U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE
Southwest Fisheries Center Honolulu Laboratory
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PROTOCOL FOR THE LONGLINE FISHERY OBSERVER PROGRAM TO COLLECT TISSUE BIOPSIES FROM SEA TURTLES FOR DNA ANALYSIS

CATEGORY A: Sampling a live sea turtle brought aboard the vessel.

1. Stabilize the turtle by turning it over and holding it still in a supine position. If available, a second person should provide assistance.

2. Using a disposable alcohol swab, clean the skin region located immediately dorsal to each of the hind flippers. The skin in this area is normally soft and smooth, and devoid of hard or enlarged keratinized scales. Skin dorsal of the hind flippers is the preferred area to biopsy. However, if for some reason it is not possible to sample this region, skin in the ventral pectoral area, at the base of the front flippers, may be used.

3. Carefully remove a new biopsy punch (Acu-Punch brand) from its sealed wrapper. Exercise care in handling as the circular cutting end of this instrument is very sharp. Use caution by holding the cutting edge away from you and other persons at all times.

4. Hold the plastic handle of the biopsy punch using your thumb and index finger. Place the circular cutting end on the smooth skin dorsal of a hind flipper and rotate the punch while pressing down with moderate force. A circular cut will rapidly be made through the skin. Continue to rotate and press down to about 5 mm depth, or until the steel blade reaches maximum penetration. For samples taken from small turtles (<25-35 cm carapace length), cutting to a depth of only 2-3 mm, or about half the length of the steel blade, will be sufficient.

5. Withdraw the biopsy punch from the skin by lifting it straight out. Use forceps to grasp and remove the thin circular plug of skin resulting from the cut made with the biopsy punch. The plug may momentarily adhere to the underlying tissue, but will easily detach when lifted away.

6. Immediately place the plug of skin in a designated vial containing harmless purified granular salt (NaCl). Shake the vial for several seconds after replacing the cap. Label the vial with the date, the turtle's flipper tag number, and/or any other unique identifying information available for the turtle. These data should correspond with information entered in your observer's logbook.

7. Using the same biopsy punch, obtain a second disk of skin from the turtle, but from the opposite hind flipper region. This should be accomplished by repeating the procedures listed in Steps 1-6.

Place the second plug of skin in the same labeled vial. Store the vial in a secure location reserved for valuable scientific specimens.

8. When both skin samples have been obtained, immediately return the biopsy punch to its protective wrapper and mark the package as "used." Return it to the National Marine Fisheries Service Honolulu Laboratory for proper disposal. Additional new biopsy punches have been supplied to each observer; therefore, the same punch should not normally be used to obtain skin samples from another turtle. However, if for some reason it becomes absolutely necessary to use a punch on a different turtle, the instrument must first be thoroughly cleaned of any tissue and then soaked in 90% alcohol for 15 minutes or longer. A pint of 90% alcohol has been supplied for this purpose.

The forceps used to grasp the skin plug must always be thoroughly cleaned of any adhering tissue and rinsed with 90% alcohol after each turtle is sampled.

9. The turtle should be released in an appropriate and safe manner after all pertinent data have been collected and the turtle has been tagged. No special treatment of the biopsy site is necessary prior to release. Slight bleeding may occur, but this will cease shortly after the turtle has been returned to the ocean.

CATEGORY B: Sampling a dead sea turtle brought aboard the vessel.

1. Follow the same protocol as described above for a living turtle (Category A, Steps 1-8).

2. Be certain that the turtle is, in fact, dead prior to freezing it for transport to a National Marine Fisheries Service Honolulu Laboratory. A comatose but live sea turtle may, in some cases, exhibit absolutely no movement or signs of life. In other cases, a unconscious turtle may show some evidence of eyelid or tail movement when touched. A turtle that shows no signs of life after 2 hours on deck (held in the shade where further damage to it won't occur) may be safely considered as dead.

CATEGORY C: Sampling a large sea turtle dead or alive in the water alongside the vessel that has been hooked or entangled.

1. Unpack the trigger-controlled tissue biopsy device (Anchor brand) and attach it to the end of the 10-ft wooden pole using the hardware that has been supplied. Attach the nylon cord to the trigger. Cock the biopsy device by pressing the lever located on top of the plastic handle. This will open the split needle and activate the trigger.

2. While firmly gripping the pole at the opposite end, position the biopsy needle and press it the full distance (about 3.5 cm) into the turtle's tissue at any accessible location, EXCEPT the carapace, plastron, head, eyes, and neck. The dorsal and ventral pectoral regions, at the base of each front flipper, are the preferred sites for this procedure. Once the needle has been inserted, pull the cord on the pole to engage the trigger. Immediately withdraw the needle and carefully lay the pole and biopsy devise on the deck.

3. Press the lever again to open the needle. Using forceps, carefully lift out the small piece of muscle tissue that was captured within the needle when the bottom and top snapped shut after the trigger was pulled. Place the sample in a labeled vial following the same instructions given in Category A, Step 6.

4. If time and circumstances permit, it is highly desirable to obtain a second sample by repeating Steps 2 and 3 using the same biopsy devise attached to the pole. If feasible, the opposite pectoral region of the turtle should be sampled.

5. Disassemble the biopsy device from the end of the pole and save the hardware for future use. Rinse the hardware and biopsy device thoroughly with fresh water and dry before storage to prevent corrosion. Replace the biopsy device in its protective wrapper, mark the package as "used," and return it to the National Marine Fisheries Service Honolulu Laboratory for proper disposal. Additional sterilized biopsy devises have been supplied to each observer. If for some reason it becomes absolutely necessary to use a biopsy device on another turtle, follow the cleaning procedure outlined in Category A, Step 8.

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January 1995