Spotted Turtle Assessment Protocol

Spotted Turtle Working Group[1]

Supported in part by State Wildlife Grants
through the USFWS Competitive State Wildlife Grants Program
and the Northeast Regional Conservation Needs (RCN) Program
www.northeastturtles.org

March 7, 2018

This document outlines a standardized and flexible methodology for sampling Spotted Turtle (*Clemmys guttata*) populations in the core of the species' range (Maine to Florida). This protocol is adapted in part from the Northeast Blanding's Turtle Sampling Protocol developed by the Northeast Blanding's Turtle Working Group (www.blandingsturtle.org) and funded by a US Fish and Wildlife Service Competitive State Wildlife Grant to the Virginia Department of Game and Inland Fisheries. The protocol is based upon an expert poll completed by representatives from Maine to Florida.

Two basic methodologies are outlined: trap-based assessments and visual assessments without traps. Two levels of trap-based assessments—**Rapid** and **Demographic**—are described. The protocol for Rapid Assessments is simply a reduced-effort version of the Demographic Assessment protocol. A visual Rapid Assessment is also described. To summarize the protocol: (1) delineate potential Spotted Turtle habitat using a geographic information system (e.g., Google Earth or ArcGIS) and recent aerial imagery; (2) place up to four 200-m radius plots centered on potential Spotted Turtle habitat with plot centroids up to 800 m apart; (3) conduct a Trap-based Rapid Assessment (TRA), Demographic Assessment (DA; trap-based), or Visual Rapid Assessment (VRA). For TRAs, place five traps ≥30 m apart within the reference plots. Traps may be set anytime during the Spotted Turtle activity season in your region. Check all traps every 24 hours for four consecutive days. For DAs, conduct the TRA protocol three times (for a total of 12 nights). For VRAs, two types of assessments are possible—**time constrained** and **unconstrained**. In both cases, a single observer visits a site three times during the survey season and during each visit, actively searches for turtles on foot. For time constrained surveys, the surveyor searches for 20 minutes per reference plot (up to 80 minutes total per visit), recording start and stop time and location of each survey. For unconstrained surveys, the surveyor walks a meandering transect anywhere within each reference plot, for as long as the survey takes, recording start and end time and GPS track.

[1] For a list of partners and additional information, see: www.northeastturtles.org or www.americanturtles.org. Protocol development sub-group: Liz Willey (American Turtle Observatory [ATO] and Antioch University New England), Mike Jones (Massachusetts Division of Fisheries and Wildlife), Patrick Roberts (University of Massachusetts and ATO), Kat Lauer (Antioch University New England), Tom Akre (Smithsonian Conservation Biology Institute), Lori Erb (Mid-Atlantic Center for Herpetology and Conservation), Derek Yorks (Maine Department of Inland Fisheries and Wildlife), Jonathan Mays (Florida Fish and Wildlife Conservation Commission), and JD Kleopfer (Virginia Dept. of Game and Inland Fisheries). For questions, contact: info@americanturtles.org.

The methodology outlined in this document is designed to be relatively simple, flexible, fit within existing research programs, and accommodate regional differences in seasonal activity period, habitat structure, and research priorities. Broad regional participation is encouraged to increase the size of the representative sample. Data collected through the regional effort are maintained in a centralized database at the American Turtle Observatory (www.americanturtles.org) for pooled analysis.

Planning Phase

Step 1: Select a wetland complex

Identify and delineate a wetland or wetland complex that is suitable for study. It may either be (A) an area known to be occupied by Spotted Turtles; (B) a data-deficient site with potentially suitable Spotted Turtle habitat; (C) randomly-selected areas of potential habitat and occurrence (to be added in Year 2; 2019). When selecting a wetland complex for surveys, remember that Spotted Turtles are associated with a *wide array* of wetland habitats that vary regionally including, but not limited to emergent marshes, deciduous shrub swamps, forested wetlands, seasonal pools, sphagnum bogs and seeps, linear ditches and canals, floodplain forests, and beaver impoundments. Whenever possible, use leaf-off or spring season aerial images when determining plot locations, as they allow greater visibility when mapping small seasonal pools in deciduous forest habitats (Fig. 1). In some cases, additional examination of leaf-on imagery may assist plot placement. Surveyors should confirm that property access is allowed by the landowner, and that the site has diverse wetland habitat suitable for Spotted Turtles, either through aerial photo interpretation or field reconnaissance. As an approximate guide, the focus area should be $\geq$800 m^2 and $\leq$2 km^2 (though if much larger, multiple groups of four reference plots could be delineated).

Step 2: Develop reference plots

Within the focus area, identify four reference points separated by 400 to 800 m using Google Earth or a similar GIS program (Fig. 1). Reference points should be centered on areas of highly suitable Spotted Turtle habitat (i.e., high potential use wetlands). Points may fall either on constellations of small wetlands (e.g., seasonal pools) or on portions of a single large wetland. Delimit 200-m radius (see distance justification, below) circular plots around reference points. All sampling should be conducted within these circular plots. Although four plots are ideal for spatial replication and to adequately sample larger landscapes, surveyors may place fewer than the recommended four reference plots if there is not enough suitable habitat available or if access is unavailable.

Step 3: Conduct an optional reconnaissance site visit

If you have not visited the site already, consider conducting a reconnaissance visit to make sure that property access is feasible and that the study plots should not be re-situated. Use this visit to identify potentially ideal trap locations and locations for visual surveys.

Figure 1. Illustration of study site delineation in Google Earth. The yellow central dots illustrate Reference Points centered on areas of suitable (or potentially suitable) Spotted Turtle habitat, surrounded by reference plots with 200-m radius.

Survey Phase

Option 1: Conduct a Trap Assessment (Rapid or Demographic Assessment)

Trap Assessment Types

Trap-based sampling may take the form of either rapid or demographic assessments. These assessment types differ in intensity (i.e., trap nights), but utilize the same trapping methodology and are therefore directly comparable.

Rapid.—Trap-based Rapid Assessments (TRA) are intended to serve as a method for quickly collecting baseline occurrence and abundance information. TRAs require four consecutive nights of trapping at a site during the Spotted Turtle active period.

Demographic.—Long-Term Trap Assessments (DA) are a more intensive method intended to facilitate the collection of population information that will allow for more precise estimates of population size, age structure, sex ratios, and additional population information via mark recapture. DA sites should be trapped for 3, 4-night trap runs (3 TRAs) for a total of at least 12 nights during the Spotted Turtle active season.

Trap Configuration

Within each of the four circular sampling plots, place five traps (recommended: ProMar TR-502 or TR-503 24or36"x12" collapsible turtle traps OR crab traps utilized in FL/GA, see equipment section, below) 0–200 m from the reference point at the plot centroid (20 traps total over the four reference plots) in high potential use areas, as determined by the researcher in accordance with

expert opinion. Ideally, all five traps within a single reference plot should be the same trap type, though different reference plots could have different trap types. The five traps per sampling plot can be placed in any number of wetlands (e.g., one large wetland or as many as five small wetlands). Ideally, traps should be placed at least 30 m intervals (the average daily movement distance of females in the spring observed by Litzgus and Mosseau [2004] in South Carolina, see movement justification, below) in different directions from the reference point (e.g., 30 m to NW; 60 m to NE, etc.); however, the configuration and wetlands and microhabitat will often preclude this strategy. In instances where the wetland configuration is a single linear feature (e.g., a ditch or canal), the traps may be placed in a line along the wetland, separated by at least 30 m, ideally. Emphasis should be placed on habitat suitability rather than strict adherence to these distance rules, but traps should be at least 15 m apart if 30 m is not possible.

Trap Placement

Microhabitat.—Traps should be located within high potential use areas as follows:

- In shallow (≤0.2 m, <trap diameter) flow channels that may direct movement of individuals
- At the edge of thick vegetation (e.g., sedges, grasses, shrubs) or structure (e.g., logs, debris)
- Proximal to basking sites
- At sites with good solar exposure
- Surrounded by cover that conceals traps

Placement.—Traps should be firmly staked into the ground (e.g., with 4' plastic-wire coated tomato stakes) or affixed to adjacent structures (e.g., using rope) at two locations to prevent animals, wind, etc. from moving them. The traps should be set so that turtles have adequate headspace to breathe. For ProMar traps, place 1–2 empty plastic bottles (16 oz, with caps on tight) within traps or pool noodles along the outside of traps to ensure breathing space. GPS coordinates should be recorded at each trap once they are placed, and traps should be flagged or marked in accordance with each researcher's preference, including the reference number and trap number. In locations where traps may be seen by the public (e.g., roadsides, boardwalks, etc.), traps can be inconspicuously labeled, instead, so as to not attract attention. On the day of trap deployment, complete the trap set-up field form including habitat suitability information. Surveyors must watch forecast weather conditions and pull or monitor traps if heavy precipitation or flooding is expected. During subsequent DA trap placements, traps should generally be placed in the same location as during the previous run, unless this is impossible due to changing water levels.

Trap Checks.—Traps should be checked every 24 hours. On each trap-check day, the trap-check field form should be completed, and the turtle individual field form should be completed for each Spotted Turtle captured in the trap (see protocol for processing individual turtles). Traps should be baited with ~½ can of sardines in oil (e.g., Beach Cliff) and rebaited every 24 hours. Air temperature should be recorded once in each reference plot and water temperature at each trap. Air temperature should be measured in the shade. Water temperature should be measured 10 cm below the surface, adjacent to a trap. For additional details, see field-form instructions.

Option 2: Conduct a Visual Rapid Assessment

Visual Rapid Assessments (VRA) serve as a second method of rapid assessment intended to facilitate population assessments in regions or terrain where trap-based assessments may not be

feasible as well as in habitats and portions of the species range where trapping appears to be less effective. VRAs and trap assessments can be applied at the same site, but *time-constrained* VRAs and trap assessments generally should not occur at the same time. However, a researcher who wishes to conduct unconstrained VRAs during trap checks (or while setting traps) could do so by recording visual survey effort between traps using tracks and processing turtles visually encountered using the unconstrained VRA protocol described below.

A single VRA is made up of three separate visits to one site within a four-week window of time in the active survey season. VRAs consist of active searching for turtles within wetlands on foot. There are two main approaches to distributing time throughout a reference plot and recording information during a VRA: Time constrained surveys and unconstrained surveys.

Time Constrained

If you are conducting a time-constrained survey, a total of 20 minutes should be spent surveying each reference plot (for a total of 80 minutes for 4 reference plots) on a given day. The information to record for each survey depends on the configuration of the wetland in the reference plot.

A) **Time Constrained 1**: For small (<0.1 ha) seasonal wetlands, observers should record the location of the wetland using GPS and the start time of the survey. The survey should continue until the entire wetland has been searched by the observer (or the water becomes too cloudy for the survey to be effective), and the end time of the survey should be recorded. The surveyor can then move on to another wetland in the reference plot until a total of 20 minutes has been spent in the reference plot on that day.

B) **Time Constrained 2:** For straight, linear wetland features (e.g., canals or ditches), the observer should record the start time and location (using GPS) of the survey, and proceed to survey the linear wetland until either 20 minutes has elapsed, the entire segment of the wetland in the reference plot has been surveyed, or the water becomes too cloudy for the survey to be effective. The surveyor should then record the time and GPS location at the end of the survey and then move on to another wetland in the reference plot, if there are any, until a total of 20 minutes has been spent in the reference plot on that day.

C) **Time Constrained 3:** For larger or amorphous wetlands that make up the majority or entirety of a reference plot, the observer records the time and GPS location of the start of the survey and surveys throughout the wetland, within the reference plot, until 20 minutes has elapsed, and the surveyor then records the time and location of the end of the survey.

For each of the time-constrained VRA approaches, each visit requires 20 minutes of active searching per reference plot for a total of 80 minutes of active searching throughout the site. If animals are processed during a survey, the clock should be stopped during processing. As noted above, the observer should keep track of the amount of time not spent actively searching for turtles (e.g., when handling turtles) per sampling plot, and GPS waypoints should be recorded at the beginning and end of each sampling plot survey. The observer should attempt to visit all wetlands within the sampling plot during the allotted 20-minute window.

Unconstrained VRA

Instead of spending 20 minutes/plot, a surveyor may choose an unconstrained visual survey approach. For this method, the surveyor records the starting time and location of a survey and

begins recording a GPS track. The surveyor then conducts a visual survey on foot anywhere within a reference plot for as long as it takes to adequately sample the plot, regardless of wetland configuration (i.e., the surveyor may move between wetlands). At the end of the reference plot survey, the surveyor records the end location and time of the survey and any processing time that occurred during the survey time, and stores the GPStrack for the survey, before moving on to the next reference plot. For unconstrained surveys, each reference plot should be surveyed 3 times.
Regardless of the approach selected (constrained or unconstrained), a VRA field form should be filled out for each site visit. Air and water temperature should be recorded once within each sampling plot.

Number of observers
For consistency and to avoid scaring turtles, we recommend that only one observer should perform each VRA site-visit, but subsequent visits should ideally be conducted by different observers to reduce observer-related bias. If two observers are in the field together, we suggest they conduct surveys in different reference plots. For example, on survey day 1, observer 1 could sample plots 1 and 2 and observer 2 could sample plots 3 and 4. On survey day 2, they could switch: observer 1 could sample plots 3 and 4 and observer 2 could sample plots 1 and 2. If it is necessary for more than one observer to conduct a survey within a single reference plot at the same time, please designate one person as the **lead observer** and note that on the field form. The lead observer should survey the wetland independently and unimpeded by the additional observer(s) who should trail behind and be sure not to influence the survey of the lead observer. The total number of turtles, as well as the number observed by the lead observer should be recorded on the VRA field form.

Protocol for Processing Individual Turtles

When a spotted turtle is captured (either during trapping or visual surveys), the turtle observation field form should be completed, and the following protocols are recommended.

Morphometrics. Record shell dimensions in mm. At a minimum, record SCLmin (straight carapace length) and SPLmin (straight plastron length). Optionally, also record: PW @ H-P seam (plastron width at humeral/pectoral seam), CW @ V3/4 (carapace width at the 2nd and 3rd vertebral line), and SH (shell height at the 2nd and 3rd vertebral line). Dial calipers 6"/500 mm are recommended.

Weight. Record animal mass in g (Pesola scale 250 g or 500 g).

Age and Plastral Wear: Assess the animal's age if new growth is visible along the medial seams and the plastral scutes are only lightly worn. Otherwise, report the minimum number of annuli visible and whether the plastral scutes are "not worn" (≤10% wear), "partly worn" (<50%), "mostly worn" (50%-90%) or "worn" (>90%).

Individual marking. Turtles should be individually notched as directed by state coordinators. Secondary recognition is recommended using photographs, injuries, deformities, PIT tags, etc.

Photographs. Photograph carapace and plastron with animal ID visible in photo (or sorted/ tagged post-capture). If possible, photograph lateral head shot and limbs/tail, as well as obvious injuries or deformities.

Injuries and general health. Note missing or injured limbs, tail, eyes, etc., as well as the presence of skin or upper respiratory tract infection or lethargic condition.

Scute morphology and other deformities. Note any major scute or other deformities, including less than or more than 12 marginals on either or both sides.

Tissue collection for genetic analysis. With approval from state coordinators, trained researchers may consider collecting blood or tissue samples for genetic sampling. See tissue collection protocol.

Required Equipment

The following equipment is required to complete the protocol: field forms, writing implements, GPS for recording trap trap locations and visual survey points/track, flagging for marking traps, calipers (~6 in), Pesola scale ≥500 g, extra slim taper triangular file (for marking turtles), camera or cell phone for photographing turtles, air and water thermometers, and 20 traps/site operated at a time with associated stakes, ties, and bait. Additional optional equipment may also be necessary including waders, polarized sunglasses, binoculars, disinfecting equipment, and/or blood sampling equipment. Because researchers currently have a range of available equipment, specifications are flexible. Any traps >0.2 m in diameter with < 3 cm mesh are acceptable, though we recommend that all five traps within a single reference plot be the same type of trap. These variations will be incorporated as a covariate in the modeling process. To help standardize future equipment purchases, we recommend medium or large sized ProMar, collapsible minnow traps (Model TR502 or TR503, 12" diameter by 24" or 36" length with 5" dual openings. https://promarnets.com/product/deep-water-crawfish-crab-nets/ [Note: we do NOT recommend the smaller, square, red ProMar model]). This model trap has been used successfully by researchers throughout the species range for over a decade. Alternatively, we recommend hard-sided crab traps like those used in Georgia and Florida (Chandler et al. 2017). Hard-sided traps are particularly useful in areas where raccoons or other predators are an issue.

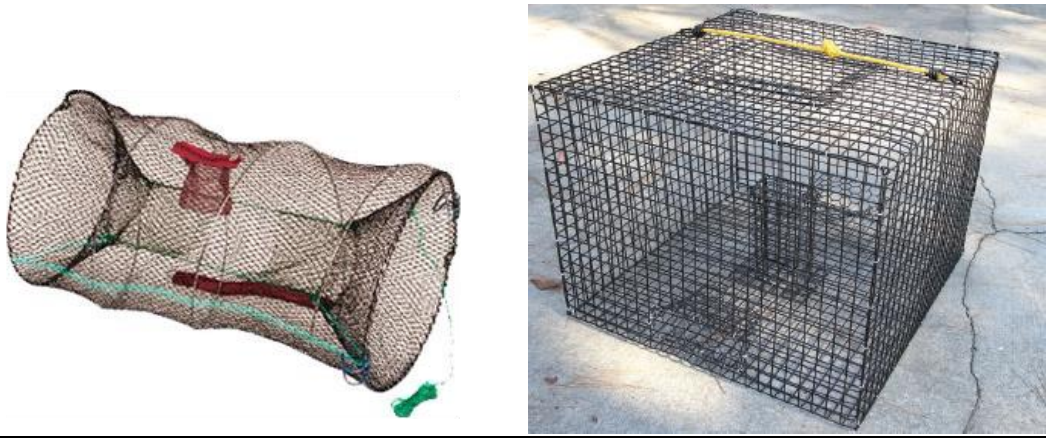

Figure 2. Promar TR502 (left) and modified crab trap from Chandler et al. (2017)

Trap identification: Assign unique ID to each trap and label trap in the field and on the corresponding field form.

Trap location/operation: Record trap ID, lat/long (decimal degrees), and functional period (mm/dd-mm/dd), and complete appropriate field form upon trap placement.

Bait: Sardines in oil (Beach Cliff or other brand).

Re-bait frequency: 24 hr (puncture can, do not open entirely, or use part of a can in a container that allows the oil to escape, but not the fish).

Trap check frequency: 24 hr with more frequent checks as required by agencies/partners or flood conditions.

General protocols to reduce likelihood of disease transfer.

Several states and research teams within the region already have a standard decontamination procedure in place to prevent the spread of disease, and teams should follow their local practices and procedures. For those teams without a decontamination protocol, we suggest several precautionary measures to prevent the spread of disease. A 3% bleach solution may be used to disinfect traps and clothing between sites. After bathing or spraying tools and clothing in the bleach solution, items should be rinsed with clean water. Captured turtles from different sites and those displaying signs of illness should be held separately during processing, and equipment should be sterilized between turtles. Calipers should be swabbed with alcohol, files can be burned, and notches should be dabbed with Betadyne. Latex gloves for handling turtles are an additional precautionary suggestion. The Northeast Partners for Amphibian and Reptile Conservation (NEPARC) Disinfection Protocol contains additional recommendations (http://northeastparc.org/disinfection-protocol).

Data Entry

For any of the protocols, enter your data onto the standardized field forms available at http://northeastturtles.org while in the field. Upon returning to the office, electronically enter data as soon as possible into the formatted Excel Worksheet also available on the website.

Data Analysis

Data will be analyzed at the regional level by CSWG partners, including American Turtle Observatory, Smithsonian Conservation Biology Institute, and Mid-Atlantic Center for Herpetology and Conservation. Rapid Assessments will be analyzed in a mixture modeling framework (Royle 2004) using the unmarked (Fiske and Chandler 2011) package in R (R Core Team 2018). Demographic Assessment sites will be analyzed in a capture mark recapture framework using the Rcapture (Baillargeon and Rivest 2011) package or spatially explicit capture recapture techniques (Royle et al. 2011) using the secr package (Efford 2017) in R.

Plot Size and Trap Night Justification

To determine appropriate plot sizes and trap distances for sampling design, we reviewed the literature to evaluate known movement distances for Spotted Turtle. Ideally, each reference plot would be independent at the scale of an entire sampling event (an active season) and therefore be larger than, but the same order of magnitude as, a spotted turtle home range, and large enough to

encompass many spotted turtle home ranges. A 200-m radius plot is equivalent to a 12.6 ha plot, slightly larger than three times the size of the average minimum convex polygon (MCP) measured via radio-telemetry by Milam and Melvin (2001), between the average size of male and female MCPs observed by Litzgus and Mosseau in South Carolina (2004), and large enough to encompass the home ranges of multiple individuals. Thirteen turtles tracked for a year in Florida by J. Mays (unpublished data) fell within a 13ha area. It should be noted that some individuals move much farther, however; Milam and Melvin (2001) tracked an individual 1125m in a year, J. Mays has tracked males in Florida that moved over 1200 m straight line over the course of a year, but both are within the order of magnitude of the reference plots we suggest.

The four combined reference plots would be equivalent to about 50 ha. In the expert poll, respondents stated that known Spotted Turtle populations from Maine to Florida range in size from 0.7 ha to over 100 ha. The proposed four-reference plot arrangement allows for a broad configuration of sites to be sampled, and encompasses all of the size classes provided by experts.

Traps themselves should be far enough away to be independent at the scale of a single trap night, so that animals are not observed in different traps on the same day, but close enough that animals might be recaptured in adjacent traps on different nights. The recommended 30 m separation distance represents the average daily movement distance observed by Litzgus and Mosseau (2004) by females during the spring season in South Carolina (Table 1). In addition, 30 m is consistent with the trap separation distance most often used by experts from Maine to Florida.

Table 1. Movement and home range distances of spotted turtles from previous studies.

Author	Location	Sample Size, method	Mean home range area (ha)	Home range length (m)	Mean Daily Movement (m/day)
Beaudry et al. 2007, Beaudry et al. 2008	Maine	40 radio-telemetry	9.3 ha (95% FKE, Range 0.3 - 64.0) or 7.9 ha (MCP, Range 0.4 - 40.0)		102 (SD = 0, range: 18– 251) using thread trailing
Milam and Melvin, 2001.	Massachusetts	26 (10M, 16F), radio-telemetry	3.5 ha (Range: 0.2-53.1)	313 (Range: 115-1125)	
Buchanan et al., 2017.	Rhode Island	12 radio-telemetry	1.95 ha (MCP) Range=0.59-4.07ha		
Litzgus and Mosseau, 2004	South Carolina	31 (9M 22F), radio-telemetry	Male: MCP = 5.15±1.13, Kernel 95% = 4.67±0.61; Gravid Female: MCP = 19.06±6.75, Kernel 95% = 10.35±2.29		Male (n=7-9): Spring =21.77±0.39, nesting = 10.7±0.22, late summer = 10.41±0.28, fall = 10.34±0.3, winter = 7.13±0.28; Gravid Female (n=16-20): Spring = 26.96±0.36, nesting = 19.89±0.17, late summer = 33.44±0.45, fall = 8.04±0.11, winter = 2.33±0.07
Mays, unpublished data	Florida	29 (11M, 18F)	MCP=2.3 (range=0.1-20.6); 95% kernel=4.5 (range= 0.4-40.3)		

Similarly, to estimate the required number of trap nights, we reviewed recent literature and compiled information from experts across the region. Across studies in Rhode Island, Massachusetts, Maryland and Florida, traps yielded an average of 0.3 Spotted Turtle captures/trap night (Table 2). The trap rapid assessment, consisting of 20 traps for 4 nights would yield an average 26 Spotted Turtles, while the 12-night long DA (consisting of 3, 4 night TRAs) would yield a total of 240 trap nights, and an estimated average of 77 Spotted Turtles, assuming consisting trap results over time. Survey returns of this magnitude would allow observers to easily differentiate between sites with high density and low density populations during TRAs and could allow for population estimates during DAs at sites with high recapture rates (for example, see Massachusetts populations from 100 traps nights in Table 2). If after the 2018 season it is determined that additional trap nights, or higher densities of traps, are necessary for better demographic estimates, the protocol will be adapted to meet those needs.

Table 2. Capture rates and population estimates with known trapping effort from previous studies

Authors	**Location**	**Total TN**	**Individuals**	**Captures**	**Turtles/TN**	**Population Estimate**	**Estimate Standard Error**
Buchanan, pers. comm.	Rhode Island	40	21	24	0.6		
Willey, Jones, Milam, unpublished data, 2014	Massachusetts Total	216	23	58	0.27		
Willey, Jones, Milam, unpublished data, 2014	MA Site 1- Hampshire Co.	109	13			11.3	SE=0.6
Willey, Jones, Milam, unpublished data, 2014	MA Site 2- Franklin Co.	107	10			21.2	SE=7.8
Mays, in Chandler et al. 2017	Florida	698		32	0.05		
Chandler et al. 2017	Georgia	866		146	0.17		
Howell, unpublished data	Maryland				0.79		
Liebgold, unpublished data	Maryland				0.02		
Approximate average (assuming equal trap effort)					0.32		

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Spotted Turtle Monitoring Protocol Overview

PLANNING PHASE

□ Select and delineate a wetland complex

- Select sites with a known population OR potentially suitable habitat
- Use leaf-off aerial imagery
- Confirm permission to access property

□ Place up to four 200-m radius reference plots centered on suitable Spotted Turtle habitat

- Reference plot centroids should be 400–800 m apart

□ Conduct a reconnaissance visit

SURVEY PHASE

□ Option 1: Trap-based assessments (rapid or long-term)

· Set five traps (recommended: ProMar TR-502 24"x12" collapsible turtle traps or FL/GA crab traps) per sampling plot (20 total per site)
· Complete the trap set-up field form
· Place traps:
- Ideally 30 m apart (no less than 15 m)
- In high potential use wetlands and microhabitat
- Such that there is adequate headspace for turtles to breathe
· Affix traps at two locations (at least) to ensure they cannot be moved by animals
· Bait traps with ½ can of sardines in oil and rebait every 24 hours
· Check traps every 24 hours
- Complete a trap check field form whenever traps are checked
- Complete an individual turtle form for each Spotted Turtle captured

□ Trap-based Rapid Assessment (TRA)
· A single trap-run (using the above methodology) consisting of four nights

□ Demographic Assessment (DA)
· Three, 4 night trap-runs, for a total of 12 trap-nights (using the above methodology)

□ Option 2: Visual Rapid Assessment (VRA)
· On foot, actively search each reference plot for 20 minutes (80 minutes per visit to a site)
A) Small seasonal pools should be searched in their entirety before moving to the next wetland in the reference plot
B) The beginning and ending points of surveys for long, linear wetland features (ditches or canals) should be recorded
C) In larger wetlands, a meandering transect survey should be conducted and GPS track should be logged.
· A VRA is complete when three surveys are conducted at a site within a four-week window
· Complete a VRA field form for each visit to a site
· Each visit should be conducted by a single observer
· Attempt to rotate observers for consecutive visits to a site to reduce bias
· Record GPS tracks as well as start and end coordinates