NOAA Technical Memorandum NMFS-SEFSC-579

SOUTHEAST FISHERIES SCIENCE CENTER SEA TURTLE RESEARCH TECHNIQUES MANUAL

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NOAA Fisheries
Southeast Fisheries Science Center
75 Virginia Beach Drive
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Cover Photo: Scanning for PIT tags (NMFS/SEFSC photo).

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Preface

This document is a compilation of the current research techniques and protocols of the National Marine Fisheries Service (NMFS) Southeast Fisheries Science Center (SEFSC). This sea turtle research techniques manual was developed in support of NMFS/SEFSC research permit applications and to provide a comprehensive training document for NMFS researchers and fishery observers. Methods vary among researchers, but the techniques described here are accepted by the SEFSC after consultation with research, academic, and veterinary colleagues.

Only authorized personnel may conduct the procedures described in this manual while working with listed threatened or endangered sea turtles. The Endangered Species Act of 1973 prohibits any person from harassing, harming, pursuing, hunting, shooting, wounding, killing, trapping, capturing or collecting any listed threatened or endangered species. Authorization to "take" (as described in the previous sentence) a listed threatened or endangered species must be granted under an ESA Section 10(a)(1)(B) permit or similar authorization. Additional state permits or import permits may be required as well. When conducting research, authorized personnel must carry all relevant permits and authorization letters and follow all terms and conditions, including reporting requirements, as outlined in the permit(s).

While this document represents the best practices currently available, sea turtle research is a dynamic field, and new techniques and technologies may become available in the future. Periodic updates will be made to this document to reflect these changes, and revised documents will be available online at:

http://www.sefsc.noaa.gov/seaturtletechmemos.jsp

This manual was made possible through the contributions of many people who provided information, photographs, and helpful comments. We sincerely thank the contributors: Larisa Avens, Lisa Belskis, Scott Benson, Joanne Braun-McNeill, Peter Dutton, Joseph Flanagan, Craig Harms, Ben Higgins, Terra Kelly, Catherine McClellan, Steve Morreale, Chris Sasso, Amanda Southwood, and Jeanette Wyneken. Ben Higgins, and the sea turtle staff at the NMFS Galveston Laboratory, and Joanne Braun-McNeill, and the sea turtle staff at the NMFS Beaufort Laboratory, were invaluable in contributing photographs, and Patrick Opay provided useful comments. We also wish to acknowledge and thank Jim Bohnsack and Alex Chester for their review of this manual.

Chapter 1: Species Identification

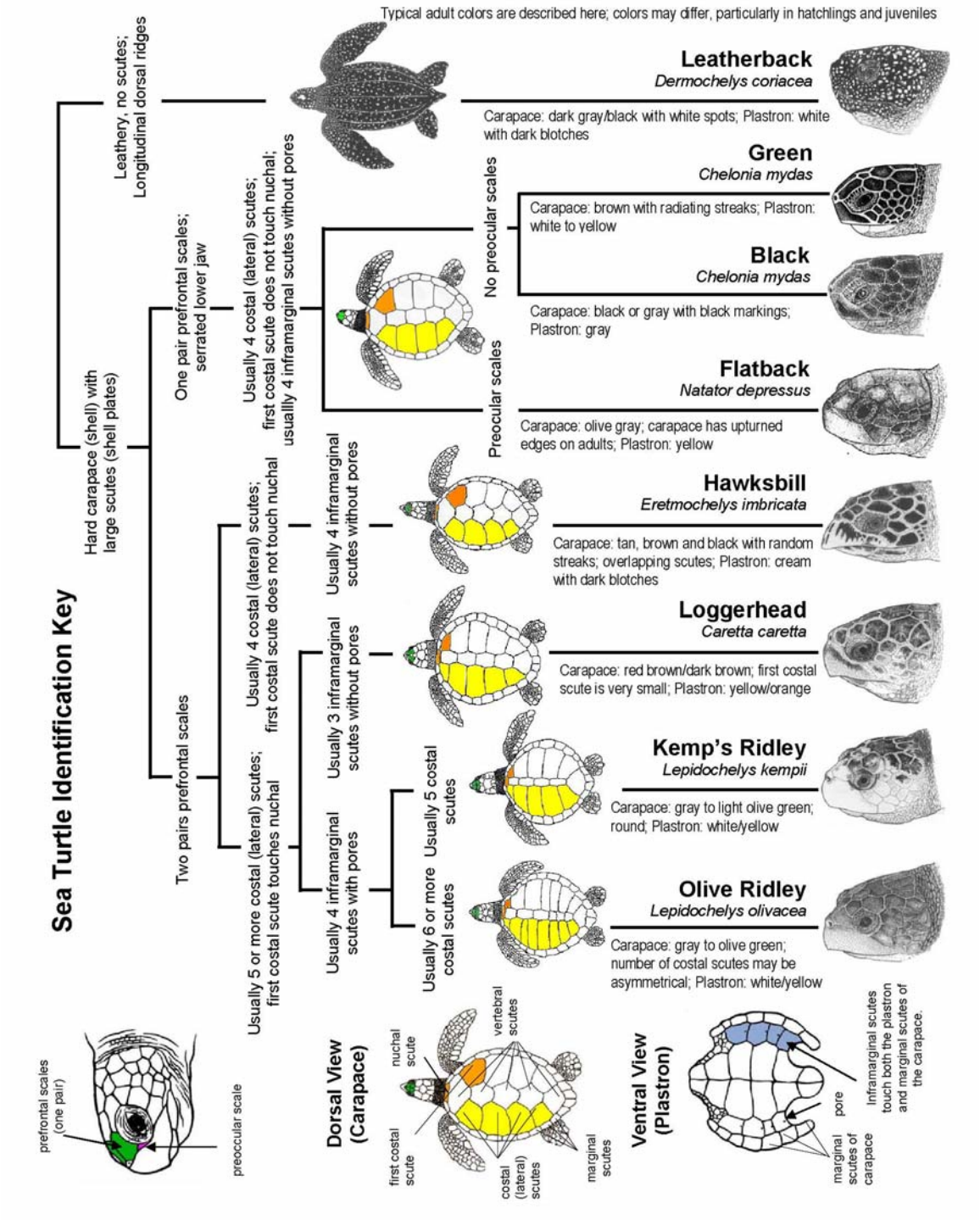


Figure 1-1. Sea turtle identification key (NMFS/SEFSC diagram modified from seaturtle.org).

Leatherback, ***Dermochelys coriacea*** (**Spanish:** Baula, Tortuga Laúd, Tora, Cardón, Tinglar; **French:** Tortue Luth; **Portuguese:** Tartaruga Gigante, Tartaruga-de-couro)
Adult Size Range: Length: 165-190+ cm/ 65-75+ in; **Weight:** 400-500 kg females, males to 900 kg/ 885-1985 lb
Range: All oceans, sub-arctic to tropical; mainly pelagic oceanic (surface dwelling in the open ocean) but found in bays and over continental shelves

Green, Black*, ***Chelonia mydas*** (**Spanish:** Tortuga Verde, Tortuga Blanca; Tortuga Negra, Prieta; **French**: Tortue Verte; **Portuguese:** Tartaruga Verde, Aruanã)
Adult Size Range: Length: 90-120 cm/ 35-45 in; **Weight:** 120-230 kg/ 265-510 lb
Range: All subtropical and tropical seas; bays and coastal waters; black form restricted to eastern Pacific Ocean; pelagic oceanic (surface dwelling in the open ocean) small juveniles; benthic neritic (bottom dwelling in coastal waters) large juveniles and adults
*The status of the black turtle or eastern Pacific green turtle as *Chelonia agassizii* or *C. mydas agassizii* as a distinct species or subspecies is not supported, although it is often treated as such.

Flatback, ***Natator depressus*** (**Spanish:** Kikila, Tortuga Aplanada, Tortuga Franca Oriental; **French:** Chelonée à dos Plat; **Portuguese:** Tartaruga de Casco Achatado)
Adult Size Range: Length: to 100 cm/ 40 in; **Weight:** to 90 kg/ 200 lb
Range: Tropical coastal Australia, including the waters up to Irian Jaya, Papua New Guinea and Java; pelagic neritic (surface dwelling in coastal waters)

Hawksbill, ***Eretmochelys imbricata*** (**Spanish:** Tortuga Carey; **French:** Tortue Imbriquée, Tortue Caret; **Portuguese:** Tartaruga-de-pente, Tartaruga de Escamas, Tartaruga Bico de Falcão, Tartaruga Verdadeira)
Adult Size Range: Length: 90-110+ cm/ 35-45+ in; **Weight:** 60-80 kg/ 130-175 lb
Range: All oceans; tropical waters, rarely subtropical; reef areas; pelagic oceanic (surface dwelling in the open ocean) small juveniles; benthic neritic (bottom dwelling in coastal waters) large juveniles and adults

Loggerhead, ***Caretta caretta*** (**Spanish:** Caguama, Amarilla, Cabezona, Tortuga Boba; **French:** Caouanne; **Portuguese:** Tartaruga Boba, Tartaruga Comum, Tartaruga Careta, Tartaruga Cabeçuda, Tartaruga amarela, Careba Dura, Careba Amarela)
Adult Size Range: Length: 90-130 cm/ 35-50 in; **Weight:** 100-180 kg/ 220-400 lb
Range: All oceans; primarily subtropical and temperate waters; often associated with structures (i.e., reefs, wrecks, platforms); pelagic oceanic (surface dwelling in the open ocean) small juveniles; benthic neritic (bottom dwelling in coastal waters) large juveniles and adults

Kemp's Ridley, ***Lepidochelys kempii*** (**Spanish:** Tortuga Lora, Cotorra; **French:** Tortue de Kemp; **Portuguese:** Tartaruga de Kemp)
Adult Size Range: Length: to 70 cm/ 28 in; **Weight:** 35-50 kg/ 80-110 lb
Range: Gulf of Mexico, eastern USA, rarely in eastern North Atlantic; coastal, primarily subtropical and temperate waters; pelagic oceanic (surface dwelling in the open ocean) small juveniles; benthic neritic (bottom dwelling in coastal waters) large juveniles and adults

Olive Ridley, ***Lepidochelys olivacea*** (**Spanish:** Tortuga Golfina, Tortuga Olivacea, Parlama, Guaraguá, Maní; **French:** Tortue Olivâtre; **Portuguese:** Tartaruga Oliva, Tartaruga Olivácea, Tartaruga Pequena, Xibirro)
Adult Size Range: Length: 70-80 cm/ 28-32 in; **Weight:** 35-60 kg/ 80-130 lb
Range: Pacific, Indian and Atlantic Oceans, rarely in eastern North Atlantic; pelagic oceanic (surface dwelling in the open ocean); most often in tropical waters

Sources:

Seaturtle.org

Pritchard, P. C. H. and Mortimer, J. A. (1999) Taxonomy, External Morphology, and Species Identification. pp. 21-38. In: Eckert, K.L., K.A. Bjorndal, F.A. Abreu-Grobois, and M. Donnelly (Editors). 1999. Research and Management Techniques for the Conservation of Sea Turtles. IUCN/SSC Marine Turtle Specialist Group Publication No. 4. (for further details see http://www.iucn-mtsg.org/publications.htm)

Wyneken, J. The Anatomy of Sea Turtles. 2001. U.S. Department of Commerce NOAA Technical Memorandum NMFS-SEFSC-470, 172 pp.

Sea turtle figures used by permission of the Marine Turtle Specialist Group (iucn-mtsg.org), Peter Pritchard and Jeanette Wyneken
Illustrations by Tom McFarland and Dawn Witherington

Chapter 2: Handling

All Turtles

After capture, every turtle should be assessed to determine their general state of health and suitability for subsequent research procedures, including an examination of the oral cavity (see Chapter 4: Oral Cavity Anatomy). Remove any attached gear if applicable (see NMFS 2008), and attempt to resuscitate all comatose turtles (see Chapter 3: Resuscitation) when necessary. Successfully resuscitated turtles benefit from being held on deck as long as possible (up to 24 hours) when conditions permit to allow stress toxins to dissipate from the body. All captured turtles should be subject to standard processing protocols before release: identification, standard measurements, weight, photographs, flipper and PIT tags, and skin biopsies (in select cases). Some may be subject to the additional procedures described in this manual, depending on the results of their general assessment and the directed research needs.

Figure 2-1. Keep the turtle moist and in the shade (NMFS/SEFSC photo).

Turtles should be protected from temperature extremes of heat and cold, provided adequate air flow, and kept moist during sampling. Keep the skin and eyes moist while the turtle is on deck; cover the animal's body with a wet towel (Figure 2-1) and periodically spray it with water or apply petroleum or water-based lubricant jelly to the skin and carapace. If using towels, pay particular attention to the ambient temperature, as evaporative cooling may chill the animal under some conditions. If the turtle is to be held out of water for an extended period of time (e.g., transport, surgery) or in cool air temperatures, use petroleum or water-based lubricant jelly on the skin as necessary to avoid drying instead of using wet toweling. Keep the turtle in the shade, maintaining its body temperature between 60° and 90° F, similar to water temperatures at capture. If air temperatures are greater than 80° F, ensure that the turtle does not overheat; conversely, if air temperatures are less than 60° F, ensure that the turtle does not become hypothermic. Safely isolate the turtle and immobilize it on a cushioned surface such as a foam pad, an automobile tire or similar. The area surrounding the turtle should not contain any materials that could be accidentally ingested.

Transport turtles in individual containers to ensure that they are unable to injure themselves or each other. Do not transport turtles in open vehicles during excessive heat or cold. Do not pick up turtles by their flippers, but rather, lift them by grasping both sides of the carapace (a better support of their weight) or use a stretcher that provides adequate support. In order to minimize the

risk of either introducing a new pathogen into a population or amplifying the rate of transmission of an endemic pathogen from animal to animal, thoroughly clean containers in which turtles are being transported with soap and water and disinfect them with a mild bleach solution.

Conduct field and laboratory procedures using latex or similar disposable gloves whenever possible. Remove the gloves following the proper method: 1) Grip one glove on the outside of the glove near the cuff. Reflect and peel it down until it comes off inside out. Cup it with your other gloved hand. 2) Place 2 fingers of your bare hand inside the cuff of the glove that is still on your hand. 3) Peel that glove off so that it comes off inside out with the first glove inside it. During these steps, take care not to snap the glove during the removal so that material could spray or aerosolize. 4) Dispose of the gloves in an appropriate container and thoroughly wash your hands with soap and water.

All equipment (tagging equipment, tape measures, etc.) that comes into contact with sea turtle body fluids, cuts, or lesions must be disinfected between the processing of each turtle. Whenever feasible, equipment that does not contact fluids, cuts, or lesions should be disinfected between the processing of each turtle as well. To disinfect field equipment, use an appropriate disinfectant such as a freshly mixed 1:10 solution of household bleach (~5 – 6 % sodium hypochlorite). To prepare 1:10 bleach solution, add one volume of household bleach (e.g., 1 cup, liter) to 10 volumes of clean water (e.g., 10 cups, liters). Spray or soak equipment for at least 2 minutes for equipment disinfection and use fresh solution each time.

Figure 2-2. Green turtle displaying fibropapilloma tumors (Photo courtesy of the Turtle Hospital, Marathon, Florida).

NOAA Fisheries researchers (including fishery observers) must maintain a separate set of sampling equipment for handling animals displaying fibropapilloma (FP) tumors or lesions (Figure 2-2). Whenever an animal suspected of having FP tumors is encountered, care must be taken to ensure that the same equipment is not later used on other turtles. For most, this means that some equipment (e.g., calipers, scales) should not be used on affected turtles because one is not likely to have a duplicate set. If a spare set of calipers is not available, use a tape measure and record curved measurement only. Quarantine the tape measure and use the spare until the original tape measure can be disinfected. Use the same protocols for tagging pliers and PIT tag injectors – quarantine the equipment and then use spares thereafter. The PIT tag scanner may be used again after removing and disposing of the plastic bag around the PIT tag reader and replacing it with a new plastic bag. NOAA Fisheries observers in the field should place contaminated equipment (used on a turtle displaying FP tumors) into a plastic bag

thorough disinfection as soon as possible. Any equipment that comes into contact with animals displaying FP tumors must be disinfected using bleach solution (as described above).

During release, turtles should be lowered as close to the water's surface as possible, in water of similar temperature as capture, when fishing gear is not in use (if applicable) and engines are in neutral.

Leatherback Turtles

Exercise extra care when handling, sampling and releasing leatherback turtles during directed capture research activities (Figure 2-3), as field and laboratory observations indicate that they have more friable skin and softer bones than hardshell turtles. Leatherback turtles shall not be turned on their backs. Precautions shall be taken to ensure that animals are supported from underneath during handling and release.

The additional recommended monitoring protocols will be taken for animals captured during directed research activities. In order to improve monitoring of the animals during directed capture and to improve our basic understanding of the biology and medical status of leatherbacks, a designated observer should be on each capture outing team. Whenever possible, this observer should be a veterinarian; however, a dedicated observer with training in the techniques required for this position is also acceptable.

<u>Recommended Monitoring Protocols:</u>

- Perform a gross examination upon capture, including assessment of body fat (subjective), activity, alertness, pre-existing injuries, weight and length.

Figure 2-3. Handling a leatherback during directed research capture activities (NMFS/SEFSC photo).

- Record respiratory rate over a two-minute period, logged every 20 minutes.
- Record response to noxious stimuli (either tail pinch or blink response), logged every 20 minutes.
- Record heart rate determined by digital or Doppler detection on femoral artery, ultrasound, rectal pulse oximiter, or EKG, logged every 20 minutes.
- Record body temperature detected by anal probe inserted 15 cm, logged every 20 minutes.
- Assure cooling by running ambient seawater over the carapace and forelimbs during the time on deck.
- Collect two tubes of blood in a clot tube and urine or feces if possible.
- Relate changes in the animal's condition to the chief scientist so that an ongoing assessment of the animal's condition can be made.

The chief scientist for each outing will be trained by a veterinarian in the following information and procedures:

- Acceptable parameters for heart rate, respiration, temperature, and responsiveness, as defined by baseline data gathered in the field as well as in collaboration with veterinarians and colleagues from NMFS/SWFSC.

- Appropriate response to changes suggesting a need to abort further animal handling and initiate release.

- Safe water reintroduction and monitoring of a turtle in possible distress.

- Appropriate first aid measures for animals in distress. These measures may include intubation, artificial respiration, and administration of pharmaceuticals to stimulate respiration and/or cardiac contraction.

During laboratory procedures, monitor each turtle manually, noting its response to stimuli (surgical stimuli, eye reflex, withdrawal reflex) and respiratory intervals. Monitor the following parameters on each turtle using instruments such as electrocardiogram (ECG or EKG), blood gases, and cloacal temperature (to allow temperature corrections for blood gases).

Adverse reactions could be indicated by cardiac arrhythmia, cardiac arrest, respiratory arrest, seizures, or severe blood gas alterations. Veterinarians are still in the process of defining normal and altered blood gas parameters by establishing baselines, but alarming values would be recognized (Dr. Craig Harms, pers. comm.). The response to adverse reactions would depend on the type of reaction, but would likely involve basic supportive therapy including intubation and assisted respirations, IV fluids (for shock and to hasten elimination of drugs through renal excretion), anti-arrhythmic drugs (e.g., IV lidocaine for VPCs), cardioresuscitory drugs (e.g., IV epinephrine for cardiac arrest), or anti-seizure medication (e.g., IV diazepam).

Avoid any animal deemed to be in distress at any time during the pre-capture period. In addition to animal monitoring, include an emergency field kit for intervention on each directed capture research outing. This kit should be available to the field team veterinary observers or the chief scientist and should include:

- Oxygen canister and a demand breathing valve
- Endotracheal tubes
- Oral speculum and appropriate sized blade
- Water-based lubricant jelly
- Betadine® ointment or similar
- Gauze sponges
- Medical tape
- Isopropyl alcohol
- Needles and syringes (various sizes)

  Doxapram, epinephrine, lidocaine, furosemide, diazepam, dexamethasone sodium phosphate, sodium bicarbonate, and saline solution

Chapter 3: Resuscitation

If a turtle appears to be comatose or unresponsive, as determined by testing for bilateral responsiveness (Figure 3-1), attempt to revive the turtle (Figures 3-2a and b) before putting it back into the water. A fully conscious turtle has bilateral reflexes and has a central (e.g., brain) recognition of the stimulus. An unresponsive turtle will not have full bilateral responses nor central recognition of a stimulus. A comatose turtle will have lost all reflexes. To test eye reflexes, check for a blink response by gently touching the corner of the eye or eyelid. Pinch both front and rear flippers and the tail to check for response; a lack of bilateral response for any of these tests may indicate the need for resuscitation. Use the method of resuscitation described on the following Sea Turtle Resuscitation Guidelines (66 FR 67495, December 31, 2001). Regulations (66 FR 67495, December 31, 2001; 50 CFR 223.206) allow a fisherman to keep incidentally captured turtles on deck up to 24 hours for resuscitation purposes.

Figure 3-1. Testing eye reflex (NMFS/SEFSC photo).

Figures 3-2a and b. Resuscitation position with 15-30° elevation on (a) a cushioned surface and (b) on a standard automobile tire (NOTE: a slightly greater angle of head inclination than depicted in photo (b) would be preferable to better facilitate water drainage.) (NMFS/SEFSC photos).

Successfully resuscitated turtles benefit from being held on deck as long as possible, when conditions permit, to allow stress toxins to dissipate from the body. Keep the skin and the eyes moist while the turtle is on deck (Figure 3-2b) by covering the animal's body with a wet towel, periodically spraying it with

water, or by applying petroleum or water based lubricant jelly to its skin and carapace. Comatose or unresponsive turtles captured during directed research activities should be transported as quickly as possible to a rehabilitation facility whenever feasible.

A turtle that has shown no sign of life after 24 hours on deck (held in the shade, kept moist and its body temperature maintained above 60° F) may be considered dead. If the turtle cannot be revived before returning to port, it should be returned to the water, preferably in a non-fishing area. Mark the turtle (spray paint it or tag it) before returning it to the water.

In the past, an alternative method of resuscitation known as plastral pumping was recommended (see FR 43 32801, July 28, 1978; 57 FR 57354, December 4, 1992). This practice involved placing the turtle on its carapace and pumping the plastron with a hand or foot. However, we strongly discourage this technique, as further study determined that it may actually do more harm than good and should not be attempted during resuscitation (per 66 FR 67495, December 31, 2001). Plastral pumping may cause the airway to block and cause the viscera to compress the lungs which are located dorsally, thereby hindering lung ventilation.

Sea Turtle Resuscitation Guidelines

If a turtle appears to be unresponsive or comatose, attempt to revive it before release. Turtles can withstand lengthy periods without breathing; a comatose sea turtle will not move, breathe voluntarily, or show reflex responses or other signs of life. In other cases, an unresponsive turtle may show shallow breathing or reflexes such as eyelid or tail movement when touched. Use the following method of resuscitation in the field if veterinary attention is not immediately available:

- **Place the turtle on its plastron (lower shell) and elevate the hindquarters approximately 15 - 30 degrees to permit the lungs to drain off water for a period of 4 up to 24 hours. A board, tire or boat cushion, etc. can be used for elevation.**

- **Keep the turtle in the shade, at a temperature similar to water temperature at capture. Keep the skin (especially the eyes) moist while the turtle is on deck by covering the animal's body with a wet towel, periodically spraying it with water, or by applying petroleum jelly to its skin and carapace. Do not put the turtle into a container with water.**

- **Do not put the turtle on its carapace (top shell) and pump the plastron (breastplate) or try to compress the turtle to force water out, as this is dangerous to the turtle and may do more harm than good.**

- **Periodically, gently touch the corner of the eye or eyelid and pinch the tail near the vent (reflex tests) to monitor consciousness.**

- **Sea turtles may take some time to revive; do not give up too quickly. Turtles that are successfully resuscitated benefit from being held on deck as long as possible (up to 24 hours) to fully recover from the stress of accidental forced submergence.**

- **Release successfully resuscitated turtles over the stern of the boat, when fishing or scientific collection gear is not in use, the engine is in neutral, and in areas where they are unlikely to be recaptured or injured by vessels. A turtle that has shown no sign of life after 24 hours on deck may be considered dead and returned to the water in the same manner.**

NMFS/SEFSC Photos

References:

Federal Register, December 31, 2001. Government Printing Office, Washington DC 66 (250), pp. 67495- 67496.

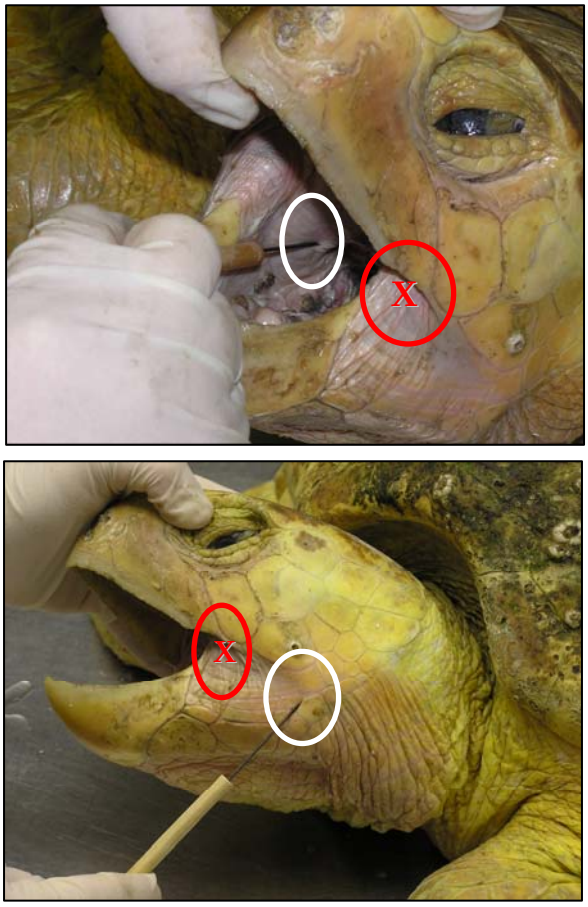
October 2008

Figure 3-3. Resuscitation guidelines (NMFS/SEFSC).

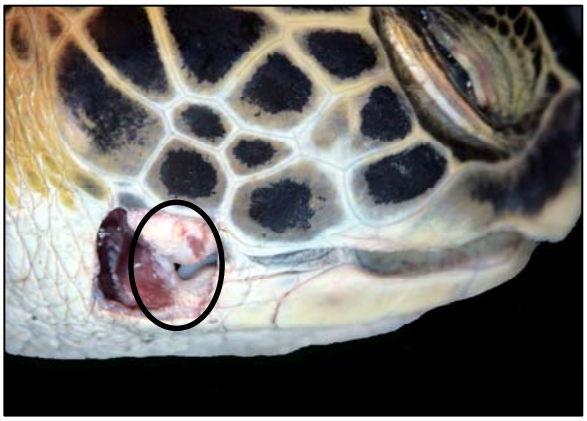
Chapter 4: Oral Cavity Anatomy

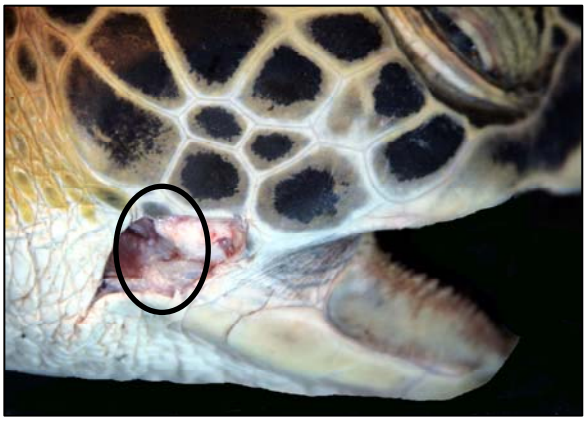
The oral cavity is described here to assist in performing general health assessments and to identify the location of hooks in incidentally captured turtles, especially to distinguish hooks that are swallowed from those lodged in the oral cavity (Figures 4-1 ─ 4-7). The anatomy details described here are intended primarily to provide the basic knowledge necessary to assess whether hook removal may cause further injury. Do not attempt to remove hooks when it appears that removal will cause further serious injury to the turtle. For example, the removal of hooks lodged in the jaw joint (Figures 4-1 and 4-2) the glottis, or in the esophagus where the insertion point is not visible may cause greater injury to the turtle than leaving the hook in place. For all hooked animals, follow the guidance in the NOAA Technical Memorandum NMFS-SEFSC-580, Careful Release Protocols for Sea Turtle Release with Minimal Injury (NMFS SEFSC 2008).

The upper and lower beak (Figure 4-3), or rhamphotheci, of hardshell sea turtles are keratinized and cover many of the bones of the upper jaw and dentary of the lower jaw. They differ among species and can be used for identification. The tongue (Figures 4-3 and 4-4) is a large, nonprotrusible, muscular organ fixed to the floor of the mouth. The glottis (Figures 4-4 and 4-5), the opening to the trachea and the valve to open and close the airway, is located at the back of the tongue. The esophagus (Figure 4-6) starts at the back of the mouth behind the tongue and links the oral cavity to the stomach. Most of the length of the esophagus is lined with sharp, keratinized papillae that angle toward the stomach. These are presumed to trap food, preventing food particles from being regurgitated when excess water is expelled. The roof of the mouth (Figure 4-7) is ventral to the braincase.



Figures 4-1a and b. Internal view (a) and external view (b) of jaw joint location, indicated by the pointer. The jaw joint should not be confused with "the corner of the mouth" indicated here in red. (NMFS/SEFSC photos).





Figures 4-2a and b. External view of jaw joint location with skin and muscle removed, shown with the jaws closed (a) and open (b). (Photos by J. Wyneken, Florida Atlantic University).

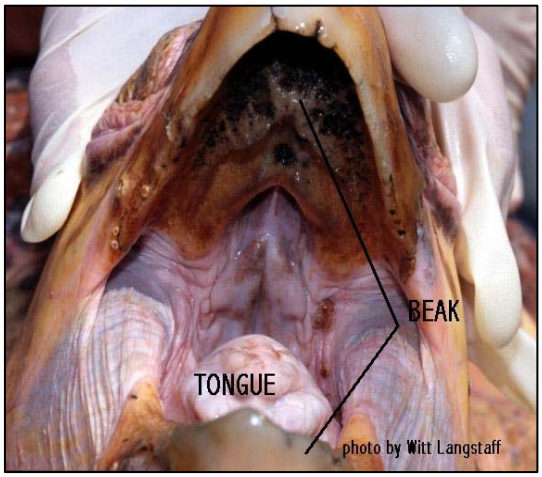


Figure 4-3. The upper and lower beak, or rhamphotheci, of a loggerhead (Photo by W. Langstaff).

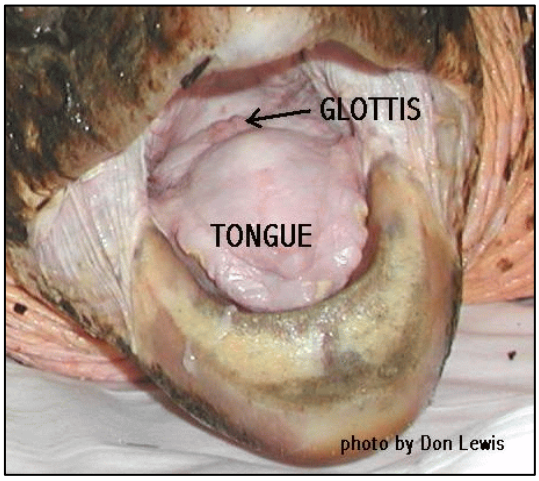
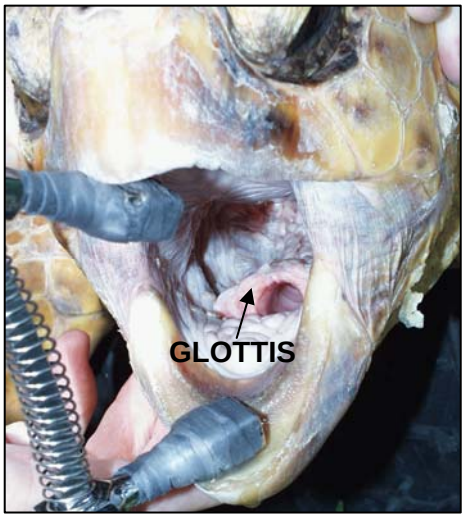
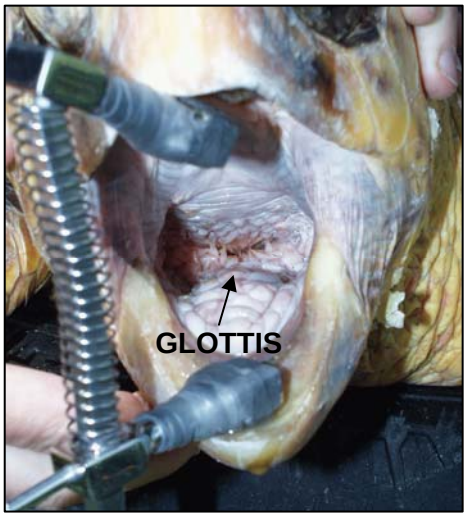


Figure 4-4. The tongue and glottis, which is closed in this photograph (Photo by D. Lewis).





Figures 4-5a and b. Glottis (a) open and (b) closed (Photos by C. Harms, N.C. State University).

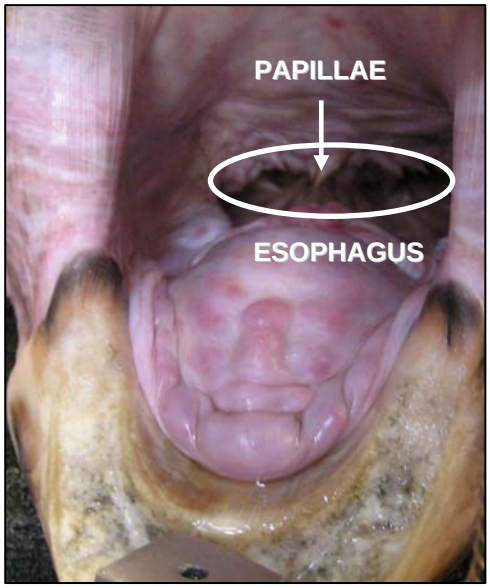


Figure 4-6. The entrance of the esophagus is marked by the presence of papillae (NMFS/SEFSC photo).

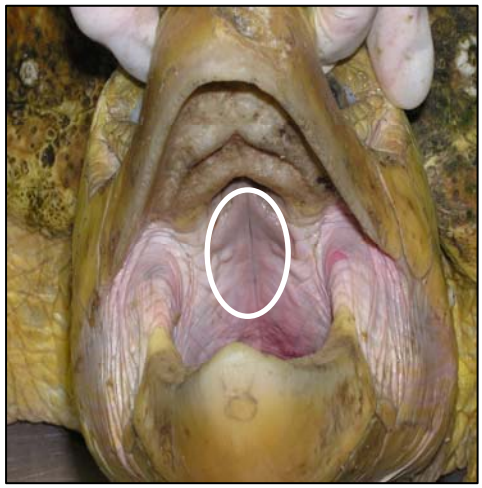


Figure 4-7. Roof of the mouth and upper jaw (NMFS/SEFSC photo).

Chapter 5: Morphometrics

Standard Measurements

If the turtle can be brought onboard or on land, take standard carapace measurements: CCL, SCL_{STD}, SCL_{MIN}, CCW, and SCW. Use a flexible fiberglass tape measure to take over-the-curve measurements and calipers for straight measurements; record in centimeters, rounded to the nearest 0.1 cm. For measurements over-the-curve (CCL and CCW), follow the curvature of the carapace. If barnacles affect these measurements, record this in the comments on the datasheet. For leatherbacks, generally only curved measurements are taken.

Figure 5-1. Curved carapace length taken with flexible fiberglass tape measure (NMFS/SEFSC photo).

Methodology to weigh turtles will differ slightly depending on the type of scale available, but in all cases, the turtle must be adequately restrained so there is no potential for injury from this procedure. The scale, sling or platform used should be disinfected between animals when practicable.

CCL – Curved Carapace Length, standard (notch-to-tip): Record the distance between the center of the nuchal scute and the posterior tip of the longest postcentral scute, following the curvature of the dorsal centerline (Figures 5-1 and 5-3). On leatherbacks, take the measurement alongside (not over the top) the central vertebral ridge (Figure 5-4).

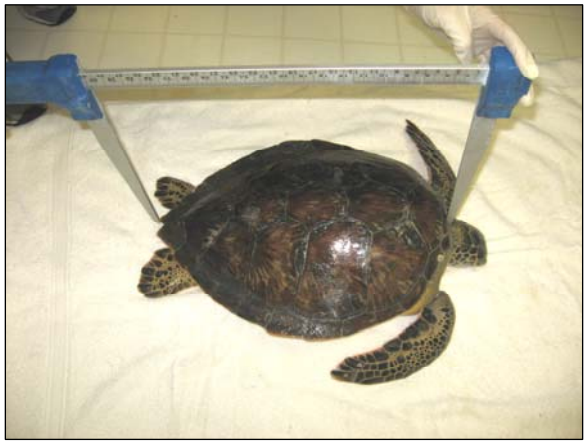


Figure 5-2. Straight carapace length (SCL) measurement, notch-to-tip (NMFS/SEFSC photo).

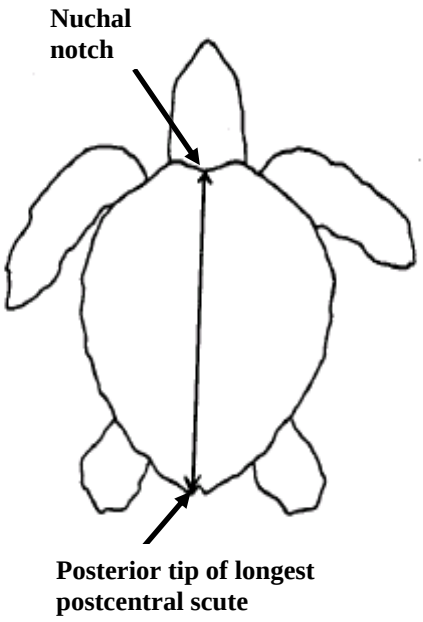


Figure 5-3. Carapace length (CCL and SCL) measurement, notch to tip [Figure modified from Bolten (1999)].

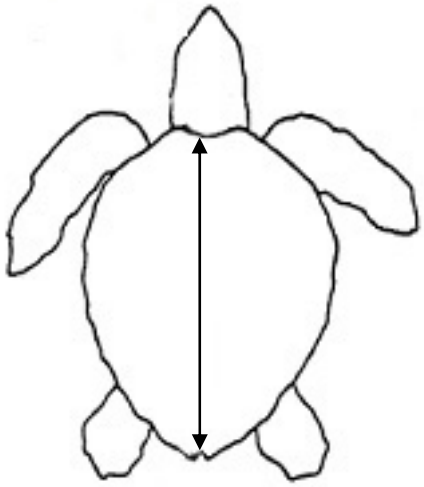


Figure 5-5. Carapace length (CCL and SCL) measurement, notch to notch [Figure modified from Bolten (1999)].

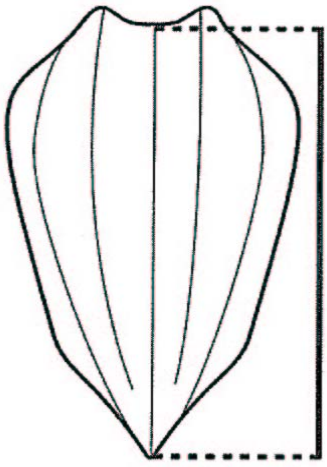


Figure 5-4. Curved carapace length (CCL) and straight carapace length (SCL) in leatherback turtles. In both cases, length is measured from the nuchal notch (anterior edge of the carapace at the midline) to the posterior tip of the caudal peduncle [Figure and caption text taken from Bolten (1999)].

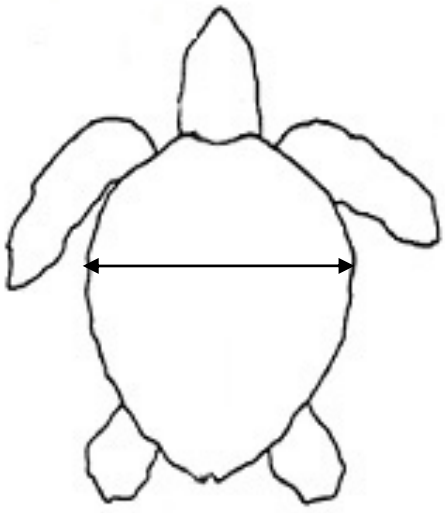


Figure 5-6. Carapace width (CCW and SCW) measurement [Figure modified from Bolten (1999)].

SCL_{STD} – Straight Carapace Length, standard (notch-to-tip): Record the distance between the center of the nuchal scute and the posterior tip of the longest postcentral scute (Figures 5-2 and 5-3).

SCL_{MIN} – Straight Carapace Length, minimal (notch-to-notch): Record the distance between the center of the nuchal scute and the notch between the two postcentral scutes (Figure 5-5).

CCW – Curved Carapace Width: On leatherbacks, measure the width from side ridge to side ridge (ridges depicted in Figure 5-4) at the widest point. On hardshell turtles, record the maximum distance between the lateral edges of the carapace, measured over the curvature of the shell, perpendicular to the longitudinal axis of the carapace at the widest point (Figures 5-6 and 5-7).

SCW – Straight Carapace Width: Record the maximum distance between the lateral edges of the carapace taken perpendicular to the longitudinal axis of the carapace at the widest point (Figures 5-6 and 5-8).

Figure 5-7. Curved carapace width (CCW) measurement (NMFS/SEFSC photo).

Figure 5-8. Straight carapace width (SCW) measurement (NMFS/SEFSC photo).

Additional Measurements

Additional measurements (maximum carapace length, maximum head width, maximum head length, body depth, plastron length, total tail length, plastron-to-vent length, vent-to-tip length, and circumference) may be taken as needed, following the protocols of Wyneken (2001).

Oral Cavity Measurements

Measures of the jaw and internal oral cavity anatomy may be taken to investigate oral cavity dimensions, particularly as they relate to a turtle's ability to swallow hooks of various sizes. All measures are taken using spring and/or dial calipers while the mouth is held open with a canine mouth gag (a type of oral speculum available from veterinary equipment suppliers). The canine mouth gag tips should be padded to reduce damage to the beak as the turtle bites down on the gag. All mouth measurement instruments should be cold sterilized using 2% chlorhexidine gluconate or similar between each use.

These oral cavity measures include:

Internal Gape Width: Measure is taken with spring calipers at the midpoint of the lateral oral commissures, the soft tissue connecting upper and lower jaws at the angles of the mouth, while the jaws are held open to their full extent with a canine mouth gag. Fixed spring caliper distance is then measured using dial calipers.

Esophagus Width: Measure is taken with spring calipers at the entrance of the esophagus (Figure 5-9), marked by the first presence of papillae. This distance is then measured with dial calipers. Note: this is a flexible opening, and the measurement represents a close approximation of the unstretched diameter of the esophagus width.

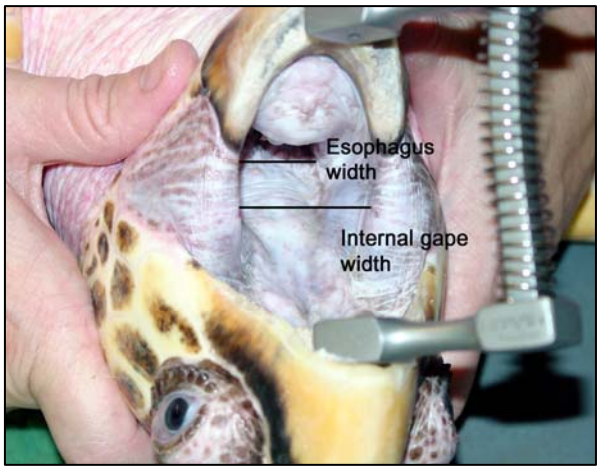


Figure 5-9. Internal oral cavity measurements: internal gape width, esophagus width (NMFS/SEFSC photo).

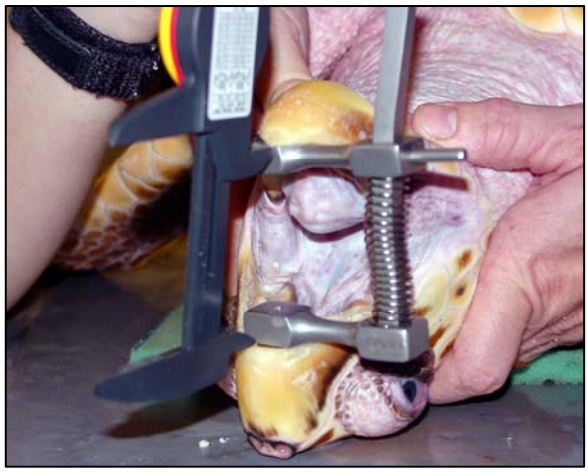


Figure 5-10. Gape Height (NMFS/SEFSC photo).

Gape Height: Measure is taken using dial calipers while jaws are held open to full extent with a canine mouth gag (Figure 5-10), representing the maximum internal distance between the distal points of the upper and lower jaw.

Upper Jaw Length: Measure is taken with dial calipers from the soft tissue at the insertion point of the rhamphotheca (keratinaceous beak) to the distal point of the upper jaw (Figure 5-11).

Lower Jaw Length: Measure is taken with dial calipers from the soft tissue at the insertion point of the rhamphotheca (keratinaceous beak) to the distal point of the lower jaw (Figure 5-12).

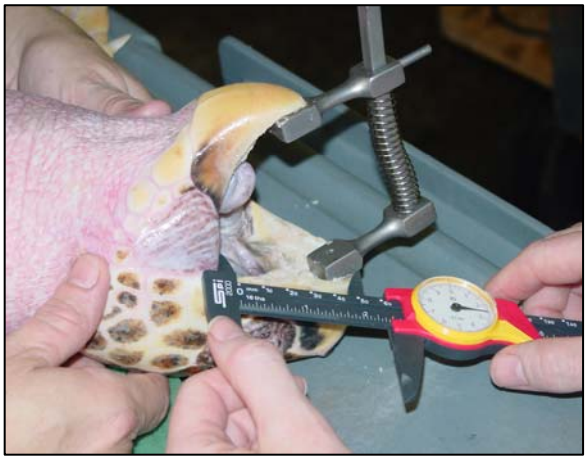


Figure 5-11. Upper jaw length (NMFS/SEFSC photo).

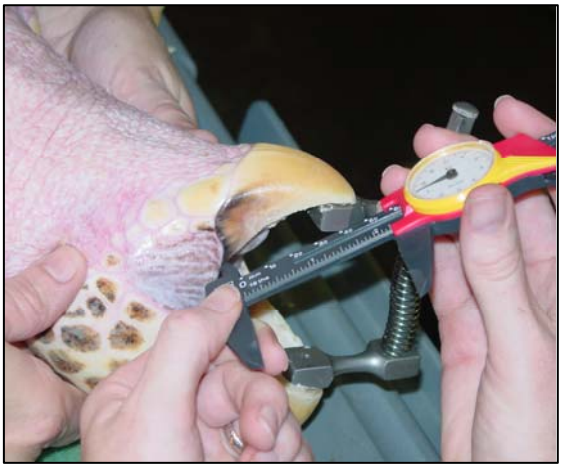


Figure 5-12. Lower jaw length (NMFS/SEFSC photo).

Chapter 6: Marking

Temporary Marking

Turtles may be temporarily marked using a non-toxic substance (e.g., paint, livestock paint sticks, non-toxic fingernail polish). No potentially harmful or toxic paints, such as xylene or toluene-based paints, or those containing tributyl tin and cyanide or copper cyanide, should be used. No reflective paints or paints with exothermic set-up reactions should be used. Paint should be applied without crossing the suture lines separating the scutes whenever possible.

Shell Etching

An etching tool such as a Dremel® with a pear-shaped bit can be used to place an etch or groove in the carapace of hardshell turtles. The bit and carapace should be disinfected before use, and the groove should not penetrate the scute. The groove could be marked with non-toxic paint if desired. Care should be exercised when choosing this technique, as discomfort may result from the procedure.

Flipper Tags

If a turtle is encountered without flipper tags, apply two new flipper tags to the trailing edge of the rear flippers just proximal to the first scale. If this site is unsuitable (lesions, scars, missing flippers, etc.), locate an alternate site along the trailing edge of a suitable flipper (i.e., the trailing edge of the front flipper(s) immediately proximal and adjacent to the first scale, or between the first and second large scales distal to the axilla). Turtles larger than 30 cm SCL should generally receive flipper tags. Experienced taggers may be comfortable tagging smaller animals in some cases. Extra care should be taken when positioning the tag in smaller animals to allow room for growth, although the tag should be positioned to allow for growth on all turtles. Check carefully for previous tagging scars on both front and rear flippers and note if present.

There may be circumstances where a previously applied tag will need to be removed prior to applying a new one. If a tag is damaged, covered in fouling organisms (e.g., barnacles) that cannot be removed, or if the tag appears to be in danger of coming off, the tag should be removed and replaced with a new tag. There may also be situations where a tag may be improperly placed (i.e., overgrown with tissue or tearing out), or injurious to the animal. In these situations, the tag should be carefully removed and replaced at the discretion of the tagger if they feel that removal will not cause further injury. Generally, the tag can be removed using two pairs of pliers to uncrimp the tip, but wire or bolt cutters may be necessary. If a previously applied tag is removed, the identification number should be recorded, and the tag should be reported to the original tagging project and the Cooperative Marine Turtle Tagging Project (CMTTP). Return the voided tag to the CMTTP or program coordinator.

To apply self-piercing, self-locking Inconel® alloy flipper tags:

Step **(1)** Remove a tag from the strip (Figure 6-1) and record its identification number on the tagging form. Be careful not to bend the tag from its original shape. Only peel back enough tape on the

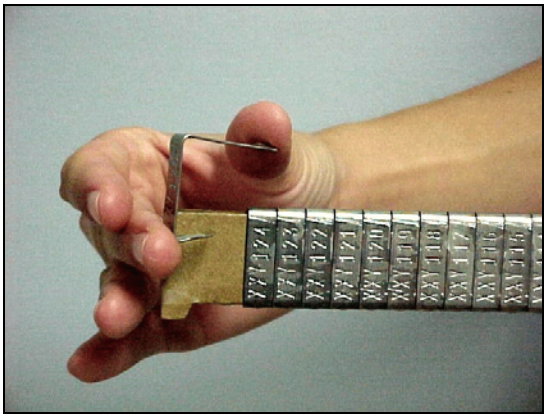


Figure 6-1. Remove cleaned tag from strip (NMFS/SEFSC photo).

Figure 6-2. Inserting the tag into the applicator (NMFS/SEFSC photo).

strip to remove one or two tags at a time to prevent loss of remaining tags. Scrub all tags with hot, soapy water to remove the oily residue present when shipped from manufacturer and disinfect with isopropyl alcohol or 10% povidone-iodine solution prior to use. Tags provided to NMFS/SEFSC observers will be cleaned before distribution.

(2) Hold the applicator in one hand. With the pointed (piercing) side of the tag facing the depression in the jaw of the pliers and with the hole placed adjacent to the depression, place the end of your index finger of the other hand inside the tag against the bend. Pull the tag straight back into the open jaws of the applicator, aligning the point opposite the small depression (Figure 6-2). A firm pull will be needed to snap the tag completely into the correct position. Take care not to squeeze the applicator together before you are ready to tag the turtle or the tag will fall out. Swab the tag, applicator tips, and tagging site with 10% povidone-iodine solution.

(3) *Rear Flipper Tagging (preferred site):* Locate the correct site (Figure 6-3) to apply the tag (the trailing edge of the rear flipper just proximal to the first scale). Juvenile and subadult hardshell turtles can be placed on their carapace to facilitate access to the tagging site. If someone is available to help, they should hold the turtle and restrain the flipper while the tag is applied. Be sure to position the tag so there will be adequate overhang (approximately 1/3 the length of the

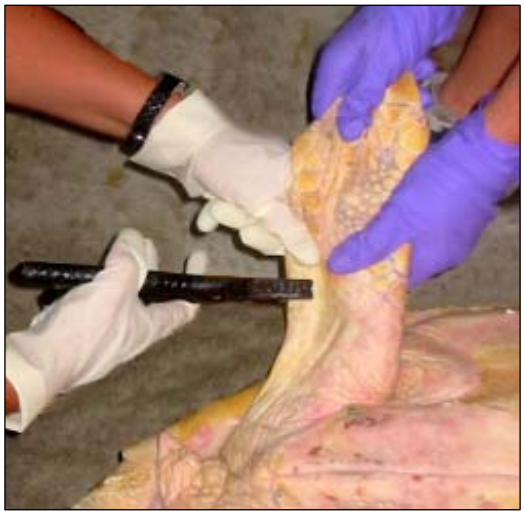


Figure 6-3: Applying an Inconel® tag to the rear flipper of a loggerhead turtle (NMFS/SEFSC photo).

tag) after it is attached to the flipper (Figure 6-4).

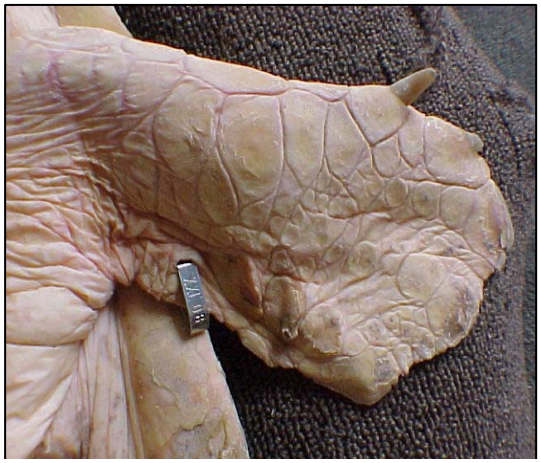


Figure 6-4. Inconel® tag applied to the rear flipper of a sea turtle (NMFS/SEFSC photo).

Front Flipper Tagging: Although the rear flipper is the preferred location, there may be circumstances where the front flipper is tagged instead. Place the turtle on its plastron and locate the correct site to apply the tag (the trailing edge of the front flipper(s) immediately proximal and adjacent to the first scale (Figure 6-5), or between the first and second large scales distal to the axilla). If someone is available to help, they should hold the turtle and restrain the flipper while the tag is applied. Be sure to position the tag so there will be adequate overhang (approximately 1/3 the length of the tag) after it is attached to the flipper.

(4) Apply the tag by squeezing the applicator together in a firm, steady manner. The tag point will pierce the flipper and lock into place with the tip bending securely over the opposite side like a staple point. Squeeze the applicator together with some force in order to fully lock the tag; it may be helpful to use both hands. If the tag does not lock, grasp it once again with the pliers and apply more pressure. You can use the tips of the pliers to pinch down on the end of the tag's tip to ensure that the tip is securely locked. If you cannot get the tag to lock, remove it and apply another tag to the same flipper. A tag that is not applied properly will be shed quickly.

Figure 6-5. Applying an Inconel® tag to the front flipper of a loggerhead turtle (NMFS/SEFSC photo).

(5) Repeat the entire procedure and apply a second tag at the same site on the other flipper (Figure 6-6). All turtles should be double tagged in this manner using consecutive tag numbers

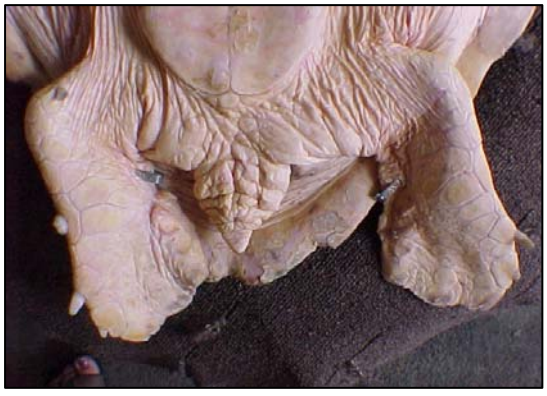


Figure 6-6. Two rear flipper tags (NMFS/SEFSC photo).

whenever possible. If a tag is damaged for any reason, please record this information on the tagging form and return the damaged tag. If the recommended tagging site has been injured or is unsuitable for tag application, use an alternate site along the trailing edge of the flipper.

PIT Tags

Currently, NMFS/SEFSC is using sterile-packed single use 125 kHz Destron PIT tags. These inert tags are 12 mm x 2.1 mm glass encapsulated RFID tags. They are positioned inside the turtle where loss or damage due to abrasion, breakage, corrosion over time is virtually non-existent (Balazs 1999).

Scanning Protocol

All turtles encountered should be checked for PIT tags. Rarely, a turtle may have more than one PIT tag. PIT tag scanners in use by the SEFSC generally are capable of reading frequencies of 125 kHz, 128 kHz, 134.2 kHz, and/or 400 kHz. Researchers should avoid using AVID encrypted tags; these encrypted tags cannot be read by all scanners, and few scanners capable of reading encrypted tags are widely in use by researchers in the field.

Figure 6-7. Scanning for internal PIT tags (NMFS/SEFSC photo).

(1) Keep the PIT tag scanner inside a plastic sealed bag at all times during use to prevent it from getting wet. Scan a sample tag to verify that the PIT tag reader is working properly. The button on the scanner needs to be continuously depressed throughout the scanning process, and the screen may display "WORKING" or similar (depending on the type of scanner) when functioning properly.

(2) Place the PIT tag scanner directly on the turtle's skin; on leatherbacks you may have to press hard into the skin with the reader, as the tag may be deep. For hardshell turtles, slowly scan the dorsal surface of both front flippers (Figure 6-7), the shoulder and neck areas, and rear flippers. Attempt to scan the ventral surfaces, especially all four flippers and the neck, as some projects tag in the rear flippers or other locations; small turtles can be turned over for access to ventral surfaces. For leatherbacks, scan the dorsal musculature of both forelimbs, the shoulder region and the top of the neck. It is important to slowly move the scanner multiple times, allowing it to cycle through different tag frequencies to avoid missing a tag.

(3) If a PIT tag is detected, record the identification code exactly as it appears on the scanner display, including any hyphens that may appear as part of the code. ID codes usually are hexadecimal (digits 0-9 and letters A-F) and are 10 bytes (125, 128, or 400 kHz tags) or 15 bytes (134.2 kHz tags) long. Double check to make sure you have recorded the ID code exactly as it appears on the reader display. Please be especially careful with letters and numbers

that easily are confused, such as the letter O and the number Ø. Record all tag IDs (there could be more than one PIT tag). If the scanner display reads "AVID" or the ID reads inconsistently, you may have detected an encrypted AVID tag. Encrypted tags may display a 16 byte alphanumeric code (0-9 and A-Z) on non-AVID reader displays. Record what you see on the viewer and insert a new PIT tag in the opposite shoulder/flipper.

(4) Wipe off the plastic bag. If a tag ID code remains on the display, press the scanner button again until it reads "no tag found" to extend the battery life, although the PIT tag scanner automatically turns itself off eventually. When not in the field, store the unit with the plastic bag open so that humidity does not accumulate and damage the unit. Replace or recharge batteries as needed, and do not store the unit for long periods with the batteries installed.

Application Protocol

Turtles larger than 30 cm SCL should receive PIT tags if scanning reveals no PIT tags present. In some cases, experienced taggers may feel comfortable tagging smaller turtles in the triceps superficialis muscle. The tag should occupy less than 20% of the muscle's total volume and 1/5 of its length, and it should not be located near the ends of the muscle (J. Wyneken pers. comm.). To determine if a small turtle should be tagged in the triceps superficialis, pinch the muscle forward and assess the tag size relative to the muscle size.

(1) Scan the PIT tag before opening the package to ensure that it is a functional tag. Double check that the number on the display matches the label.

(2) Record the PIT tag number on the datasheet and peel off the self-adhesive label on the PIT tag package, if available, and attach it to the datasheet.

(3) Remove the loaded needle from the sterile wrapper and insert it into the injector, or remove the preloaded syringe and needle if using these, taking care not to depress the plunger.

(4) Swab the PIT tag injection location and the end of the injector with 10% povidone-iodine solution

(5) Place the tag into musculature, where it will become encapsulated. Leatherbacks should be tagged in the center of the dorsal musculature (triceps complex) of the forelimb (Figures 6-8 and 6-9); insert the entire needle perpendicular to the skin (Note: The preferable site for leatherbacks is the musculature above the right forelimb, as some nesting research projects only scan the right side). Hardshell turtles should be tagged in the triceps superficialis muscle (Figures 6-10 and 6-11); pierce the skin of the flipper with the needle and insert the entire needle parallel to the surface just under the skin and into the muscle. Slide the plunger forward. (Note: The preferable site for Kemp's ridleys is the left triceps superficialis muscle to maximize the chances of tag detection, as the nesting project in Rancho Nuevo scans the left front flipper).

(6) Put your thumb over the injection site and apply pressure while carefully removing the needle. Dispose of the needle in a sharps container. If the

injection site bleeds, swab it with 10% povidone-iodine solution and apply pressure until the bleeding stops.

(7) Scan the flipper for the PIT tag to ensure that it is functioning in the turtle.

Figure 6-8. Leatherback turtle PIT tagged in the dorsal musculature (Photo courtesy of S. Eckert, Duke University).

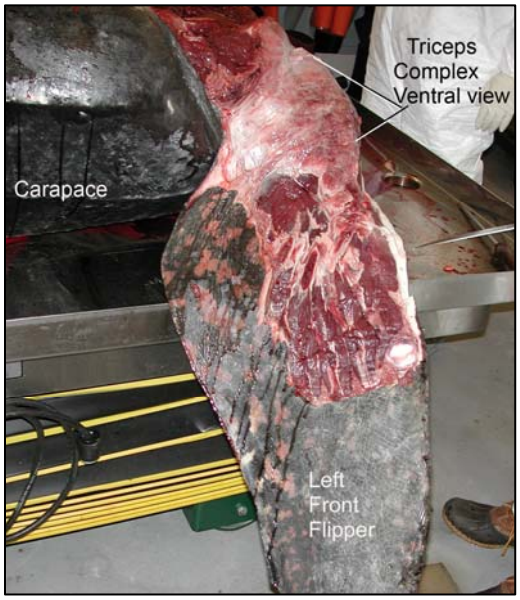
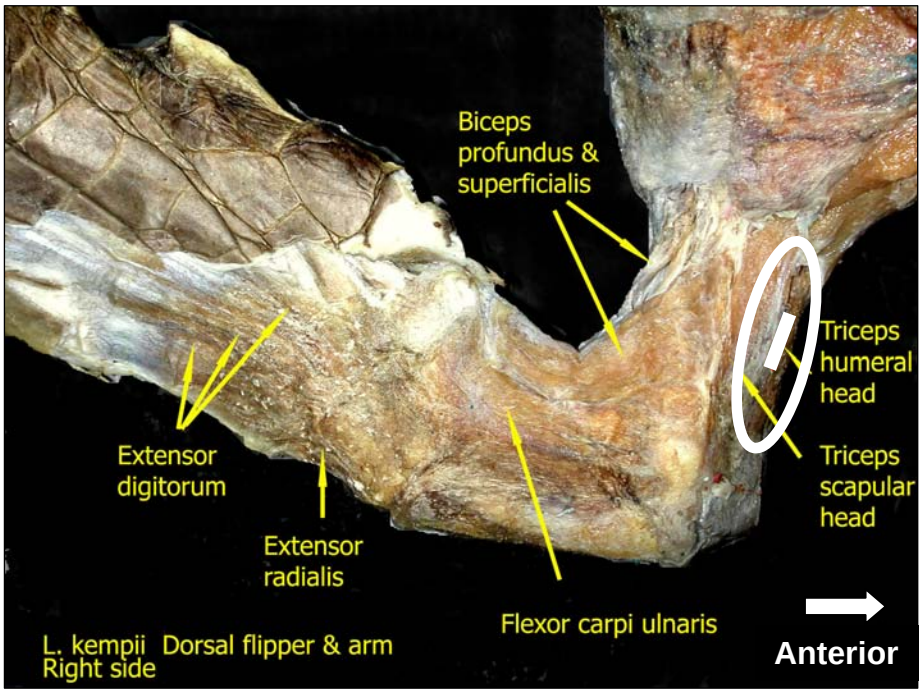
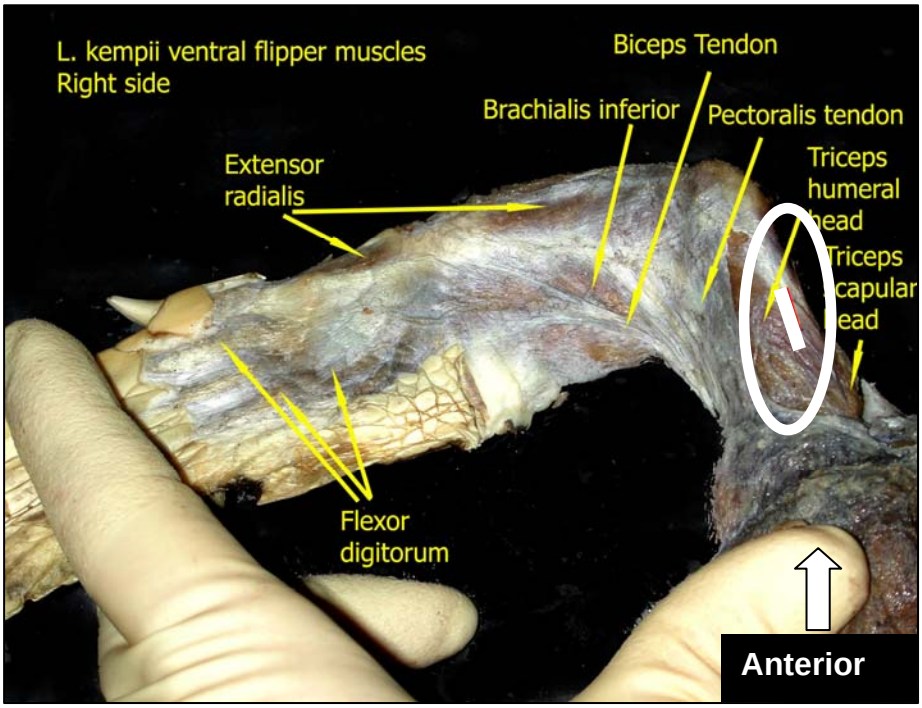


Figure 6-9. Annotated leatherback musculature depicting the triceps complex. (Photo courtesy of J. Wyneken, Florida Atlantic University).

Figure 6-10. Inserting PIT tag into the triceps superficialis muscle of a Kemp's ridley (NMFS/SEFSC photo).





Figures 6-11a and b. PIT tag placement (white line) in hardshell turtles, shown in dorsal view (a) and ventral view (b) of a dissected Kemp's ridley flipper (Photos and annotations by J. Wyneken, Florida Atlantic University).

Carapace Tagging

Tagging leatherbacks externally allows researchers, observers and fisherman to report tag sightings even when they are unable to bring the turtle onboard. Color-coded tagging, such as Floy® dart tags, would allow for the easy identification of an animal that had interacted with a fishery when encountered again on the high seas or on a nesting beach. These tags have a stainless steel applicator tip and a nylon dart head. Further detailed descriptions of Floy® dart tags can be found at: http://www.floytag.com/images/floycatalog.pdf. The tagging protocol is relatively simple and has been used for a number of years in marine and freshwater fish species.

1) Clean harpoon applicator tip and dart anchor thoroughly with 10% povidone-iodine wipes.

2) Load tag into applicator.

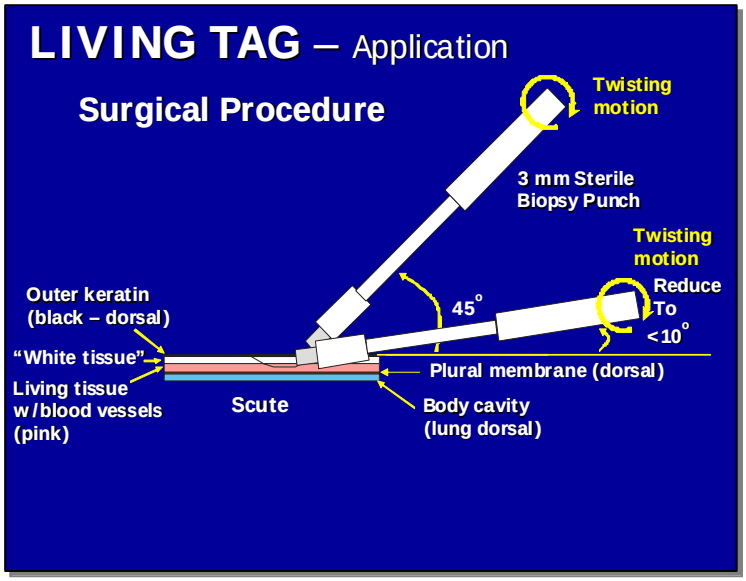
3) Tag animal with a quick, forceful jab high on carapace adjacent to the central ridge to optimize visibility.

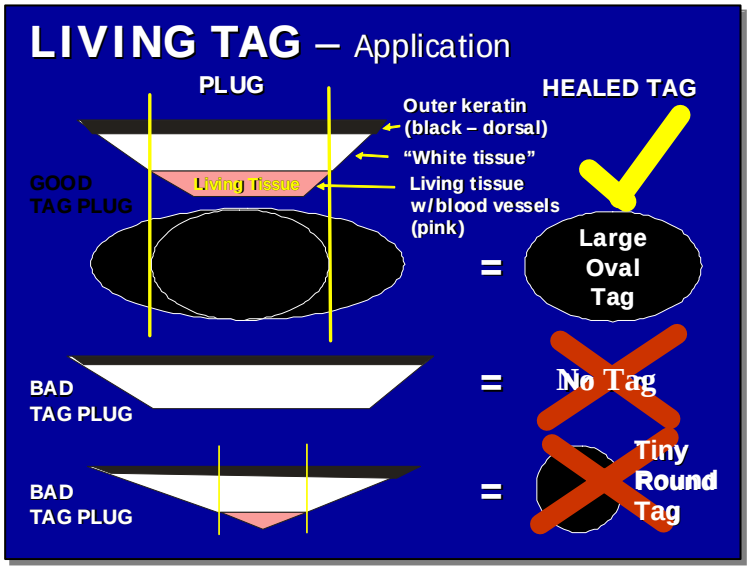
Living Tags

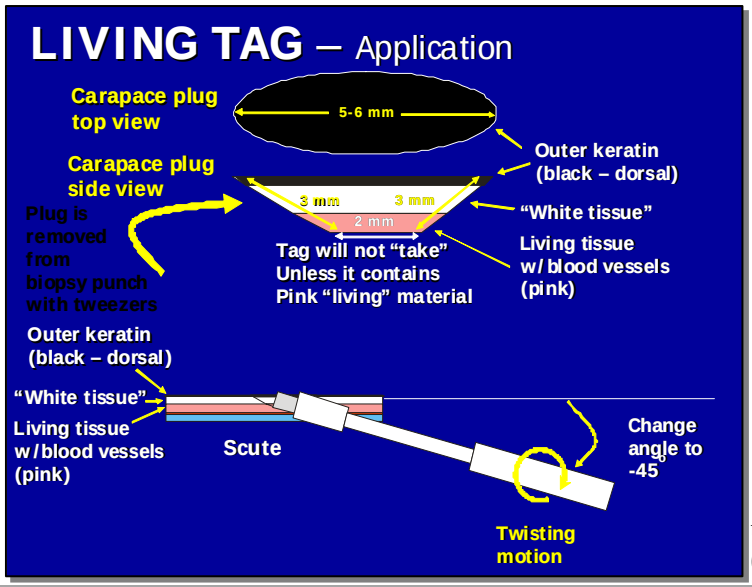
Living tags provide a permanent marking method for sea turtles, and they are particularly useful with post hatchlings and small juveniles that cannot be marked using traditional tagging methods. A living tissue plug is removed from the plastron and transplanted into the carapace, leaving a permanent, identifiable light spot that grows with the animal on the contrasting dark carapace.

At least 24 hours prior to tagging, thoroughly scrub the carapace and plastron with clean water, antibacterial soap, and a scrub brush (e.g., toothbrush). Flakes of keratin, if present, can be scraped off with a scalpel blade held perpendicular to the carapace. Clean the area with fresh water and dry with a paper towel just prior to tagging (Figures 6-12a and b). Wear latex or similar disposable gloves and keep the area and equipment clean. Select a standard scute location on the carapace to receive the living tag plug. The ideal location is usually fairly central in the scute, and the topography of the carapace should match that of the plastron plug (i.e., do not take a plastron plug from a flat area and then take a peak from the carapace; a flat carapace location should receive a flat

Figures 6-12a and b. Cleaning the (a) carapace and (b) plastron (NMFS/SEFSC photos).







Figures 6-13a-c. Living tag procedure (NMFS/SEFSC diagrams).

plastron plug). For loggerheads, NMFS/SEFSC has found that the best tags were taken from the relatively thick tissue of the humeral or pectoral scutes on the plastron, so that bone was not sampled.

The order that the living tissue is obtained does not matter; the carapace or the plastron can be sampled first. The goal is to minimize the time required for the procedure. First, the method of taking carapace tissue is described. Place the blade of a sterile 3 ─ 6 mm biopsy punch at the carapace surface forming an approximate 45º angle. Using moderate force and a twisting action (twisting reduces the amount of force required to cut through the carapace material), let the biopsy punch cut into the carapace material to a depth of approximately 1 ─ 2.5 mm (Figure 6-13a).

Depth control is critical to obtaining a good plug. You must reach the area containing "pink" living tissue (only one mm thick on a seven-month-old Kemp's ridley, two mm in a 120 g loggerhead) that is vascularized. If you go too deep, you may puncture the lung (carapace) or enter body cavity (plastron). Once at the correct depth, reduce the angle of the biopsy punch to approximately five degrees, push forward two to three mm, and then angle back up to the surface, creating a plug that is 5 to 6 mm in length, three mm wide and 2.5 mm in depth (Figure 6-13b).

The side profile of the plug should be layered (Figures 6-13b and c) with a layer of black or brown/white keratin, layer of "white" bone/cartilage, and a thin layer of "pink" vascularized (blood vessel) material. Take care to ensure that no pigmented keratin material contaminates the white and pink layers of the plug, and if the plug is temporarily placed aside, it should be placed with the keratin (shell) side down to avoid contaminating the "living" areas of the plug. Only tag plugs with suitable living tissue will form good living tags; tag plugs with no living tissue will not take, and those with little living tissue will form small, potentially undetectable healed living tags. Depending on the skill level of the tagger, oval shaped tags may remain oval or heal in a circular shape. The shape, size and area of the living tissue on the tag plug and tag hole dictate the final shape and size of the living tag. Unless reciprocal transplants are to be done, discard the carapace plug (Figure 6-14); attempting to transplant carapace plug into plastron hole is rarely successful on Kemp's ridleys, but has been found to work well on loggerheads.

Figure 6-14. Removing the carapace tissue plug (NMFS/SEFSC photo).

Next, select an all white or cream-colored scute from the plastron matching the profile of carapace surface where the tag will be placed as the source of the living tag plug. Use a 3 ─ 6 mm biopsy punch to remove a plastron plug, and use forceps disinfected with 70% isopropyl alcohol to handle the plug (Figure 6-15). Clean off forceps in alcohol between handling the carapace plug and the plastron plug. The presence of moisture will cause tissue adhesive to foam or clump; excess moisture (including blood) should be blotted from the area to receive glue. Use veterinary

Figure 6-15. Removing the plastron tissue plug. (NMFS/SEFSC photo).

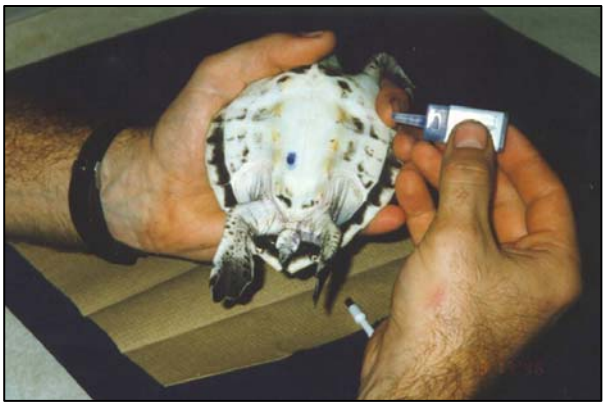


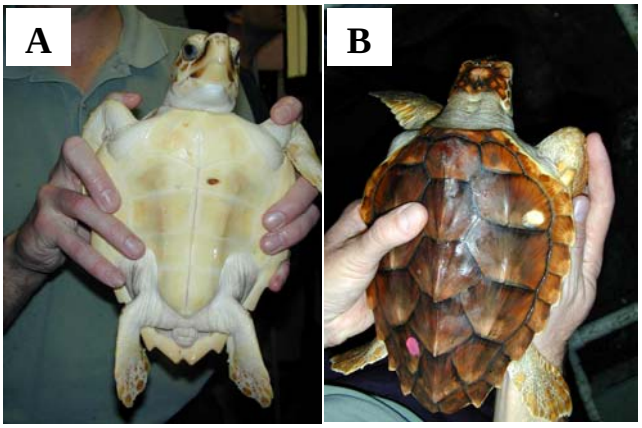
Figure 6-16. Sealing plastron with tissue glue (NMFS/SEFSC photo).

Figure 6-17. Inserting the plastron tissue plug onto the carapace (NMFS/SEFSC photo).

quality tissue adhesive (such as Nexaband® or Vetbond®) to fill and seal the empty plastron donor hole where the plug was taken if there is no reciprocal transplant, ensuring that all edges are sealed with one continuous film (Figure 6-16).

Insert the plastron plug into the carapace hole and press it into place. Rotate the plastron plug in the carapace hole to get the best fit possible, and then press down the plug to expel any liquid that might have pooled in the hole (Figure 6-17). If the plastron plug is a good match for the carapace hole, pressing down will create a slight vacuum that will hold the tag in place until it can be glued. Blotting with a paper towel helps remove any excess liquid that will interfere with the tissue glue. Apply veterinary quality tissue adhesive (such as Nexaband® or Vetbond®) around the perimeter of the tag, taking care not to allow the glue to flow over the complete surface of the tag or get under the plastron plug. If glue gets under the plastron plug, or if too much glue is used and the surface of the tag is completely covered, the tag will not "take." Use only enough glue to seal the perimeter of the plastron plug to the edges of the carapace hole, and do not try to wipe off excess glue, as the tag may stick to the wiping object and pull out.

Turtles should be left out of the water for 15 – 30 minutes after tagging to allow the glue to dry. It takes about six weeks for the living tag to heal before it can be determined whether or not the tissue graft was successful (Figures 6-18a and b).



Figures 6-18a and b. A 14-month-old loggerhead showing living tags on (a) the plastron and (b) the carapace. (Photos by J. Wyneken, Florida Atlantic University).

Wire Tags

Successfully tagging large numbers of hatchlings presents a significant challenge. Coded Wire Tags (CWTs) provide a reliable method for marking hatchlings, and they have been used extensively in captive reared and wild Kemp's ridley hatchlings (Higgins et al. 1997). CWTs have also been used in larger turtles, such as yearling Kemp's ridleys from the NOAA Kemp's ridley headstart project (1978-1992). A small section of coded wire is injected using a specialized tag injector into the dorsal surface of the front flipper near the claw (Figure 6-19).

The tags may be either non-magnetized or magnetized at the time of insertion, but the wire tag must be magnetized for detection with a handheld magnetometer. A non-magnetized tag can be magnetized immediately before detection by passing a magnet over the front flippers where the tags would be implanted, or before tagging by using a pre-magnetized roll of wire or using a magnetized head on the tag injectors (Higgins et al. 1997, Fontaine et al. 1993).

CWTs may be inscribed with binary or decimal coding. They each may be coded with a unique label or more often, each tag on a spool of coded wire is identical, allowing for the identification of groups and not individuals. To read the code on a CWT, dissection and examination under a microscope is necessary. Therefore, the code on CWTs is only obtained when a turtle is recovered dead, allowing for dissection and removal of the tag.

CWTs are detected using a wand type tag detector (magnetometer) or by x-ray radiography. To detect a tag using a wand tag detector, make sure that there are no metal objects (e.g., jewelry, watches) in the area, as they can interfere with magnetic tag detection. Test the unit by passing it over a metal with a known magnetic content and confirm an audible beep. If possible, position the turtle at least one meter away from the ground, sand, metal equipment, vehicles, electronic circuits, walls with pipes or reinforcement steel, etc. Immobilize the turtle and extend the flipper away from the turtle's body and hold the detector perpendicular to the leading edge of the flipper next to the body. Pass the wand over the surface of the flipper keeping it perpendicular to the leading edge of the flipper, keeping it as close to the flipper surface as possible without touching it (Figure 6-20) . Scan each surface of both flippers at least three times up and down the length of the flipper. If no tags are detected after three passes along the entire flipper, try several short passes back and forth in the area of the claw. If a suspected tag is detected (the wand beeps), carefully pass the wand over the suspected tag site to confirm consistent multiple readings. Check around the flipper to make sure there are no metal sources that could cause a false positive reading.

If no tag is detected after examining both surfaces of the front flippers, pass a magnet over the flippers in an attempt to magnetize a non-magnetized wire tag (Figure 6-21). The magnet should be passed in only one direction in parallel sweeps from the leading edge of the flipper towards the trailing edge in overlapping sweeps. Take care not to sweep the magnet perpendicular from the leading edge to the trailing edge, as this could result in a failure of the tag to take a magnetic charge or the un-magnetization of a previously magnetized tag. After passing the magnet over each flipper surface, follow the previously described procedure for tag detection.

A detailed description of the protocol for wire tagging and detection (Higgins et al. 1997) can be found at: http://www.sefsc.noaa.gov/seaturtletechmemos.jsp and http://galveston.ssp.nmfs.gov/publications/pdf/279.pdf.

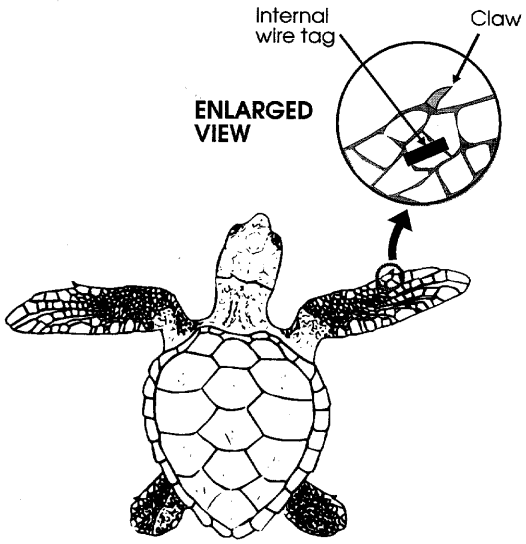


Figure 6-19. Implantation location of internal wire tag in hatchling flipper (Diagram from Higgins 1997).

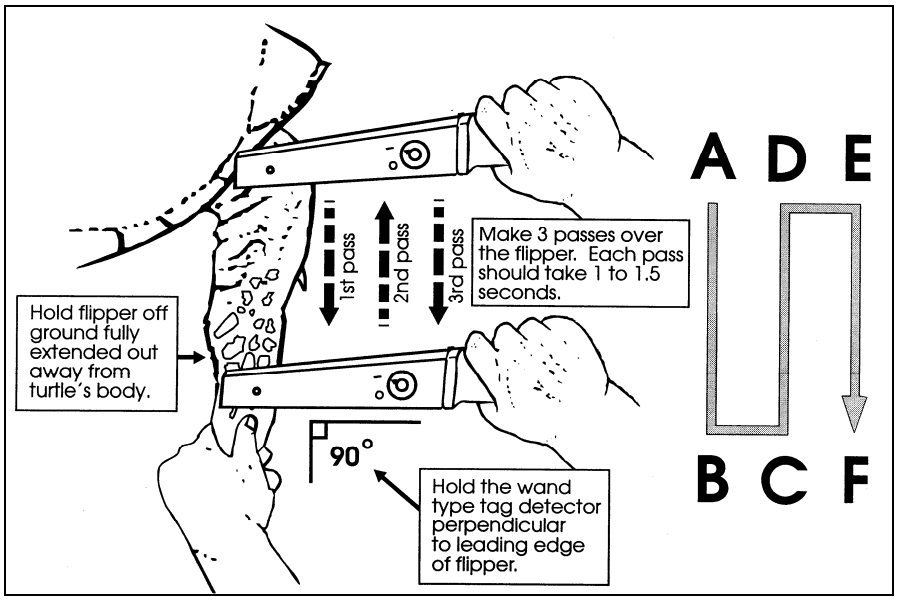


Figure 6-20. Proper positioning of wand over the turtle's flipper for magnetized wire tag detection (Diagram from Higgins 1997).

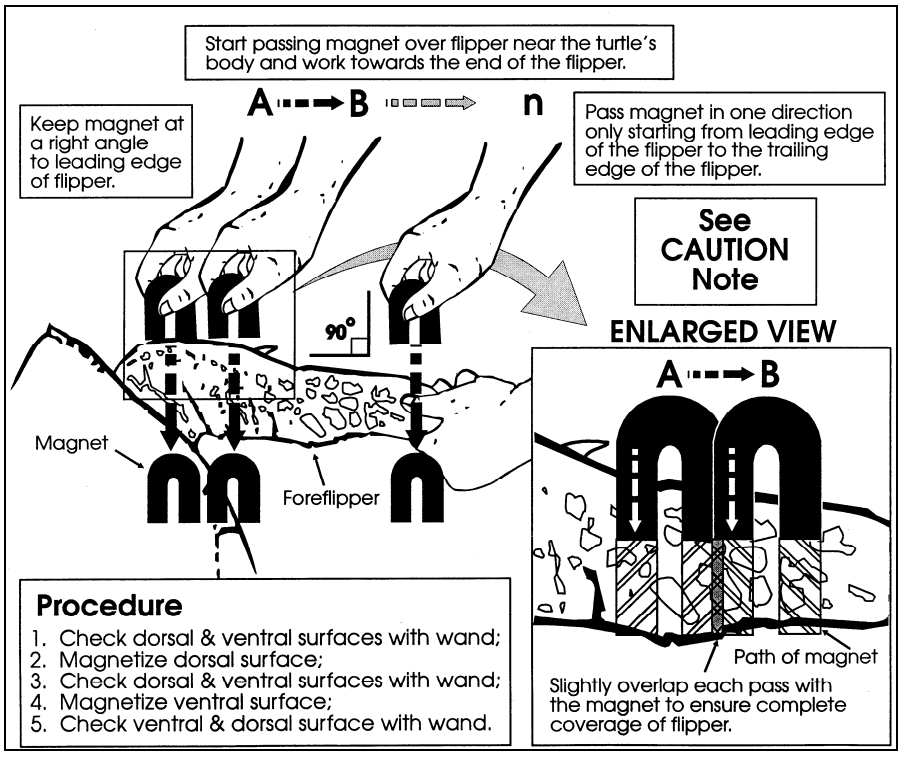


Figure 6-21. Proper technique for magnetizing a previously non-magnetized wire tag in a flipper (Diagram from Higgins 1997).

Oxytetracycline Marking

In certain circumstances, sea turtles may be injected with the antibiotic oxytetracycline (Figure 6-22). Oxytetracycline marks the bones of the sea turtle at the time of injection so they can be used in future aging studies if the turtle strands dead. One dose administered prior to hook removal, skin biopsy, and tagging could offer the same beneficial prophylactic effects as presurgical antibiotics may offer in preventing post-surgical infections as well.

The quantity of tetracycline to be administered depends on the weight of the animal, which can be estimated from its straight carapace length (SCL_{N-T}) if the actual weight is unknown. Estimated dosage quantities are provided on the Dosage Cards for Kemp's ridleys (Table 6-1), loggerheads (Table 6-2), and green sea turtles (Table 6-3). These values are based on length-weight regression models derived from morphometric data collected from wild-caught turtles in the coastal waters of North Carolina. As such, we do not recommend using the estimated dosage provided on turtles outside the Atlantic Ocean and Gulf of Mexico to account for potential differences in length-weight relationships among populations. Measure the straight carapace length of the turtle in cm and use the Dosage Cards to find the corresponding dosage (25 mg/kg assuming an oxytetracycline concentration of 200 mg/ml). If the actual weight of the turtle is known, or if you are using a different concentration of the drug, calculate the dosage using the formula:

Dosage (ml) = Weight (kg) x 25 (mg/kg) / concentration (mg/ml).

Verify that product has not expired, as this product can become toxic after expiration, and confirm the product's concentration. Wear disposable gloves and draw the necessary dosage from the bottle with a disposable syringe. Use a 3-cc syringe for antibiotic quantities 0.6 – 2.9 ml and a 5-cc syringe for larger quantities.

Figure 6-22. Oxyetracycline injection (NMFS/SEFSC photo).

On a restrained turtle, clean the injection site with 10% povidone-iodine solution. Insert the needle in the right front dorsal shoulder musculature (latissimus dorsi, terres major, and deltoides) in a single injection site using a sterile, disposable syringe and a 20-gauge 1" needle. Animals with a SCL > 70 cm should have their dosage split into two equal volumes to administer in each shoulder. Before injecting the tetracycline, pull back on the syringe plunger to make sure the needle is not in a blood vessel. If there is no blood coming into the syringe, apply continuous force to the plunger to administer the antibiotic. If blood does enter the syringe, readjust the needle placement by partially retracting the needle

(do not remove entirely) and changing the angle of insertion. Check again to verify the needle is not in a blood vessel before administering the antibiotic. After removing the needle, apply pressure with a 10% povidone-iodine swab in the area to stop any bleeding and prevent infection. Dispose of the needle and syringe in a sharps container.

OXYTETRACYCLINE DOSAGE FOR KEMP'S RIDLEY SEA TURTLES

SCL N-T (cm)	Drug Dosage (ml)		SCL N-T (cm)	Drug Dosage (ml)
20	0.2		43	1.4
21	0.2		44	1.5
22	0.2		45	1.6
23	0.3		46	1.7
24	0.3		47	1.7
25	0.3		48	1.8
26	0.4		49	1.9
27	0.4		50	2.1
28	0.5		51	2.2
29	0.5		52	2.3
30	0.5		53	2.4
31	0.6		54	2.5
32	0.6		55	2.6
33	0.7		56	2.8
34	0.8		57	2.9
35	0.8		58	3.0
36	0.9		59	3.2
37	0.9		60	3.3
38	1.0		61	3.5
39	1.1		62	3.6
40	1.1		63	3.8
41	1.2		64	3.9
42	1.3		65	4.1

Table 6-1. Oxytetracycline dosage card for Kemp's ridley sea turtles assuming a dose of 25 mg/kg and an oxytetracycline concentration of 200 mg/ml. Dosage (ml) = Weight (kg) x 25 (mg/kg) / concentration (mg/ml). Dosage calculations are based on estimated weight from Kemp's ridley morphometric data regressions where: Weight (kg) = $0.0006(SCL)^{2.6121}$ with $R^2 = 0.894$.

OXYTETRACYCLINE DOSAGE FOR LOGGERHEAD SEA TURTLES

SCL N-T (cm)	Drug Dosage (ml)		SCL N-T (cm)	Drug Dosage (ml)
40	1.3		73	6.7
41	1.4		74	7.0
42	1.5		75	7.3
43	1.6		76	7.5
44	1.7		77	7.8
45	1.8		78	8.1
46	1.9		79	8.4
47	2.0		80	8.7
48	2.1		81	9.0
49	2.2		82	9.3
50	2.4		83	9.6
51	2.5		84	9.9
52	2.6		85	10.2
53	2.8		86	10.5
54	2.9		87	10.8
55	3.1		88	11.2
56	3.2		89	11.5
57	3.4		90	11.9
58	3.5		91	12.2
59	3.7		92	12.6
60	3.9		93	12.9
61	4.1		94	13.3
62	4.3		95	13.7
63	4.5		96	14.0
64	4.7		97	14.4
65	4.9		98	14.8
66	5.1		99	15.2
67	5.3		100	15.6
68	5.5		101	16.0
69	5.8		102	16.4
70	6.0		103	16.8
71	6.2		104	17.3
72	6.5		105	17.7

Table 6-2. Oxytetracycline dosage card for loggerhead sea turtles assuming a dose of 25 mg/kg and an oxytetracycline concentration of 200 mg/ml. Dosage (ml) = Weight (kg) x 25 (mg/kg) / concentration (mg/ml). Dosage calculations are based on estimated weight from loggerhead morphometric data regressions where: Weight (kg) = $0.022\ (SCL)^2 - 1.1789(SCL) + 22.751$ with $R^2 = 0.897$.

OXYTETRACYCLINE DOSAGE FOR GREEN SEA TURTLES

SCL N-T (cm)	Drug Dosage (ml)		SCL N-T (cm)	Drug Dosage (ml)
20	0.1		57	2.6
21	0.2		58	2.8
22	0.2		59	2.9
23	0.2		60	3.1
24	0.2		61	3.2
25	0.2		62	3.4
26	0.3		63	3.5
27	0.3		64	3.7
28	0.3		65	3.8
29	0.4		66	4.0
30	0.4		67	4.2
31	0.5		68	4.4
32	0.5		69	4.6
33	0.6		70	4.8
34	0.6		71	4.9
35	0.7		72	5.1
36	0.7		73	5.4
37	0.8		74	5.6
38	0.8		75	5.8
39	0.9		76	6.0
40	1.0		77	6.2
41	1.0		78	6.5
42	1.1		79	6.7
43	1.2		80	7.0
44	1.3		81	7.2
45	1.3		82	7.5
46	1.4		83	7.7
47	1.5		84	8.0
48	1.6		85	8.3
49	1.7		86	8.6
50	1.8		87	8.8
51	1.9		88	9.1
52	2.0		89	9.4
53	2.1		90	9.8
54	2.3		91	10.1
55	2.4		92	10.4
56	2.5			

Table 6-3. Oxytetracycline dosages for green sea turtles assuming a dose of 25 mg/kg and an oxytetracycline concentration of 200 mg/ml. Dosage (ml) = Weight (kg) x 25 (mg/kg) / concentration (mg/ml). Dosage calculations are based on estimated weight from green sea turtle morphometric data regressions where:
Weight (kg) = $0.0002(SCL)^{2.861}$ with $R^2 = 0.777$.

Chapter 7: Electronic Tags

Electronic tags allow researchers to remotely monitor information such as position, movement patterns, dive behavior, survival, and environmental parameters. Satellite tags are used to collect data on location, depth, and/or temperature. Deployment length is dependent on battery size and will vary depending on research question and animal size. Sonic tags emit an acoustic signal that can be received underwater with a hydrophone. Triangulation of the acoustic signal allows researchers to determine an animal's location. Radio tags emit a radio signal on a specific frequency that can be detected by an antenna when a turtle surfaces. Radio tags provide location information via triangulation of the signal above the water.

Electronic tags, including sonic, radio, satellite transmitting, and archival tags are attached to sea turtles via two methods: direct and tethered. All tags and attachment materials should weigh less than five percent of a turtle's weight, and tags should be streamlined to minimize any effects of drag. Researchers must make attachments as hydrodynamic as possible. Tag dimensions vary by manufacturer and tag type, but should be proportional to turtle size and consistent with weight restrictions. Each attachment must be made so that there is no risk of entanglement. The lanyard (if used) length must be less than 1/2 of the carapace length of the turtle to avoid entanglement in the turtle's front flippers and prevent the turtle from biting the tag. It must include a corrodible, breakaway link that will corrode and release the tag-transmitter after the tag-transmitter life is finished. Adequate ventilation around the head of the turtle must be provided during the attachment of tags if attachment materials produce fumes. To prevent skin or eye contact with harmful chemicals used as attachment materials, turtles must not be held in water during the application process. Ideally, turtles will be and held no longer than two hours; however, there may be weather or logistical events that may require bringing turtles to shore for tagging and temporary holding. In that event, turtles should be released as weather permits, no more than one day after capture.

Electronic Tag Specifications

Below is a list of tag types currently in use or being considered for use by the NMFS/SEFSC. Specific manufacturers and models are not listed here because of the dynamic nature of this field of technology. Attachment methods are constantly refined and improved by researchers; the methods defined here have been tested and approved by NMFS/SEFSC, but do not constitute an exhaustive list of potential acceptable methods. Argos transmitting satellite and archival tags operate within approved frequencies, 401.618 MHz to 401.680 MHz. Sonic tags operate in the 25 – 80 kHz range, and radio tags use a range of 164 – 166 MHz.

Tag Attachment Protocols

Tether Attachment Protocol (see Epperly et al. 2007)

Hardshell turtles

Tethers for satellite, radio, or sonic tags are attached to the trailing edge at the rear of the carapace (Figure 7-1) to reduce drag while

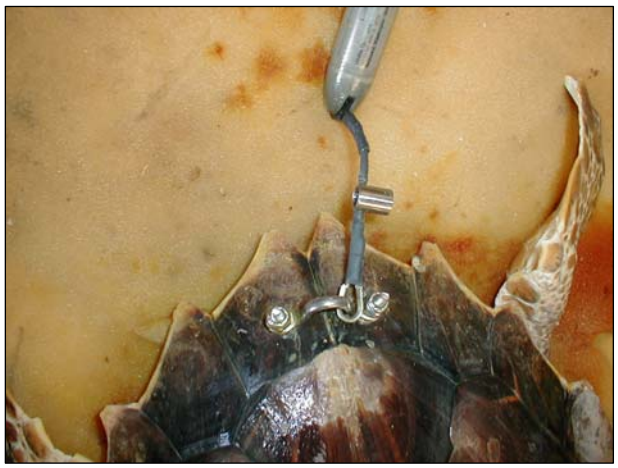


Figure 7-1. Attachment of an archival satellite tag on a hardshell turtle (NMFS/SEFSC photo).

being towed by the turtle. Tags are streamlined and as light as possible (< five percent of body weight) to keep drag to a minimum. When handling the turtle and equipment, use disposable gloves and change them often to maintain the most sterile environment possible.

After removing epibionts and thoroughly scrubbing the area with water and povidone-iodine disinfectant, drill a 0.5 cm hole through one of the turtle's pygal bones, as well as the overlying scutes, with a drill bit soaked in povidone-iodine disinfectant for 15 minutes prior to use. Pass a plastic electrician's tie through the hole and secure. Transmitters should be housed in bullet-shaped buoys (approximately 10 cm diameter and 10 cm in height) secured to one end of a tether that consists of one mm diameter stainless steel fishing leader. Connect the tether to the plastic electrician's tie in the turtle's shell with a ball-bearing swivel and two short lengths of either 30 lb braided fishing line (e.g., Spiderwire®) or 30 lb test monofilament fishing line. This configuration will allow the turtles to break free if either the buoy or tether becomes entangled in submerged or floating debris or bottom structure.

1. Immobilize the turtle and clean dorsal and ventral surfaces of postcentral scutes using a scouring pad and scrub brush, and if needed for barnacle removal, a chisel. Activate tag.

2. Pour ~ two oz 10% povidone-iodine to thoroughly soak the hardware into a bag containing the hardware, exclusive of the nylon parts, and a new drill bit, and soak for at least 15 minutes, agitating frequently. Use alcohol swabs to clean the nylon parts, as iodine breaks down nylon over time, while alcohol is inert.

3. Saturate sterile gauze sponges with 10% povidone-iodine and cleanse the dorsal and ventral surfaces of the postcentral scutes several times over a 15 minute period.

4. Install a 3/16" titanium drill bit into the portable drill and align the eyestrap (pad eye) on the postcentral scutes. Be sure to position the eyestrap as far forward (toward head) as possible on the postcentral scutes to capture the underlying bone. However, be cognizant that you will be drilling at an angle; do not drill so far up as to intercept the integument on the ventral surface (Figure 7-1). Using the holes of the eyestrap as a guide, drill once quickly through the scute. Use a blood clotting gel such as Clotisol® or ferric subsulfate to stop bleeding, if necessary, after first cleaning the dropper tip with an alcohol swab.

5. Flood the area thoroughly with 10% povidone-iodine.

6. Select an appropriate length 1/8" bolt and insert it through the eyestrap. If the bolts are too long, insert them from the bottom so that they can be trimmed later. Use nylon washers against the carapace and the plastron and a stainless washer between the eyestrap and the nut or head of the bolt.

7. Thread thimble of tag tether over eyestrap before inserting the second bolt.

8. Repeat steps six and seven for the second hole.

9. Turn the turtle onto its carapace, being careful to protect the tag (try to keep it in the PVC sleeve), and secure the bolts with washers and lock nuts using a wrench.

10. Use bolt cutters to cut off any excess length of the bolts if necessary.

Leatherback Turtles- Pygal Tether Attachment (Figure 7-2)

1. Immobilize the turtle and activate the tag. When handling the turtle and equipment use disposable gloves, changing them often to maintain the most sterile environment possible.

2. Pour enough 10% povidone-iodine into a hardware bag containing two 1/4" X 1 5/8" acetal polyoxymethylene resin (e.g., Delrin®) disks, a new 5/16" drill bit, and 1/4" outer diameter surgical tubing to coat items. Agitate the bag frequently to disinfect the hardware.

3. Saturate sterile gauze sponges with 10% povidone-iodine or use 10% povidone-iodine scrubs and cleanse the dorsal and ventral areas of turtle in the pygal region (Figure 7-2). Do this several times.

4. Install the drill bit into a portable drill and drill a single hole through the center of the pygal region. Use a blood clotting agent such as Clotisol® or ferric subsulfate to stop bleeding, if necessary, after first cleaning the dropper tip with an alcohol swab.

5. Flood hole thoroughly with 10% povidone-iodine.

6. Swab outside of surgical tubing with a triple antibiotic ointment such as Neosporin® and pass surgical tubing through the hole until it is flush at the top.

7. Cut excess surgical tubing flush at the bottom using scissors or line cutters.

8. Thread monofilament tether through an acetal polyoxymethylene resin (e.g., Delrin®) disk that has been swabbed with triple antibiotic ointment on the bottom.

9. Pass monofilament through surgical tubing. Lubricate monofilament with triple antibiotic ointment if needed.

10. Secure monofilament at the bottom with the second acetal polyoxymethylene resin disk (swabbed with topical antibiotic ointment) and a crimp below the disk so that the tether is tight and secure. Cut off any excess monofilament.

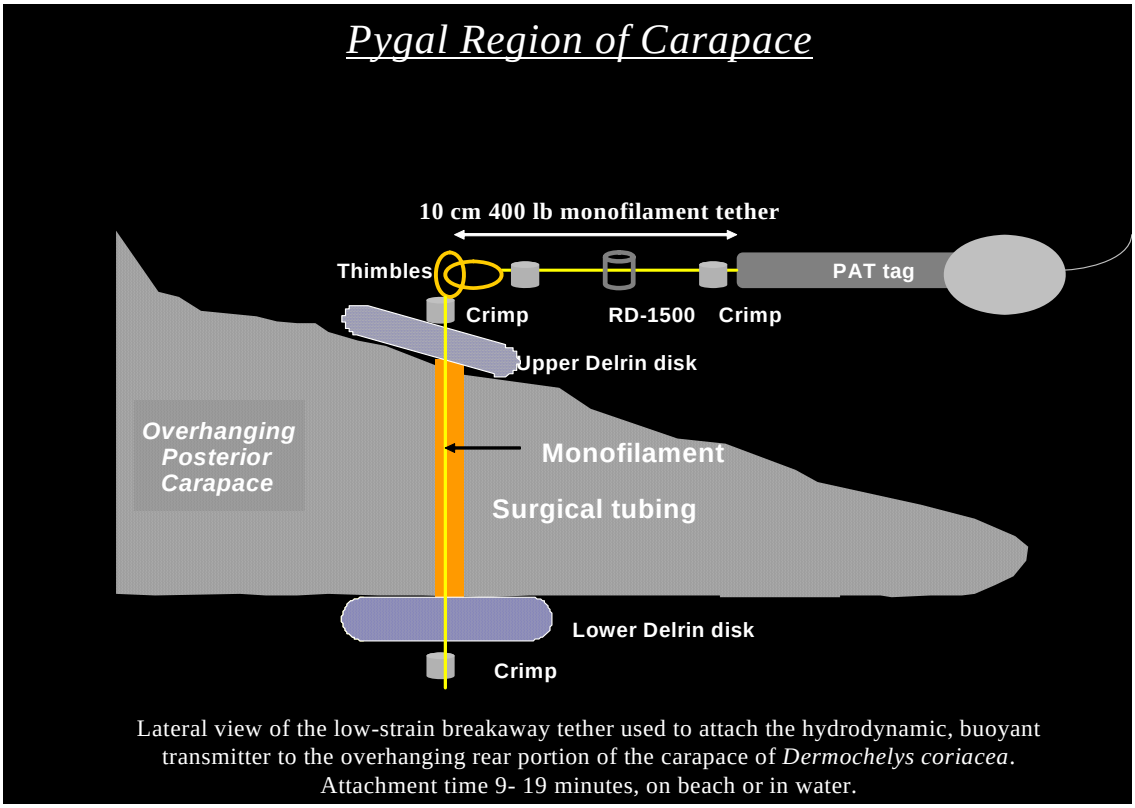


Figure 7-2. Attachment of archival tag using a tether through the pygal region of a leatherback (NMFS/SEFSC diagram).

Harness Attachment Method (Figure 7-3, Method developed by Scott Eckert and adapted from Eckert and Eckert 1986)

Note: Recent concerns about the effects of drag have been raised regarding this harness attachment method. Drag effects are currently being researched, and new materials and attachment methods are currently under investigation. NMFS **does not** currently use or endorse this method of attachment pending futher research.

1. Activate satellite tag.

2. Place plastron strap under the posterior end of plastron (approximately 10 – 20 cm from the edge.

3. Feed each end of the plastron strap through the loop of their respective vinyl tube covered shoulder strap.

4. Center the elastic tubes with the four D-rings on the carapace with two D-rings forward for the shoulder straps and one D-ring to each side for the plastron strap.

5. Secure the plastron strap at each end to the D-rings, making sure that tension of elastic tubes is not too great and allows for growth of the turtle without allowing movement of the harness. The attached loops for the shoulder straps should be just below the D-ring.

6. Feed the vinyl tube with each shoulder strap under the front flippers and curve the tubes over the turtle's carapace.

7. Secure the shoulder straps to the remaining D-rings until an appropriate amount of tension is present in the elastic tubes which will allow for growth of the turtle yet ensure the harness will remain in place. Some trimming of the vinyl tubes may be needed to properly secure the shoulder straps.

8. Attach the transmitter plate to the vinyl tubes ahead of shoulder D-rings and loosely attach with four large cable ties. Do not secure yet, as some adjustments may still be needed for the harness.

9. Check overall tension on shoulder straps, the plastron strap, and the elastic tubes. Make any needed adjustments at this time. Do not over tighten the harness. The harness should be secure on the turtle but still allow for growth.

10. Once all the straps are properly adjusted, secure the shoulder strap loops to the plastron strap on each side of the turtle several centimeters below the D-rings for the plastron strap using small cable ties. A hole will need to be punched with an awl (or similar tool) through the hole in the loop and the plastron strap for the cable ties.

11. Next, secure the plastron strap below each of the D-rings with a cable tie by punching a hole with the awl through the tensioned plastron strap and its loose end below the D-ring.

12. Secure the shoulder straps with cable ties below their D-rings.

13. Tighten cable ties for the transmitter plate to the vinyl tubes.

14. Trim all excess strap material and cable ties.

Figure 7-3. Satellite tag attached using the harness attachment method (NMFS/SEFSC photo).

Direct Attachment Protocol

Epoxy Attachment for Satellite Tags on Hardshell Turtles (see Godley et al. 2002)

Holding – Use a tub to safely hold the turtle in a natural prone position while attaching the transmitter. The tub size will vary based on the size of the animal (e.g., a plastic fish box for small animals or a plastic pool or tank for large animals). Place a cushioned pad on the bottom of the tub to cushion the turtle. The tub will serve to comfortably restrict movement of the turtle during the attachment procedure and can be used aboard a boat or on land. A wet cloth draped over the turtle's eyes to completely block vision often reduces the turtle's desire to move. Shelter the turtle from

direct sunlight, wind, and rain with a tarp during the attachment procedure.

Preparing the carapace – Remove epibionts (barnacles, algae, etc.) from the carapace at the mounting and bonding site of transmitter. In general, the ideal location to place the transmitter is the point where the first and second vertebral scutes meet (Figure 7-4). This section of the carapace rises to a maximum point above the sea surface each time the turtle breathes, and the base antenna on the transmitter will break the plane of the water's surface. Alternatively, transmitters may be attached directly to the second vertebral scute on the carapace (Papi et al. 1997, Polovina et al. 2000, Griffin 2002). Attachment media may also encompass sections of the first and third vertebral scutes, as well as the first and second costal scutes. Thoroughly scrub these areas with a scrub brush and 10% povidone-iodine, rinse with fresh water, dry with a towel, and then lightly sand with sandpaper. When smooth, lightly wipe the entire area with an alcohol pad or a small amount of acetone.

Mounting the transmitter on the carapace – Activate the transmitter in the lab prior to entering the field. Coat all surfaces of the transmitter except the bottom with anti-fouling paint if desired, and cover saltwater switches with electrical or masking tape. The size and weight of the satellite transmitter used will depend on the size of the turtle. Large tags will be attached to the carapace using a two-part epoxy, or a combination of two-part epoxy and fiberglass resin and cloth (< 200 g). The tag and attachment materials should not exceed five percent of the turtle's body weight.

Use a two-part cool setting epoxy (e.g., Power-Fast®) to secure the transmitter on to the carapace. The epoxy components are discharged from the cartridge in equal amounts via a caulk gun, and are incorporated in a specialized mixing nozzle so no modification of amounts is required. There is no danger of setting too quickly. Use a small amount of epoxy (< 50 g) to create an even base for the transmitter to rest and to secure it to the carapace. Drying time will vary between 20 – 60 minutes depending on ambient temperature and humidity. Secure small tags with the epoxy alone; apply additional epoxy or two coats of fiberglass material on larger transmitters to ensure a long attachment life (i.e., one year). When the base has hardened, fiberglass cloth and resin (e.g., Power-Fast® or Bondo®) may be used to further secure the transmitter to the carapace from the edges and/or top to the surrounding scutes. If using fiberglass cloth and resin, use 20 drops of catalyst to two oz of fiberglass resin and mix liberally for about 15 seconds.

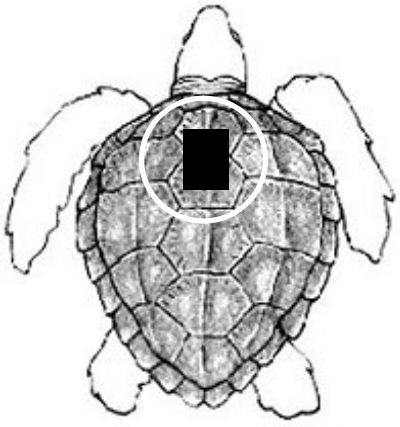
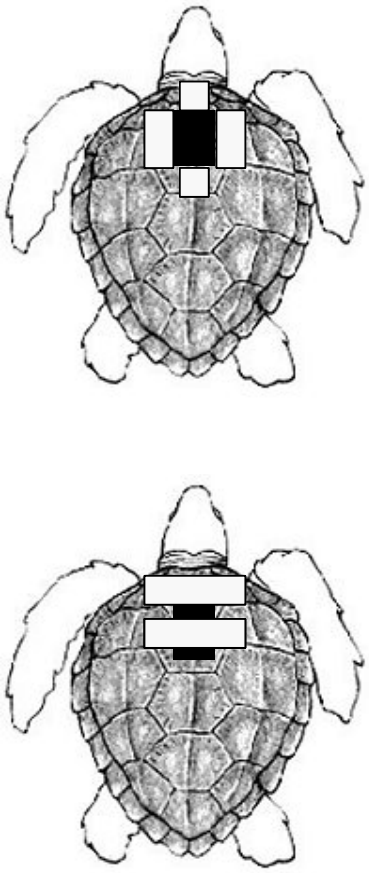


Figure 7-4. Position of satellite transmitter attachment on turtle's carapace (Diagram by C. McClellan, Duke University).

The amount of catalyst may change based on ambient temperatures and humidity, and will be tested in advance to determine hardening time. Use a liberal coat of mixed resin on the transmitter and carapace where pre-cut strips



Figures 7-5a and b. Placement of (a) the first layer and (b) the second layer of fiberglass (Diagrams by C. McClellan, Duke University).

of fiberglass cloth will be applied in two layers over the transmitter, allowing each layer to dry completely (approximately 15 – 20 minutes). Use two five cm wide by 11 cm wide by 5 cm long squares of fiberglass cloth in the first layer, one piece on each edge of the tag (Figure 7-5a). The second layer consists of two 5 cm wide by 13 cm long strips of fiberglass cloth, one over the tag and one across the front of the tag (Figure 7-5b).

Take care to prevent fiberglass resin from running off the shell or coming in contact with the turtle's skin or eyes by wiping up drips immediately. Maintain adequate ventilation while using fiberglass media (e.g., Bondo®). A coat of fiberglass anti-fouling paint may be applied over attachment media to prevent fouling on these materials. When the attachment materials are dry, remove the tape from the saltwater switches and polish with sandpaper to remove any residual grime. Sand the fiberglass as well to remove any sharp edges. Release the turtle at or near the point of capture. Ideally, turtles will be tagged on the boat and held no longer than 1.5 hours, barring unforeseen weather or logistical events (Figure 7-6).

An alternative attachment method is to use a roll of 1.0 cm diameter adhesive (e.g., Sonic Weld®, Ed Greene and Company) around the bottom edge of the transmitter to form a well, followed by application of epoxy resin (e.g., Foil Fast®, Rawlplug Company) epoxy to the entire bottom surface of the transmitter within the well using a glue gun. Heat generated by curing epoxy has not been noticed by researchers during the application process. Preparation and setting time is approximately one hour, after which turtles are released in close proximity to where they were collected.

Figure 7-6. A satellite tagged loggerhead ready for release (Photo by C. McClellan, Duke University).

Direct Satellite Tag Attachment for Leatherbacks

1. Immobilize the turtle and activate the tag. When handling the turtle and equipment, use disposable gloves and change them often to maintain the most sterile environment possible. Attachment methods may vary depending on tag design; one suggested attachment method is described here.

2. Pour enough 10% povidone-iodine to thoroughly soak the hardware (e.g., four 1/4" X 1 5/8" acetal polyoxymethylene resin (e.g., Delrin®) disks and a new three mm drill bit) in a bag. Agitate the bag frequently to disinfect the hardware.

3. Saturate sterile gauze sponges with 10% povidone-iodine or use 10% povidone-iodine scrubs and cleanse the central ridge area of turtle (Figure 7-7). Do this several times.

4. Install drill bit into a portable drill and drill two small holes through the ridge. If necessary, use a blood clotting agent such as Clotisol® or ferric subsulfate to stop bleeding by applying drops into the holes after first cleaning the dropper tip with an alcohol swab. The hole will only penetrate a few millimeters horizontally through the carapace ridge and will not enter the body cavity.

5. Flood holes thoroughly with 10% povidone-iodine.

6. Thread one monofilament or coated wire tether through an acetal polyoxymethylene resin disk that has been swabbed with triple antibiotic ointment (e.g., Neosporin®) on the bottom.

7. Swab outside of the tether monofilament with triple antibiotic ointment and pass through the hole.

8. Once passed through the hole, secure the monofilament with a second acetal polyoxymethylene resin disk (swabbed with triple antibiotic ointment) and a crimp so that the tether is tight and secure. Cut off any excess monofilament.

9. Repeat steps six through eight for the second monofilament tether.

Figure 7-7. Direct carapace attachment on leatherback (Photo courtesy of Sandra Ferraroli, Centre National de la Recherche Scientifique).

Sonic and Radio Transmitter Attachment

General information – Transmitters will be programmed by the manufacturer and tested in the lab prior to entering the field. Activation of the transmitter simply involves removing a magnet. Coat the transmitter with anti-fouling paint before attaching to the turtle.

Holding the turtle in a prone position – Use a container to safely hold the turtle in a natural prone position while attaching the transmitter. The container size will vary depending upon the size of the animal and could range from a

plastic fish box for small animals to a plastic pool or tank. The container will serve to comfortably restrict movement of the turtle to a minimum during the attachment procedure and can be used aboard a boat or on land. Place a cushioned pad on the bottom of the container and shelter turtles from direct sunlight, wind, or rain with a tarp during the attachment procedure. A wet cloth draped over the turtle's eyes to completely block vision often reduces the turtle's desire to move.

Mounting the sonic transmitter on the carapace – In general, locating the transmitter on the posterior section of the carapace will reduce drag and will keep the transmitter submerged even when the turtle surfaces to breathe (Figure 7-8). Sonic transmitters are available in various sizes enabling us to tag both small and large sea turtles (loggerhead, green, and Kemp's ridley). Given that the transmitter and attachment materials cannot exceed five percent of the turtle's body weight, transmitters will be placed only on turtles > 20 cm SCL.

Attachment media will encompass sections of the last vertebral scute as well as the last costal scute. Remove epibionts (barnacles, algae, etc.) from the carapace at the site of transmitter attachment using a hoof pick or other blunt instrument. Thoroughly scrub these areas, rinse with fresh water, dry, and then lightly sand with sandpaper. When smooth, lightly wipe the entire area with an alcohol pad or a small amount of acetone. Use a two-part cool setting epoxy (e.g., Power-Fast®) to secure the transmitter on to the carapace. The epoxy components are discharged from the cartridge in equal amounts via a caulk gun and are incorporated in a specialized mixing nozzle, so no modification of amounts is required, and there is no danger of setting too quickly. Use a small amount of epoxy (< 20 g) to create an even base for the transmitter to rest and to secure it to the carapace. Taper the attachment media to prevent it from catching on rocks or fishing nets. Drying time will vary between 20 – 60 minutes, depending on ambient temperatures and humidity. When the attachment materials are dry, release the turtle at or near the point of capture.

Mounting the radio transmitter on the carapace – Radio transmitters are available in various sizes, enabling tagging of both small and large sea turtles. The transmitter and attachment materials should not exceed five percent of the turtle's body weight. Therefore, transmitters should be placed only on turtles > 20 cm SCL. Small (e.g., ~30 g cylindrical) transmitters can be attached directly to the carapace of smaller turtles (25 – 40 cm SCL) or tethered to the posterior end of the carapace of larger turtles (> 40 cm SCL). Larger (e.g., ~60 g rectangular) transmitters can be attached directly to the carapace of larger turtles (> 40 cm SCL).

Use a two-part cool setting epoxy (e.g., Power-Fast®) to secure the transmitter to the

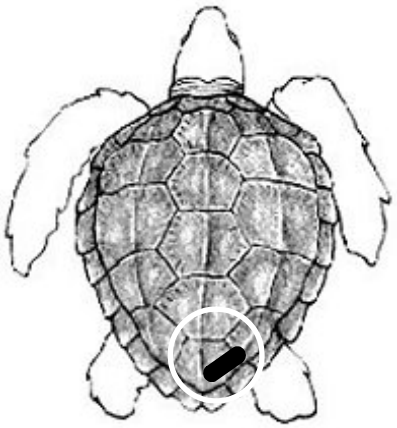


Figure 7-8. Position of sonic transmitter attachment (Diagram by C. McClellan, Duke University).

carapace. The epoxy components are discharged from the cartridge in equal amounts via a caulk gun and are incorporated in a specialized mixing nozzle, so no modification of amounts is required, and there is no danger of setting too quickly. Use a small amount of epoxy (< 20 g) to serve the dual function of creating an even base for the transmitter to rest and securing it to the carapace. Taper the attachment media to prevent it from catching on rocks or fishing nets. Drying time will vary between 20 – 60 minutes, depending on ambient temperatures and humidity. When the attachment materials are dry, release the turtle at or near the point of capture.

Stomach Temperature Pill

Satellite-linked data recorders (e.g., Mk10-AL, 93 x 51 x 22 mm, 125 g; Wildlife Computers Redmond, WA) and stomach temperature pills (e.g., STP3, 21.5 mm diameter, 63 mm length; Wildlife Computers, Redmond, WA) may be deployed in turtles > 105 cm in length. Currently these devices are used to record temperature as described here, but future advancements may allow multiple parameters to be sampled.

Adult loggerhead turtles maintain internal body temperatures several degrees higher than ambient water temperature (T_W) (Sato et al. 1994). Ingestion of prey at ambient T_W has the effect of rapidly lowering stomach temperature (T_S), such that fluctuations in T_S may be used to identify a feeding event. The magnitude of the decrease in T_S and time necessary for T_S to recover to previous levels following prey ingestion reflects both prey size and ambient T_W. The STP3 possesses four thermistors to detect T_S, and a transmitter to relay T_S data to a satellite-linked data recorder, such as the MK10-AL, mounted on the turtle's carapace. The T_S data are intercepted and archived by the MK10-AL instrument. Data recognition software then analyzes the T_S data to identify large fluctuations indicative of ingestion. When one of these events is recognized, software then pick 6 points that characterizes the fluctuation, records the time the fluctuation occurred, the depth at which the fluctuation was recorded, and the ambient Tw. This information is transmitted, along with location data and dive behavior data, the next time the Mk10-AL uplinks to an Argos satellite when the turtle surfaces to breathe.

The satellite-linked data recorder will be attached to the turtle's carapace between the first and second vertebral scutes using Power-Fast® marine epoxy or similar. Stomach temperature pills will be inserted in animals (must be > 105 cm CCL) to a depth of 20 – 25 cm into the esophagus using a lubricated flexible rubber tube. Generally it is not necessary to restrain the turtle with a net while inserting the STP3. Rather, use nylon webbing straps to hold the mouth open. One person holds strap on upper jaw and another person holds strap on lower jaw while a third person uses the lubricated rubber tube to push the pill into the turtle's esophagus.

The insertion of the pill is a quick procedure, and the turtle's mouth is usually held open for less than one minute. A damp cloth is placed over the turtle's eyes to keep it calm during the procedure. Ensure that the pill is properly sized for the animal to prevent potential intestinal blockage. Previous studies have shown that an STP3 inserted in this manner is eventually pushed into the stomach by peristaltic action and food ingestion (Southwood et al. 2005), causing no residual effects.

Chapter 8: Biopsy Sampling

Biopsies, the sampling of single or multiple tissues, are routinely collected to:

- Provide information relative to the life history of the population being studied – Skin biopsies have been collected for genetic studies, while bone biopsies have been collected for aging studies;

- Better understand the nature of a lesion and determine the most appropriate therapy – Single or multiple samples are collected, determined by the type of lesion biopsied;

- Determine sex – Small pieces of gonadal tissue can be evaluated histologically to determine the sex of the animal;

- Evaluate the animal for contaminants – Both fat and liver biopsies provide a way to monitor organochlorine contaminants in wildlife populations. Biopsies also may be obtained from other visceral structures, usually through a laparoscopic incision;

- Conduct stable isotope analysis – Analysis of stable isotope levels of carbon and nitrogen provides insight into diet, foraging behavior and potentially distributional patterns; and

- Conduct biochemical analyses – Muscle biopsies can be evaluated to determine aerobic and anaerobic metabolic capacity, thermal tolerance, or stable isotope analysis.

Skin Biopsy

Protocol for Turtles Boated or on Land

Small hardshell turtles should be turned onto their carapaces briefly to facilitate skin biopsy sampling; this may not be possible for large turtles. The sample site should be along the posterior edge of a rear flipper in soft tissue, not a scale. If a rear flipper is not accessible, samples can be taken from the front flippers as well. Thoroughly soak and scrub the area with 10% povidone-iodine solution followed by an isopropyl alcohol wipe, then thoroughly swab again with 10% povidone-iodine solution prior to sampling. A new, sterile biopsy tool should be used for each turtle to prevent cross-contamination.

The researcher should wear gloves to protect the hand that is holding the flipper and the sampling surface. A vial cap, plastic dive slate, or other plastic surface cleaned with 70% isopropyl alcohol should be placed beneath the sampling site as a hard surface against which to press. Press a new biopsy punch firmly into the flesh just along the posterior edge and rotate one complete turn, cutting all the way through the flipper to the plastic surface (Figure 8-1). Repeat the tissue punch process with the same punch to obtain two plugs from each animal. An alternative method is to remove a plug of skin from the shoulder region using a sterile 6 mm biopsy punch to cut a skin plug and forceps and surgical scissors to extract and trim the sample.

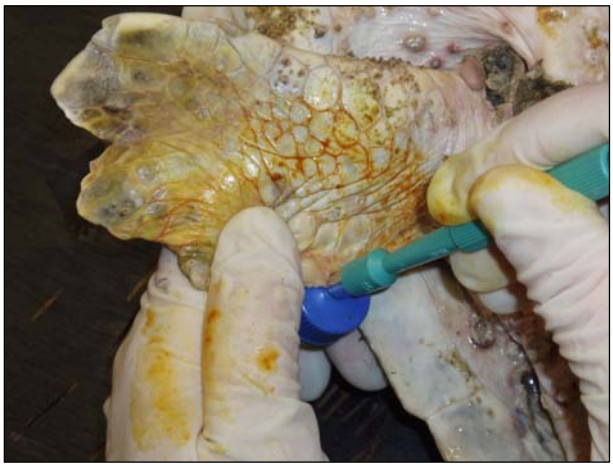


Figure 8-1. Skin biopsy taken from trailing edge of rear flipper (NMFS/SEFSC photo).

Place the tissue plugs into the vial containing a suitable storage solution, such as saturated NaCl solution with or without 20% DMSO. If the sample does not come out of the corer easily, place it into the vial by inserting a new, clean wooden applicator stick through the hollow handle of the biopsy punch, shaking the punch in the vial, or snapping the tip off of the biopsy punch and placing the entire tip in the vial. Wipe the punched area with 10% povidone-iodine solution. If necessary, a blood clotting agent, such as ferric subsulfate or Clotisol®, or a cyanoacrylate tissue glue such as Nexaban® (Veterinary Products Lab, Phoenix, AZ, USA) or an over-the-counter equivalent such as Super-Glue® or Krazy-Glue® can be used for hemostasis. Using a pencil, label a piece of waterproof paper with the date, species, id, master tag, and trip number if applicable, and place in the vial. Label the outside of the vial using a permanent marker with date, species, id, and master tag and seal the label with clear tape. To prevent spillage, wrap laboratory sealing film, such as Parafilm®, around the cap of the vial. Place vial within a labeled sample bag (e.g., Whirl-pak®) and close.

Wear gloves each time you collect a sample and handle the buffer vials. The NMFS/SEFSC observer programs currently use a saturated sodium chloride solution for tissue sample storage, but some programs may use 20% dimethyl sulfoxide (DMSO) buffer saturated with sodium chloride instead. If you are using DMSO buffer, it is nontoxic and nonflammable, but handling the buffer without gloves may result in exposure, producing a garlic/oyster taste in the mouth along with breath odor. This substance soaks into skin very rapidly along with any dissolved contaminants. Do not store the buffer where it will experience extreme heat, and do not freeze the sample. The buffer must be stored at room temperature or cooler, such as in a refrigerator.

Protocol for Turtles Not Boated

When a turtle that cannot be boated is alongside the vessel, a corer attached to a biopsy pole is used to obtain a biopsy sample. The sampling gear consists of a 12’ anodized aluminum breakdown biopsy pole, such as the NOAA/Epperly Biopsy Pole, or similar biopsy harpoon and a disinfected stainless steel biopsy corer.

Assemble the pole sections together if necessary to attain the desired pole length. The corers should be stored in ethanol-cleaned vials. Clean the end of the threaded stud on the biopsy pole section with an alcohol swab. Carefully remove the corer from its vial and screw it tightly on the end of the stud of the biopsy pole.

No more than two biopsies should be conducted per animal, and if you are unsuccessful obtaining a sample after two

attempts, no further attempt should be made (as required by permit conditions). Suitable sampling sites for hardshell turtles include the flippers, shoulders, and pelvic regions. A forceful jab perpendicular or oblique to the body is needed to penetrate the skin of most turtles (Figure 8-2). There are nerve bundles high on the shoulders near the carapace that should be avoided, as should the heavily vascularized armpit area. The best method to obtain biopsy samples from leatherbacks is to scrape a ribbon of tissue from the carapace with the corer, leaving a gray superficial scar that will heal well over time. Do not target the carapace, head and neck, or limbs with a jabbing motion when sampling leatherbacks.

Due care should be taken not to strike anyone when handling the pole onboard. Unscrew the corer from the pole, and place the entire corer with tissue sample into the sample vial. Do not attempt to remove the tissue from the corer. Clean the adapter stud with an alcohol swab and label the vial as previously described.

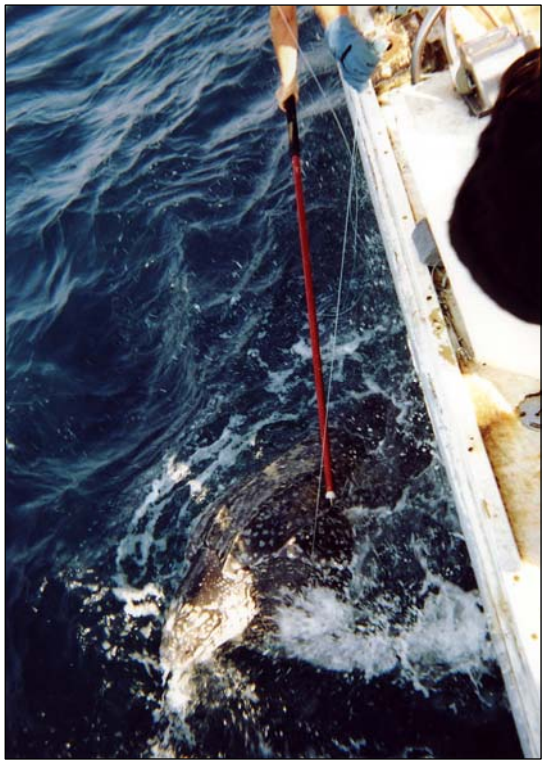


Figure 8-2. Taking a biopsy from a leatherback not boated (NMFS/SEFSC photo).

Lesion Biopsy

Samples may be taken to better understand the nature of a lesion and determine the most appropriate therapy. Single or multiple samples are collected, determined by the type of lesion biopsied. The methods used to collect and preserve the sample vary, depending upon the nature of the lesion and which diagnostic tests will be performed. For histologic evaluation, samples are fixed in 10% neutral buffered formalin (NBF). Samples to be examined for microbial isolation attempts are first cleansed with sterile saline before being placed in an appropriate transport media or sterile container for shipment to a diagnostic laboratory. Never freeze tissues undergoing histologic examination to preserve them, as this will result in tissue damage due to crystallization.

Fat Biopsy

Subcutaneous fat is collected from the inguinal region (Figure 8-3). Only a veterinarian or other highly trained individual using sterile surgical instruments should conduct this procedure. This procedure should not be performed on any compromised animals (e.g., those that are emaciated, with heavy parasite loads or bacterial infections) unless medically advised or necessary based on the experimental design of a health related study. After manually restraining the turtle, scrub the inguinal area with 10% povidone-iodine solution.

Infuse lidocaine hydrochloride (e.g., Phoenix Pharmaceuticals, Inc., St. Joseph, MO, USA), up to 2 mg/kg, intradermally and subcutaneously around the proposed incision sites in the inguinal areas ten minutes prior to the procedure to block any pain and discomfort to the turtle. Pull the rear flipper on the side of the incision back and toward the opposite side, causing the skin to remain taut. Make a two cm incision in the inguinal fo ssa using a disposable scalpel blade; blunt dissection of the connective tissue will be accomplished using surgical scissors. After grasping the connective tissue layer with forceps, use the surgical scissors to cut sharply down into the subcutaneous fat. Use the connective tissue layer to assist with gripping the fat with the forceps (as the consistency of the fat makes it difficult to seize it), and excise an approximately 0.4 – 4.0 g (~0.44 –4.4 cc) of the fat, which will then be placed in hexane-rinsed aluminum foil and immediately frozen at -80°C.

To close the incision, use a buried, simple continuous (or continuous horizontal mattress) subcuticular pattern using a monofilament nominally absorbable suture, such as one of the three following (Govett et al. 2004): polyglyconate (e.g., Maxon™, US Surgical, Norwalk, CT, USA), or poliglecaprone 25 (e.g., Monocryl™, Ethicon, Somerville, NJ, USA), or polydioxanone (e.g., PDS II™, Ethicon), followed by cyanoacrylate tissue glue on the surface. Depending on the size of the biopsy, it may be necessary to close the fat layer to eliminate dead space and reduce the chances of seroma or hematoma formation.

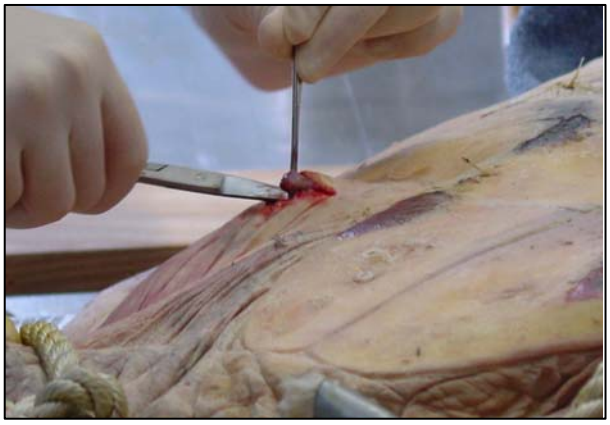


Figure 8-3. Taking a fat biospy sample (NMFS/OPR photo).

To reduce post-surgical complications (i.e., infections), a single dose of antibiotic (Table 8-1) may be administered prior to surgery. A non-steroidal, anti-inflammatory drug (e.g., ketoprofen at 2 mg/kg IM, MacLean et al. 2008) may be administered to reduce post-operative pain. If administered to green turtles, be especially watchful, as an older related anti-inflammatory compound, flunixin meglumine (e.g., Banamine®), can be lethal to green turtles (D. Mader, pers. comm.).

Drug	Dosage	Source
ceftazidime	20 mg/kg IM	Stamper et al. 1999
oxytetracycline	25 mg/kg IM	Harms et al. 2004
enrofloxacin	20 mg kg oral	Jacobson et al. 2005
ticarcillin	50 or 100 mg/kg IM	Manire et al. 2005
amikacin	5 mg/kg IM	Carpenter 2005

Table 8-1. Several antibiotic choices to reduce post-surgical complications.

Muscle biopsy

Surgical muscle biopsy (Southwood et al. 2003, Southwood et al. 2006)

Muscle tissue may be collected for biochemical analyses to determine aerobic and anaerobic metabolic capacity, thermal tolerance, or stable isotope analysis. Muscle tissue may be obtained from either the iliotibialis muscle of the rear flipper (Figure 8-4a) or the deltoidus muscle (Figure 8-4b), which protracts and abducts the front flippers during swimming. Only a veterinarian or other highly trained individual using sterile surgical instruments should conduct this procedure. This procedure should not be performed on any compromised animals (e.g., those that are emaciated, with heavy parasite loads or bacterial infection) unless medically advised or necessary based on the experimental design of a health related study.

Figures 8-4a and b. Muscle tissue may be excised from (a) the iliotibialis muscle or from (b) the deltoidus muscle (Photos from A.L. Southwood).

Thoroughly clean the incision area with 95% ethyl alcohol and 10% povidone-iodine solution. Inject up to 2 mg/kg 2% lidocaine (e.g., Vetoquinol Inc., Lavaltrie, QC) intramuscularly, intradermally, and subcutaneously into the incision area 10 minutes before the sample is to be taken. Make a 1.5 cm incision in the skin using a disposable scalpel blade and use surgical scissors for blunt dissection to expose muscle. Grasp muscle tissue with tissue forceps and use surgical scissors to excise approximately 200-300 mg of muscle tissue. Wrap the excised tissue in aluminum foil or place in a suitable storage vial and freeze in liquid nitrogen immediately. Use monofilament absorbable suture (e.g., polygloconate, MaxonTM, US Surgical, Norwalk, CT, USA; polydioxanone, Ethicon PDS IITM, Piscataway, NJ, USA; or poliglecaprone, Ethicon MonocrylTM) to close the incision area. A simple interrupted pattern with 3-0 suture may be used to pull muscle tissue together and horizontal mattress using 2-0 suture may be used to close the skin incision. Treat incision area with topical antibiotic cream (e.g., povidone-iodine ointment or triple antibiotic ointment) and give the turtle a single dose of antibiotic (Figure 7-4) at a site other than the incision site to reduce the risk of infection. Samples should be stored in an ultrafreezer at -80ºC.

Non-Surgical Muscle Biopsy

When a small sample is sufficient, an alternative non-surgical method, which is

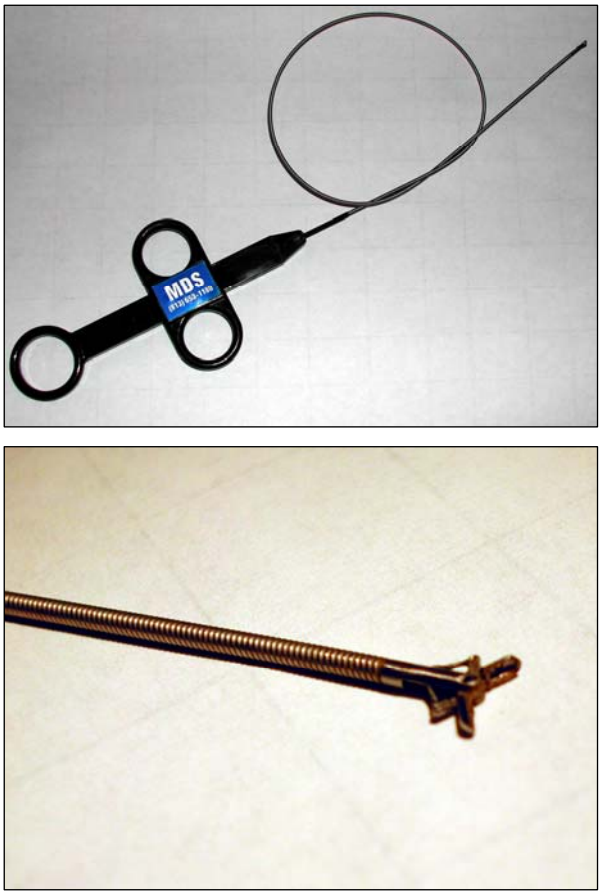
possible to conduct in the field, is to take a muscle biopsy sample in the shoulder region after thoroughly cleaning the area with 10% povidone-iodine solution and alcohol. Collect one sample on each side of the neck using a sterile 6 mm biopsy punch to the depth of the corer. Hold the sample with forceps and trim using surgical scissors. The samples should be placed in a suitable storage vial and stored in an ultrafreezer at -80ºC. If bleeding occurs, a blood clotting agent, such as ferric subsulfate or Clotisol® can be used, or the region may be cauterized or sutured if necessary.

Biopsies Taken During Laparoscopy

Laparoscopies (Chapter 15: Laparoscopy) are performed to identify the sex of the animal, as well as to collect tissues for health assessments and for histology to confirm sex identification. It is possible to sample tissues such as the gonads, liver, kidney, spleen, and mesenteric fat, as well as any lesions. Below we describe in detail the methods for two of these; the methods for the other tissues will be done with similar care and attention to the well being of the turtle.

Gonad Biopsy

This procedure can be performed in the course of laparoscopy for sex determination, but should only be conducted by a veterinarian or other highly trained individual. This procedure should not be performed on any compromised animals (e.g., those that are emaciated or having heavy parasite loads, bacterial infections, etc.) unless medically advised or necessary based on the experimental design of a health related study. Propofol may be administered (5 mg/kg IV, MacLean et al. 2008) as a short-acting (depending on ambient temperature considerations) general anesthetic prior to the procedure. A nonsteroidal anti-inflammatory drug (e.g., ketoprofen, 2 mg/kg IM, MacLean et al. 2008) may be administered to reduce post-operative pain with no sedation, but special care should be taken with green turtles, as an older related anti-inflammatory compound, flunixin meglumine (e.g., Banamine®), can be lethal in that species (D. Mader, pers. comm.). A single pre-surgical dose of antibiotic (Table 8-1) may be administered to reduce the chances of post-surgical infections.



Figures 8-5a and b. Endoscopic cup biopsy forceps used for gonad biopsies (Photo by J. Vaughan, Florida Atlantic University).

Follow the procedure for laparoscopy in Chapter 15. Once the gonad is identified, extend the incision about three to four mm, attach the biopsy guide over the scope or open a biopsy port if the trocar is so equipped, and feed the biopsy tool into its port. Using an endoscopic cup biopsy forcep (Figures 8-5a and b), sample a one to two mm piece of the side of the cranial 1/3 of the gonad (about 1/3 the way down), avoiding vascular areas (the gonad sits on top of some of the renal blood vessels). Also, make sure the paramesonephric duct (i.e., the oviduct in females) is not lying on the sampling site. Sampling 1/3 of the way down from the cranial pole of the gonad will avoid accessory ducts (epididymus, vas deferens, Wolfian ducts, etc.), thus allowing access to the greater concentrations of follicles in the caudal ends of the ovaries. In addition, if one were to sample all the way cranially, this may disrupt the epididymus/vas deferens of males. Using a clean hypodermic needle, retrieve samples from the forcep cup, place into microcentrifuge tubes (e.g., Eppendorf®) filled with 10% buffered formalin, and store at room temperature. If any bleeding occurs (it is exceedingly rare for it to bleed beyond the surface sampling site), administer 10 ml/kg of intracoelomic fluids (e.g., Lactated Ringer's solution, 0.9% saline solution). After completing the examination, remove all air prior to suturing the wound. Close the incision as described in Chapter 15. Label the biopsy sample tubes with a permanent marker on the top and the side and properly package them prior to shipping.

Liver Biopsy

Liver biopsy samples for toxicology analysis may be collected in the course of laparoscopy for sex determination. This procedure should not be performed on any compromised animals (e.g., those that are emaciated or having heavy parasite loads, bacterial infections) unless medically advised. Propofol may be administered (5 mg/kg IV, MacLean et al. 2008) as a short-acting (depending on ambient temperature considerations) general anesthetic prior to the procedure. A nonsteroidal anti-inflammatory drug (e.g., ketoprofen, 2 mg/kg IM, MacLean et al. 2008) may be administered to reduce post-operative pain with no sedation, but special care should be taken with green turtles, as an older related anti-inflammatory compound, flunixin meglumine (e.g., Banamine®), can be lethal in that species (D. Mader, pers. comm.). A single pre-surgical dose of antibiotic (Table 8-1) may be administered to reduce the chances of post-surgical infections.

Follow the procedure for laparoscopy in Chapter 15. After laparoscopic examination of the gonads (if applicable), leave the laparoscope and sleeve in place and make a second one cm skin incision in the same inguinal space as the laparoscope. Advance a second trocar into the body cavity at a location that can be verified by the laparoscope as safe from any internal organ contact. Once the trocar is in the body cavity, advance a 4-mm cup biopsy instrument into the field of view and guide it to the liver. Take the biopsy at a location at the margin of the liver with minimal observable vascularity, avoiding the vascular areas (the gonad sits on top of some of the renal blood vessels). Make sure the paramesonephric duct (that will be the oviduct in females) is not lying on the sampling site. Using an endoscopic cup biopsy forcep, sample a one to two mm piece of the liver by firmly clamping the desired tissue with the cutting cup biopsy tip and retracting until the

tissue comes away. Obtain two biopsies of approximately 0.1 g (one to two mm) each from each turtle. Use a hypodermic needle to get the samples out of the forcep cup and into microcentrifuge tubes (e.g., Eppendorf®) filled with 10% buffered formalin. Observe the biopsy site directly for hemorrhage; if clotting fails to occur rapidly, insert a small piece of absorbable gelatin sponge hemostatic device (e.g., Gelfoam® Pharmacia & Upjohn, Kalamazoo, MI, USA) via the instrument port, and apply to the biopsy site to promote clotting. Close the incision as described in Chapter 15.

Release

Hold turtles receiving propofol out of water for at least one hour following the conclusion of the procedure, and do not return to the water until fully responsive. Hold all animals temporarily in tanks to ensure normal swimming and diving activity have returned prior to release.

Chapter 9: Blood Sampling

Blood samples should be taken by experienced personnel, and care should be taken to ensure no injury results from sampling. Turtles that are severely injured or compromised should not be sampled unless specifically authorized or during treatment by a veterinarian. Make a maximum of two attempts on each side of the neck, limiting needle insertion attempts to a total of four. Extract a maximum of 3 ml blood/kg of body weight; this cumulative blood volume should not be exceeded within a 45-day period of time. If the turtle cannot be adequately immobilized for blood sampling, efforts to collect blood must be discontinued.

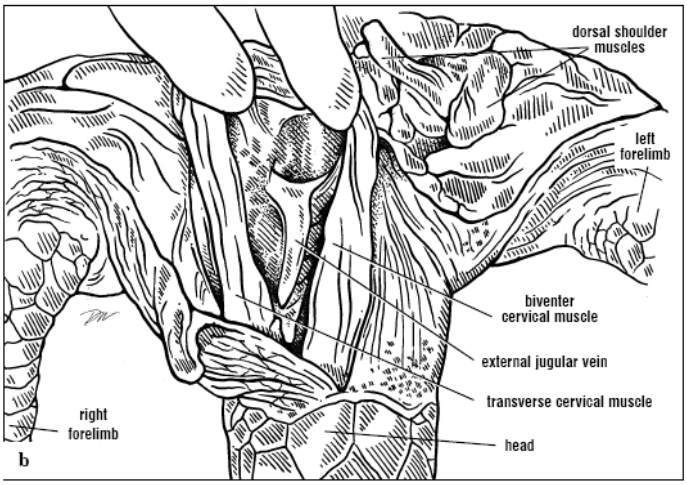


Figure 9-1. External jugular vein, biventer cervical and transverse cervical muscles in green turtle (Diagram from Wyneken 2001).

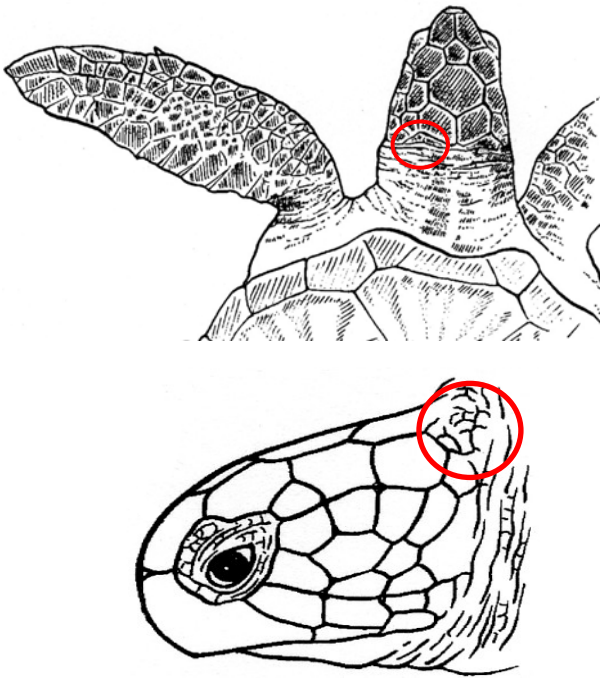
Hardshell Turtles

The external jugular vein (often termed the dorsal cervical sinus) is a commonly used blood collection site in sea turtles (Wyneken 2001). This vein is located relatively dorsal and superficial in the neck, and the biventer cervical and transverse cervical muscles are good landmarks for its location (Wyneken 2001). Rinse and clean the neck region with water and 10% povidone-iodine solution, isopropyl alcohol or other antiseptic prior to sampling. Restrain the turtle and pull the head gently forward and downward until it is fully outstretched to facilitate the filling of the bilateral cervical sinus. Use a new, disposable syringe and needle or a vacuum tube, needle, and holder system to collect the sample. A general guideline for needle selection to obtain blood samples is: for turtles less than

0.5 kg, use a 23-gauge 0.5" needle; for turtles 0.5 – 5 kg, use a 21-gauge 1" needle; for turtles larger than 5.0 kg, use a 21-gauge 1.5" needle.

Insert the needle on either side of the midline of the neck (depending on the size of the turtle, from 0.5 – 3.0 cm lateral to the midline) about 1/3 to 1/2 of the distance between the back of the head from the anterior edge of the carapace (Figure 9-2).

Figure 9-2. Blood sampling from the bilateral cervical sinus using a vacuum tube and needle (Photo courtesy of J. Wyneken, Florida Atlantic University).



Figures 9-3a and b. Blood sampling site for green sea turtles (Diagram from Wyneken 2001, annotations by A. Southwood)

In green and hawksbill sea turtles, the external jugular is smaller in diameter and branches little when compared with the anatomy in other cheloniids (Wyneken 2001). Some researchers have found that particularly with green turtles, the ideal sampling site is anterior to this position just behind the head (Figures 9-3a and b). To draw blood from this site, insert the needle at a very shallow angle (≤ 30°) lateral to the supraoccipital crest at the base of the skull where the scales form a noticeable "V" shape, directing the needle at shallow angle straight back towards the carapace along the medial line (A. Southwood, pers. comm.). The correct location (Figure 9-4) is within the "valley" between the biventer and transverse cervical muscles (Figure 9-1).

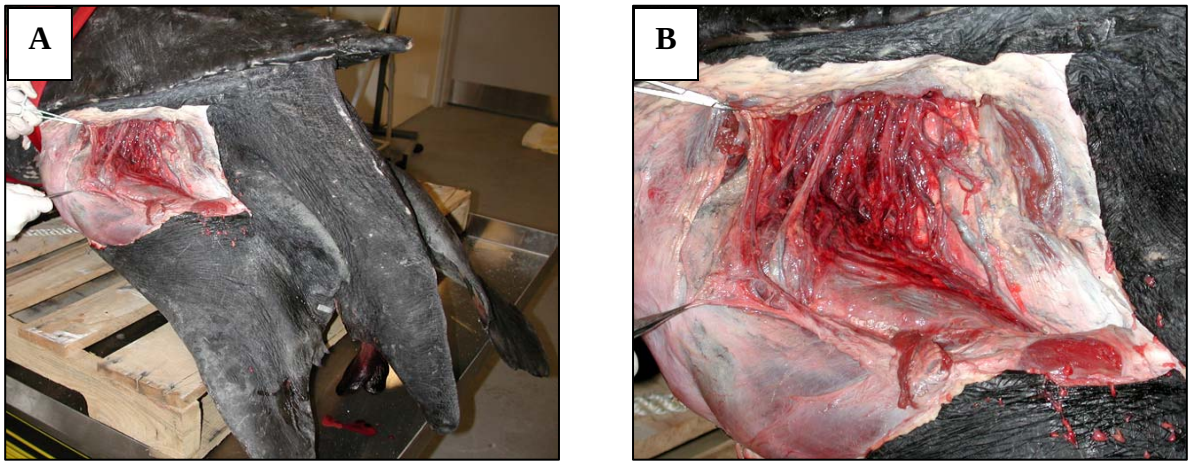
Insert the needle approximately 45 – 90° to the plane of the neck and do not move the needle laterally to locate the vessel, as this will cause tissue damage. Once the needle is inserted, apply suction and move the needle slowly up and down until the sinus is located. Do not remove the needle from the neck while still applying suction, as this can contaminate the sample. Once the blood has been obtained, withdraw the needle and insert it into the rubber tip of an appropriate vacuum tube to transfer the sample. Dispose of the needle and syringe in a sharps container.

Place the samples on ice until they can be processed. Place the vials adjacent to, but not directly on the ice to prevent freezing and lysis of red blood cells, which adversely affects plasma biochemistry analysis and some serological assays. In the laboratory, spin down blood samples for 10 min in a centrifuge and then pipette the separated plasma into a cryogenic vial. Wash the remaining red blood cells with an equal amount of 2.5% saline solution and then pipette into a second cryogenic vial. The samples should be stored at -80°C.

Figure 9-4. Blood sampling from superficial veins lateral to the supraoccipital crest in a green sea turtle (Photo courtesy of A. Southwood).

Leatherbacks

Leatherbacks are generally blood sampled in the rear flipper (Figures 9-5a and b) in a nexus (vein bundle) located approximately five cm from the edge of the carapace and one cm interior medial of the tibia (Dutton 1995, Wyneken pers. comm.). Alternatively, blood can be sampled from the interdigitary vessels, with optimal needle insertion points approximately one inch distal to the junctions of each pair of phalanges (Wallace and George 2007). Sampling from the dorsal cervical sinus is not recommended by some in leatherbacks, as it is relatively deep compared with other species, but it can be performed using an 8.9 cm (3.5") spinal needle (Harms et al. 2007) if the researcher is experienced with this procedure.



Figures 9-5a and b. Location of the rear flipper nexus (a) for blood sampling and (b) a closeup view (Photos courtesy of J. Wyneken, Florida Atlantic University).

Chapter 10: Cloacal and Microbiologic Lesion Sampling

In order to conduct bacterial culture and/or antimicrobial susceptibility testing, cloacal and lesion samples may be examined. Temporarily overturn each turtle to be sampled onto its carapace and restrain. First, scrub the external opening of the cloaca with 10% povidone-iodine to disinfect the area. After securely gripping the tip of the tail, insert a sterile culturette tip (e.g., BBL CultureSwab™, Becton Dickinson and Company, Sparks, MD) approximately one inch into the cloaca. For culture of external lesions, gently insert the culturette tip into the deepest area of the wound. In both cases, rotate the culturette tip approximately 360° and remove it from the cloaca or lesion (Figure 10-1). Immediately place the culture into a sterile transport medium (e.g., BBL Port-A- Cul™ Tubes, Becton Dickinson and Company, Sparks, MD) for overnight shipment to a laboratory for bacterial culture and antimicrobial susceptibility testing. Some media tubes also may be stored between at -80° C in liquid nitrogen prior to testing.

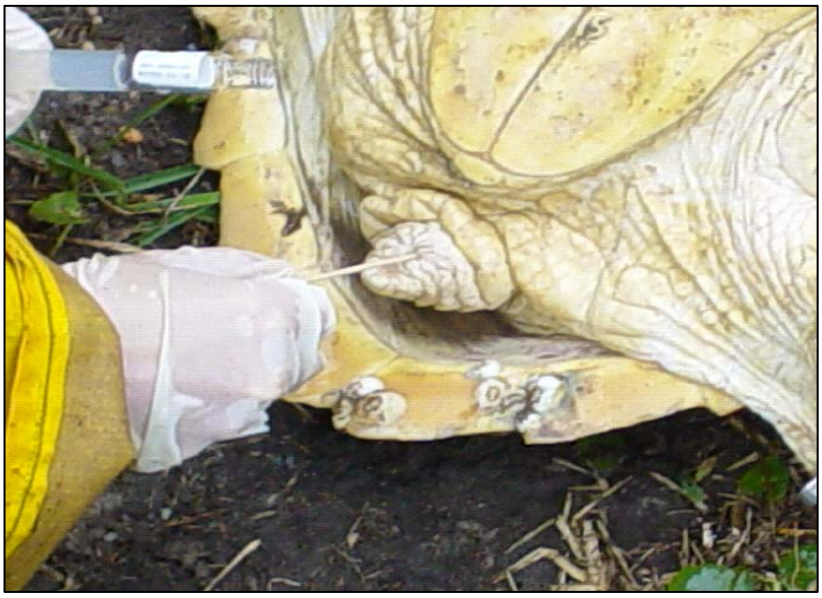


Figure 10-1. Cloacal culture (NMFS/SEFSC photo).

Chapter 11: Fecal Sampling

Fecal samples may be collected for diet analysis or to evaluate the presence, diversity, and species composition of any internal parasites using sodium nitrate flotation and sedimentation. When sampling for diet content analysis, turtles will be held in a suitable holding pool (in a shaded area with water temperatures similar to those of the water temperatures at capture) for a period of up to 48 hours to obtain a defecated sample.

Sampling for parasite analysis will occur either after turtles have defecated during biological sampling or by digital extraction of feces from the cloaca. Those turtles that do not defecate during the sampling period may be temporarily overturned onto the carapace and restrained.

After cloacal cultures have been obtained, fecal samples can be taken if desired (see Chapter 10: Cloacal and Microbiologic Lesion Sampling). Insert one finger while wearing lubricated latex gloves into the cloaca of the turtle to feel for the presence of a fecal mass (Figure 11-1). If one is detected, remove it, place it into either a polyethylene bag or a conical centrifuge tube and place it on ice. Label bags and tubes (e.g., turtle identification number, date, and species), and then ship the sample on ice to the laboratory.

Figure 11-1. Collection of fecal sample for parasite analysis (NMFS/SEFSC photo).

Chapter 12: Epibiota Sampling

Using a hoofpick/scraper, rounded putty knife or other blunt instrument, carefully pry off barnacles or other epibiota from the turtles' carapace, taking care not to remove the underlying scute (Figure 12-1). Barnacles may also be removed by placing the narrow tip of a wooden skewer at the base of the barnacle and gently rotating so that the skewer tip lodges between the barnacle and the carapace of the turtle. Apply gentle leverage in order to pry the barnacle from the carapace. If necessary, several entry/leverage points may be used to ensure that the barnacle is removed from the carapace without causing damage; however, in some instances, the keratin to which the barnacles are attached may already exist in a 'sloughing' state and become dislodged. For epibiota present in areas other than the carapace, use the corner of a plastic putty knife or forceps tips to gently pry up the edge of the specimen in question, and then pull the entire organism away from the epithelium. If bleeding occurs, apply pressure to the affected area using a 10% povidone-iodine swab. Seal the sample in a freezer-style plastic storage bag, and using a permanent marker, label the bag (e.g., turtle identification number, date, and species). The epibiota also can be placed in sample jars filled with ethanol.

Figure 12-1. Epibiota sampling from loggerhead carapace (NMFS/SEFSC photo).

Chapter 13: Keratin Sampling

Keratin may be collected from the outermost edge of the eight most posterior marginal scutes of the carapace for mercury analysis (Day 2003). Scutes free of fouling organisms/epibiota, and those that appeared to have keratin of sufficient thickness and texture to provide a sufficient sample mass while minimizing the risk of penetrating through the keratin layer, should be targeted for sampling (Figure 13-1). A relatively thin edge of keratin, where the keratin and underlying bone can be discriminated, is usually present where the dorsal and ventral surfaces of a scute meet. Thus, it is possible to avoid scraping too deeply, causing injury to the turtle and contaminating the sample with untargeted tissues.

To obtain a keratin sample, place each turtle on its plastron briefly on a slightly elevated platform with approximately 15 – 20 cm of the posterior edge of the carapace overhanging the edge of the platform. While one researcher is restraining the turtle's rear flippers, two other researchers will prepare to collect the sample. Before taking the keratin sample, scrub two cm or more of carapace dorsal and ventral to the edge of the scutes vigorously with a plastic scrubbing pad to remove sloughing keratin. If there are no areas free of epibiota, use a plastic scraper to clear the target area as thoroughly as possible prior to scrubbing. Afterward, rinse the scrubbed area with high purity distilled water and isopropanol, and then remove any remaining foreign matter and debris using cellulose based cleanroom wipes or cotton gauze, distilled water, and isopropanol or high purity 95% ethanol. Finally, remove the lateral edge of the prepared marginal scutes by shaving off the edges of the scutes parallel to the edge being sampled using a disposable, sterile scalpel blade. Keratin splinters may also be collected by carefully sliding a sterile biopsy punch along the outer edge of the scute, parallel to the body axis. Allow the shavings to fall directly into a polyethylene sample bag held by a second researcher wearing Kevlar gloves to prevent injury.

Figure 13-1. Collecting keratin sample. Turtle in this photo is positioned on its carapace to better highlight the sampling region (Photo by M. Godfrey, North Carolina Wildlife Resources Commission).

Typically, the posterior lateral corner of each scute will yield the thickest sample without penetrating the keratin and contaminating the sample with untargeted tissue. This should yield small shavings or splinters of keratin ~ one mm in thickness totaling ten cm total of one mm thick shavings. Label the outside of the polyethylene bag (e.g., turtle identification number, date, and species), and then freeze the sample at -20 °C.

Chapter 14: Gastric Lavage

(Adapted from Forbes 1999)

Although there are several procedures to analyze the feeding habits of wild sea turtles, the preferred method is gastric lavage or stomach flushing (Forbes 1999). Gastric lavage allows for the retrieval of undigested food from the esophagus and anterior portions of the stomach for content analysis. This simple technique to sample the gut contents of wild turtles has been conducted on hardshell turtles ranging in size from 25 to 115 cm curved carapace length (Forbes 1999).

Restrain turtles by placing them briefly in an inverted position on a cushioned surface, such as an automobile tire or padded slant board. If using a tire, it should be placed on a platform raised several feet above the ground to allow easier access to the turtle during the lavage process. Prior to lavage, adjust the turtle's position so that the cranial anterior part of the body is lower than the caudal posterior to allow gravity to assist with collection of esophageal contents. Depending on the size and activity level of the turtle being lavaged, one or two individuals will restrain the flippers and hold the head so that the neck and esophagus remain in line with the longitudinal axis of the body.

Prompt the turtle to open its mouth by gently tugging on the skin of the throat, offering a bite block, or working two lengths of soft, large-diameter rope in between the jaws to hold the jaws apart from one another (see NMFS 2008 for mouth opening tools and techniques). Once the mouth is open, insert an appropriately sized standard veterinary canine oral speculum just posterior to the anterior tip of the rhamphotheca to keep the jaws from closing. The powerful jaws of larger loggerheads may necessitate use of a short length of 5 cm diameter PVC or similar bite block to keep the mouth open. Both the bars of the oral speculum and any pipe used for this purpose should be wrapped with soft, rubber tape or tubing to prevent damage to the rhamphotheca.

Once the mouth is securely open and the turtle's position has been stabilized, prepare to insert two lengths of clear, flexible, vinyl tubing lubricated with vegetable oil or water-based lubricating gel into the esophagus, passing to either side of the oral speculum. The first tube, used to retrieve food items from the esophagus, should be one m in length with a wall thickness of two mm and inner diameter of three to five cm, depending upon the size of the turtle. The second tube, used to introduce water into the esophagus to flush out food particles, should be three m in length with a wall thickness of two mm and inner diameter of five mm. Round the ends of both lengths of tubing by melting them with a flame and allowing them to cool prior to use to ensure that the tubing will not damage the walls of the esophagus during insertion. Align the tubes exterior to the turtle to pre-measure the distance to the caudal margin of the pectoral scute of the plastron, roughly corresponding to the level of the stomach, and mark the distance on the tube for that particular turtle with either tape or erasable marker. The tubes should be passed no farther than this mark, or no farther than they will pass without resistance. Although the lengths of tubing may partially obstruct the glottis during the lavage process, take care not to

Figure 14-1. Gastric lavage (NMFS/SEFSC photo).

accidentally introduce the ends of the tubing into the glottis opening. An alternative method will be to lubricate a soft plastic veterinarian's stomach tube with vegetable oil and cautiously insert it into the mouth and throat area.

To initiate lavage, pump freshwater into the esophagus using a double-action, veterinary stomach pump while the introduction tube is gently moved up and down the length of the esophagus (Figure 14-1). If water does not begin to flow from the retrieval tube within a few seconds after introducing water into the esophagus, or if return water flow is low, adjust the position of the retrieval tube to remove any obstruction. If the situation does not resolve, water flow into the esophagus should be stopped. Barring any such difficulties, gently move the retrieval tube up and down the esophagus for 30 – 45 seconds while water flowing through the tube is collected in a bucket. The actual lavaging of an individual turtle should not exceed three minutes. Strain the bucket contents through a fine-mesh sieve, and preserve any food particles in 10% buffered formalin for future analysis.

After completion of lavage, stop the water flow and elevate the posterior of the turtle slightly to allow the introduction and retrieval tubes to drain. Once the tubes have drained, remove the introduction tube first, followed by the retrieval tube and the mouth gag or PVC pipe. At this point, elevate the anterior part of the turtle's body slightly relative to the posterior to allow any remaining water to drain into the esophagus, away from the glottis, so that the turtle can take a breath.

Equipment (e.g., lavage tubes) must be disinfected between animals. Lavage tubes must be thoroughly cleaned prior to disinfection. Disinfectants should be used according to directions, ensuring that contact time with disinfectant according to label directions is sufficient. Disinfection can be compromised if items are contaminated with debris and/or have rough or porous surfaces. Researchers should clean items prior to disinfection and increase the exposure time for rough or porous items. Additionally, a separate set of equipment must be used for healthy animals and those with health problems (e.g., fibropapilloma tumors).

Chapter 15: Laparoscopy

Laparoscopies are performed to identify the sex of the animal and for health assessments (Figures 15-1a – c). Only individuals thoroughly trained in the laparoscopy of marine turtles, or directly supervised by individuals so trained should conduct this procedure. Aseptic techniques should be used at all times to prevent infection. This procedure should not be performed on any compromised animals (e.g., those that are acutely emaciated or obese, overheated, or have heavy parasite load or severe bacterial infection).

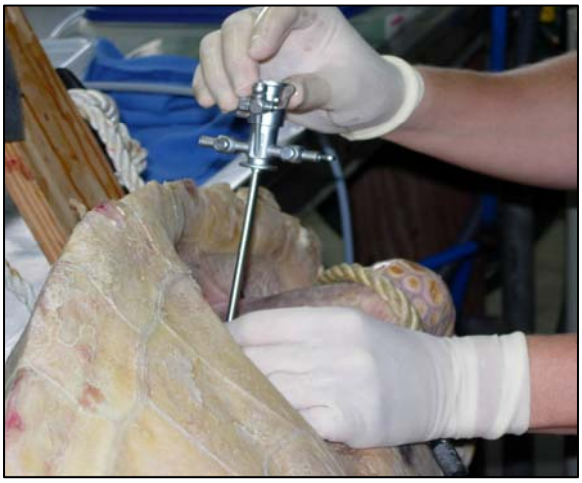
Large Juvenile and Adult Turtles

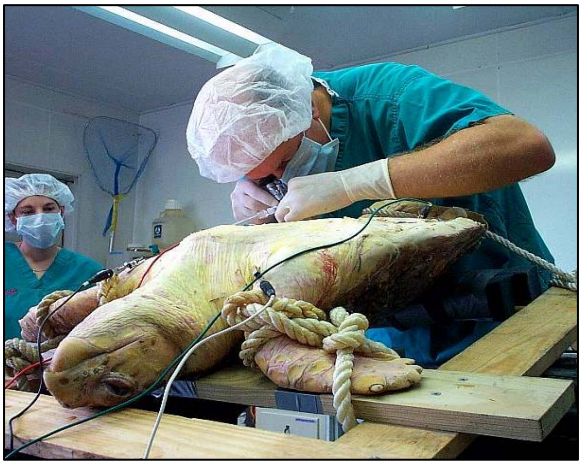
Maintain the turtle at temperatures similar to capture temperature and restrain the turtle briefly on its carapace in an inverted or lateral position for surgery (Figure 15-1). Following a surgical scrub (either three alternating applications of 70% ethanol and surgical iodine scrub, 70% isopropanol, povidone-iodine scrub, and chlorohexidine wipe), inject a local anesthetic (lidocaine, maximum of 2 mg/kg) into the muscle and dermis of the peritoneal wall of the prefemoral fossa. At operating temperatures above 78º F, allow a minimum of 10 minutes and a maximum of 45 minutes after the lidocaine injection prior to surgery. For lower temperatures, allow greater drug effect onset times (e.g., allow a minimum of 15 – 20 minutes when operating between 72 – 78° F).

Make a one to two cm incision just through the skin; use the trocar and sleeve to push through the muscles and peritoneal wall into the body cavity. Be careful to avoid an entry that is too far posterior (where the trocar might strike the kidney) or an entry that goes too deep (where the trocar might strike the lung or intestine). After achieving entry into the peritoneal cavity, verify the location of the trocar with the laparoscope prior to inflating the body cavity with filtered air. Inflation (known as insufflation) is sometimes necessary to visualize the internal organs.

After completing the examination, remove all air prior to suturing the wound. Intracoelomic fluids (sterile 0.9% saline or other IV fluid solution at up to 3% body weight or 30 ml/kg) may be administered as supportive peri-operative care to maintain fluid and electrolyte balance and to help displace air in the body cavity during evacuation.

Use a single deep suture and two superficial sutures to seal the wound using a monofilament nominally absorbable suture (Govett et al. 2004), such as: polyglyconate (Maxon™, US Surgical, Norwalk, CT, USA), or poliglecaprone 25 (Monocryl™, Ethicon,
Somerville, NJ, USA), or polydioxanone (PDS II™, Ethicon). The suture size depends on the size of the turtle, but will be 2-0, 3-0 or 4-0. The deep suture is a horizontal mattress pattern to eliminate dead space, and the superficial sutures may be either a buried, subcuticular horizontal mattress or external simple interrupted, horizontal mattress, or cross mattress, depending on surgeon preference.





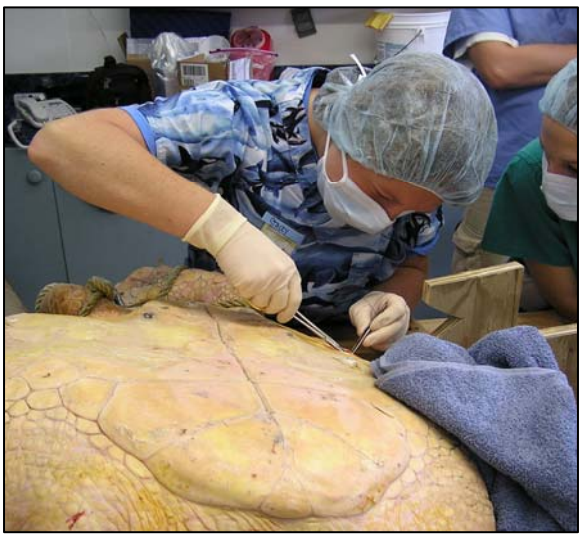


Figure 15-1a, b and c. Laparoscopy for sex identification (Photos by M. Godfrey, NCWRC).

Drug	Dosage	Source
ceftazidime	20 mg/kg IM	Stamper et al. 1999
oxytetracycline	25 mg/kg IM	Harms et al. 2004
enrofloxacin	20 mg kg oral	Jacobson et al. 2005
ticarcillin	50 or 100 mg/kg IM	Manire et al. 2005
amikacin	5 mg/kg IM	Carpenter 2005

Table 15-1. Several antibiotic choices to reduce post-surgical complications.

A single pre-surgical dose of antibiotic (Table 15-1) may be administered to reduce the chances of post-surgical infections. Propofol may be administered (5 mg/kg IV, MacLean et al. 2008) as a short-acting general anesthetic prior to the procedure. Turtles that receive propofol should be held out of water for at least one hour following the conclusion of the procedure and should not be returned to water until it is established that they are fully responsive. A nonsteroidal anti-inflammatory drug (e.g., ketoprofen, 2 mg/kg IM, MacLean et al. 2008) may be administered to reduce post-operative pain with no sedation; be especially attentive if used on green turtles, as an older related anti-inflammatory compound, flunixin meglumine (e.g., Banamine®), can be lethal in that species (D. Mader, pers. comm.). All wild turtles should be held in tanks temporarily to ensure that normal swimming and diving activity have returned prior to their release.

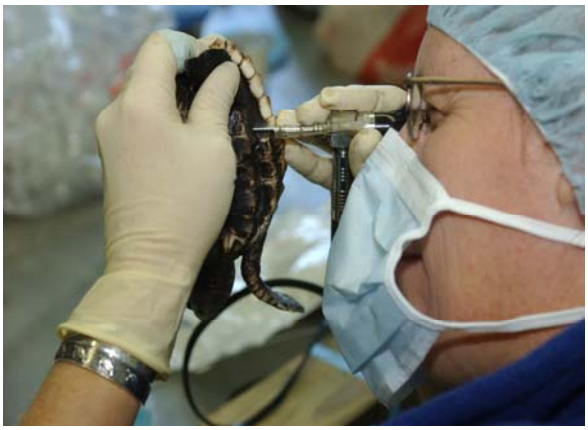
Post-hatchling and Small Juvenile Turtles (Wyneken et al. 2003, 2007)

The laparoscopic method for sex determination has been modified for use in sea turtles as small as 120 g (Figures 15-2a and b). When post-hatchling turtles reach a minimum size of 120 g, withhold food for 24 hours and give one to two drops of infant simethecone orally 12 – 24 h prior to the procedure. Prior to laparoscopy, clean the turtle posterior to

the head with disinfectant soap and water, then give them a surgical scrub (either three alternating applications of 70% ethanol and surgical iodine soap, or soap and water, 70% isopropanol, povidone-iodine scrub, and chlorhexidine wipe). A minimum of 10 – 15 minutes prior to surgery, and no longer than 40 min prior to surgery, inject 0.25 ml 10% lidocaine (1mg/ml) for 120 g turtles (1.3 – 2.1 mg/kg) around the incision site in the anterior inguinal fossa to block any pain or discomfort the turtle might experience during the procedure.

Right handed observers usually use the right prefemoral fossa while left handed people may prefer to enter through the left inguinal fossa. Depending on state permit conditions, the analgesic butorphanol may be administered (0.1mg/kg SQ) approximately 10 minutes prior to surgery. When deemed appropriate, an antibiotic such as ceftazidime (20 mg/kg IM) may be administered in the shoulder musculature to reduce the risk of post surgical infection.

It is simplest to hold small turtles by hand and position each so the head is facing down; the viscera are displaced by gravity away from the incision site and are not



Figures 15-2a and b. Laparoscopy for sex identification in loggerhead post-hatchling (Photo 15-2a by J. Foote, Mote Marine Lab and 15-2b by S. Taylor, Duke Marine Laboratory).

covering the gonads. For post-hatchlings, omit the use of a trocar and sleeve, as they require a larger incision. Use a simple longitudinal incision to open the skin with a simple 0.5 cm cut and follow with a stab incision made with closed 4.5” straight sharp-point surgical scissors. This does the least damage to the inguinal muscle.

Examine the internal organs, especially the gonads and gonadal ducts of the turtles using a 2.7 mm 30º rigid orthopedic endoscope (e.g., Medical Diagnostic Systems, Brandon, Florida). Landmarks for

gonads and ducts are established by the lung tip and kidney, posteriorly. Internal anatomical characteristics, such as relative size, color, shape, attachment of the gonad, and accessory duct (Müllerian duct) form are then recorded. Remove the scope and close the incision with one to three simple interrupted sutures.

Use absorbable suture material [e.g., Maxon™ (polyglyconate), Monocryl™ (polyglyconate) and/or Vicryl™ (polyglactin) 000 or 0000 size with an attached cutting needle works well] often with cyanoacrylate tissue glue, closing both the muscle and skin at the same time. The cut edges are everted slightly. Finally, apply triple antibiotic ointment to the site to prevent any post-surgical infection, and coat each turtle with a water-based lubricating gel to prevent the turtle from drying out. Return the turtles to the water the next day and feed. Typically, the post-hatchling turtles eat enthusiastically following this procedure. Release into oceanic waters one to three weeks following surgery. If a turtle does not feed and/or floats with its flippers out to the sides, seek veterinary assistance quickly, as the turtle may have developed an infection.

Chapter 16: Imaging

Turtles may be examined using a variety of imaging techniques [e.g., ultrasonography (U/S), radiography (x-rays), computed tomography (CT), and magnetic resonance imaging (MRI)] to assess their health and to gather baseline images. These imaging devices are similar to those used in human medicine, and the procedures are non-invasive. Transport, handling, restraint and positioning for imaging shall be consistent with safe handling procedures detailed in Chapter 2: Handling. Wet towels may interfere with image clarity, but may be used on non-affected areas of the turtles, depending on ambient air temperatures. Dry towels used over the head and eyes or for restraint may calm the turtle, will not interfere with the image, and do not pose the risk of evaporative cooling. It may be advised to fast the animal for 48 hours, depending on the animal's condition and veterinary guidance.

Figure 16-2. Turtle restrained for MRI procedure (NMFS/SEFSC photo).

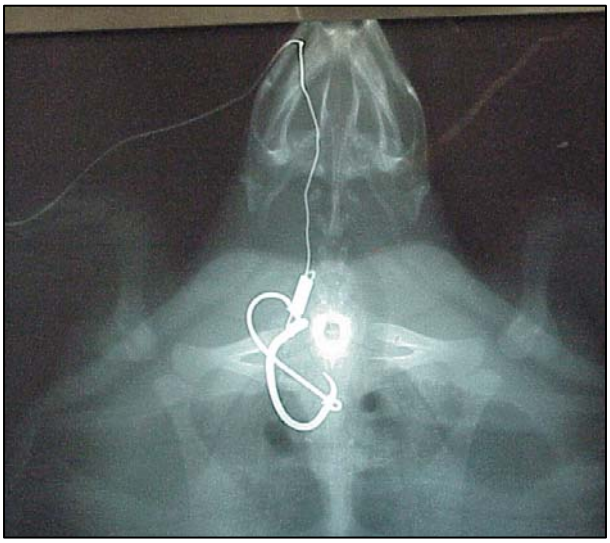


Figure 16-1. X-ray image of ingested fishing hook (Photo courtesy of J. Flanagan, Houston Zoo).

Ultrasonic examination is non-invasive, quick, and generally does not require an anesthetic. Ultrasonic imaging has been used widely on sea turtles (Owens 1999), primarily to evaluate a turtle's gonadal condition and determine reproductive status. Radiography (Figure 16-1) has wide ranging application for health assessment, including diagnosis after injury or ingestion of foreign materials (e.g., fishing hooks, plastic). CT scans and MRIs (Figure 16-2) offer high resolution images. Methodology for these imaging procedures will differ depending on the technology used and operator preference; therefore, no specific guidelines for these procedures are detailed here. Standard radiation safety protocols for personnel shall be followed for radiographic and CT imaging.

In some cases, the turtle may need to be physically or chemically restrained or anesthetized [e.g., 3 - 5 mg/kg propofol IV; or mededtomidine (0.15 - 0.18 mg/kg IV) / ketamine (5 - 6 mg/kg IV) reversed with atipamezole (0.7 - 0.9 mg/kg IM) (after Chittick et al. 2002)], at the discretion of the attending veterinarian. Ultimately, determining the need for sedation, drug selection and dosage is solely the decision of the attending veterinarian. While sedated, the animal's heart rate should be monitored (Figure 16-3) using an ultrasonic doppler flow detector (e.g., Model 811-BTS, Parks Medical Electronics, Inc., Aloha, Oregon, USA) or other appropriate methods whenever possible.

Figure 16-3. Monitoring heart rate (NMFS/SEFSC Photo)

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