Autonomous measurement of ingestion and digestion processes in free swimming sharks

Carl G. Meyer* and Kim N. Holland

Hawaii Institute of Marine Biology, University of Hawaii, PO Box 1346, Kaneohe, Hawaii 96744

Short Title: Autonomous measurement of shark feeding

Key words: Feeding ecology, digestive physiology, elasmobranch, tiger shark, sandbar shark

Summary

Direct measurement of predator feeding events would represent a major advance in marine trophic ecology. To date, devices available for empirically quantifying feeding in free-swimming fishes have relied on measuring stomach temperature, pH or physical motility, each of which has major, practical limitations. We hypothesized that the considerable physical changes which occur in the stomachs of carnivorous predators during the processes of ingestion and digestion should be quantifiable using Bulk Electrical Impedance measured across paired electrodes. We used a prototype archival data logging tag (Wildlife Computers Inc. Redmond, WA) to record changes in impedance inside the stomachs of captive, free swimming tiger and sandbar sharks over multiple, successive feeding events. Feeding and digestion events produced characteristic changes in electrical impedance of the stomach contents identifiable as 5 successive phases: (1) Pre-ingestion (empty stomach), (2) Ingestion, (3) Chemical 'lag' phase, (4) Mechanical 'chyme' phase, and (5) Stomach emptying phase. The duration of the chyme phase was positively related to meal size.

Introduction

In marine ecosystems, the advent of electronic tags has provided unprecedented new insights into movements of highly mobile sharks and fishes (Meyer et al., 2007; Meyer et al., 2010), but we still know very little about where and when these animals are feeding or how much they are eating (Papastamatiou and Lowe, 2004; Bestley et al., 2008). We need this information both to understand the ecology of individual species and also to gain a broader understanding of patterns of energy flow through marine food webs. Attempts to infer feeding by marine animals directly from their spatial behavior (e.g., specific dive patterns or shape, tortuous segments of tracks, etc.) have assumed that these behaviors are reliable proxies for feeding (Robinson et al., 2007; Papastamatiou et al., 2009). However, in the case of sharks and highly mobile fishes (except southern bluefin tuna, *Thunnus maccoyii*, Castelnau – see below), such interpretations are completely speculative and empirical validation of feeding is lacking (Bestley et al., 2008; Weng et al., 2008).

In the case of southern bluefin tuna, stomach temperature telemetry was successfully used to empirically quantify feeding in free-ranging individuals and demonstrated that time spent within an area had no clear relationship with feeding success (Bestley et al., 2008). Thus, the utility of stomach temperature telemetry alone for quantifying feeding in fishes and sharks appears to be limited to a small number of endothermic species which either utilize visceral warming to aid digestion (Bestley et al., 2008) or maintain relatively warm stomach temperatures and ingest cool prey (and cool sea water) during feeding (Goldman, 1997; Papastamatiou and Lowe, 2004). However, even in endothermic fishes, interpretation of cold spikes in stomach thermal records could be confounded by fishes drinking cool seawater (Wood et al., 2007; Anderson et al., 2006; Anderson et al., 2009) without ingesting food.

A more broadly applicable and unequivocal measure of feeding is clearly needed and in recent years stomach pH tags were successfully used to identify feeding events and quantify meal size in captive poikilothermic sharks (Papastamatiou et al., 2008). Unfortunately, these devices also proved to have major shortcomings including a limited lifespan for measuring pH (maximum 16 days), sensor drift, inherent fragility due to the incorporation of a glass electrode and a maximum depth limit of 100m (Papastamatiou and Lowe, 2004). To substantially advance the field of marine trophic ecology we need a more-

robust device with solid-state sensors capable of providing long-term, quantitative feeding data from a wide variety of free-ranging sharks and fishes.

Most sharks and large predatory fishes initially ingest prey whole, or in large pieces, along with seawater. In the stomach this food is chemically and mechanically processed into semi-liquid chyme (an opaque slurry consisting of partially digested food) (Papastamatiou and Lowe, 2004). We hypothesized that these considerable physical changes in the stomach contents should be detectable using bulk electrical impedance. To test this hypothesis we used a prototype electronic archival tag (Wildlife Computers Inc. Redmond, WA) to monitor the stomach environment of captive sharks during successive feeding and digestion events.

Materials and methods

Bulk electrical impedance (hereafter 'impedance') of the stomach environment was measured using a circuit incorporating two stainless steel electrodes separated by a distance of 1.3 mm. Impedance (Ohms) and stomach temperature were sampled at 1-sec intervals and stored in the data logger's archive. Electronic components of the tag were encased in hard plastic resin and the tag was encased in a small block of syntactic foam (device overall length =15cm, width = 2.5cm, depth = 2cm, volume = 75ml, weight in air = 56g) to facilitate recovery after regurgitation (i.e. to ensure the device floated). The tags were hidden inside pieces of food (e.g. a large mackerel) and fed to a captive tiger shark (*Galeocerdo cuvier*, Péron and Lesueur; 217 cm Total Length, calculated weight 51kg) and sandbar shark (*Carcharhinus plumbeus*, Nardo; 191 cm Total Length, calculated weight 61kg) in an outdoor holding facility (a semi-enclosed lagoon) at the Hawaii Institute of Marine Biology. Captive sharks each retained the data logger in their stomachs for between 8 and 22 days, during which they were fed during daytime at intervals of 1 to 3 days. Meals weighing 0.4 to 3.8 kg (0.8-7.5% of body weight) consisted of previously frozen fish, shark and squid tissue. To create thermal reference points in the data logger temperature records (i.e. 'cold spikes' indicating time of food ingestion; see also Papastamatiou and Lowe 2004), some meals were below ambient seawater temperatures (26.7-29.0 ^{o}C) when fed to sharks, whereas other meals were at ambient temperature to control for possible cold effects on physiological or sensor responses.

Results and discussion

Ingested data loggers recorded changes in impedance and temperature directly correlated with feeding and digestion events (Figs 1, 2). Background variation in temperature was also evident as a diel cycle with a 2^{o}C amplitude (minimum nighttime low of 26.7 ^{o}C, maximum daytime high of 29.0 ^{o}C) (Figs 1, 2). Cold spikes lasting up to 2h were seen whenever sharks were fed food below ambient temperature (Figs 1, 2), but there was no thermal evidence of feeding or digestion (no visceral warming) in sharks given food at ambient temperature.

In both shark species, feeding and digestion events produced characteristic changes in electrical impedance of the stomach contents, identifiable as 5 successive phases: (1) Pre-ingestion (empty stomach), (2) Ingestion, (3) Chemical 'lag' phase, (4) Mechanical 'chyme' phase, and (5) Stomach emptying phase. Empty stomachs were characterized by a baseline, 'medium' level of impedance (Figs 1, 2). Ingestion (feeding) events were characterized by a rapid drop and low variance in impedance (Figs 1, 2), presumably because of the intake of high-conductivity seawater along with the food (Papastamatiou and Lowe, 2004). Electrical impedance remained low for a period of 7-13 hours after ingestion of food. This 'lag phase' was consistent with a post-prandial period of low stomach motility previously described by Papastamatiou et al. (2007), during which ingested food is steeping in an increasingly acidic mix of seawater, stomach acid and enzymes, but the stomach is not contracting (Papastamatiou et al., 2007). The lag phase was followed by a marked increase in the variance, mean and maximum levels of electrical impedance (Figs 1, 2). The start of this 'chyme' phase is consistent with a transition from low to high stomach motility described by Papastamatiou et al. (2007), and likely reflects the onset of stomach contractions where mechanical action begins to break up large pieces of food into smaller pieces to form chyme. Average electrical impedance increased over time during this phase, possibly reflecting increased lipid content and increasingly uniform emulsification of the chyme (see Kuzmina et al., 2008). The end of the chyme phase was characterized by a relatively rapid (1-4 h) decline in electrical impedance to baseline (empty stomach) levels. This was presumably caused by chyme being passed from the stomach down into the spiral valve of the intestine. Regression analyses indicated a significant positive linear relationship between meal size and the duration of the chyme phase in both sandbar and tiger sharks (Fig 3).

Although the temperature sensors only detected the arrival of artificially cold food in these captive experiments, they are nonetheless still potentially valuable sources of corroborative evidence of feeding and digestion detected by electrical impedance sensors. For example, even in poikilothermic sharks, the temperature sensor could record natural cold spikes associated with animals making yo-yo dives to feed in deep, cold waters. Yo-yo diving behavior is common among sharks (e.g. Nakamura et al., 2011) and although water temperature generally decreases with increasing depth, stomach temperature is likely to lag due to the insulating effects of the shark's body mass. Thus, a deep diving poikilothermic shark will probably have a relatively warm stomach (reflecting ambient temperatures in the warmer, surface mixed layer) at depth unless cold prey and seawater are ingested during feeding (producing a cold spike). Such cold spikes could be cross-referenced with changes in stomach content impedance records to confirm prey ingestion.

Bestley et al. (2008) noted their bluefin tuna visceral warming technique could not distinguish between 1 kg ingested over a few minutes and 1 kg eaten over a number of hours. Thus, repeated ingestion of multiple prey items over a period of several hours could only be identified as a single feeding 'event', thereby limiting conclusions about actual feeding times. By contrast, the rapid drop in impedance associated with ingestion in the current experiments suggests that individual feeding events (even on small items) can be detected. Our new approach provides a more precise indication of the time of initial food ingestion, but further experimentation is required to determine the maximum resolution of multi-sensor stomach data loggers (i.e., whether we can detect individual consumption of multiple small prey items over relatively short periods of time). It is possible that combining multiple sensors may allow us to distinguish individual prey ingestion events. For example, impedance sensors could be combined with accelerometers, with the latter potentially able to quantify distinctive signatures associated with chasing, capturing and swallowing prey. The average impedance values and patterns of variability associated with each phase may be sufficiently distinct to permit on board identification of feeding events and remote recovery of processed data via satellite uplink or acoustic modem in future generations of stomach tags.

Acknowledgements and Funding

We thank Wildlife Computers for providing the prototype stomach data loggers utilized in these experiments. We are grateful to T. Lindstrom for his technical advice. We thank

Monterey Bay Aquarium for providing funds for capture and maintenance of captive sharks used in experiments.

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Figure legends

Figure 1. Detail of two consecutive feeding events recorded in the stomach of a 217 cm Total Length tiger shark (*Galeocerdo cuvier*). Black arrows at top indicate time and size (kg) of meals. Bottom panel: Raw impedance (red points) plus 5% and 95% percentile (upper and lower black lines) values. Vertical dashed lines denote start and end of digestive phases. For reference, the impedance of seawater at 24^{o} C = 35Z. Middle panel: Hourly variance (green line), median (black line) and mean (brown line) impedance. Top panel: Temperature (blue line). Note cold spikes associated with ingestion of meals.

Figure 2. Detail of two consecutive feeding events recorded in the stomach of a 191 cm Total Length sandbar shark (*Carcharhinus plumbeus*). Black arrows at top indicate time and size (kg) of meals. Bottom panel: Raw impedance (red points) plus 5% and 95% percentile (upper and lower black lines) values. For reference, the impedance of seawater at 24^{o} C = 35Z. Middle panel: Hourly variance (green line), median (black line) and mean (brown line) impedance. Top panel: Temperature (blue line). Note cold spikes associated with ingestion of meals.

Figure 3. Regression analyses of chyme phase duration (h) versus meal size (g) in sandbar (top) and tiger (bottom) sharks.

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