RECOMMENDED SURVEY PROTOCOL FOR THE ALLIGATOR SNAPPING TURTLE (*MACROCHELYS TEMMINCKII*) AND SUWANNEE ALLIGATOR SNAPPING TURTLE (*MACROCHELYS SUWANNIENSIS*)

Alligator snapping turtle (*Macrochelys temminckii*)
Photo credit: Luke Pearson

Suwannee alligator snapping turtle (*Macrochelys suwanniensis*)
Photo credit: Greg Brashear

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Introduction

The alligator snapping turtle (*Macrochelys temminckii*; henceforth, AST) and the Suwannee alligator snapping turtle (*M. suwanniensis*; henceforth, SuwAST) were petitioned for listing under the Endangered Species Act (ESA) in 2012. The U.S. Fish and Wildlife Service (Service), after a review of the best available scientific information, found that listing these species was warranted. Accordingly, on November 9, 2021, the Service published a proposed rule to list the AST as a threatened species with a special rule issued under section 4(d) of the Act (86 FR 62434). On June 26, 2024, the Service published a final rule listing the SuwAST as threatened (89 FR 53507). The Service expects to publish the final rule to list the AST as threatened with a special 4(d) rule in late 2024 or early 2025.

This survey protocol document is adapted from the Department of Defense Partners in Amphibian and Reptile Conservation (DoD PARC) Recommended Best Management Practices for the AST on Department of Defense Installations (DoD PARC 2021) and modified to meet Service priorities and needs and provide environmental and implementation flexibility. These protocols are intended to serve as guidelines for the Service and other conservation agencies to assist in planning, prioritizing, and conducting monitoring and conservation actions that provide a conservation benefit to the AST and SuwAST. Implementation of these protocols should 1) standardize survey efforts and recorded data across the species' ranges, 2) be used as training materials to support safe and efficient trapping and handling of a threatened turtle species, and 3) align with existing efforts among Service programs and other stakeholders to conserve and recover these species. For more information on the ecology, life-history, threats, and population status of the AST and SuwAST, please refer to the respective Species Status Assessments: Alligator Snapping Turtle SSA and Suwannee Alligator Snapping Turtle SSA.

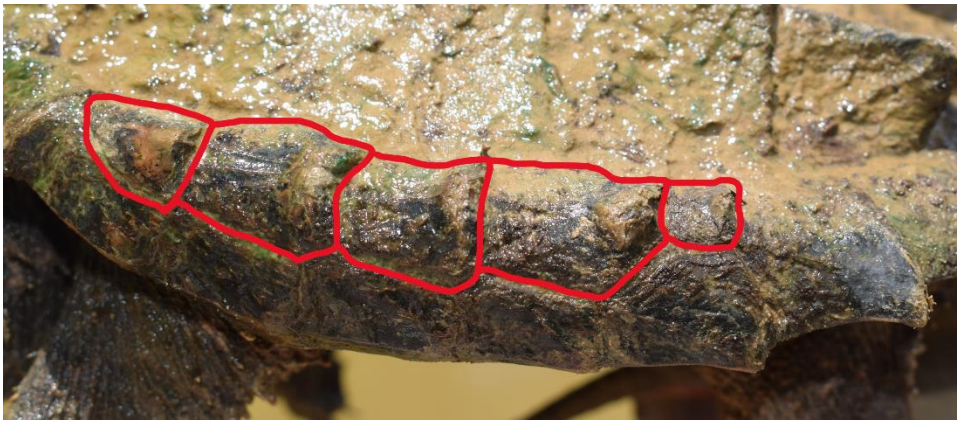
SPECIES PROFILE

Description

The AST is the largest freshwater turtle in North America, with the largest currently recorded wild individual measuring 29.5 inches (in) (75 centimeters (cm)) midline carapace (upper shell) length and 211.2 pounds (95.8 kilograms (kg); Rosenbaum et al. 2023). The largest wild SuwAST measured 28.1 in (71.3 cm) midline carapace length and was estimated to weigh approximately 168 pounds (76.4 kg; Johnston et al. 2023). The carapace has three strongly keeled ridges and is distinguished from the eastern snapping turtle (*Chelydra serpentina*) by the presence of 2 to 5 supramarginal scutes (Figure 1). AST shell coloration is grayish-brown to brown as are the head, legs, and tail. The AST Apalachicola lineage, which inhabits the Apalachicola River drainage, as well as the SuwAST can be golden in color. The plastron (lower shell) is reduced in size and cruciform in shape in both species.

The head and jaws are large with the upper jaw strongly hooked. The AST generally has a more "slender" head than the AST Apalachicola lineage and SuwAST, with skull width narrower than skull length. The AST Apalachicola lineage and the SuwAST generally have skull widths equal to or greater than the skull length (Murray et al. 2014). A worm-like lingual appendage that may be pinkish, light gray to white, or dark purple in color is found in the lower jaw and visible when the mouth is opened (Glorioso et al. 2023). Eyes are located on the side of the head in AST and SuwAST, while eye placement in the eastern snapping turtle is towards the top of the head. The head has a series of fleshy tubercles, and the eyes are ringed with small fleshy tubercles. The tail is quite long in relation to body length. Adult males attain a larger size than females, with female maximum mass reaching about 83 pounds (37.7 kg; L. LeBlanc, pers. comm. 2024). Females attain maturity between 13–21 years of age and 32.7–37 cm straight carapace length (SCL) and males between 11–21 years of age and 37.8–41.0 cm SCL (Tucker and Sloan 1997).

Figure 1. An adult AST (top right) compared to an adult eastern snapping turtle (top left). Diagnostic characteristics for the AST include three prominent ridges along the carapace, the presence of supramarginal scutes between the pleural and marginal scutes (highlighted below), strongly hooked mandible and triangular head viewed from top, and lateral placement of eyes. Diagnostic characteristics for the eastern snapping turtle include a relatively smooth carapace, lack of supramarginal scutes, placement of eyes towards top of head, and a much longer neck and faster strike.



Distribution

The AST is found in southeastern river systems that flow into the Gulf of Mexico from the Chattahoochee-Flint-Apalachicola River system of Georgia and Florida west to the San Antonio River in Texas (Figure 2). The species' historical range includes Alabama, Arkansas, Florida, Georgia, Illinois, Indiana, Kansas, Kentucky, Louisiana, Mississippi, Missouri, Oklahoma, Tennessee, and Texas. However, the species is considered extirpated in Indiana, and reintroductions to augment extirpated populations have occurred in Illinois and Kansas.

The SuwAST is endemic to the Suwannee River system of Florida and Georgia. A disjunct population has been documented in the Homosassa River, approximately 45 miles south of the Suwannee River. This population likely originated from animals that escaped captivity from a wildlife attraction decades prior to this area becoming a Florida state park (K. Enge pers. comm. 2024).

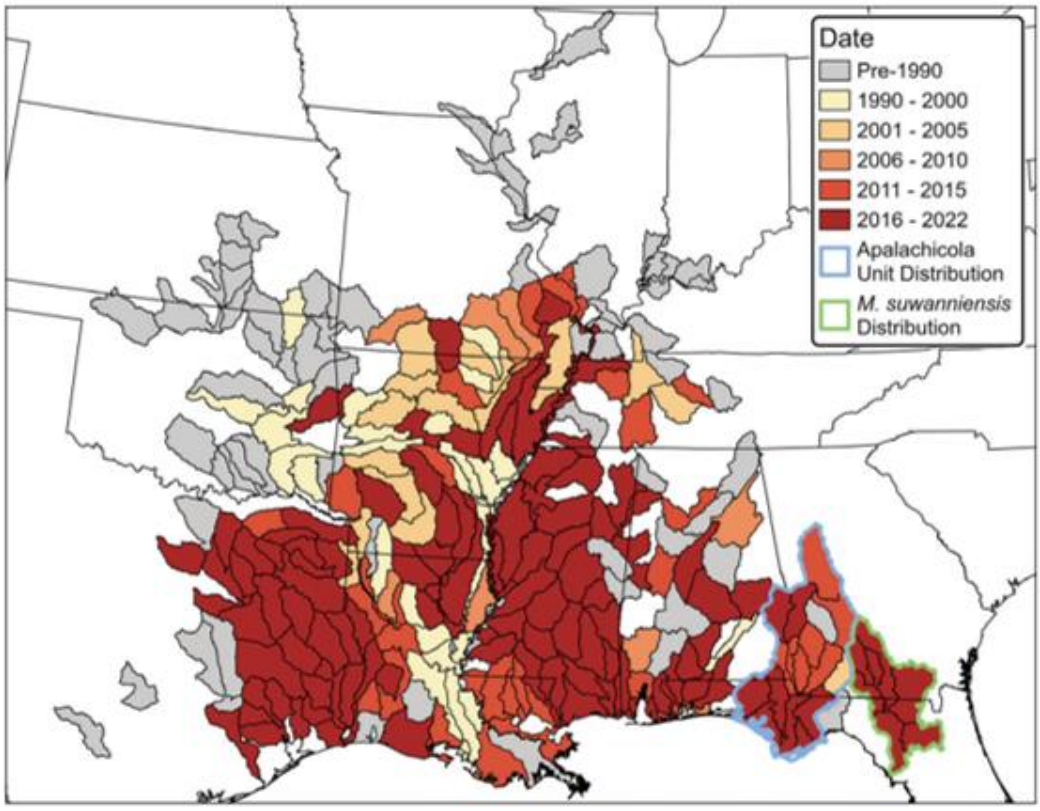


Figure 2. Distribution map of AST and SuwAST by watershed (HUC-8) and color-coded by year-range observed. Blue outlined area indicates Apalachicola lineage, and green outlined area represents SuwAST distribution. Source: Carr et al. (2023a)

Habitat

AST and SuwAST inhabit a range of freshwater lotic and lentic habitats from small streams to large rivers, oxbows, springs, swamps, bayous, lakes, and canals with water clarity that ranges from clear to murky and turbid (Ernst and Lovich 2009). During high water events, turtles will move out of deeper waters and river channels and into adjacent inundated flood plains (Delisle et al. 2023; Cozad et al. 2023; Thomas et al. 2023). Tidally influenced, brackish water habitats are also utilized (Godwin et al. 2023). Shaded stream banks with intact riparian tree cover, an abundance of submerged logs, trees, and other in-stream structure appear to be favored. In bayous, oxbow lakes, and swamps, vegetated microhabitats with plants such as cypress, tupelo, buttonbush, and floating aquatic vegetation are occupied (Harrel et al. 1996; Riedle et al. 2006; Shipman and Riedle 2008; Howey and Dinkelacker 2009). Occupied habitat substrate includes soft mud, clay, sand, gravel, and rocks. Juvenile and adult turtles prefer an abundance of coarse woody debris, such as submerged root masses, log jams, and entangled branches to use as shelter and sites to ambush prey.

Behavior

The AST and SuwAST are highly aquatic species that are rarely observed moving overland. Basking has been documented for juvenile individuals (Ewert 1976; Carr et al. 2011). Nesting by females is the main terrestrial activity of the species (Carr et al. 2023b). The annual activity cycle of AST and SuwAST has not been studied (Ernst and Lovich 2009). Individuals have been captured in baited nets in several southern states between March and November. Bogosian (2010) suggests turtles in Louisiana may be inactive from October to February, although Boundy and Kennedy (2006) trapped significant numbers in October and November. Radio-telemetry studies have observed reduced movements during the winter; however, individuals are still documented making short-distance movements during these periods of inactivity (Delisle et al. 2023).

Nesting in Georgia, Florida, Mississippi, and Louisiana occurs from April to May and may extend to June in other parts of the range (Ernst and Lovich 2009; K. Enge pers. comm. 2024; L. Pearson pers. comm. 2024). Females lay between 9 and 61 eggs in a nest generally within 20 meters (m) of the water and at least 1 m above the waterline in sand or sandy soil mixed with silt and organic material, although nests have also been documented in clay soils.

Nesting females tend to avoid open sandbars and low forested ground with matted roots (Ewert 1976; Miller et al. 2014; Ewert and Jackson 2023).

Both species consume a wide variety of food sources with fish as a primary prey item. Other items ingested such as crustaceans, mollusks, snakes, turtles, birds, mammals, and vegetation indicate that feeding is also opportunistic and scavenging behavior occurs (Elsey 2006; Ernst and Lovich 2009). This genus is unique in the feeding behavior using the lingual appendage that resembles a worm and functions as a lure to attract prey (Glorioso et al. 2023).

Threats and Conservation Status

The AST was petitioned by the Center for Biological Diversity in 2012 to be assessed for federal listing. The Service determined that the petition presented substantial scientific information indicating that a status assessment of the AST is warranted (Service 2015). Accordingly, the Service proposed to list the AST as a threatened species with a rule issued under section 4(d) of the Act (Service 2021a), with a final decision due by late 2024 or early 2025. In 2014, the SuwAST was elevated to a new species based on differing morphology and a unique genetic lineage (Thomas et al. 2014). Thus, the Service determined that a species' status assessment was warranted and proposed to list the SuwAST as threatened on April 7, 2021 (Service 2021b). The SuwAST was finalized as a threatened species pursuant to the Endangered Species Act on June 27, 2024 (Service 2024a). The International Union for the Conservation of Nature (IUCN) lists the AST as Vulnerable, while the SuwAST has not been assessed.

Threats to both the AST and SuwAST include bycatch mortality on fishing gear (e.g., trotlines, hoop nets), hook ingestion, habitat and hydrologic alterations (e.g., impoundments, channelization, desnagging), pollution of streams and wetlands, collection for foreign and domestic sale, and nest depredation by mammals and birds (Service 2021c, 2024b). AST and SuwAST have experienced drastic population declines due to these threats. The AST's range historically included 14 states, one of which is considered extirpated (Illinois), and recent reintroductions have occurred in Kansas, although determining if these reintroduced individuals create a viable and breeding population will take time. The AST is considered a "species of greatest conservation need" (SGCN designation) in 12 states, but recreational harvest is allowed in Louisiana and Mississippi until time of federal listing. The range of the SuwAST includes the Suwannee River drainage of Florida and Georgia, and the species is classified as threatened in both states.

INVENTORY AND MONITORING TECHNIQUES FOR ALLIGATOR SNAPPING TURTLE

Alligator Snapping Turtle Assessment Protocol

The 2021 DoD PARC AST survey protocol for military installations is the only formally established protocol. Otherwise, most surveys in the AST and SuwAST range have followed similar survey methodologies. AST and SuwAST presence at a site often go unnoticed because, while a large and impressive turtle, it is cryptic in its behavior. Individuals seldom bask and females are seldom observed while nesting; therefore, traditional visual encounter surveys (VES) are ineffective. VES surveys conducted using time-lapse game cameras may be a non-invasive method of estimating population size when trapping surveys are not feasible (P. Delisle, pers. comm. 2024).

Trap-based surveys are the preferred methodology for monitoring of *Macrochelys*. Trap-based assessment involves the use of baited traps to capture AST and SuwAST in their natural habitat. This method may be conducted as either a 1) rapid assessment (40 to 80 trap nights; e.g., 15 traps set for 1 night = 15 trap nights) or 2) long-term trap assessments (re-trapping the same site at standard intervals over a multi-year timespan). Rapid assessments are intended to serve as a method for quickly collecting baseline occurrence and coarse abundance information, whereas long-term trap assessments are intended to facilitate the collection of population demographic information to determine more precise estimates of population size via mark-recapture analyses, age structure, sex ratios, survivorship, and growth rates.

Mark-recapture

Mark-recapture remains the most widely used method to census turtle populations. This technique uses permanent marks on shells such as notching or drilling the marginal scutes to provide long-term visual indicators to distinguish unique individuals. Use of Passive Integrated Transponder (PIT) tags should also be used to identify individuals in conjunction with physical scute notching. Researchers can perform mark-recapture surveys monthly, seasonally, annually, or as time and funding allow, depending on the objective of the surveys. Mark-recapture studies are typically long-term monitoring projects to assess population size, viability, survivorship, growth rates, and reproductive ecology of one or multiple populations. These long-term studies are vital in understanding the ecology and population trends of long-lived species that react slowly to environmental and anthropogenic alterations. There are few long-term studies

published on AST and SuwAST (Trauth et al. 2016; Folt et al. 2016; Godwin et al. 2023; King et al. 2016), with additional ongoing long-term studies being conducted for AST in Texas (Munscher et al. 2023) and Mississippi (L. Pearson pers. comm. 2024), and for SuwAST in Georgia (C. Coppola pers. comm. 2024) and Florida (K. Enge pers. comm. 2024).

Telemetry

Radio-telemetry is a method in which a radio transmitter is attached to an animal with the unique radio frequency detected by a receiver. Use of radio-telemetry allows researchers to follow the movements and behavior of study animals. Juvenile and adult AST and SuwAST are large enough that a radio transmitter with multi-year battery life can easily be attached to the shell. With regular tracking (at least weekly) of individual AST, data may be collected on movements, habitats used, and seasonal activity patterns. Several studies have used radio-telemetry to monitor AST and SuwAST including Cozad et al. (2023), Delisle et al. (2023), Munscher et al. (2021), Spangler et al. (2021), Carr et al. (2010), Bass (2007), Hyder et al. (2021), Kessler (2020), Sloan and Taylor (1987), Moore et al. (2014), Riedle et al. (2006), Harrel et al. (1996), Shipman and Riedle (2008), Thomas et al. (2023).

Acoustic and satellite telemetry are other methods to document turtle movement patterns and home ranges and identify potential nesting locations. Acoustic transmitters ping stationary acoustic receivers anchored to river bottoms (passive data collection) as turtles pass nearby (Micheli-Campbell et al. 2017). Turtles can also be actively tracked using towable and directional hydrophone receivers (Enge et al. 2023). However, acoustic receivers do not function out of water, so identifying nesting locations can be challenging. Acoustic receivers have been used for SuwAST in Florida with success (Enge et al. 2023). Satellite/GPS telemetry passively collect data via the use of GPS coordinates but only work when the satellite/GPS unit is above water, making it ideal for identifying nesting locations but mostly inoperable for in-stream movements. Currently, satellite/GPS transmitters are being preliminarily tested on AST in Texas to identify nesting locations (E. Munscher, pers. comm. 2024).

Environmental DNA (eDNA)

Environmental DNA is organismal DNA found in the environment that originates from cellular material shed by organisms (via skin, excrement, etc.) into aquatic or terrestrial environments. eDNA can be sampled and monitored using new molecular methods. Testing is easily repeatable, relatively inexpensive, and may aid in targeting or prioritizing locations for

surveying efforts, particularly in locations where AST or SuwAST are presumed extirpated or have extremely low densities. Several AST-specific eDNA primers have been developed and field tested successfully (Feist et al. 2018a, 2018b; Kessler et al. 2021; P. Dehaan pers. comm. 2024; Sternhagen et al. 2024).

ALLIGATOR SNAPPING TURTLE ASSESSMENT PROTOCOL

This document provides guidelines for a standardized and flexible methodology for sampling alligator snapping turtle (*Macrochelys* sp.) populations.

The methodology outlines a trap-based assessment with descriptions of two assessment levels: rapid and demographic. The rapid assessment protocol represents a lower intensity effort compared to the demographic assessment protocol, but both yield comparable information. To summarize the methodology: (1) delineate potential AST habitat using a geographic information system (GIS) (e.g., Google Earth or ArcGIS) and recent aerial imagery; (2) conduct a trap-based rapid assessment (RA) or demographic assessment (DA). For RAs, set 10 to 25 traps > 100 m apart along the stream stretch using one or both banks of the stream channel. Rebait all traps every 24 hours and check traps for 40 to 80 trap nights or for at least five consecutive days. The same type of trap and bait should be used throughout the time period of the demographic assessment to ensure data compatibility. For DAs, repeat trapping surveys monthly, seasonally, or annually.

The methodology outlined in this document is designed to be relatively simple and flexible, fit within existing research programs, and accommodate regional differences in seasonal activity, habitat structure, and research priorities. Broad regional participation is encouraged to increase the size of the representative sample.

Planning Phase

Step 1: Select a stream section

Identify a stream section that is suitable for study. It may either be (A) a stream known to be occupied by AST; (B) a data-deficient site with potentially suitable AST habitat; (C) randomly-selected stream of potential habitat and occurrence. When selecting a stream for surveys, remember that AST are associated with streams and tributaries of a wide range of sizes, cypress swamps seasonally connected to streams, coastal marshes, and beaver impoundments. The focus area should generally be 3 to 12 stream miles (smi) (5 to 20 stream kilometers (skm)) in length.

Step 2: Develop reference stream sites

Within the focus area, identify reference trap sites separated by at least 100 m using Google Earth or a similar GIS program. Reference trap sites should be within stream sections of highly suitable AST habitat. Reference trap sites may be along one or both banks of a stream.

Step 3: Conduct an optional reconnaissance site visit

If you have not recently visited the stream, consider conducting a reconnaissance visit to make sure stream access is feasible and reference trap sites represent highly suitable AST habitat. You should also identify potential trap locations at this site visit. Traps may be set either from a boat, canoe, or foot access along the stream bank. If a boat is to be used, then an important goal with a reconnaissance site visit is to assess boat ramp condition and proximity to selected stream sections. If traps are to be carried from a land vehicle to the stream bank for setting, then accessibility from nearest road and ease of trap transport needs to be assessed.

Survey Phase

Option 1: Conduct a 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., number of trap nights) but utilize the same trapping methodology and are therefore directly comparable.

Rapid.-Rapid Assessments (RA) are intended to serve as a method for quickly collecting baseline occurrence and abundance information. During the AST active period (April to September), RAs require 40 to 80 trap nights at a site to document presence/absence or relative abundance. The number of trap nights varies depending on location within the species range and the density of AST in the area, with presumed low-density locations requiring up to 80 trap nights (i.e., northern states, range periphery, headwaters) and presumed higher-density locations requiring fewer trap nights (i.e., southern states, known records nearby, protected lands) to acquire a high confidence of AST presence or absence.

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 via mark-recapture, age structure, sex ratios, and additional population demographics (e.g., survivorship, growth rates). Trapping efforts at DA sites can vary based on trapping intensity, number of site visits, and frequency of recaptures. If surveys are intensive (>

100 trap nights) and produce reliable recaptures, then less surveys are necessary per year. However, if surveys are low intensity (< 50 trap nights) or are not reliably producing recaptures, then more surveys are necessary per year. These DA sites should be visited for at least two years, although longer durations (> five years) are preferred.

Trap Configuration

Large hoop nets are the preferred trap type for AST and SuwAST with the basic configuration being a hoop net of four fiberglass hoops, 1.2 m in diameter covered with #36 nylon twine netting with a square mesh size of 4.45 cm, with body length of the trap 2.4 m, and total length 3 m (Figure 3). The Arkansas-style (flat throat) is the preferred throat type for AST and SuwAST trapping, as this design prevents entanglement of captured animals in the throat twine and allows the user to untie the back of the trap to release AST and alligators. An alternative throat design is a fingered throat, with funnel length of 0.75 m with an inner throat diameter of 35 cm. The downside of a fingered throat is the propensity to drown alligators in the twine fingers of the throat and the inability to untie the back of the trap to release AST and alligators. Fiberglass hoops are preferred because fiberglass will not rust when exposed to saline waters and are less likely to be damaged by alligators. A minimum of 10 traps, and up to 25 traps, should be set during each sampling period and spaced a minimum of 100 m apart.

Traps can be modified in several ways, including increasing the number of hoops to seven, adding a second throat funnel, and increasing the total length of the trap up to 4.3 m total length. The advantage to using the longer double-throated trap is the ability to set the opening in deeper water while having the tail exposed to air and likely better retention of trapped turtles. The disadvantage to having longer traps is finding appropriate locations to set traps in smaller, shallower river systems where space can be limited. If space is heavily limited, or the researcher is exclusively sampling in shallow microhabitats, a subset of the traps can have three fiberglass hoops with a diameter of 0.9 m, an Arkansas-style (flat) throat, and a total length of approximately 1.5 m with the same mesh and twine dimensions as listed earlier. These traps are capable of capturing AST up to 56 kg (123 lbs), although space inside the trap is limited and increases chances of drowning very large turtles.

Baited single hoop net traps (Figure 3) are used in streams with directional flow and can also be used in coastal tidally influenced waters, cypress swamps, and beaver ponds that lack directional flow. A second trap configuration can be used in areas that lack directional flow:

paired (tandem) hoop nets with lead lines (trammels) (Figure 4). Lead nets are 1.2 m in height and 13.7 m in length with the end of the lead net tied into the throat of a hoop net. The intervening lead net functions as a drift fence to intercept and direct turtles into the hoop nets. These tandem hoop nets can be baited or unbaited.

Commercial net sources are available. The following currently available sources are provided for convenience and not endorsed by the Service: Memphis Net and Twine, Memphis, TN (https://www.memphisnet.net/), Miller Net in Memphis (https://millernets.com/), and Nets and More (formerly The Fish Net Company, Jonesville, LA (https://www.netsandmore.com/).

Figure 3. *Top*: A single hoop net with 7 fiberglass hoops and two throats set alongside a submerged log. *Middle and bottom:* Single hoop net with 3 fiberglass hoops, back tied to secure structure (tree/cypress knees) and front attached to PVC pipe anchored into substrate. Note that the tail is tied above water line with the opening facing downstream. All nets should have buoys for floatation. Photo credit: Jim Godwin (*top*); Luke Pearson (*middle, bottom*).

Figure 4. Paired hoop nets with interconnecting lead net (trammel). Funnel openings of the hoop nets face one another with the lead net acting as an aquatic drift fence directing active turtles into one of the hoop nets. No bait is needed with this trap configuration but may be used if desired. Photo credit: Luke Pearson

Trap Placement

Microhabitat: Traps should be set within high potential use areas as follows:

- In streams with steady flow, parallel to bank, with throat opening pointing downstream;
- Upstream of structural features (e.g., logs, trees, large limbs, undercut banks, large rock) that may be used by AST or SuwAST;
- Adjacent to riverbanks with intact riparian zones that have trees overshadowing the stream;
- Non-stream settings parallel to shore in shallow (approximately. 1 m) water; and
- Non-stream setting near structural features including downed trees, cypress knees, or stands of cypress or tupelo, if available.

Placement:

Trap placement will depend upon the aquatic setting with stream placement differing from non-stream placement.

Stream:

In streams, tie the tail of the trap to a stout anchor, such as a tree trunk or thick limb, with the opening (throat funnel) of the net facing downstream. Tail of the trap should be above water to allow trapped turtles access to atmospheric air with the throat funnel (open end) of the trap facing downstream. To minimize the chances of turtles drowning due to unexpected increases in water levels, buoys should be placed at the tail end of the trap. The front (downstream side) of the trap can also be 1) tied to structure, if present; 2) a rope loop can be attached to the hoop and placed around a PVC pipe hammered into the substrate; or 3) a long rope can be attached to a heavy weight (anchor or railroad tie plates) and sunk to the bottom.

Trap set should be parallel or near parallel to the bank and upstream of suitable microhabitat features. Preferable flow conditions are those in which some flow is present but not strong enough to float the net from off the stream bottom. ***The downstream hoop must be touching the ground.*** If using the Arkansas-style (flat) throat, confirm that the throat is oriented horizontally, parallel to the water surface. An easy way to do this is to mark the "top" of the last hoop with a trap ID tag so that when the trap ID tag is visible above the water surface, then the flat throat is oriented horizontally. Bait (fresh chopped fish is preferred; frozen tilapia, invasive carp, catfish nuggets, canned sardines, or any other fish-based bait may be substituted) is placed in a 1-liter bottle with holes cut in it. Alternatively, a 3-inch diameter PVC pipe with two grated caps on either end and holes drilled into the pipe may be used and is more durable than a plastic bottle. Bait volume in bottle should approximate half to two-thirds the bottle volume or approximately 1 to 2 large fish steaks (cross-section, including bone); this allows water to freely enter and flow through the bottle to carry the bait scent downstream. The bottle is tied and hung in the rear of the trap and must be submerged when the trap is set. Bait is usually attached to the 3rd hoop if running a 3-hoop trap; attached to the 3rd hoop if running a 4-hoop trap; attached to the 6th hoop if running a 7-hoop trap. ***If conducting long-term mark-recapture surveys, it is recommended to also hang a piece of fish outside of the bottle. AST and SuwAST can become very trap-shy after capture, especially if they cannot access bait.*** A buoy should be placed in the back of each hoop net.

Non-stream:

Non-stream habitat generally has soft mud substrates. Single hoop nets, as described above, can be used in non-stream habitats. Paired funnel traps with intervening lead lines can also be used and are anchored with PVC pipes (1.5-inch diameter, 7.5 to 10 ft length) driven into

the soft mud substrate. Four poles are used for one tandem hoop net configuration, which includes two hoop nets plus the lead net. One pole anchors the hoop opening with a second pole anchoring the tail of the net, with this arrangement repeated at the other hoop net. A buoy is placed in the rear of each hoop net to maintain an air space for trapped turtles. In cypress swamps, trees and cypress knees may be suitably spaced to tie and anchor nets. In swampy and marshy settings where alligators are abundant, the dual hoop net configuration with intervening lead net can be used without bait to reduce the likelihood of attracting alligators. However, alligators can tangle in the lead net, and similar to single hoop nets, drownings are possible if alligators enter the hoop net.

Trap data and turtle species captured should be recorded on the trap sheet (example included below). Minimum trap data includes site (river, site), latitude/longitude (decimal degrees), trap ID, trap type, and bait type. Traps should be labelled according to Federal and/or State regulations. Collecting data on the turtle assemblage associated with AST or SuwAST is strongly advised. During subsequent DA trap placements, traps should generally be placed in similar locations as during the previous sampling event, unless this is impossible due to changing water levels.

Trap Checks

For the safety of captured turtles and other animals, all traps should be checked daily. ***Under no circumstances should traps be left unchecked for longer than 24 hours.*** All traps should be rebaited every 24 hours, if possible. Surveyors must watch forecasted weather conditions and stream flow parameters, if available (https://waterdata.usgs.gov/nwis/rt) and pull or monitor traps if heavy precipitation or flooding is expected. On each trap-check day, the trap sheet should be completed and the AST or SuwAST data sheet should be completed for each AST or SuwAST captured in the trap (see protocol for processing individual turtles). Other species of trapped turtles may be processed at the surveyor's discretion but at minimum, turtle species and sex should be recorded on the trap sheet.

Disinfection and Disease Transfer

There are several precautionary measures to prevent the spread of disease. A 3% bleach solution may be used to disinfect traps, boats, and clothing between sites, if time does not permit fully drying of traps in direct sunlight. After bathing or spraying equipment with the bleach solution, items should be rinsed with clean water. Captured turtles from different sites, and those

displaying signs of illness, should be held separate during processing, and equipment should be sterilized between turtles. Calipers and drill bits should be swabbed with alcohol, bleach solution, or be exposed to open flame. The Northeast Partners for Amphibian and Reptile Conservation (NEPARC) Disinfection Protocols contain additional recommendations: http://www.northeastparc.org/products/pdfs/NEPARC_Pub_2014-02_Disinfection_Protocol.pdf

Protocol for Processing Individual Turtles

When an AST or SuwAST is captured, the AST data sheet (example included below) should be completed, and the following protocols are recommended. This sheet has been designed specifically for the AST and SuwAST. Placing large juvenile and adult turtles on the carapace with the head hanging free (edge of boat decks or seat work well) allows the researcher to easily collect ventral morphometric data and tissue samples. This technique has been employed by AST researchers for over 25 years with > 1,250 turtles having been handled and measured with no individuals exhibiting signs of harm afterward.

MORPHOMETRICS

Record shell dimensions in centimeters (cm). At a minimum, record SCL (straight carapace length) down the midline, MCL (maximum carapace length), CH (carapace height at the 2nd and 3rd vertebral suture line), CW (carapace width at 2nd and 3rd vertebral suture line), SM (supramarginal scutes) for each side, and PL (midline plastron length). PCL (pre-cloacal tail length, from posterior edge of plastron to cloaca) is measured in millimeters (mm). PCL is used to identify sex, with mature females generally having PCL < 120 mm and mature males having PCL ≥ 120 mm. *Tip: Very large females (≥ 55 pounds) can have PCL up to 130 mm and subadult or immature males can have PCL < 120 mm. If uncertain, document your uncertainty. You can also subjectively identify sex based on turtle behavior, with females being more active, more difficult to handle, and constantly trying to escape or fight. Males will fight for a short period of time and then mostly stop moving.*

Captured turtles may span a very broad size range from small juveniles to mature adults; MCL of males can be up to 80 cm. To properly measure turtles across this spectrum, several caliper sizes are needed. At minimum, dial calipers 150 mm (6 in) and Haglof tree calipers 65 cm (15.75 in) can be used, but access to Haglof tree calipers 80 cm (31.5 in) may be necessary.

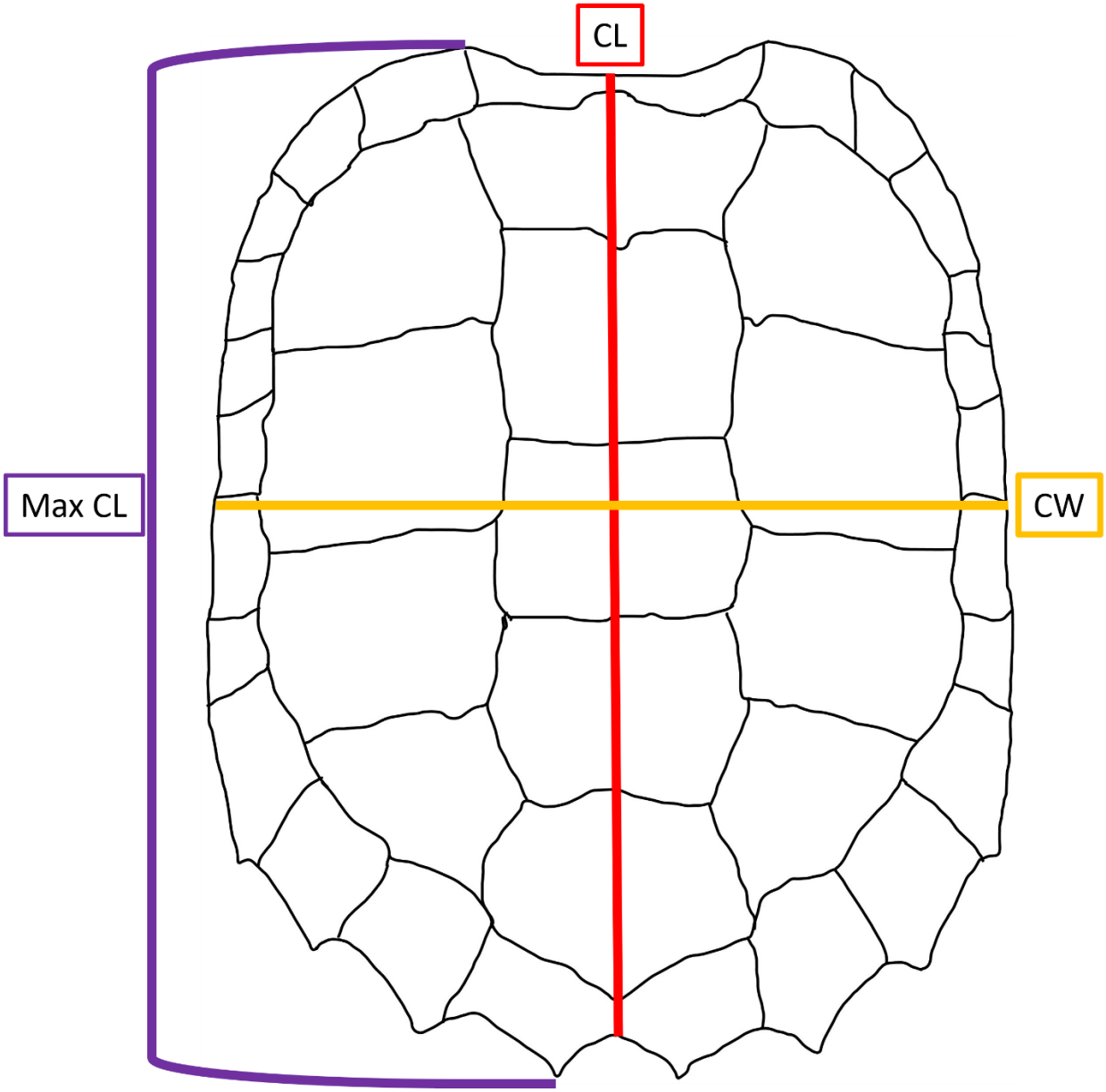


Figure 5. Diagram showing carapace morphometric measurements for the alligator snapping turtle. CL: midline carapace length from the nuchal scute (above the head) to the caudal notch. Max CL: maximum carapace length of the shell, usually from the first marginal scute/nuchal scute suture to the posterior tip of the 12th marginal scutes. CW: carapace width at the suture of 6th and 7th marginal scutes.

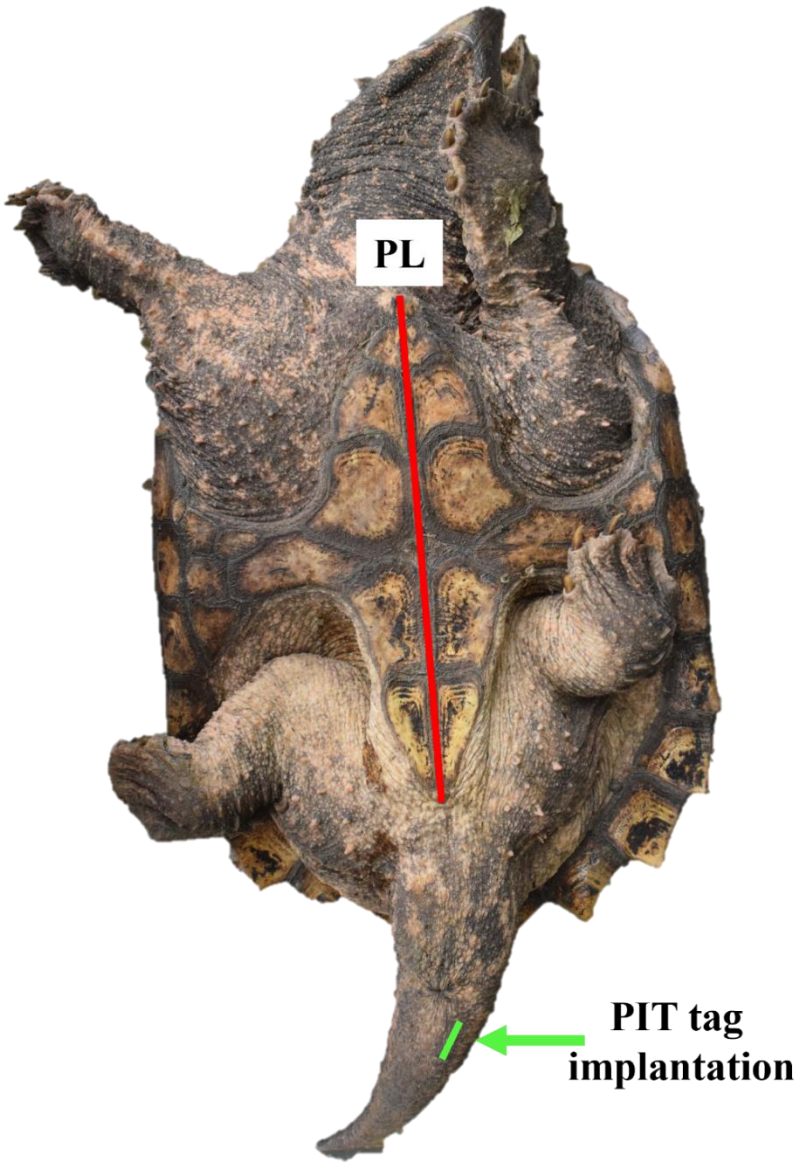
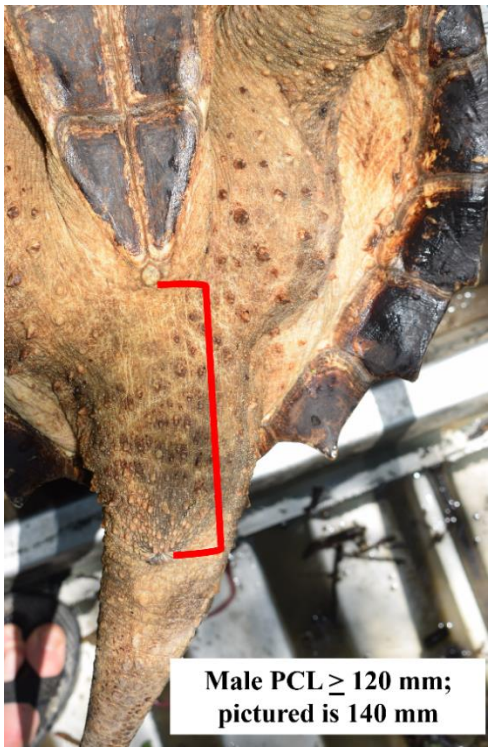


Figure 6. Plastron length (PL) is measured from the anterior point of the plastron to the posterior point of the plastron. The green mark on the tail shows a location of PIT tag implantation for most individuals ≥ 2 kg. PIT tags are placed lateral to the cloaca (right or left side) and approximately 1.5 needle lengths below the cloaca. PIT tags are placed ***subcutaneously*** so the implanter needs to be shallowly inserted and directed towards the lateral side of the cloaca. Photo credit: Luke Pearson



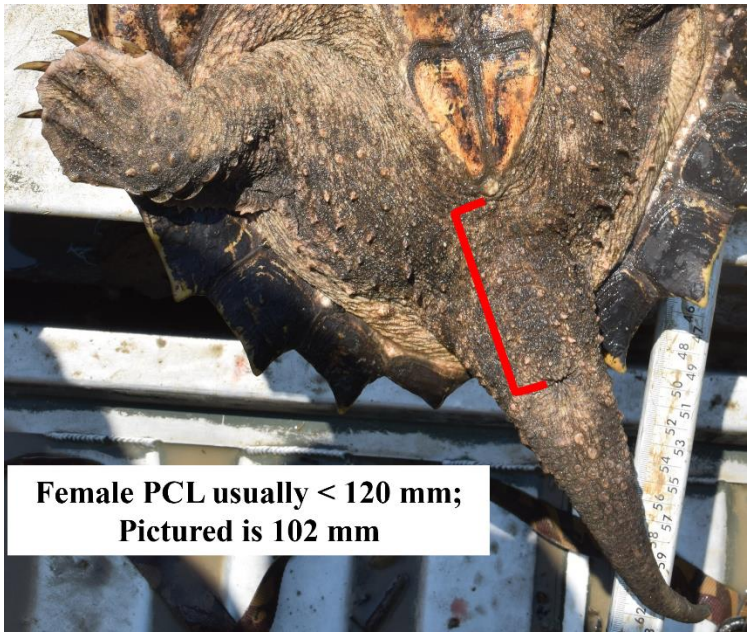


Figure 7. Pre-cloacal tail length (PCL) is measured from the posterior tip of the plastron to the cloaca using calipers. This measurement is used to identify sex, with mature females generally having PCL < 120 mm and mature males having PCL ≥ 120 mm. *Tip: Very large females (≥ 60 pounds) can have PCL up to 130 mm and subadult or immature males can have PCL < 120 mm. If uncertain, document your uncertainty. You can subjectively identify sex based on turtle behavior, with females being more active, more difficult to handle, and constantly trying to escape or fight. Males will fight for a short period of time and then mostly stop moving.* However, testosterone analyses can accurately identify sex if unsure of sex identification in the field. Photo credit: Luke Pearson (top); John Tupy (bottom).

WEIGHT

Record animal mass to nearest 0.1 kg. This will require a set of Pesola scales (at minimum, 10 kg, 50 kg) or a dial or electronic scale capable of weighing > 50 kg. Most turtles will be less than 40 kg. If the goal is to collect accurate data on all specimens, a scale capable of measuring in excess of 50 kg will be necessary. Constriction or ratchet straps are needed in weighing large turtles. The strap is tightened around the shell with the scale attached to the strap and a metal rebar attached to the scale for easy pickup between two people. ***Warning: AST will spin when lifted. Be careful of where the head is facing when weighing turtles using this method.***

Figure 8: Example of how to weigh large turtles with ratchet strap around turtle, attached scale, and lifting using a rebar with two people.

PIT (Passive Integrated Transponder) Tags

All AST and SuwAST should be PIT tagged, if possible, with implantation occurring laterally in the base of the tail or parallel to the tibia/femur in the back leg (if small juvenile). All turtles PIT tagged should also be physically scute notched. Disinfection of PIT tag injector should occur after contact with an individual turtle. Disinfection can be open flame, diluted bleach, or ethanol/isopropyl alcohol.

Figure 9. PIT tag implantation occurring laterally (left) and 1.5 needle lengths below the cloaca. Use liquid bandage after implantation to seal the puncture site and use a PIT tag reader to confirm the PIT tag was implanted successfully. Record the PIT tag number on the AST or SuwAST data sheet. This is also a method of stretching the tail to acquire pre-cloacal tail length (PCL) measurements. Photo credit: Calvin Rezac.

SCUTE NOTCHING

Turtles should be uniquely scute notched, using a marking scheme as directed by the lead researcher. The marking scheme shown on the provided AST data sheet is a modified Ernst et al. (1974) scheme only using the rear 8–12 marginal scutes to avoid the head. This scute notching scheme can uniquely identify up to 454 AST without further modification. Scute notching can occur in a variety of ways, but all tools used to scute notch turtles must be disinfected following contact with an individual turtle using bleach, ethanol/isopropyl alcohol, or an open flame to reduce disease transmission. Use of a numbered list for these scute notching techniques are for convenient reference only and do not imply prioritization.

1. Individuals weighing above approximately 2 kg can be marked with stainless steel screws in the marginal scutes. Select the unique number to be used. Use a portable drill with a 1/8 in drill bit to begin a starter hole and screw a Phillips pan head stainless steel screw (#10 x ½ in) into the hole until tight with screw head flush to shell surface (Figure 10). This marking method uses easily obtained and inexpensive materials and provides an immediate visual cue that the turtle has been previously captured and marked. This scheme has led to positive identification after a decade in Alabama.

Figure 10. Alligator snapping turtle has been marked on the 2nd and 3rd left marginal scutes with #10 stainless steel screws. Photo credit: Jim Godwin.

2. All individuals can be scute notched using a mini, hand-held hacksaw to cut triangular notches in the appropriate marginal scutes (Figure 11). This scheme has led to positive identification after six years, with notches still in perfect condition, in Mississippi.

Figure 11. Triangular scute notches using a mini (10 in) hacksaw. Photo credit: Patrick Delisle.

3. All individuals can have holes drilled into the appropriate marginal scutes, with the size of the drill bit varying based on size of the individual being marked (Figure 12). This marking method has led to positive identification after a decade in Florida.

Figure 12. Hole drilled in 9th right marginal scute. Photo credit: Patrick Delisle.

PHOTOGRAPHS

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

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

Figure 13. Injuries sustained by a male AST, consistent with male-male combat. Triangular bite to underside of neck (*left*) and large wound on tail (*right*). Photo credit: Luke Pearson.

TISSUE OR BLOOD COLLECTION

With approval and proper permits, trained researchers may consider collecting tissue or blood samples for genetic sampling or hormone analysis. Tissue samples can be easily acquired from webbing of the back feet, tip of the tail, or from tubercles on the back legs. Scissors need to be disinfected between tissue samples, either with an open flame (e.g., lighter), diluted bleach, or ethanol/isopropyl alcohol. Tissue samples must be preserved in 95% ethanol or higher

concentration, preferably in a screw-top tube with an O-ring on the lid (e.g., Fisherbrand threaded end microcentrifuge tubes). Place a hand-written (e.g., pencil) or pre-printed Rite-in-Rain label into the tissue tube. Tissue samples in 100% ethanol can be stored at room temperature, although storage in a freezer is preferred to minimize evaporation rate of ethanol. Blood samples can be acquired from the dorsal coccygeal vein in the tail between the upper-most vertebrae. Blood samples can be preserved in several ways, including 95% or higher ethanol, a lysis buffer, or SED buffer (Salt, EDTA, and DMSO buffer; Seutin et al. 1991). Blood samples should be stored in a freezer. Training is necessary to take blood samples, and appropriately sized needles (gauge and length) are needed to acquire blood from larger individuals.

Required Equipment

The following equipment is required to complete the protocol:

Trapping equipment:

- 10 to 25 hoop nets
- Bait
- Bait containers
- PVC pipes (1.5 inch, 7.5–10ft long; at minimum, 1/trap unless determined otherwise)
- Extra rope
- Zip ties (fixing trap) and twine (hanging bait)
- Sledgehammer (3–4 pounds)
- GPS with extra batteries
- Knife/machete if bait is not pre-cut
- Trap ID tags

Data equipment:

- Data sheets (Rite-in-Rain paper) in binder
- Writing implement: mechanical pencil, Bic pens
- Calipers: 15 to 65 cm, minimum
- Scales: Pesola or electronic, capable of reading > 50 kg
- Ratchet strap or sling to weigh turtles
- Rebar to weigh turtles between two people
- 5-gallon bucket to weigh smaller turtles
- Camera/cell phone for photographing turtles

Tissue/blood equipment:

- Needles (22 to 26 gauge, 1-to-1.5-inch length)
- Syringes (1 to 3 mL)
- Screw-top tubes with O-rings (up to 2 mL)
- Ethanol (95 to 100%)
- Tissue dissecting scissors
- Tweezers
- Sharps container
- Disinfecting supplies (e.g., lighter, diluted bleach, ethanol)
- Rite-in-rain tissue labels (tissue) or sharpie (blood)

Marking equipment:

1. Drill/screw method:
    - Portable drill with batteries
    - Drill bit set
    - Stainless steel screws
    - Screwdriver
2. Hacksaw method:
    - Mini/compact 10-inch hand-held hacksaw

    - Extra blades
    - Basic tools to replace blade, if needed
3. PIT tagging:
    - PIT tags
    - PIT tag reader
    - Implanters/syringes
    - Ethanol
    - Liquid bandage

Other equipment:

- Boat with trailer or canoe
- Gas
- Batteries (AA, AAA, boat battery)
- Paddles (minimum 2)
- Lifejackets
- Type 4 throwables
- Fingerless gloves
- First aid kit which includes trauma kit (gauze, band aids, wraps, etc.)
- Sunscreen
- Flashlight/headlight

Trap identification: Assign unique ID to each trap and label trap on the corresponding trap sheet.

Trap location/operation: Record trap ID, latitude/longitude (decimal degrees), date, river, and site on appropriate trap sheet upon trap placement.

Bait: Fresh (or frozen) fish. Specify what type of fish (buffalo, carp, catfish, etc.)

Re-bait frequency: Every 24 hours if possible. No longer than 48 hours. If permitted by appropriate agency, non-game, non-threatened fish captured in traps may also be used by tying into rear of trap and making several deep cuts to release blood and scent.

Trap check frequency: No longer than 24 hours (daily) with more frequent checks as required by agencies/partners or flood conditions.

Data Entry

Upon returning to the office, or if possible, in the field at the end of each day, electronically enter data as soon as possible into a formatted Excel Worksheet.

List of species potentially co-occurring with AST and SuwAST is below. The subset of species will vary by drainage and state. Species may be selected from this list to include in the "Species captured" column of the trap sheet in order to record data on the turtle assemblage associated with the AST and SuwAST.

Apalone ferox	*Apalone mutica*
Apalone spinifera	*Chelydra serpentina*
Chrysemys dorsalis	*Chrysemys picta*
Deirochelys reticularia	*Graptemys barbouri*
Graptemys ernsti	*Graptemys flavimaculata*
Graptemys geographica	*Graptemys gibbonsi*
Graptemys nigrinoda	*Graptemys oculifera*
Graptemys ouachitensis	*Graptemys pearlensis*
Graptemys pseudogeographica	*Graptemys pulchra*
Graptemys sabinensis	*Kinosternon baurii*
Kinosternon subrubrum	*Pseudemys alabamensis*
Pseudemys concinna	*Pseudemys floridana*
Pseudemys nelsoni	*Sternotherus carinatus*
Sternotherus intermedius	*Sternotherus minor*
Sternotherus odoratus	*Sternotherus peltifer*
Trachemys scripta	

EXAMPLE TRAP DATA SHEET:

Location: **GPS:** **Trap ID:** **Trap Type:** **CL:** midline carapace length **PL:** plastron length
Date: **CW:** carapace width **M:** Mass (g or kg)
Bait Type: **CH:** carapace height

Species:	ID #	Sex	CL	CW	CH	PL	M	Tissue (Y/N)	Date	Notes (e.g., injuries)

Page: _____ of _____

- **Location:** site name; for example, Savage – Coldwater River
- **Date**: day trap set; Day/Month/Year
- **GPS:** coordinates; latitude/longitude in decimal degrees
- **Trap ID**
- **Trap Type**: 0.9 m vs. 1.2 m trap; flat throat vs. fingered throat
- **Bait type**: fresh buffalo, frozen catfish, etc.
- **Page number:** for example, 10 traps set, so the first trap would be Page: 1 of 1.

Morphometric information:

- **Species**: turtle species captured in trap; see species list above.
- **ID**: turtle ID (001, 002, 003, etc.)
- **Sex:** male, female, juvenile
- **CL**: carapace length down middle of carapace from nuchal scute to caudal notch
- **CW**: carapace width at 6th marginal scute
- **CH**: carapace height behind 2nd vertebral keel
- **PL**: midline plastron length
- **M**: mass in g or kg.
- **Tissue or blood sample**: Yes or No
- **Date**: day turtle captured; Day/Month/Year
- **Notes:** additional information about turtle (injuries, deformities), PIT tag information, or bait used to rebait trap (NB [new bait] – fresh carp).

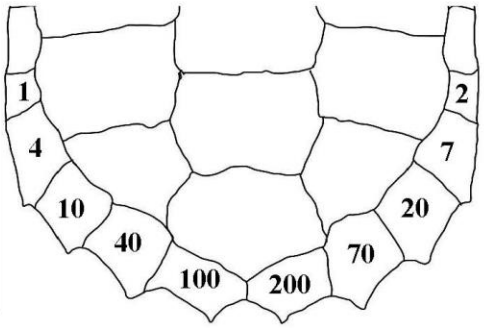
EXAMPLE AST DATA SHEET:

Macrochelys temminckii

Date:

Location:

GPS:



Trap Information:

Trap #:

Trap Type:

Turtle ID:	Notes
Sex:	
CL:	
Max CL:	
CW:	
CH:	
PL:	
M:	
Pre-Cloacal Length:	
Tissue (Y/N):	
PIT #:	
Lure:	
Supramarginal L:	
Supramarginal R:	

This datasheet can be duplicated and placed on a single page, so each page can have data for two AST. This sheet includes all previously mentioned morphometric measurements, except for:

- **Max CL**: maximum carapace length
- **Pre-cloacal length**: length between posterior point of plastron and cloaca with tail straight
- **Lure:** lingual lure color; can be white, pink, light/dark gray, purple, mottled, red, multi-colored, present as a nub (missing most of lure), or absence altogether (no lure)
- **Supramarginal L/R**: count of supramarginal scutes on left and right side of carapace
- **PIT #:** Passive integrated transponder (PIT) tag identification number

SAFETY TIPS, TRICKS, AND WARNINGS

1. AST can cause serious injury. We are aware of at least one officially documented case of an amputated finger (Johnson et al. 2016) and many other anecdotal stories of people losing fingers to this species. ***Always pay attention to where an AST's head is, where your hands and forearms are, and where other people in the boat are.*** Spatial awareness and communication among partners are key in preventing serious injuries to you or the turtle. Some AST have very sharp claws that can rip through clothing and cause significant scratches.

2. Most people worry about being injured by large AST and SuwAST. However, most bites and close calls are actually from juveniles. These smaller turtles have larger ranges of motion of their head, can physically lunge forward, and can turn extremely quickly. ***Always be aware of your surroundings and the turtle's orientation.***

3. Traps with multiple ASTs are one of the most high-risk situations and have substantial potential to result in injuries to surveyors and turtles. In these situations, awareness and communication with partners is key. Surveyors can either bring the trap into the boat or drag the trap to shore to remove turtles. One person should use a paddle as a barrier between ASTs to prevent turtles from biting each other or the surveyor. The handle/hook end of the paddle can also be used to move or position an AST while in or outside of the trap. Always remove the easiest and safest turtle first, regardless of size or weight. This turtle could be the 100-pound male that falls out of the trap when the tail end is untied, or it could be a 2-pound juvenile. ***Always pay attention to where your hands and forearms are, where your partners are, where the other turtles are, and do what is safest for you and the turtles.***

4. Alligators can be incidentally captured in hoop nets when targeting ASTs, and this is easily observable if the trap is thrashing around. If this situation occurs, do not grab the hoop to check the trap. Instead, use a rope attached to the hoop or the handle/hook end of the paddle to begin the process of checking the trap. ***NEVER bring a hoop net with a captured alligator into the boat.*** If necessary, the hoop net can be pulled onto shore; however, as long as the tail end of the hoop net can be untied and opened, all captured alligators can be safely removed with the hoop net in the water and the surveyors on the boat. If an AST is also captured in the trap with the alligator, the surveyor has two choices:
    a. Untie the back of the trap and release both the alligator and the AST; or
    b. Attempt to separate the AST and confine it to the front of the trap using the fiberglass hoops, while maneuvering the alligator to the tail end of the trap for untying and release. ***Never physically touch the alligator during this process.*** Use paddles, hooks, or other devices to assist in this endeavor. This process can be time-consuming and has taken up to 40 minutes to remove a single 7ft alligator while retaining four ASTs in the trap.

5. Flipping large AST (> 70 pounds) for plastron length, pre-cloacal tail length, tissue samples, and weighing can be a challenge. To safely flip these turtles, place one hand near marginals 10 and 11 and another hand near marginals 6 and 7, and flip the turtle in the direction you want them to go. ***This must be a fast, explosive movement,*** otherwise the turtle will immediately stop your momentum and right itself. Once flipped on the carapace, make sure the turtle is level (not leaning to one side) and tap the plastron or legs until the turtle stops attempting to right itself.

6. There are different ways to hold AST depending on the size and weight of the turtle:
    a. If the turtle is small enough, surveyors can hold the turtle on the posterior carapace where the back legs are or can pinch-hold the posterior plastron (see below).

Photo credit: Luke Pearson

Photo credit: Luke Pearson

    b. Larger turtles will require one hand (or several fingers) holding the anterior carapace behind the head, while the other hand will be holding the posterior carapace (see below). Some AST do not like being held in this manner. These AST will either pull their heads back and smash the hand holding the carapace behind the head or will fully extend the neck and reducing the amount of grip of the surveyor. Generally, the surveyor will know quickly if an AST will do this when the turtle is handled for the first time. ***If this happens, do not drop the turtle; swiftly lower the turtle to the ground before releasing.*** Wearing fingerless gloves is one way to reduce any minor

injuries to the surveyor's hands from scratches or smashing.

Photo credit: Brad 'Bones' Glorioso

7. Taking pictures with AST is fun and encouraged. However, remember that you and the AST are facing the person taking the pictures. Have a plan for where picture-takers will move in order to avoid an AST that won't cooperate.

Acknowledgements

We would like to thank Jim Godwin (Alabama Natural Heritage Program) and DoD PARC for drafting their Recommended Best Management Practices for the Alligator Snapping Turtle on Department of Defense Installations document (https://www.denix.osd.mil/legacy/denix-files/sites/33/2022/06/Alligator-Snapping-Turtle_BMP_Final_508.pdf), of which was modified to create this document. We also thank the various people who allowed us to use their photos for reference material, and the U.S. Fish and Wildlife Service's alligator snapping turtle Core Team for reviewing the document.

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