Conservation Plan for the Wood Turtle in the Northeastern United States

Tissue Collection Protocol

Wood Turtle Tissue Collection Protocol

Northeast Wood Turtle Working Group

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Background and Overview: As part of a regional conservation effort to assess Wood Turtle (*Glyptemys insculpta*) populations in the northeastern United States and improve the survival of the species, the Northeast Wood Turtle Working Group has worked to identify and protect genetic variation among Wood Turtles. Biologists collected over 1,800 tissue samples from populations throughout 12 northeastern states in order to undertake a fine-scale, region-wide, conservation-driven genetic analysis for the Wood Turtle. The objectives of this study were to (1) assess genetic structuring and diversity of populations, (2) identify and prioritize highly distinct or highly diverse populations for conservation, and (3) create a database and tissue repository that can be used to evaluate the origin of individuals confiscated from illegal pet trade. The findings of this study can be found in *Conservation Plan for the Wood Turtle in the Northeastern United States* (2018). This document outlines the Northeast Wood Turtle Working Group's recommended methodology for Wood Turtle tissue collection.

Guidelines:

Step 1. Select an Appropriate Population for Study. Ideally, blood or tissue (as described below) should be collected from 20 turtles per site. However, the chances of collecting tissue from 20 turtles is low for most populations throughout the Northeast; therefore, individual turtles, especially those in data-deficient or marginal areas (crossing roads, dead on road, during surveys at low-density sites, etc.) should also be sampled in the appropriate manner. The following scenarios are provided as examples:

a.) **Long-Term Reference Sites.** In most cases, genetics sites will correspond to **Long-Term Reference Sites** at which Wood Turtle populations are assessed using standardized criteria (see "Wood Turtle Population Assessment Protocol"; www.northeastwoodturtle.org). However, this is not necessary. Samples may be collected from other sites.

b.) **Populations of Regional Interest, Targeted for Tissues Only.** In other cases, a site may not be a priority Long-Term Reference Site, but is a priority for tissue collection. Examples include remote locations that are logistically difficult to survey over extended periods and watersheds in the Ohio-Monongahela basins.

c.) **Incidental Observations.** In the course of regular field work, researchers encounter Wood Turtles incidentally, on roads and in natural environments. Single turtles of known origin should be sampled for inclusion in certain analyses.

d.) **Confiscations of Unknown Origin.** Wood Turtles confiscated in the USA or abroad can be sampled in the same manner as known-origin turtles for attempted assignment to known populations.

Step 2. Capture up to 20 Wood Turtles. If possible (see above), capture 20 unrelated Wood Turtles (i.e., preferably adult and never hatchlings from the same nest). Adult animals are ideal, as they may be less likely to be from the same nest cohort. When possible, locate turtles following the Wood Turtle Population Assessment Protocol (www.northeastwoodturtle.org).

Step 3. Collect Genetic Material. From each individual turtle (males and females), collect genetic material following the most appropriate protocol. Blood is preferred, but tissue may be collected by several methods:

Figure 1. Blood collection from the dorsal coccygeal vein of a young Blanding's turtle, showing the needle insertion site. The tail must be gently pulled straight out, and then gently pulled dorsally. Note that the Wood Turtle protocol requires gloves.

a.) **Blood Collection from Dorsal Coccygeal Vein (preferred).** Obtain 0.1 to 0.5 cc of blood by drawing blood from the dorsal coccygeal vein using new 1.0 mL syringes and 25 gauge (5/8") insulin needles. Store the blood in screw-cap 2.0 mL Cryovials (Chemglass CLS-4762-020, 2.0 mL sterile round bottom) provided by UMass to cooperating states. The tubes should be half-filled with 95% ethanol. If cryovials are not available, blood may be stored dry on filter paper. Blood should be refrigerated prior to shipment and frozen if it will be stored for extended periods. A detailed protocol follows (adapted from Rhymer 2011 and Hughe 2010):

i.) All blood collection must occur in coordination with state wildlife officials, with all necessary permits;

ii.) All personnel undertaking blood draws must be trained by qualified veterinarians or other experts;

iii.) Researchers should strive to maintain a sterile work environment and follow the most current NEPARC/SEPARC decontamination procedures;

iv.) Prior to collecting blood, confirm that 0.5 mL will total less than 2% blood volume. Use the following estimate based on body mass: 4–8% of total body mass is blood; <2% of blood volume should be drawn (Perpinan 2013). One mL of blood is approximately 1.03 g. Following this equation, 0.5 mL may be drawn from turtles larger than 650 g. Correspondingly less must be drawn from smaller turtles.

v.) Prior to collecting blood, confirm that the animal has not already been bled. To do so, maintain a running list of all bled animals that may be shared across field personnel working at any given site.

vi.) Write the turtle's unique identification number, species, date, site code, sex, and tissue type on the label of a storage buffer vial (using fine-point Sharpie).

vii.) Remove syringe and needle from packaging and prepare for blood draw.

viii.) Collect blood from a restrained turtle by firmly grasping the tail with a gauze pad for traction. Gently pull the tail out in a straight line extension, and then pull it over the plastron (see Figure 1). It is important that the tail is fully extended straight out before gently flexing it over plastron.

ix.) Swab the needle insertion site, and collect up to 0.5 mL blood using a new 25 gauge 5/8" 1 cc syringe/needle. Do not re-stick the turtle if the insertion site if blood is not obtained on the first attempt. Insert needle at a 45º angle directly on mid-line of the tail between the first and second caudal vertebrae. When the needle barely touches the spinous process, back off very slightly.

x.) Once blood appears in the syringe, apply gentle pressure for the duration of the blood collection.

xi.) Replace cap on needle by laying the cap on the ground (do not use your hand) and remove needle from the syringe as quickly as possible. Inject the blood sample into the buffer tube.

xii.) Swab turtle's tail with an alcohol pad and release turtle at the capture location.

xiii.) Dispose of the used needle and syringe in a hard-shelled biohazard sharps box, if available.

xiv.) Place the labeled blood samples in a refrigerator upon returning from the field.

b.) **Toenail Collection**. Toenail clippings are preferable to bone shavings (which were recommended in previous years) but less preferable than blood. If toenail clippings appear to be the most appropriate method, follow these protocols:

i.) Write the unique identification number (carapace notch code or unique ID if not notching), species, date, site code, sex, and tissue type on the lid of a screw-cap storage Cryovial half-filled with ethanol (using fine-point Sharpie).

ii.) Restrain the turtle. Hold hind foot gently.

iii.) Swab toenails and the turtle's foot with an alcohol prep pap. Use surgical (or very sharp, fine) scissors or fingernail clippers to clip approximately 2 mm or 10% of the longest toe nail into the ethanol vial. Use caution, because the toenail fragment is easily lost. Swab collection site and release turtle at capture location. The goal is to avoid blood

vessels in the toenail. In our experience with state partners, the extreme end of the toenail, typically ≤2 mm in adult emydids, can be safely clipped without the nail bleeding. If bleeding is observed, the nail should be swabbed with Betadyne solution and less nail material should be collected in subsequent collections. Where possible, use a different nail clipper at each distinct site. Where feasible, clean the scissors/clipper in 50% household bleach solution with a wire brush.

c.) **Tissue Collection from Dead Turtles.** As appropriate, collect multiple forms of tissue from Wood Turtles found dead on roads, killed by mowers, etc. Liver tissue is preferable, but you may also collect toenails, soft tissue, or bone.

Step 4. Ship Samples to UMass. If the samples were refrigerated or frozen, ship them overnight in a styrofoam cooler with ice packs. If they were stored in ethanol at room temperature, ship them overnight without refrigeration.

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