Journal of Experimental Marine Biology and Ecology 393 (2010) 188–192

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Non-lethal method to obtain stomach samples from a large marine predator and the use of DNA analysis to improve dietary information

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ARTICLE INFO

Article history:
Received 16 February 2010
Received in revised form 24 July 2010
Accepted 27 July 2010

Keywords:
Diet
Gastric lavage
Notorynchus cepedianus
Shark
Stomach flushing

ABSTRACT

Dietary information of apex predators is crucial to understanding community dynamics and ecosystem processes. However, as dietary studies traditionally involve lethal sampling, obtaining this essential information can have repercussions on predator populations and the structure and functioning of marine ecosystems. With stronger emphasis being placed on conservation of species that are vulnerable to overexploitation, the need for non-destructive methods of sampling is imperative, as is the requirement to maximize the information obtained from each sample. Stomach flushing (gastric lavage) and DNA analysis of stomach contents methods were tested on the broadnose sevengill shark *Notorynchus cepedianus* Peron 1807. Acoustic tracking and recaptures of sharks implied high survivorship post-fishing and stomach flushing. From 85 prey items collected, 36 (43%) could be identified to species level using morphological analysis. After DNA analysis, a further 35 items were identified to species level, doubling the information obtained from these stomachs. The number of *N. cepedianus* that were confirmed to have eaten gummy sharks *Mustelus antarcticus* Gunther 1870 also doubled after DNA analysis. Without DNA analysis (of stomach contents) the importance of *M. antarcticus* in the diets of *N. cepedianus* would have been substantially underestimated. In addition, the non-lethal approach provides an opportunity to obtain meaningful information from non-harvested, endangered or rare species or sampling of species within protected areas.

1. Introduction

Stomach content analysis is a key technique in animal ecology and fisheries research (Hyslop, 1980; Light et al., 1983; Hartleb and Moring, 1995). Dietary studies of fish traditionally involve lethal sampling and to ensure sampling integrity, large sample sizes are usually required to accurately measure a species' diet (Hartleb and Moring, 1995; Kamler and Pope, 2001). However, for many shark species, there is growing concern about killing these animals, and the conservation of shark species is becoming a well publicized topic (Heupel and Simpfendorfer, in press). Nevertheless, the conservation status of most shark species is classified as being data deficient; highlighting the need for basic biological and ecological data to make adequate conservation decisions (Heupel and Simpfendorfer, in press). In addition, as many sharks are apex predators, dietary information is also needed to help assess the role of these large predators in marine communities, but accurate information is still lacking for the majority of species (Braccini, 2008; Heithaus et al., 2008).

Therefore, while considering both conservation and the need for information, scientists are presently exploring non-lethal methods of biological and ecological data collection (see Heupel and Simpfendorfer, in press). A non-destructive technique previously used in diet studies of fish is stomach flushing, also referred to as gastric lavage (Light et al., 1983). This technique involves pumping water via a tube down the throat of the animal into the stomach, and expelling the stomach contents via the mouth.

Although well known amongst shark researchers, only one study has reported the use of stomach flushing to extract stomach contents from a shark species (Medved, 1985). Sandbar sharks *Carcharinus plumbeus* were flushed to investigate gastric evacuation rates. The method was verified by dissecting 18 individuals after flushing, to discover that all stomachs contained no food and very little water. Despite the technique showing promise, to date, no study has addressed the possible long term effects from stomach flushing. Other non-lethal methods have also been used to extract stomach contents from elasmobranchs. For instance, forceps have been used to evert the stomachs of small sharks (<136 cm TL) (Schurdak and Gruber, 1989; Cortes and Gruber, 1990; Webber and Cech, 1998; Bush,

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doi:10.1016/j.jembe.2010.07.022

2003). However, since this method involves either restraining the shark upside down or anaesthetizing the animal before reaching into its mouth with forceps and pulling the stomach out, it is not appropriate for larger species. Thornback skates *Raja clavata* and lesser spotted dogfish *Scyliorhinus canicula* in captivity have also been injected with a emetic to induce stomach eversion (Andrews et al., 1998; Sims et al., 2000). Vomiting occurred up to 10 min after the injection and in some cases the animal everted its stomach multiple times (e.g. nine times for one skate) (Sims et al., 2000). However, this method is not appropriate for wild animals that cannot be kept in tanks until they vomit. Therefore gastric lavage may be the most promising method for extracting stomach contents from large sharks in the field.

Regardless of the method used to gather stomach contents, accurately quantifying stomach contents from morphological characteristics often fails to achieve species level identifications due to a lack of hard remains, soft bodied prey and well digested remains (Haywood, 1995; Reñones et al., 2002). In many cases, prey can only be identified to a broad taxonomic category. These broad categories may not be sufficient to give accurate information on species interactions and underestimate the importance of certain taxa as prey. This is particularly relevant for generalist predators or when a predator preys upon a large number of species from similar taxonomic groups (Symondson, 2002). In these cases, DNA based methods can improve the probability of species level identification (Jarman et al., 2004).

DNA analysis has been used to identify prey from faecal material for a number of seal species (Purcell et al., 2004; Orr et al., 2004; Kvitrud et al., 2005; Parsons et al., 2005; Casper et al., 2007), whales (Jarman et al., 2002; Jarman et al., 2004), whale sharks (Jarman and Wilson, 2004), lobsters (Redd et al., 2008) and penguins (Jarman et al., 2002; Jarman et al., 2004; Deagle et al., 2007). Two studies have also used molecular techniques to investigate the stomach contents of predatory fish (Rosel and Kocher, 2002; Smith et al., 2005). All the studies above with the exception of Smith et al. (2005) (predatory fish) and Deagle et al. (2007) (penguins) developed group-specific PCR primers to amplify specific target species. In general, this usually requires some prior knowledge of the likely diet (Valentini et al., 2008). For predators with diverse diets, a universal primer approach (primers designed to amplify a wide range of taxa) is more appropriate (Valentini et al., 2008). With the universal approach DNA sequences are normally run through a barcode database (e.g. GenBank) to see if they match with sequences previously deposited in the system. For example, universal primers identified prey items from predatory fish from western equatorial Pacific with 95–100% accuracy using GenBank (Smith et al., 2005). Both group-specific and universal primer sets have also been used to study penguin diets (Deagle et al., 2007), and, both methods produced similar results, demonstrating the effectiveness of universal primers in dietary analysis (Valentini et al., 2008). However, to avoid biased conclusions caused from universal primers failing to amplify all prey species, samples should be analyzed with multiple universal primer sets to allow cross validation (Deagle et al., 2007).

Another limitation to using barcoding data bases is the misidentification or non-identification due to the reference database not containing a comprehensive list of the species in a group that is being studied (Deagle et al., 2007; Valentini et al., 2008). However, with the ever increasing number of sequence data (barcoding markers) continually added to databases and the improved quality and rigorous design of new databases (e.g. Barcode of Life Data Systems, BOLD) this problem should be negligible for future DNA barcode dietary studies (see Valentini et al., 2008 for a review on the use of barcoding in ecology).

This study aims to test the use of stomach flushing to acquire dietary samples for sub-adult and adult (size range 150–290 cm TL) sevengill sharks *Notorynchus cepedianus* and evaluate post-flushing survival rates. The use of universal primers for DNA dietary analysis to improve the quantity and quality of dietary information was also investigated for this species.

2. Methods

2.1. Field methods

Stomach samples were collected from the Derwent Estuary and Norfolk Bay south east Tasmania, Australia (43.00°S; 147.76°E). *N. cepedianus* were caught using bottom-set longlines that were set for 4–6 h. Soak times of 4 h were used as it normally takes 2–4 h to attract a number of sharks to the lines (Pers. obs.). Once landed, their stomachs were flushed, total length measured, and they were tagged in the dorsal fin with plastic Jumbo tags (Daltons, Henley-on-Thames, England) (20 individuals were also acoustically tagged, see below for detail) and returned to the water.

Stomach flushing was undertaken by restraining the shark, while a plastic hose (~3 cm diameter) attached to a submersible electric pump was inserted through the mouth into the stomach. Seawater was pumped into the shark's stomach. Once the stomach was filled with water, which was evident by the expansion of the stomach region, the hose was removed and gentle pressure applied to the abdominal region causing the water and any food items to be regurgitated (~3 min per flush). Any material regurgitated was collected in a sieve, bagged, labeled and placed on ice for subsequent morphological and molecular analysis. Ten sharks were dissected after flushing to validate the effectiveness of this flushing method. If prey species that are vulnerable to capture from longlines showed no signs of digestion, they were judged to be recently ingested and were excluded from analysis due to the likelihood that they were eaten from the longline.

2.2. Survival after capture and stomach flushing procedures

To assess the survivorship of sharks after fishing and stomach flushing, we recorded the number of sharks recaptured. In addition to the use of conventional tags (recapture data), 10 individuals were tagged with acoustic transmitters after being stomach flushed, and their post-release survivorship compared to 10 other individuals that were also tagged but not stomach flushed. Acoustically tagged sharks were released within an acoustic monitoring array consisting of 72 receivers deployed throughout the study area. Coded acoustic transmitters (VEMCO Ltd., Halifax, Canada) were inserted though a ~2 cm incision in the abdominal wall into the body cavity. The incision was closed with a surgeon's suture. The entire procedure was normally accomplished in 3 to 5 min. Running water was pumped over the shark's gills throughout the procedure to ensure the gills remained wet.

2.3. Dietary analysis

In the laboratory, stomach contents were identified to their lowest taxonomic level using morphological characteristics. For stomach samples containing prey that was sectioned in multiple pieces ($n = 38$), we first had to determine the number of prey. As with most dietary studies, there was the potential for some inaccuracy in determining the number of prey at this stage, however, we used a methodology aimed at minimising the risk of identifying multiple pieces of the same prey as separate prey items. This was done by piecing body parts together and looking for changes in the size or repetition of body parts (e.g. two sets of jaws would indicate two of that particular prey species present or disproportionate body parts would indicate more than one individual). Stomach contents that only contained a small amount of a single tissue type e.g. <50 g of muscle were assumed to be from a single animal. For prey that could not be positively identified to species level, a small piece of tissue (approximately 2 mm^3) was removed for molecular analysis. This