

EASTERN BOX TURTLE POPULATION ASSESSMENT PROTOCOL

NORTHEAST EASTERN BOX TURTLE WORKING GROUP

Supported by the Northeast Regional Conservation Needs (RCN) Program

www.northeastturtles.org

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This document outlines the 2019 Eastern Box Turtle (*Terrapene carolina carolina*) Population Monitoring Protocol developed by the Northeast Eastern Box Turtle Working Group. This standardized sampling protocol is intended to provide a framework for assessing Eastern Box Turtle populations throughout the northeastern United States. The basic elements of the protocol include Rapid Assessments (RA) and Demographic Assessments (DA). RAs are intended to allow for the efficient assessments of relative abundance at a given site, while DAs, which require more intensive sampling using the same protocol, provide a means for estimating population size and other demographic parameters. Two sampling options are described: (Option 1) circular-plot based sampling (strongly encouraged), (Option 2) feature polygon-based sampling. Early sampling will primarily be focused on a visual survey approach, but we also recommend the evaluation and potential future inclusion of two additional approaches (i.e., trap-assisted and dog-assisted surveys). This standardized population monitoring protocol is designed to be flexible, and to allow use in a variety of habitat and project types throughout the northeastern United States and elsewhere throughout the species range.

Goal:

Provide a flexible and efficient framework for detecting and monitoring Eastern Box Turtle populations that will facilitate the assessment of distributional trends, patterns of occupancy and abundance, long-term population trends, and effects of habitat management throughout the northeastern United States.

Objectives:

1. Assess Eastern Box Turtle occupancy and relative abundance throughout the northeastern United States.
2. Provide a framework for tracking trends in occupancy over time.
3. Quantify population densities for a subset of sampled populations.
4. Provide a framework for tracking trends in population density over time.
5. Assist in the evaluation of the effects of habitat management actions on Eastern Box Turtle populations.
6. Provide a flexible, yet standardized monitoring framework that is compatible with monitoring efforts throughout the range, including citizen science efforts.

GUIDELINES

Site Selection

A survey site may be any area containing habitat that could potentially support Eastern Box Turtles (e.g., early successional, forest, or ecotone conditions). Ideally, survey sites should be located >1,200 m apart (approx. twice the average annual movement distance in Massachusetts [Willey 2010]). Alternatively, sites can be chosen <1,200 m apart, but should be separated by a clear barrier to movement (e.g., lake or 4 lane highway). For sites that are unfamiliar to surveyors, performing a reconnaissance site visit is advisable to assess site access and current ground conditions of survey areas. In an attempt to ecologically and geographically stratify sampling efforts across the region, provisional sampling targets for physiographic areas and states are provided at the end of this document (Appendix B). It is also recommended that, where possible, surveyors select survey sites along a gradient of rural-urban conditions, habitat patch sizes, and habitat types.

Below we describe methods for defining your specific survey area within survey sites for three different survey methods. **Option 1 (circular plot survey) is the recommended method** at this time. Option 2 (feature polygon survey) is provided for surveyors that do not have access to specialized mapping program (beyond Google Earth) and/or GPS units.

Option 1: Circular plot survey

This option is strongly preferred for Rapid Assessments. Within your site, place four 28-m radius (1/4 ha) **circular plots** centered within suitable habitat or potentially suitable Eastern Box Turtle habitat (Fig 1). For example, suitable habitat may be a field-forest ecotone, section of a power

line corridor, old gravel pit, or a patch of forest. The Northeast Eastern Box Turtle Working Group (NEEBTWG) recommends that surveyors target **early-successional habitat adjacent to mature forest**, but surveyors may also consider other areas frequently used by Eastern Box Turtles during the spring months in your region (e.g., forested habitats). The four paired circular sampling plots should be non-overlapping and no more than 350 m from each other (approximately $\frac{1}{2}$ the average distance between overwintering location and early successional habitat in Massachusetts [Willey 2010]). Circular plots are strongly preferred, however, square plots $\frac{1}{4}$ in size could also be used.

Option 2: Feature polygon survey

This option is best for Demographic Assessment sites. Within a selected site, delineate a polygon encompassing a **feature polygon** that will be surveyed (Fig 2). A “feature” is defined as any component/aspect of the landscape consisting of suitable or potentially suitable Eastern Box Turtle habitat. For example, suitable habitat may be a field-forest ecotone, section of a power line corridor, old gravel pit, or a patch of forest. The NEEBTWG recommends that surveyors target **early-successional habitat adjacent to mature forest**, but surveyors may also consider other areas frequently used by Eastern Box Turtles during the spring months in your region (e.g., forested habitats). Delineated feature polygons should be 2–4 ha in size and take on any shape. Multiple features may be designated at a single large site (e.g., state park with multiple patches of field/forest ecotone habitat patches) if they are separated by >1,200 m OR a barrier to movement (i.e., 4-lane highway, lake, larger river).

Visual Encounter Surveys

Rapid Assessment:

Conditions for Surveys

- *Sampling period:* mid-April – June (recommended). In more southern locations (mid-Atlantic region and south), mid-April may work for habitat with thin and/or low growing vegetation and June surveys may also be effective. Optimal survey dates may vary by geographic location and yearly variation in spring weather conditions.
- *Time of day:* 7:00 AM to 3:00 PM
- *Weather conditions:* Surveys can take place under most weather conditions, but avoid extended (>3 days) cold (< 60 degrees) and hot (> 85 degrees) periods.

Options 1: Circular Plot Surveys (strongly preferred)

- *Sampling area:* Sampling plots should be searched as evenly and thoroughly as possible. Surveyors may find it useful to create and upload plot boundary points or plot center points into a GPS unit, or use Google Earth on their cell phone (if cell reception is available) to help guide them during the survey. If the center points in a GPS, surveyors can use the “go to” feature to stay within 28 m of the center point.

- *Number of surveys:* Each set of plots should be surveyed **3 times within a single season**.
- *Survey effort:* Each ¼ ha sampling plot should be searched for 11 min with one surveyor (approximately 0.75 person hrs/ha and a total of 45 min of active search time for 4 plots). This excludes time spent processing turtles. If two surveyors are surveying the plot, they would search for 5.5 minutes each. No more than 2 surveyors should be used at a given plot. It is recommended that no more than 2 surveyors sample a site during each survey. However, when >2 surveyors are used they should survey different plots so that no more than 2 surveyors search a single plot. All sampling plots within the same site should be surveyed during the same day and at least 48 hours should separate any two sampling events at a given site.
- *Survey effort for **thickly vegetated sites** (see Fig 3 and 4):* The time to survey each plot can be extended up to 22 min per plot in instances where the vegetation is very thick and it is difficult to see the ground. This may be the case at particular sites throughout the sampling period (shrubby locations), only during surveys at the tail end of the sampling period when the grasses and forbes are taller and thicker, OR may not be necessary during any of your surveys depending on the site.
- *Data:* It is highly recommended to record tracks during surveys. GPS unit or app can be used to save an independent set of tracks for each survey. Please see the data forms for track naming convention. Please see the data forms for track file naming convention. The start time, end time, weather conditions, and habitat features will be noted. Survey field forms can be found in Attachment B and at northeastturtles.org.
- See Appendix A for step-by-step Survey Instructions

Option 2: Feature Survey

- *Sampling area:* The entire feature should be surveyed as evenly and thoroughly as possible. Surveyors may find it useful to create and upload feature boundary points into a GPS unit, or use Google Earth on their cell phone to help guide them during the survey.
- *Number of surveys:* Each feature should be surveyed **3 times within a single season** and least 48 hours should separate any two sampling events at a given feature.
- *Survey effort:* During each survey, surveyors should spend 0.75 person hours per hectare searching for turtles. This excludes time spent processing turtles. It is recommended that no more than 2 surveyors be used during a single survey, however if additional surveyors are used the survey time should be modified accordingly (see Table 2).

- *Survey effort for **thickly vegetated sites** (see Fig 3 and 4):* The time to survey each plot should be doubled to 1.5 person hours per hectare. Double the times in Table 2.
- *Data:* It is highly recommended to record tracks during surveys. GPS unit or app (e.g., GAIA, Avenza) can be used to save an independent set of tracks for each survey. Please see the data forms for track naming convention. Please see the data forms for track file naming convention. Survey start time, end time, weather conditions, turtles observed, and habitat features will be noted. Survey field forms will be provided.
- See Appendix A for step-by-step survey instructions

Demographic Assessment:

Visual Surveys (preferred)

For demographic assessments, features will be delineated as described above, and the Rapid Assessment methodology will be followed. A minimum of four to six additional survey events will also be required for a total of $\geq 7-9$ independent surveys (dependant on the number of recapture events) at demographic sites within a two-year time frame.

Data Management:

Data should be entered into the regional database using the Data Entry Excel spreadsheet. For GPS track data collected please label each track with the following convention: SItelD_YYMMDD. The turtle photos should be labeled as follows: StateCode_Site ID_TurtleID_YYMMDD_C or P. Photos of the carapace should end with a C and photos of the plastron should end with a P.

Alternative Methodologies Under Evaluation

Trap-Based Surveys:

Where time and resources allow it would be valuable to evaluate trapping with use of drift fences and passive unbaited box traps with adjustable wings (Fig 5) as a potential alternative method for a demographic assessment. We recommend use of 2-4 drift fences of 56 m in length (equivalent to the diameter of a $\frac{1}{4}$ ha circular plot) (Fig 6). Silt fencing material would work well for the drift fence. Trap density should be 12 traps/plot with traps placed on either end of the drift fencing and on both sides of the fencing (Fig 7) as well as approximately every 10 m along the drift fence on both sides. Traps should be deployed for X trap nights and checked daily.

Dog-Assisted Surveys:

Dog-assisted surveys should be evaluated as an additional optional survey method for both RA and DA population assessments. The protocol would follow the same survey conditions and sampling methods as the visual encounter surveys with one exception. Surveyors should perform at least 4 surveys per site. We recommend a comparison study between the visual encounter surveys and dog-assisted surveys. This would be done by alternating human versus dog-assisted survey at each site. For example, you would conduct a dog-assisted survey during your first and third site visit and a human survey during your second and fourth visit. Handlers should follow behind the dog and any turtles missed by the dog and found by the handler should be counted as turtles found off the clock and recorded on the survey form under “#Off-clock”.

Since dogs may search the entire plot or feature more quickly than humans, we recommend noting in the comments field how much time you think it took for the dog to adequately search the survey area. However, the dog should continue to search the area for the full recommended time (11 minutes for a plot or 0.75 person hrs/ha). This data collected during year 1 (trial) will be used to determine if we need to adjust the recommended survey time for the dog-assisted surveys.

Other Survey Requirements

All participants must have permits from their state wildlife agency, IACUC protocol if necessary (for University associated research), and follow the NEPARC disinfection protocol (http://www.northeastparc.org/products/pdfs/NEPARC_Pub_2014-02_Disinfection_Protocol.pdf).

Literature Cited

- Efford M.G. and R.M. Fewster. 2013. Estimating population size by spatially explicit capture-recapture. *Oikos* 122(6):918-928.
- Pledger, S., K.H. Pollock and J.L. Norris. Open capture-recapture models with heterogeneity: I. Cormack-Jolly-Seber Model. *Biometrics* 59(4):786-794.
- Royle, J.A. 2004. *N*-mixture models for estimating population size from spatially replicated counts. *Biometrics* 60:108-115.
- Royle J.A., and J.D. Nichols. 2003. Estimating abundance from repeated presence-absence data or point counts. *Ecology* 84(3):777-790.
- Wiley L.L. 2010. Spatial ecology of eastern box turtles (*Terrapene c. carolina*) in central Massachusetts. Dissertation, University of Massachusetts, Amherst, USA.

Figures

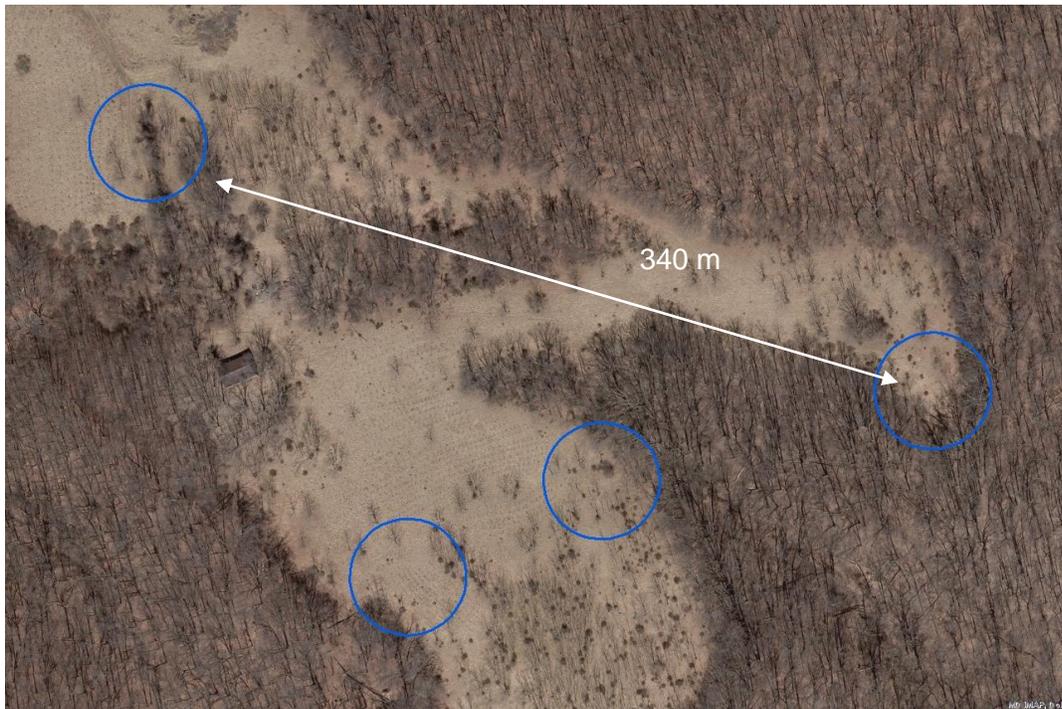


Figure 1. Four $\frac{1}{4}$ ha sampling plots (blue) within suitable Eastern Box Turtle Habitat. Each plot has a 28 m radius and the two plots furthest from each other are within 350 m of each other.

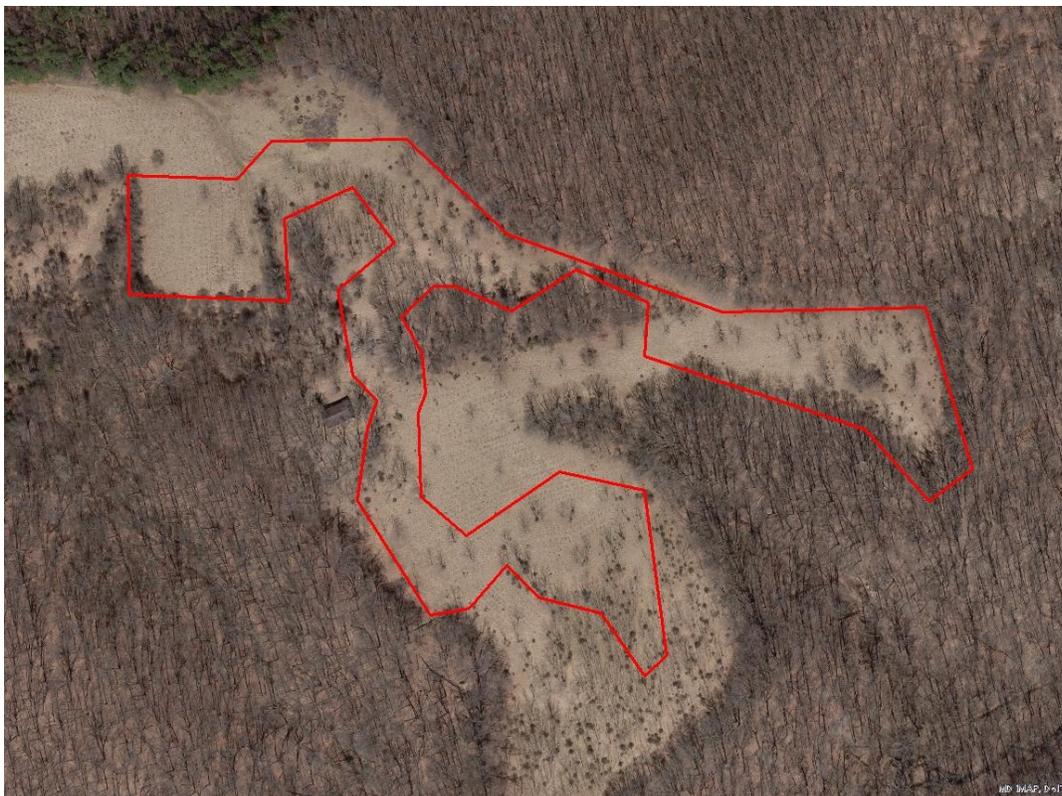


Figure 2. A feature polygon (red) within a site.



Figure 3. Image of a thinly or regularly vegetated habitat on the left and thickly vegetated habitat on the right.



Figure 4. Graphics of thinly vegetated habitats on the left and thickly vegetated habitats on the right.



Figure 5. A photo of a passive box trap with adjustable wings.

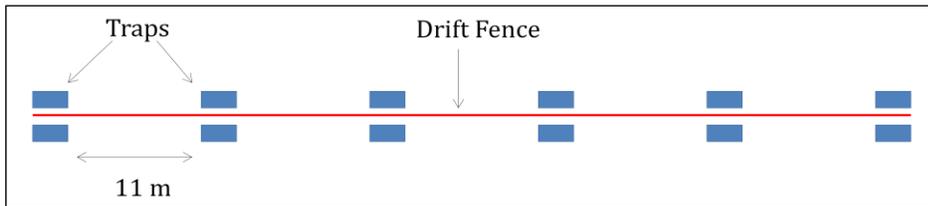


Figure 6. A diagram of one drift fence and 12 trap set up.



Figure 7. Four 56 m long drift fences set up at a site.

Tables

Table 1. Survey time chart to calculate the number of minutes needed to reach a 0.75 person hours/ha of effort given the number of surveyors and area to be surveyed.

| Size of Site (ha) | Number of Surveyors | | | | |
|-------------------|---------------------|----------|----------|----------|----------|
| | <i>1</i> | <i>2</i> | <i>3</i> | <i>4</i> | <i>5</i> |
| <i>1</i> | 45 | 23 | 15 | 11* | 9* |
| <i>1.25</i> | 56 | 29 | 19 | 14* | 11* |
| <i>1.5</i> | 68 | 35 | 23 | 17 | 14* |
| <i>1.75</i> | 79 | 40 | 26 | 19 | 16 |
| <i>2</i> | 90 | 46 | 30 | 22 | 18 |
| <i>2.25</i> | 101 | 52 | 34 | 25 | 20 |
| <i>2.5</i> | 113 | 58 | 38 | 28 | 23 |
| <i>2.75</i> | 124 | 63 | 41 | 30 | 25 |
| <i>3</i> | 135 | 69 | 45 | 33 | 27 |
| <i>3.25</i> | 146 | 75 | 49 | 36 | 29 |
| <i>3.5</i> | 158 | 81 | 53 | 39 | 32 |
| <i>3.75</i> | 169 | 86 | 56 | 41 | 34 |
| <i>4</i> | 180 | 92 | 60 | 44 | 36 |

*Surveys should not be less than 15 min in length.

APPENDIX A: STEP BY STEP SURVEY INSTRUCTIONS

SITE SELECTION

- Select your sampling site (e.g., a 4-6 ha patch of field/forest edge habitat within a state park).
- Map the survey area (using 28-m radius circles or a feature polygon) using Google Earth, ArcGIS or some other mapping app.
- Visit the site to determine if the mapped area is appropriate habitat for eastern box turtles, feasible to survey and determine if any modifications need to be made to the survey area mapping. You may want to flag/mark the survey area boundary while you're there in preparation for surveys at later time.
- Send an electronic copy (as shp or kmz file) of the final survey area boundary to the state project lead.

OTHER FIELD SEASON PREPARATIONS

- Obtain appropriate state permits (or letters of authorization) and landowner access permissions.
- Contact your state lead to obtain turtle notch codes and a diagram of that state's marking scheme (only for those with permission to notch turtles).
- Determine field equipment needs (clip board, calipers, pesola scales, etc) and obtain needed equipment.
- Print site maps and/or upload circular plot center point coordinates (or survey area boundary points) into a GPS unit or mapping app on your phone.
- Print data sheets. Consider using weatherproof paper if you'll be surveying during rain events.
- Clean and disinfect all sampling equipment with 10% bleach solution and rinsed well. For sensitive equipment like pesola scales you can wipe down the tool with a paper towel wetted in 10% bleach.
- Review the survey protocol, procedures, and survey sheets.

SURVEY PROCEDURES

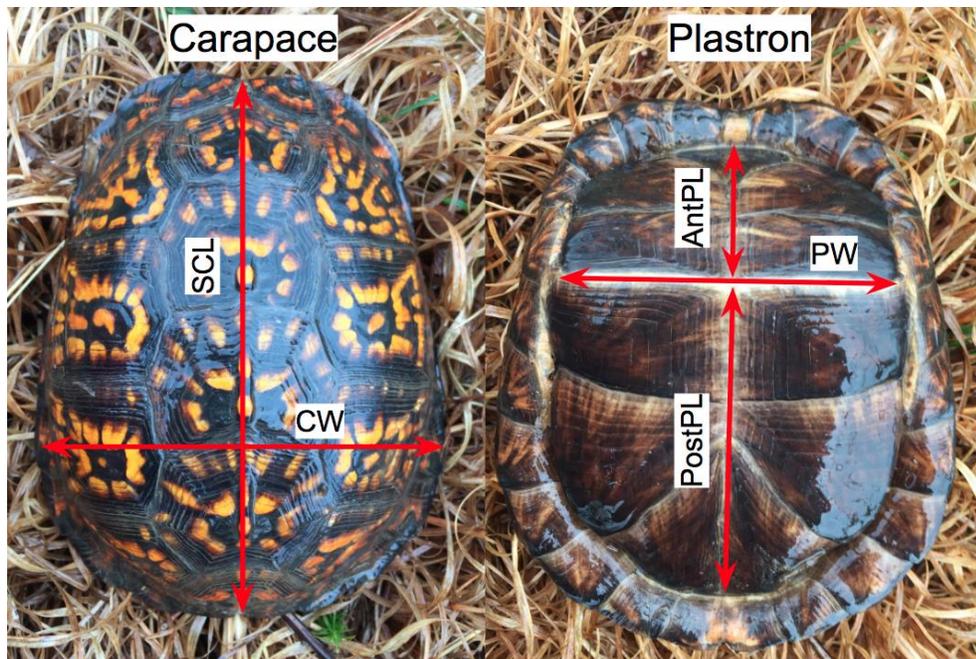
- Use maps, GPS or phone app to navigate to the site.
- If needed determine and mark the survey area boundary. If doing this immediately prior to the survey try not to walk through the survey area as little as possible.
- If you plan to use a GPS unit or a mapping app to track your survey path (recommended when possible), turn the GPS unit/app on, clear the previous track and start tracking.
- Fill out the *Box Turtle Visual Rapid Assessment* field form.

- Review the field form and fill in what you can prior to the survey (e.g., site name, site code, date, annual visit, observers, cloud cover, rain, etc.).
- Survey option descriptions:
 - Non-random sites = survey areas that were non randomly selected.
 - Random site = survey areas selected using a randomized point generator in ArcGIS or some other mapping app.
 - Full Random = survey areas where the effect of habitat management actions is being monitored and points were randomly placed within the management area or entire park/property.
- When you are ready to start surveying, set a timer or stopwatch.
 - Optional - If you find it helpful, each time you stop the survey for any reason (e.g. process turtles, answer a phone call), record the stop time on the *Site Visit Log*, and then record the time when you resume the survey. Keep track of total time spent looking for turtles on this form (see example below).
- Walk/survey the entire survey area as evenly as possible, but giving slightly more time to thickly vegetated areas and less time to open areas where visibility is good. Your total survey time should equal approximately 0.75 person hrs/ha (e.g. 45 min per circular plot assuming one surveyor). You will be walking at a fairly brisk pace. For larger survey areas (> 2 ha) it helps to visually divide the feature into sections and time your survey of each section. For example, if you have a square 4 ha feature you can divide it into quadrates and time your survey of each quadrate making sure you finish each within 45 minutes (assuming one surveyor).
- At the end of the survey, record the end time and complete the *Box Turtle Visual Rapid Assessment* form. Also record the end time on the *Site Visit Log* (if used).
- *Save your track and label it "SiteID_YYMMDD."*

TURTLE PROCESSING

- Complete a *Box Turtle Individual Form* for each turtle found (including recaptures).
- Record the following information
 - *site name*
 - *site code (optional)*
 - *Survey type* (options are feature or plot). For plot surveys please note which of the four plots you are surveying by checking the appropriate box.
 - *visit* - note whether this is your first, second, or third visit. An additional option is available if you visit the site more than 3 times.
 - *observer(s)* that found the turtle (full name)
 - *date* of the survey (mm/dd/yyyy)
 - *time* the turtle was found
 - *turtle ID#* (coordinate with you state lead for a notch code system)
 - *sex* (male, female, unknown)
 - *age* (A=adult, J=juvenile)
 - *waypoint ID* (where appropriate)

- *unmarked, marked 1st capture, within yr recap* – unmarked is for turtles that have not been marked to date. Marked 1st capture is for turtles that were marked in previous years but where this is the first time they were captured during the field season. Within yr recap is for any turtles that were captured previously during the same field season.
- *coordinates* of the location where the turtle was found. Please use decimal degrees for the lat long (dd.dddd)
- *SCLmin (mm)* – straight line carapace length measure down the middle of the carapace. See the diagram below.
- *CW (mm)* – measure of the widest point of the carapace
- *AntSPL (mm)* – measure down the middle of the anterior portion of the plastron
- *PostSPL (mm)* – measure down the middle of the posterior portion of the plastron
- *SPLmin (optional)* - if the turtle hinge is completely open and you are able to get a straight line measure of the full plastron length you may use this field opposed to the AntSPL and PostSPL.
- *PW (mm)* – measure the width at the humeral-pectoral seam
- *SH (mm)* – measure the maximum height of the carapace (typically at the hinge)



- *mass (g)*
- *photo file names (optional)* if it helps you organize your photos at the end of the field season. Always take a full frame photo of the carapace and plastron for each capture. Please also take photos of any dead turtle or carcasses that you find and provide any relevant information in the comments field.
- *PIT number (optional)* if you PIT tag the turtle
- *wear class* of the plastron scute



0% wear—distinct deep growth rings



<50% wear—growth rings less distinct but most visible



≥50% wear—many rings lightly visible



≥90% wear—growth rings not visible or only barely

- *visible annuli* - the number of annuli that are visible
- *gravid* or *not gravid* for females that you are able to palpate
- *general health* of the turtle including any signs of sickness (lethargy, nasal discharge, swollen eyes, etc.)
- *injuries* observed including missing limbs or toes, eye wounds, or stub tails.

- *Scute morphology* - note if the turtle has a normal number of marginal, vertebral and costal scutes. If not normal please note what irregularity is present.
- use the shell sketch to note the notches and any injuries
- *comments* to provide any additional information that may be important
- Check with your state lead to determine how to handle notching of turtles with an irregular number of marginal scutes (e.g. 11 or 13) on one or both sides of the carapace. Researchers use several ways to count marginal scutes including from the anterior to the posterior, head to the bridge and tail to the bridge, and posterior to anterior.

EQUIPMENT LIST

- Field Forms
- Survey Protocol and Instructions
- Camera
- Transect tape 28 m or longer (to set up circular plots)
- Flagging (optional; to mark survey area boundaries)
- Site maps (optional)
- GPS unit or mapping app (optional)
- Clip board
- Pencil or pen
- Thermometer
- Caliper(s) or ruler (e.g., 200 mm)
- Pesola scale(s) (optional; e.g., 300 g and 1000 g;)
- Small triangle file(s)