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Variations in gastric acid secretion during periods of fasting between two species of shark

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Abstract

Vertebrates differ in their regulation of gastric acid secretion during periods of fasting, yet it is unknown why these differences occur. Elasmobranch fishes are the earliest known vertebrates to develop an acid secreting stomach and as such may make a good comparative model for determining the causative factors behind these differences. We measured gastric pH and temperature continuously during periods of fasting in captive free-swimming nurse sharks (*Ginglymostoma cirratum*) using autonomous pH/temperature data-loggers. All nurse sharks secreted strong gastric acids (minimum pH 0.4) after feeding; however, for most of the sharks, pH increased to 8.2–8.7, 2–3 days after feeding. Half of the sharks also exhibited periodic oscillations in pH when the stomach was empty that ranged from 1.1 to 8.7 (acid secretion ceased for 11.3 ± 4.3 h day^{-1}). This is in contrast to the gastric pH changes observed from leopard sharks (*Triakis semifasciata*) in a previous study, where the stomach remains acidic during fasting. The leopard shark is a relatively active, more frequently feeding predator, and continuous acid secretion may increase digestive efficiency. In contrast, the nurse shark is less active and is thought to feed less frequently. Periodic cessation of acid secretion may be an energy conserving mechanism used by animals that feed infrequently and experience extended periods of fasting.

Keywords: Data-loggers; Digestion; Energetics; Foraging ecology; Gastric pH; Leopard sharks; Nurse sharks; Stomach

1. Introduction

Vertebrates possess multiple physiological and behavioral adaptations to feeding, and these adaptations are strongly related to foraging mode. Feeding frequency in the wild has been shown to influence the physiological performance and response of the gastrointestinal tract to periods of fasting in terrestrial vertebrates. While work has focused on the influence of feeding frequency and fasting on organ (heart, stomach, intestine, liver) morphology, intestinal uptake rates and metabolic rates (Secor and Nagy, 1994; Secor and Diamond, 1998; Andersen et al., 2005), far less is known of its influence on gastric digestion.

The stomach is the first organ used for major digestive action and is primarily tasked with the role of initiating protein digestion as well as sterilizing ingested prey. Concentrated hydrochloric acid (HCl) converts the inactive zymogen pepsinogen into the active protease enzyme pepsin, in addition to assisting in the physical breakdown of skeletal elements and other chitinous materials. Studies have shown that vertebrates differ in their regulation of gastric acid secretion during periods of fasting. Continuous gastric acid secretion is seen in fasting humans and rats (Sachs, 1994), while dogs (Sachs, 1994) and Burmese pythons (*Python molurus*) (Secor, 2003) appear to cease acid secretion when not feeding. Continuous acid secretion during periods of fasting is thought to provide sterilizing conditions, preventing the growth of bacterial flora on the gastric mucosa (Hunt, 1988; Joseph and Kirschner, 2004), but this would not explain why some vertebrates periodi-

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cally cease acid secretion when fasting. Unfortunately, continuous measurements of gastric pH have only been obtained from vertebrates in widely different taxonomic groups, yet it is unclear how variable gastric acid secretion may be between closely related groups.

Elasmobranch fishes are considered to be the earliest vertebrates to develop an acid secreting stomach (Smolka et al., 1994), and because they exhibit a wide variety of foraging modes (Motta, 2004), may provide a good comparative model for examining the causative factors that affect the gastric acid secretion response during fasting. Previous studies of gastric pH from surgically restrained or dead animals suggest that some species of elasmobranchs may exhibit continuous acid secretion during fasting while others exhibit a discontinuous mode of acid secretion (Sullivan, 1905; Babkin et al., 1935; Williams et al., 1970). Single sample measurements of gastric pH from dead nurse sharks (*Ginglymostoma cirratum*) indicated that acid secretion may not be continuous in this species, although the study did not take into account diet, meal size and time since feeding (Caira and Jolitz, 1989). Continuous measurements of gastric pH have only been made in one species, the leopard shark (*Triakis semifasciata*), which maintained low pH values of 1.54 ± 1.42 (± 1 S.D.) during periods of fasting (Papastamatiou and Lowe, 2004). Leopard sharks are known to be active foragers and may feed nightly, whereas nurse sharks are less active and may feed less frequently (Castro, 2000; Kao, 2000). Therefore, we hypothesize that the differences in gastric acid secretion during periods of fasting are related to feeding frequency and activity of these two species. The goals of our study were to (1) measure gastric pH continuously in free-swimming nurse sharks during periods of fasting, (2) compare changes in gastric pH over time (pH profiles) with those from leopard sharks, and (3) relate differences in the foraging modes of the two species, with the respective gastric pH profiles.

2. Materials and methods

We maintained four nurse sharks (*G. cirratum,* Bonnaterre 1788) of total length (TL) 154.3 ± 10.3 cm (mean ± 1 S.D.) and mass 24 ± 4.3 kg (Table 1) in large seawater tanks at the Mandelay Bay Shark reef Las Vegas, or the Long Beach Aquarium of the Pacific, CA, USA. Prior to the start of experiments, sharks were fed three to four times a week, but there was no fixed schedule with regards to feeding intervals or times.

In order to measure gastric pH continuously, we used autonomous pH/temperature data-loggers (earth & Ocean Technologies, Kiel Germany). The pH data-loggers consist of a microelectrode, a pressure compensating reference electrode with a free-diffusion liquid junction, an attached temperature sensor (accuracy ± 0.1 °C), and an 8-bit data-logger (Peters, 1997a,b). The data-loggers have dimensions of 11 cm long × 2 cm in diameter and a mass in air of approximately 80 g. Before deployment each pH data-logger was calibrated with four NBS standard pH buffers (1.68, 4.01, 6.86, 10.01). Water temperatures in the aquarium tanks were monitored continuously by aquarium life support personnel.

Table 1
Summary information for sharks fitted with autonomous pH/temperature probes

Shark #	Sex	Total length (cm)	Mass (kg)	Retention time (days)	Minimum pH	Maximum pH	Water temperature (°C)
N1	M	148	27	8	1.2	8.3	24
N2	F	145	19	13	0.4	1.4	26
N3	M	168	28	3	1.3	8.2	25
N4	M	147	18	13	1.1	8.7	26
L1	F	145	16	16	1.1	3.8	17
L2	F	149	10	5	0.6	1.3	19
L3	F	128	7.7	7	1.5	2.6	18
L4	F	125	7.3	11	1.5	1.6	18

N1–4 are nurse sharks (*Ginglymostoma cirratum*) while L1–4 are leopard sharks (*Triakis semifasciata*). Retention time is the duration the pH probe remained in the shark's stomach. Water temperatures in the tanks were kept constant. Minimum and maximum pH, represents values during periods of fasting (>48 h after feeding). Data for leopard sharks L1–4 are from Papastamatiou and Lowe (2004).

Sharks were force fed the pH data-loggers using the technique described by Papastamatiou and Lowe (2004). Sharks were anaesthetized using MS 222 (0.15 g/L), and a lubricated 3 cm diameter PVC tube was gently inserted through the mouth and into the stomach. The data-logger was then dropped down the tube into the stomach, followed by thawed fish to help prevent premature regurgitation. Sharks were measured, sexed and weighed before being revived and returned to the seawater tanks. The pH data-loggers provide accurate data for periods up to 16 days, depending on the rate of electrolyte outflow from the reference electrode (see Peters, 1997b). During the next 10–16 day period, sharks were fed 1–2 meals of squid at 1% of their body weight (%BW). Feeding times were designed so that the sharks went 5–7 days without eating between meals, so that we could measure changes in pH during periods of fasting. Nurse shark #1 was fed one meal during deployment, after which it was starved for 6 days (Fig. 1a). Nurse shark #2 and 4 were fed twice during deployment, with 8 days of fasting in between meals (Fig. 1b,d). Nurse shark #3 regurgitated the pH data-logger 3 days after deployment, before feeding could occur (Fig. 1c; Table 1). For the remaining sharks, the pH data-loggers were manually retrieved by anaesthetizing the shark as before and inserting a flexible magnetic retrieval device through the mouth into the stomach, which would attach to the end of the data-logger, allowing it to be gently pulled from the shark's stomach (Peters, 1997a, earth & Ocean Technologies). After retrieval, the pH data-loggers were re-calibrated in four NBS buffers (1.68, 4.01, 6.86, 10.01) in order to quantify any drift of the pH electrode. The data-loggers

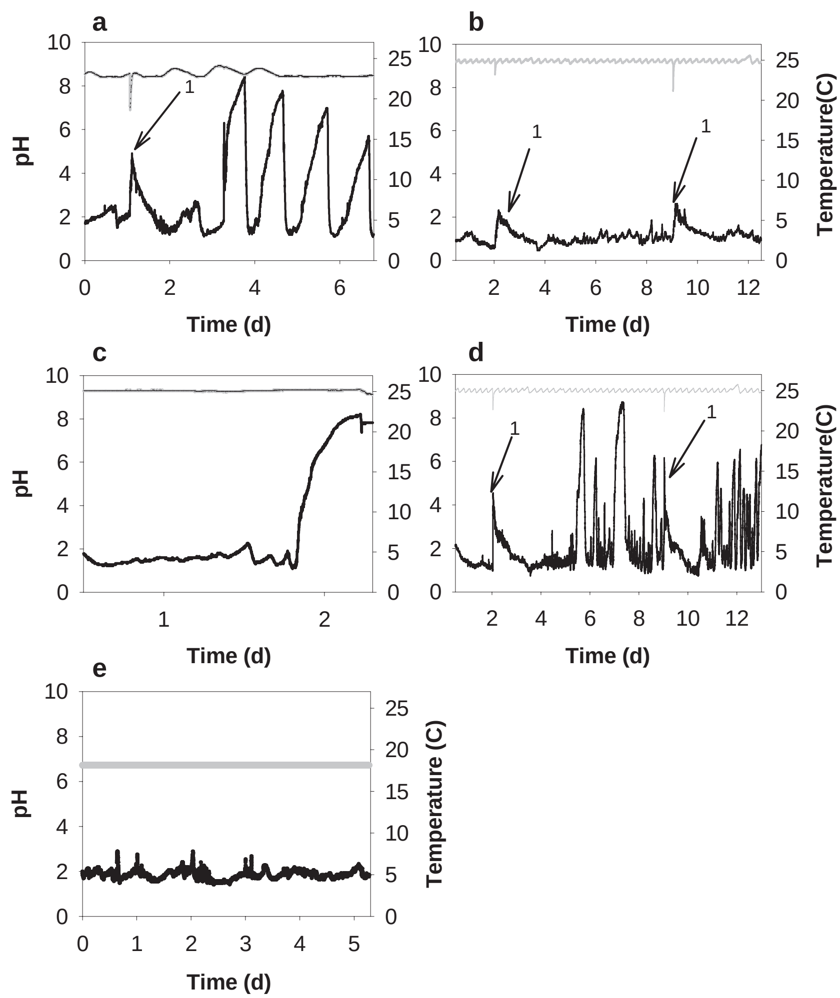

Fig. 1. Continuous measurements of gastric pH (lower black line) and temperature (upper grey line) in four free-swimming nurse sharks (*Ginglymostoma cirratum* (a–d represents nurse sharks #1–4, respectively) and one representative leopard shark #L1 ((*Triakis semifasciata*), e, data taken from Papastamatiou and Lowe, 2004). Data were obtained by using stomach placed autonomous pH/temperature data-loggers. Arrows indicate feeding events, and numbers above arrow are ration size expressed as a percentage of the shark's body mass.

were then downloaded and analyzed using Multi-trace pHG software (Jensen software), which interpolates and corrects for drift of the pH electrode as well as correcting gastric pH for any changes in gastric temperature (see Peters, 1997a). Using the pH drift model described by Peters (1997a), we determined that resolution of pH measurements was <0.062 units and accuracy was <±0.15.

We qualitatively compared pH profiles of fasting nurse sharks with pH profiles previously determined from fasting leopard sharks (Papastamatiou and Lowe, 2004). Maximum gastric pH levels of nurse and leopard sharks during periods of fasting were also compared using Student's *t*-test.

3. Results

Water temperatures in the aquarium tanks were maintained at constant levels (Table 1). Nurse shark stomach temperatures remained similar to ambient seawater (mean stomach temperature: 24.9±0.6 °C), although there were regular periodic oscillations. Stomach temperature decreased by 3.9±1.3 °C after feeding, as sharks were consuming frozen or recently thawed squid.

Nurse sharks secreted gastric acid with pH values as low as 0.4, with feeding causing a rapid increase in mean pH of 2.5±0.9 (±S.D.) followed by a more gradual decrease to mean baseline pH levels of 1.1±0.3. However, in three nurse sharks, mean pH rose to 8.4±0.3, within 2–3 days after feeding (Fig. 1a,c,d). After the initial rise in pH, two nurse sharks then showed regular oscillations in pH, with values ranging between 1.1 and 8.7, and acid secretion ceasing for 11.3±4.3 h day^{-1} (Fig. 1a,d). The rate of decline in pH that occurred during the oscillations during fasting periods (0.069±0.009 pH units min^{-1}) was greater than the rate of decline that occurred after feeding events (0.002±0.001 pH units min^{-1}) (t'=2.45, $p<0.0001$). A clear diel periodicity can be seen in the last 4 days of data from nurse shark #1 (N1, Fig. 1a), with pH slowly increasing throughout the day and evening, before rapidly declining at dawn, even though no feeding occurred during this period. There was also a linear decline in the maximum pH reached each day ($r^2=0.98$, $p=0.01$) (Fig. 1a). Nurse shark #2 did not exhibit the same profile as the others, with pH remaining low (~0.4) during 5 days of fasting (Fig. 1b). Nurse sharks had significantly higher maximum pH levels 3 days after feeding (mean 6.7±3.5) than leopard sharks (mean 2.3±1.1) ($t'=1.96$, $p=0.02$, Table 1).

4. Discussion

Gastric acid secretion does not appear to be continuous in nurse sharks. The slow rise in pH seen in three of the four sharks during periods of fasting suggests that acid secretion had ceased, rather than a sudden introduction of alkaline fluids (e.g. consumption of seawater). However, pH in the stomach increased above that of seawater, indicating that some alkaline fluids were accumulating in the stomach. This increase in alkaline fluids could possibly come from bicarbonate secretion by the gastric mucosa or duodenal reflux (Debellis et al., 1998). In addition, this pattern of elevated gastric pH during periods of fasting was also seen in nurse sharks that where caught on a long-line and their stomach pH sampled (Caira and Jolitz, 1989). Nurse sharks with empty stomachs had significantly elevated gastric pH levels (7.19±0.65), while those with food in their stomachs had lower pH levels (2.56±0.43) (Caira and Jolitz, 1989). Continuous monitoring of gastric pH from two nurse sharks suggests that although acid secretion may cease, it does so only for a short while and is regularly punctuated by bursts of acid secretion. Why one nurse shark continuously secreted gastric acid when fasting (Fig. 1b) is unclear, although acid secretion could be influenced by the health of the shark, gastric bacteria levels, and the energetic state of the individual (e.g. Joseph and Kirschner, 2004). Although the presence of the data-loggers themselves may have influenced the pH profiles of nurse sharks, in a previous study we found that the data loggers had no influence on acid secretion of leopard sharks (Papastamatiou and Lowe, 2004). The causative factors behind the remarkable periodicity in gastric pH seen in one of the nurse sharks (N1, Fig. 1a) during fasting are also unclear, although they may be related to some endogenous rhythm or feeding behavior. However, nurse sharks were not fed on any predictable time schedule prior to the initiation of the experiments.

Leopard sharks previously studied maintained acidic stomachs up to 5 days after feeding. In addition, we never recorded a pH value above 4.5 during continuous monitoring of six leopard sharks, or during single sampling events of an additional 13 leopard sharks (Papastamatiou and Lowe, 2004). However, leopard sharks were maintained in cooler water (17 °C) than the nurse sharks (25 °C). Although temperature can influence pH, the difference in stomach pH between these two species clearly exceeds the physical effects of temperature on pH.

Due to the fact that the species are from separate orders, it is possible that the variation in the response of gastric acid secretion to fasting is the result of a phylogenetic difference in physiology. Determining the influence of phylogeny will require measuring gastric pH in a wide variety of species, followed by phylogenetically independent contrast analysis (e.g. Secor and Diamond, 2000). The differences may also be explained by inter-specific variation in diet, intestinal morphology and enzymatic secretions. The morphology of the elasmobranch spiral valve is known to differ between species (Holmgren and Nilsson, 1999), and there may be inter-specific differences in the enzymes secreted by the stomach and the intestine (e.g. Holmgren and Nilsson, 1999; Gutowska et al., 2004). Thus, differences in the response of gastric acid secretion may also represent the use of alternative digestive enzymes with varying pH optima.

If the inter-specific differences in fasting gastric pH are the result of genetic differences between these two species, then one possible mechanism for these different responses may be attributed to feeding periodicity or foraging activity. Leopard sharks have been shown to feed relatively frequently in the wild (approximately every 35 h; Talent, 1976; Kao, 2000), move over large areas when foraging (Manley, 1995; Ackerman et al., 2000), and can often be seen foraging along the benthos (personal observation). While little is know about the feeding ecology of nurse sharks, it is a comparatively inactive species (Carrier, 1987), and a large proportion of nurse sharks caught in the wild have been found to have empty stomachs (Castro, 2000). In addition, the jaw morphology and feeding mechanics of nurse sharks are well suited to a lifestyle as an ambush predator (Motta et al., 2002; P. Motta, personal communication). Although qualitative, this implies that nurse sharks may feed less frequently and be less active than leopard sharks. Discontinuous acid secretion may be an energy conserving mechanism, whereby nurse sharks minimize costs by periodically ceasing acid secretion while fasting. Occasional bursts of acid secretion may be used to sterilize the gastric mucosa. Although prior feeding frequency or diel rhythms may influence periods of gastric acid secretion and cessation in nurse sharks while fasting, periodicity and frequency of acid secretion varied among individuals (Fig. 1a–d). Therefore, it is unclear what cues may regulate this response during fasting. More frequently feeding leopard sharks may maintain continuously acidic gastric conditions that increase digestive efficiency, while reducing digestion time. This may be advantageous to a frequently feeding opportunistic predator, allowing it to be physiologically prepared to digest a meal soon after a feeding event (Papastamatiou and Lowe, 2004).

While the mechanisms involved in regulation of acid secretion during periods of fasting are still unclear, the evolution of this ability is not limited to lower vertebrates and there may be some evidence that this ability may be used by other species that go relatively long periods between feeding events. For example, acid secretion has been shown to cease after gastric evacuation is complete in the Burmese python, a species that can often go two or more months without feeding (Secor, 2003). Acid secretion is thought not to be continuous in domestic dogs; however, this ability may be due to their evolutionary history of feeding on large prey with extended periods of fasting (Mech, 1970; Sachs, 1994). Humans and rats, both of which continuously secrete gastric acid, feed much more frequently, often more than once a day (Sachs, 1994). These findings further support our hypothesis that different species may have evolved abilities to regulate acid secretion during periods of fasting depending on their feeding ecology.

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