

# A new acoustic pH transmitter for studying the feeding habits of free-ranging sharks

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**Abstract** – Little is known about the feeding habits of large free ranging fish, due in large part to lack of an appropriate technique for quantifying feeding variables. A previous study demonstrated that changes in gastric pH can be used as a proxy for feeding events in free-ranging sharks. Here we describe the development of a new acoustic pH transmitter to remotely measure gastric pH in sharks in the field. The transmitter consists of a dual sensor (pH and temperature) continuous pinger, and was tested in captive adult blacktip reef sharks (*Carcharhinus melanopterus*). The transmitter was retained in the shark's stomach for between 5–12 days. The empty stomach had a low pH ( $1.6 \pm 0.2$ ) and feeding induced a rapid increase in gastric pH, which was clearly distinguishable from baseline levels. Meal size showed a significant linear relationship with the magnitude of the pH changes. Measurement accuracy of the pH transmitter ranged from 0.05–0.9, although resolution of the VR100 receiver that decoded the transmitter signal was 0.1 units. The pH transmitter can be used to determine when free-ranging sharks in the field are feeding and hence quantify feeding chronology, frequency and daily ration.

**Key words:** Acoustic telemetry / Blacktip reef sharks / Digestion / Foraging / Gastric pH

## 1 Introduction

Sharks are widely thought to play an important role in structuring marine communities but to date most evidence for this comes from analyzing stomach contents of dead sharks and laboratory experiments to determine gastric evacuation rates of juvenile sharks (Wetherbee et al. 1990; Cortes 1997). Stomach content analyses traditionally involve sacrificing a large number of animals in order to achieve adequate sample sizes and this is increasingly undesirable because shark populations in many areas are already in rapid decline due to over-harvesting (Cortes 1997; Baum et al. 2003). Yet in order to better understand the full impact of shark predation on marine ecosystems, and to predict what may happen if shark populations are depleted, we need to know what sharks eat, how often they feed and how much they typically consume (Wetherbee et al. 1990; Papastamatiou and Lowe 2004).

The stomach is the first site of digestion in vertebrates, and is responsible for the breakdown of prey items into semi-liquid chyme, which it achieves by secreting concentrated hydrochloric acid (HCl) and the inactive enzyme pepsinogen. The HCl breaks down prey hard parts and also converts pepsinogen into pepsin, a proteolytic enzyme (see Papastamatiou and Lowe 2004, 2005). Due to these physiological principals, a specific

change in gastric pH should occur after a shark consumes a prey item. Studies with captive sharks using autonomous pH data-loggers have shown that by measuring gastric pH continuously, time of feeding can be determined and meal size estimated (Papastamatiou and Lowe 2004, 2005). However, the pH data-loggers used in the captive studies are not practical for field studies because the data-logger must be retrieved for the data to be collected. Here we describe the development of an acoustic pH transmitter, which will enable gastric pH to be measured remotely in free-ranging sharks, hence allowing the technique to be applied to the field.

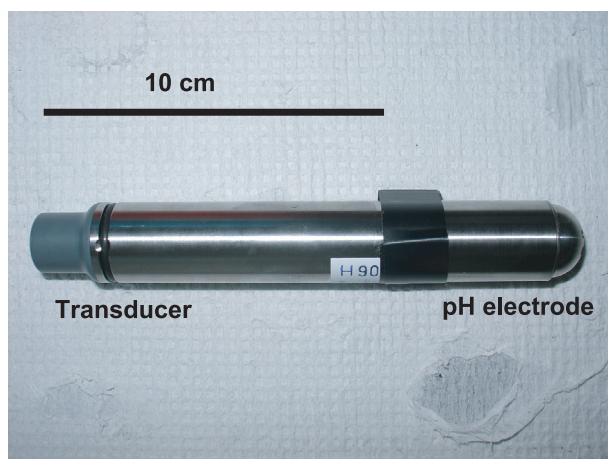
## 2 Methods

### 2.1 Study site

We maintained captive adult blacktip reef sharks (*Carcharhinus melanopterus*) in a pen situated in a lagoon at the Hawaii Institute of Marine Biology (HIMB). The water in the pen is tidally flushed and habitat consists of coral rubble, coral ledges, and sand with a maximum depth of 3 m. Sharks used for experiments were moved through a gate to an adjacent experimental pen (10 × 20 m), where they were acclimated for a week or until they resumed normal feeding. No more than two sharks were maintained in the experimental pen at any one time.

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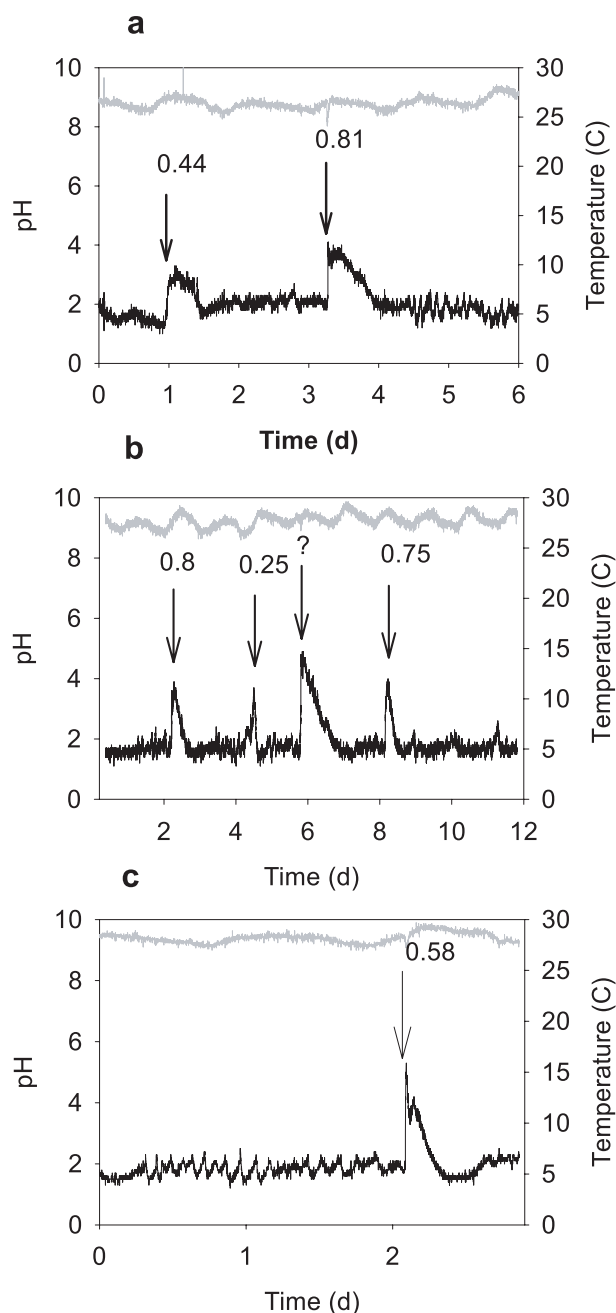
**Fig. 1.** Acoustic pH transmitter, with transducer and pH electrode labeled.

## 2.2 pH transmitter

We measured gastric pH using a pH/temperature dual sensor continuous pinger (Vemco Ltd, Nova Scotia). The sensor consists of a glass micro-electrode, and a reference electrode with a free-diffusion liquid junction connected to a piston-driven reservoir of electrolyte (Peters 1997a). The piston ensures a continuous supply of fresh electrolyte is passed over the reference electrode, greatly reducing measurement drift over a 16 d period, or until the reservoir runs out of fluid (Peters 1997a,b).

The reference electrode was coupled to a transducer, a 3.6 V lithium battery, and all electronics were encased within a titanium shell (total length of pH transmitter:  $17 \times 2.5$  cm, mass in air = 170 g, nominal battery life 50 d, Fig. 1). A temperature sensor encased in the electronics also recorded temperature. Both pH and temperature recordings were converted by the transducer into an acoustic signal with a carrier frequency of 60 kHz and a power output of 157 dB. The transmitter was a two channel continuous pinger, which transmits a continuous sequence of pings with three time intervals; a fixed interval of 1150 ms, followed by a signal for temperature and pH respectively. Changes in either temperature or pH result in a change of the interval of the emitted signal. The sensor measures pH between 0–9 units, and temperature between  $-5$ – $35$  °C. Due to the large amount of drift associated with measuring pH, the sensor had to be calibrated before every use. We calibrated the sensor using NBS standard pH buffers (1.68, 4.01, 6.86) and programmed the calibration parameters into a Vemco VR100 receiver. We deployed an omnidirectional hydrophone connected to the VR100 receiver, in the experimental pen. The VR100 recorded and stored all data received and was downloaded daily to a laptop computer.

To insert the pH transmitter, we restrained sharks on a stretcher, inverted them to induce tonic immobility (a trance like state), and anaesthetized them with a  $0.15 \text{ g L}^{-1}$  solution of MS-222. We then inserted a lubricated PVC pipe through the mouth into the stomach and dropped the transmitter through the pipe with the sensor pointing towards the caudal fin. The shark was then revived, released and fed meals of mackerel (*Scomber spp.*) over the next 5 to 12 d. After each shark



**Fig. 2.** Continuous measurements of gastric pH and temperature in three free-swimming blacktip reef sharks (*Carcharhinus melanopterus*, a-c equivalent to shark # 1-3). Lower line in each graph is gastric pH, upper line is gastric temperature. Data was obtained using a dual sensor pH/temperature transmitter. Arrows point to feeding events and meal size is expressed as a percentage body weight. “?” indicates a meal of unknown size.

regurgitated the pH transmitter, we re-calibrated the electrode in pH buffers, and used the pH drift model described by Peters (1997b) to estimate the uncorrected errors associated with drift of the electrode during deployment.

In order to quantify the affect of meal size on gastric pH changes, we determined the area underneath the feeding induced change in the pH-time curve using ArcView (ver. 3.2).

**Table 1.** Summary information for blacktip reef sharks used in experiments. Error analysis for the pH electrode is also given over three hypothetical pH values using the uncorrected pH drift model described by Peters (1997a).

Shark #	Total length (cm)	Mass (kg)	Sex	Deployment duration (d)	pH drift		
					2	4	6
1	155	26	F	7	0.45	0.24	0.92
2	140	19	M	12	0.38	0.27	0.15
3	150	24	F	5	0.14	0.05	0.24

We then used multiple regression analysis to determine the influence of stomach temperature and meal size (expressed both in g and percentage body weight %BW) on the area under the pH-time curve. Temperature and meal size were also set as an interactive term.

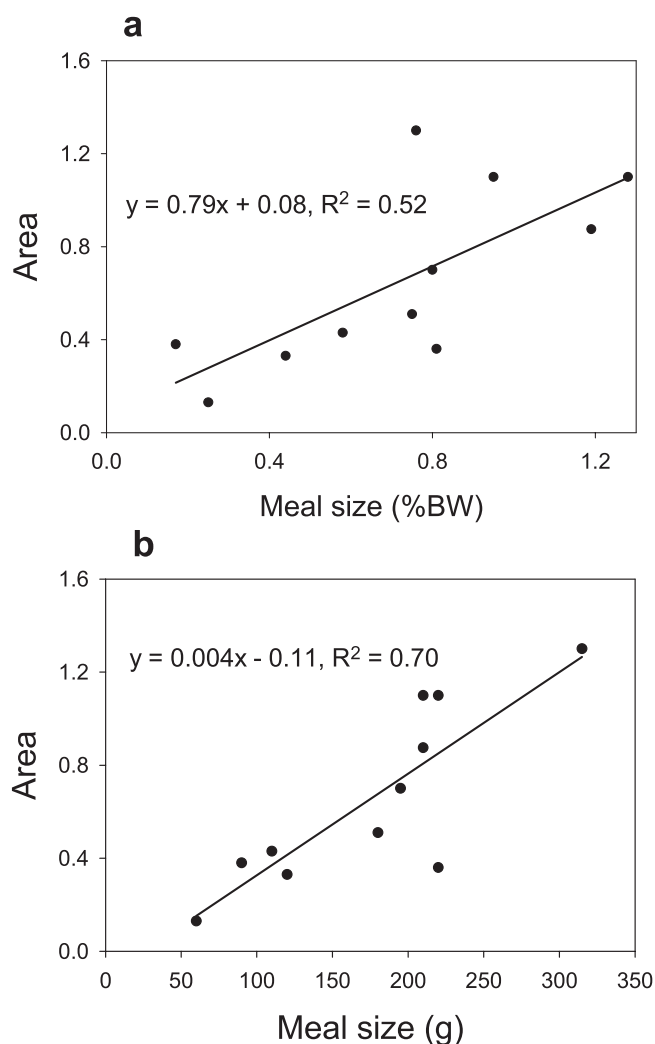
### 3 Results

We deployed the pH transmitter in three blacktip reef sharks (Total Length (TL) =  $148.3 \pm 7.6$  cm, mass =  $23 \pm 3.6$  kg) for periods ranging from 5–12 d. During deployment, pH error within the range measured in the stomach was between 0.05–0.45 (Table 1), although resolution of the VR100 receiver was 0.1 units. However, drift of the pH electrode was not linear as error ranged up to 0.92 in the pH 6 range, for one shark.

Stomach pH averaged  $1.6 \pm 0.2$  ( $\pm 1$  SD) during periods of fasting but increased rapidly to a peak of  $4.1 \pm 0.8$  following ingestion of food, and then declined more gradually back to baseline levels (Fig. 2). Multiple regression analysis revealed that meal size (g) influenced area under the pH-time curve ( $p = 0.001$ ,  $t = 3.36$ ), but stomach temperature had no significant affect ( $p = 0.881$ ,  $t = 0.15$ ). When analyzed independently, meal size had a significant influence on area under the pH time curve when expressed as % BW ( $p = 0.01$ ,  $r^2 = 0.52$ ,  $F = 9.89$ ), and in g ( $p = 0.001$ ,  $r^2 = 0.70$ ,  $F = 20.9$ , Fig. 3).

### 4 Discussion

The pH transmitter provides viable data on changes in gastric pH for up to 12 d. Measurement drift (0.05–0.45) was generally low within the typical range of pH values observed in the blacktip stomach (pH 1–4). Custom made software can be used to interpolate and correct for drift of the electrode if the transmitter is recovered (see Peters 1997a,b), although such recoveries are unlikely in field studies of sharks. The sharks used in the present study could only dive to a maximum depth of 3 m, and animals diving to greater depths may cause additional drift of the electrode. The flow rate of electrolyte out of the capillary junction of the reference electrode is crucial to the performance of the electrode, and suboptimal flow rates or electrolyte depletion, will increase measurement drift. Therefore, a larger electrolyte reservoir could increase the length of time over which the electrode can accurately measure pH. The



**Fig. 3.** Linear regression analysis between meal size and area under the pH-time curve from blacktip reef sharks, *Carcharhinus melanopterus*. Meal size is expressed as % BW (a) and in g (b). Data was obtained using a pH/temperature transmitter.

current pH transmitter has a depth rating of a 100 m, but embedding all electronic components in epoxy will greatly increase the depth rating (Peters 1997b).

Regardless of measurement accuracy, feeding events were clearly distinguishable from background variation in pH. Feeding resulted in a rapid increase in pH followed by a more gradual decrease to baseline levels. The rapid increase in pH is caused by seawater and the prey items buffering the small amounts of gastric acid present in the stomach during fasting (Papastamatiou and Lowe 2004). The presence of prey items in the stomach then causes distention of the mucosa wall and the release of secretagogues, all of which increase gastric acid secretion and the subsequent re-acidification of stomach contents (Papastamatiou and Lowe 2004, 2005). As meal size increases, a greater mass of prey (most of which are weakly acidic or neutral in pH) is in the stomach, and hence the time taken for gastric contents to re-acidify also increases.

The diet of blacktip reef sharks in the field consists primarily of reef fish (teleosts), crustaceans, cephalopods, and in some locations reptiles (Lyle and Timms 1987; Cortes 1999). Although daily ration has not been determined for this species, for other carcharhinids it has been estimated at approximately 1–2% BW/day (Wetherbee et al. 1990), within range of the linear regression calculated in this study, relating meal size to the postprandial changes in gastric pH. Extrapolating the regression to zero on the  $y$ -axis, predicts that the smallest meal size estimated using the transmitter would be 25 g. However, some caution will be required when applying the technique to the field, as gastric evacuation rates (and presumably post-prandial changes in gastric pH) are thought to be a function of prey lipid content (e.g. Anderson 1999). The prey used in the current experiments (mackerel) most likely have higher lipid contents than prey consumed by blacktip reef sharks in the field, potentially underestimating meal size (high lipid prey will have longer gastric evacuation times).

The changes in gastric pH and the linear response of time taken to re-acidify stomach contents, suggests that feeding chronology, frequency and meal size can be determined in free-ranging sharks. Although other similar sized marine organisms may differ in their gastric physiology, it is likely that the transmitter can also be used with large teleosts, marine mammals and birds. The primary factor limiting application of the technique is the size of the species in question, as it is unlikely than an animal smaller than 1 m will tolerate the transmitter. However, size of the transmitter can be reduced by selecting for a smaller transducer and battery (also reducing life span of the unit and signal strength).

## 5 Conclusion

We describe the development and testing of an acoustic pH transmitter. The pH transmitter can be used to quantify changes in gastric pH in free ranging sharks, which can be used as a proxy for feeding in the field. The pH transmitter can be used to quantify feeding frequency, chronology and daily ration in free ranging sharks.

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