

Postprandial response of gastric pH in leopard sharks (*Triakis semifasciata*) and its use to study foraging ecology

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Summary

Changes in gastric pH of leopard sharks *Triakis semifasciata* were quantified as an indicator of feeding frequency and ration size. Continuous *in situ* measurements of gastric pH were made in captive adult leopard sharks using an autonomous pH/temperature probe for periods ranging from 5–16 days. Instrumented sharks were fed meals of squid at different ration sizes. Gastric fluid samples were also taken from non-instrumented juvenile leopard sharks at different time intervals after feeding, and the pH measured to quantify effects of the pH probe in the stomach. Continuous *in situ* measurements of pH show that empty stomachs have a low pH of 1.54 ± 1.42 (mean \pm S.D.) and that feeding causes

a rapid increase in pH to 3.11 ± 0.71 , followed by a gradual decrease back down to baseline levels. There was a positive relationship between changes in pH and meal size ($r^2=0.72$, $P=0.001$). There were no significant differences in pH between continuous *in situ* and laboratory serial sample measurements. Together these findings indicate that gastric acid secretion may be continuous in leopard sharks, and that changes in gastric pH may be used to estimate feeding chronology, frequency and ration size of leopard sharks in the field.

Key words: digestion, feeding chronology, acid secretion, gastric evacuation, leopard shark, *Triakis semifasciata*.

Introduction

Many sharks are apex predators and serve an important role in the transfer of energy from lower trophic levels in the marine ecosystem (Wetherbee et al., 1990; Lowe, 2002). However, an understanding of how sharks influence lower trophic levels requires knowledge of their feeding habits, in particular how much and how often they eat. Despite numerous studies on the feeding ecology and behavior of sharks, little is known regarding the feeding chronology, frequency of feeding, and daily ration for many shark species (Medved et al., 1988; Cortes, 1996; Bush, 2002).

Estimates of feeding chronology and frequency as well as daily ration, stem from stomach content analysis and determination of the state of digestion of ingested prey items (Medved et al., 1988; Cortes, 1996; Kao, 2000; Bush, 2002). Unfortunately, the analysis of stomach contents requires the capture, killing and extensive sampling of animals within the population. Presently, there is no established non-invasive method of quantifying feeding chronology, frequency and daily rations of marine ectotherms.

In contrast, for marine endotherms, frequency and timing of feeding events have been made by recording associated changes in stomach temperature using gastric temperature data-loggers or telemetry devices (Wilson et al., 1992; Gremillet et al., 2000; Gunn et al., 2001). Unfortunately, this technique is only

effective for quantification of feeding events for marine ectotherms when the body temperature of the ingested prey differs considerably from the predator's own body temperature.

One characteristic typical of most carnivorous vertebrates is the production of hydrochloric acid (HCl) within the stomach. In higher vertebrates, such as humans, it has been shown that an increase in HCl secretion rate is initially caused by distention of the stomach lining as food enters, and further regulated by the activation of secretagogues (e.g. gastrin, histamine and acetylcholine), produced by the stomach (Johnson, 1985). The acidic fluids secreted by the stomach cause the cleavage and conversion of the inactive zymogen pepsinogen, into the active protease enzyme pepsin, initiating the digestion of proteins in the stomach. Although the mechanisms of acid secretion in fishes have not been as well studied, stomach distention (Smit, 1967) and secretagogues, including histamine and gastrin, are known to play a role in the control of acid secretion after feeding (e.g. Bomgren and Jonsson, 1996; Hogben, 1967; Vigna, 1979). This suggests that the characteristic changes in gastric pH associated with meal digestion could be used to indicate when feeding has occurred.

The few studies that have examined feeding-induced changes in gastric pH of elasmobranchs have yielded different results. Sullivan (1906) and Caira and Jolitz (1989) found that

Table 1. Summary information for adult leopard sharks *Triakis semifasciata* fitted with pH/temperature probes, including residence time of pH probe in the shark's stomach

Shark number	Total length (cm)	Mass (kg)	Sex	Ration size (% M_b)	Residence time in stomach (days)	Water temperature (°C)
1	145	16.3	F	1.0, 0.3, 2.1	16	17.5
2	152	15.2	F	1.0*, 1.0	9	17.2
3	149	10.4	F	2.0	5	19.4
4	128	7.8	F	2.0	7	18.0
5	125	7.3	F	2.0, 1.0, 0.3	11	18.0
6	150	15.8	F	2.0, 1.0, 0.1, 0.1, 1.0*	10	17.8

M_b , body mass.

Ration sizes are for meals of squid except for *, which represents a meal of capelin (*Mallotus villosus*).

some shark species with food in their stomachs had acidic pH values (pH 2–3.5), while those with empty stomachs had neutral pH values (pH 6–7), indicating that acid was only being secreted when food was in the stomach. However, studies by Williams et al. (1970) and Menon and Kewalramani (1959) on a number of other elasmobranch species, revealed that the stomach pH remained acidic (2.5–4.0) regardless of the presence or absence of food, indicating that acid secretion is continuous. Unfortunately, none of these studies continuously monitored postprandial gastric pH.

Leopard sharks *Triakis semifasciata* are a well-studied coastal species of shark, ranging from Oregon to Baja California (Love, 1996), and were chosen for this study because of their abundance, large size and rapid acclimation to captive conditions. The objectives of this study were to (1) obtain continuous measurements of gastric pH, (2) quantify feeding-induced changes in gastric pH, and (3) investigate the possible use of changes in gastric pH as an indicator of feeding episodes in free-ranging leopard sharks.

Materials and methods

Continuous measurements

To continuously monitor gastric pH, we used six adult female leopard sharks *Triakis semifasciata* (Girard 1854), total length TL 141.5 ± 11.9 cm (mean \pm 1 S.D.), mass 12.1 ± 4.1 kg (Table 1). Sharks were maintained in large seawater tanks, either at the Long Beach Aquarium of the Pacific (LBAOP: 5.4×10^5 l) or the Scripps Institute of Oceanography Marine Laboratory (SIO: 7.6×10^3 l).

We continuously measured gastric pH and temperature using an autonomous stomach pH probe with a pressure-equalizing reference electrode (Peters, 1997a,b). The probe was initially designed to measure gastric pH and temperature in free-diving seabirds and consists of a microelectrode, a reference electrode with free-diffusion liquid junction and a data-logger encased within a titanium shell (earth & Ocean Technologies, Kiel, Germany; Peters 1997a,b). The data-logging probe (11 cm \times 2 cm, length \times diameter; mass in air = 80 g) was programmed to record gastric pH and

temperature every 30 s. Before deployment, the probe was calibrated using three NBS standard buffers of pH 1.7, 4.0 and 6.8. Further details of probe design and preparation can be found in Peters (1997a,b).

Kao (2000) fed adult leopard sharks (>120 cm TL ; kept at 13–18°C) meals of innkeeper worms (*Urechis* sp.) and sculpins (Cottidae) at 1–1.7% of the sharks' body mass (M_b) and found that it took the sharks 27–30 h to empty their stomachs. Therefore before deploying the probe, we fasted all sharks for a minimum of 3 days to ensure that their stomachs were empty. The first probe deployment was carried out by concealing the pH probe within a herring, and then feeding the instrumented bait to an adult female shark. However, subsequent attempts to feed the probe to sharks failed because sharks would not swallow the fish whole. Consequently, all remaining sharks were force-fed the probe after the sharks were anaesthetized using MS-222 (70 mg l^{-1}) and a 3 cm diameter lubricated PVC pipe was gently inserted through the mouth into the stomach. The probe was then carefully passed down the pipe into the stomach, after which the pipe was retracted. Sharks were revived, returned to the holding tank, and observed for 15 min to ensure that they had recovered and did not prematurely regurgitate the probe. In all cases, the pH probe weighed $<1.1\%$ of the shark's body mass (M_b) in air.

Every 2–3 days we fed sharks squid meals ranging from 0.1 to 2.1% of the shark's M_b . Because gastric evacuation rates in sharks are known to vary with temperature (Schurdak and Gruber, 1989, Wetherbee et al., 1990), water temperatures were recorded in the tanks at the LBAOP and SIO (mean water temperature: $18.0 \pm 0.8^\circ\text{C}$).

The pH probe can provide accurate pH data for periods up to 16 days, depending on electrolyte outflow rate. Therefore, we removed the probe from the sharks after a 6–16 day period. Probe removal was carried out by anaesthetizing the sharks as before, reinserting the 3 cm diameter PVC pipe into the stomach until the probe was felt, and then tipping the shark's head downwards, allowing the probe to fall out through the pipe. Sharks were then revived and returned to the holding tanks. Two sharks regurgitated the probe after a 5 and 7 day period (Table 1).

Immediately after recovery, the probe was recalibrated in pH

buffers (1.7, 4.0 and 6.8). The data were then downloaded and analyzed using pHG 2.0 software (Jensen Software Systems, Service earth & Ocean Technologies) to interpolate and correct for drift of pH (see Peters, 1997a). The software also corrects pH for any corresponding changes in stomach temperature. Measurement accuracy was determined by using the pH drift model described by Peters (1997a). Resolution of pH measurements for each probe deployment was calculated by determining the gradient of the calibration curve before and after deployment.

We pooled pH data for all six sharks into hourly intervals and compared pH before and after feeding using a one-way analysis of variance (ANOVA). A Tukey's pairwise comparison was used to determine the location of any hourly differences in pH before and after a meal. The rate of increase in pH after a meal, and the subsequent rate of decrease after peak pH had been reached, were calculated. The two rates were compared using a *t*-test for unequal variances.

Titration time is defined as the time taken for gastric pH to return to baseline following a meal. Titration time was determined using the technique described by Gardner et al. (2002), where we calculated the percentage of time within 10 min intervals that pH remained below 2.0. A pH of 2.0 was determined to be a baseline measurement for humans (Gardner et al., 2002), and appeared appropriate for leopard sharks as well, based on our examination of the gastric pH data. We used the 10 min intervals beginning 2 h prior to consumption of a meal and until pH returned to baseline levels. The first of two consecutive 10 min intervals, where pH <2.0 for only 5% of the interval, was designated P₁. The first of two consecutive intervals, where pH <2.0 for 90–100% of the time, was designated as P₂. Titration time was then calculated as P₂–P₁. A linear regression was used to determine any relationship between titration time and meal size (expressed both in kg and %M_b).

We calculated the areas under the feeding-induced profiles of gastric pH using Arcview GIS (3.2). A linear regression was used to identify the relationship between meal size (as % M_b and kg) and the area under the feeding-induced pH curve.

Time-series sampling

In order to determine whether the pH probes themselves were triggering acid secretion in the adult leopard sharks, we quantified gastric pH changes in non-instrumented juvenile sharks. For time-series sampling, 12 juvenile leopard sharks were obtained from local aquaria and maintained in 900 l tanks containing recirculating seawater at California State University Long Beach Shark Laboratory. Sharks were measured, weighed, sexed, tagged and allowed a minimum of 1 week to adjust to captive conditions before starting the experiments (seven females: five males, mean total length 50.6±6.3 cm and mass 0.63±0.26 kg). Sharks were fed a meal of anchovies and then fasted for a minimum of 72 h to ensure that their stomachs were empty. Sharks were then fed squid meals at 1% of the shark's M_b and gastric fluid subsequently sampled at 1, 7, 12, 24, 48, 72 and 96 h following feeding. Gastric fluid samples were obtained by netting sharks and quickly inverting them,

which places them into tonic immobility. Sharks were removed from the water, tipped head down to drain water from their mouth area, and a flexible (5 mm diameter) plastic tube was then inserted through the mouth and into the stomach. We used an attached syringe to remove 0.5–2 ml of gastric fluid and measured the fluid pH using a calibrated bench-top pH meter.

After we obtained a fluid sample, the sharks were fed a meal of anchovies and allowed 3 days to recover before a second sample was obtained at another sampling time, after a subsequent feeding. 3 days of fasting also allowed sufficient time for all food to leave the stomach prior to subsequent feeding events (Kao, 2000). In this way, each shark was only sampled once after each meal on any given day, and therefore each shark was fed and sampled a total of seven times. The mean water temperature in the shark tank was 21.6±0.8°C (range: 20.6–23.0°C).

To ascertain the effect of a second meal on gastric pH, six sharks were fed a second meal of squid (at 1% M_b) 24 h after the first meal. 1 h after the second meal, a sample of gastric fluid was obtained and the pH measured.

In order to compare probe and laboratory measurements, all laboratory pH measurements were corrected for temperature to a standardized value of 18°C (mean during probe trials was 18.0±0.8°C), using the technique described by Brower et al. (1998). A one-way ANOVA was used to compare pH among specific sampling times (1, 7, 12, 24, 48, 72 and 96 h after feeding). A Tukey's pairwise comparison was then used to determine the location of any pairwise differences.

To determine any effects of the automated pH probe on acid secretion, we compared pH values from sharks fitted with stomach probes that had consumed squid at 1% M_b, to pH values from juvenile sharks kept in laboratory tanks, using a *t*-test for equal variances. A Kolmogorov–Smirnov test was used to determine if data sets were normally distributed. Data were compared for pH values at 1, 7, 12 and 24 h after a meal. The maximum time interval between meals for sharks that had been fitted with a stomach pH probe and consuming a meal of 1% M_b, was 24 h.

Results

Continuous measurements

Shark stomach temperatures were very similar to the ambient water temperatures except during feeding events, when ingestion of frozen squid caused a sudden decrease in stomach temperature. Hence, a decrease in temperature of 0.3–1°C was observed during these times. Resolution and accuracy of temperature measurements were ±0.1°C.

For all sharks, gastric pH was relatively acidic (pH <4.5) at all sampling times. Mean gastric pH 1 h prior to feeding sharks with empty stomachs (i.e. sharks fasted >48 h) was 1.54±1.42, although there was a large amount of variability among sharks (range: 0.4–2.16). Ingestion of food items induced a rapid increase in gastric pH (0.051±0.045 pH units min⁻¹) peaking approximately 1 h post-feeding, followed by a more gradual decrease of 0.0058±0.0058 pH units min⁻¹, until baseline

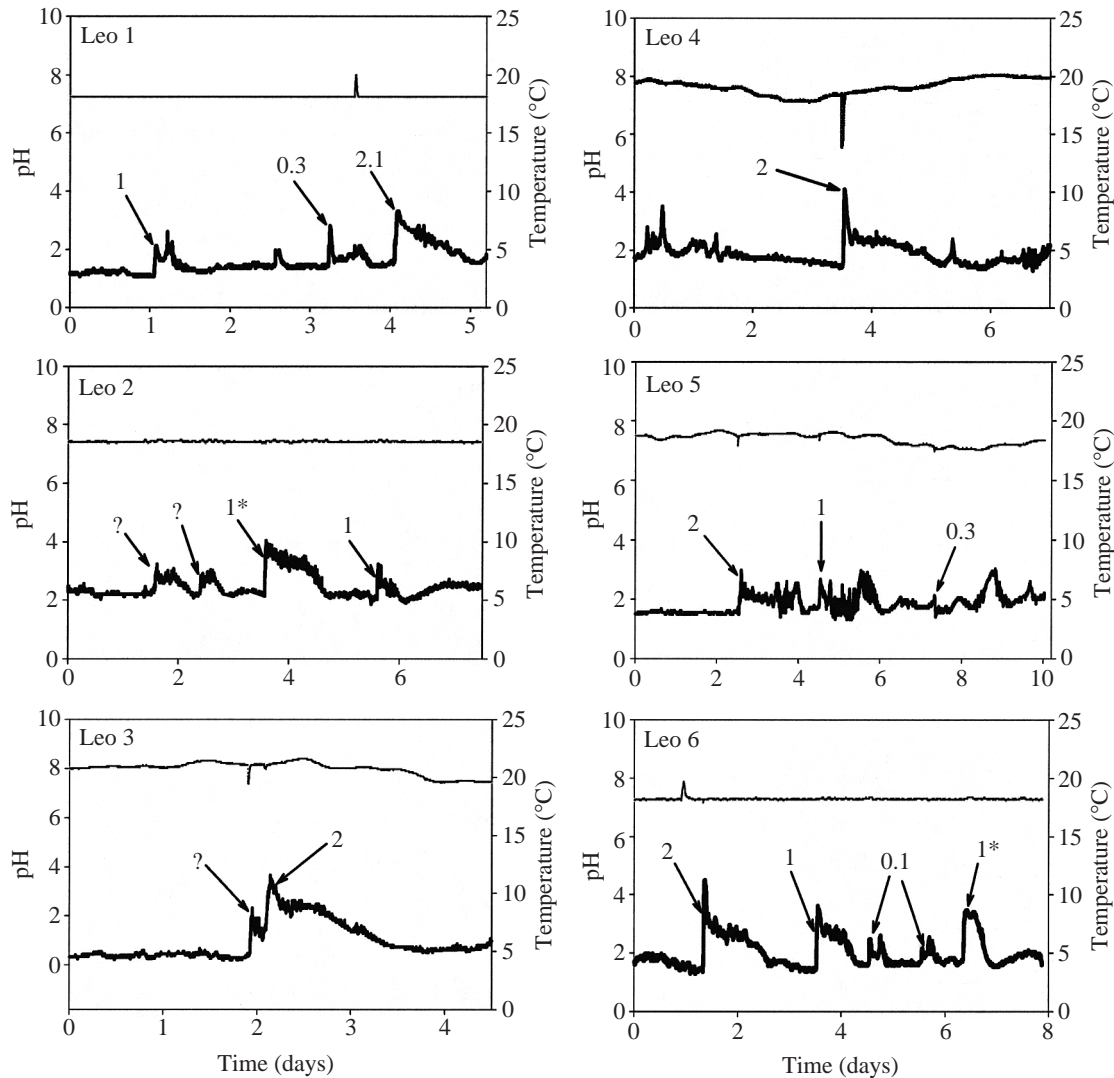


Fig. 1. Continuous gastric pH measurements from six adult female leopard sharks *Triakis semifasciata* (Leo 1–6) fitted with a pH/temperature probe. Temperature is depicted in grey, pH in black. Arrows point to feeding events; numbers above arrows represent meal size (percentage of the shark's body mass). '?' indicates the point where a meal of unknown mass was consumed. All meals were squid except for that marked with an asterisk, where a meal of capelin *Mallotus villosus* was consumed.

levels were reached (Fig. 1). The rate of pH increase was significantly greater than the rate of decrease (*t*-test for unequal variances, $F=65.8$, $P=0.009$). After a meal, pH increased on average by 1.55 ± 0.81 units, with the rates of increase a function of meal size expressed either as % M_b ($r^2=0.5$, $P=0.02$) or in kg ($r^2=0.48$, $P=0.02$). Pooled data for all six sharks showed a significant difference in pH from 1 h before a meal up to 6 h after a meal (Tukey's test, $F=6.23$, $P<0.0001$), and pH had returned to baseline after 7 h.

We found the integrated area under the pH profile to be significantly related to meal size, expressed as a % M_b ($r^2=0.63$, $P=0.007$) and in kg ($r^2=0.61$, $P=0.006$). The percentage of time that the pH remained below 2.0 varied significantly with meal size. Titration time also significantly varied as a function of meal size expressed as a percentage of the shark's M_b ($r^2=0.69$, $P=0.006$, Fig. 2A) and in kg ($r^2=0.73$, $P=0.003$, Fig. 2B).

Error analysis of probe data indicated that continuous pH

measurements were within a range of ± 0.12 – 0.45 units for the duration of the deployments, for the majority of sharks (Table 2). Resolution of pH measurements for all sharks was typically 0.068 pH units.

Time-series sampling

Twelve juvenile leopard sharks were sampled over the seven sampling times; however, not all sharks could be sampled at some of the later sampling times as insufficient gastric fluid was obtained for pH measurements. There was a decrease in the pH of gastric fluid in the hours following a feeding event, with a minimum being reached between 12–24 h after the meal (Fig. 3). Leopard sharks had gastric fluids with a mean pH of 3.31 ± 0.35 1 h after feeding, which had decreased to 1.75 ± 0.24 96 h after the meal; however there were very small amounts of fluid left in the stomach >48 h after a meal. A one-way ANOVA revealed a significant difference in pH among the

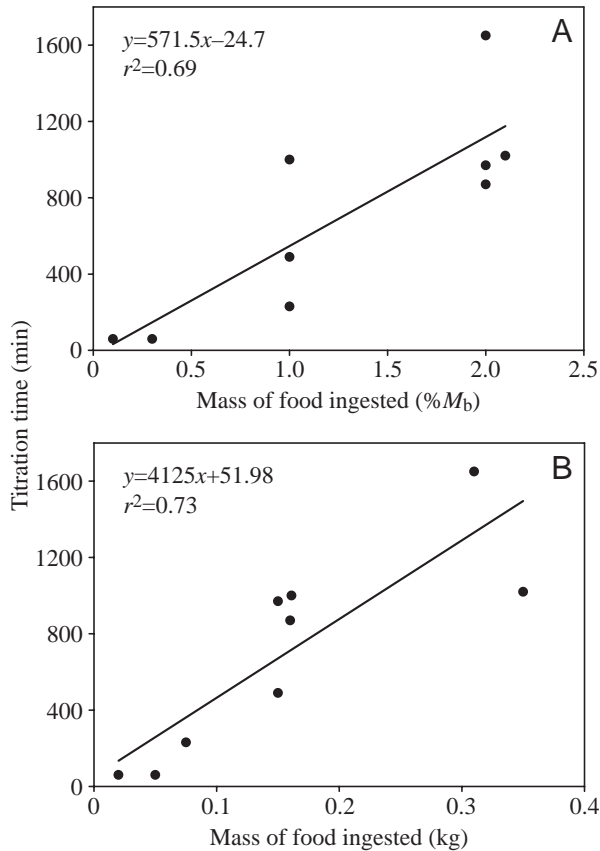


Fig. 2. Regression correlations between titration time and meal size in six adult leopard sharks. Sharks were fitted with pH/temperature probes and fed meals of squid. Meal size is expressed as both a percentage of the shark's body mass (A) and in kg (B).

sampling times ($F=20.62$, $P<0.001$). A Tukey's pairwise comparison revealed a significant difference between the pH at the 1 h sampling time and the pH of all subsequent sampling times ($P<0.001$, Fig. 3). In addition, gastric pH at 7 h post-feeding was significantly different from the values at 12, 24, 72 and 96 h ($P<0.001$).

The ingestion of a second meal, 24 h after the first, caused

Table 2. Summary information of automated pH/temperature probe performance for each individual adult leopard shark *Triakis semifasciata*

Shark number	Resolution		Drift error (pH)			pH	
	Start	End	2	4	6	Min.	Max.
1	0.068	—	—	—	—	1.07	3.79
2	0.090	0.101	1.26	1.21	1.17	1.98	3.93
3	0.081	0.079	0.49	0.47	0.45	0.23	3.66
4	0.067	0.057	0.28	0.17	0.22	1.44	4.10
5	0.061	0.076	0.28	0.2	0.53	1.13	3.04
6	0.063	0.064	0.14	0.14	0.15	1.42	4.50

Resolution before and after deployment is included, as well as error analysis over a range of pH values.

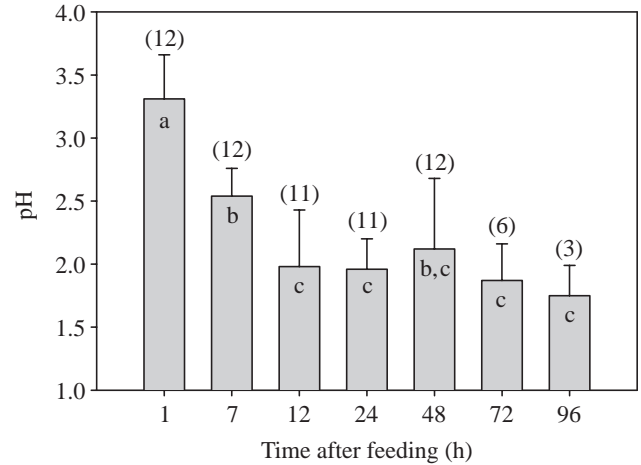


Fig. 3. Change in gastric pH of juvenile leopard sharks following ingestion of squid at 1% body mass. Samples of gastric fluid were removed from the shark's stomach, and pH readings corrected to a standardized value at 18°C. Values are means \pm 1 S.D.; numbers above bars are sample sizes. Bars with the same letter are statistically insignificant from each other (see text for details).

the pH of the gastric fluid to rise (Fig. 4). 1 h after the second meal the pH was 3.4 ± 0.34 , and this was significantly greater than the pH at all other sampling times (7, 12, 24, 48, 72, 96 h) except at the initial 1 h sampling time (Tukey's test, $P<0.001$).

There were no significant differences observed between continuous probe and laboratory measurements of gastric pH 1 h, 7 h, 12 h and 24 h ($P>0.264$) after a meal.

Discussion

Gastric pH changes

Gastric pH of leopard sharks was acidic (pH <3.5) at all times except immediately following a feeding event. The postprandial increase in gastric pH is undoubtedly due to the mixing of ingested food and seawater with the acidic stomach fluids. The pH of gastric fluid returned to baseline levels, presumably as acid secretion increased, thereby re-acidifying stomach contents (James, 1957; Johnson, 1985).

Stomachs of leopard sharks remained strongly acidic (pH <2.0) for at least 4 days following meal ingestion. However, the average gastric evacuation time estimated for adult leopard sharks is approximately 28 h (Kao, 2000), indicating that leopard sharks continuously secrete acid. Although acid secretion is continuous, the variation in acid secretion rates following gastric evacuation is unknown. Nevertheless, larger amounts of gastric fluid were obtainable from non-instrumented sharks 1–12 h after a meal, while only very small amounts of fluid could be obtained 24–96 h after feeding. Therefore, it is hypothesized that the ingestion of food causes an increase in acid secretion triggered by the distention of the stomach (Smit, 1967), and is further regulated by chemical secretagogues (Hobgen, 1967; Vigna, 1979).

Similarities in feeding-induced pH change between

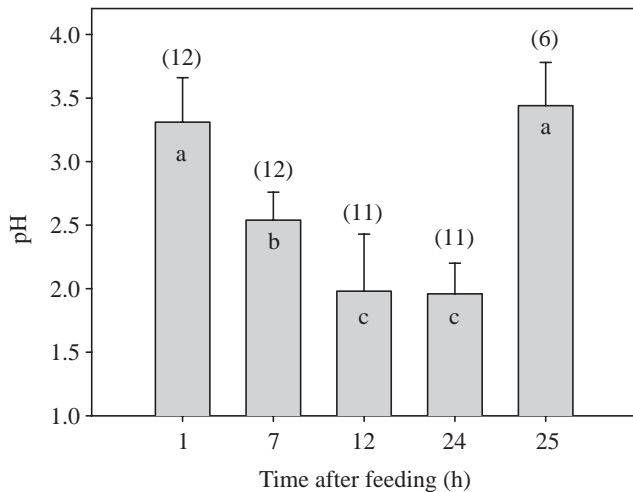


Fig. 4. Gastric pH changes in juvenile leopard sharks fed a meal of squid at 1% body mass M_b . Six sharks were fed a second meal of squid (1% M_b) 24 h after the first. The pH of the gastric fluid was determined 1 h after the second meal. All pH readings were corrected to a standardized temperature of 18°C. Values are means \pm 1 S.D.; numbers above bars represent sample sizes. Bars with the same letter are statistically insignificant from each other (see text for details).

instrumented and non-instrumented sharks indicate that it is unlikely that the physical presence of the pH probe within instrumented sharks stimulated long-term additional acid secretion. However, three of the sharks fitted with a pH probe did exhibit a slight decrease in gastric pH followed by a gradual increase over a 1 day period following the introduction of the probe; therefore there may be some stimulatory effects of the probe during the first day following deployment. For the smallest shark used (mass = 7.3 kg), a low stomach volume:probe volume ratio may have been responsible for the observed fluctuations in gastric pH, which made it difficult to discern feeding events (see Fig. 1; Leo 5). In addition to having negligible physiological effects, the pH probe also appeared to have negligible behavioral effects on the leopard sharks utilized in this study. Although a majority of the sharks had to be force-fed the probe, all sharks resumed active feeding within 1 day of deployment of the probe and behaved similarly to non-instrumented sharks.

Digestive physiology

Other studies on elasmobranchs have found similar changes in gastric pH to those seen in leopard sharks. Two skate species (*Raja clavata* and *R. naevus*) were found to maintain an acidic gastric environment (pH 1.0–4.0), regardless of the presence or absence of food (Williams et al., 1970). Babkin et al. (1935) found that fasting skates secreted very small amounts of acidic fluid, with gastric pH ranging from 2.5–3.5. Menon and Kewalramani (1959) found gastric pH values for three species of elasmobranchs (*Chilloscyllum griseum*, *Dasyatis warnak*, *Rhinobatus halavi*) of 2.6–3.5 and 3.3–4.5, 1 and 7 days following feeding, respectively, indicative of continuous acid secretion. Dobreff (1927; cited in Barrington, 1957) was able

to obtain small quantities of acidic fluid from the fasting stomach of an elasmobranch for up to 112 days after a meal, although there was a gradual rise in pH during this period.

Not all elasmobranchs exhibit this pattern of gastric pH response to food consumption. Sullivan (1906) found that empty stomachs of eight different species of elasmobranchs exhibited neutral pH values, whereas those containing food were acidic. Caira and Jolitz (1989) found that nurse sharks *Ginglymostoma cirratum*, collected in the field with food in their stomachs, had a mean pH of 2.56 ± 0.43 , whereas those with empty stomachs had a gastric pH of 7.19 ± 0.65 , indicating that acid secretion may not be continuous for this species.

There are also different patterns in pre- and postprandial gastric pH changes for teleosts. A similar pattern in feeding-induced gastric pH changes to those seen in leopard sharks has also been observed for a number of coral reef fishes (e.g. *Caranx ignobilis*, *Acanthurus nigrofuscus*), where those with empty stomachs had lower gastric pH values than those containing food (Lobel, 1981; Montgomery and Pollak, 1988). However, some coral reef fishes have been shown to exhibit the opposite trend, with lower gastric pH when food was present in the stomach (e.g. *Acanthurus triostegus*; Lobel, 1981).

A great deal more information exists regarding postprandial gastric pH for terrestrial vertebrates. Gastric pH has been measured continuously for snakes (Secor, 2003), birds (Peters 1997a,b; Gremillet et al., 2000), ruminants (Enemark et al., 2003) and humans (e.g. Evans et al., 1988; Gardner et al., 2002). As with leopard sharks, a rise in gastric pH with meal ingestion, followed by a decrease to baseline levels, has been observed for birds (Duke et al., 1975; Peters, 1997a; Gremillet et al., 2000) and humans (James, 1957; Gardner et al., 2002). Humans continuously secrete acid, with an unstimulated (i.e. empty) stomach possessing an acid secretion rate 10–15% that of a stimulated stomach (i.e. food present). Thus, in the absence of food the human stomach has a pH of 1.0–2.0 (Johnson, 1985; Evans et al., 1988). While the postprandial pattern of gastric pH for leopard sharks is similar to that of humans, it differs from that seen in some snakes. The empty stomach of Burmese pythons *Python molurus* maintains a neutral pH and becomes acidified upon the ingestion of food items (Secor, 2003).

These studies suggest that there are differences in the pattern and mechanisms of gastric acid secretion among vertebrates. The variation in gastric pH changes between species may be due to differences in methodology, such as measurement technique, sampling interval, meal size and meal composition. Nevertheless, digestive physiology is likely to be strongly influenced by a species feeding ecology. Feeding frequency has been shown to be responsible for inter-specific differences in the digestive physiology for a number of reptile species (Secor et al., 1994; Secor and Diamond, 1998) and it is possible that this may be true for fish as well.

Continuous secretion of acidic fluid is likely to be energetically expensive, as suggested by the high number of mitochondria found within oxyntic and oxyntopeptic cells (Secor, 2003; Rebolledo and Vial, 1979), and the reasons for

maintaining a continuously acidic gastric environment are unresolved. It has been suggested that humans continuously secrete gastric acid to prevent growth of pathogenic bacterial flora on the empty stomach mucosa (Secor, 2003), although there have been few quantitative studies to test this hypothesis. We therefore propose two additional hypotheses to explain the continual secretion of gastric acid in leopard sharks.

Elasmobranchs are the earliest known vertebrates to possess a stomach capable of secreting acid (Smolka et al., 1994), and continual maintenance of a low gastric pH in leopard sharks may be a primitive mechanism to increase gastric evacuation rate, causing a more rapid return of appetite (Wetherbee et al., 1990; Sims et al., 1996). This may be important, as many sharks are known to be opportunistic in their feeding habits (Wetherbee et al., 1990), and maintaining low gastric pH would facilitate rapid digestion of a subsequent meal. 1 h after a meal, the pH in the leopard shark stomach ranges from 2.5 to 4.5, and this may be an optimum range to cause both the conversion of pepsinogen into pepsin (occurs at pH <5.0; Johnson, 1985) and an increase in acid secretion rates through the action of acid-stimulating secretagogues such as gastrin. In mammals, gastrin is secreted when gastric pH rises above 3.0 (Johnson, 1985). Although there is limited information on gastrin secretion and its regulation in fish, cod have been shown to exhibit a higher gastric acid secretion rate when stomach content pH is increased (Bomgren and Jonsson, 1996). In addition, the exogenous introduction of gastrin has been shown to stimulate increased acid production by the isolated dogfish mucosa (Vigna, 1983).

Maintenance of low gastric pH may also be attributed to the energetic cost of regulating acid secreting oxynto-peptic cells. Based on the gastric evacuation time for adult leopard sharks and the percentage of sharks caught with empty stomachs, it appears that leopard sharks feed approximately once every 35 h (Talent, 1976; Kao, 2000). Therefore, for leopard sharks, the time interval between gastric emptying and the introduction of a new meal is relatively short, hence it may be energetically more expensive to completely downregulate and then upregulate acid secretion, than to merely reduce the acid secretion rate. Similarly, snakes that feed frequently are found to maintain their intestinal tract in a state of physiological readiness (Secor et al., 1994), in anticipation of the next meal.

Continuous gastric acid secretions may place considerable strain on the gastric mucosa. The mechanisms by which the gastric mucosa in leopard sharks resists damage from the acid are unknown. Quigley and Turnberg (1987) found that in humans there was a steep gradient in pH ranging from the stomach lumen across the mucus membrane. While the lumen contents were always acidic (pH 2.01 ± 0.17), the mean juxtamucosal pH was 4.84 ± 0.37 and was often close to neutral. Although the pH of the leopard shark gastric mucosa was not measured in this study, a mucus layer could play a similar role in protecting the gastric mucosa for the leopard shark.

Application for studying foraging ecology

In addition to furthering the current knowledge of gastric

acid secretion for an elasmobranch fish, this study also demonstrates the potential use of continuous gastric pH measurements as a tool in the study of leopard shark foraging ecology. Presently the only technique used to directly quantify feeding events and meal size in marine endotherms is *in situ* measurement of stomach temperature. Stomach temperature is relatively easy to measure, and changes markedly as food enters the stomach (Wilson et al., 1992; Gremillet et al., 2000; Gunn et al., 2001; Klimley et al., 2001). However, changes in stomach temperature can only be observed if the predator has a different body temperature than that of its prey. This obviously limits this technique, given the lack of a temperature difference between ectothermic fishes and their ectothermic prey. In contrast, gastric pH changes when food items are ingested, independent of prey temperature.

The observed feeding-induced changes in gastric pH for leopard sharks made it easy to identify individual feeding events and to distinguish them from background fluctuations in gastric pH. Discrete feeding events could be resolved within 4 h of each other (Fig. 1; Leo 3), which is important because some species of sharks may eat multiple meals over a short period before fasting for a number of days (Cortes and Gruber, 1990; Wetherbee et al., 1990).

Another finding of this study was the correlation between gastric pH change and meal size. The larger the meal, the greater the volume of acid that needs to be secreted and the longer the duration before gastric pH returns to baseline values. However, titration time and pH changes are likely to be strongly affected by changes in gastric evacuation times. Gastric evacuation rates in sharks are temperature dependent (Schurdak and Gruber, 1989; Wetherbee et al., 1990; Bush, 2002), and also sensitive to food type (Jackson et al., 1987), prey energy content (Wetherbee and Gruber, 1990), and prey fat content (Schurdak and Gruber, 1989). Changes in titration times may be particularly susceptible to crustacean prey items containing exoskeletons and hard parts (see Jackson et al., 1987) and further studies are required to determine how different food types can effect gastric pH changes.

This study demonstrates that the monitoring of gastric pH can be used to quantify feeding chronology, frequency and daily ration of leopard sharks in the field. Incorporation of the pH probe with a telemetry transmitter (acoustic or radio) would allow researchers to continuously monitor the gastric pH of free-ranging animals and identify those changes in pH associated with feeding (see Nelson, 1974, 1990; Lowe and Goldman, 2001).

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