

# Eastern Box Turtle Tissue Sampling Protocol

## Appendix C

### Northeast Eastern Box Turtle Working Group

*Supported by the Regional Conservation Needs Grant Program*

The Northeast Eastern Box Turtle Working Group (NEEBTWG) is coordinating a project to develop a status assessment and conservation action plan for the Eastern Box Turtle (*Terrapene carolina carolina*) throughout the northeastern United States, from West Virginia to Maine. A central component of this effort was the development of a standardized population monitoring protocol to assess the relative status of populations throughout the range. Although a genetic analysis is not a specified component of the overall project, the NEEBTWG identified the development and opportunistic implementation of a tissue sampling protocol for future genetic analyses as a valuable outcome of this coordinated effort. This document provides general guidelines for (1) selecting sites for genetic sampling and (2) tissue collection and storage. The information provided largely draws from protocols developed for Wood Turtle (*Glyptemys insculpta*) and Spotted Turtle (*Clemmys guttata*).

#### Site Selection

Within the context of this protocol, a “site” should be considered any geographic area where Eastern Box Turtles are regularly interacting with one another. Biologists should consider taking into account three primary factors when considering where to collect genetic samples:

1. *Likelihood of collecting an ideal sample size (20) at a given site.* Because this is the ideal sample size for analyses, biologists should prioritize those sites that are more likely to yield at least 20 turtles.
2. *Amount of genetic sampling equipment available.* Although 20 samples should be the goal for most sites, if ample genetic sampling equipment is available, biologists should consider collecting samples even when 20 samples are unlikely to be collected.
3. *Relative significance of the samples/population.* Certain populations may be of higher priority for genetic sampling even if 20 samples are unlikely to be collected. For example, island populations, otherwise geographically isolated populations, and populations within unique or rare ecological contexts should be considered.

Specific site/sample types that should be considered for genetic sampling:

- A. *Populations of potential/likely genetic significance.* As described above, certain populations may be priorities for genetic sampling due to their potential genetic significance. Examples include populations in unique ecological contexts that could foster novel adaptations or potentially reproductively isolated populations (e.g. island populations).

- B. *Populations of regional significance.* Regionally significant populations might include particularly large populations or other sites of high conservation value.
- C. *Demographic Assessment sites.* Sites that are targeted for a Demographic Assessment using the Eastern Box Turtle Monitoring Protocol ([northeastturtles.org](http://northeastturtles.org)).
- D. *Long-term reference sites.* Sites that have been the subject of long-term population monitoring studies, but are not Demographic Assessment sites for this project.
- E. *Data deficient zones.* Portions of the region that are lacking genetic information.

### Sample Collection

Ideally, samples will be collected from turtles captured using the standardized Eastern Box Turtle Monitoring Protocol ([northeastturtles.org](http://northeastturtles.org)). However, samples will also be accepted in instances where the monitoring protocol is not used (e.g., when turtles are incidentally captured at other sites). Biologists should prioritize sampling adult Eastern Box Turtles because they are less likely to come from the same nest cohort. Genetic samples should not be collected from turtles from the same nest. All collection of genetic material must occur in coordination with state wildlife officials, with all necessary permits. All personnel collecting samples must be trained by qualified veterinarians or other experts. For each individual turtle, collect genetic material using one of the following methods:

*Blood collection from dorsal coccygeal vein (preferred).* Draw up to 0.3 cc of blood from the dorsal coccygeal vein using sterile 1.0 mL syringes and 25 gauge (5/8") insulin needles (see blood volume guidelines below). Ideally, store blood in screw-cap 2.0 mL vial (e.g., Chemglass CLS-4762-020, 2.0 mL sterile round bottom; Fig. 1) that have been half-filled with 95% ethanol. If vials are not available, blood may be stored dry using 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. Biologists and technicians should strive to maintain a sterile work environment and follow the most current NEPARC/SEPARC decontamination procedures.
- II. Prior to collecting blood, confirm that 0.3 mL will total less than 2% blood volume. Use the following estimate based on body mass: 4% of total body mass is blood; <2% of blood volume should be drawn (Perpinan 2013; one mL of blood is approx. 1.03 g). Also see our recommended blood volume guidelines below.
- III. 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.
- IV. Using fine-point Sharpie, write the date of collection, species (four digit code TECA for *Terrapene carolina*), site name/code, state (2-digit code), unique turtle ID, sex, and sample type (and buffer solution) on the label of a storage vial (Fig.1). Write the state and unique turtle ID on the vial cap.
- V. Remove sterile syringe and needle from packaging and prepare for blood draw.
- VI. 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 (Fig. 2). It is important that the tail is fully extended straight out before gently flexing it over plastron.

- VII. Swab the needle insertion site, and collect blood using a sterile 25 gauge 5/8" 1 cc syringe/needle (Fig. 2). Do not re-stick the turtle at the insertion site if blood is not obtained on the first attempt. Insert needle at a 45° 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.
- VIII. Once blood appears in the syringe, apply gentle pressure in the syringe for the duration of the blood collection.
- IX. Inject the blood sample into the buffer tube.
- X. Swab turtle's tail with an alcohol pad and release the turtle at the capture location.
- XI. Dispose of the used needle and syringe in a hard-shelled biohazard sharps box, if available.
- XII. Place the labeled blood samples in a refrigerator upon returning from the field.

Blood volume guidelines:

- $\geq 75\text{g}$  turtle  $\leq 0.30$  ml limit
- 50g turtle  $\leq 0.20$  ml limit
- 25g turtle  $\leq 0.10$  ml limit
- No turtles under 25 g should be bleed

*Toenail or shell shaving collection.* Toenail and shell shaving samples have been shown to be less effective for acquiring adequate DNA samples (Dana and Whiteley 2018); however, if blood sampling is not an option, biologists should consider collecting toenail tissue or the shell shavings produced when notching a turtle. Toenail samples can be collected with scissors or toenail clippers. To increase the likelihood that samples are viable for analyses, collectors should attempt to obtain soft tissue within the toenail without drawing blood. Similar to blood collection above, samples should be stored in screw-cap 2.0 mL Cryovials (Chemglass CLS-4762-020, 2.0 mL sterile round bottom) that have been filled with 95% ethanol.

*Tissue collection from dead turtles.* As appropriate, collect tissue from any Eastern Box Turtles found dead (e.g. road or agricultural mortality events).

### Supplies and Materials

- 1.0 mL syringes and
- 25 gauge (5/8") insulin needles
- 2.0 mL Cryovials (Chemglass CLS-4762-020, 2.0 mL sterile round bottom)
- 95% Ethanol as buffer solution
- Gauze pads
- Used needle disposal container

- Alcohol wipes
- Storage container for samples (e.g., lunch box with ice packs)
- Rite-in-the-Rain paper
- Fine-point Sharpie
- Pencil
- Sample collection form

### **Ship Samples to University of Massachusetts**

Ship refrigerated or frozen samples overnight in a styrofoam cooler with ice packs. If they were stored in ethanol at room temperature, ship them overnight without refrigeration. Send samples to the following address and make sure to alert Patrick Roberts of the shipment via email ([hprobert@umass.edu](mailto:hprobert@umass.edu)) before sending.

Attn: Patrick Roberts  
Department of Environmental Conservation  
Holdsworth Building  
University of Massachusetts  
Amherst, MA 01003

### **Literature Consulted**

- Diehl et al. 2001. A good practice guide to the administration of substances and removal of blood, including routes and volumes. *J. Appl. Toxicol.* 21:15-23.
- Haskell, A. and M. Pokras. 1994. Nonlethal blood and muscle and tissue collection from Redbelly turtles for genetic studies. *Herpetological Review* 25(1): 11-12.
- Hughe, E. 2010. Protocol for the sampling and storage of painted turtle (*Chrysemys picta*) blood and tissues for genetic analysis. Genetic sampling protocol of the CRD Species at Risk Information and Collaborative. Available at website:  
<http://speciesatrisk.hat.bc.ca/index.php/western-painted-turtle/12-western-painted-turtle/research-a-survey-methods/14-genetic-sampling-protocol>
- Perpinan, D. 2013. Blood collection in turtles. *Vetcom* 52: 38–39.
- Rhymer, J. 2011. Blanding's turtle blood collection protocol. Unpublished document; University of Maine; Orono.

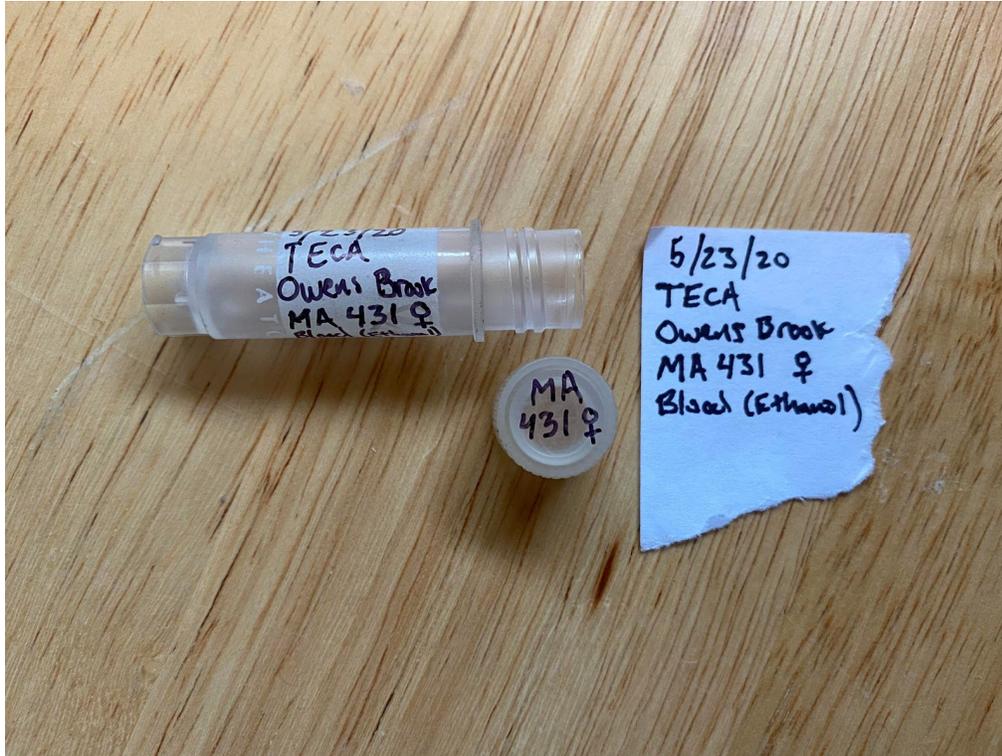


Figure 1. Example of a labeled vial with date, species code (TECA), site name, state, turtle ID, sex, tissue type, and buffer solution provided. Vial cap should be labeled with state and turtle ID.



Figure 2. Blood collection from the dorsal coccygeal vein. Tail should be gently pulled straight out from the shell and then downward toward the plastron.