FINAL PERFORMANCE REPORT
South Carolina State Wildlife Grants Project
South Carolina Department of Natural Resources
Project period: October 1, 2019 – March 30, 2022

Project Title: Integrating crayfish into aquatic assessments from the uplands to the coast

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Project Location: Counties within the Savannah watershed in South Carolina

Project Type: Implementation

Project Goal: To develop faunal assemblage-level data for crayfish through aquatic resource assessments in the Savannah River Basin.

Progress: Completed

Accomplishments:
Coastal environments have a strong connection to their upland watersheds with freshwater streams integrating their drainage area due to the cumulative nature of hydrologic systems, with the consequences of poor land management (e.g. siltation, excessive nutrients, flow disruption) eventually impacting downstream systems. Upstream water quantity and quality can have important influences on the productivity of coastal ecosystems (Elliott and Whitefield 2011) by influencing phytoplankton biomass (Kimmerer et al. 2018), blue crab abundance (Sanchez-Rubio et al. 2011), as well as overall fisheries productivity (Gillson 2011). Integrative watershed approaches to water quality have been shown to be effective and efficient ways of maintaining robust and healthy ecosystems across the continuum of aquatic habitats from mountains to the coast, being used by governments across the world (e.g. Nichols et al. 2017).

Aquatic resource assessments such the South Carolina Stream Assessment (SCSA), Small River Assessment (SRA), and Headwater Streams Assessment were developed to determine the status of aquatic resources in streams and rivers throughout the State using fish communities to assess resource condition. Such approaches have been shown to be broadly effective in the assessment of biological resources (e.g. Barbour et al. 1999). In addition to fish communities, less mobile invertebrate communities have also been used in a complimentary manner alongside fish assessments as indicators of water quality (e.g. Carter et al. 2017). Crayfish are a representative invertebrate taxon that may be able to enhance existing state-wide assessments of aquatic resources due to their sensitivity to environmental conditions and status as priority conservation species in South Carolina. Additionally, given the inclination of some crayfish species to use terrestrial habitats during parts of their life history, they could have different sensitivities to land use patterns than strictly in-stream species. There are currently 40 species of crayfish documented from South Carolina (38 native and 2 introduced). Recent research in the coastal plain has shown that distributions of native crayfish have been changing, in part due to their displacement by non-native species (SWG report SC-T-F18AF00962).

The purpose of this project was to develop a broad spatial-scale assessment to provide new data on the distribution and life history for priority crayfish species, representing a necessary first step for building plans to better assess their conservation status and effectiveness as indicators of water quality.

Objective 1: Collect crayfish samples from a subset of state-wide biological assessment sampling locations

To better assess the distribution and abundance of crayfish in the Savannah River basin, SCDNR biologists georeferenced locations of 145 potential sampling sites within the South Carolina portion of the Savannah River watershed, selected from previous SCSA and SRA sampling locations. A subset of 55 sites was selected for sampling, with sites distributed relatively equally in a north-west to south-east direction across South Carolina's portion of the Savannah basin. Sampling at all 55 sites was conducted using dipnets by teams of 2-3 biologists from January-March 2021 and encompassed all of South Carolina's physiographic regions (Figure 1). Dip nets and hand searching under rocks were used for specimen collection. We recorded date, latitude, longitude, start time of sampling, length of sampling, dominant riparian vegetation, and estimated percent canopy cover. Water quality (water temperature, dissolved oxygen, conductivity, salinity) was recorded using YSI ProDO. Geospatial data, including the 2016 National Land Cover Database and physiographic province information, were included for each sampling location (Dewitz 2021).

Objective 2: Identify all crayfish collected to the lowest practical taxonomic level and maintain a genetic archive of gill tissues for future analyses

Crayfish identifications were completed for all 55 sampled sites and sorted to species level based on morphology using dichotomous keys (e.g. Eversole and Jones 2004). Species identifications and associated conservation priority statuses are documented in Table 1. Compilation of genetic material in ethanol for archiving purposes is complete.

Objective 3: Crayfish data and patterns of biodiversity

For sites where species-level data were available, linear regression and analysis of variance (ANOVA) techniques were used to compare patterns of species richness and Shannon diversity against environmental and geospatial data. Land cover was attributed to each sampling location based on the National Land Cover Database (Dewitz 2021) into Agricultural Vegetation, Developed/human use, Forest & Woodland, Recently disturbed/modified. Land use was described at each sampling location by the field team into the category of 'rural', 'forest', and 'farmland' based on the dominant land use in the immediate area around the sampling site. Physiographic province was attributed to each sampling location based on available geospatial data for 'Blue Ridge', 'Piedmont', and 'Coastal Plain' provinces.

Land cover was not a significant driver of crayfish richness ($F_{3,33} = 1.358; P = 0.273$) but was marginally significant for Shannon diversity ($F_{3,33} = 2.043; P = 0.127$). Land use was not a significant driver of crayfish richness ($F_{3,33} = 0.76; P = 0.525$) or Shannon diversity ($F_{3,33} = 0.753; P = 0.529$). Temperature was not a significant driver of crayfish richness ($P = 0.847$) or Shannon diversity ($P = 0.910$). Field-assessed canopy cover was a significant driver of richness ($R^2 = 0.20; P = 0.003$) and Shannon diversity ($R^2 = 0.23; P = 0.001$). Physiographic province was a significant driver of richness ($F_{2,34} = 9.71; P < 0.001$) and Shannon diversity ($F_{2,34} = 12.99; P < 0.001$). Field-assessed canopy cover did differ significantly across physiographic provinces ($F_{2,34} = 6.322; P = 0.005$), with sites in the Coastal Plain having significantly greater canopy cover than Piedmont ($P = 0.009$), but no significant difference was found between sites in the Coastal Plain and Blue Ridge ($P = 0.09$) or site in the Piedmont and Blue Ridge ($P = 0.50$).

For fish surveyed at these same sites, Land cover was also not a significant driver of fish richness ($F_{3,46} = 0.618; P = 0.607$) or Shannon diversity ($F_{3,46} = 0.403; P = 0.752$). Land use was not a significant driver of fish richness ($F_{4,45} = 0.21; P = 0.930$) or Shannon diversity ($F_{4,45} = 0.367; P = 0.831$). Physiographic province was also not a significant driver of richness ($F_{2,47} = 0.082; P = 0.921$) or Shannon diversity ($F_{2,47} = 0.457; P = 0.636$). Due to the differences in timing of collection for crayfish and fish, field-assessed condition (i.e. temperature and canopy cover) were not included in analyses for fish.

Discussion

By incorporating crayfish into a subset of the existing SCSA and SRA sampling locations, this project developed up-to-date distributions for crayfish in SC and began the process of integrating some of these species into biological assessments. South Carolina currently has 24 species of crayfish in its SWAP, many of which are designated as data-deficient species. Data and specimens collected through the course of this project are valuable additions to the limited pre-existing data already recorded for some of these conservation priority species, especially when considered in conjunction with the assemblage patterns observed during this project. Through the concurrent collection of fish assemblage data (already collected by the SCDNR) and crayfish assemblage information (as collected as part of this project) in the Savannah River basin, we show how assemblage patterns differ across physiographic provinces for these two taxonomically distinct groups. Further, combining information on distribution and abundance of multiple taxa groups (e.g., crayfish, finfish, mussels) may provide a broader and more comprehensive view of aquatic habitat quality and common stressors in the State.

This research builds on our understanding of the abundance and distribution of crayfish in South Carolina, including conservation priority species (Table 1). Information on the abundance and distribution of these taxa are valuable for future assessments on the conservation status of these species and is vital to developing effective wildlife management plans. Additionally, non-native crayfish, such as the red swamp crayfish *Procambarus clarkii*, are currently expanding their range in the state, and this project facilitates a broad-scale understanding of the distribution and potential impact of invaders on conservation priority native crayfish species. The red swamp crayfish was only collected at a single sampling location in the Savannah River basin during this study. These findings are juxtaposed against the high prevalence of red swamp crayfish recently documented in the Pee Dee basin of SC (>50%, see project SC-T-F18AF00962). The relatively low prevalence of invasive crayfish in the Savannah River basin, as compared to the Pee Dee basin, coupled with the limited range of many crayfish species in SC, suggests that localized management strategies may be an important component for reducing the spread and impacts of invasive species. This is of particular importance given that two species collected during this project, *Cambarus chaugaensis* and *Cambarus reflexus*, are listed as the 'Highest' conservation priority status in the State Wildlife Action Plan. Each species had less than ten specimens collected cumulatively across all 55 sampling efforts, and each was only documented in a single physiographic region.

In comparing patterns and drivers of crayfish assemblages in the Savannah basin, it becomes apparent that the underlying physiographic province is a strong predictor of both crayfish richness and diversity in the sampled sites. This contrasts with the richness and diversity of finfish assemblages where physiographic province was not a predictor of these metrics. In crayfish, the association of richness and diversity with physiographic province could be driven by several factors. Sampling and identification artifacts are two important considerations in understanding the patterns in these data. Crayfish identification based on morphology is sometimes difficult. This is especially true since morphological keys used to identify crayfish in this study were based on first form male (F1 male) specimens which may not be present at every sampling location. If F1 specimens were not available in a sample, identification was still made to the lowest taxonomic resolution based on morphology of F2 male and female specimens. If species in the Coastal Plain, for instance, where the highest richness and diversity were found, are more readily distinguished than specimens from the Piedmont and Blue Ridge, this ability to identify specimens could artificially contribute to the patterns observed in this study. Additionally, sampling was restricted to the months of January-March. The timing of this sampling could have affected the catchability of some taxa more than others. For instance, the colder conditions of sites in the Blue Ridge or Piedmont may have reduced the catchability of crayfish in these habitats during this time period, whereas in the slightly warmer waters of the Coastal Plain, catchability may have been higher. It will be important to take these factors into account in future studies, and ensure that taxonomic resolution of identifications is made consistently across physiographic regions and that catchability is not a major factor in driving patterns of diversity or richness.

Assuming these factors are minimal drivers of the observed patterns, this study points to important considerations for understanding distribution and niche use by crayfishes in SC. For one, they demonstrate a steep shift in site-specific species richness across the downstream gradient: only one species (*Cambarus bartonii*) was collected in the Blue Ridge physiographic region during this project. Similarly, only seven species of the thirteen collected during the project were collected from the Piedmont; and, of these, only one was restricted solely to that region. In contrast, the Coastal Plain physiographic region, despite containing fewer study sites than the Piedmont, resulted in nine separate species as well as the greatest species diversity. Were it only a watershed-driven pattern, it would be probable that such a trend would also reflect itself in state finfish records as well. However, as that is not the case, additional variables are likely at play.

Given that physiographic regions are defined by geomorphological conditions that occur along a gradient (Fenneman 1916), the transition from one region to the next corresponds with changes in soils, slope, underlying geology. Crayfishes and fishes may respond differently to these factors. With the tendency of crayfishes to burrow into the sediment and directly interact with both water and soil, rather than being strictly confined to waterbodies as are fishes, findings of this project may reflect the importance of terrestrial and/or geological factors to crayfish assemblage structure. The site-specific patterns of richness and diversity juxtaposed across fish and crayfish assemblages in this study provide the foundation for synthesizing biodiversity data from multiple taxa groups (e.g. fish, crayfish, mussels, insects) into biological assessments of aquatic resources.

Studies investigating the ecological and genetic biogeography of fish populations have shown that geologic events can result in genetically similar species existing on opposite sides of the world, while also restricting movement of species when resulting in isolating barriers (Bermingham and Avise 1986; Griffiths 2006). However, when considering documented trends in fish taxa throughout the Savannah basin, it appears more subtle geomorphological transitions such as those from one physiographic region to the next impact fish taxa richness and diversity far less than one might expect. In contrast, the apparent relationship between crayfish richness and abundance and the characteristics defining the physiographic regions explored during the study are much more obvious. Additional research into more specific parameters (e.g. soil type, geological structure, associated terrestrial habitat) are necessary to identify specific drivers of this trend.

Physiographic province is known to be an important factor driving species distribution for many aquatic invertebrates such as caddisflies (Harris et al. 1991) and crayfishes (Schuster et al. 2022). Additional research into richness and diversity patterns across physiographic regions of other movement-restricted taxa within this scope would also be beneficial in determining whether this pattern is singular to crayfishes or more widespread across additional taxa that also rely more heavily on subtle geological variation than finfishes. Understanding the role of physiographic province on driving patterns of biodiversity for natural resources in SC may facilitate the use of geospatial analyses to assess species range data, thus more efficiently managing conservation priority species.

Significant deviations: None

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Table 1. Species collected as part of this sampling effort and their respective conservation priority rankings. G/S-rank refers to the Global (G) Conservation Status Ranks, as assigned by NatureServe, and the Subnational (S) conservation status rank for South Carolina as assigned by the South Carolina Department of Natural Resources.

Scientific name	**Common name**	**G/S-rank**	**SWAP Priority status**	**Blue ridge**	**Piedmont**	**Coastal plain**
Cambarus bartonii	Appalachian brook crayfish	G5/S4	None	13	62	0
Cambarus chaugaensis	Chauga crayfish	G2/S2S3	Highest	0	7	0
Cambarus latimanus/striatus	Variable Crayfish	G5/S4	None	0	200	66
Cambarus reflexus	Pine Savannah Crayfish	G4/S3	Highest	0	0	1
Faxonella clypeata	Ditch Fencing Crayfish	G5/S2?	Moderate	0	0	9
Procambarus acutus	White River Crawfish	G5/SNR	None	0	0	72
Procambarus clarkii	Red Swamp Crawfish	G5/Non-native	None	0	24	0
Procambarus enoplosternum	Black Mottled Crayfish	G4/SNR	Moderate	0	0	21
Procambarus hirsutus	Shaggy Crayfish	G4/S4	Moderate	0	5	19
Procambarus pubescens	Brushnose Crayfish	G4/S3?	Moderate	0	0	26
Procambarus raneyi	Disjunct Crayfish	G4/S2	None	0	60	26
Procambarus Spiculifer	White Tubercled Crayfish	G5	SNR	0	33	0
Procambarus troglodytes	Eastern Red Swamp Crayfish	G5/S4	None	0	0	162

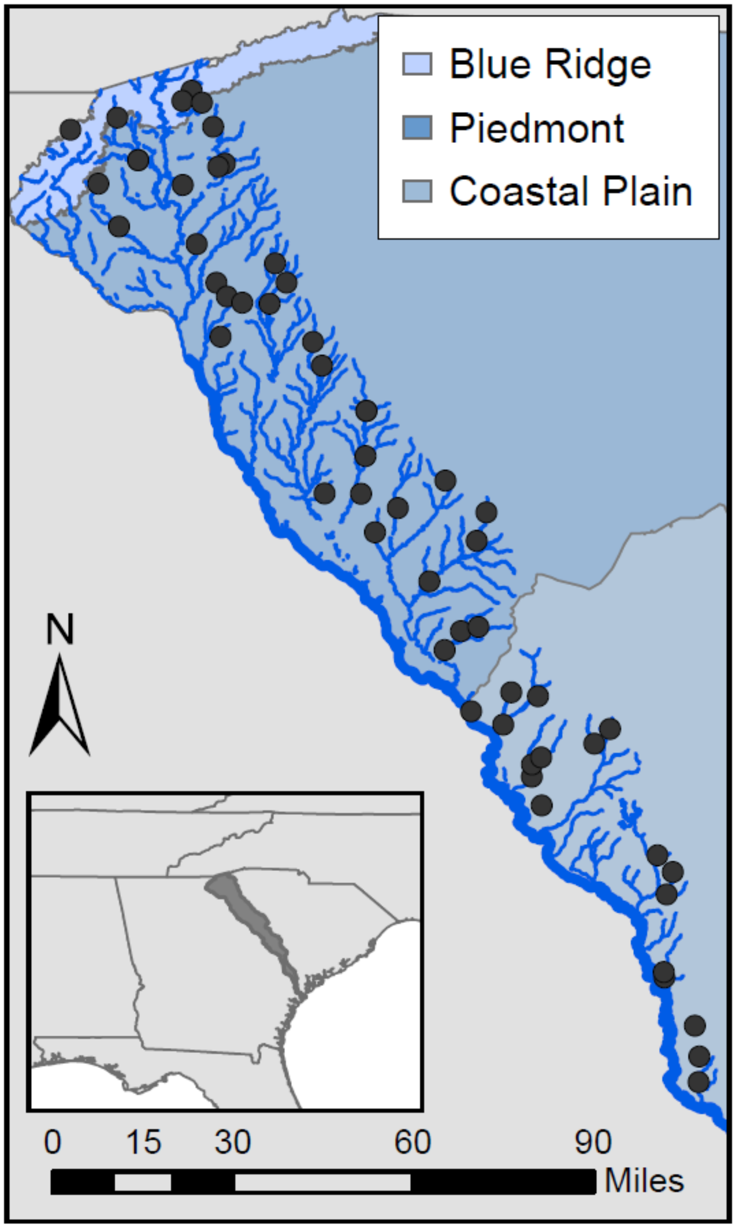

Figure 1. Sampling sites in the Savannah River basin across each of the 3 major physiographic provinces of SC.

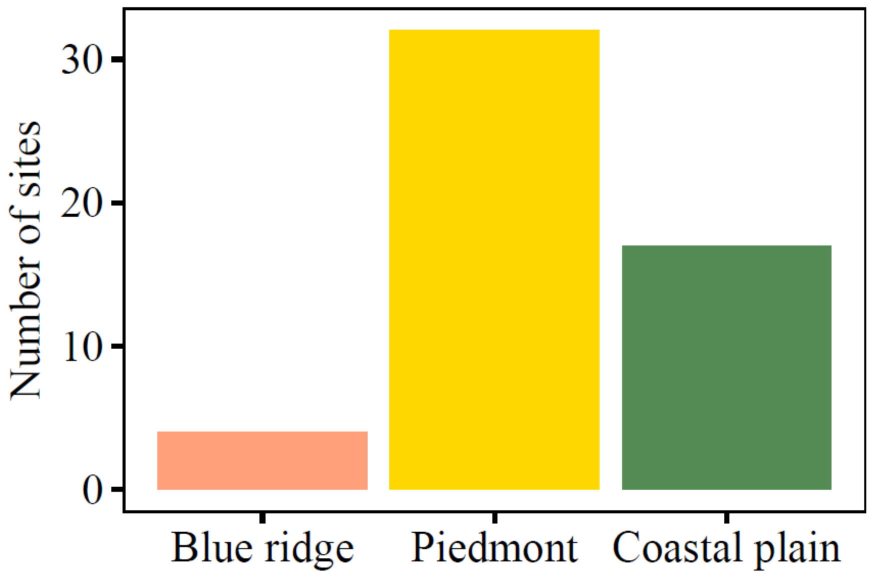

Figure 2. Distribution of sampling sites across physiographic provinces.

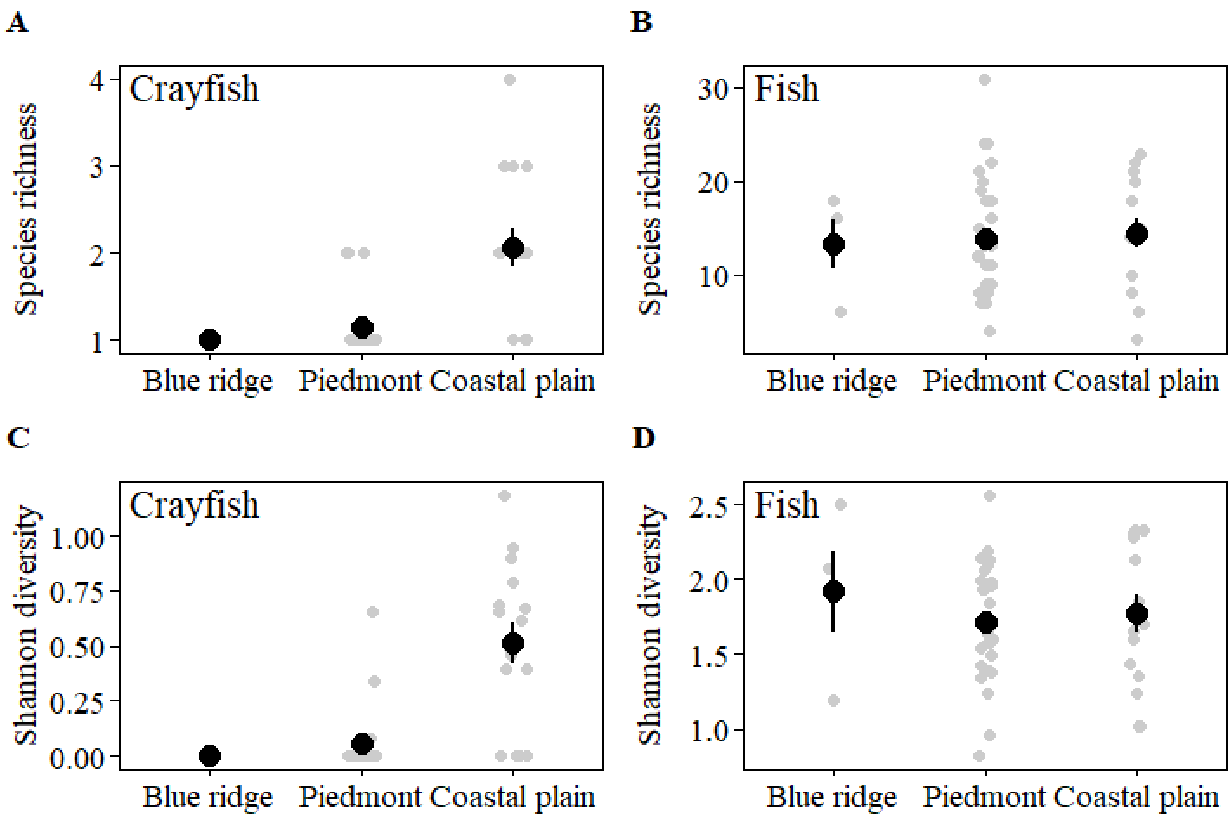

Figure 3. Comparison of species richness (top panels) and Shannon diversity (bottom panels) for both crayfish (left panels) and fish (right panels) across physiographic provinces for sites sampled in SC's Savannah River basin. Mean values (±SE) are presented as black circles (and error bars) with site-level data presented as grey circles.