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The response of gastric pH and motility to fasting and feeding in free swimming blacktip reef sharks, *Carcharhinus melanopterus*

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Abstract

In many fish and reptiles, gastric digestion is responsible for the complete breakdown of prey items into semi-liquid chyme. The responses of the stomach to feeding and to periods of fasting are, however, unknown for many lower vertebrates. We inserted data loggers into the stomachs of free-swimming captive adult blacktip reef sharks (*Carcharhinus melanopterus*) to quantify gastric pH, motility and temperature during fasting and following ingestion of food. Gastric acid secretion was continuous, even during long periods of fasting, with a mean pH of 1.66±0.40 (±1 SD) when the stomach was empty. Stomach contractions were greater following meals of mackerel than for those of squid. Gastric motility following feeding on mackerel, was positively influenced by ambient temperature, and followed a quadratic relationship with meal size, with maximum motility occurring after meals of 0.8–1.0% body weight. Diel changes in gastric motility were apparent, and were most likely caused by diel changes in ambient temperature. Gastric digestion in blacktip reef sharks is affected by both biotic and abiotic variables. We hypothesize that behavioral strategies adopted by sharks in the field may be an attempt to optimize digestion by selecting for appropriate environmental conditions.

Keywords: Data-loggers; Foraging ecology; Gastric digestion; Motility; Optimal foraging; pH; Sharks

1. Introduction

Gastric digestion in carnivorous vertebrates is responsible for the breakdown of ingested prey items into semi-liquid chyme. The role of the stomach is particularly important in lower vertebrates such as fish and reptiles, many of which ingest their prey whole with little mastication (e.g., Secor, 2003; Motta, 2004). Two components to gastric digestion occur: chemical digestion accomplished by the secretion of concentrated hydrochloric acid (HCl) and digestive enzymes, and mechanical digestion accomplished by muscular contractions of the stomach wall (Mayer, 1994; Holmgren and Holmberg, 2005).

Elasmobranch fishes are one of the earliest groups of carnivorous vertebrates to have evolved a functional stomach and (based on the identification of H^+–K^+ ATPase in acid secreting cells) probably one of the first to have evolved an acid secreting stomach (Smolka et al., 1994). In addition, the morphology of the stomach permits only the passage of semi-liquid chyme into the intestine, yet many species of elasmobranch ingest their prey whole, highlighting the importance of the stomach

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to food breakdown (Andrews and Young, 1993; Motta, 2004).

Elasmobranchs are capable of secreting highly acidic gastric fluids (down to pH 0.4, Papastamatiou and Lowe, 2004, 2005). Distention of the stomach wall as food enters is the initial stimulus for increased acid secretion (Smit, 1967), followed by the action of secretagogues such as gastrin and histamine, although the interactions between hormones and acid secretion are not well known (Hogben, 1967; Vigna, 1983). There are inter-specific differences among elasmobranchs in the response of gastric acid secretion to fasting, with some species continuously secreting acid while others periodically cease secretions during fasting (Barrington, 1942; Papastamatiou and Lowe, 2004, 2005). Secreted HCl aids in the physico-chemical breakdown of the hard parts of prey and contributes to enzymatic digestion by converting the inactive zymogen pepsinogen into the proteolytic enzyme pepsin (Guerard and Le Gal, 1987; Holmgren and Nilsson, 1999). Some elasmobranchs are also capable of secreting chitinase enzymes, which also have optimal function at low pH, and breakdown chitin-containing exoskeletons (Fange et al., 1979).

To date, gastric motility has only been measured in euthanized or anaesthetized elasmobranchs, although results suggest that gastric motility is under the control of both nervous and hormonal mechanisms (Andrews and Young, 1993; Holmgren and Nilsson, 1999; Buddington and Krogdahl, 2004). A variety of neurotransmitters have been identified in elasmobranch gut neurons (Nilsson and Holmgren, 1988) and it appears that there is both nervous inhibition and excitation of the stomach muscles (Campbell, 1975; Andrews and Young, 1993). Elasmobranchs are known to have relatively slow gastric evacuation rates (Wetherbee et al., 1990), and electrical stimulation of the splanchnic nerve in lesser spotted dogfish, *Scyliorhinus canicula*, induced gastric contractions but peristalsis did not move the stomach contents into the small intestine (Andrews and Young, 1993). Presently, it remains unclear whether gastric motility in elasmobranchs only functions to mix food items and to pass chyme out of the stomach, or if motility is also involved in mechanical trituration. Gastric evacuation rates (and presumably motility) in elasmobranchs are influenced by a variety of factors including: meal size, surface area of ingested prey, prey lipid composition, the presence of skeletal or chitin containing hard-parts, and feeding periodicity (Wetherbee et al., 1990; Schurdak and Gruber, 1989). In summary, it is probable that species specific differences in stomach motility and patterns of acid secretion are shaped by species specific diet and feeding strategies.

Presently, very little is known of the response of gastric acid secretion and motility following feeding and during fasting in free-swimming elasmobranchs. Obtaining such data under semi-natural conditions is important as it enables the physiological response of the stomach to be put into an ecological context, and subsequently applied to the study of the feeding strategy and optimal foraging behavior of the animal in the wild. Our goals were to quantify changes in gastric pH, motility and temperature in a captive free-swimming elasmobranch, the blacktip reef shark (*Carcharhinus melanopterus*), using autonomous data-loggers under semi-natural conditions. The blacktip reef shark was chosen as a model species because it is an abundant predator on coral reefs in tropical and semitropical regions of the Pacific and Indian Oceans (Compagno et al., 2005), is large enough to retain gastric data-loggers, and feeds and behaves normally while in captivity. Our specific objectives were to: (1) determine the post-prandial changes in gastric pH and motility in free-swimming captive blacktip reef sharks; (2) quantify the influence of meal size, meal type, and temperature on gastric motility; (3) determine the response of pH and motility during periods of fasting; and (4) determine if there were any diel changes in the profiles of gastric pH and motility. Because many species of shark are considered nocturnal foragers (Wetherbee et al., 1990), we hypothesize that diel differences in gastric digestion will occur.

2. Methods

2.1. Study animals

Tests were conducted with five captive adult blacktip reef sharks (*Carcharhinus melanopterus*, Quoy and Gaimard, 1824), total length 145.6±6.8 cm (mean± 1 SD) and mass 21.8±3.1 kg (Table 1). All sharks were maintained at the Hawaii Institute of Marine Biology in a sectioned off lagoon (120×20 m) consisting of coral rubble, coral, and sand, with a maximum depth of 3 m. The lagoon is tidally flushed and contains a fish and

Table 1
Summary information for adult blacktip reef sharks (*Carcharhinus melanopterus*) used in experiments with pH and motility data-loggers

Shark #	TL (cm)	Mass (kg)	Sex	Min pH	Max pH	Mean pH
1	139	19	F	1.2	3.6	1.7
2	144	21	F	0.8	3.4	2.0
3	140	19	M	0.4	5.3	2.0
4	150	24	F	1.2	4.0	1.9
5	155	26	F	–	–	–

invertebrate community typical of Kaneohe bay, Oahu, Hawaii. Prior to testing, sharks were fed to satiation two to three times a week with mackerel (*Scomber* spp.). Animals used in experiments were moved into a smaller rectangular section (approximately 10×20 m), with similar habitat characteristics as the rest of the lagoon. No more than two sharks were maintained in the testing area at any one time. Sharks were acclimated to the test area until they resumed feeding, after which they were fasted for a week before the experiments began. Sharks were fitted with one of two types of data-logger measuring either stomach pH or gastric motility.

2.2. pH data-loggers

To measure gastric pH and temperature in free-swimming blacktip reef sharks, we used autonomous pH/temperature data-loggers (earth & Ocean Technologies, Kiel Germany). The data-loggers are cylindrical (11×2 cm), weigh approximately 80 g in air and consist of a pH micro-glass electrode, a reference electrode with a free-diffusion liquid junction and a 12-bit data-logger encased in a titanium shell (Peters, 1997a,b). The reference electrodes are designed to compensate for any pressure changes associated with diving (Peters, 1997b). A sensor on the data-logger also measured temperature (resolution of 0.1 °C). Before deployment, the pH data-loggers were programmed to record pH and temperature every 30 s and were calibrated in NBS standard pH buffers (1.68, 4.01, 6.86, and 10.01).

To deploy the pH data-loggers, we netted a shark and inverted it in a stretcher to induce tonic immobility (see Papastamatiou and Lowe, 2005). Additional anesthesia was induced by inserting a 2 cm diameter siphon into the mouth and applying a solution of MS 222 (0.15 g l^{-1}) to the gills. It took between 5 and 10 min before the shark was anaesthetized to a level of immobility, after which we inserted a lubricated 3 cm diameter PVC pipe through the mouth into the stomach. The pH data-logger was dropped down the pipe with the pH sensor pointing towards the caudal fin (i.e. at the base of the cardiac portion of the stomach), followed by pieces of bait fish to prevent premature regurgitation of the data-logger. We then removed the pipe and measured and sexed the shark before reviving it by manually 'swimming' the animal through the lagoon water. Each shark revived within approximately 10–15 min, after which it was observed for an additional 15 min to ensure normal swimming behavior. We determined shark mass using the length-weight regression $\text{Weight} = 1.004 * 10^{-6}(\text{Total length})^{3.39}$ (Stevens, 1984). During the period that the pH data-logger was retained in the stomach, we fed each shark meals of mackerel (*Scomber* spp.) at a variety of ration sizes. Two sharks were also fed meals of reef fish (various *Acanthurus* and *Chaetodon* species), and one shark was also fasted for 12 days. The data logger was deployed in each shark only once.

The pH data-loggers can record accurate pH data for up to 16 days, depending on electrolyte outflow rate (see Peters, 1997b). If the shark had not regurgitated the data-logger within 16 days, then we restrained the shark as described above and used a magnetic device to remove it from the stomach. After retrieval, the data-loggers were re-calibrated with the same NBS standard pH buffers used prior to deployment. The data were then downloaded and analyzed using pHG 2.0 software (Jensen Software Systems), which interpolates and corrects pH data for any drift of the electrode and also for changes in stomach temperature (Peters, 1997a). Error analysis of pH electrode performance was determined using the pH drift model described by Peters (1997a).

We determined titration time for each meal that each shark consumed, with titration time defined as the time taken for pH to return to 2.0 (baseline) (Gardner et al., 2002). To determine the time of onset of a response, we first established a baseline by analyzing gastric acidity in the two hours prior to feeding. This period was divided into 10 min blocks and onset of a response (P_1) was defined as the first of two consecutive 10 min intervals where pH was <2.0 for only 5% of the time. We analyzed the 24 h period following feeding in the same way and defined the end of the response (P_2) as the first of two consecutive 10 min intervals where pH was >2.0 for less than 10% of the time. Titration time was calculated as $P_2 - P_1$. We used linear regression analysis to quantify the relationship between meal size and titration time. For each meal, we also measured the area under the pH curve using ArcView GIS (ver 3.2). A linear regression was used to compare meal size to area under the pH–elapsed time curve.

2.3. Gastric motility

We measured gastric motility using a motility/temperature data-logger (14×1.9 cm, length×diameter, 45 g in air, earth & Ocean Technologies, Kiel, Germany). The sensor consists of a piezoelectric film encased in a flexible silicon bulb, connected to an 8-bit data-logger. Movement of the piezoelectric film generates a voltage, the size of which is a function of the extent and speed of deflection (Peters, 2004). The motility sensor provides a cumulative measure of stomach muscle activity over

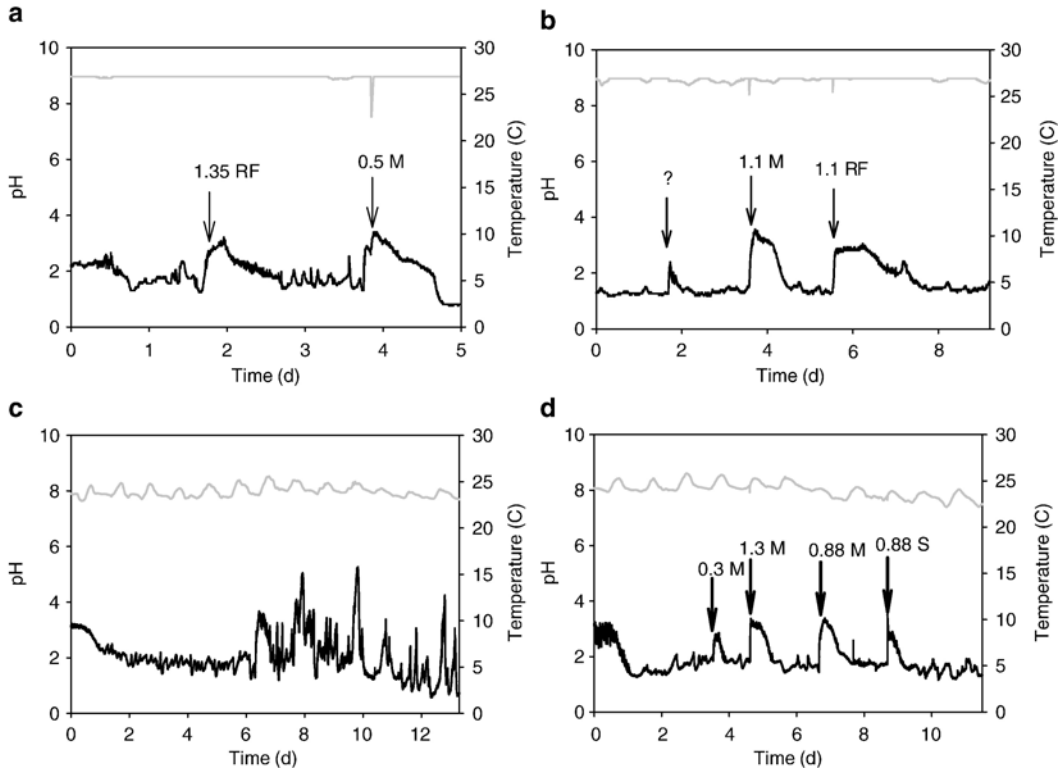

Fig. 1. Continuous measurements of gastric pH and temperature in free swimming blacktip reef sharks (*Carcharhinus melanopterus*). Lower line is gastric pH; upper line is gastric temperature. Arrows indicate time of feeding, and the number above each arrow represents meal size expressed as % BW. "?" indicates that a meal of unknown size was consumed. Meal codes are "M" for mackerel (*Scomber* spp.), "RF" for reef fish (*Acanthurus*, *Chaetodon*), and "S" for squid (*Loligo* spp.). Data from individual sharks are shown in separate panels: (a) Shark #1, (b) shark #2, (c) shark #3 (fasted for entire duration of deployment), (d) shark #4.

time. In our case, the data-logger was programmed to record stomach motility every 15 s. A temperature sensor coupled to the data-logger also enabled simultaneous measurements of stomach temperature (resolution 0.1 °C).

The stomach motility and temperature (SMT) data-loggers were deployed as described above for the pH data-loggers. However, the former were deployed with the sensor pointing towards the mouth. To evaluate any spatial differences in gastric motility within the stomach, one shark had the SMT data-logger deployed with the motility sensor pointing towards the caudal fin. During deployment, sharks were fed squid (*Loligo* spp.) or mackerel (*Scomber* spp.) at a variety of ration sizes. SMT data-loggers were either regurgitated by the shark or we retrieved them as described. After retrieval, data from the SMT data-loggers were downloaded and analyzed using pHG 2.0 software (Jensen Software System).

We used a General Linear Model (GLM) to evaluate the effects of temperature, meals size and meal type on gastric motility. In all cases, motility was the dependent variable while meal size, temperature, and meal type were covariates. Meal size and temperature were also set as interactive variables. Two measures of motility were used: (1) the mean over the first 7 h after feeding, and (2) the mean over the first 24 h after feeding. We used 7 h in addition to 24 h because there appeared to be an approximate 7 h delay between feeding and the onset of the strongest contractions and we wanted to test if there were differences in motility related to feeding during the 7 h "lag" period (see Results). Because the GLM showed that motility differed between mackerel and squid, we used multiple regression analysis for mackerel and squid meals separately. Motility values were not normally distributed, so we applied a square root transformation. The effect of meal size on motility appeared to be best described by a quadratic equation, so meal size was squared. In all cases, the residuals from the GLM and regressions were examined to ensure that all assumptions of the models were met. All GLM and multiple

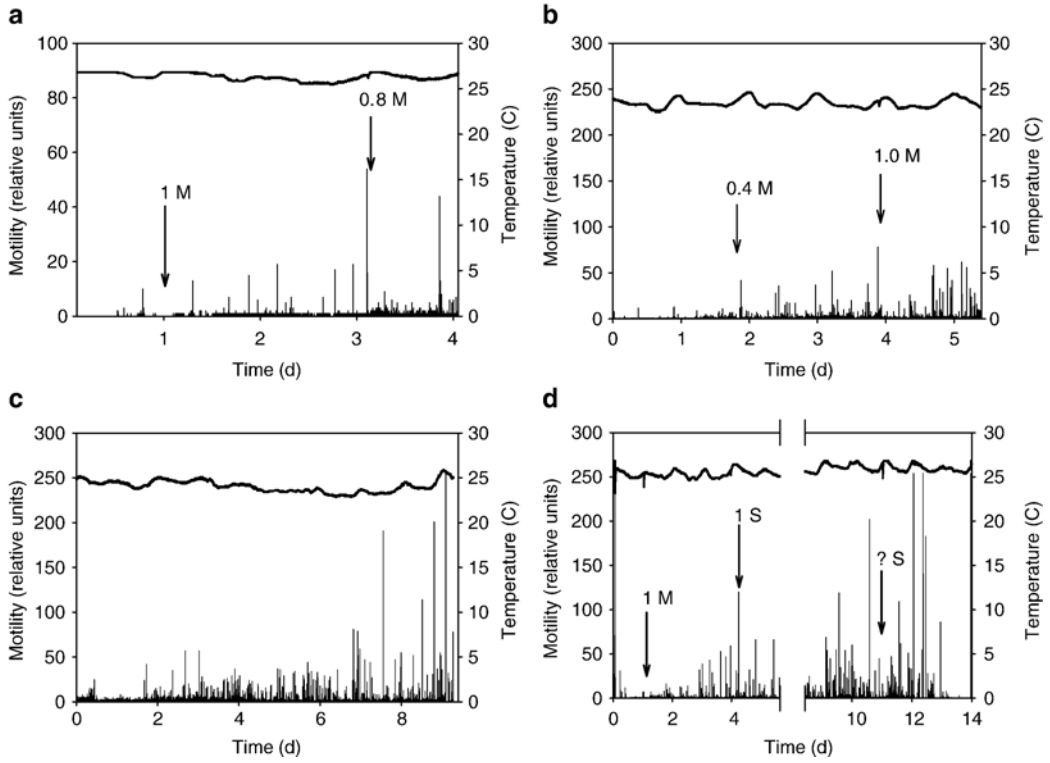

Fig. 2. Continuous measurements of gastric motility and stomach temperature in free swimming black tip reef sharks (*Carcharhinus melanopterus*). The upper trace shows stomach temperature. Data from individual sharks are shown in separate panels: (a) Shark #5 (In this instance the data logger was deployed with sensor pointing towards caudal fin whereas all other sharks had sensor deployed pointing towards mouth.), (b) shark #4, (c) shark #3 (fasted for entire deployment), (d) shark #2 (The gap in data set was due to data-logger failure.). Arrows indicate time of feeding, and number above arrow represents meal size expressed as a percentage of body mass. "?" indicates a meal of unknown size was consumed. Meals codes are "M" for mackerel (*Scomber* spp.), and "S" for squid (*Loligo* spp.). High contractions towards end of deployment seen in panels c and d are most likely attempts to regurgitate data-logger.

regression analysis were performed using Minitab (ver. 14).

Due to the logistics associated with maintaining large adult sharks in captivity, we only had five sharks with which to deploy data-loggers (Table 1). As a consequence, we deployed the motility logger in each shark on two separate occasions (with the exception of shark # 2, in which it was only deployed once). Although this constitutes a degree of pseudo-replication, we visually checked the distribution of all data points to ensure that statistical analyses were not strongly influenced by data from one individual (by examining maximum and minimum data points).

We used time series analysis to determine if there were any cyclical patterns in gastric motility, applying a Fast Fourier Transformation (FFT) which converts time-series data into frequencies, thereby facilitating the identification of temporal periodicity in the dataset. The FFT produces a power spectrum with the power of each frequency being dependent on how well the data fit the sinusoidal wave of that particular frequency (Chatfield, 1996). The time period of the event could then be calculated as the inverse of frequency, with each block of data equivalent to 15 s (the sampling rate of the data-logger). For example, there are 5760 data blocks (each equivalent to 15 s) in a 24 h period, which translates to a frequency of 0.00017. All motility data were smoothed using a Hamming window before running the FFT (Chatfield, 1996). FFT analysis was performed using Statistica (ver.7).

3. Results

3.1. Gastric pH

Drift of the pH electrodes were generally low, with resolution varying between 0.004 and 0.06 pH units and error between 0.02 and 0.4. Regardless, the blacktip reef sharks maintained an acidic stomach at all times (maximum pH: 5.3, Fig. 1, Table 1). During periods of

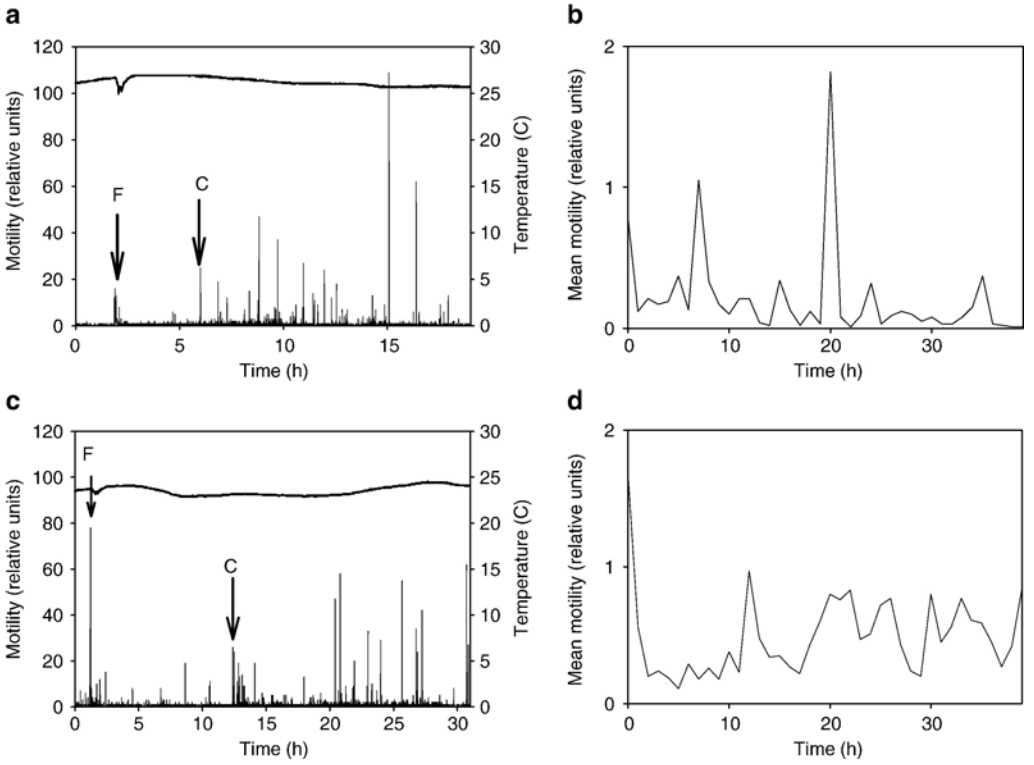

Fig. 3. Examples of lag in motility following feeding in two free swimming blacktip reef sharks (*Carcharhinus melanopterus*). Upper line in each graph is stomach temperature. Results from sharks #2 and #4 show raw data (a, c) and running average (b, d). "F" indicates time of feeding, whereas "C" shows time of strong contractions.

fasting (>48 h after feeding), gastric pH was 1.66±0.40 (mean±1 SD). In all sharks, feeding caused a rapid increase in gastric pH (decrease in acidity) of 1.66±0.41 units to a peak value of 3.15±0.41, followed by a gradual decrease back down to baseline (more acidic) levels. The rate of increase in pH following feeding (0.027±0.019 pH units/min) was faster than the subsequent decrease (0.0015±0.0005 pH units/min, t test paired sample for means, $t=3.77$, $p=0.007$). There was no significant effect of meal size on titration time ($p=0.26$, $F=1.93$) or area under the pH/time curve ($p=0.34$, $F=1.25$). However, the regression was strongly influenced by one outlier point. When this point was removed, meal size (expressed in g) affected both titration time ($p=0.04$, $F=21.7$, $r^2=0.87$) and area ($p=0.04$, $F=36.0$, $r^2=0.92$).

Shark #3 was fasted for 12 days and showed pH profiles with two separate phases (Fig. 1c). For the first seven days of fasting, pH remained relatively stable

Table 2
Multiple regression of square root transformed seven hour motility against stomach temperature, meal size and meal size^2

Predictor	Coefficient	SE coefficient	T	P
Constant	−0.9409	0.5684	−1.66	0.196
Meal size	−0.0676	0.1197	−0.56	0.612
Temperature	0.0719	0.0219	3.29	0.046
Meal size^2	−1.3468	0.3429	−3.93	0.029
$S=0.105$	$R^2=91.2\%$	R^2 (adj.)=82.3%		

Data are from blacktip reef sharks fed meals of mackerel (*Scomber* spp.).

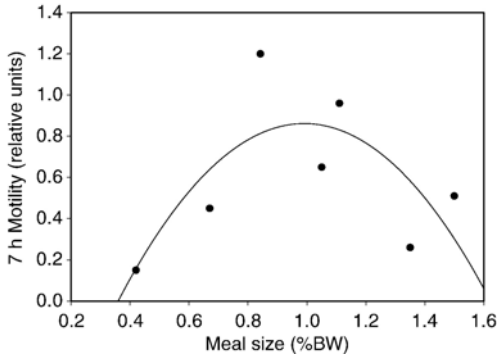

Fig. 4. Effect of meal size on mean motility in blacktip reef sharks (*Carcharhinus melanopterus*) during the seven hours following consumption of mackerel (*Scomber* spp.), measured using a motility data-logger. The solid line is a curve fitted using a quadratic equation ($r^2=0.53$, $p=0.029$). Maximum motility occurs after sharks consume meals of 0.8–1.0 % of body weight.

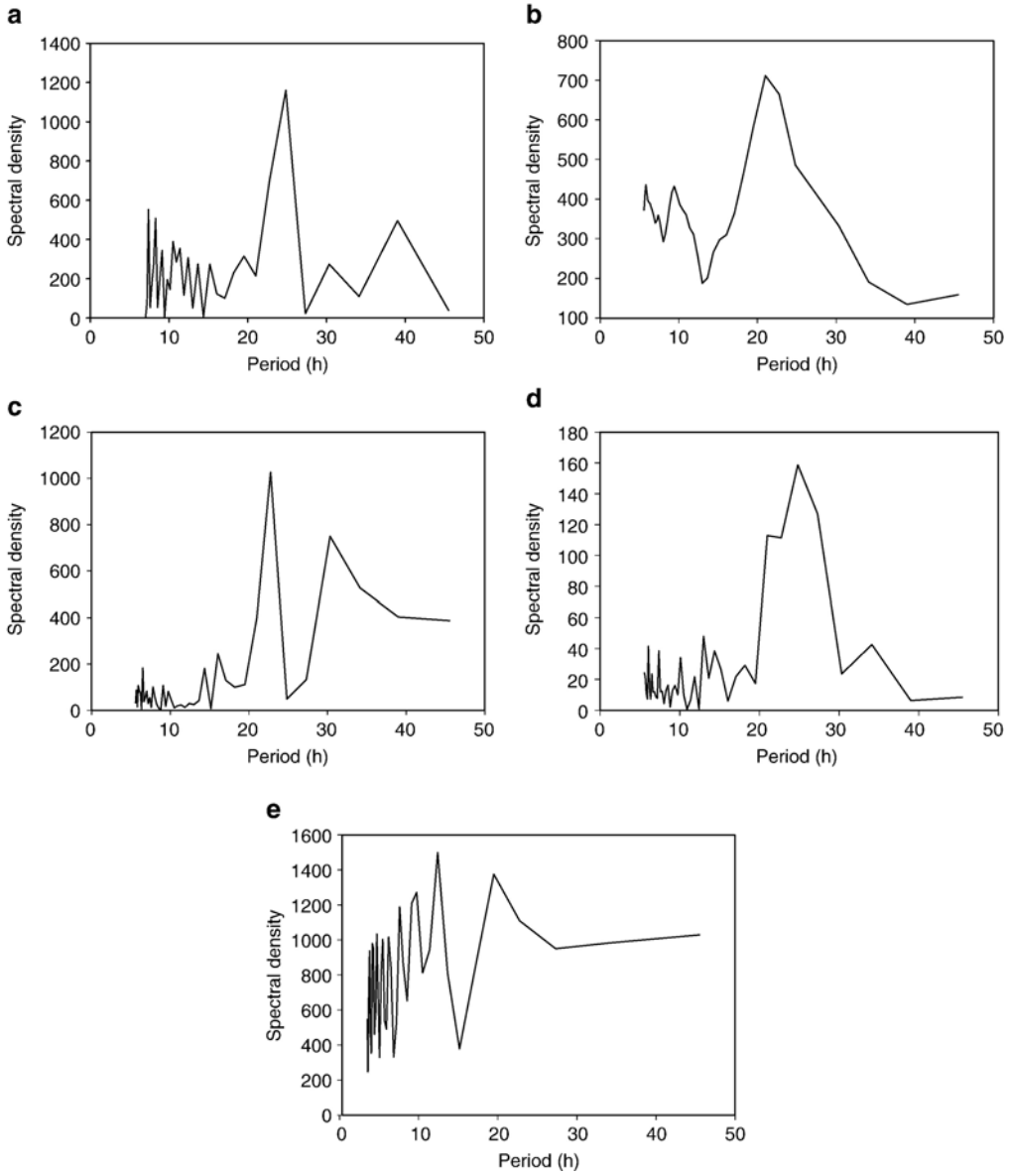

Fig. 5. Spectral analysis (FFT) of gastric motility data from blacktip reef sharks. Data are from sharks # 1–5 (panels a–e respectively). All sharks had the motility sensor pointing towards the mouth, except for shark #5 (e) which had the sensor pointing towards the caudal fin. Different scales were used on the *y*-axis to clarify data presentation.

between 1.4 and 2.1, but after day seven, pH started to fluctuate between 5.3 and 0.4 even though no feeding occurred. FFT analysis was used to analyze both these phases in shark #3. As expected, no major peaks in the density spectrum were observed during the first phase (when pH remained stable). The second phase produced two peaks however; one at 27.8 h and one at 41.7 h. We interpret this as a diel fluctuation in pH, with pH being lowest between 0600 and 0800 in the morning and highest during the late afternoon.

3.2. Gastric motility

Motility appeared to be reduced during the first two days of deployment and consequently motility data were only used from meals given to sharks >2 days after deployment of the logger. However, gastric motility was generally low for all sharks (0.43±0.18 relative units, Fig. 2). All sharks showed a delay of 7–12 h following feeding, before the onset of strong contractions (Figs. 2, 3). The results of the GLM showed that both meal type

($F=13.79$, $p=0.006$) and stomach temperature ($F=6.44$, $p=0.035$) affected motility during the 7 h post-prandial period, while only meal type ($F=9.16$, $p=0.019$) affected motility during the first 24 h post-feeding. There was no significant interaction effect between meal size and temperature on 7 h post-prandial motility ($F=3.43$, $p=0.101$). Meals of mackerel elicited stronger contractions (0.60±0.37 relative units) than meals of squid (0.21±0.07 relative units, $F=13.79$, $p=0.006$). Multiple regression analysis for mackerel meals showed that meal size^2, and stomach temperature affected motility during the 7 h following feeding ($r^2=82.3$, $F=10.31$, $p=0.043$, Table 2), but not over the 24 h following feeding ($F=3.95$, $p=0.145$). Stomach temperature positively correlated with 7 h post-prandial motility, while the effect of meal size on 7 h motility was best described by a quadratic equation ($r^2=0.53$, Fig. 4). Temperature and meal size did not affect motility during the first 7 or 24 h following consumption of squid meals ($F=0.68$, $p=0.572$).

The FFT spectra showed a motility peak for all sharks at a frequency of approximately 0.0002, regardless of whether the shark ate during that time period (Fig. 5). This frequency translates to a time period of 23.4±1.8 h. Shark #5 (the one animal that had the sensor pointing towards the caudal fin) did not show any peaks in the frequency spectra (Fig. 5e). All sharks that fed showed a second peak in motility with a period of 2.0±0.3 h, but this peak was absent from sharks that were fasted (Fig. 6).

4. Discussion

The use of intra-lumenal data-loggers to measure digestive variables appears to be a viable technique in medium to large sized sharks. Although we did not quantify the effects of the data-logger on acid secretion in blacktip reef sharks, previous work with leopard sharks (*Triakis semifasciata*) showed no effect of the data-loggers on gastric acid secretion (Papastamatiou and Lowe, 2004). All blacktip reef sharks behaved similarly to non-instrumented sharks, and resumed feeding within one day of deployment.

4.1. Gastric pH

Blacktip reef sharks are capable of secreting highly acidic gastric fluid (minimum measured pH 0.4). Gastric

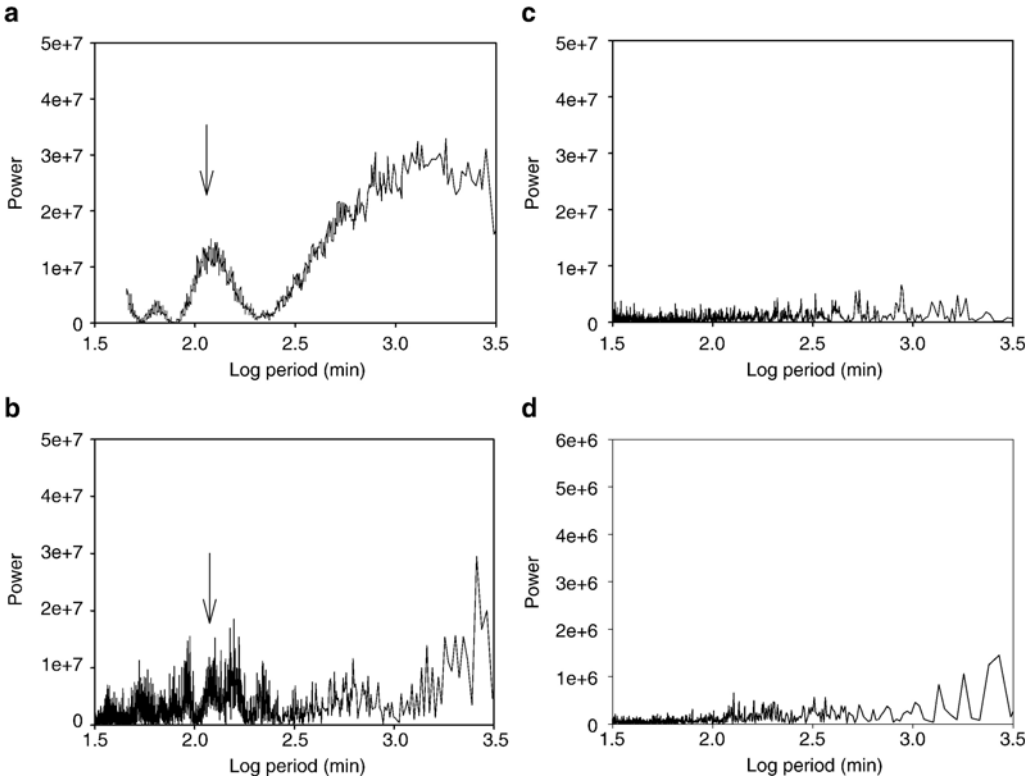

Fig. 6. FFT of gastric motility data from sharks #2 (a), #1 (b), #3 (c), and #4 (d). Sharks #3 and #1 were fed during deployment of the data-logger, while sharks #4 and #5 were fasted. The arrow indicates peaks in the gastric motility spectrum equivalent to a period of 2.0±0.3 h in fed sharks. Note the absence of any peaks in sharks that were fasted. Different scales were used on the *y*-axis in panel d to clarify data presentation.

acid secretion appears to be continuous in this species because low pH values were recorded even after long periods of fasting (e.g. shark #3 was fasted for 12 d, see Fig. 1c). Maximum pH recorded for any blacktip was 5.3, and pH remained at this level for only a short period of time before returning to low levels. It has been proposed that shark species that feed frequently in the wild continuously secrete gastric acid during fasting thereby enabling them to be in a state of physiological readiness for the next meal, whereas sharks which feed less frequently (e.g. nurse sharks, *Ginglymostoma cirratum*) may periodically cease acid secretion while the stomach is empty as an energy conserving technique (Papastamatiou and Lowe, 2004, 2005; Papastamatiou, in press). Although little is known about the feeding habits of blacktip reef sharks in the wild, they are an active continuously swimming species that lives in semi-tropical and tropical waters, and spend a considerable amount of time searching over sand flats and along reef ledges (Papastamatiou and Lowe, unpublished data; Stevens, 1984). In combination, these factors suggest that blacktip reef sharks probably have high energy requirements and may have to feed frequently. If this is the case, the present result appears to agree with the hypothesis that feeding frequency influences gastric acid secretion patterns in sharks.

Following feeding, a rapid increase in gastric pH occurred with a subsequent gradual decrease back to baseline levels. We interpret the rapid increase in pH as being caused by seawater and the food items themselves (most of which are alkaline) entering the stomach and diluting or buffering the small amounts of gastric fluids that are present in the stomach. After feeding, an increase in gastric acid secretion is presumably triggered by stomach distention and the action of secretagogues such as histamine and gastrin (e.g. Smit, 1967; Hogben, 1967; Vigna, 1983) resulting in re-acidification of the stomach. This interpretation is supported by the fact that the amount of time taken for the stomach to re-acidify appears to be a function of meal size.

Gastric pepsin and chitinase enzymes have optimum activity at low pH. For example, pepsin from the lesser spotted dogfish (*Scyliorhinus canicula*) has an optimum pH of 2.5 (Guerard and Le Gal, 1987), while chitinase enzymes from several species of shark and skate show optimal activity at pH of approximately 1.6 (Fange et al., 1979). Although gastric enzymes have not been identified in blacktip reef sharks, it is highly likely that at least one, if not both, of these enzymes are present and the observed gastric conditions would be optimal for both enzymes (especially in the 12– 24 h following feeding).

4.2. Gastric motility

In all blacktip reef sharks there appeared to be a delay in heightened stomach activity of 7–12 h following feeding. A delay in active gastric contractions following feeding has also been seen in teleosts such as bluefin tuna, *Thunnus thynnus*, and rainbow trout, *Oncorhynchus mykiss* (Carey et al., 1984; Olsson et al., 1999). The delay in active contractions following feeding (also known as gastric accommodation or relaxation), is the initial response following distention of the stomach wall, and is thought to allow for more space in the stomach and for the accumulation of gastric fluids before mixing (Mayer, 1994; Holmgren and Holmberg, 2005). The delay in motility observed in the current experiment may be related to the post-prandial lag in gastric evacuation of stomach contents observed in other elasmobranchs such as juvenile sandbar sharks, *Carcharhinus plumbeus* (Medved, 1985), and scalloped hammerhead sharks, *Sphryna lewini* (Bush and Holland, 2002).

The results from the present study show that gastric motility is a function of abiotic and biotic variables. Meals of squid elicited lower levels of stomach contraction than similar sized meals of mackerel (*Scomber* spp.), which is contrary to predictions based on data from other vertebrates. Stomach motility in vertebrates is thought to be sensitive to lipid levels, with high-lipid prey taking longer to evacuate from the stomach than low-lipid prey (Anderson, 2001; Mayer, 1994). The mackerel used in the present study have higher lipid levels than those found in squid (e.g. Mackerel is 4.72% lipids, as opposed to squid which is 1.72%; O'Neal Scientific Services Inc., MO) and should have elicited weaker contractions than squid. These two food items also differ in their physical digestibility however. Squid contains collagen fibers which increase the tissue's resistance to digestive action (Jackson et al., 1987). Our results agree with studies of gastric evacuation rates in elasmobranchs. Gastric evacuation rates for little skate (*Raja erinacea*) fed meals of squid were slower than those fed high lipid sand lance, or lipid poor krill (Nelson and Ross, 1995), while blue sharks (*Prionace glauca*) took longer to evacuate squid than they did anchovies (Tricas, 1979). The reduced motility after squid meals increases the time of exposure of squid tissue to HCl and gastric enzymes, required for the breakdown of collagen fibers (Jackson et al., 1987).

We also found that stomach temperature positively correlated with increased gastric contractions for meals of mackerel, but not squid. It is well established that gastric evacuation in fish is positively influenced by temperature (e.g. Nelson and Ross, 1995; Bush and Holland, 2002),

but it is unclear why temperature did not influence gastric motility patterns for squid meals in blacktip reef sharks, although the small sample size may have compromised our results. The movement patterns of some elasmobranchs in the field may be for behavioral thermoregulation (e.g. Carey and Scharold, 1990; Matern et al., 2000). Moving into warmer water should increase gastric evacuation rates (theoretically lowering digestive efficiency), but our results suggest that this deficit may be countered by improved mixing of stomach contents.

The magnitude of gastric contractions during the 7 h following feeding on mackerel was best modeled to meal size using a quadratic equation. Gastric motility increased with meal size until the sharks were consuming 0.8–1.0% of their body weight (BW), after which there was a decline in motility. It is thought that gastric motility in vertebrates increases as a function of distention of the stomach wall (Mayer, 1994). Although this has never been explicitly tested in fish, preliminary results from dab (*Limanda limanda*) suggest that gastric motility is a function of the cube root of the size of stomach contents (Jobling, 1974). Previous studies have shown that increased meal size also increases gastric evacuation time in elasmobranchs (Sims et al., 1996; Bush and Holland, 2002). In lemon sharks (*Negaprion brevirostris*), initial processing of prey occurred faster when meal size increased, but total gut transit time also increased, suggesting that the rate of digestion remained constant (Wetherbee and Gruber, 1990). Our results agree with those of Wetherbee and Gruber (1990) because motility during the 7 h following ingestion increased with meal size but total motility during the 24 h following feeding did not. However, we were not able to determine what influence the data-logger itself may have had on gastric motility, especially in relation to meal size.

Based on our results, we hypothesize that optimum gastric digestive efficiency in blacktip reef sharks occurs when meal size is 0.8–1.0% of BW. Daily ration has not been measured in blacktip reef sharks, but for other carcharhinid sharks it has been calculated as approximately 1–2% of BW day^{-1} (Wetherbee et al., 1990). The observed decrease in gastric motility at high ration levels may be due to stomach fullness reducing stomach contractions and consequently mixing. Gross conversion efficiency (the efficiency by which ingested prey items are converted into predator tissue) in elasmobranchs is thought to decrease at high ration levels (Cortes and Gruber, 1994; Duncan, 2006). Optimal gross conversion efficiency was achieved at relatively high ration levels (e.g. 5.1% BW/day for scalloped hammerhead sharks, Duncan, 2006), but those studies were conducted using juvenile animals with higher mass specific metabolic rates than adults. We found that a large proportion of the variability in gastric motility following feeding could be attributed to meal size and stomach temperature (at least for meals of mackerel), but we did not measure surface area of prey items or changes in seawater dissolved oxygen concentration, both of which can influence motility (Schurdak and Gruber, 1989; Mayer, 1994).

The results of the FFT suggest that diel changes in gastric motility exist regardless of whether the sharks fed. The 23.4 ± 1.8 h periodicity in motility is most likely a result of the diel fluctuations in stomach temperature, which in turn are related to daily fluctuations in ambient water temperature. Motility was highest in the afternoon when water temperatures were also highest and lowest during the early morning hours when water temperatures were lowest (see stomach temperature data in Fig. 2). Based on the data from one fasting shark that showed lower pH values during early morning hours (between 0600 and 0800), we hypothesize that blacktip reef sharks preferably forage during periods (or areas) of lower temperature (in our study such conditions occurred in early morning, 6–8 AM) although they may feed opportunistically at all times of the day. As such, the period of gastric accommodation (low motility) following feeding coincides with periods of low temperature, with increased gastric motility occurring during periods of increased temperature. Our sharks also showed a periodicity in motility with a frequency of 2.0 ± 0.3 h, which, because this cycle was absent from sharks that were fasted, may represent regular periods of stomach contractions involved with mixing stomach contents and passing chyme into the small intestine. In vertebrates, gut motility during the interdigestive state (fasting) is characterized by migrating motor complexes (MMC), which consist of periods of quiescence (phase I), periods of irregular single contractions (phase II), and periods of strong contractions (phase III, Mayer, 1994; Holmgren and Holmberg, 2005). The interdigestive state in blacktip reef sharks was not characterized by long periods of quiescence, nor was there an obvious transition between phases II and III, which are similar to the results found for rainbow trout (Olsson et al., 1999).

In conclusion, gastric digestion in blacktip reef sharks appears to be a function of abiotic and biotic variables. While foraging behavior (and subsequent optimal foraging theory) is a function of the tactics used to capture prey, it also may optimize digestive efficiency or energy extraction from prey items (Hume, 2005). By quantifying the effects of prey type, meal size, and stomach temperature on gastric digestion in sharks under semi-natural conditions, we can make predictions of foraging strategies in the field. Subsequent studies will aim to

quantify gastric processes in free-ranging sharks in the field to test these hypotheses.

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