# Final Report: Quantifying the contribution of sustainable forest practices to at-risk species and terrestrial and aquatic communities

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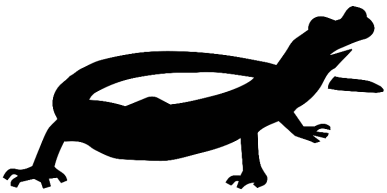

TANGLED BANK CONSERVATION™

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## Scope of Work:

We conducted thorough surveys on the WCI properties of selected sites using a 3-pronged approach in order to assess the presence of 31 target species. This multi-faceted approach should provide important insight into total diversity present and community health on WCI properties.

## **Method 1**: Freshwater turtle visual surveys and trapping

*Approach:*
We used two primary methods to sample for freshwater turtles. Map turtles are best surveyed with visual counts of basking individuals because map turtles are easily observed basking. Pronounced sexual dimorphism allows for most basking individuals to be categorized by sex. We surveyed for Alabama Red-belly Turtles and Alligator Snapping Turtles using hoop nets. In rivers with consistent downstream, we used flow baited hoop nets to survey Alligator Snapping Turtles. In slow moving, tidally influenced waters, we used unbaited hoop nets connected with a lead net (hoop and fyke net) to survey for Alabama Red-belly Turtles. The lead net type functions as an aquatic drift fence. We took data on all freshwater turtle species observed during visual surveys or captured in hoop nets to provide information on overall freshwater turtle assemblage.

*Results:*

- Turtle trapping was conducted in Spring and Fall of 2021 and Spring of 2023. Figure 1 below shows the location of all trapping and visual basking survey efforts
- Total trap effort (2021 & 2023) was 28 trap-nights
- 6 species of turtle were captured during 2021 and 2023 trapping efforts. Figures 2 and 3 below show which species were detected at each site
    - Pond Slider (*Trachemys scripta)* was most abundant with catch-per-unit effort (CPUE) of 0.86 turtles/trap-night
    - Alligator Snapping Turtle was second most abundant with CPUE of 0.25
        - See Supplemental Table 2 for all Turtle Trap CPUE values
    - Turtle assemblage trapped, in decreasing order of abundance, Slider (*Trachemys scripta*) 24; Alligator Snapping Turtle (*Macrochelys temminckii*) 7; Spiny Softshell (*Apalone spinifera*) 2; Stripe-necked Musk Turtle (*Sternotherus peltifer*) 2; Common Snapping Turtle (*Chelydra serpentina*) 1; Intermediate Musk Turtle (*Sternotherus intermedius*) 1
- Traps were deployed at 20 sites along Sizemore Creek, Little River, Styx River, and Pigeon River in spring and fall of 2021
    - 1 target species, the Alligator Snapping Turtle (*Macrochelys temminckii*), was trapped at 4 locations
- Traps were deployed at 2 sites along Pigeon and Persimmon Creek in spring of 2023
    - 1 target species, the Alligator Snapping Turtle (*Macrochelys temminckii*), was trapped at 1 location
    - 1 target species, the Escambia Map Turtle (*Graptemys ernsti*) was visually observed at 2 locations: Pigeon Creek and Persimmon Creek

Although trap results are limited, the reported turtle species suggests streams support unaltered turtle assemblages because most of the expected species were documented.

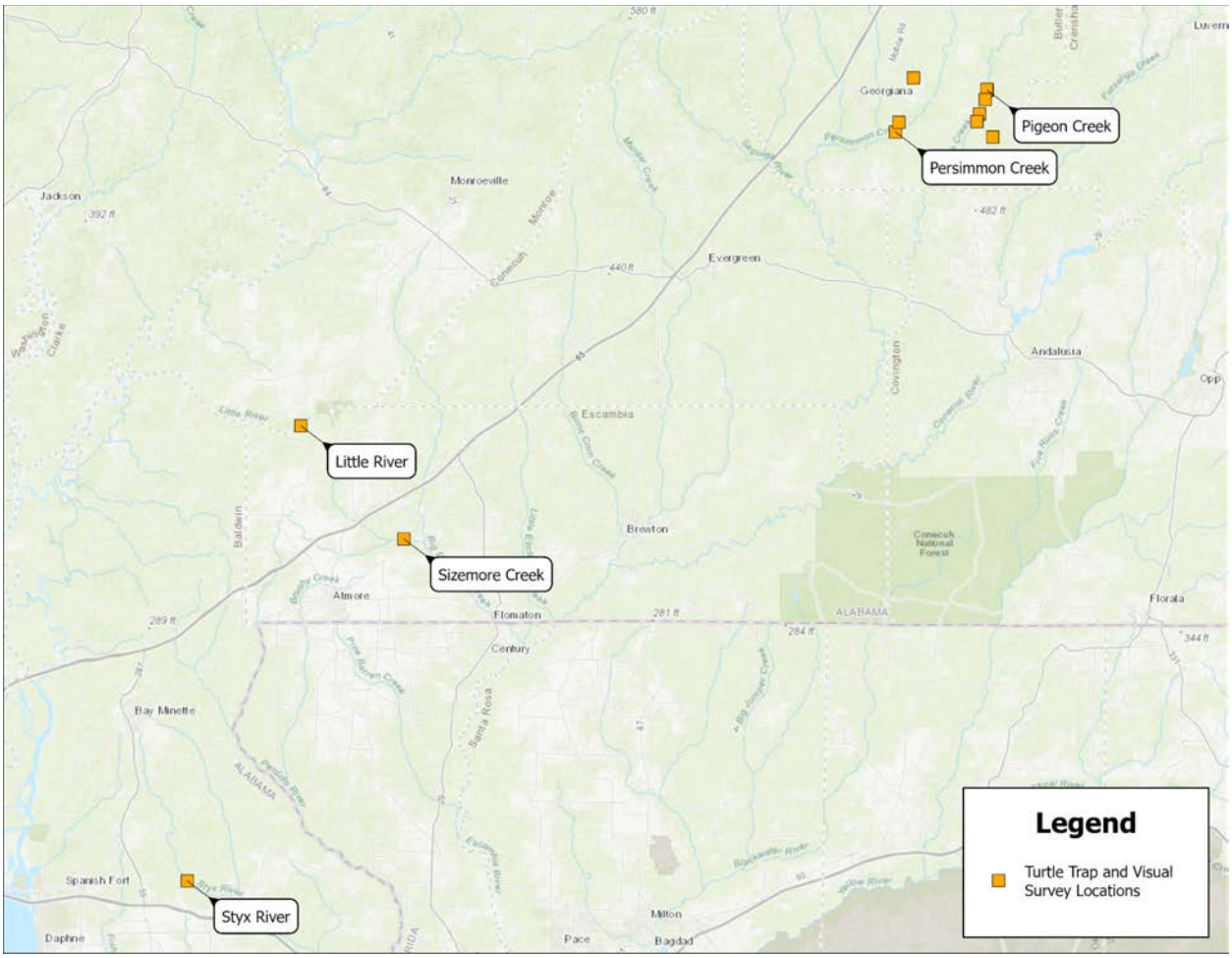

Figure 1. The locations of freshwater turtle visual survey and trapping efforts.

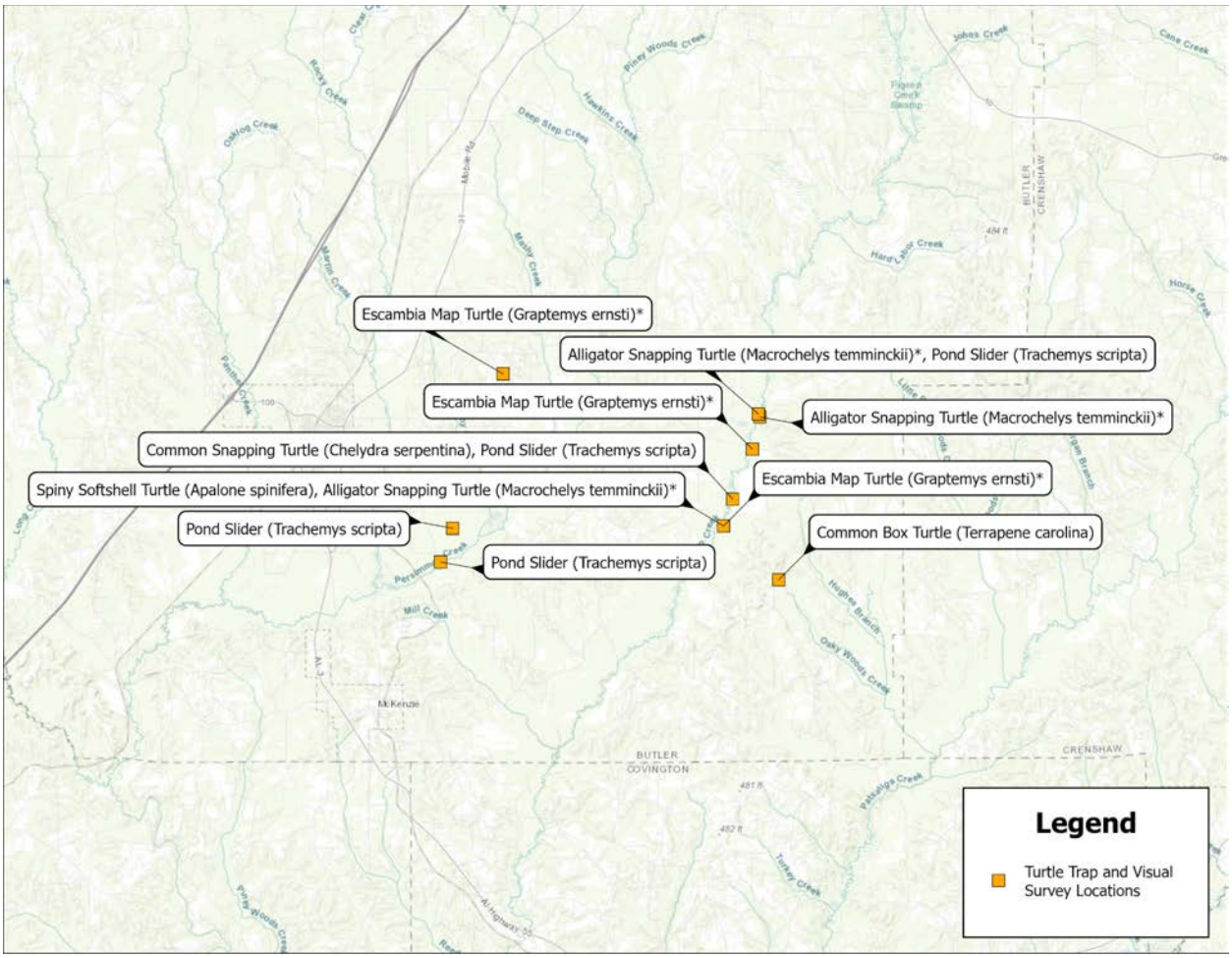

Figure 2. A closeup of the visual survey and trapping locations along Pigeon and Persimmon Creeks. Species trapped or visually detected are listed for each site. An asterisk indicates a target species.

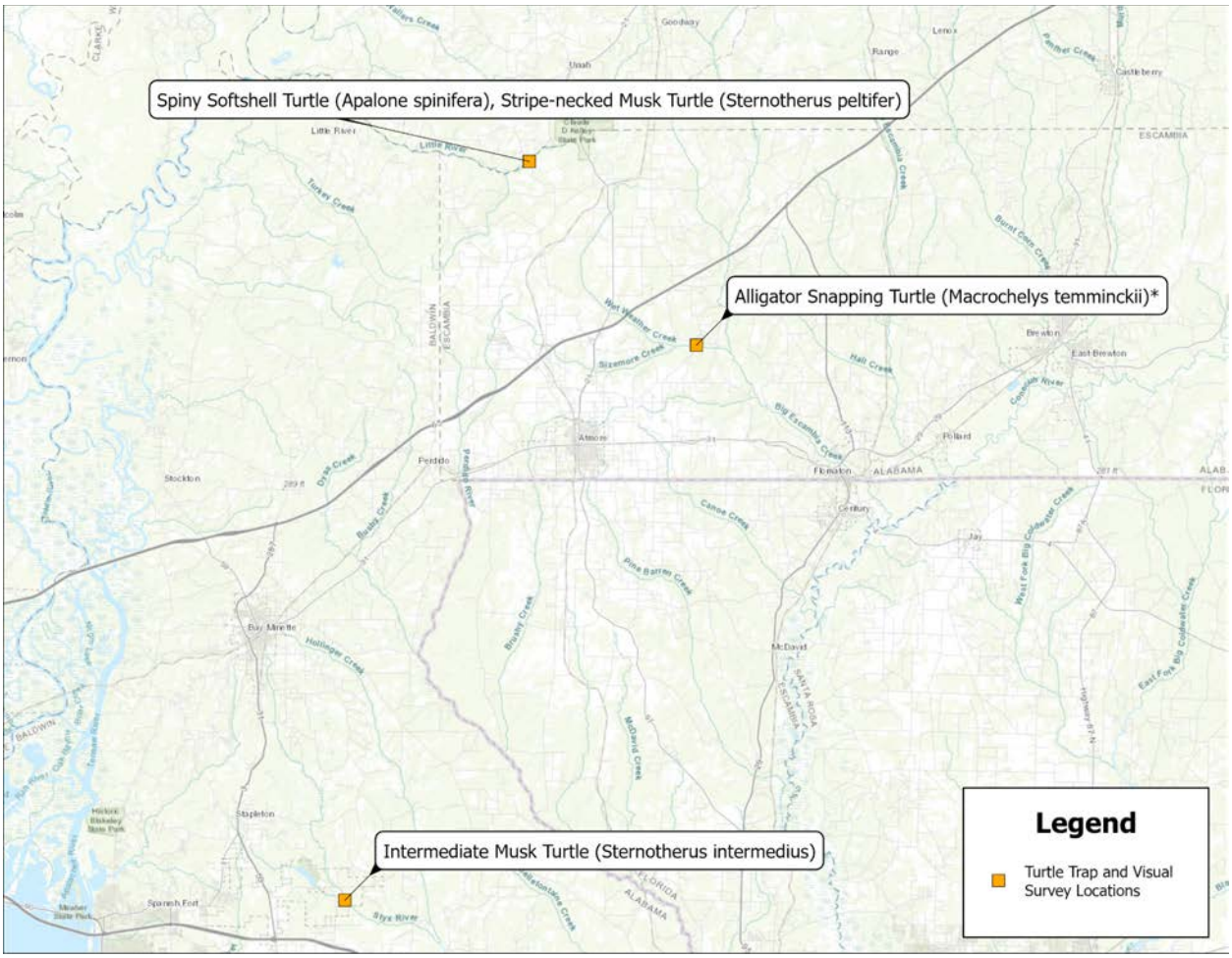


Figure 3. A closeup of the visual survey and trapping locations along Styx River, Little River, and Sizemore Creek. Species trapped or visually detected are listed for each site. An asterisk indicates a target species.

## **Method 2:** Camera trapping arrays and visual terrestrial surveys for rare amphibians and reptiles

*Approach:*
Drift fence arrays with traps have traditionally been used to assess herpetological diversity, but are extremely time consuming and expensive given the frequency at which they must be checked. However, newly developed arrays that employ game cameras are far more efficient and effective. They have been shown to capture species that tend to escape bucket and box traps (e.g. pinesnakes) and also capture many small non-target mammals and birds, giving a broader picture of diversity. We constructed camera trap arrays by mounting a game camera pointing downwards in an upside-down bucket with entrance and exit holes cut into it, and attached drift fences to funnel animals through the bucket. We deployed these camera arrays across target sites. In addition, we conducted visual surveys to target species that are not easily documented with camera drift fence arrays (i.e. Red Hills Salamander, Gopher Tortoise, and Eastern Diamond-backed Rattlesnake).

*Camera Trapping Results:*

- Camera trap arrays were deployed across 60 sites for durations of 66-218 trap nights (average 130.67), totalling 6,403 trap nights between March 2021- June 2023. All camera trapping locations can be seen below in Figure 4
- 470 occurrences of a total of 46 species, including 1 target species (Eastern Diamond-backed Rattlesnake, *Crotalus adamanteus*), were detected in camera trap imagery
- Black Racer (*Coluber constrictor*) was the most abundant species detected with catch-per-unit effort (CPUE) of 0.0151 individuals per trap-night
    - See Supplemental Table 2 for all Camera Trap Array CPUE values
- Noteworthy non-target species detected via camera trap efforts include:
    - Black Pinesnake (*Pituophis melanoleucus lodingi*), Federally Threatened, Alabama Ranked S2, Globally Ranked T1
    - Long-tailed Weasel (*Mustela frenata*), Alabama State Ranked S3
    - Coal Skink (*Plestiodon anthracinus pluvialis*), Alabama Ranked S3

Figures 5-7 visually represent species detected per trap night within 10-km grid cells across the study area. In Figure 5, the total trap nights across all cameras are summarized per grid cell. Note that some cameras only have data for 49 trap nights. These cameras are still deployed, but their SD cards were swapped out after 49 days. In Figure 6, the total number of species detected across all cameras is summarized per grid cell. Figure 7 displays the calculated species-per-trap-night values for each 10-km grid cell.

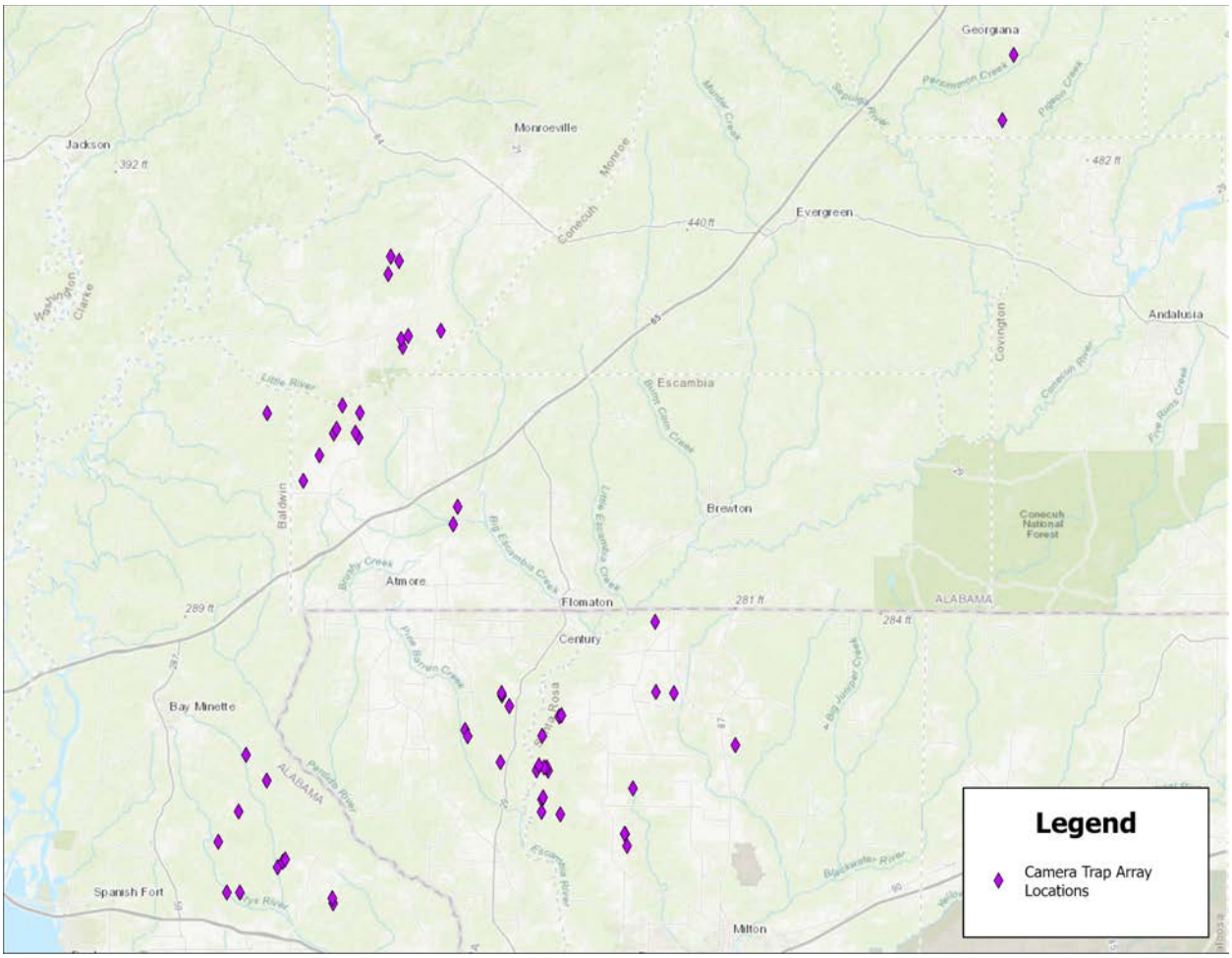

Figure 4. Locations of camera trap arrays deployed in 2021-2023.

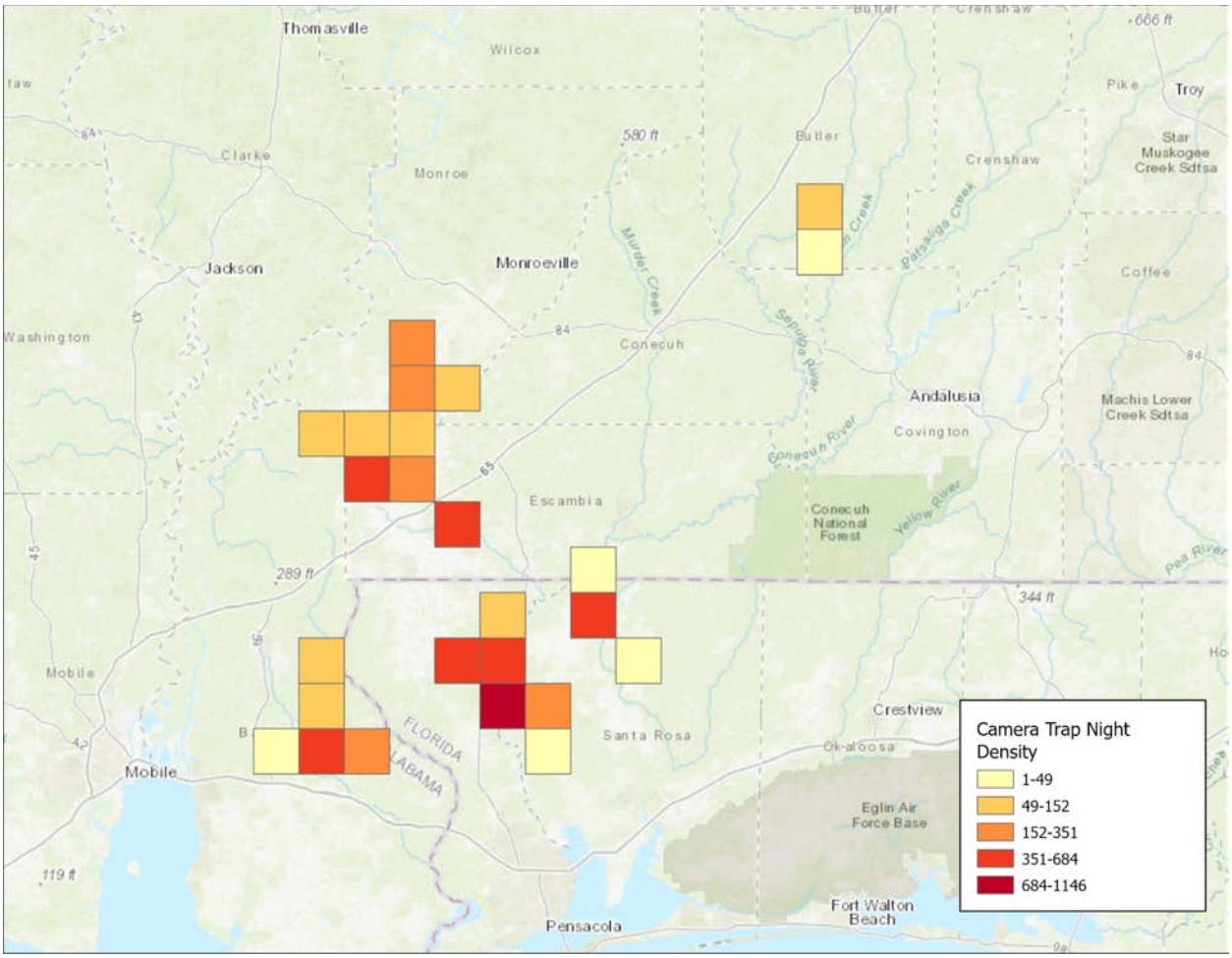

Figure 5. Density of camera trap nights within 10-km grid cells across the study area.

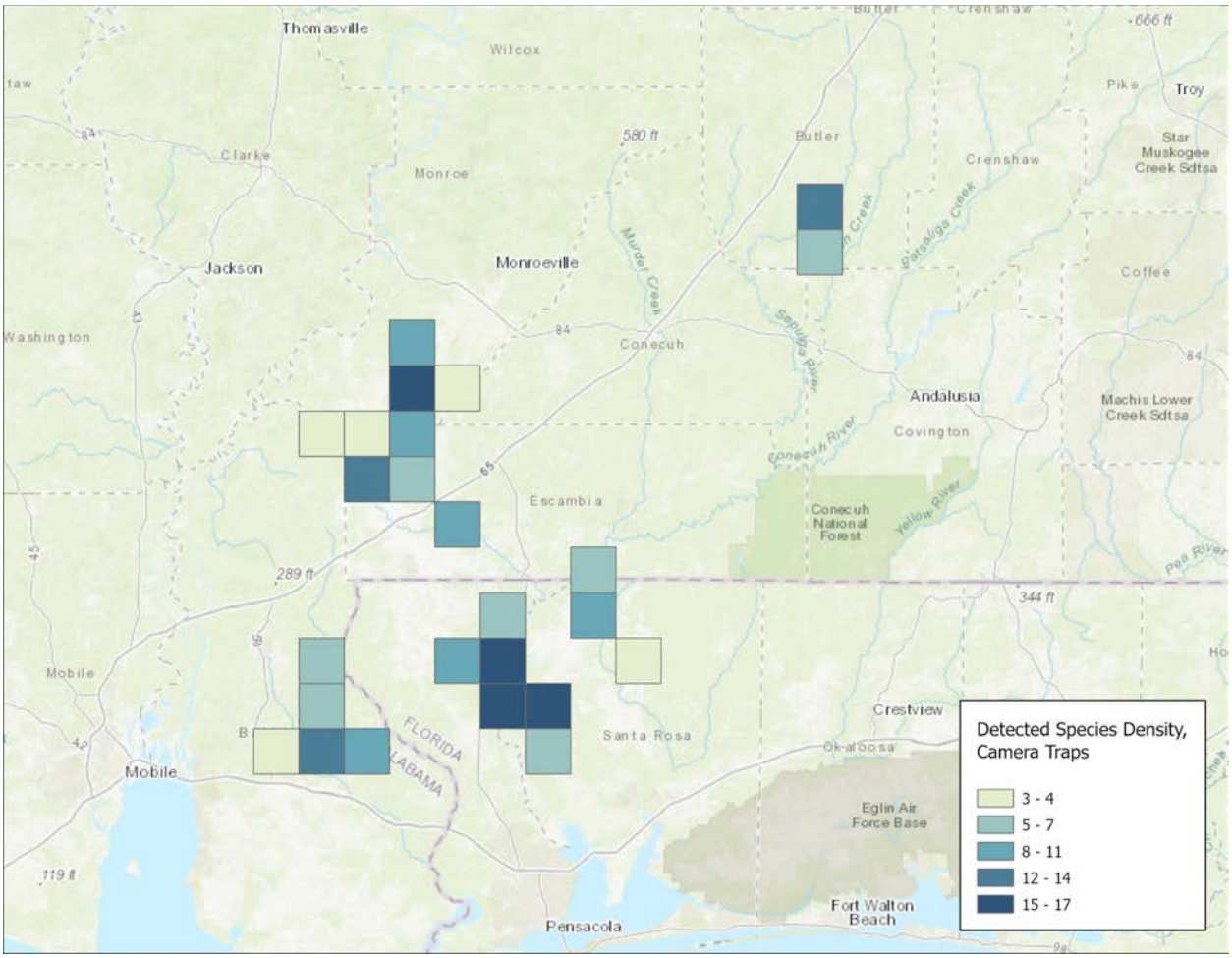

Figure 6. Density of species detected via camera traps within 10-km grid cells across the study area.

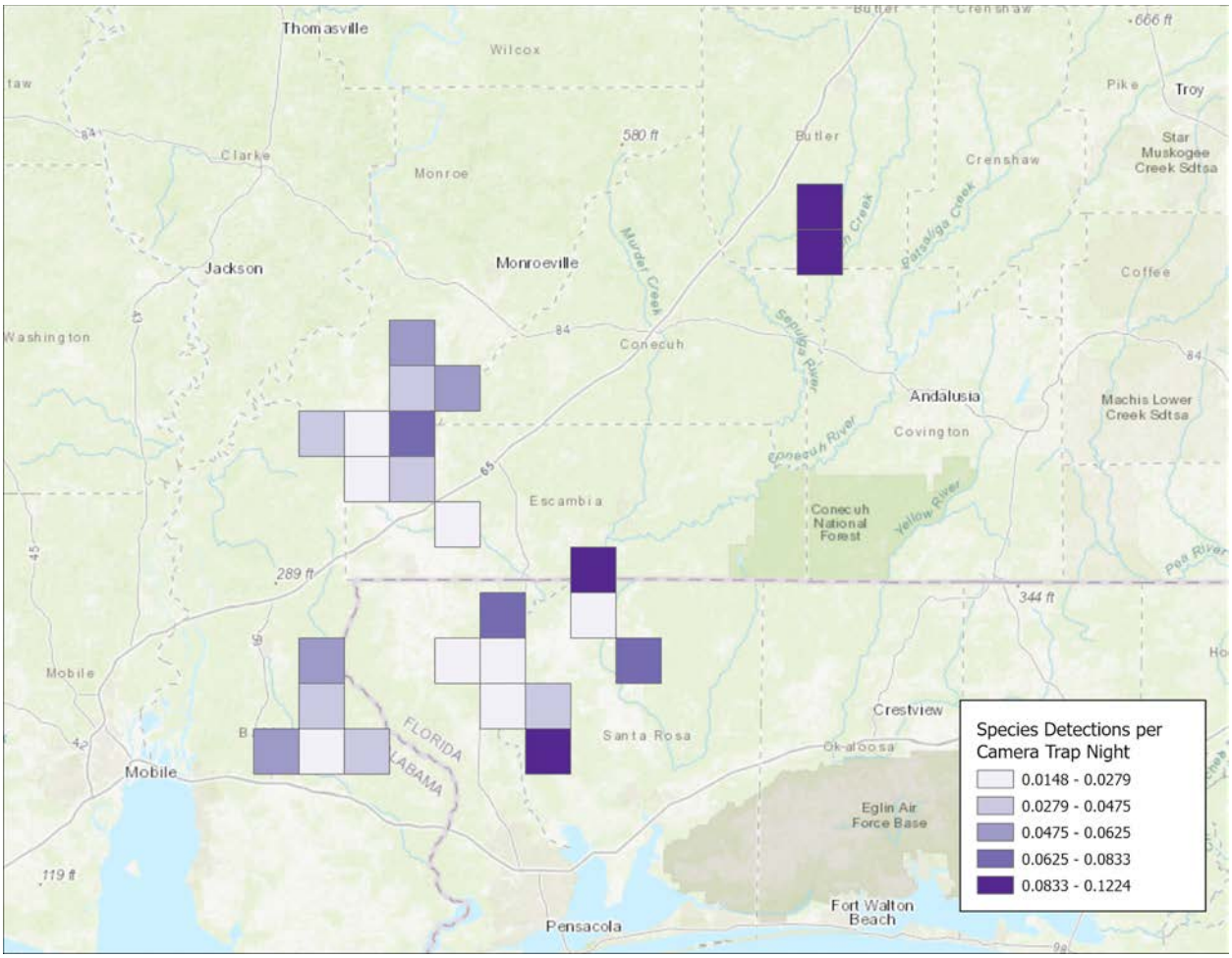

Figure 7. Number of species detections per camera trap night, summarized by 10-km grid cells.

*Visual Survey Results:*

- 45 species have been documented across 266 locations between April 2021- April 2023
    - Detected species density per 10-km grid cell can be seen below in Figure 8
- 5 target species have been identified during visual survey efforts
    - Eastern Diamond-backed Rattlesnake (*Crotalus adamanteus*)
    - Gopher Tortoise (*Gopherus polyphemus*)
    - Escambia Map Turtle (*Graptemys ernsti*)
    - Florida Pinesnake (*Pituophis melanoleucus mugitus*)
    - Red Hills Salamander (*Phaeognathus hubrichti*)
- See Figures 15-23 under “Target Species Summary" for all target species occurrences

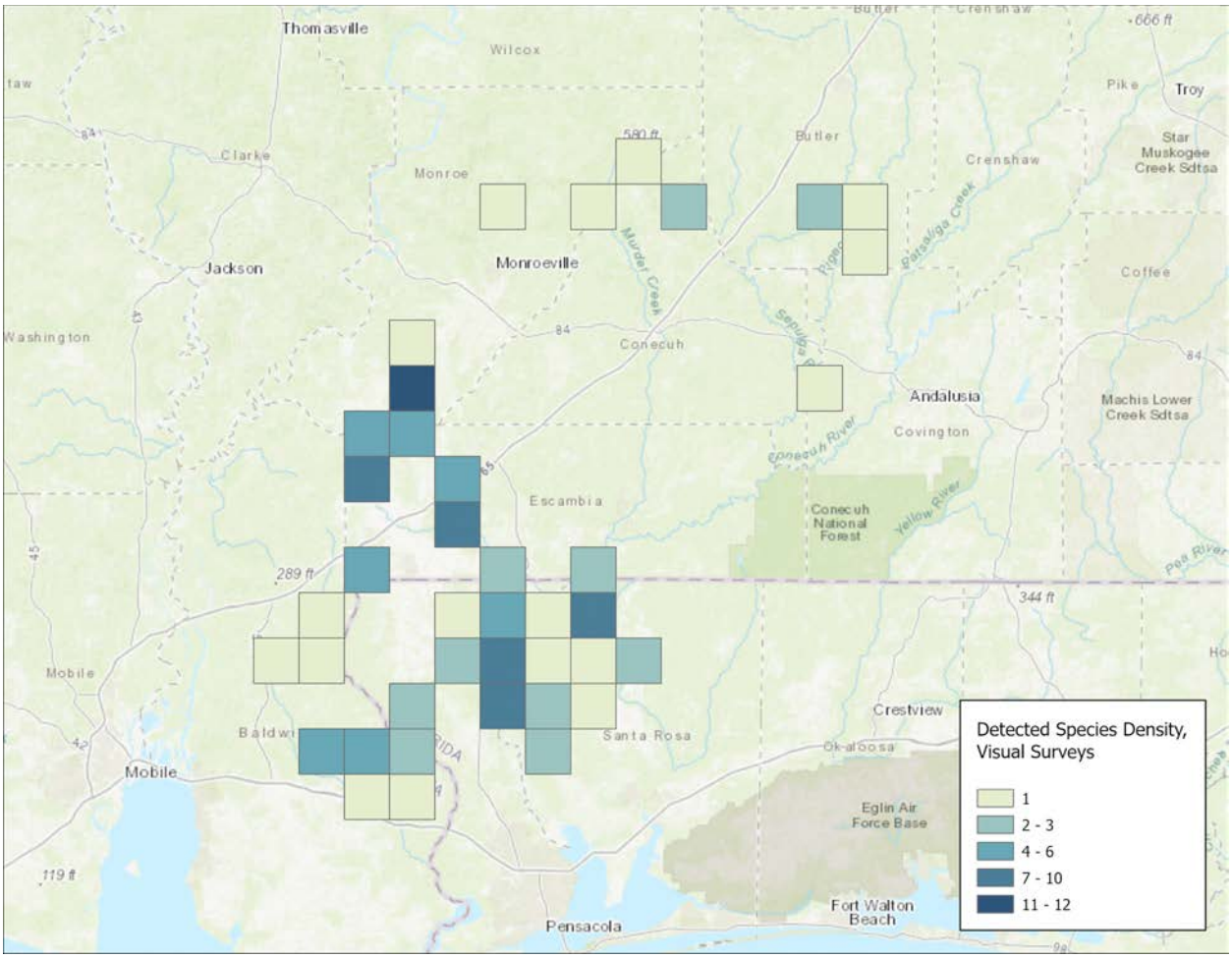


Figure 8. Density of species detected via visual surveys within 10-km grid cells across the study area.

**Method 3:** Employing environmental DNA and metabarcoding approaches for aquatic and semiaquatic organisms followed by ground truthing surveys in subsequent years of the study

*Approach:*
Sequencing environmental DNA (eDNA) has revolutionized biological surveys over the past decade. Sequencing eDNA is useful to paint a broad picture of the biological diversity in an area. eDNA surveys can be a cheap and efficient way to determine where to prioritize limited resources for more in depth field surveys, making surveys more effective, efficient, and economical. We sequenced eDNA using metabarcoding and next generation sequencing to survey for aquatic target species. Metabarcoding uses general primers to amplify all DNA in the water at a specific gene, then compares the sequences generated to a reference database to determine which species are present. Tangled Bank Conservation uses this approach for several projects and has shown it to be an effective technique for a surprising number of taxa, including bats. Thus metabarcoding provides a picture of overall aquatic biodiversity, although is susceptible to false negatives.

*Site Selection for freshwater mussel eDNA sampling effort:*
Because of the diversity and imperilment of freshwater mussels in the southeast, we aimed to quantify freshwater mussel populations on WCI property. We selected sites for exploratory surveys based on historical mussel occurrence records of target species provided by the USFWS, the Florida Museum of Natural History at the University of Florida in Gainesville, and the North Carolina Museum of Natural Sciences in Raleigh, NC, as well as peer reviewed publications of recent surveys within the target area.

*Results:*
- 251 water samples were collected from 101 sites between April 2021 and June 2023, then extracted. All sample collection locations can be seen below in Figure 9
- We made 9,096 observations identified to at least genus of 212 total taxa from 245 of the 251 samples. These 212 taxa are comprised of:
    - 101 fishes
    - 30 birds
    - 28 amphibians
    - 24 mammals
    - 17 bivalves
    - 8 reptiles
    - 5 turtles
- Rough Shiner (*Notropis baileyi*) was most abundant with catch-per-unit effort (CPUE) of 0.0916 detections/ sample
    - See Supplemental Table 2 for all Metabarcoding CPUE values
- We detected federally endangered target species Alabama Pearlshell (*Margaritifera marrianae*) in Jordan Creek
- We detected Rayed Creekshell (*Strophitus* [= *Anodontoides*] *radiatus*) in Stringer Creek. The Rayed Creekshell is a target species and is under review for federal listing
- Multiple samples had positive *Pleurobema sp.* detections. The sequences could not be assigned with confidence to a single species, but could represent *Pleurobema decisum*, *Pleurobema perovatum*, or *Pleurobema strodeanum*, which are target species

- Multiple samples had positive *Elliptio* sp. detections. The sequences could not be assigned with confidence to a single species, but could represent *Elliptio arca* or *Elliptio arctata*, which are target species
- We detected three other, non-target, species that were or are petitioned for listing: Alligator Snapping Turtles (*Macrochelys temminckii*), Pinesnakes (either a Florida or Black Pinensnake, *Pituophis melanoleucus*), and Alabama Shad (*Alosa alabamae*)

Figures 10-12 visually represent species detected per eDNA sample within 10-km grid cells across the study area. In Figure 10, the total samples collected are summarized per grid cell. In Figure 11, the total number of species detected via metabarcoding across all samples is summarized per grid cell. Figure 12 displays the calculated species-per-eDNA sample values for each 10-km grid cell.

*Validation:*
In general, metabarcoding with eDNA can have a high false negative rate, but usually a low false positive rate (Ficetola et al. 2015)). False negatives can be influenced by stream flow dynamics, seasonal changes in detectability, and the amount of DNA shed by an individual (Spear et al. 2015; Zinger et al. 2019; Klymus et al. 2021). Increasing the number of times a sample is sequenced can decrease the false negative rate (Ficetola et al. 2015). Still, it should be noted that the absence of detection does not always indicate a true absence.

Despite the false negative rates, even with rare and endangered mussels, metabarcoding has proven a powerful technique to detect most, if not more, species compared to visual encounter surveys. Numerous studies have paired eDNA surveys with visual encounter surveys to test the efficacy of eDNA-based surveys. In general, eDNA surveys for mussels are comparable to visual encounter surveys, while other surveys actually documented more species than found in visual encounter surveys (Klymus et al. 2021; Prié et al. 2021).

Given the high potential for false negatives, we will work to conduct visual encounter surveys at these locations to verify our results.

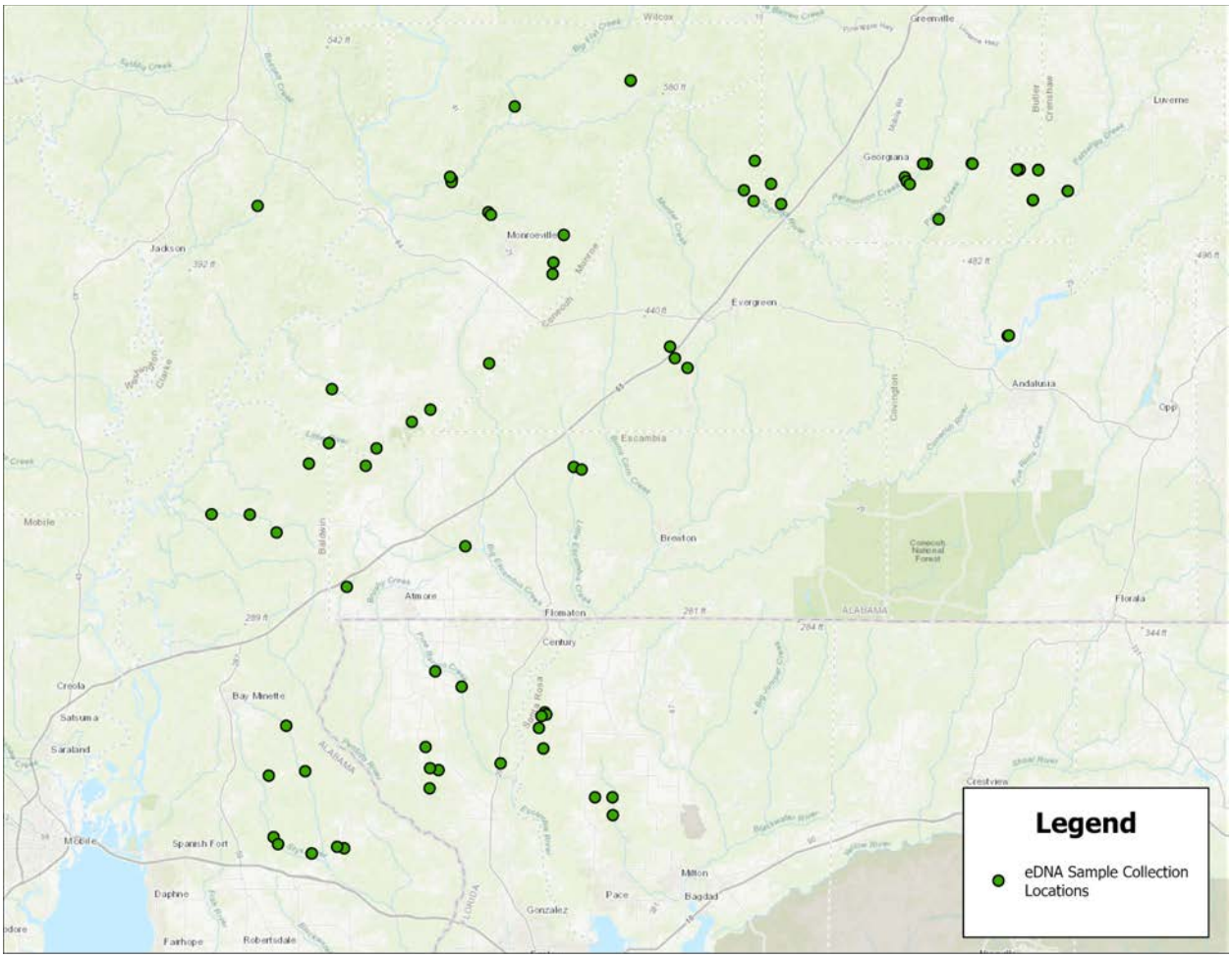

Figure 9. eDNA sample collection locations from 2021-2023.

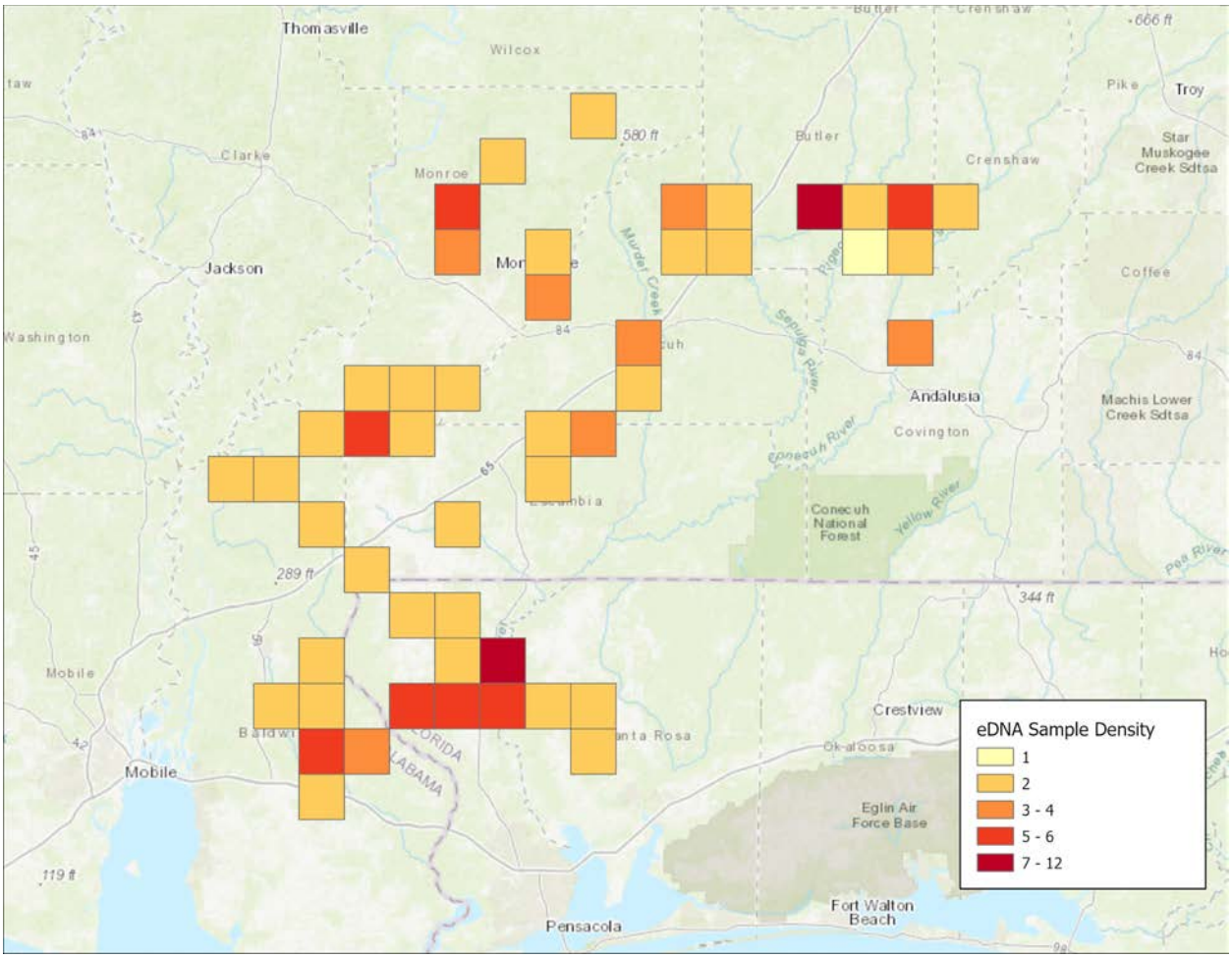

Figure 10. Density of eDNA samples collected within 10-km grid cells across the study area.

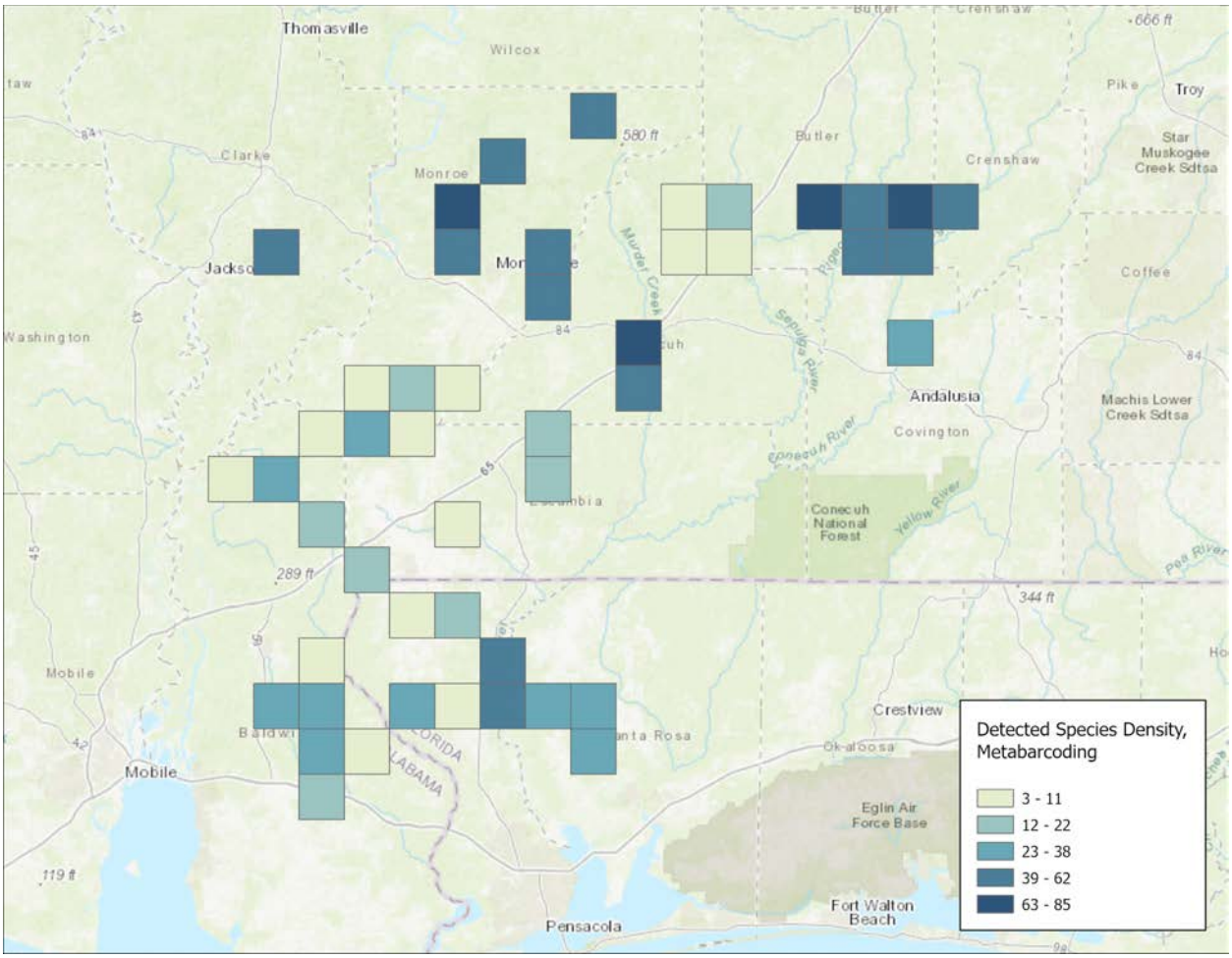

Figure 11. Density of species detected via eDNA (metabarcoding) within 10-km grid cells across the study area.

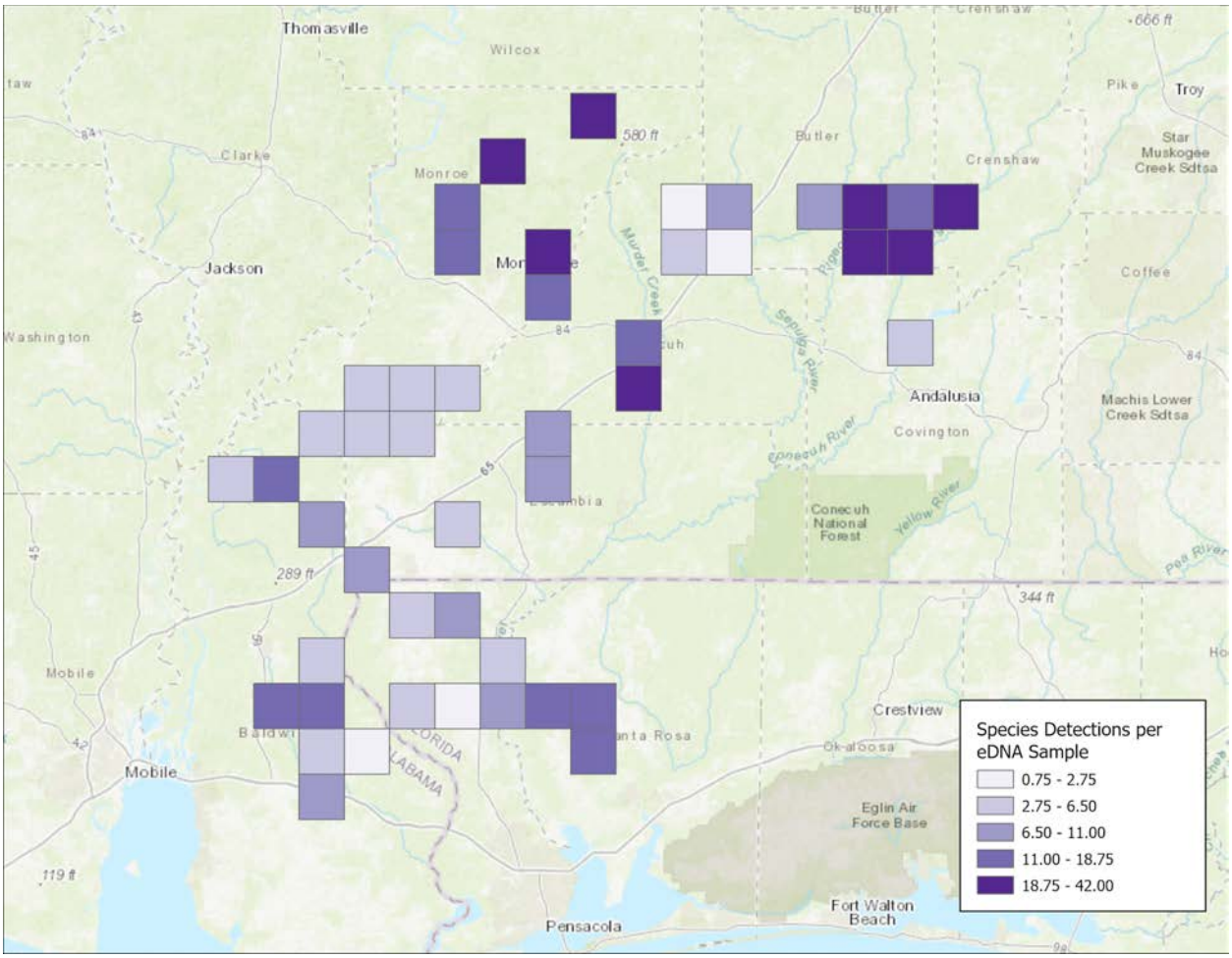

Figure 12. Species detection per eDNA sample, summarized by 10-km grid cells.

## **Method 4:** Area constrained upland pine surveys

*Approach:*
Our main goal for this approach was to document gopher tortoise (*Gopherus polyphemus*) occurrences, though there are several potential target species that occupy the same habitat. We targeted appropriate gopher tortoise xeric soils for area constrained active surveys.

*Results:*

- Occurrences or evidence of gopher tortoises (burrows, etc.) were recorded at 110 locations within the study area
    - See Figure 18 under “Target Species Summary" for all gopher tortoise occurrences
- Eastern diamond-backed rattlesnakes, another target species, were identified at 10 sites
    - 2 individuals were found within gopher tortoise burrows
    - See Figure 17 under “Target Species Summary" for all eastern diamond-backed rattlesnake occurrences

## All Methods Summary:

Across all efforts, we documented 3,871 occurrences of 271 species on WCI properties. Figure 13 shows a density map of all species detections within 10-km grid cells. A full list of all species detected across all survey methods, including their Federal, Global, and Alabama State listing status, can be found in Supplemental Table 1. A list of all species detections and their associated detection methods can be found in Supplemental Table 2.

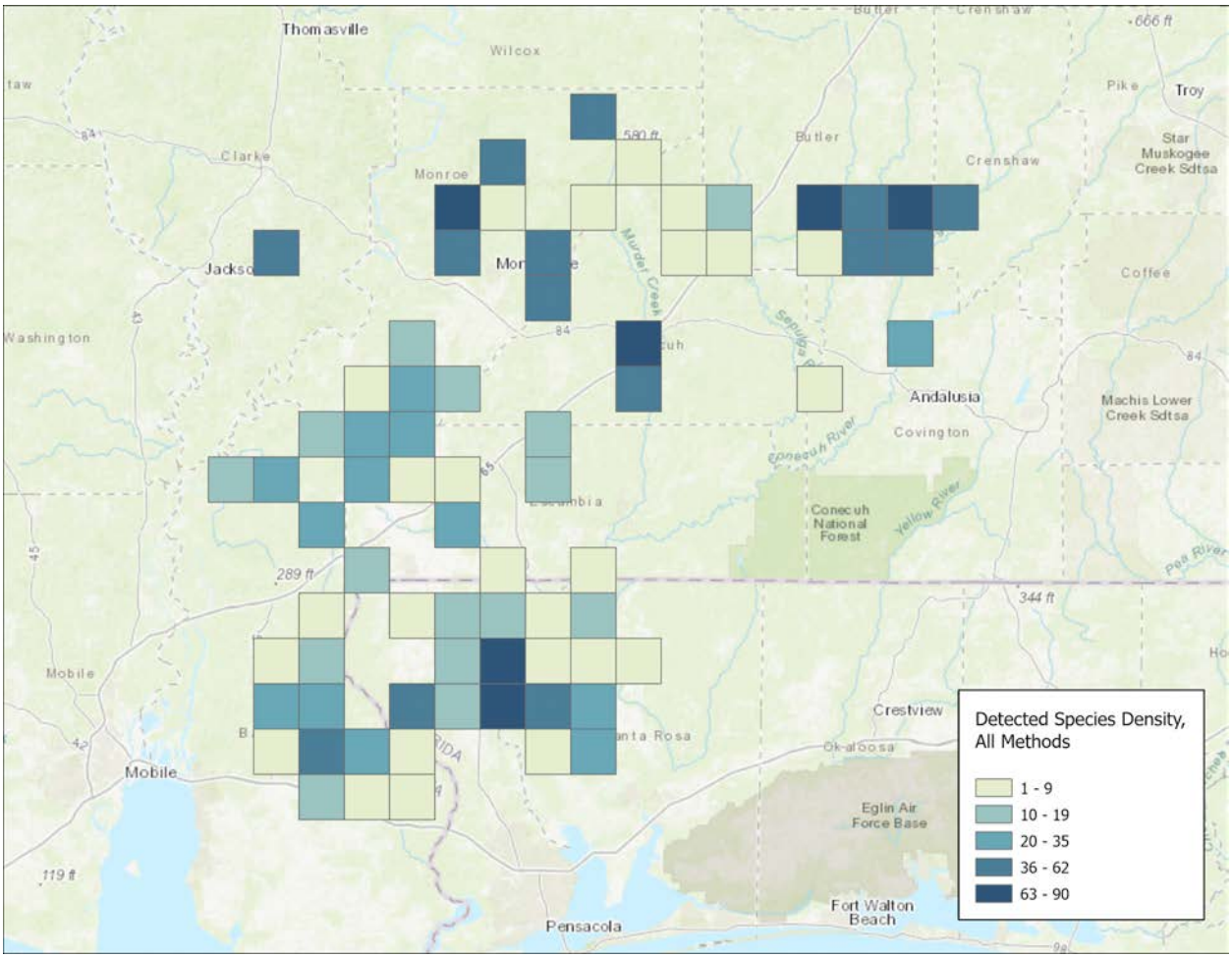

Figure 13. Density of all species detected within 10-km grid cells across the study area.

*Species Accumulation Curves:*
In order to visualize how efficiently we assessed species richness across the sites, we created species accumulation curves (see Figure 14). We binned sites that were within the same square kilometer so that we could determine how many additional species we would detect by surveying an additional square kilometer. We separated our species detections by method, then calculated the mean number of expected species for a given square kilometer using the Mao Tau estimate of species-based rarefaction (Coleman et al. 1982). We found that metabarcoding detected the greatest number of species with the least effort. Moreover, because visual surveys and camera traps were constrained in the area that they could be deployed and the number of species they

could detect, they underestimated the total biodiversity present. Put together, however, these methods can make up for some of their respective shortcomings and detected nearly 250 species. Note: because these curves represent the mean number of expected species per square kilometer, the mean number of expected species for "all methods combined" is less than just the metabarcoding because the all methods combined curve is lowered by the low numbers of expected species using camera traps and visual surveys.

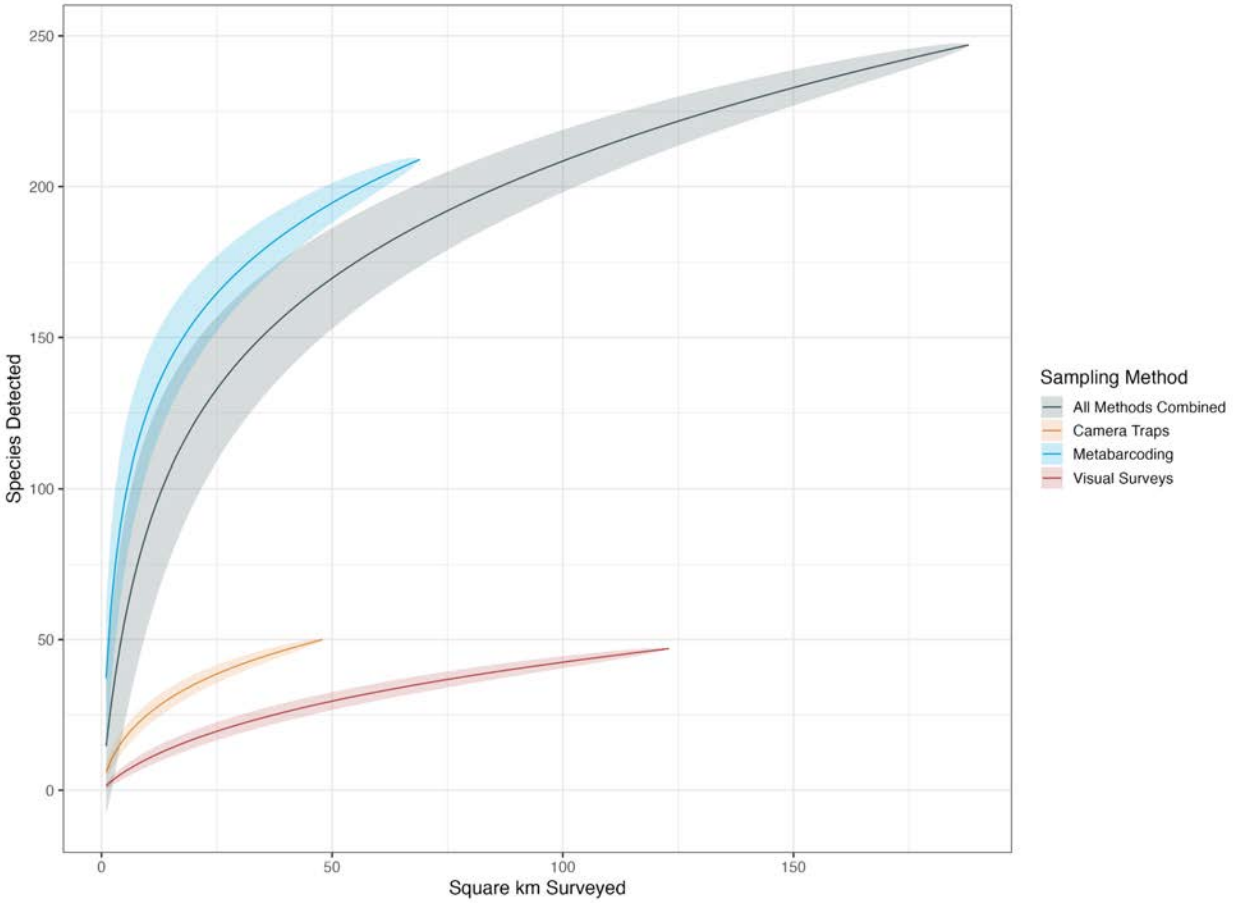


Figure 14: Species accumulation curves, separated by method, across each square kilometer surveyed.

*Target Species Summary:*

To date, 9 of 31 target species have been identified across the study area. Additionally, positive detections of 2 mussel genera that could not be confidently assigned to species may represent target species. All target species and detected target species are shown below in Table 1. Maps showing all target species detection locations can be found below in Figures 15-23.

| Target Species- Common Name | Scientific Name | Detected |
|---|---|---|
| Atlantic Sturgeon (Gulf Sturgeon) | *Acipenser oxyrinchus desotoi* | |
| Alabama Shad | *Alosa alabamae* | Yes |
| Rayed Creekshell | *Strophitus (Anodontoides) radiatus* | Yes |
| Eastern Diamond-backed Rattlesnake | *Crotalus adamanteus* | Yes |
| Eastern Indigo Snake | *Drymarchon couperi* | |
| Alabama Spike | *Elliptio arca* | *Elliptio* sp. detected |
| Delicate Spike | *Elliptio arctata* | *Elliptio* sp. detected |
| Narrow Pigtoe | *Fusconaia escambia* | |
| Gopher Tortoise | *Gopherus polyphemus* | Yes |
| Escambia Map Turtle | *Graptemys ernsti* | Yes |
| Black-knobbed Map Turtle | *Graptemys nigrinoda* | |
| Alabama Map Turtle | *Graptemys pulchra* | |
| Southern Sandshell | *Hamiota australis* | |
| Orangenacre Mucket | *Hamiota perovalis* | |
| Southern Hog-nosed Snake | *Heterodon simus* | |
| Carolina Gopher Frog | *Lithobates capito* | |
| Alligator Snapping Turtle | *Macrochelys temminckii* | Yes |
| Alabama Pearlshell | *Margaritifera marrianae* | Yes |
| Gray Bat | *Myotis grisescens* | |
| Choctaw Bean | *Obovaria choctawensis* | |
| Alabama Hickorynut | *Obovaria unicolor* | |
| Red Hills Salamander | *Phaeognathus hubrichti* | Yes |
| Florida Pinesnake | *Pituophis melanoleucus mugitus* | Yes |
| Southern Clubshell | *Pleurobema decisum* | *Pleurobema* sp. detected |
| Ovate Clubshell | *Pleurobema perovatum* | *Pleurobema* sp. detected |
| Fuzzy Pigtoe | *Pleurobema strodeanum* | *Pleurobema* sp. detected |
| Inflated Heelsplitter | *Potamilus inflatus* | |

| | | |
|---|---|---|
| Alabama Red-belly Turtle | *Pseudemys alabamensis* | |
| Southern Kidneyshell | *Ptychobranchus jonesi* | |
| Round Ebonyshell | *Reginaia rotulata* | |
| Alabama Sturgeon | *Scaphirhynchus suttkusi* | |

Table 1. All target species identified for the project.

In addition to the target species identified for this project, other noteworthy species of conservation concern detected during survey efforts include:

- Coal Shiner (*Percina brevicauda*)- AL S2, USFWS Under Review, Global G2
- Black Pinesnake (*Pituophis melanoleucus lodingi*)- AL S2, USFWS Threatened, Global T1
- Flatwoods Creekshell (*Strophitus williamsi*)- AL S2, Global G2
- Greater Siren (*Siren lacertina*)- AL S1, Global G5
- Florida Sandshell (*Lampsilis floridensis*)- AL S2, Global G4
- Cherryfin Shiner (*Lythrurus roseipinnis*)- AL S2, Global G5
- Southeastern Myotis (*Myotis austroriparius*)- AL S2, Global G4

A full species list, including Alabama State, Federal, and Global rankings, can be found in Supplemental Table 1.

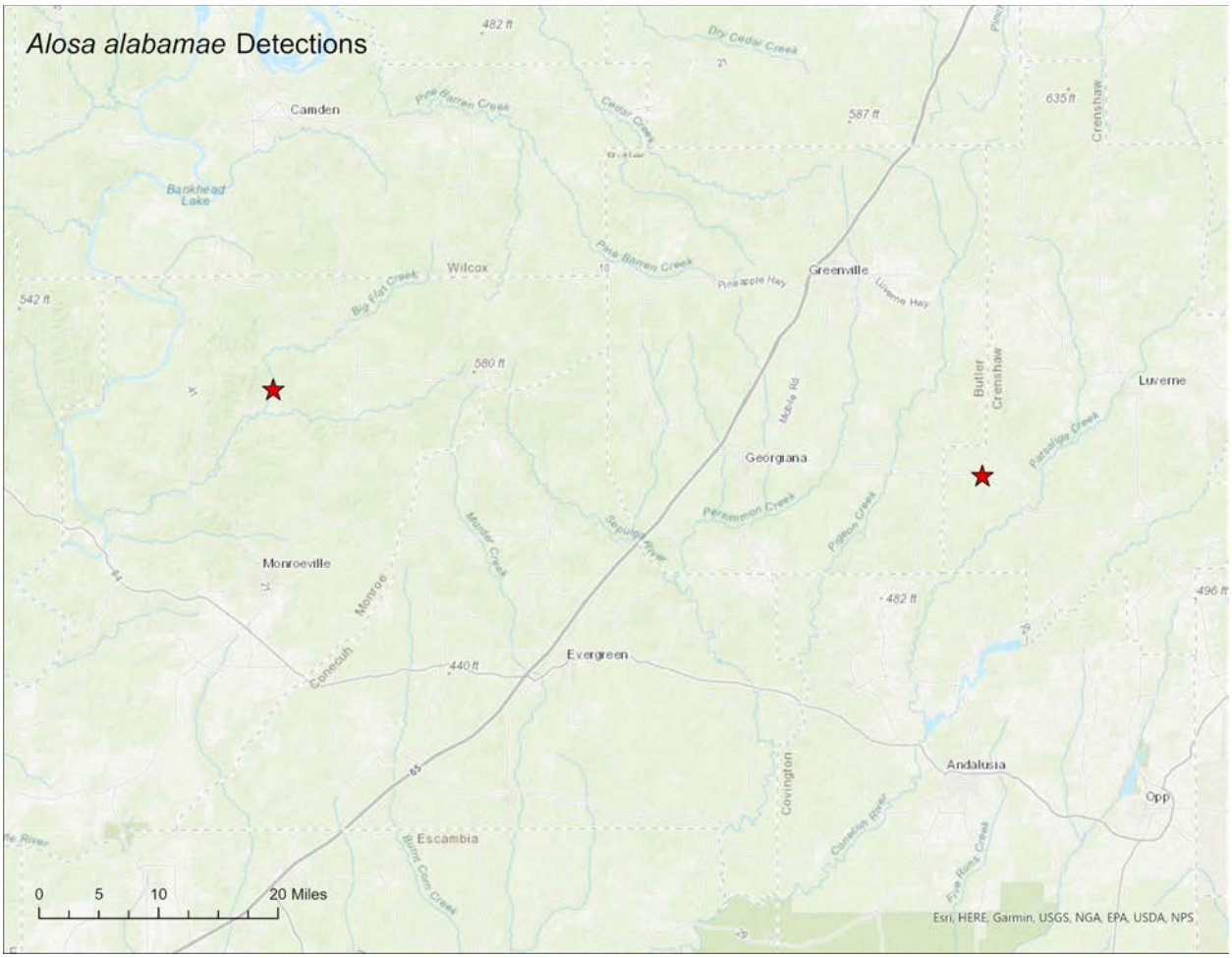

Figure 15. All Alabama Shad (*Alosa alabamae*) detections.

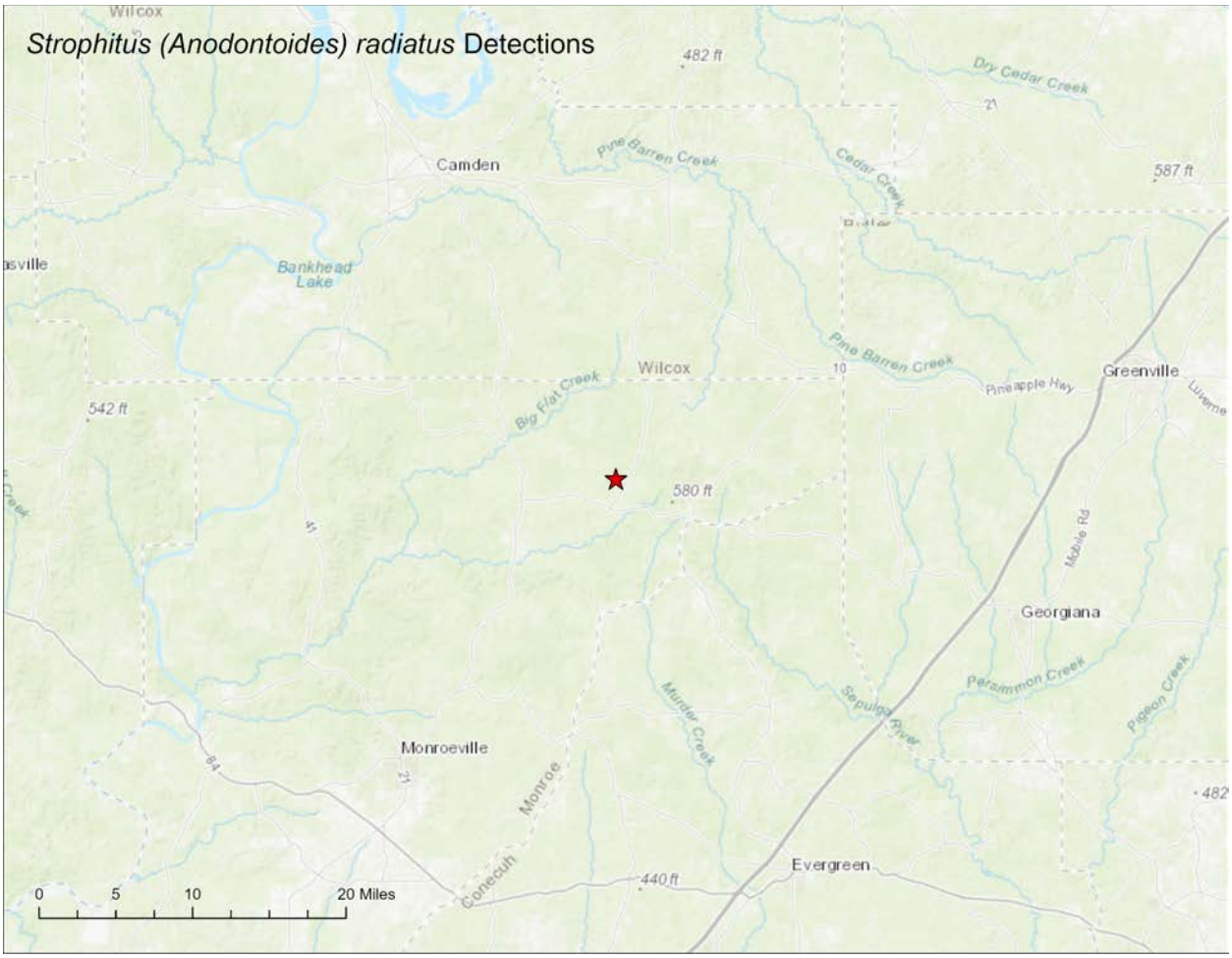

Figure 16. All Rayed Creekshell (*Strophitus (Anodontoides) radiatus*) detections.

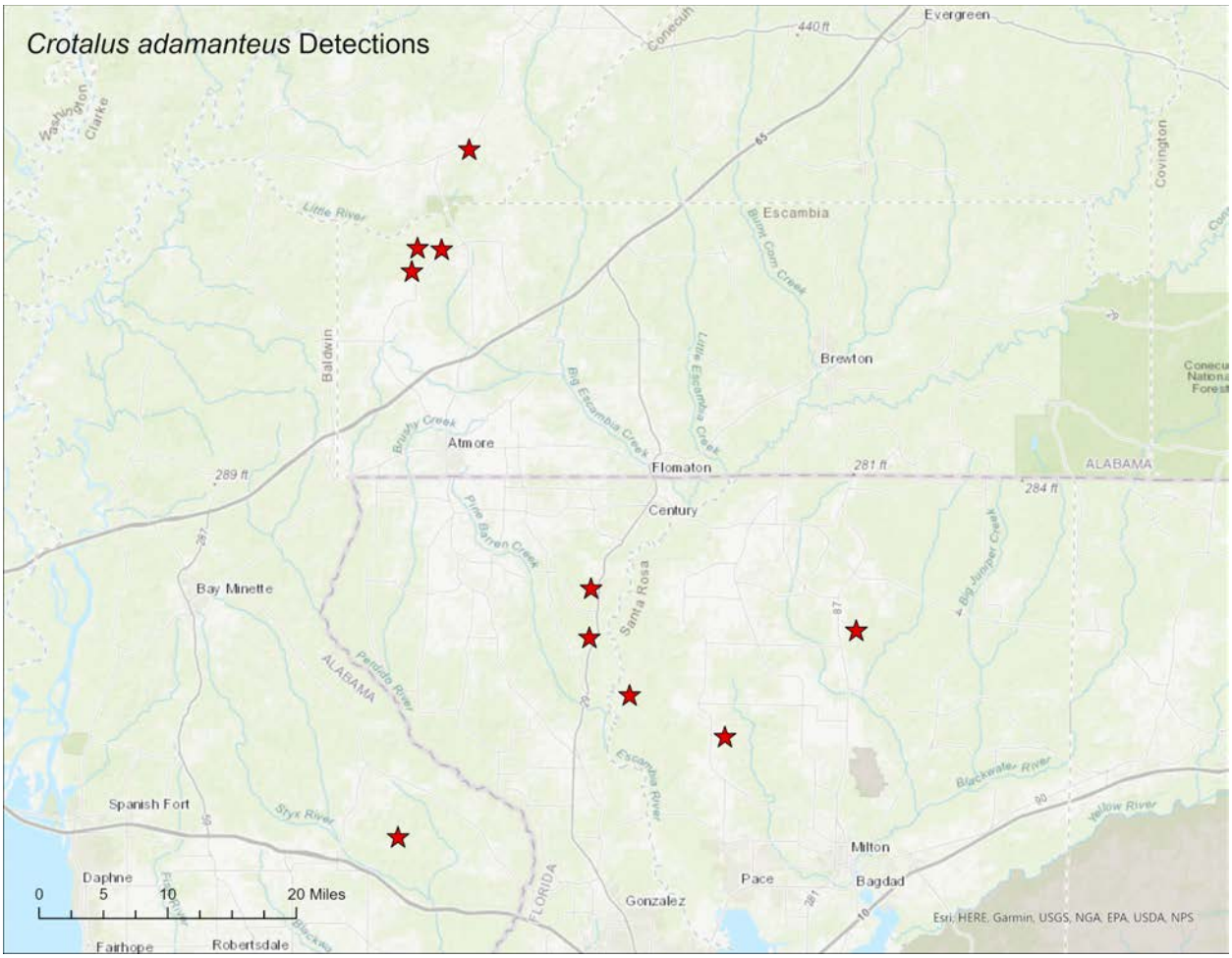

Figure 17. All Eastern Diamond-backed Rattlesnake (*Crotalus adamanteus*) detections.

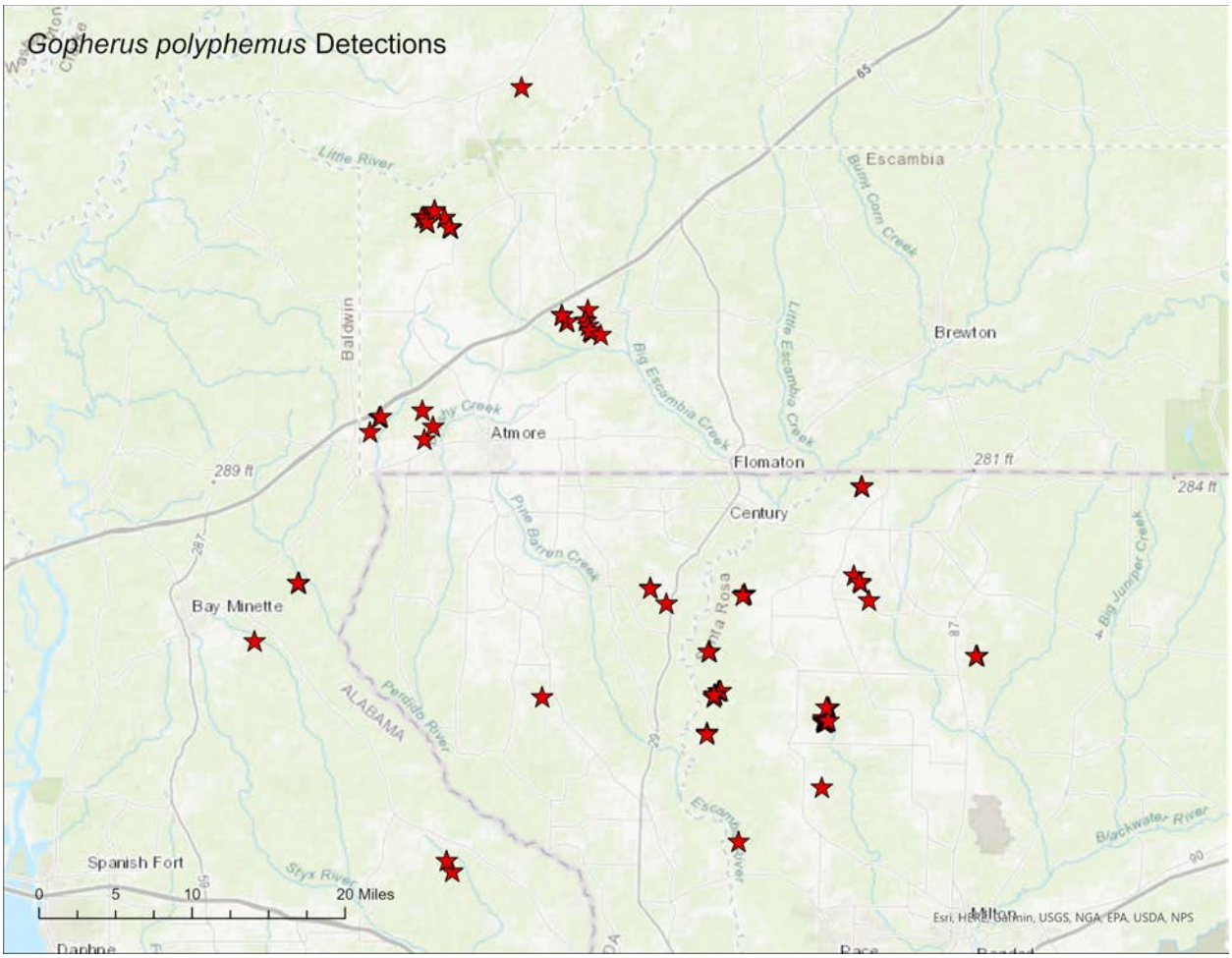

Figure 18. All Gopher Tortoise (*Gopherus polyphemus*) detections.

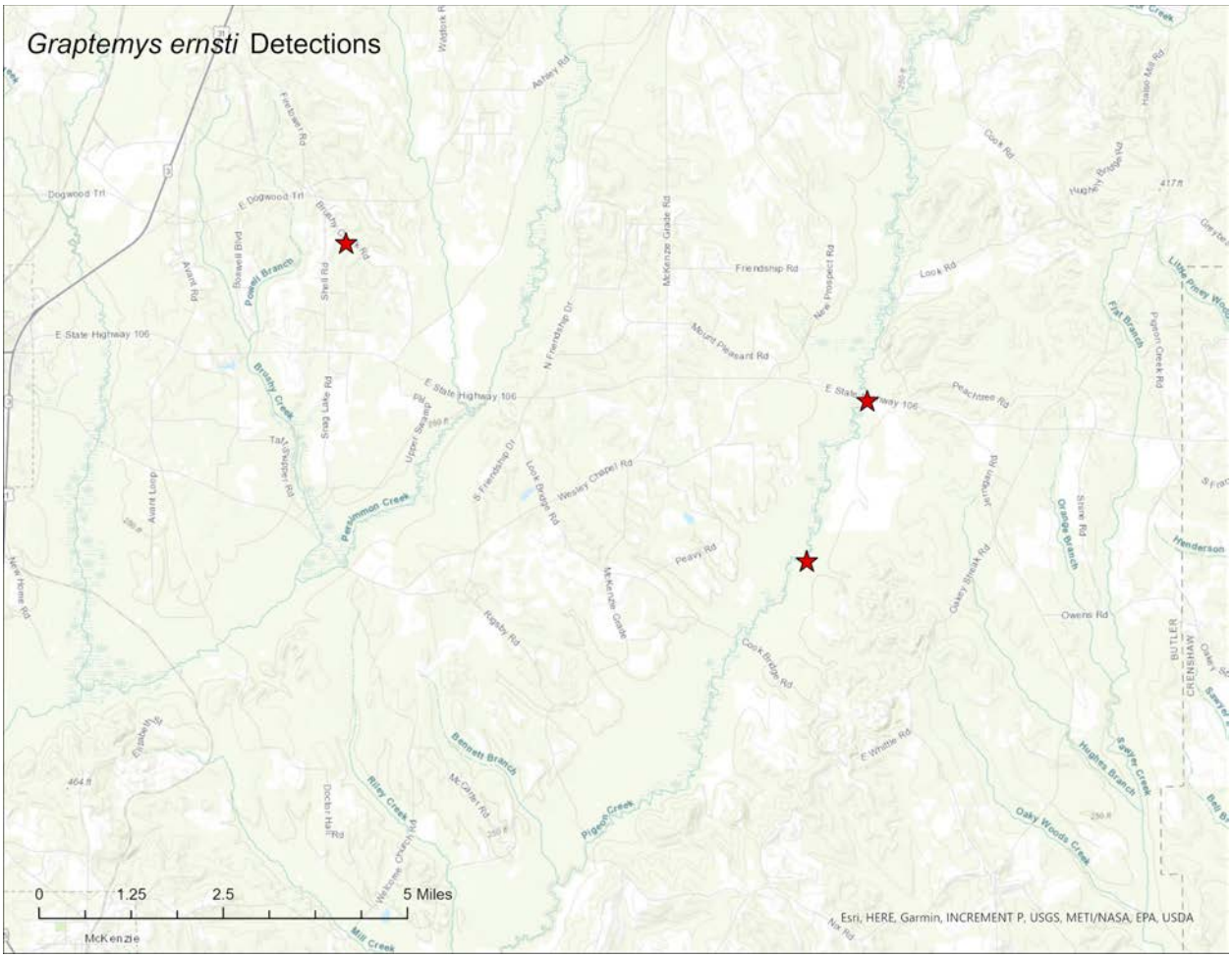

Figure 19. All Escambia Map Turtle (*Graptemys ernsti*) detections.

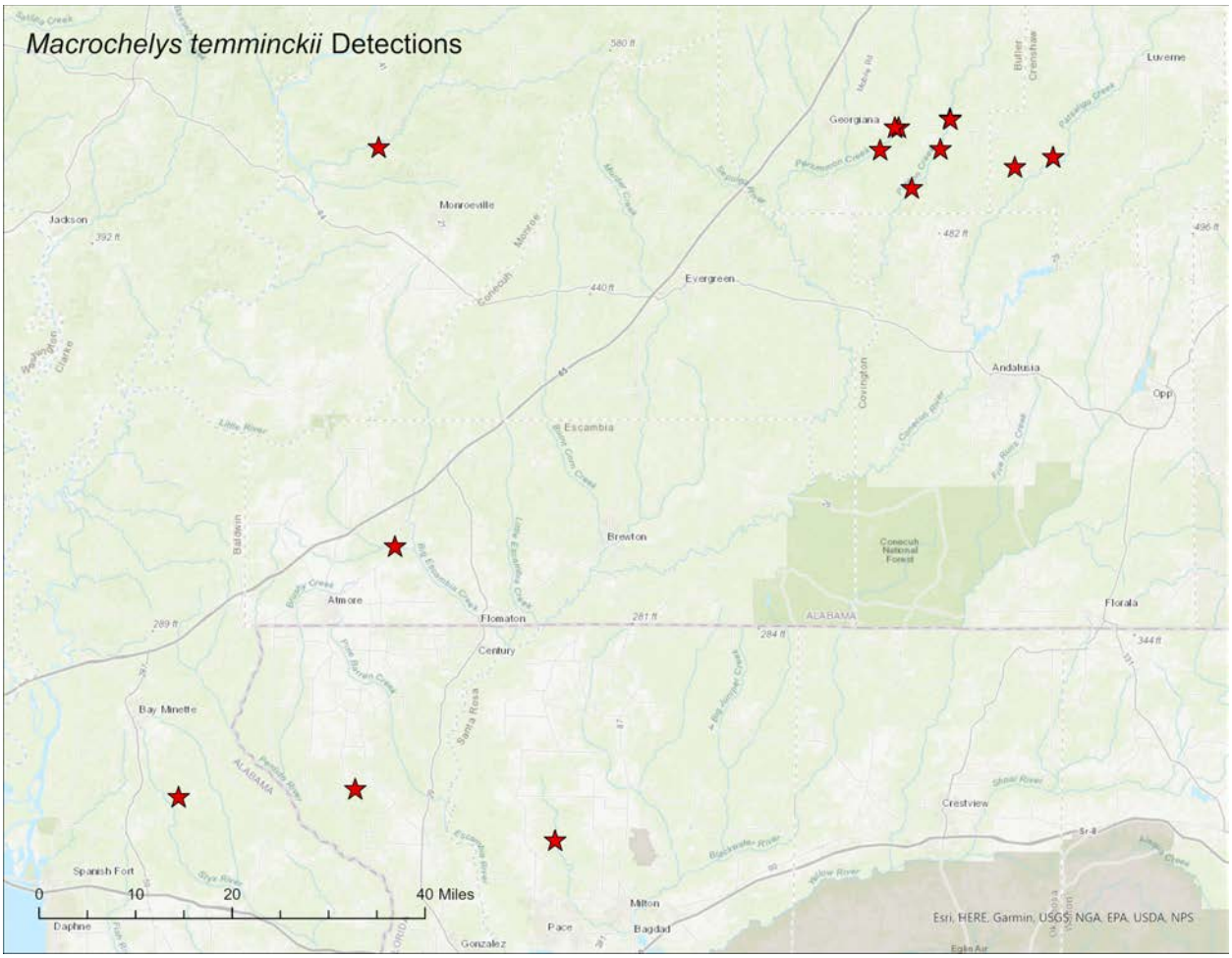

Figure 20. All Alligator Snapping Turtle (*Macrochelys temminckii*) detections.

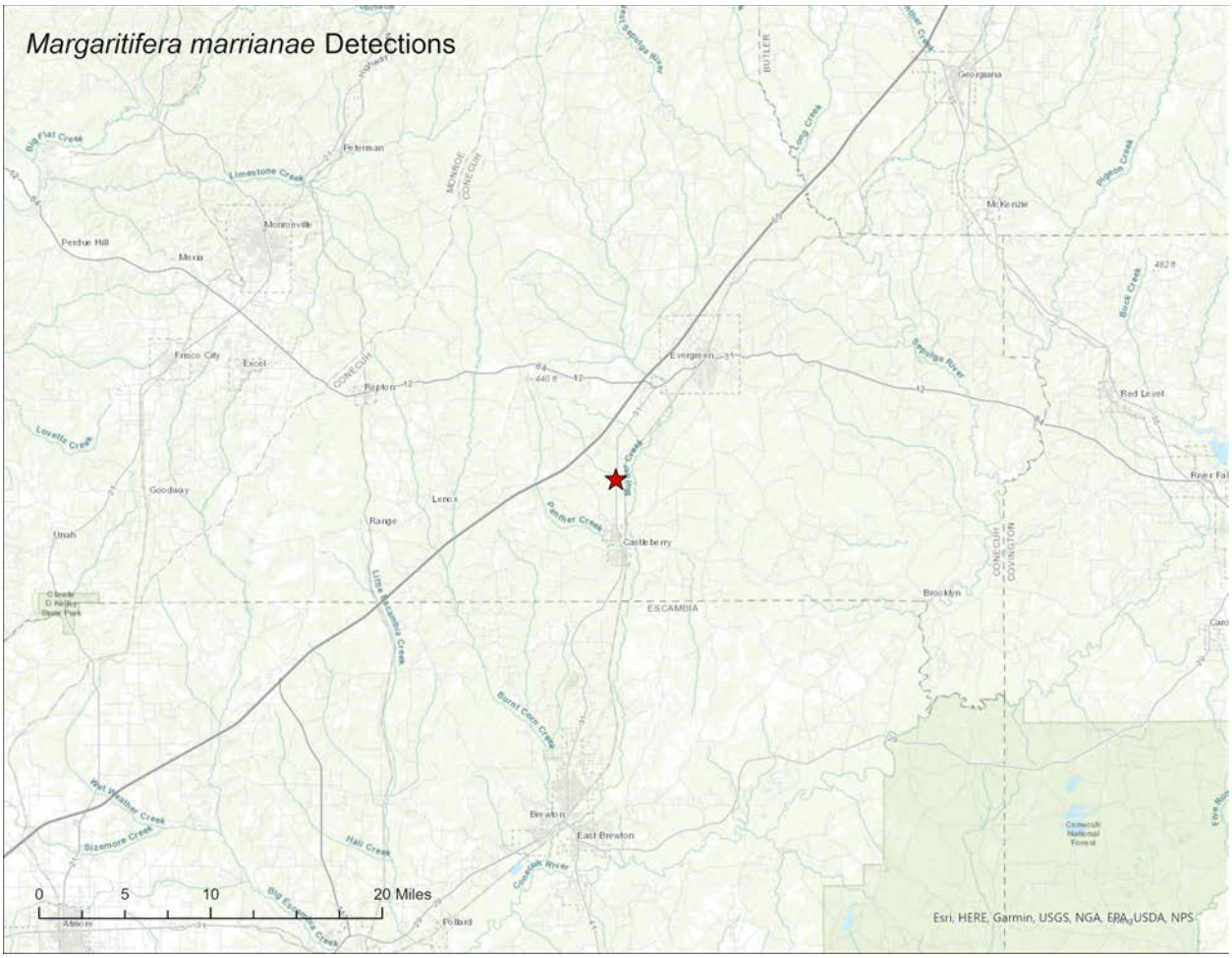

Figure 21. All Alabama Pearlshell (*Margaritifera marrianae*) detections.

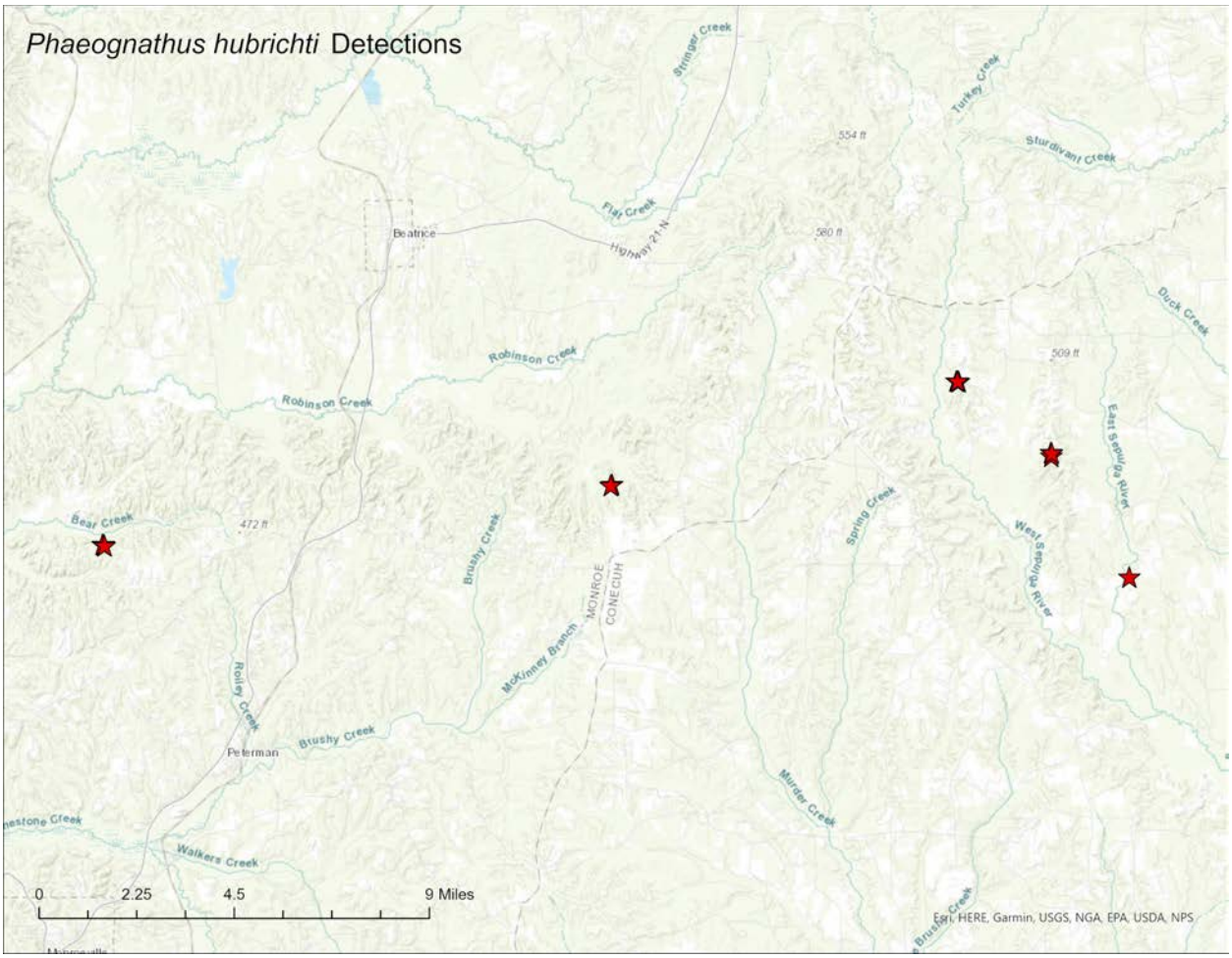

Figure 22. All Red Hills Salamander (*Phaeognathus hubrichti*) detections.

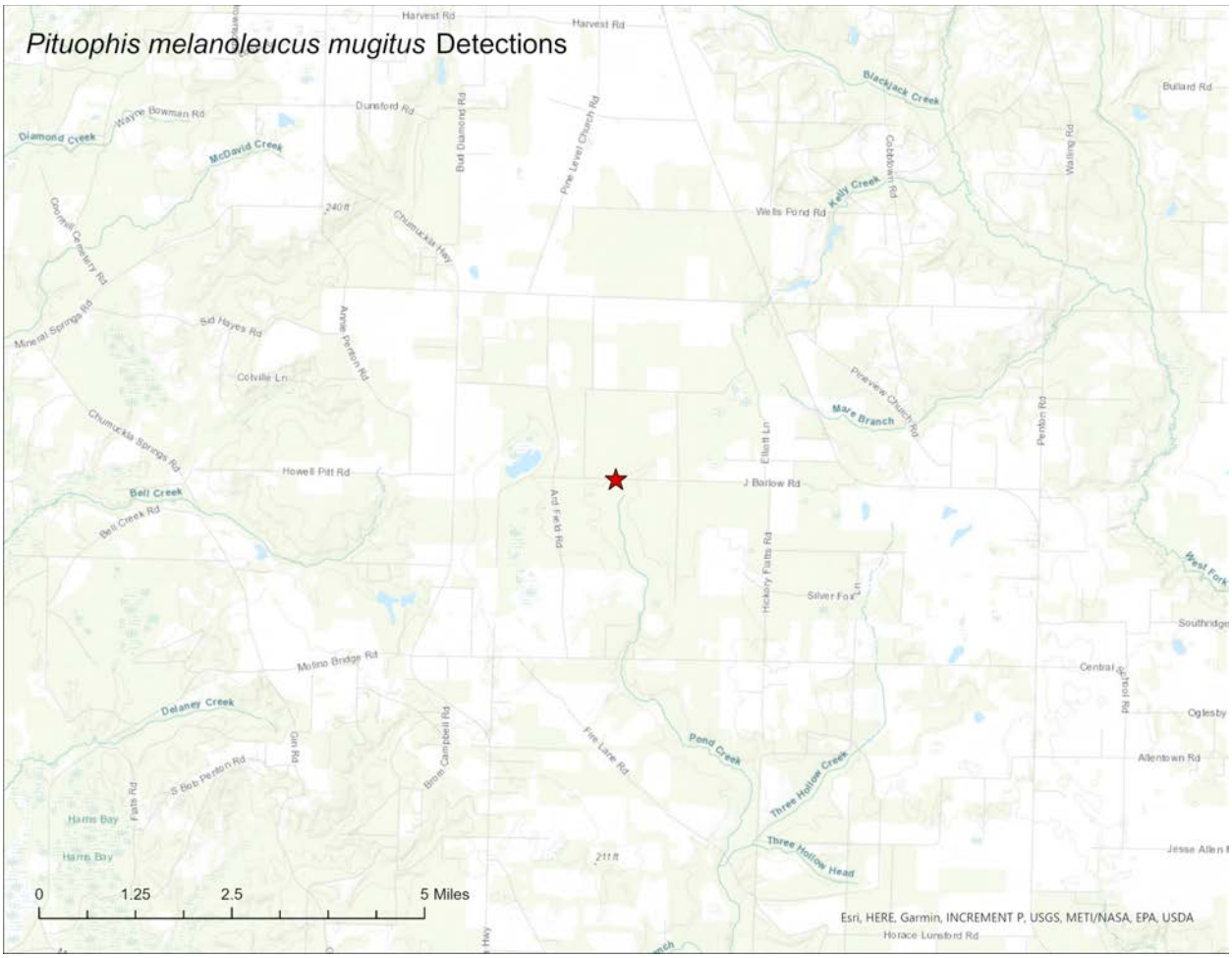

Figure 23. All Florida Pinesnake (*Pituophis melanoleucus mugitus*) detections.