed out with forceps into separate, pre-weighed 0.5-ml micro-centrifuge tubes. These samples were immediately refrigerated for several hours until they could be processed for measurements of pH, titratable base, and Cl^- concentration. Each tube was weighed to determine the mass of the gastrointestinal contents, then received 200 μl of distilled water, was thoroughly mixed by vortexing, allowed to sit for 30 min, and finally centrifuged at $13\,000 \times g$ for 5 min. The supernatant was analyzed for pH and ions as outlined below. Whenever possible, bile samples were also collected from the gall bladder and analyzed directly for pH.

2.3. Gut content chemistry

To determine average gut content chemistry of fish in the wild, fish were dissected soon after being seined from FSL. These measurements were made on the same stock of FSL fish as used by Wood et al. (2002a) for drinking rate determina-

tion. Cl^-, pH, and total base content were measured at the field site using aliquots from the freshly separated supernatants. The remainder of the sample was then frozen, and Na^+, K^+, and Ca^{2+} were assayed after shipping the frozen samples back to McMaster University. Repetition of the Cl^- measurement ensured that there had been no change in the hydration state of the samples. Water from FSL in which the fish were living was analyzed in an identical fashion. Forty fish in total were sampled, but it was not possible to obtain all samples from every fish or to make all measurements on every sample because of gut rupture during sampling and sample volume limitations, respectively. The most difficult measurements, for titratable alkalinity (=titratable base), were made on a complete set of samples for nine fish.

Cl^- was measured on a Radiometer CMT 10 coulometric chloridometer with a silver electrode. The pH of gut supernatant was measured with a Radiometer pH micro-electrode (E5021) on a Radiometer pHM 71 meter. The electrode temperature was maintained at 36 °C with a circulating bath. Analysis of total titratable base content of gut samples was done by the double-titration method as recommended by Hills (1973) and described by Wilson et al. (1996). Radiometer GK24O1C electrodes were used, attached to Radiometer pHM 71 or pHM 82 meter. Specifically, a 50-μl aliquot of supernatant was diluted in 10 ml of 40 mM NaCl, and then the continuously aerated solution was titrated to pH<4.0 with 0.02 mol l^{-1} HCl to remove HCO_3^- and CO_3^- as CO_2 gas, then back-titrated to the initial pH using 0.02 mol l^{-1} NaOH. The difference in the number of moles of acid and base required to return to the initial pH was equal to the number of moles of HCO_3^- equivalents in the original aliquot of supernatant. Na^+, K^+, and Ca^{2+} were measured by a flame attachment on a Varian 1275 AA atomic absorption spectrophotometer. Ion concentrations (Na^+, Ca^{2+}, K^+, Cl^-, and titratable base or acid) are presented both as mequiv kg^{-1} of whole gut content and also as mequiv l^{-1} of gut water content. In all cases, values were corrected for the 200 μl of distilled water added to the samples, and the known volumes of supernatant removed prior to particular analyses. Gut water content was determined in the following series.

2.4. Gut water content

Ten additional fish were dissected soon after being seined from FSL. Solid material plus fluids from each gut section were collected as above into pre-weighed 0.5-ml micro-centrifuge tubes. Tubes were re-weighed to obtain wet mass of contents, after which the contents were dried within the open tubes in the sun (approx. 50 °C) at the field site until stable dry weights were obtained. Percent water was calculated as the difference between wet weight and dry weight. Values for water content are presented as both percent water values and ml H_2O g^{-1} dry mass.

2.5. Drinking vs. feeding, stomach pH, and gut retention time

To determine the internal distribution of water that was imbibed by the fish in the presence of food, seven tilapia (8–12 g) were placed in 4 l of FSL water labeled with 120 μCi [^{3}H]PEG-4000, a drinking marker (Wood et al., 2002a), together with algae-covered rocks to serve as a food source. The fish were allowed to eat and/or drink ad libitum for 20 h, then quickly anaesthetized with metomidate-HCl and rinsed in radioisotope-free FSL water. The gut sections were separately ligated with suture: stomach, anterior foregut, posterior foregut, midgut, and hindgut. Each ligated section was processed for scintillation counting by methods identical to those described by Wood et al. (2002a) for whole-animal drinking rates. Values for the relative distribution of label in each section of the tract were calculated as percentages of the entire ingested [^{3}H]PEG-4000 load.

To determine if a diurnal stomach pH and/or fullness cycle existed in *A. grahami*, we re-analyzed stomach pH and content data collected in the gut content chemistry studies outlined above. The time of sampling over the 24-h day was noted. Stomach fullness was rated qualitatively as empty, some food present, or full of food, and possible relationships with stomach pH were examined.

To determine gut retention time, colored food was fed to previously fasted fish, and the gut contents were examined in individuals sequentially killed over time. In detail, fish (N=7, 2–7 g) were caught at FSL at 18.30 h and held for 17 h without food to allow the gut to clear of any previously ingested material. Commercial Tetramin cichlid pellets were ground with red food dye and

repelleted. At 11.30 h fish were fed approximately 30% body weight (i.e., a great excess) of this colored food. After 5 h, assuming fish had filled their stomachs, the food was removed, and fish were placed into clean FSL water, and then sampled 1 h, 2 h, 3 h, 5 h 30 min, and 24 h later. The presence or absence of any food, its color, and location in the gut were recorded.

2.6. Statistical analyses

Data are generally expressed as means ± 1 S.E.M. (N = number of different fish). Differences in ion concentrations and other parameters among gut sections were compared using a one-way ANOVA followed by an LSD procedure (Ramsey and Schafer, 1997) using Minitab Statistical Software, Version 12.0. Regressions were fitted by the method of least squares and the significance of the r^2 value was assessed. A significance level of $P < 0.05$ was used throughout.

3. Results

3.1. Gut morphology

A. grahami at the Fish Springs Lagoon site have a long gut typical of herbivores, which is some three times the total body length (mean gut length = 233.3 ± 12.8 mm and mean total body length = 80.3 ± 3.3 mm; $N = 68$). The regression equation for gut length (GL, in mm) based on total length (TL, in mm) from FSL fish ($N = 56$) is: GL = −44.1880 + 3.6344TL with an r^2 of 0.68 ($P < 0.001$). The great majority of this length (>80%) is composed of the various sections of the intestine.

The esophagus is short and its mucosa is surrounded by a layer of striated muscle intermingled with smooth muscle fibers. The stomach branches laterally from the base of the esophagus forming the vertical leg of a 'T' intercepting at right angles the straight line formed by the esophagus and the duodenum, the first segment of the gut (Fig. 1a). The stomach contains the expected outer longitudinal and inner transverse layers of smooth muscle, but no skeletal muscle. There is no discrete sphincter between the esophagus and the stomach. However, due to the presence of striated and smooth muscles in the esophageal wall, peristaltic contraction appears to impede any back-flow of chyme from the stomach. The pyloric sphincter is very

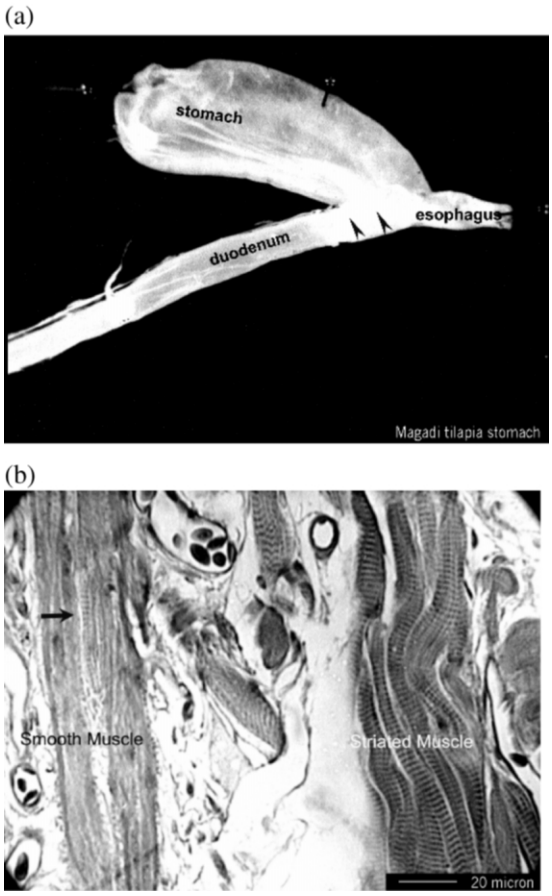

Fig. 1. (a) The trifurcated region (esophagus, stomach, duodenum) of the anterior digestive tract of the stomach of *A. grahami*. Arrowheads: the pyloric sphincter. (b) Histology of the pyloric sphincter. Smooth and striated muscles are closely associated (arrow).

distinct. On fresh tissue it appears as a prominent white circular band located below the stomach opening (Fig. 1a). In addition to the usual smooth muscle, there is the unusual presence of a tunica of striated muscle contributing to the pyloric sphincter, which is clearly visible in histological sections (Fig. 1b). Smooth and skeletal muscles are in close proximity here.

We have experimentally observed the operation of this functional trifurcation in relation to stomach filling. When the stomach is partially or totally full, it is distended, and the pyloric sphincter is shut and can be forced open only by strong retrograde saline injection from the duodenum (Fig. 2a). Anterograde infusion of saline down the esophagus passes partially into the stomach and partially through the sphincter, suggesting that at

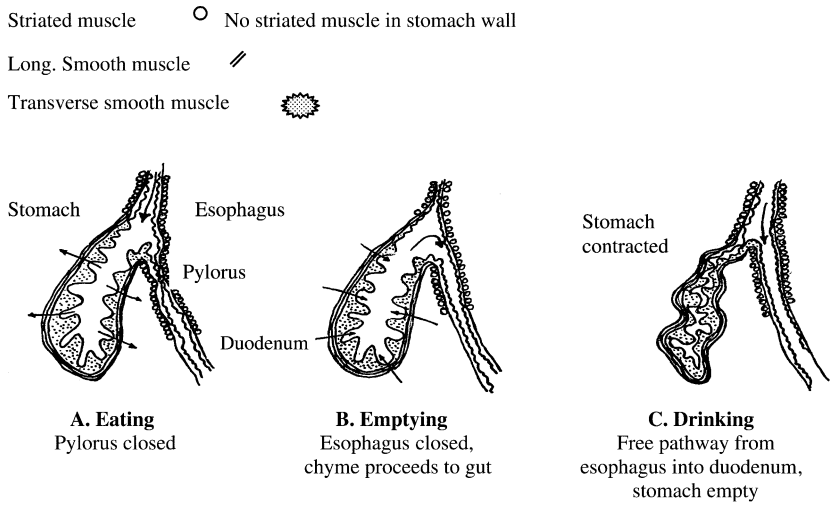

Fig. 2. Diagrammatic representation of the functioning of the trifurcated region (esophagus, stomach, duodenum) of the anterior digestive tract in *A. grahami* with particular reference to the role of the pyloric sphincter. (a) Eating. (b) Emptying of the stomach. (c) Drinking. Conditions (a) and (c) were directly observed; condition (b) is postulated.

least a partial bypass may occur at this time. When the stomach is empty, it is contracted, and the pyloric sphincter is open (Fig. 2c). Saline injection in either direction flows freely through the pyloric sphincter, and not into the contracted stomach. Under such conditions, drinking water could clearly flow directly from the esophagus into the foregut and bypass the stomach. This means that the pyloric sphincter is usually shut when the stomach is filling or full but open when the stomach is empty. The one exception would be when the stomach is emptying; at this time the pyloric sphincter presumably opens, while peristalsis in the esophagus prevents backflow (Fig. 2b). To support these conclusions, water was never found in the stomachs in the absence of food, but clear fluid or fluid chyme was routinely found in the foreguts of such fish.

In general, the contents of the full or partially full stomach appeared drier than those of the foregut. Thereafter, the contents appeared progressively less moist, and by the time the material reached the rectum, it was packaged in dry mucus-coated pellets. These trends in moisture content were later confirmed by direct measurement (Fig. 5c). The gall bladder was engorged with bile in fish lacking food in the stomach and foregut, but only partially full or collapsed in fish that had recently been eating. Mean bile pH was 7.10 ± 0.16 ($N = 17$) with a range of 5.53–8.00. Fourteen of the fish from which samples were obtained had a full gall bladder in correlation with empty, shrunken stomachs.

3.2. Drinking, feeding, stomach pH, and stomach retention time

The distribution of the drinking marker [^{3}H]PEG-4000 in different parts of the gut varied according to whether the fish had been eating. In four fish in which the stomach was full of food, 43% of the label was found in the stomach and lesser amounts were detected in various sections of the intestine (Fig. 3a). This contrasts with only 4% of the label found in the stomachs of three fish where the stomach was empty (Fig. 3b). In the latter, anterior and posterior foregut and midgut contained the greatest amounts of label. These observations indicate that water does enter the stomach when the fish is feeding, but in the absence of feeding imbibed water bypasses the stomach.

In captive fish fed red-dyed food in the laboratory by approximately 6 h from the time when the fish were first given access to it, some food was still present in the stomach, but most of it had moved into the foregut. By 7–8 h, some food had reached the hindgut, and by 10 h 30 min from the start of feeding (i.e., 5 h 30 min after the end of feeding), no food remained at all in the stomach.

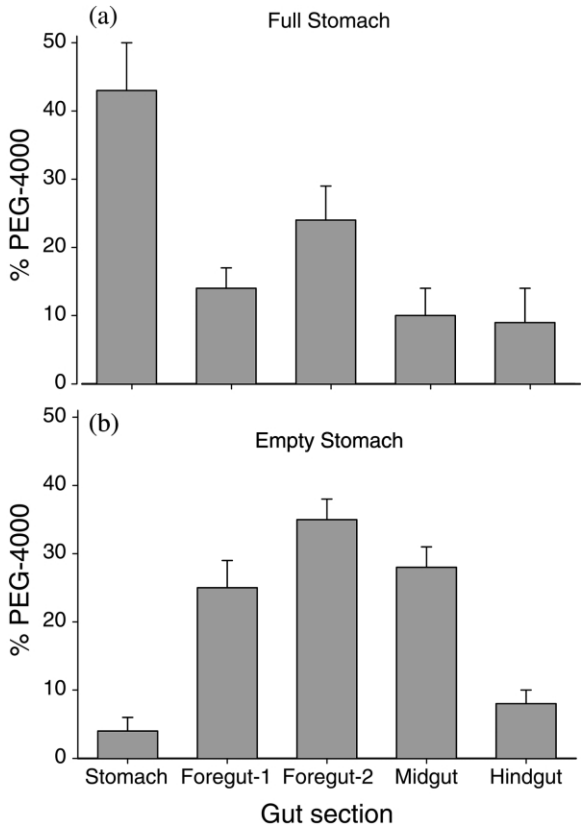

Fig. 3. Relative distribution of [^{3}H]PEG-4000, a drinking marker, in *A. grahami* stomach, anterior foregut (Foregut-1), posterior foregut (Foregut-2), midgut, and hindgut, comparing (a) fish that had been eating, i.e., stomach full of food ($N=4$) to (b) those that had not been eating, i.e., stomach empty ($N=3$). Means ± 1 S.E.M.

Food was present in the hindgut for 29 h (i.e., 24 h after the end of feeding).

In the laboratory, captive fish were observed to feed mainly during the day, and gut dissections found fluid in the intestines both during the day and during the night, suggesting that drinking occurred at all times. At the FSL field site, our visual observations of the fish indicated that most feeding occurred during the day. Observations at night, aided by the brief use of flashlights, indicated that feeding was generally suspended during the hours of darkness, but continued on nights when the moonlight was bright. Fish collected in the early morning tended to have only fluid and little or no food in their intestines, suggesting that drinking predominated over eating during the night. Fish collected throughout daylight hours had both food and fluid in their intestines, suggesting that drinking continued during the day, in concert with feeding. However, overall, there was no clear trend in stomach fullness relative to time of capture in wild fish, probably because feeding had started by the time the first fish were caught (06.00 h; Fig. 4a). There was also no clear diurnal trend in stomach pH (Fig. 4b), which averaged 3.72 ± 0.31 ($N=17$). However, there was a definite trend for stomach pH to be lower in fish with full stomachs and higher in fish with empty stomachs (Fig. 4c). Absolute observed range was 1.90 (very full) to 6.58 (small amount of food present).

3.3. Gut content chemistry

This series was conducted in parallel to the determinations of drinking rate (8.01 ± 1.29 ml

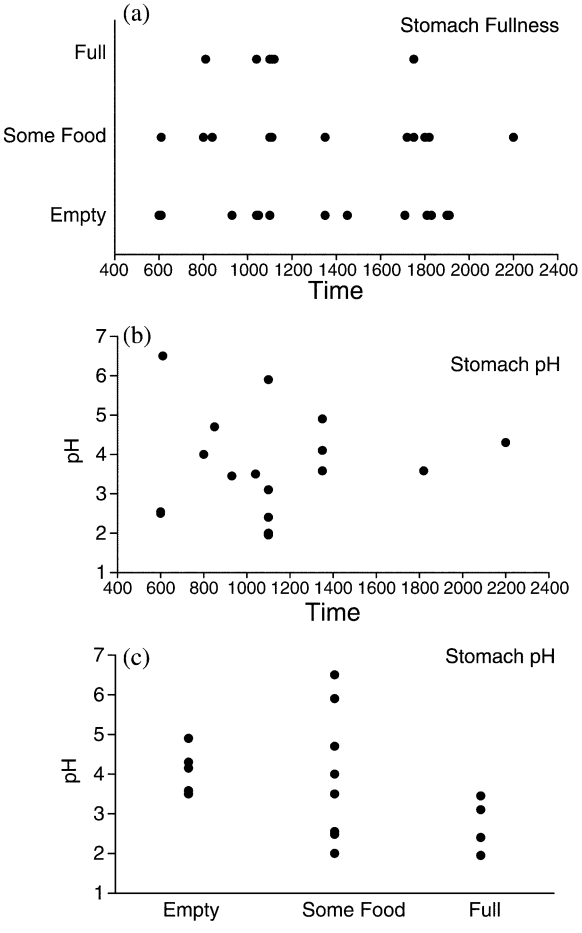

Fig. 4. (a) Relative stomach fullness in relation to time of capture; (b) pH of the stomach contents in relation to time of capture; (c) pH of the stomach contents in relation to stomach fullness in *A. grahami* collected from Fish Springs Lagoon.

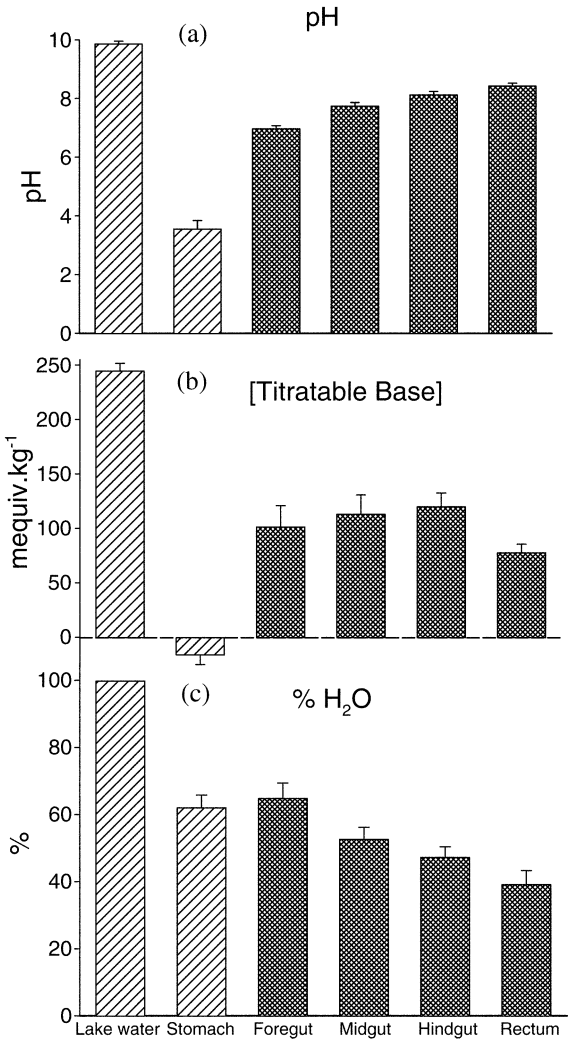

Fig. 5. (a) pH ($N=15$–28); (b) titratable alkalinity ($N=9$); and (c) percentage water content ($N=7$–10) of the gastrointestinal contents in the stomach, foregut, midgut, hindgut, and rectum of *A. grahami* relative to lake water. Means ± 1 S.E.M.

kg^{-1} h^{-1}, $N=16$) reported by Wood et al. (2002a). The pH of the gut contents was very different from that of the environmental water (9.85). In this series, the mean pH of the stomach contents was 3.55 ± 0.29 ($N=15$; Fig. 5a), similar to the value of 3.72 measured in the previous series. The mean pH in the foregut was significantly higher at 6.96 and this rose progressively along the remainder of the intestinal tract to 7.74 in the midgut, 8.12 in the hindgut, and 8.42 in the rectum (Fig. 5a). The corresponding changes in titratable alkalinity are shown in Fig. 5b. The concentration of water in the gut contents was 62.0% in the stomach, 64.8% in the foregut (the average of 68.3% in the first half of the foregut and 61.3% in the second half), and then progressively decreased along the remainder of the tract to 52.6% in the midgut, 47.2% in the hindgut and 39.1% in the rectum (Fig. 5c). For both pH and water concentration (Fig. 5a,c), the changes from foregut to hindgut and rectum were significant, as were the changes from midgut to rectum. For titratable alkalinity, the lower value in the rectum was significantly different from all others, as was the negative value in the stomach (Fig. 5b).

Expressing water concentration on a percentage basis tends to disguise the magnitude of the changes in actual water content that occur along the length of the gut. When these same data were expressed as milliliter of H_2O per gram dry matter of gut contents, water content increased from 1.80 ml/g dry matter in the stomach to 2.73 ml/g dry matter in the anterior half of the foregut, and then progressively decreased along the other sections to only 0.64 ml/g dry matter in the rectum (Fig. 6).

In the lake water in which these experiments were conducted, the major cation was Na^+ (355 mequiv l^{-1}); K^+ (2.2 mequiv l^{-1}) and Ca^{2+} (1.3 mequiv l^{-1}) made very minor contributions. The major anion was titratable base (245 mequiv l^{-1}) with Cl^- (108 mequiv l^{-1}) also making a significant contribution. Considered together, these measured ions yielded good overall charge balance (Figs. 7 and 8).

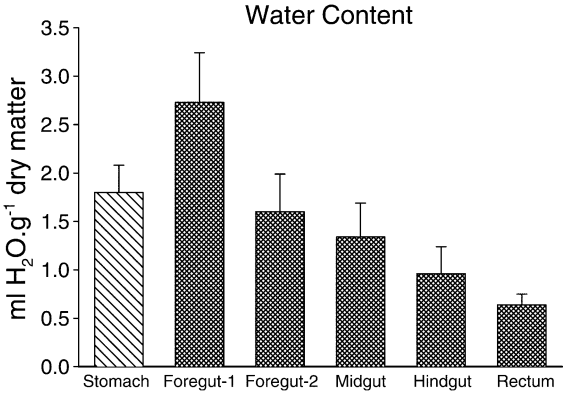

Fig. 6. Absolute water content per gram dry matter in the gastro-intestinal contents in the stomach, anterior foregut (Foregut-1), posterior foregut (Foregut-2), midgut, hindgut, and rectum of *A. grahami*. Means ± 1 S.E.M. ($N=7$–10).

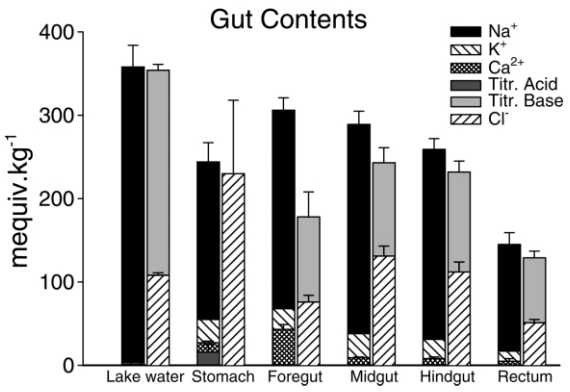

Fig. 7. Concentrations of ions in the whole gastro-intestinal contents (in mequiv kg^{-1}) in the stomach, foregut, midgut, hindgut, and rectum of *A. grahami* relative to Lake Magadi water. Data are presented as paired stacked bars with positive (left) and negative (right) charged ions. Left: Na^+ (black); titratable acid (dark gray); K^+ (back-slashed); and Ca^{2+} (cross-hatched). Right: titratable base (light gray); and Cl^- (forward-slashed). Means $\pm$ 1 S.E.M. (N=9–33).

Fig. 7 displays measured gut ions per kilogram wet weight of whole gut contents. Ionic composition of the stomach contents was fundamentally different from that of environmental water. Na^+ still carried most of the positive charge (173 mequiv kg^{-1}), but contributions from K^+ (28 mequiv kg^{-1}), Ca^{2+} (27 mequiv kg^{-1}) and titratable acid (16 mequiv kg^{-1}) were now important as well. On the anionic side, there was no titratable base, and Cl^- (230 mequiv kg^{-1}) was the only measured anion, again yielding reasonable overall charge balance (Fig. 7).

In contrast to the stomach, samples taken from all sections of the intestine more closely resembled the environmental water (Fig. 7). Na^+ was the major cation, and titratable base was the major anion, maintaining levels equal or greater than those of Cl^- throughout the intestine. Na^+ stayed more or less constant at approximately 240 mequiv kg^{-1} from foregut to hindgut, but fell significantly to 128 mequiv kg^{-1} at the rectum. There was no titratable acid, but K^+ was present at about the same levels as in the stomach (approx. 25 mequiv kg^{-1}) from foregut to hindgut, falling significantly to 12 mequiv kg^{-1} at the rectum. Ca^{2+} levels in the foregut (43 mequiv kg^{-1}) were even higher than in the stomach, but then fell significantly to approximately 8 mequiv kg^{-1} in midgut and hindgut and 5 mequiv kg^{-1} at the rectum. Titratable base was more or less constant at approximately 110 mequiv kg^{-1} from foregut to hindgut, and then fell significantly to 78 mequiv kg^{-1} at the rectum. In contrast, Cl^- was initially low in the foregut (76 mequiv kg^{-1}), rose significantly to levels comparable to those of titratable base in midgut and hindgut, then fell significantly to 51 mequiv kg^{-1} at the rectum.

Note that there was a significant charge imbalance (128 mequiv kg^{-1} excess of positive charge) only in the foregut, and that this diminished to non-significant levels in the other sections of the intestine (Fig. 7). Likely this was explained by unmeasured negative charge on organic molecules (amino acids, polypeptides, fatty acids) liberated by digestion. These would be titrated by H^+ (and thereby masked) at the low pH in the stomach, but would be revealed at the high pH in the foregut. Their removal by absorption in this and subsequent regions would explain the disappearance of this ‘anion deficit’.

In Fig. 8, these same ion data have been reported per liter of water in the whole gut contents. Expressing the data in this manner reveals that Na^+ concentration (279 mequiv l^{-1}) in stomach water was lower than in environmental water (355 mequiv l^{-1}), and that any titratable base had been completely replaced with Cl^- (370 mequiv l^{-1}), which was 3.4-fold higher than in the environmental water (108 mequiv l^{-1}). However, in the water throughout the intestine, Na^+ levels were generally

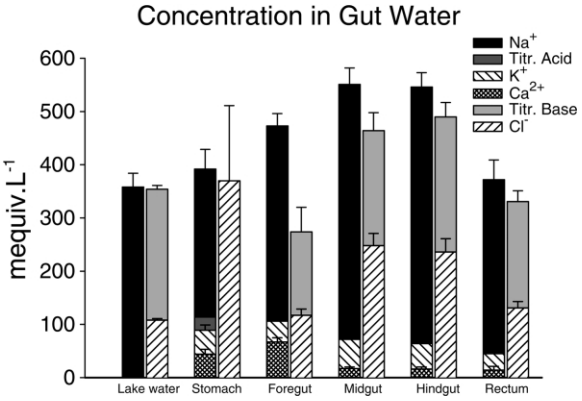

Fig. 8. Concentrations of ions in the water phase of the gastro-intestinal contents (in mequiv l^{-1}) in the stomach, foregut, midgut, hindgut, and rectum of *A. grahami* relative to Lake Magadi water. Data are presented as paired stacked bars with positive (left) and negative (right) charged ions. Left: Na^+ (black); titratable acid (dark gray); K^+ (back-slashed); and Ca^{2+} (cross-hatched). Right: titratable base (light gray); and Cl^- (forward-slashed). Means $\pm$ 1 S.E.M. (N=9–33).

similar to or higher than those in the ingested water. Na^+ increased from environmental concentrations in the foregut (367 mequiv l^{-1}) to higher levels in midgut and hindgut (approx. 480 mequiv l^{-1}), and then decreased back to environmental levels in the water in the rectum (341 mequiv l^{-1}). Titratable base concentrations in the water of the foregut (157 mequiv l^{-1}) were approximately 65% of environmental levels (245 mequiv l^{-1}), and then increased to approximate environmental levels along the remainder of the tract, with a slight fall at the rectum (208 mequiv l^{-1}). Cl^-, however, started at levels (117 mequiv l^{-1}) comparable to those in the imbibed water in the foregut, and then more than doubled along the tract, reaching 236 mequiv l^{-1} in the hindgut, before falling to 136 mequiv l^{-1} in the water of the rectum. Ca^{2+} fell from relatively high levels in the water of the stomach (44 mequiv l^{-1}) and foregut (66 mequiv l^{-1}) to much lower concentrations (14–16 mequiv l^{-1}) in the other sections of the intestine. In contrast, K^+, which was comparably high in the water of the stomach (45 mequiv l^{-1}) and the foregut (39 mequiv l^{-1}), remained high (33–55 mequiv l^{-1}) throughout all the other sections.

4. Discussion

4.1. Gut structure, stomach pH, and periods of feeding and drinking

These studies confirm that the gut structure and physiology in *A. grahami* are adaptive for life in highly alkaline water and differ from those of most other freshwater and marine teleosts. The unusual configuration in which the esophagus, stomach, and duodenum form a 'T' (Fig. 1a) is consistent with the requirements of this fish for drinking alkaline water and its pattern of eating and drinking (Fig. 2). Ingesting very alkaline water into an empty, highly acidic stomach would needlessly inflate the stomach and upset neutral buoyancy with the production of excess CO_2, in addition to wasting energy used to secrete stomach H^+. The [^{3}H]PEG-4000 experiment demonstrated clearly that when the fish is not feeding, water can almost completely bypass the stomach and enter directly into the intestine (Fig. 3). When the fish is feeding, simultaneous intake of water with the food into the stomach may be unavoidable; nevertheless, at least some of the imbibed water can be shunted past the stomach directly into the intestine so that the stomach pH can remain low during digestion. Indeed, the full stomach is more acidic than the empty stomach (Fig. 4c). Likely, titration of some of the secreted stomach acid by the carbonate and bicarbonate in lake water is unavoidable, resulting in the production of some CO_2. Interestingly, in *A. grahami*, the Bohr effect on the blood dissociation curve is absent (Narahara et al., 1996), presumably to protect the O_2-carrying ability of the hemoglobin. The lack of Bohr shift in the blood may be one way *A. grahami* is able to cope with blood pH changes induced by the excess CO_2.

Typically, in freshwater teleosts, the esophagus enters the anterior part of the stomach, with the intestine attached at the posterior end of the stomach separated by the pyloric sphincter (see Bertin, 1958). In *O. niloticus*, which inhabits freshwater lakes and is thought to be distantly related to *A. grahami* (Seegers et al., 1999), and in several other tilapines the esophagus enters the stomach close to, but noticeably separate from, the anterior intestine, forming a 'Y' configuration (Moriarty, 1973). This is thought to allow certain foods requiring greater mechanical and acid digestion to be held for longer periods in the blind terminal region of the stomach (Caceci et al., 1997) and/or to allow indigestible material to quickly bypass the stomach for regurgitation or evacuation (Morrison and Wright, 1999). The 'T' configuration of stomach, esophagus and foregut, which provides a functional trifurcation in *A. grahami*, is unique to our knowledge, but likely derived from the 'Y' modifications seen in other tilapines, and is consistent with the requirement for drinking of alkaline lake water. The modification is, therefore, in accord with the first hypothesis of Smith (1982); (see Introduction) that a 'bypass' system is operative. Indeed, the presence of striated muscle in the pyloric sphincter (Fig. 1b) suggests an element of voluntary control (Fig. 2).

Digestion in tilapines is considered unique because of the very low stomach pH values during the gastric phase of digestion—frequently as low as pH 1.0–1.25 (Moriarty, 1973; Bowen, 1976, 1982; Payne, 1978). Moriarty (1973) found that algae were lysed most efficiently when the stomach pH was less than 2.0 in *O. niloticus*. In marine fish, typical gastric pH is 2.2–4.3 (Horn, 1998). The mean pH for stomach contents in *A. grahami* (3.55 and 3.72 in the two series) was higher than those reported for other tilapines but comparable

Table 1
Intestinal processing, and net absorption rates of Na^+, Cl^-, titratable base ($HCO_3^-/CO_3^=$) and water per milliliter of Lake Magadi water ingested by *A. grahami* at a drinking rate of 8.01 ml kg^{-1} h^{-1}

	Ingested (μmol/ml)	Absorbed (μmol/ml)	Efficiency (%)	Net absorption rate (μmol kg^{-1} h^{-1})
Na^+	355	279	78.5	2231
Cl^-	108	77	71.7	621
Base	245	198	80.8	1586
H_2O	1.00 (ml)	0.766 (ml)	76.6	6.14 (ml kg^{-1} h^{-1})

to those in marine teleosts despite the high alkalinity of Lake Magadi water, more than 100-fold greater than the alkalinity of seawater.

Our observations on field-collected fish, in combination with our laboratory measurements of gut retention time, suggest that feeding is reduced or stopped during the hours of darkness, although drinking continues. The water at Fish Spring Lagoons is extremely hypoxic at night due to cyanobacterial respiration, and the temperature falls from typical daytime levels of 36–40 °C to as low as 23–25 °C (Narahara et al., 1996). Because of the very high Q_{10} (approx. 6.0 in this range) in *A. grahami*, metabolic rate falls during night-time to a small fraction of the daytime values (Narahara et al., 1996). It is, therefore, logical that *A. grahami* directs most of its energy into drinking, assimilation, and absorption in the intestine during the night, and refrains from new feeding until oxygen and temperature levels rise with sunlight in the morning (Narahara et al., 1996).

4.2. Gastro-intestinal processing of ions and water

Drinking rates of 8.01 ± 1.29 ml kg^{-1} h^{-1} were reported by Wood et al. (2002a) for this same batch of *A. grahami* in the same water quality, which has an osmolality equivalent to that of approximately 50% seawater. These rates were considerably higher than the mean drinking rates for most euryhaline teleosts in 40–50% seawater, and indeed were close to the upper end of the range (2–10 ml kg^{-1} h^{-1}) for teleosts in 100% seawater (Maetz, 1974; Perrott et al., 1992; Evans, 1993). The morphological observations and ionic composition measurements made along the gastro-intestinal tract in this study indicate that this imbibed water bypasses the stomach much of the time, and thereby passes directly into the foregut. Based on the fact that the composition of the gut contents decreases from 2.73 ± 0.51 to 0.64 ± 0.09 ml water per gram dry matter from foregut to rectum (Fig. 6), we can conclude that at least 76.6% of this imbibed water, or 6.14 ml kg^{-1} h^{-1} is absorbed. Indeed, this must be a conservative estimate for three reasons. Firstly, the calculation employs the dry matter of the intestinal contents as a marker, and this in itself likely decreases as the chyme moves along the tract because nutrients are absorbed. Secondly, the calculation does not account for any water absorbed in the esophagus prior to reaching the foregut. Thirdly, the calculation assumes that the availability of imbibed water for absorption is the same as that of the water inside algal cells, whereas if anything, the former is likely greater.

In the intestine of typical seawater fish, water absorption is dependent on ion transport, particularly Na^+ and Cl^- (Evans, 1993; Loretz, 1995). The present measurements allow an analysis of ion and water transport in the intestine of the Magadi tilapia. Using our conservative estimate of 76.6% absorption of imbibed water, the net absorption of various ions (Table 1) can be estimated from the measurements of the ionic composition of the water in the rectal contents and the environmental water (Fig. 8). These calculations demonstrate that the net absorption of Na^+ (with 78.5% efficiency) is accompanied by the absorption of both titratable base and Cl^- in approximate proportion to their concentrations in the environmental water, but that the net absorption of base is slightly more efficient (80.8%) than the net absorption of Cl^- (71.8%). The calculated average composition of the 'absorbed fluid' is, therefore, 364 mequiv l^{-1} Na^+, 101 mequiv l^{-1} Cl^-, and 259 mequiv l^{-1} titratable base, which represents a solution with approximate charge balance and an osmotic pressure likely close to that of the environmental water. Thus, in contrast to typical marine teleosts, where water absorption across the intestinal tract is driven by the absorption of Na^+ and Cl^- in

approximately equal amounts due to the presence of a co-transport mechanism (Evans, 1993; Loretz, 1995), more than 70% of the intestinal flux in the Magadi tilapia appears to represent some sort of Na^+ plus base co-transport system, and less than 30% can be attributed to Na^+ plus Cl^- co-transport.

There has been one previous study of intestinal ion and water processing in the Magadi tilapia (Skadhauge et al., 1980), but that study did not measure the principal anion (titratable base) and was compromised by poor health of the fish and the use of electron probe microanalysis for ions, which produced exceptionally high plasma Cl^- levels that could not be confirmed in later studies (Eddy and Malloiy, 1984; Wright et al., 1990; Wood et al., 2002a,b). An accompanying error in gut Cl^- analysis is probably the reason why reported Cl^- levels in all parts of the intestine were two- to three-fold higher than Cl^- levels measured in our study. Nevertheless, if we disregard the anion data, the cation data are in accord with the present study, indicating a much lower Na^+ level in the stomach than in the environmental water, and $>80\%$ absorption of both Na^+ and water during their passage through the tract.

The calculated net intestinal absorption rates of these ions in this study are also instructive. The estimated net Na^+ absorption rate of approximately 2200 μequiv kg^{-1} h^{-1} (Table 1) through the digestive tract nicely matches the difference between radiotracer measurements of unidirectional influx (2100 μequiv kg^{-1} h^{-1}; Wright et al., 1990), and efflux rates (4000 μequiv kg^{-1} h^{-1}; Eddy et al., 1981; Eddy and Malloiy, 1984) of Na^+ through the gills of *A. grahami* in similar water quality. Thus, Na^+ absorbed at the intestine appears to be mainly excreted at the gills, as in typical marine teleosts (Maetz, 1974; Evans, 1993). For Cl^-, the net intestinal absorption rate of approximately 600 μequiv kg^{-1} h^{-1} (Table 1) may be compared with a unidirectional branchial efflux rate of approximately 1650 μequiv kg^{-1} h^{-1} (Eddy et al., 1981); unidirectional Cl^- influx rates have apparently not been measured in the Magadi tilapia, but presumably are approximately 1000 μequiv kg^{-1} h^{-1}. The net uptake of basic equivalents through the intestine (approx. 1600 μequiv kg^{-1} h^{-1}; Table 1) represents a fundamental difference from marine teleosts where a large net secretion of basic equivalents into the tract has been recently documented and is thought to help water absorption by precipitating divalent cations out of solution as carbonate salts (Wilson et al., 1996, 2003b; Wilson, 1999; Grosell et al., 2001).

In the Magadi tilapia, the *absorption* rather than the secretion of base directly promotes water uptake by osmotic attraction. Given the low availability of Cl^- and the virtual absence of Ca^{2+} (and Mg^{2+}; Wood et al., 1989) in the environmental water, this intestinal uptake of base is probably both unavoidable and adaptive for osmoregulatory homeostasis. Nevertheless, it will likely pose a substantial challenge to the acid–base regulatory system of the fish. Some authors (e.g., Meijer et al., 1990; Atkinson, 1992) have suggested that such uptake of environmental HCO_3^- may simply provide a substrate for metabolic urea production (thereby 'eliminating' the base load). However, experimental tests of this hypothesis on *A. grahami* have proven negative (Wood et al., 1994). At present, there is no direct information on how the gills handle the base load, though Laurent et al. (1995) have proposed a scheme whereby base is co-transported outwards with Na^+ by the branchial chloride cells. Essentially, this model envisages that rather than Cl^- entering the cell with Na^+ on the basolateral Na^+, K^+, $2Cl^-$ co-transporter and leaving through an apical channel, it is base (HCO_3^- or $CO_3^=$ or OH^-) which fulfills this role in substitution for Cl^-, in analogy with the Na^+ plus Cl^- co-transport mechanism of typical marine teleosts (Evans, 1993).

Our data also allow calculation of the rates of absorption of water and specific ions in various portions of the gastrointestinal tract (Fig. 9). The results of these calculations should be interpreted with caution for several reasons. Again, they employ the dry matter of the intestinal contents in various sections as a marker. Since the calculations are based on the net removal of the substance in question in each preceding section of the tract, the absorption calculated for the foregut (which is based on midgut composition measurements) also includes any contribution by the esophagus. Furthermore, the analysis does not explicitly consider the role of the stomach in absorption. However, the stomach is likely a site of net secretion, where the production of HCl renders Cl^- as essentially the only inorganic anion present in the gastric contents (Figs. 7 and 8).

This analysis suggests that almost 70% of the net water absorption occurs in the foregut (and esophagus), whereas only approximately 15%

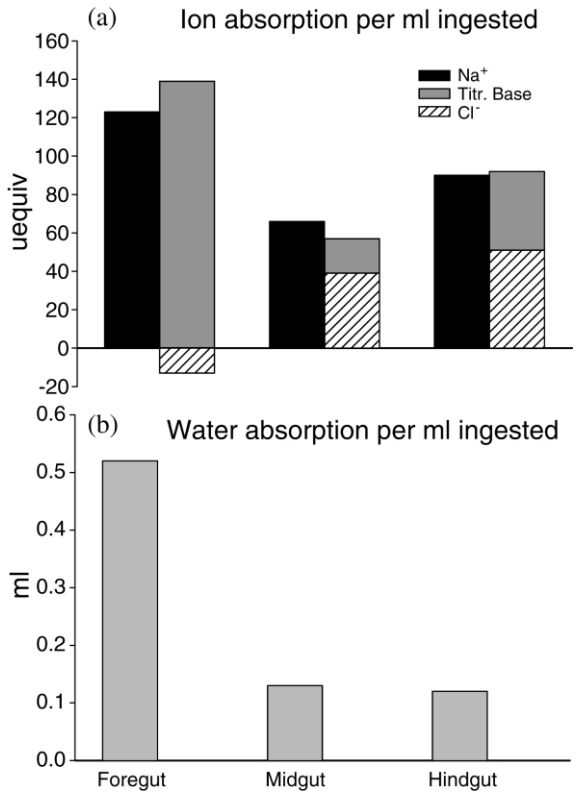

Fig. 9. (a) Calculated ion absorption (in μequiv.) per milliliter of Lake Magadi water ingested for Na^+ (black), titratable base as HCO_3^-/CO_3^{2-} (gray), and Cl^- (slashed). (b) Calculated water absorption (in milliliter) per milliliter of Lake Magadi water ingested in the foregut, midgut and hindgut of *A. grahami*.

occurs in each of the midgut and hindgut. For Na^+, approximately 45% of the absorption occurs in the foregut, with 23% in midgut and 32% in hindgut. Interestingly, Na^+ absorption appears to be completely coupled to base absorption in the foregut (indeed, there is a small net secretion of Cl^- here), but in the other two sections, Na^+ absorption is matched by approximately one-third base and two-thirds Cl^- absorption. Thus, the sites of base absorption and Cl^- absorption appear to be spatially separated to some extent. Ca^{2+} or K^+ are not listed in Table 1 or Fig. 9 because there is no net absorption of these electrolytes from imbibed water. Slightly more Ca^{2+} and K^+ are excreted in the rectal water than actually enter in drinking. The high concentrations of both these ions in the water of the stomach and foregut (Fig. 8), therefore, probably originate from ingested algae, though it is also possible that they are secreted by the esophagus or stomach. Regardless, application of a similar approach suggests that Ca^{2+} is absorbed at an overall rate of approximately 400 μequiv kg^{-1} h^{-1} in the remainder of the tract, and that approximately 90% of this occurs in the foregut, with only 5% in each of the midgut and hindgut. For K^+, the comparable rate is approximately 200 μequiv kg^{-1} h^{-1}, distributed more evenly over the three sections, with 16% in the foregut, 45% in the midgut, and 39% in the hindgut. Future studies may profitably employ in vitro pharmacological and radioisotopic examination of the various portions of the tract (e.g., Loretz, 1995; Grosell et al., 2001) to evaluate all of these tentative conclusions.

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