# Quantifying the Contribution of Sustainable Forest Practices to at-risk Species and Terrestrial and Aquatic Communities

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# Table of Contents

# Background

The Endangered Species Act (ESA), passed by the 93rd Congress and signed into law by President Nixon in 1973, was a major milestone in our nation's efforts to stem the loss of biodiversity. It is credited with preventing the extinction of hundreds of species and has served as a model for biodiversity legislation globally. For 50 years, it has effectively created an "ESA emergency room" that has allowed us to largely prevent the extinction of some of our most imperiled species. It has been a lifeline to some of our most treasured taxa by addressing acute threats and through the protection of vital habitat. In short, it has been an astounding conservation success story. And yet, it is an incomplete one. The most notable shortcoming of the Act is its failure to recover species. Less than 3% of listed species ever achieve recovery, and thus the number of listed species continues to grow exponentially, overextending the resources available to recover a species, and shrinking the chances of recovery for any given species.

There is a growing consciousness that the ESA and the United States Fish and Wildlife Service (USFWS) cannot recover species on their own. At this pivotal time, as we face a global biodiversity crisis, the conservation community needs to leverage new paradigms, partnerships, and approaches if we aim to save our native species. In other words, we need to create a collaborative approach to conservation. Collaborative conservation can be loosely defined as an inclusive public/private partnership where parties with different motivations work on an equitable basis to address a shared conservation problem. It is important to note here that collaborative conservation differs from the model of cooperative conservation, where federal or state governments provide regulations and guidelines and landowners and other conservation practitioners faithfully follow them. In collaborative conservation, each partner must have their own objectives and both sides must work towards a common goal with an equal voice. Cooperative conservation is a useful model for protection and for mitigating losses, but collaborative conservation is a recipe for progress towards true recovery.

One such collaborative conservation effort is the Wildlife Conservation Initiative (WCI). The WCI is a voluntary, collaborative partnership between the National Alliance of Forest Owners (NAFO), the U.S. Fish and Wildlife Service (USFWS), and the National Council for Air and Stream Improvement, Inc. (NCASI) to conserve fish and wildlife species on private working forests. Given that NAFO members manage more than 46 million acres of forest in the United States, this partnership provides a massive conservation opportunity. To put it in perspective, the federal government owns and manages 37 million acres east of the Mississippi River. This prospect is even more exciting when one considers that since the mid 1990's, NAFO members have voluntarily managed their lands in accordance with third party certification, such as the Sustainable Forestry Initiative (SFI). Third party certification standards are designed to help protect water quality, biodiversity, and wildlife habitat, and thus have potentially been contributing to species recovery for roughly 3 decades. However, the large-scale implementation of these standards and their impact on imperiled species and total levels of biodiversity has yet to be assessed at a landscape scale.

There are many barriers to a landscape scale assessment of the effects of 3rd party certification standards. The first is the fragmented nature of private land ownership. It is simply difficult to obtain access and compile data across various landowners. The second is the sheer difficulty in collecting sufficient biological data at scale given the resources available. The former problem is largely solved by the creation and implementation of the WCI. The latter problem is what this project aims to solve.

The difficulty in collecting even simple biological data, such as presence/absense records, is well known. Especially for ephemeral or species that are difficult to detect. This phenomenon is so well known that it has been dubbed the “Wallacean Shortfall”. The Wallacean Shortfall is an acknowledgement that for the vast majority of species on earth we know frustratingly little about their distributions, much less about population sizes or trends, making it difficult to assess how management practices on private land are contributing to biodiversity preservation and recovery. This problem is compounded when cost is considered, as even the most basic surveys are prohibitively expensive, even at the local scale. However, recent advances in species detection have made the prospect of assessing the contribution of working forest lands to biodiversity recovery at the landscape scale possible.

In this study, we combined four complementary survey methods that, to our knowledge, have never been combined in a biological survey of this size, much less on private lands. The four techniques are a combination of classic active survey techniques and technology driven novel passive techniques, they include: freshwater turtle trapping, camera trap arrays, environmental DNA (eDNA), and visual surveys. Freshwater turtle surveys included both basking surveys and hoop net trapping. Camera traps were deployed to detect terrestrial species such as snakes, mammals, and other small vertebrates. eDNA was used to detect aquatic species using metabarcoding methods. Lastly, visual surveys were conducted to supplement the other survey methods. Visual surveys included targeted Red Hills salamander surveys, upland pine surveys, and general opportunistic surveys. Together, these methods support a comprehensive assessment of biodiversity on working forest lands.

## Scope of Work

As part of the Wildlife Conservation Initiative (WCI), this collaborative project between the U.S. Fish and Wildlife Service (USFWS), National Alliance of Forest Owners (NAFO), NCASI, Sustainable Forestry Initiative (SFI), Tangled Bank Conservation (TBC), and additional partners aims to assess the conservation value of working forest landscapes for species at risk, listed species, and broader aquatic and terrestrial communities. Surveys were conducted across WCI properties using a multi-pronged approach to detect the presence of 31 target species and provide insight into total species diversity and ecosystem health.

# Methods

## Survey Methods

Field surveys were conducted across 148 visits between March 17th, 2021 to February 18th, 2025. Survey sites were selected in collaboration with NCASI and other partners. In March 2021, 32 transects were surveyed for Red Hills salamanders across five known occurrence sites. Turtle trapping was conducted across 28 trap nights between 2021 and 2023, focusing on appropriate riverine habitats of target species. Upland pine surveys were conducted throughout the duration of the study, with timing and effort adjusted seasonally to match target species activity. 69 camera trap arrays were deployed across the study area for a total of 11,759 trap days. Lastly, 262 eDNA samples were collected across 131 sites throughout the study area, with duplicate samples collected at each location.

### Red Hills Salamander Transect Surveys

Transect surveys targeting Red Hills Salamander (*Phaeognathus hubrichti*) burrows were conducted at historically occupied sites. At each site, multiple linear transects were established in suitable habitat. Observers walked slowly along each transect, visually scanned the surrounding soil banks and leaf litter for characteristic burrow entrances, and recorded all *P. hubrichti* burrows detected within a fixed distance from each transect line.

### Freshwater Turtle Visual Surveys and Trapping

Two primary methods were used to sample 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. Alabama Red-belly bellied Turtle and Alligator Snapping Turtle were surveyed using hoop nets. In rivers with consistent downstream flow, flow baited hoop nets were used to survey Alligator Snapping Turtles. In slow moving, tidally influenced waters, unbaited hoop nets connected with a lead net (hoop and fyke net) were used to survey for Alabama Red-belly Turtles. The lead net type functions as an aquatic drift fence. Data was collected for all freshwater turtle species observed during visual surveys or captured in hoop nets to provide information on overall freshwater turtle assemblage.

### Upland Pine Visual Surveys

Visual surveys were conducted using both areas-constrained and opportunistic methods. Area-constrained surveys were specifically targeting gopher tortoises (*Gopherus polyphemus*), although there are several potential target species that occupy the same habitat. Opportunistic visual surveys were conducted as time allowed between eDNA sampling and camera trap array deployment methods.

### Camera Trap Array Deployment

Camera trap arrays were deployed across the study area in order to detect small terrestrial animals. These arrays are constructed of two bucket camera traps with an attached drift fence to guide animals into the traps. The bucket camera traps are upside-down buckets fitted with entrance and exit holes cut into each side. A motion-activated game camera sits on the inside of the bucket pointing downwards to capture photos of animals entering and exiting the bucket. This method improves upon traditional drift fence trapping, as it reduces labor intensity and increases detection of elusive species that may avoid or escape physical traps such as pinesnakes. It also captures a broader range of non-target wildlife, including small mammals and birds.

### Environmental DNA Sample Collection

Environmental DNA (eDNA) samples were collected across the study area to detect aquatic target species and assess overall aquatic biodiversity. In order to increase the likelihood of detecting target mussel species, target sites were selected based on known species occurrence records provided by the USFWS, the Florida Museum of Natural History, the North Carolina Museum of Natural Sciences, and recent peer-reviewed surveys within the region. Additional sampling locations were selected in order to provide representative coverage of WCI properties across the full extent of the study area.

At each selected site, duplicate water samples were collected following standardized field protocols designed to minimize contamination. Control samples were collected and filtered every 10 field samples. Sampling locations included streams, rivers, ponds, and wetlands across WCI properties. All water samples were collected from the surface at representative points within each site, stored on ice, and filtered the same day or shortly thereafter. Filters were preserved by freezing or desiccation and then shipped to the Tangled Bank Conservation lab for laboratory analysis using metabarcoding techniques.

## Metabarcoding Methods

We followed manufacturer’s protocols to extract DNA from each eDNA filter using the Zymo QuickDNA Water kit (Zymo Research). Following extraction, we amplified DNA with four primer sets in four reactions. We used AcMDB07 F and R 12S primers (Bylemans et al. 2018), and L14912 and H15149 cytb primers (Miya and Nishida 2000) following published thermocycler protocols to target vertebrate diversity. We used PfaCOI2_Degen COI primers and ND1_Mini_F4_Degen ND1 primers (Klymus 2020) to target mussels. For each set of primers, the PCR cocktail consisted of 10uL Kappa Hifi ReadyMix, 1uL each forward and reverse primer (at 5uM) and 8uL of eDNA template was used in the reaction. Post amplification, we treated the samples with ExoSAP-it Express (ThermoFisher Scientific) following their protocol and increased the ExoSAP-it to 4uL with 10uL of PCR product.

We individually labelled each library with unique barcode indexes via a PCR reaction using a Kapa Hifi Hotstart Readymix with i5 and i7 primers ordered from the BadDNA lab (University of Georgia). We cleaned samples using Speed Beads at 1.0x concentration, then samples were run on a gel electrophoresis and quantified via Qubit 3.0 Fluorometer (Fisher Scientific) to confirm successful amplification. We normalized and pooled libraries, then did a final clean up with a 1.0X concentration of SpeedBeads. We sequenced the libraries on an Illumina NovaSeqX sequencer with paired-end 150 bp configuration.

We used QIIME2 for metabarcoding bioinformatics (Bolyen et al. 2019). For each primer set, we compared the unknown sequences to on reference sequence reads downloaded from GenBank (NCBI). We downloaded sequences from the gene of origin that contained the primer site, and were vertebrates or mussels (depending on the primerset). We used the plugin rescript (Robeson II et al. 2021) to download sequences and taxonomy. To identify species in our raw, unknown eDNA sequences we first filtered the sequences, then classified them against the reference sequence databases. Briefly, we used Dada2 (Callahan et al. 2016) to denoise samples, remove chimeras, trim primers, and truncate reads for analysis. Next, we used the QIIME2 function “classify-consensus-blast” (Camacho et al. 2009; Bokulich et al. 2018) to classify query sequences against the GenBank reference dataset, retaining matches that had more than 90% identity with the reference sequence.

## Data Processing

To examine wildlife diversity on lands managed under the Wildlife Conservation Initiative (WCI), we excluded detections of humans (*Homo sapiens*), domestic animals (*Bos taurus*, *Felis sp*., *Gallus sp.*, and *Canis lupus familiaris*), and plants (*Serracenia sp.)* from analyses. We also excluded detections of feral swine (*Sus scrofa*), which were identified only by metabarcoding, because they cannot be distinguished from domestic pigs using available metabarcoding.. All taxa detected were classified to the highest taxonomic level possible, following the current guidelines for taxonomic classification per class (as per Grenié et al., 2023), including the eBird/Clements checklist for birds (v2024; Clements et al., 2024), The American Society of Mammologists Mammal Diversity Database (v1.13, Mammal Diversity Database, 2024), The Reptile Database (Uetz et al., 2024), Amphibian Species of the World (v6.2; Frost, 2024), FishBase (v10/2024; Froes & Pauly, 2024), and the World Register of Marine Species for mollusks (WoRMS Editorial Board, 2025). The state- and global-level conservation status of each species was determined from NatureServe (2025). All data visualization and analysis for this project were conducted in program R (v4.3.1; R Core Team, 2023).

## Compiling Historical and Crowdsourced Records

To identify species that were newly inventoried in this study compared to publicly available records from within the same study area, we compiled historical and crowdsourced records of wildlife identified to the species-level through data available via NatureServe and iNaturalist. We compiled historical species records via NatureServe Explorer (2025) by first defining the study area using the drawing tool on the mapping interface and exporting all species records. We then compiled crowdsourced species records using the package **rinat** (v0.1.9; Barve & Hart, 2022) and filtering all records that intersect with WCI-managed properties to be of Research grade and with a positional accuracy of under 5 km. Because in some cases the taxonomy of the same species differed among each data source, we consolidated all species records to use the same taxonomy based on the most up to date classifications described above.

## Retrieving Environmental Covariates

To explore spatial patterns of diversity across the study area, we extracted environmental covariates commonly associated with biodiversity from publicly available data. First, we extracted hydrological data for the study area–including the boundaries of sub-basins (HUC8), major waterbodies, and stream networks–from the NHDPlusTools database using the **nhdplusTools** package (Blodgett & Johnson, 2023). For each site, we also retrieved land cover class using the 2021 National Land Cover Database at a 30-meter resolution (NLCD; Dewitz 2023) and aggregated land cover classes under their major categorizations. Because local climatic conditions may influence biodiversity, we calculated average daily temperature and annual precipitation across a 5-year period spanning the study period— January 1, 2019 through December 31, 2023. We retrieved daily precipitation and maximum and minimum temperature data over this period for each site via Daymet V4 (Thornton et al., 2021), a dataset of estimated daily meteorological conditions at a 1-km spatial resolution. From the minimum and maximum daily temperature records accessed using package **daymetr** (v1.7.1; Hufkens et al., 2018), we calculated mean daily temperature (Thornton et al., 2000) and averaged these values over the full 5-year period.

## Estimating the Proportion of Biodiversity Inventoried

We created community matrices, indicating detection/non-detection per species per site for each of the two surveying methods, from the camera trapping and eDNA metabarcoding data. From these matrices, we calculated naïve estimates of taxon-specific species richness per study site: the number of unique species detected per major taxonomic group. We also estimated regional species richness across the study area using species accumulation curves (SACs) per major taxonomic group for each survey method using package **vegan** (Oksanen et al. 2025). For the metabarcoding and camera trap survey approaches, we constructed pooled sample-based SACs using function specaccum(). Because sampling effort (number of trap days) was unequal among sites for the camera trap approach, the SAC for each taxonomic group from camera trap data was weighted by number of days deployed per camera. From these SACS, we used the Chao2 estimator (Chao, 1987), appropriate for incidence data and

robust to the presence of rare species, to estimate the proportion of total biodiversity detectable by these methods that was inventoried during the study period via the specpool() and poolaccum() functions.

### Patterns in Local Species Richness

To examine variation in local species richness detected by camera traps among sites, we constructed two Generalized Linear Models (GLMs; Dobson & Barnett, 2018)—one for amphibians and one for reptiles—in package 'lme4' (v1.1-35.1; Bates et al., 2015), with naïve species richness as the dependent variable in each model. Because sampling effort was unequal among camera traps and the day of year (DOY) that each camera was deployed varied among sites, we included the interaction between the log effect of number of days deployed and a quadratic effect of DOY as independent variables in each model. We also included mean daily temperature, mean annual precipitation, mean relative humidity, latitude, land cover class, and distance from the nearest major waterbody as independent variables. Continuous independent variables were centered and scaled to facilitate model convergence. We used a Poisson error distribution for both models, appropriate for count data like number of species detected per site.

Prior to modelling, we checked for collinearity among covariates by fitting a global Poisson GLM for each model, then calculating the Variance Inflation Factor (VIF) for each covariate with package 'car' (v3.1-2; Fox et al., 2012). We used a cutoff of VIF > 3 for exclusion of collinear covariates from candidate models (Zuur et al., 2010). Because two pairs of covariates— the 5-year average annual precipitation and mean humidity, as well as latitude and mean temperature— were collinear in both models, we included only annual precipitation and mean temperature in the models moving forward, as we believe these two variables provide more direct measures of conditions relevant to biodiversity at each site. To assess overdispersion and goodness-of-fit of the models, we used diagnostic residual plots generated in package **DHARMa** (v0.4.6; Hartig & Hartig, 2017). Model fits were satisfactory and overdispersion was not detected in the model for amphibians ($\hat{c}$ = 1.00, Pearson's $\chi^2$ = 56.25, $p$ = 0.47) or reptiles ($\hat{c}$ = 0.89, Pearson's $\chi^2$ = 49.59, $p$ = 0.715).

### Community Compositional Dissimilarity among Sites

To examine patterns in community composition—measured here as the species identified per major taxonomic class—we created an incidence-based detection/non-detection matrix for each eDNA sampling site, using only those taxa identified to the species level. Although ideally taxa would be identified to the species level for every detection, some detections can only be classified from metabarcoding to higher taxonomic levels, such as genus. Rather than conducting dissimilarity analyses at the genus level and ignoring inter-species differences, we instead omit detections that were not identified to species, as this has been shown to be a more accurate approach for understanding community dissimilarity (*sensu* Pos et al. 2014). Thus, using these detections, we calculated per-taxon Sørensen's Dissimilarity Indices (Sørensen 1948)—the most commonly used index for incidence-based dissimilarity of species assemblages in biodiversity research—for all eDNA sampling sites and parsed their

species turnover and nestedness components (Baselga 2010, 2012) using the beta.multi() function in package **betapart** (Baselga et al. 2023).

We also estimated per-taxon pairwise differences in species composition among sites via nonmetric multidimensional scaling (NMDS; Kruskal 1964a) using the metaMDS() function in the **vegan** package. For ordination, we used the Jaccard index, appropriate for detection/non-detection data and verified that stress-levels when limiting NMDS to only 2 dimensions (k=2) did not exceed a value of 0.2, the recommended cutoff value for risking false interpretation of results (Kruskal 1964b, Clarke 1993). The goodness-of-fit for each ordination was further evaluated using the goodness() and stressplot() functions in **vegan**. Following ordination, we first visualized ecological distances on the NMDS axes to examine any clustering among and variation within sub-basin. Finally, we conducted Mantel tests (Mantel 1967; Mantel & Valand, 1970), which compare pairwise distance matrices, to determine whether dissimilarity in taxon-specific community composition is correlated with spatial distance between each pair of sites, using the vegdist() and mantel() functions in **vegan**.

# Results

## Biodiversity on WCI-managed lands: Species Inventory

Across all survey methods employed, 5,098 detections of 240 wildlife taxa were recorded during the study period (Supplementary Table 1). The taxa detected belong to 6 major taxonomic groups– fish (N=88), reptiles (N=48), birds (N=32), amphibians (N=29), mammals (N=27), and bivalves (N=16) – and span 80 families (Figure 1). Of those taxa identified to the species level, 41 are of state- and/or global-level conservation concern, including 1 designated Critically Imperiled, 7 Imperiled, and 3 Vulnerable at the global level by NatureServe (Table 1; NatureServe 2025).

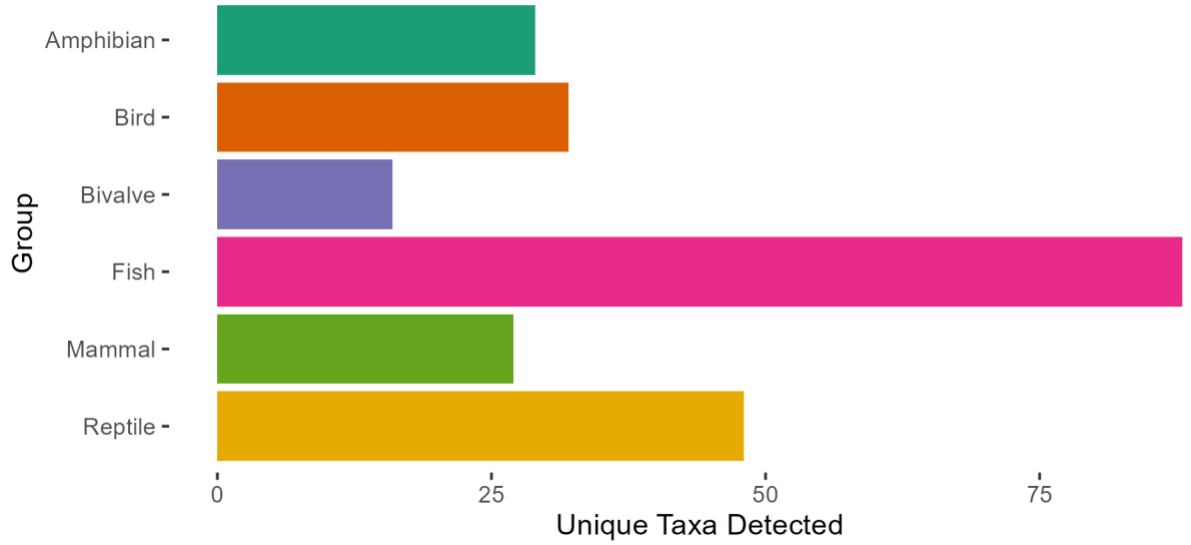
Amphibian
Bird
Bivalve
Fish
Mammal
Reptile
Group
0
25
50
75
Unique Taxa Detected


**Figure 1.** The number of taxa belonging to each of the 6 major taxonomic groups (top) and 80 families within those groups (bottom; colloquial names in parentheses) among the 240 total taxa recorded from 5,098 detections by eDNA metabarcoding, camera arrays, basking surveys of turtles, turtle traps, and visual surveys for target species between 2019 and 2024 on WCI-managed land in the southeastern USA.

**Table 1.** Species of state- and/or global-level conservation concern detected on WCI-managed land during the study period, 2019-2024. The status of each species was extracted from NatureServ, where a prefix of “S” or “G” indicates state or global conservation ranks, respectively. Suffixes indicate the following rankings at the given spatial level: 1 = Critically Imperiled, 2 = Imperiled, 3 = Vulnerable, 4= Apparently Secure, 5 = Secure, NR = Not Ranked, X = Extirpated (state) or Extinct (global), and H = Presumed Extirpated (state) or Presumed Extinct (global). State-level rankings are only reported for states in which the species was detected. All rankings extracted from NatureServe (2025).

| Taxonomic Group | Scientific Name | Common Name | FL Status | AL Status | Global Status |
|---|---|---|---|---|---|
| Amphibian | *Acris crepitans* | Northern Cricket Frog | S3 | S5 | G5 |
| | *Amphiuma means* | Two-toed Amphiuma | S4 | S3 | G5 |
| | *Phaeognathus hubrichti* | Red Hills Salamander | | S2 | G2 |
| Bivalve | *Hamiota australis* | Southern Sandshell | S1 | S2 | G2 |
| | *Lampsilis floridensis* | Florida Sandshell | S4 | S2 | G4 |
| | *Margaritifera marrianae* | Alabama Pearlshell | | S1 | G1 |
| | *Strophitus radiatus* | Rayed Creekshell | | S3 | G2 |
| | *Strophitus williamsi* | Flatwoods Creekshell | | S2 | G2 |
| Fish | *Alosa alabamae* | Alabama Shad | | S2 | G2 |
| | *Ammocrypta bifascia* | Florida Sand Darter | | S3 | G4 |
| | *Anguilla rostrata* | American Eel | S3 | S5 | G4 |
| | *Elassoma evergladei* | Everglades Pygmy Sunfish | SNR | S3 | G5 |
| | *Etheostoma histrio* | Harlequin Darter | | S3 | G5 |

| | | | | | |
|---|---|---|---|---|---|
| | *Etheostoma parvipinne* | Goldstripe Darter | S2 | S4 | G4 |
| | *Fundulus blairae* | Western Starhead Topminnow | S1 | S3 | G4 |
| | *Fundulus dispar* | Starhead Topminnow | SNR | S2 | G4 |
| | *Fundulus notatus* | Blackstripe Topminnow | | S3 | G5 |
| | *Heterandria formosa* | Least Killifish | | S3 | G5 |
| | *Lythrurus atrapiculus* | Blacktip Shiner | S2 | S4 | G4 |
| | *Lythrurus roseipinnis* | Cherryfin Shiner | SNR | S2 | G5 |
| | *Perca flavescens* | Yellow Perch | | S3 | G5 |
| | *Percina brevicauda* | Coal Shiner | | S2 | G2 |
| | *Percina vigil* | Saddleback darter | S1 | | G5 |
| | *Pteronotropis signipinnis* | Flagfin Shiner | | S3 | G5 |
| Mammal | *Myotis austroriparius* | Southeastern Myotis | | S2 | G4 |
| | *Neogale frenata* | Long-Tailed Weasel | | S3 | G5 |
| | *Tamias striatus* | Eastern Chipmunk | S3 | S5 | G5 |
| Reptile | *Agkistrodon contortrix* | Northern Copperhead | S2 | S5 | G5 |
| | *Apalone spinifera* | Spiny Softshell Turtle | | S3 | G5 |
| | *Crotalus adamanteus* | Eastern Diamondback Rattlesnake | S3 | S3 | G3 |

| | | | | | |
|---|---|---|---|---|---|
| | *Gopherus polyphemus* | Gopher Tortoise | S3 | S3 | G3 |
| | *Graptemys ernsti* | Escambia Map Turtle | | S2 | G2 |
| | *Heterodon platirhinos* | Eastern Hognose Snake | S3 | S5 | G5 |
| | *Lampropeltis getula* | Common Kingsnake | S1 | SNR | G5 |
| | *Macrochelys temminckii* | Alligator Snapping Turtle | S3 | S3 | G3 |
| | *Nerodia erythrogaster flavigaster* | Plain-Bellied Water Snake | S3 | | G5 |
| | *Pituophis melanoleucus mugitus* | Florida Pinesnake | S3 | | G4 |
| | *Plestiodon anthracinus* | Coal skink | | S3 | G5 |
| | *Plestiodon anthracinus pluvialis* | Coal skink | | S3 | G5 |
| | *Plestiodon inexpectatus* | Southeastern Five-Lined Skink | S4 | S3 | G5 |
| | *Tantilla coronata* | Southeastern Crowned Snake | S2 | | G5 |

Among the 31 target species we aimed to inventory during this study (Supplementary Table 2), we detected 10 to species-level and an additional 8 to genus-level at least once during the study period on land managed by WCI (Table 2). The spatial distribution of these detections varied among target taxa (Figure 2; individual species panels provided in Supplementary Figure 1). The remaining 13 target species were not detected in this study.

**Table 2.** The detection history of 31 target species across all sampling approaches in a study aiming to inventory biodiversity on WCI-managed lands in the southeastern USA, 2019-2024. If a detection by metabarcoding from environmental DNA that was classified to the genus level shared the same genus with a target species that was otherwise not detected to the species level during the study, this unknown species sharing the same genus as the target species is indicated in the "Detection" column.

| **Common name** | **Taxon** | **Detection history** |
|---|---|---|
| Atlantic (Gulf) Sturgeon | *Acipenser oxyrinchus desotoi* | |
| Alabama Shad | *Alosa alabamae* | Yes |
| Carolina Gopher Frog | *Aquarana (=Lithobates) capito* | *Aquarana* sp. detected |
| 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* | |
| Round Ebonyshell | *Fusconaia (=Reginaia) rotulata* | |
| 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 (=Lampsilis) australis* | Yes |
| Orangenacre Mucket | *Hamiota (=Lampsilis) perovalis* | *Lampsilis sp.* detected |
| Southern Hog-nosed Snake | *Heterodon simus* | *Heterodon sp.* detected |
| Alligator Snapping Turtle | *Macrochelys temminckii* | Yes |

| | | |
|---|---|---|
| Alabama Pearlshell | *Margaritifera marrianae* | Yes |
| Gray Bat | *Myotis grisescens* | |
| Choctaw Bean | *Obovaria (=Villosa) 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* | |
| Alabama Sturgeon | *Scaphirhynchus suttkusi* | |
| Rayed Creekshell | *Strophitus radiatus* | Yes |

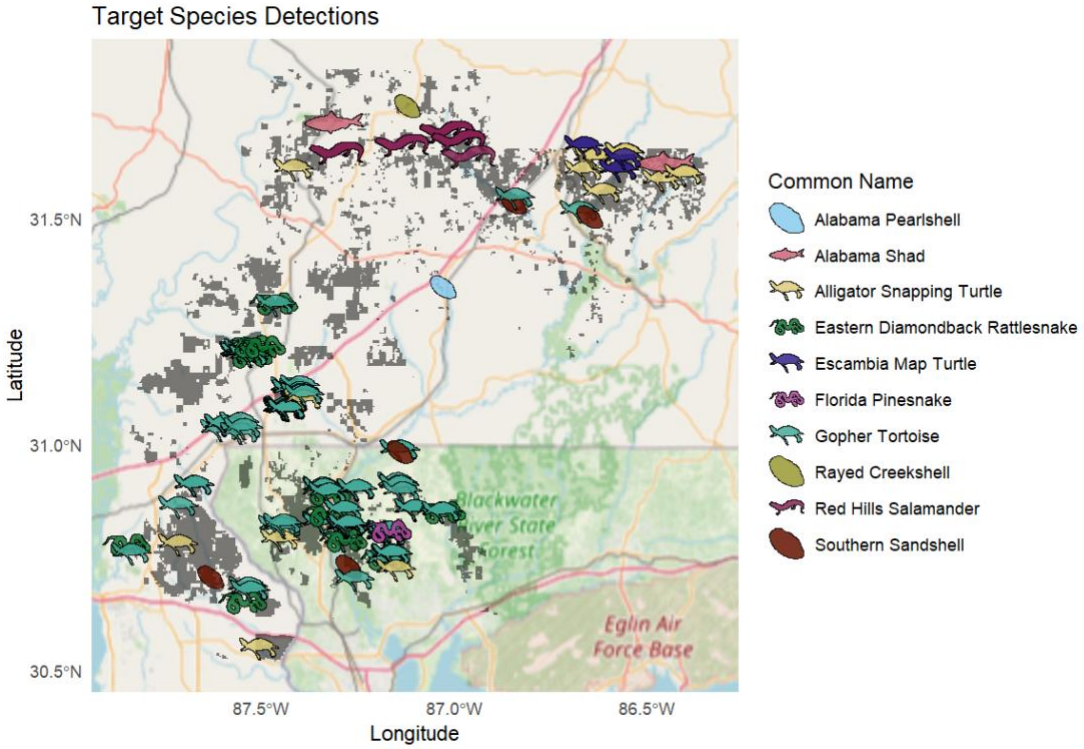


**Figure 2.** The locations of target species detections on WCI-managed lands, 2019-2024. The shape of each data point represents the taxonomic order of each detection (Bivalves = Unionida, Salamanders = Caudata, Snakes = Squamata, and Turtles = Testudines) and color indicates species. Wildlife silhouettes are downloaded from PhyloPic (www.phylopic.org) and used here with permission under a Creative Commons Universal Public Domain Dedication License (CC0 1.0). Individual species panels provided in Appendix 1 (Supplementary Figure 1).

## Species Inventoried by TBC Compared to Historical and Crowdsourced Records

To identify species that were newly detected in this study by Tangled Bank compared to those with previous publicly available detections, we compared the inventory accumulated here to historical records available through NatureServe and crowdsourced observations on iNaturalist for the same geographic area. We documented 138 species for which we found no previous records through NatureServe or iNaturalist within the study area (Supplementary Table 3). Overall, we identified 231 wildlife species in the study area among the major taxonomic groups of amphibians, birds, bivalves, fish, mammals, and reptiles, whereas 99 and 141 total species among the same taxonomic groups had publicly available records through NatureServe or iNaturalist, respectively (Table 3).

**Table 3.** The number of amphibian, bird, bivalve, fish, mammal, and reptile species detected in the study area by Tangled Bank Conservation (TBC), historical records available on NatureServe, and

crowdsourced observation from iNaturalist. The total number of unique species in these taxonomic groups detected by at least one of the data sources provided in the rightmost column.

| Taxonomic Group | TBC | NatureServe | iNaturalist | Total Unique Across All Sources |
|---|---|---|---|---|
| Amphibian | 29 | 9 | 22 | **41** |
| Bird | 29 | 20 | 46 | **76** |
| Bivalve | 15 | 12 | 5 | **24** |
| Fish | 86 | 23 | 19 | **108** |
| Mammal | 27 | 10 | 10 | **36** |
| Reptile | 45 | 25 | 39 | **67** |
| **Total** | **231** | **99** | **141** | **352** |

## Red Hills Salamander Transect Surveys

We conducted transect surveys for endangered Red Hills salamander (*Phaeognathus hubrichti*) burrows on March 20 and 21, 2021. Among 5 sites with known *P. hubrichti* presence, we surveyed 32 total transects, for an average of 6.4 transects per site (minimum = 5, maximum = 9). The lengths of transects varied, averaging 22.2 meters (minimum = 14.3m, maximum = 30.5m). We recorded all *P. hubrichti* burrows within 1.5m of either side of the transect line. We detected 31 total *P. hubrichti* burrows, averaging 0.97 burrows per transect (minimum = 0, maximum = 5). The average density of burrows across all sites was 1.49 burrows per 100 square meters (minimum = 0.54 burrows/100$m^2$, maximum = 2.7 burrows/100$m^2$; Figure 3). These targeted transects were the only method to detect *P. hubrichtii* during the study.

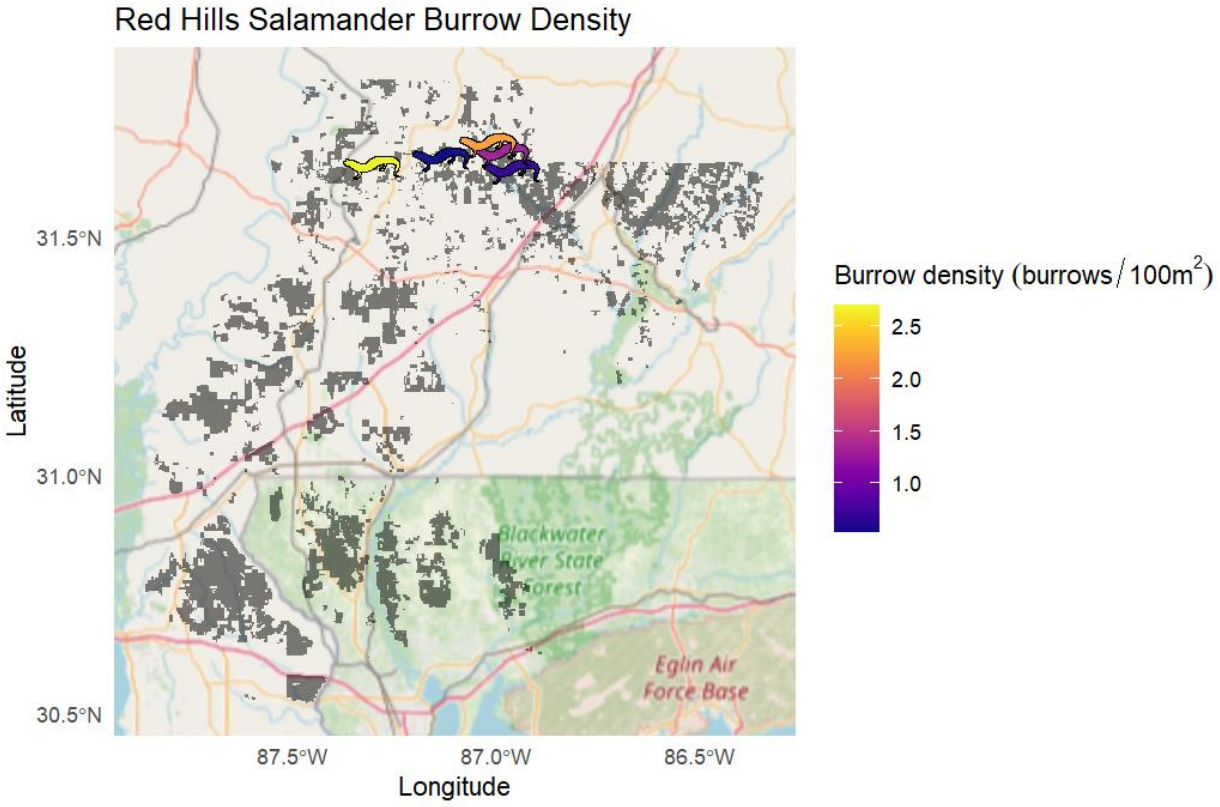


**Figure 3.** Mean Red Hills salamander (*Phaeognathus hubrichti*) burrow density detected in transect surveys at five sites in the southeastern USA, March 20—21, 2021. Survey locations are indicated by each salamander icon and colored by observed burrow density per 100 square meters. Salamander silhouette is downloaded from PhyloPic (www.phylopic.org) and used here with permission under a Creative Commons Universal Public Domain Dedication License (CC0 1.0).

## Freshwater Turtle Visual Surveys and Trapping

Between 2021 and 2023, we deployed hoop net traps targeting freshwater turtle species (N=28 trap nights) and conducted visual surveys for basking individuals on WCI-managed lands. From these methods, we detected six species: Spiny Softshell Turtle (*Apalone spinifera*), Common Snapping Turtle (*Chelydra serpentina*), Alligator Snapping Turtle (*Macrochelys temminckii*), Intermediate Musk Turtle (*Sternotherus intermedius*), Stripe-necked Musk Turtle (*Sternotherus peltifer*), and Pond Slider (*Trachemys scripta*). Across all six survey methods used in this study, visual basking surveys were the only approach to detect *G. ernsti* and *T. scripta*, while turtle traps were the only approach to detect *A. spinifera* and *S. intermedius* (Supplementary Table 4). The detection versus non-detection of these species by visual surveys and trapping varied across the study area (Table 4 and Figure 4).

**Table 4.** Summary table for sampling effort (trap nights) and the detection ("x") versus non-detection (blank cell) from visual surveys and hoop nets targeting freshwater turtle species across WCI-managed lands in the southeastern United States, 2021-2023. (*A. spinifera* = *Apalone spinifera*, *T. scripta* = *Trachemys scripta*, *C. serpentina* = *Chelydra serpentina*, *S. peltifer* = *Sternotherus peltifer*, *S. intermedius* = *Sternotherus intermedius*.)

| **Location** | **Trap nights** | ***A. spinifera*** | ***M. temminckii*** | ***T. scripta*** | ***C. serpentina*** | ***S. peltifer*** | ***S. intermedius*** |
|---|---|---|---|---|---|---|---|
| Little River | 2 | x | | | | x | |
| Persimmon Creek | 4 | | | x | | | |
| Pigeon Creek | 15 | x | x | x | x | | |
| Sizemore Creek | 4 | | x | | | | |
| Styx River | 3 | | | | | | x |

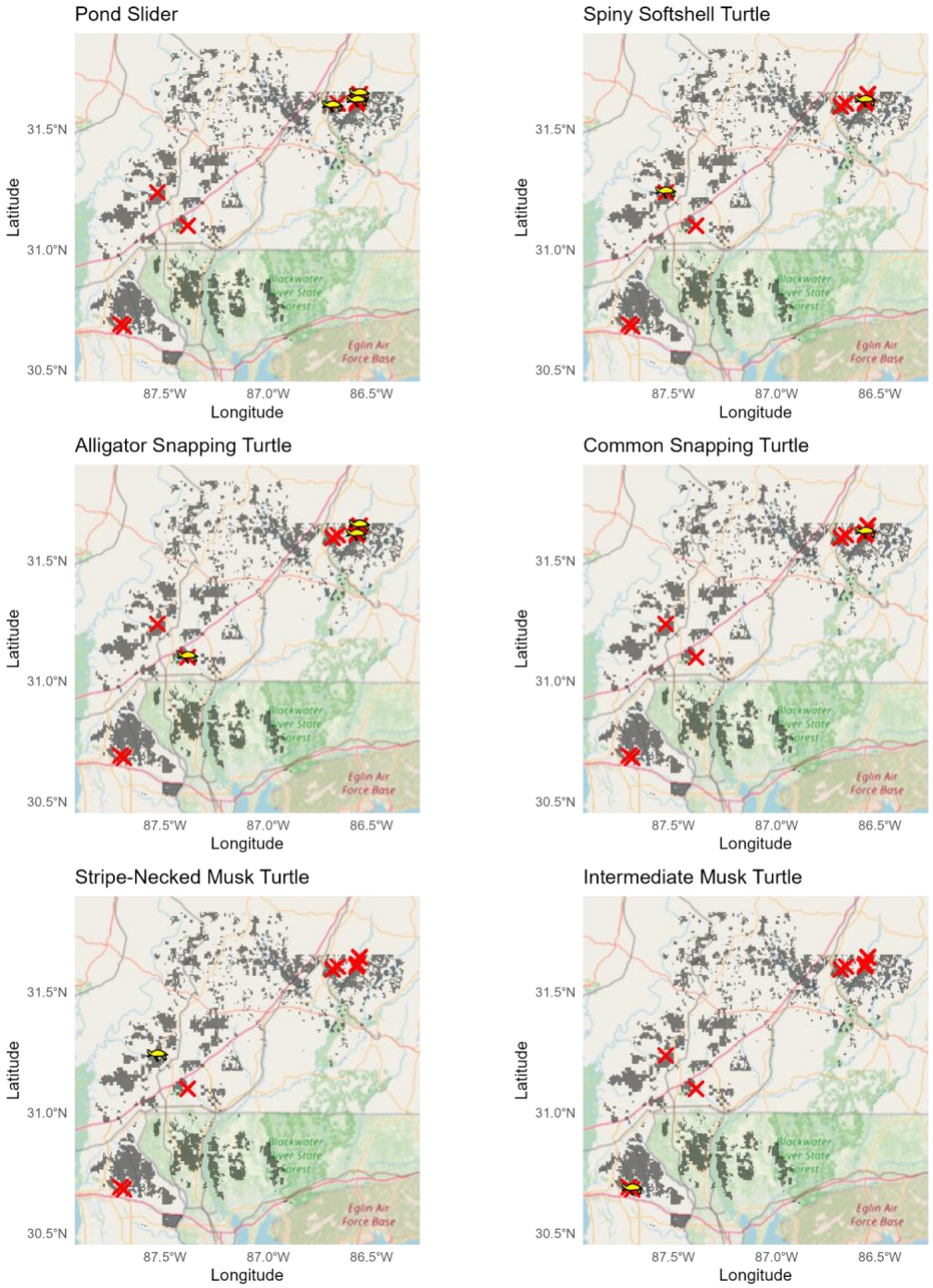


**Figure 4.** Site-level detection histories for the six species observed during turtle trapping and basking surveys on WCI-managed lands in southeastern USA, 2021-2023: Pond Slider (*Trachemys scripta*), Spiny Softshell Turtle (*Apalone spinifera*), Alligator Snapping Turtle (*Macrochelys temminckii*), Common Snapping Turtle (*Chelydra serpentina*), Stripe-Necked Musk Turtle (*Sternotherus peltifer*), and Intermediate Musk Turtle (*Sternotherus intermedius*). For each panel, a yellow turtle icon indicates that the focal species was detected at least once at a site, while a red "X" indicates that, although trapping and visual surveys were conducted at that site, the focal species was not detected during the study period.

## Upland Pine Visual Surveys

We recorded visual detections of all wildlife species from upland visual surveys and opportunistic observations throughout the study period on WCI-managed lands. Between April 16, 2021 and October 10, 2024 we recorded 354 visual detections of wildlife in the study area. These detections spanned 44 total taxa and 3 major taxonomic groups: amphibians, mammals, and reptiles. Among all survey methods employed in this study, 8 taxa were uniquely detected in upland visual surveys and/or from opportunistic visual observations (Supplementary Table 4): the Oak Toad (*Anaxyrus quercicus*), Cope's Gray Treefrog (*Dryophytes chrysoscelis*), Green Treefrog (*Dryophytes cinereus*), Southeastern Dwarf Salamander (*Eurycea quadridigitata*), North American River Otter (*Lontra canadensis*), Florida Cottonmouth (*Agkistrodon conanti*), Mud Snake (*Farancia abacura*), and Dusky Pygmy Rattlesnake (*Sistrurus miliarius barbourin*).

## Camera Trap Arrays

Between March 3, 2021 and February 18, 2025, 69 camera traps were deployed in the study area. The deployment and retrieval dates for each camera trap differed, with the shortest deployment lasting 48 days and the longest lasting 780 days, resulting in a total of 11,759 trap days over the study period. Trap days were distributed across the year to capture potential seasonality in wildlife occurrence and detection (Figure 5), with the lowest number of trap days occurring in February (N=903) and highest in July (N=1,208).

Motion detection imagery by camera traps yielded 40,720 photos (225 GB) during the study period. From these photos, we identified 63 wildlife species, 24 of which were unique to this survey method (Supplementary Table 4). The number of unique species detected by each camera trap varied over the study area (Figure 6), ranging between 0—6 for amphibians and 0—15 for reptiles.

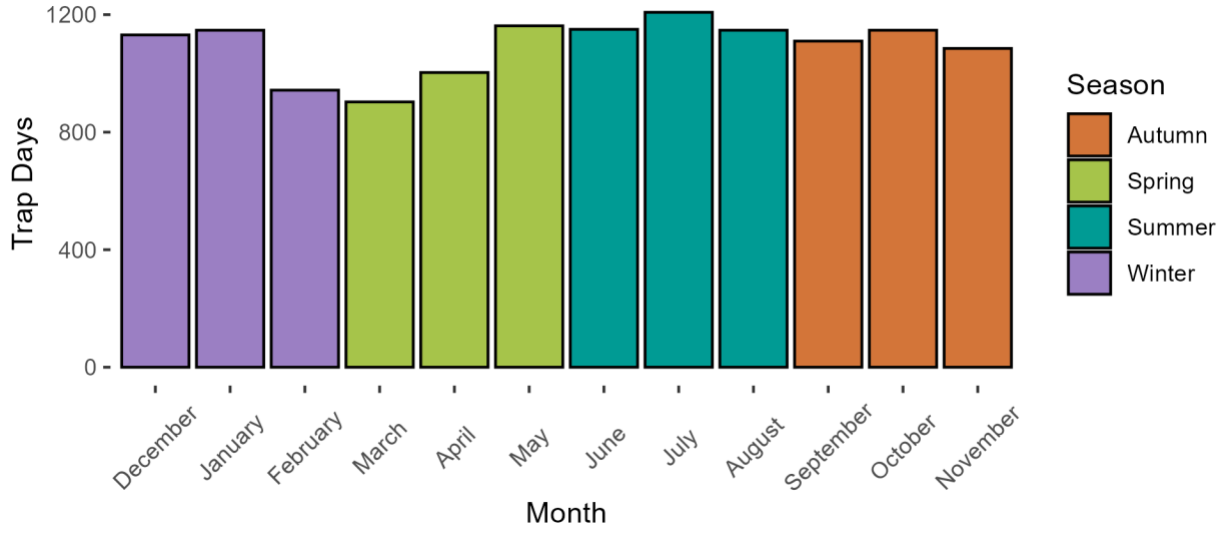


**Figure 5.** The frequency of days in each month that camera traps were deployed during the study period, 2019—2024. Monthly bins are colored by seasons based on a 3-month season classification, where Spring = March—May; Summer = June—August; Autumn = September—November; and Winter = December—February.

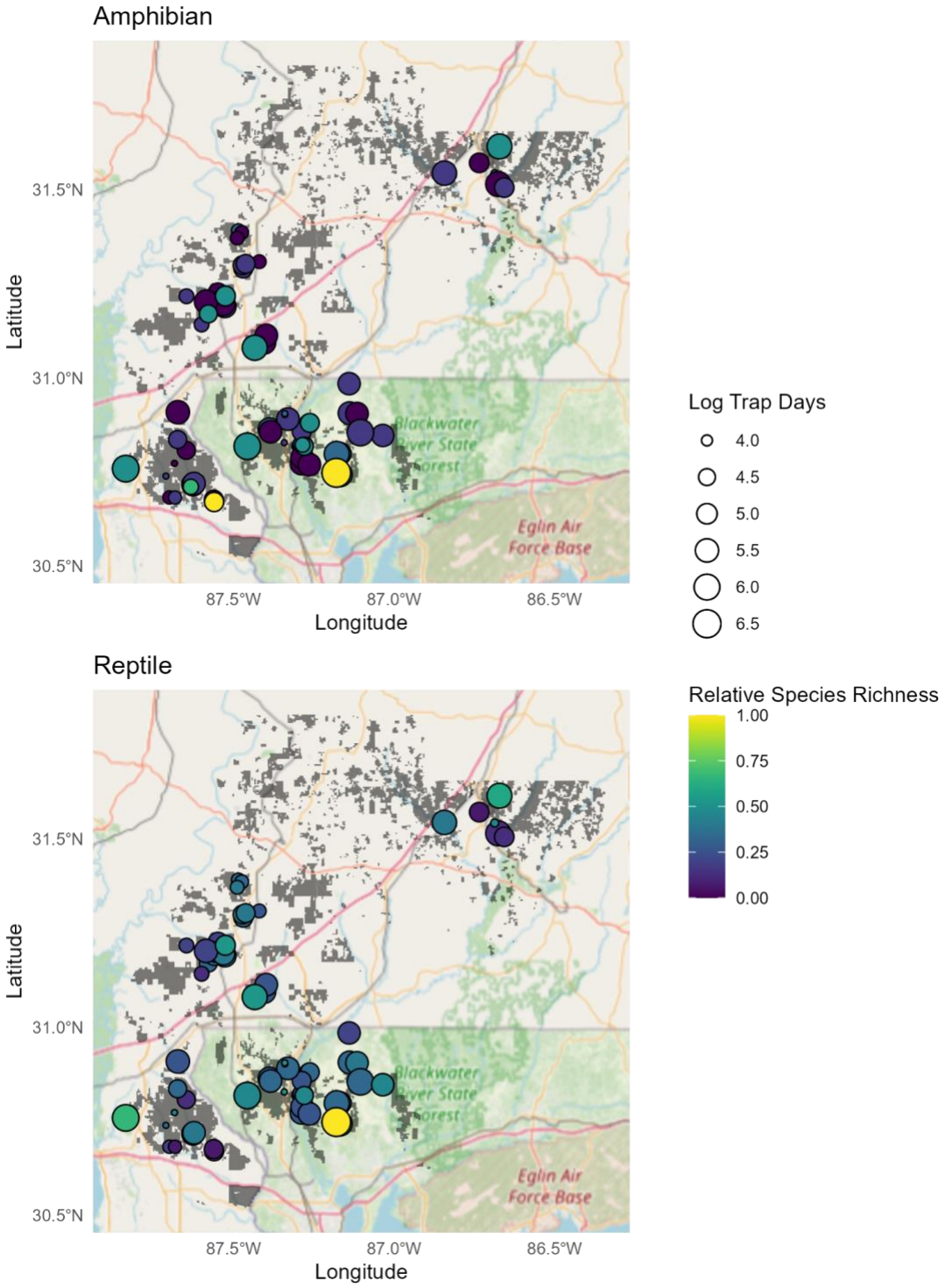


**Figure 6.** The number of unique species per major taxonomic group detected by camera traps. The size of data points indicate sampling effort: the natural log of total days deployed per trap. Colors represent the number of species detected at each site relative to the maximum number of species detected at any site during the 2019-2024 study period (indicated in yellow) for amphibians (top, N=6) and reptiles (bottom, N=15). WCI-managed lands are shaded in grey and based on 2021 boundaries.

## Environmental DNA and Metabarcoding

We collected 262 water samples for environmental DNA (eDNA) metabarcoding across 131 sites (2 samples per site) on WCI-managed lands between December 4, 2020 and June 14, 2024. From these samples we generated 1.03 billion reads of sequencing data (123 Gb of hard drive space). From those reads and samples, we detected 197 total taxa, of which 157 were species uniquely detected in the study by metabarcoding. Among the surveying methods deployed, metabarcoding was the only method to detect taxa from all 6 major taxonomic groups documented in this study; no other method deployed in this study detected fish or bivalve taxa (Supplementary Table 4). The number of unique taxa detected varied among major taxonomic groups and sampling sites (Figure 7).

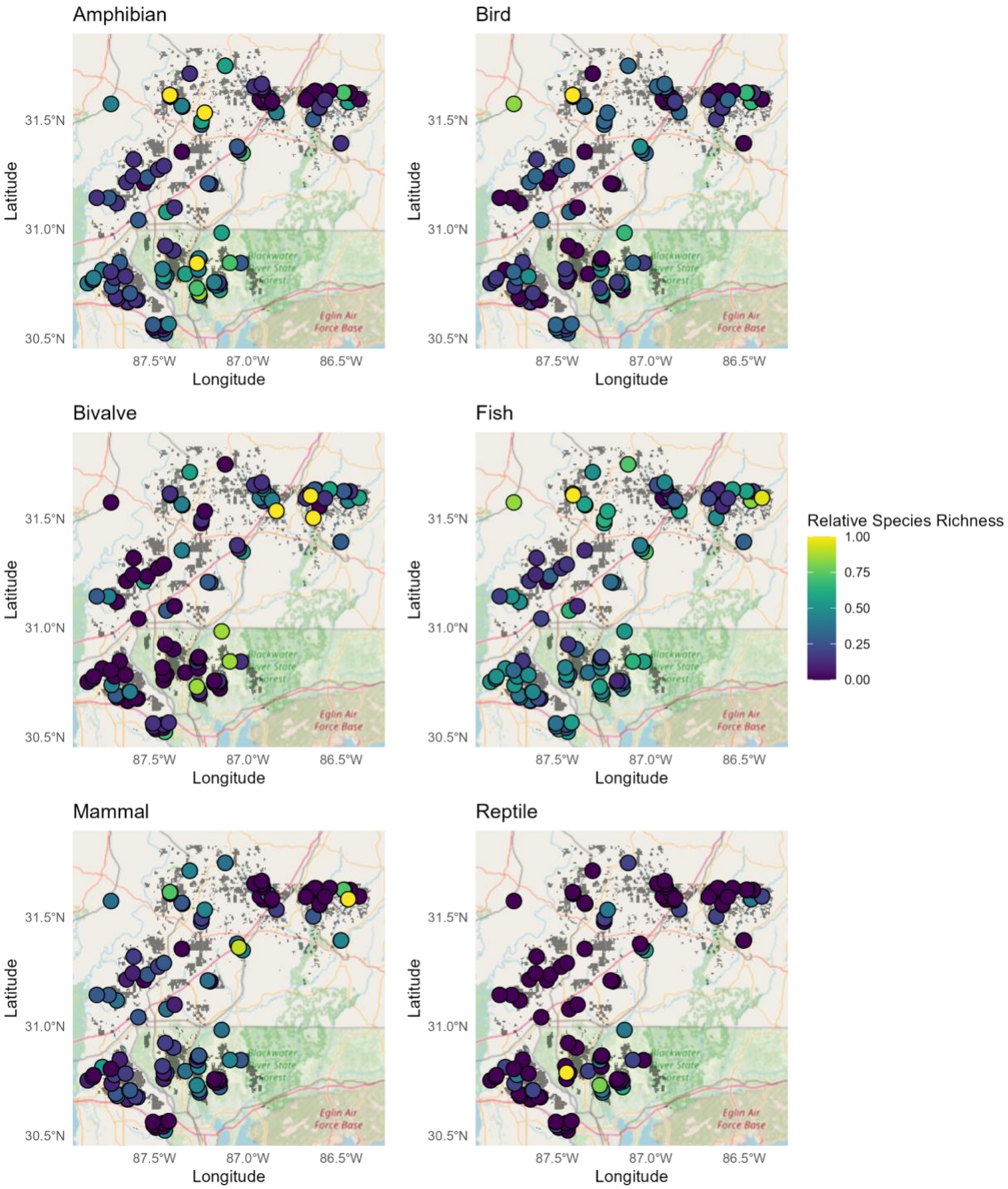


**Figure 7.** The number of unique species per major taxonomic group detected by environmental DNA metabarcoding. Colors represent the number of species detected at each site relative to the maximum number of species detected at any site during the 2019-2024 study period (indicated in yellow) for amphibians (N=7), birds (N=6), bivalves (N=7), fish (N=45), mammals (N=11), and reptiles (N=5). WCI-managed lands are shaded in grey and based on 2021 boundaries.

## Estimating the Proportion of Biodiversity Inventoried per Sampling Method

From Chao2 indices extrapolated from accumulation curves constructed for all species-level detections by camera trapping for each taxonomic group (Figure 8), we estimate that we inventoried 90% of reptiles (33 detected; 95% CI = 37[30 – 44]) detectable by this sampling method in the study area. Due to the high number of amphibian and mammals detections that were singletons and doubletons–those species detected at only one or two sites across the entire study period, respectively–we were unable to calculate unbiased estimates of extrapolated species richness and therefore do not report this index for amphibians or mammals. Additionally, because we detected only one bird species–the gray catbird (*Dumetella carolinensis*)–via bucket camera trapping, we did not calculate Chao2 indices for birds or for bivalves or fish, the latter of which are not detectable by this sampling method. However, using the Chao2 estimator to asymptotically estimate true species richness for detections via eDNA metabarcoding (Figure 8), we estimate that we detected 77% of amphibians (20 detected; 95% CI estimated true richness = 27 [20 – 35]), 57% of birds (32 detected; 95% CI = 58 [41 –76]), 81% of bivalves (16 detected; 95% CI = 21 [16 – 26]), 74% of fish (89 detected; 95% CI = 122 [96 – 149]), 57% of mammals (19 detected; 95% CI = 35 [21 – 48]), and 88% of reptiles (14 detected; 95% CI = 17 [14 – 20]) detectable by this method in the study area.

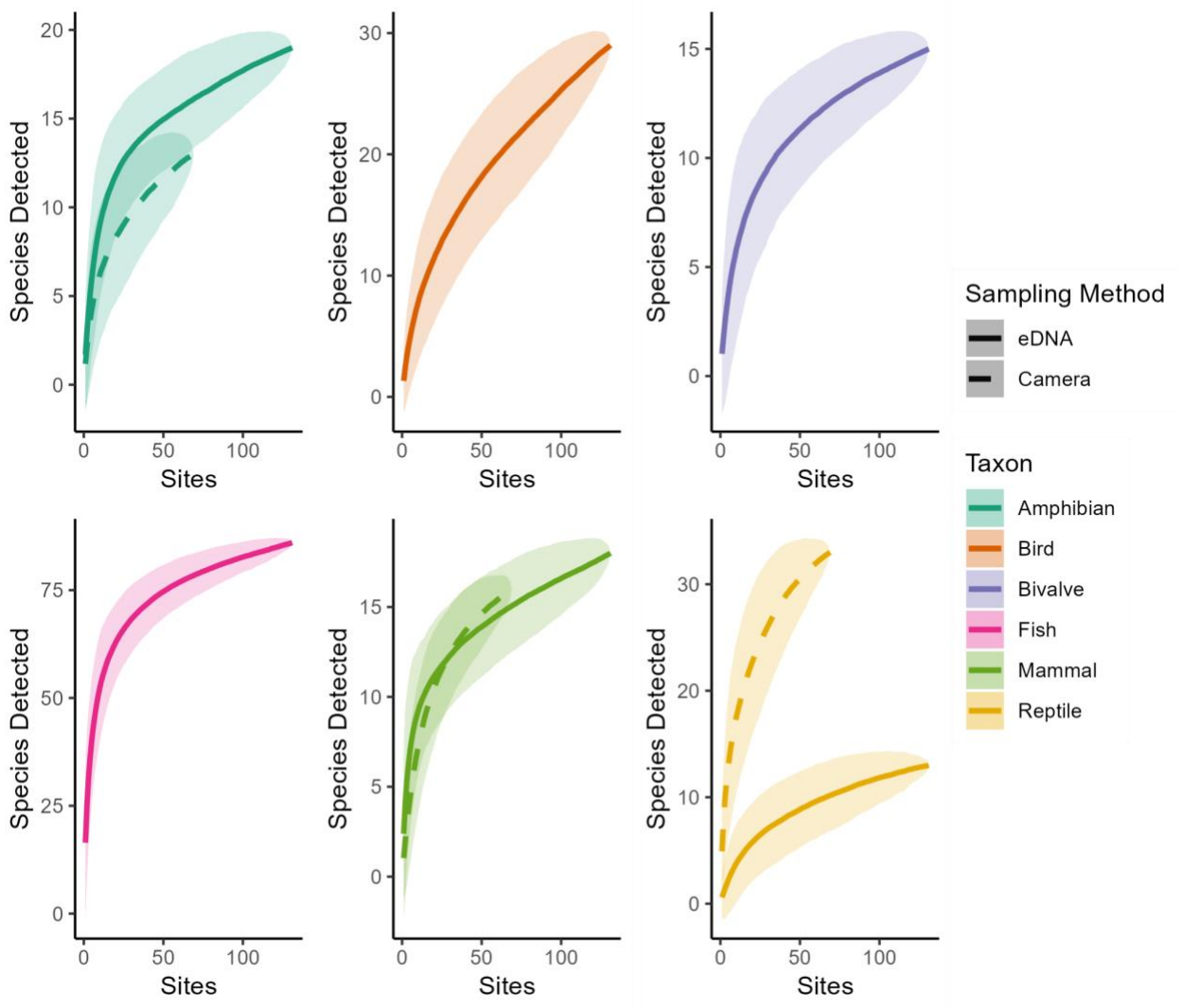


**Figure 8.** Pooled sample-based species accumulation curves for detections by metabarcoding (solid lines) and camera trapping (dotted lines) during the study period. Shaded areas represent 95% confidence intervals. These curves were constructed and colored by major taxonomic group. For panels without dotted lines, these taxonomic groups were not detected frequently enough or at all by camera traps to fit curves.

## Patterns of Species Richness among Sites

Camera trapping sites spanned a range of latitudes and environmental conditions, including mean daily temperature, annual precipitation, mean daily humidity, land cover class,

and distance from the nearest major waterbody (Figure 9). From Poisson GLMs including these environmental covariates, we found that annual precipitation and distance to the nearest major waterbody were significantly and positively correlated with the number of amphibian species detected at each site at an alpha level of 0.05 (annual precipitation: *IRR* = 1.52, *p* = 0.02; negative exponent of nearest major waterbody: *IRR* = 1.72, *p* = 0.04; Table 5). Conversely, we found that only covariates associated with detectability—seasonality, number of days deployed, and their interaction—were significantly correlated with the number of reptile species detected at each site (DOY + $DOY^2$: *IRR* = 1.00, *p* = 0.001; log total trap days: *IRR* = 0.90, *p* = 0.41; log total trap days x (DOY + $DOY^2$): *IRR* = 1.00, *p*=0.001; Table 5). However, despite satisfactory model fit and the significance of these covariates, the $R^2$ values, which indicate the proportion of variation in naïve species richness explained by the covariates, were fairly low for both models (amphibian $R^2$ Nagelkerke = 0.40; reptile $R^2$ Nagelkerke = 0.44). This suggests that examining other factors, such as management actions in the study area, may further our understanding of the unexplained variation in observed species richness across the study area.

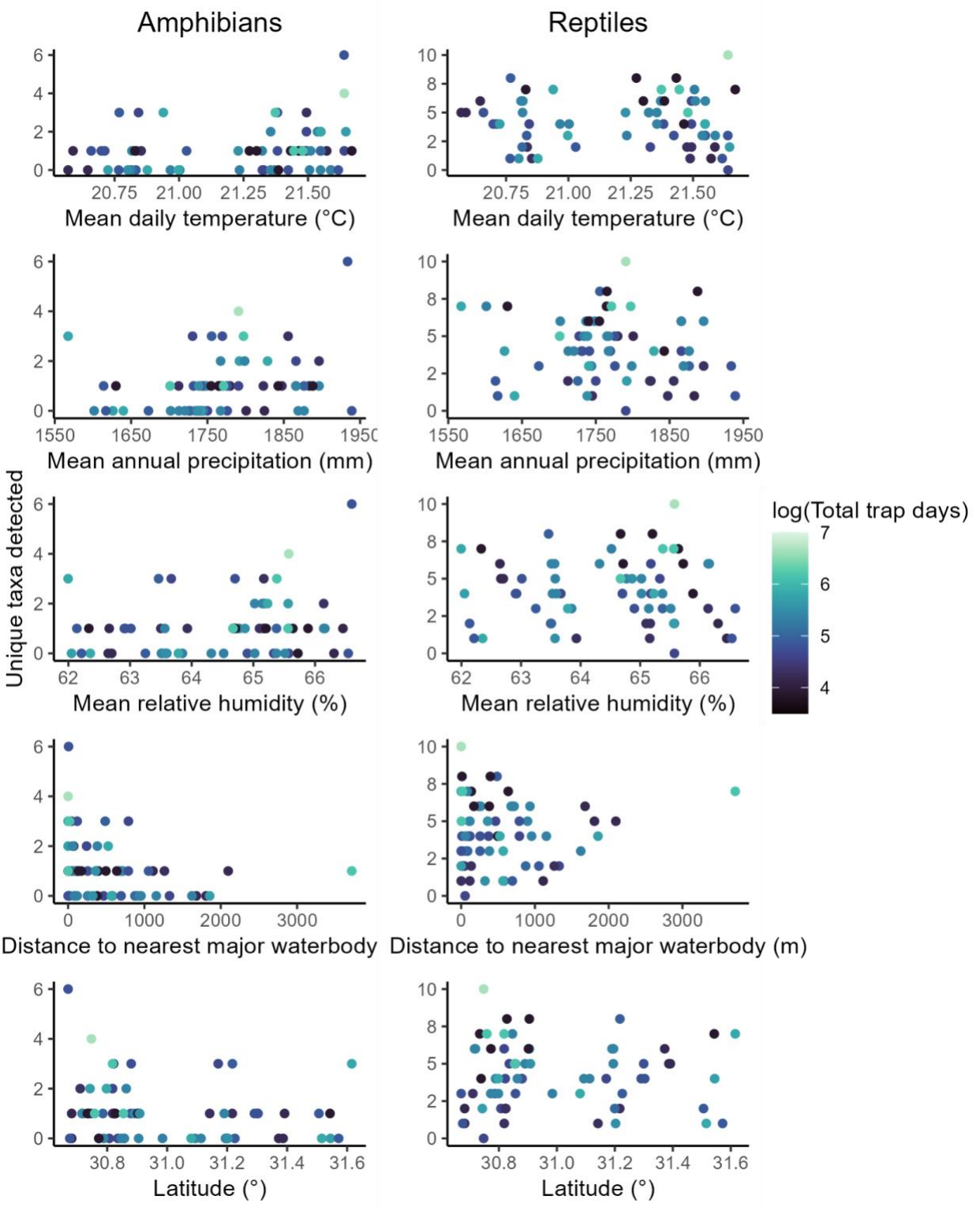


**Figure 9.** Covariates of Poisson GLMs for the number of unique amphibian (left) or reptile (right) species detected in camera arrays during the study period. Color indicates sampling effort, measured here as the natural logarithm of the number of days the camera was deployed.

**Table 5.** Output of two Poisson GLMs for number of unique species—amphibian or reptile—detected from camera trap arrays. Coefficient estimates for continuous variables are reported here based on their scaled values. Predictors estimated as significant ($p$<0.05) are indicated in bold.

| | Amphibian species richness | | | Reptile species richness | | |
|---|---|---|---|---|---|---|
| **Predictor** | ***IRR*** | **95% *CI*** | ***p*** | ***IRR*** | **95% *CI*** | ***p*** |
| intercept | 0.12 | 0.01 – 1.62 | 0.12 | 9.25 | 2.52 – 34.02 | **<0.01** |
| log(trap days) | 1.40 | 0.83 – 2.37 | 0.20 | 0.90 | 0.68 – 1.16 | 0.412 |
| DOY + DOY$^2$ | 1.00 | 1.00 – 1.00 | 0.44 | 1.00 | 1.00 – 1.00 | **<0.01** |
| mean temperature | 0.85 | 0.56 – 1.28 | 0.42 | 1.00 | 0.84 – 1.21 | 0.961 |
| mean precipitation | 1.52 | 1.08 – 2.19 | **0.02** | 0.71 | 0.44 – 1.11 | 0.153 |
| exp(-nearest waterbody distance) | 1.72 | 1.03 – 2.94 | **0.04** | 1.4 | 0.99 – 1.97 | 0.053 |
| NLCD: herbaceous | 0.76 | 0.22 – 2.04 | 0.62 | 0.78 | 0.53 – 1.12 | 0.191 |
| NLCD: shrub scrub | 1.06 | 0.48 – 2.18 | 0.88 | 0.99 | 0.85 – 1.16 | 0.908 |
| NLCD: wetland | 1.11 | 0.56 – 2.15 | 0.75 | 0.98 | 0.78 – 1.23 | 0.872 |
| log(trap days)*(DOY + DOY$^2$) | 1.00 | 1.00 – 1.00 | 0.59 | 1.00 | 1.00 – 1.00 | **<0.01** |
| | N=66 | | | N=66 | | |
| | $R^2$ Nagelkerke = 0.40 | | | $R^2$ Nagelkerke = 0.44 | | |

## Community Compositional Dissimilarity: Species Turnover and Nestedness

Community composition varied considerably among eDNA sampling locations, indicated by the Sørensen's Dissimilarity Index ($D_{SOR}$) calculated for each taxon (Table 6), in which values approaching 1 indicate complete dissimilarity in taxon-specific species assemblages among sites and a value of 0 indicates total uniformity. Estimated dissimilarity was high for all taxa detected by this method: amphibians ($D_{SOR}$ = 0.9839), birds ($D_{SOR}$ = 0.9859), bivalves ($D_{SOR}$ = 0.9844), fish ($D_{SOR}$ = 0.9751), mammals ($D_{SOR}$ = 0.9808), and reptiles ($D_{SOR}$ = 0.9866). Parsing the relative contributions of species turnover and nestedness to the overall estimated per-taxon Sørensen's Dissimilarity Indices (Table 6) indicates that this dissimilarity is driven largely by species turnover, with the percentage of dissimilarity contributed by turnover ranging from 93.6—97.7% while the percentage contributed by nestedness correspondingly ranged from 2.3—6.4%.

**Table 6.** Sørensen's Dissimilarity Index per taxon among sites with species detections from eDNA metabarcoding. Here, a value of 1 indicates total dissimilarity of species assemblages among sites, while values approaching 0 indicate more similarity among sites. We also report the respective contributions of species turnover vs nestedness to the calculated Sørensen's Dissimilarity Index, with the percentage of each contribution to overall dissimilarity indicated in parentheses.

| Taxon | Sørensen's Dissimilarity Index | Species turnover component | Species nestedness component |
|---|---|---|---|
| Amphibian | 0.9839 | 0.9555 (97.1%) | 0.0284 (2.9%) |
| Bird | 0.9859 | 0.9629 (97.7%) | 0.0229 (2.3%) |
| Bivalve | 0.9844 | 0.9414 (95.6%) | 0.0430 (4.4%) |
| Fish | 0.9751 | 0.9482 (97.2%) | 0.0268 (2.8%) |
| Mammal | 0.9808 | 0.9294 (94.8%) | 0.0514 (5.2%) |
| Reptile | 0.9866 | 0.9230 (93.6%) | 0.0636 (6.4%) |

Through NMDS ordination of per-taxon species detection histories of sites sampled with eDNA metabarcoding, we calculated pairwise ecological distances among sites to examine landscape-level variation in community composition (Figure 10). NMDS identified 2 ordination axes for each taxon (stress < 0.20). These axes are unitless and instead indicate relative differences among sites in species assemblages. While some clustering does occur for samples within the same sub-basin (HUC8), the spread of ecological distances also suggest variation within sub-basins and overlapping among sub-basins, indicating that other unexplored factors may contribute to the dissimilarity observed among eDNA sampling site species assemblages.

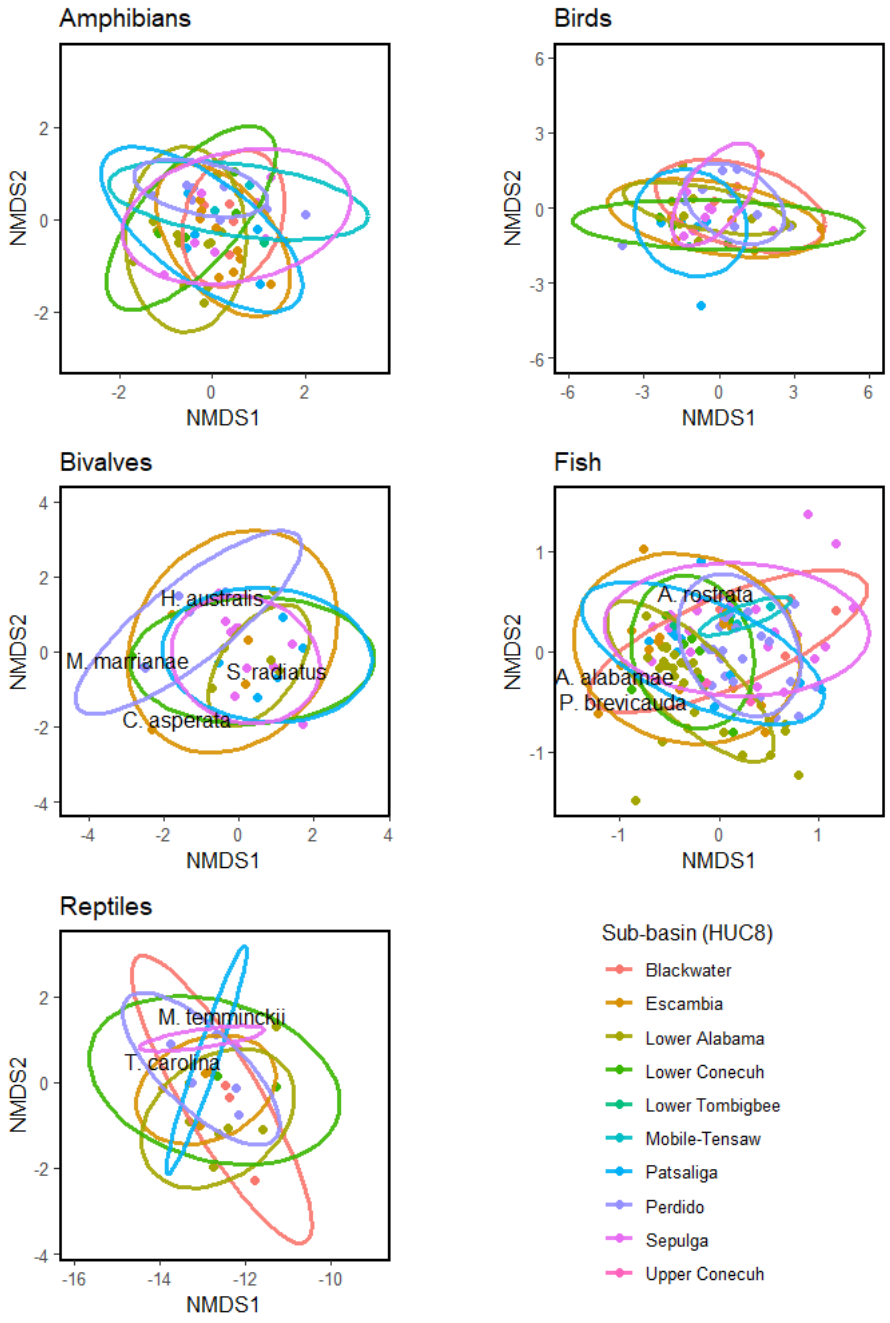


**Figure 10.** Per-taxon ecological distances among eDNA sampling sites estimated via nonmetric multidimensional scaling (NMDS), colored by HUC8 sub-basin. Axes are unitless and representative of rank differences among sites based on differences in species assemblages. Ellipses were fit by sub-basin at an 0.90 alpha level to aid in visualization. Species placement on NMDS axes are included for species of conservation concern per taxon when detected by metabarcoding, including: Southern Sandshell (*Hamiota australis*), Alabama Pearlshell (*Margaritifera marrianae*), Rayed Creekshell (*Strophitus radiatus*), Alabama Orb (*Cyclonaias asperata*), American Eel (*Anguilla rostrata*), Alabama shad (*Alosa alabamae),* Coal Shiner (*Percina brevicauda*), Alligator Snapping Turtle (*Macrochelys temminckii*), and Eastern Box Turtle (*Terrapene carolina*).

Finally, we examined the correlation between pairwise differences in geographic (Euclidean) distance and Jaccard dissimilarity in species composition among eDNA sampling sites (Figure 11). From Mantel tests based on Spearman rank correlations, we found weak yet positively significant relationships for all taxa except mammals, for which this relationship was not significant (Table 7). Mantel test statistics (*r*) can range from -1 to 1, with 1 indicating a strong positive correlation between geographic and ecological distance and -1 correspondingly estimating a strong negative correlation. Thus, the Mantel statistics estimated here, which range from 0.07—0.18, indicate that although pairwise geographic distances among sites are correlated with differences in species composition for all taxa detected in this study except for mammals, there may be additional local-scale environmental drivers of biodiversity on WCI-managed lands.

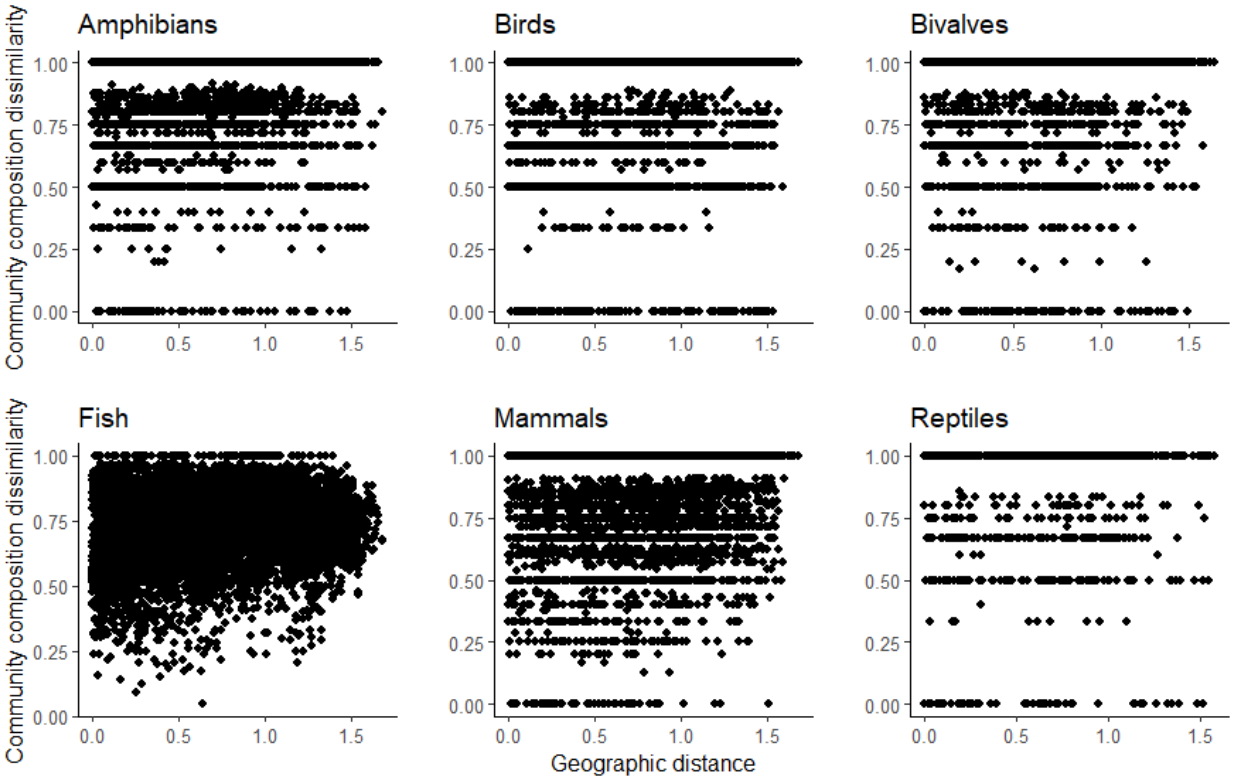


**Figure 11.** Pairwise differences between Jaccard dissimilarity of per-taxon species composition and Euclidean geographic distance among eDNA sampling sites.

**Table 7.** Results of Mantel tests determining the correlation between geographic (Euclidean) and Jaccard dissimilarity of incidence-based species composition for each taxon detected via eDNA metabarcoding in the study area. Only sites with at least one detection for each taxon (N) were included in Mantel tests. Test statistics (Mantel *r*) were based on Spearman's rank and range from -1 to 1, with a value of 1 indicating a strong positive correlation between geographic and ecological distance and a value of -1 correspondingly indicating a strong negative positive correlation. Significant relationships ($p<0.05$) indicated in bold.

| Taxon | N | Mantel *r* | *p* |
|---|---|---|---|
| Amphibian | 87 | 0.13 | **0.001** |
| Bivalve | 71 | 0.18 | **0.001** |
| Bird | 85 | 0.07 | **0.003** |
| Fish | 128 | 0.09 | **0.001** |
| Mammal | 91 | -0.02 | 0.689 |
| Reptile | 43 | 0.12 | **0.007** |

# Recommendations for Future Research

Between 2019 and 2024, we documented biodiversity across WCI-managed lands using several inventorying methods, including visual surveys, eDNA metabarcoding, camera trapping, and targeted surveys for rare species. These detections spanned 6 major taxonomic groups: amphibians, birds, bivalves, fish, mammals, and reptiles. Among those taxa, we identified several species of conservation concern, including 4 endangered species: the American Eel (*Anguilla rostrata*), Alabama Pearlshell (*Margaritifera marrianae*), Coal Shiner (*Percina brevicauda*), and Red Hills Salamander (*Phaeognathus hubrichtii*).

Although the number of unique taxa and site-level species assemblages detected by our sampling methods varied across the study area, the analyses presented here indicate that much of this variation is still unexplained by the covariates included in our models, which are commonly correlated with detection and patterns of biodiversity. Thus, to better understand how the conservation actions conducted on WCI-managed lands correspond with landscape-level patterns of species richness and community composition, we recommend further analyses incorporating local- and regional-level indices of management, such as stand age, presence and distance from logging roads, trees per acre, basal area, tree species, stage of management, soil type, streamside management zone (SMZ) area, and SMZ age. Incorporating these data as covariates in the analyses presented here will further our understanding of the relationships among management actions and wildlife diversity on WCI-managed lands.

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