Research and Management Techniques for the Conservation of Sea Turtles
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Diet Sampling and Diet Component Analysis

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The ability to sample the diet of sea turtles allows studies of the feeding ecology and physiology of these animals. Data from such studies can provide insight into questions relating to habitat utilization, digestive physiology, energetics, diet contaminants, trophic ecology, endoparasites, and the relative health of an individual turtle. Additionally, knowledge of the breadth of the diet of a turtle population allows conservation efforts to be directed to protect areas that provide such foods.

The feeding habits of wild turtles can be determined by a variety of methods, but the preferred technique is gastric lavage or stomach flushing. This comparatively simple and reliable technique has been used successfully to sample the gut contents of various vertebrate groups without harm to the animal. A system of stomach flushing of sea turtles has been developed (Forbes and Limpus, 1993) that allows rapid retrieval of large volumes of undigested food from the esophagus and anterior stomach regions of sea turtles. The technique described below has been widely and successfully used on green turtles, hawksbills, flatbacks, olive ridleys, and loggerheads ranging in size from approximately 25 to 115 cm curved carapace length (CCL). The technique should be equally successful on leatherbacks, if they could be lifted and moved as required throughout the procedure.

It is useful to note that other procedures (other than gastric lavage) also offer research potential, but they are not without their shortcomings. In analyzing samples from dead or moribund turtles, care should be exercised in the interpretation of results as the diets of these animals may not reflect the diets of healthy individuals. Diet may also be inferred from observations of turtles feeding in the wild. However, the difficulties of approaching and observing free-ranging sea turtles underwater preclude such studies under most circumstances. Collection of food fragments from the mouths of captured wild turtles can provide insight into diet, but the sample may represent only those dietary items that are hard to swallow *(e.g.,* the tentacled hydrozoan *Physalia* spp.) or are caught on various mouth structures such as the nasal choanae. The sampling bias inherent in this technique would be difficult to overcome.

Data on the food habits of wild sea turtles can also be obtained from direct underwater surveys, or from the examination of feces. Underwater surveys aimed at finding and evaluating evidence of turtle feeding activity require that an investigator locate physical evidence of turtle cropping, such as seagrass grazing plots or bite marks in sponges and gorgonians. The reliability of this technique depends on the ability of the observer to locate and accurately identify turtle cropping marks on sessile benthic organisms. Collecting fecal samples is problematic and time consuming. Additionally, the quantitative data available from fecal analyses are limited by the differential digestibilities of various dietary components which affect their representation in the feces when measured by either volume or weight.

The examination of digestive tract contents from healthy turtles captured in the wild and then sacrificed is one of the best determinations of diet. However, the ecological and moral implications of sacrificing sea turtles generally preclude this technique unless the turtles are taken in fisheries activities.

Gastric Lavage Technique

Turtles are placed on their carapace at a height which allows the head to be positioned lower than the dome of the carapace while allowing unencum-

bered access to the animal's head. The carapace should be supported to prevent the animal from rocking. Placing the turtle on a small automobile tire laid flat (wheel removed) in a wheelbarrow provides an excellent surface for support, restraint, and subsequent transport of the animal. For optimal drainage, the posterior end of the turtle should be elevated slightly higher than the head. It is rare for turtles to struggle once secured as described. Small turtles can be hand-held in the lap. Gyuris and Limpus (1986) have described a method for restraining the front flippers of large turtles.

The mouth is opened by holding the head securely and gently inserting a thin stainless steel pry bar between the maxilla and mandible. Pry bars can be easily made from flat steel stock but care should be taken to round and smooth all surfaces to reduce the risk of damage to the mouth cavity (Table 1). Although pry bars are the most effective and safe instruments, other common items such as wide blade screwdrivers and steel scalpel handles can be modified as a temporary pry bar although care must be used to prevent harm to the turtle.

The pry bar is inserted vertically between the maxilla and mandible and a gentle downward pressure is applied until the pry bar can be felt butting against the palate. At this point, the free end of the bar should be rotated downward (towards the cranium). This motion should be made gently as the intent is not to force the mouth open but to provide an irritating pressure which will cause the turtle to open its mouth. Attempting to force the jaws open will result in damage to the jaws and may hinder the animal's ability to feed. As the turtle opens its mouth, the bar is slid rapidly across the mouth cavity and out the other side at which time it is held in place at both ends until a mouth gag can be placed into position (Figure 1). Caution must be exercised to avoid striking the internal nares while passing the pry bar through the mouth.

A standard veterinary canine mouth gag is inserted into the mouth while the pry bar is held in place by an assistant (Figure 1). The gag should be inserted at the anterior end of the mouth and then expanded. The gag should be checked for stability before removing the pry bar. The gag should be expanded only to the point at which it is secure and not as far as the mouth will open as this will tear the soft dermal tissues at the junction of the mandible and maxilla. Should the turtle open its mouth further, the gag's tension spring will automatically expand the gag.

If veterinary gags are not available, polyvinylchloride (PVC) water piping can be used as a tubular gag for small to medium sized turtles. Thick-walled (4.0 mm) PVC pipe is cut into lengths of 1.5 cm. The inside diameter (ID) of the PVC pipe will be determined by the size of the turtle. Turtles >65 cm CCL require an ID of at least 4.5 cm, turtles 40-65 cm CCL an ID of 3.5 cm, and turtles <40 cm CCL an ID of 2.0 cm. Extremely large animals and loggerheads may require a tubular gag made from steel piping rather than PVC. Steel gags should have a soft coating, such as tire inner tubes, bonded to their surface to prevent slipping and damage to the mouth. The PVC or steel tubular gag should be positioned so that its opening is in line with the esophagus. It is more difficult to open the mouth wide enough to secure the tubular gag than with the adjustable veterinary gag.

Following the insertion of the gag, two flexible clear plastic tubes are inserted into the esophagus, one on each side of the gag. The first tube inserted is the retrieval tube that carries the displaced stomach contents into a mesh collection bag. The second tube is the water injection tube that carries the lavage water into the turtle. The retrieval tubing should have a wall thickness of 2.0 mm. A thinner wall may allow the tubing to collapse while a thicker wall will not provide enough flexibility. The largest diameter of tube possible should be used as large pieces of food may clog the retrieval tube (Table 1). The water injection tube should be 5.0 mm ID with a wall thickness of 1.0-1.5 mm and 3 m in length. Turtles <40 cm CCL require a tube of 3.5-4.0 mm ID. The ends of all tubes should be sanded or melted with a flame to provide smooth, rounded ends.

A mesh collection bag is fitted at one end of the retrieval tube. This bag can be made from fiberglass window screen netting or similar small mesh material. The top of the collection bag is equipped with purse draw strings that allow the bag to be drawn tightly against the tube. To prevent the bag

Table 1. Recommended dimensions of pry bars and retrieval tubes for three size classes of sea turtles. CCL is curved carapace length; ID is inside diameter.

CCL (cm)	Pry Bar	Retrieval Tube
25-50	2.0 mm x 12 mm x 15 cm	12 mm ID x 1.0 m
50-60	2.5 mm x 20 mm x 20 cm	16 mm ID x 1.5 m
>60	2.5 mm x 25 mm x 20 cm	20 mm ID x 1.5 m

from slipping off the tube, several cable ties or automotive hose clamps should be permanently placed on the outside of the tube 2-4 cm from the end. Markings are made on both tubes at 10 cm intervals from the insertion end to monitor the length of tubing inserted into the esophagus.

Before inserting the retrieval tube, one person must firmly grasp the head and extend the neck fully while keeping the head in line with the mid-line of the plastron and level with the plane of the plastron. This position must be maintained throughout the flushing procedure to prevent harm to the animal.

The tip of the retrieval tube should be dipped in a lubricant such as vegetable oil and then gently placed into the anterior end of the esophagus. If the glottis hinders the entrance of the tube, it can be depressed with the pry bar. Resistance from a muscle group near the anterior of the esophagus is frequently felt once the tube passes the glottis. If careful manipulation of the tube into the esophagus is not made at this point, delicate dermal tissues could be damaged and slight hemorrhaging could occur as evidenced by drainage of blood into the tube. As adult turtles may have large and partially convoluted trachea that hamper the insertion of the tube, they may require external manipulation of their trachea to facilitate passage of the tube.

Once the retrieval tube has passed the esophageal muscle group, the lubricated injection tube is slid in laterally along the retrieval tube (Figure 2). Lateral positioning of this tube will reduce the risk of entering the trachea which should already be sealed by the retrieval tube. Both tubes are now passed down the esophagus simultaneously until resistance is felt from either the food bolus or the junction of the esophagus and the stomach. This junction occurs ventral to the heart. In feeding turtles, a food bolus will normally be encountered before the junction. The distance to this junction can be determined prior to tube insertion by laying the tube along the midline of the plastron and measuring from the junction of the humeral and pectoral scutes to the tip of the mouth. The stomach flushing procedure should not begin at a depth greater than this measured distance.

Fresh or saltwater is now delivered through the injection tube. The flow valve to the water delivery system must be close by so that it can be turned off rapidly. If water delivery is through a pressurized domestic system, an optimal delivery pressure to the injection tube is 10-25 psi (9 liters/min). Delivery pressures for turtles <40 cm CCL should fall in the low end of this range. Delivery pressures can be de-

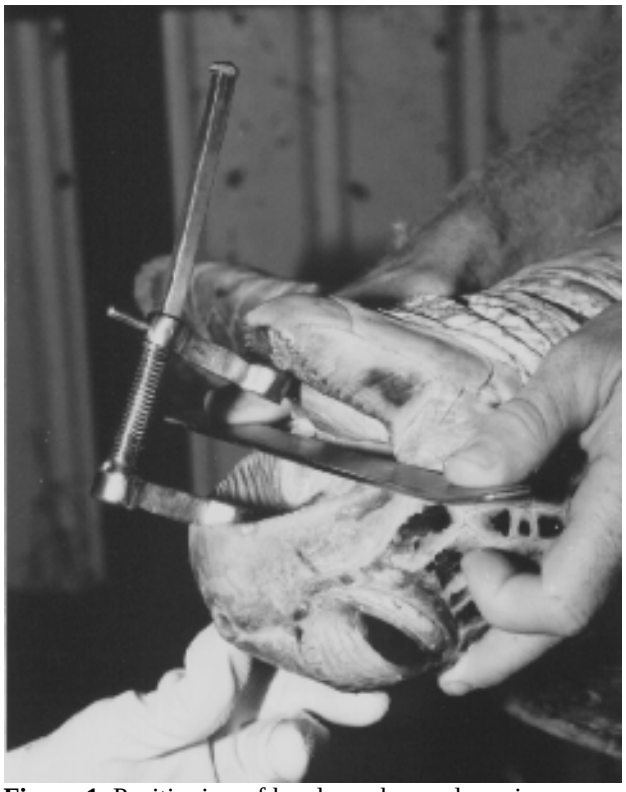

Figure 1. Positioning of head, pry bar and gag in a green turtle.

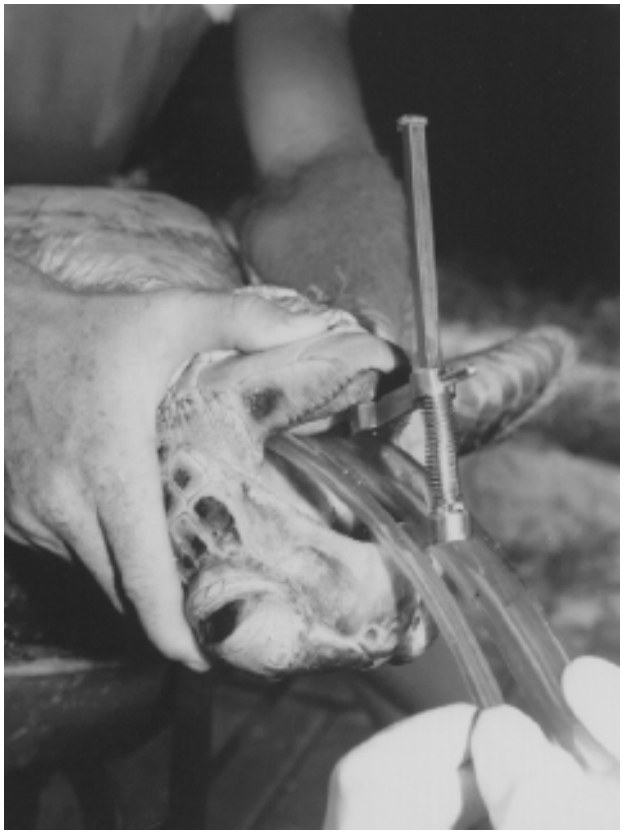

Figure 2. Lateral positioning of injection tube (left) and rerieval tube in a green turtle, *Chelonia mydas.* Note alignment of head with plastron.

termined easily with the installation of an inexpensive in-line pressure gauge placed just upstream from the flow valve. In lieu of a pressurized system, hand operated bilge pumps have been used quite successfully. Water must not be delivered at pressures or volumes greater than what can be expelled easily through the retrieval tube as the accumulation of excess water pressure within the turtle could cause it serious injury or death.

As water enters the turtle, return flow should begin within seconds through the retrieval tube. The exit flow volume should equal the delivery flow. If this is not the case, the retrieval tube should be withdrawn slightly to allow free entry of water into the tube as the tube may be obstructed. If water does not exit or the flow rate is low for more than 15-20 sec at any time during the lavage, stop the entry of water and reinsert both tubes. Once proper return water flow is achieved, food particles should be seen traveling within the tube. If particles are not present or to increase the quantity, while holding the injection tube in place, move the retrieval tube firmly against the bolus and then withdraw several centimeters to allow the dislodged particles to enter the tube. If food is not entering the tube, do not increase the force of the forward movement of the tube as the tube most likely is against soft tissue rather than the bolus. Instead, the tube should be withdrawn several centimeters, rotated slightly and reinserted until food particles begin to exit.

Although the entrance to the trachea should be sealed by the retrieval tube, the actual lavage should not exceed 3 minutes to reduce the chance of the turtle inhaling. Once the desired quantity of sample has been collected, the water to the injection tube is turned off and water and food are allowed to drain until all flow has stopped. The posterior of the turtle can be elevated slightly at this point to assist in drainage. Complete drainage is important prior to removing the retrieval tube as the turtle may breathe as the tube is removed and the airway must be free of standing water to prevent aspiration. The injection tube should be removed first and then the retrieval tube. Immediately after removing the tubes, the gag should be removed rapidly and the head elevated slightly to drain any remaining water clear of the glottis and back into the esophagus. The head should be held in this position until the first breath is taken which should be almost immediately. At this point the procedure is complete.

Proper lavage technique may yield up to 1 liter of food from healthy and actively feeding adult green turtles and 500 ml from subadults. Subadult hawksbills may yield up to 200 ml. Lavage samples should be preserved in a 6.5% buffered formalin/seawater solution. Stronger formalin solutions will discolor most plant matter as well as some animal matter making identification more difficult.

Many individual turtles have been lavaged more than three times without any known detrimental effect. Individuals have been recaptured from the day after the procedure up to three years later and appear to be quite healthy and feeding. Laparoscopic examination of the intestines following the procedure has not detected any swelling or damage to the intestines. The entire technique can be performed in less than 10 minutes and is rarely unsuccessful.

This system has proven to be a quick, safe, and inexpensive method by which sea turtles' stomach samples can be obtained in the field without injury to the animal. The technique is readily learned and proficiency can be achieved in a short time. However, care should be taken in the interpretation of the significance of the sample retrieved. The sample contents are a function of the size of the retrieval tube used, the size of the diet components in the anterior digestive tract, the duration of the lavage, the distance or depth at which the digestive tract was sampled, and the experience of the person performing the lavage.

Diet Component Analysis

Once a diet sample has been collected by gastric lavage or any other technique, the next step is to analyze the contents. A simple qualitative list of the components present in the diet sample may be all that is desired, or a detailed quantitative analysis of diet composition and the relative contribution of each diet component may be required. A reference collection of potential diet items should be established by preserving the diet items in 6.5% buffered formalin/ seawater solution in clear plastic vials stored in darkness to reduce color fading.

The two most common methods of quantifying a diet component's contribution to the diet is to determine either its weight or volume relative to the total diet sample. Attempting to quantify a component's importance to the diet by its gravimetric or weight contribution has several drawbacks. The importance of diet items with a high ash content and therefore high relative weight *(e.g.,* calcareous algae, sponge spicules, exoskeletons) will be overestimated in a gravimetric analysis while low ash content items will

be underestimated.

If a gravimetric procedure is used, diet components can be freeze dried or oven dried until a constant weight is obtained. Freeze drying is the preferred method if biochemical analyses are to be performed on the components as heating may damage heat labile compounds. If freeze drying is not possible, samples should be dried at 60°C to avoid heat damage. After drying, the diet components should be maintained in a desiccating chamber with silica gel to prevent rehydrating prior to weighing.

The relative volume of each dietary component can be determined with two techniques. One technique uses water displacement. Each diet component is placed in a graduated cylinder containing water, and the increase in volume recorded in the graduated cylinder is the volume of the diet component. For reasonable accuracy, the size of the graduated cylinder should be appropriate for the volume of the sample; that is, displacement of a 1 ml sample should not be measured in a 100 ml graduated cylinder.

The second technique uses the principles of microstereology (Weibel *et al.*, 1966; Schaefer, 1970) and a quantification technique (Forbes, 1996). For this approach, each lavage sample is emptied into a large tray and mixed until visually homogeneous. A subsample sufficient to cover the bottom of a Petri dish is removed and spread across the dish to a depth at which substage light can still be transmitted through the sample in amounts sufficient to illuminate the sample. The sample is then viewed under a dissecting microscope with wide-field ocular lenses fitted with a Weibel graticule consisting of 21 straight lines arranged in 3 rows of 7 lines. Although the Weibel pattern is the most efficient sampling graticule (from Bunton Instrument Company, 9607 Doctor Perry Road., Suite 99, Ijamsville, Maryland 21754 USA), a variety of grid patterns can be used. Filamentous species of algae can be viewed with substage lighting transmitted through a blue filter to enhance cellular definition.

Sampling field locations should be marked and numbered sequentially every 4 cm along the circumference of the Petri dish with a permanent marker. The Petri dish is rotated within a stage mounted template until the sampling field lines up with an indicator line on the stage template. The template is made by cutting a hole (equal to the diameter of the Petri dish) out of cardboard or plastic. Each diet component's contribution to the volume of a sample is determined by counting the number of graticule line endpoints that it intercepts relative to the total number of intercepts counted for all components combined.

The power of magnification will be determined by the resolution required to identify the specimen. However, all intercepts should be counted at the same magnification. If higher magnification is required, the diet item can be removed carefully from the Petri dish and viewed under a compound microscope. The number of fields required to ensure an adequate analysis of the lavage sample is determined by sampling a series of the most diverse lavage samples. The results are plotted to determine (1) the point at which there is no significant increase in the number of components added with the addition of another sampling field and (2) the point at which the cumulative percent contribution of each component levels off without significant change with the addition of another sampling field.

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