Chapter 7: Biopsy Sampling

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

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

(2) 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 preserve the sample vary, depending upon which diagnostic tests will be performed. For histologic evaluation, samples are fixed in neutral buffered 10% 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 crystallization;

(3) Determine sex – Small pieces of gonadal tissue can be evaluated histologically to determine the sex of the animal; and

(4) 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.

Skin Biopsy

Protocol for Turtles Boated or on Land

Small hardshell turtles should be turned onto their carapaces 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 followed by an isopropyl alcohol wipe, and then thoroughly swab again with 10% povidone-iodine 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 14-1). Repeat the tissue punch process with the same punch to obtain two plugs from each animal. 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. Push out the tissue plugs into the vial containing 20% DMSO saturated with NaCl by inserting a new, clean wooden applicator stick through the hollow handle of the biopsy punch, or by shaking the punch in the vial. Wipe the punched area with 10% povidone-iodine. If necessary, 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 20% DMSO buffer within the vials 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; topical medical grade DMSO is commonly used to alleviate muscle aches. Do not store the buffer where it will experience extreme heat; the buffer must be stored at room temperature or cooler, such as in a refrigerator. Do not freeze the sample.

Figure 7-1. Skin biopsy taken from trailing edge of rear flipper, note tag scar (NMFS/SEFSC photo).

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.

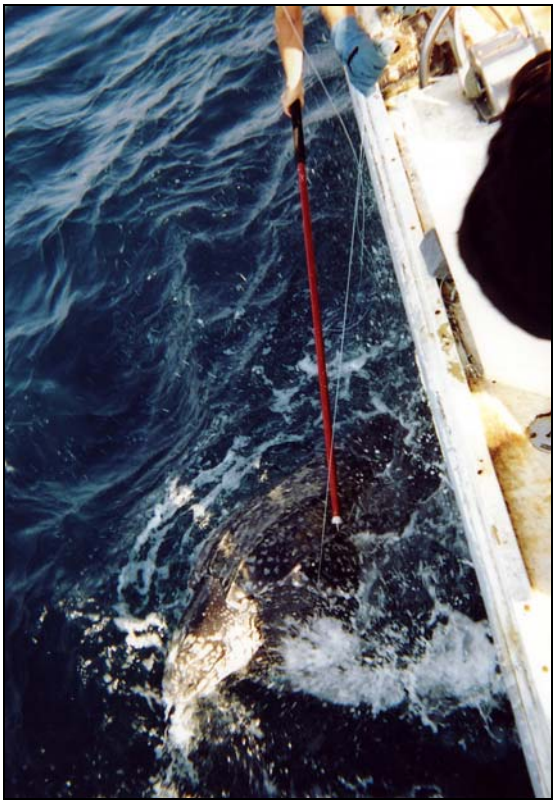

Figure 7-2. Taking a biopsy from a turtle not boated (NMFS/SEFSC photo).

Assemble the pole sections together to attain the desired pole length. The corers are 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. Suitable sampling sites for hardshell turtles, and for leatherbacks when a carapace scrape is not possible, 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. There are nerve bundles high on the shoulders near the carapace that should be avoided, as should the heavily vascularized armpit area. The preferred 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 (Figure 14-2). Do not target the carapace with a jabbing motion.

Due care should be taken not to strike anyone when handling the pole onboard, as the corers are sharp. Unscrew the corer from the pole, and place the entire corer with tissue sample inside into a vial of NaCl saturated 20% DMSO buffer. 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.