Attachment 4
PPBEP COMMUNITY GRANT FINAL RESEARCH REPORT FORM

Agreement No.:	FY2425-02		
Grantee Name:	University of South Alabama		
Grantee Address:	600 Clinic Drive, Mobile, AL 36608		
Grantee's Representative:	Charles Martin	**Telephone No.:**	251 460-7136
Project Title:	Coastal Rivers as Unrecognized Winter Fish Habitat: Quantifying Oligohaline Habitat Use in Pensacola and Perdido Bays		
Please submit any high-resolution photos related to the project (include photo credit for possible use by PPBEP for use in our e-newsletter, annual report, social media, or website) with your report as image files to mbwalkinshaw@ppbep.org.			

ABSTRACT: Limit to 250 words. The abstract should include background and a statement of the problem or issue, followed by a description of the research method(s) and design, the major findings, and the conclusions reached.

Fisheries monitoring programs are essential for establishing fish community baselines in dynamic estuarine environments like Pensacola and Perdido Bays (PPB). Seasonal sampling has become increasingly vital as climate change alters local fish communities. For instance, increased catches of Common Snook, a tropical sportfish, in Perdido Bay have led Alabama agencies to establish size/creel limits for this species. Shorter, milder winters necessitate enhanced cold-season (late fall to early spring) monitoring to detect such changes in species phenology and early detection of novel species, especially for Snook that use coastal rivers as thermal refugia. This project was designed to enhance baseline data collection and supplement state agency monitoring efforts and novel species detection by conducting electrofishing, environmental DNA, and habitat surveys in river systems feeding into the PPB from December 2024 to April 2025. Our objectives for cold season sampling in the Perdido and Pensacola River systems were to: 1) determine overwintering fish community metrics (diversity, richness, composition); 2) identify any differences in overwintering fish communities at different river sections (upper versus lower); and 3) evaluate overwintering species associated with various habitat types (e.g., marsh edge, submerged vegetation, unvegetated bottom). Overall we observed 55 species in electrofishing transects over a collective 6.54 river miles samples. Our eDNA sampling returned 126 species including Common Snook and one Snook was successfully tagged. This research successfully supplemented state monitoring, and data generated will help inform future fish and habitat monitoring efforts in the PPBEP area.

INTRODUCTION: Provide necessary background information, describe the purpose of the project, and state the key objectives.

Fisheries monitoring programs are crucial for establishing baseline data on fish communities in highly dynamic estuarine environments, such as Pensacola and Perdido Bays (PPB). Rigorous seasonal sampling has become increasingly important in these temperate estuaries, as climate change alters seasonal patterns and disrupts traditional and scientific understanding of local fish assemblages (Pinsky et al., 2013; Poloczanska et al., 2016). Additionally, the decline in the frequency of local cold events (Fodrie et al., 2010; Purtlebaugh et al. 2020) has resulted in introductions of subtropical/tropical species into the PPB area, posing a risk to local communities by introducing novel predators and competitors to native habitats (Anderson et al., 2022). The shift towards shorter, less intense winters with lower cold snap frequency presents a need to enhance cold-season (late fall to early spring) monitoring of PPB habitats to detect changes in local species' phenology as well as for early detection of novel native species (i.e., organisms that were formerly more restricted in range due to naturally occurring environmental barriers; Forgione et al., 2022) or non-native species (i.e., organisms introduced to an area by humans, either intentionally or accidentally). As other nearby estuaries have experienced a recent rise in catches of tropical sportfish due to range expansion, determining whether these 'novel native' species are overwintering in the PPB area is of great interest.

The aim of this project was to enhance the work of state agencies currently monitoring fish communities in the PPB systems by conducting electrofishing surveys during the cold weather and spring seasons (October 2024 to April 2025). This research was conducted to assist with updating baseline data collection concerning overwintering freshwater and estuarine fish species and the habitats they occupy, and enhancing detection of overwintering novel species. To do so, we conducted electrofishing, environmental DNA (eDNA), and habitat surveys from the upper portions of the river systems feeding into the PPB down to the upper estuaries. Our objectives for additional cold season sampling in the Perdido and Pensacola River systems were to: 1) determine the overwintering fish community (composition, abundance, richness); 2) identify any differences in overwintering fish communities at different river sections (upper versus lower); and 3) evaluate habitat types associated with fish communities sampled (e.g., structural habitat, submerged vegetation, unvegetated bottom). An additional objective related to detecting the overwintering presence of the novel native species, the Common Snook (*Centropomus undecimalis*), a popular sportfish species. Biological data were collected and Snook were tagged with external dart tags printed with contact information to encourage reporting if the fish was recaptured at a later date. Furthermore, the project included several community outreach objectives to enhance the knowledge of stakeholders in the PPB concerning novel and non-native fish species. To achieve this, an online survey to sample local knowledge was developed and distributed among PPB community members, community engagement events such as local fishing tournaments and festivals were attended by the project team, and project related social media pages were established to disseminate information about the study. The informational products generated from this project will provide state agencies much needed data to determine whether enhanced cold season monitoring can help detect any phenological shifts in local communities and build upon efforts to detect novel native species crucial for initiating management plans for potentially impacted native fisheries.

METHODS: Provide sufficient detail for how the project was conducted and data were collected, including specific materials and methodologies/protocols.

Agency Communication: The research team met with biological scientists from the Florida Fish and Wildlife Conservation Commission (FWC) based in the Milton, FL office to discuss the project design, previous and current sampling efforts, sampling locations and areas of interest, methods, and dissemination of results. Consultation with FWC resulted in preliminary sampling with FWC biologists in Bayou Chico, Bayou Texar, and Trout Bayou, all of which exhibited high conductivity that precluded sampling using electrofishing gear. In discussion with FWC, the research team identified areas of the primary systems sampled that were accessible to electrofishing. FWC also provided information and data concerning recent electrofishing sampling efforts in the Perdido, Escambia, and Yellow Rivers. The Perdido River was most recently sampled by FWC in spring of 2017 and 2020. The Escambia and Yellow Rivers have been sampled with higher frequency in the past. The Escambia River has been sampled by FWC every three years in the fall of 2017, 2019, 2021, 2022, and 2023. Similarly, the Yellow River has been sampled by FWC each fall from 2016-2023. The locations sampled by FWC were largely environmentally similar to the sites sampled for this project. Based on this sampling regime, all data generated from this project serve to supplement and enhance state efforts by sampling in the winter season of regularly sampled systems and sampling in systems that are not routinely monitored.

Environmental Sampling: Four river systems within the Pensacola and Perdido Bays watersheds were selected for surveys: the Perdido and Styx Rivers, Escambia River, Pond Creek (tributary of the Blackwater River) and Blackwater River, and Yellow River (Figure 1). Sampling was conducted in the freshwater reaches of the rivers as electrofishing sampling is less effective in salinity above 1-1.5ppt. Each river system was sampled from upstream to downstream locations moving toward the upper reaches of each bay. Sampling activities were conducted in ideal weather, (e.g., no precipitation, low wind, and in areas of moderate river flow). All river systems sampled are tidally influenced, and therefore occasions where a salt wedge was present negatively affected our abilities to sample those areas.

Site characteristics: The Styx River is a tributary of the Perdido River. Both rivers flow into the top of Perdido Bay, are minimally developed, and relatively wide (~150ft across in most places). Sampling locations along both rivers were located along the Styx River Wetlands Forever Tract. The Escambia River is the fourth largest river in Florida and feeds into Escambia Bay. The Escambia River is also relatively undeveloped along its edge. However, several industrial operations are located along its reach before it meets the bay including Ascend Performance Materials, the Huntsman Corporation, and Cerex Advanced Fabrics located in one industrial complex. The complex is located along the river at our sampling sites, denoted "Escambia River Industrial Complex". The Escambia River is also ~150 ft wide in the study area. The Blackwater River is a major river flowing into Blackwater/East Bay. Pond Creek is a small tributary that flows into Blackwater River close to the river mouth entering the bay. Pond Creek is a narrower stream (~50-80 ft wide) with multiple ponds formed around each bend of the river. The Blackwater River is a deeper and wider system (~600 ft wide and usually greater than 20 ft in depth in the main river channel) than Pond Creek with higher and greater flow velocity and volume. Both rivers are fairly developed along their banks, more so than other systems. The Yellow River feeds into the eastern side of Blackwater/East Bay south of the Blackwater River. The Yellow River was the least developed river system in the study. The areas sampled are located within the Yellow River Wildlife Management Area. The Yellow River is a relatively wide and high-flowing system comparable to the Perdido, Styx, and Escambia Rivers (~100-150 ft wide and greater than 10 ft in depth in most areas).

Electrofishing: In order to sample the fish community in each system, the University of South Alabama's 20 ft aluminum electrofishing vessel was used. This vessel is equipped with a generator-powered ETS ® electrofisher that delivers and controls electrical output to the vessel's bow-mounted anode arrays. Electrical charges were adjusted for varying conditions of water conductivity and temperature, but were only ever high enough to produce the desired sublethal effect of temporarily incapacitating fish in the nearby (<15 ft circumference) zone of effective influence (Figure 2). The incapacitated fish were collected by one of two "netters" standing at the bow of the electrofishing boat, who placed the fish in the onboard livewell, which is equipped with a flow through water system. At the end of each survey, each species of fish collected in that transect was identified to the lowest possible taxonomic level, measured for total length (mm) and weight (g), and number of individuals per species was counted. These fish were then returned to the area they were collected. Additional care was taken to ensure that larger fish were properly resuscitated before release. Each system was sampled on at least three separate days from December 2024 to April of 2025. Sampling trips occurred roughly once per month depending on weather conditions (Table 1). A target of at least four transects per river was to be completed in each system, though less transects were completed if salinity was too high or if transects were long in length and/or duration. Transects were selected along the river's edge (Figures 3-6). Structured habitat along the shoreline with a depth of at least 4-6 ft was targeted. Habitat types typically included downed trees, flooded bottomlands, and/or submerged aquatic vegetation (SAV), although our targeted habitats did not exclude unvegetated sandy bottom habitat or shallow edges (2-3 ft depth) from being sampled within transects. Targeted minimum duration for transects was at least 15-20 minutes which corresponded to continuous electrofishing along a riverbank for about 350-450 ft. All environmental, community, and habitat data collected for these field efforts were recorded on field-appropriate datasheets (i.e., Rite-in-the-Rain paper) and all data were entered into a Microsoft Excel® spreadsheet after each field day. Original copies of the data sheets were retained in a secure indoor location and scanned into digital files for storage.

<u>Habitat Surveys</u>: Small-scale and meso-scale habitat and geomorphology (flow characteristics and depth) descriptions of each survey transect were recorded. Estimates of habitat quantity and quality were determined using a rubric that was standardized for this project, based on percent cover of submerged/emergent habitats over the length of the electrofishing transect to achieve a relative measure of percent of preferred fish habitat per transect meter. The "preferred" fish habitat qualifier was based on several metrics including submerged structure type (hardened, woody, herbaceous, etc.), availability of each structure type (amount), structural complexity (highly complex to little complexity), submerged aquatic vegetation (SAV) presence/absence, and proximity to shoreline development. Edge habitat was assessed in the field along each transect following each electrofishing run. A handheld Garmin was used to estimate the length of habitat types. Habitat types (flooded hardwood, submerged aquatic vegetation or SAV, sand bottom, etc.) were assessed visually in the field, recorded on a data sheet, and entered into a database following each field day.

<u>Snook Tagging</u>: For any Snook collected during electrofishing, each individual was to be measured for total and fork length and tagged with a T-bar fish tag that was inserted into the fish's dorsal fin rays. Each tag was labeled with a custom printed identity number and contact details that will allow anglers to report a fish that has been recaptured so that Fish tag ID and location of recapture can be used to estimate seasonal ranges of individual fish. Tag numbers and fish metrics were recorded on data sheets in the field and entered into a database.

<u>Abiotic Data Collection</u>: Abiotic data including salinity (ppt), temperature (°C), dissolved oxygen (DO) (mg/L and % saturation), and specific conductivity (SpCond) (µS/cm) were collected using a YSI ProDSS multimeter. Depth was recorded using a Garmin fish finder mounted on the stern

of the electrofishing boat. Abiotic data were recorded at the surface, mid-depth, and bottom sections of the water column at each transect site as well as additional sites along each river to capture variation in water quality (salinity, temperature, dissolved oxygen, specific conductivity) and potential salinity and temperature stratification in the water column from upstream to downstream on each river. Mid-depth measurements were not required for verifying stratification if depth was less than 4 ft and thus occasionally excluded at shallow water sites. Air temperature data for each sampling day was sourced from the National Weather Service recorded from the station at Pensacola International Airport. Discharge data for each river system (where available) from December 2024 through April 2025 was sourced from the United States Geological Survey's Water Data for the Nation (WDFN) database. Tide charts for each sampling day were sourced from the National Oceanic and Atmospheric Administration's Tides and Currents database (NOAA, 2025a; NOAA, 2025b; USGS, 2025).

<u>eDNA Sampling</u>: Nine sites were chosen along each river system and an additional Snook-positive site was added at Soldier Creek, for a total of ten sites sampled for eDNA: Upper Styx River, Upstream Perdido River, Lower Perdido River, Upper Escambia River, Lower Escambia River, Upper Pond Creek, Lower Pond Creek, Upper Yellow River, Lower Yellow River, and Soldier Creek. Samples were collected using eDNA kits provided by Jonah Ventures, an eDNA analysis company. All samples were processed in the field. At each site, a horizontal Niskin bottle was used to collect water from mid-depth in the water column. Each water sample was filtered in the field from the Niskin bottle using a syringe and filters (45 µm). Filters were preserved in the field through injection with sodium dodecyl sulfate solution. All sampling and filtering was done wearing latex gloves, and gloves were changed between each replicate sample site. Sampling surfaces were wiped down with 10% bleach solution prior to collection at each site. The Niskin bottle was submerged in a cooler filled with 10% bleach solution prior to collection and between each sampling site. These methods were modified from those used by the EPA Gulf Ecosystem Measurement and Modeling Division (GEMMD) Laboratory in Gulf Breeze, FL (Reschke, personal communication). Samples were stored at room temperature following instructions from Jonah Ventures and refrigerated prior to shipment. Samples were shipped within five days of collection. The universal MiFish primer was used to sequence the samples. This primer targets the mitochondrial 12S rRNA gene, and has become a standard tool for fish community assessment through eDNA metabarcoding (Miya et al., 2015). The primers amplify a short region of the gene that provides good taxonomic resolution while working effectively with the degraded DNA typically found in environmental samples. The MiFish primer offers excellent sensitivity for detecting fish presence without invasive sampling, making it ideal for large water bodies where traditional survey methods are challenging or costly. It has been able to detect over 230 subtropical marine species (Miya et al., 2015). Recent comparative studies indicate that MiFish consistently outperforms other primers, detecting the highest number of fish species across diverse aquatic ecosystems including marine, river, wetland, lake, and reservoir systems (Xu et al., 2024).

<u>Social Media Campaign</u>: An Instagram and Facebook page were created for the project and managed by the project participants (i.e., project lead and others listed on this grant). The account handles were "Northern Gulf of Mexico Snook Research Team" and "@ngomsnookresearchteam" on Facebook and Instagram respectively. The accounts were designed for a weekly posting structure including posts and reels about project efforts to inform

the public about research conducted through project efforts. The accounts were also geared towards educating the public regarding potential overwintering of Common Snook and encouraged people to tag their posts with "#hookedasnook" along with the date and location of their Snook catches to help track sightings. The team worked with PPBEP staff to help increase the reach of the social media posts. Content was posted on Wednesday-Friday between the hours of 11-2pm or 4-6pm based on recommendations from PPBEP staff and online resources. Facebook and Instagram insights were used to analyze views and reach of posts. Hashtag use was tracked manually using the Facebook and Instagram search functions (no external software was needed to track hashtag use). A post-by-post and overall insights report was generated to examine effectiveness of the social media accounts. Posts were shared by PPBEP on their social media accounts as well as by the University of South Alabama Stokes School of Marine and Environmental Science accounts.

Online Survey: The survey was developed using Qualtrics through the University of South Alabama. Questions were targeted to gather data on shifting baselines and were developed so that the survey time did not exceed roughly 15-20 minutes. The project team communicated with PPBEP as well as stakeholder agencies for feedback on the questionnaire. Once finalized the survey was submitted for Institutional Review Board (IRB) approval through the University of South Alabama. The survey was distributed via the PPBEP newsletter and stakeholder groups were contacted for additional distribution by email. A question asking how individuals received the survey was included in the questionnaire to help track the reach of the survey through these different avenues. The survey began distribution upon IRB approval in April 2025 . Data were anonymized through Qualtrics, downloaded, and analyzed on a secure computer using Microsoft Excel®. A report of the final data is included below.

RESULTS: Present and describe key results from your research project. This section should accurately describe all data collected, including data summaries, significant observations, and trends (if applicable). Please attach a separate file with map(s), tables, and figures.

Electrofishing: Approximately 6.54 total river miles were sampled over the duration of the study period with an average of 1.64 miles of shoreline sampled in each system (Table 2). Some overlap occurred in transects sampled across subsequent sampling days but no area was sampled more than once per day. Overall, 55 species were recorded across all four major systems reflecting approximately 20 hrs of shocking time. We reported species collected in all systems and those observed in only one system. A total of 24 species were observed within the Perdido and Styx River system, with 305 individuals captured over the study period (Table 3). The highest catch of any one sampling event was 170 individuals on 03/21/2025 likely reflective of higher fish activity in warmer conditions combined with a large number of transects completed on this date (n = 8). (Table 4). The most abundant taxonomic group for this system was sunfish (*Lepomis* sp.), specifically juvenile sunfish with 81 individuals captured, followed by Bluegill

(*Lepomis macrochirus*) with 52 individuals captured. Three species were observed in the Perdido and Styx River system and no other system: Gizzard Shad (*Dorosoma cepedianum*), Redspotted Sunfish (*Lepomis miniatus*), and Speckled Darter (*Etheostoma stigmaeum*). A total of 30 species were observed within the Escambia River system and 419 individual fish were captured overall. Higher catch numbers were observed in lower reaches of the river, possibly due to more ideal sampling locations with lower flow. The species with the highest overall abundance was Redear Sunfish (*Lepomis microlophus*) with 96 individuals captured followed by Bluegill with 60 individuals captured overall. Nine taxa were observed in the Escambia River and no other system: Black Crappie (*Pomoxis nigromaculatus*), Blue Catfish (*Ictalurus furcatus*), Flathead Catfish (*Pylodictis olivaris*), Highfin Carpsucker (*Carpiodes velifer*), a Madtom Catfish (*Noturus sp.*) (<3cm in length), Skipjack Herring (*Alosa chrysochloris*), Striped Bass (*Morone saxatilis*), Threadfin Shad (*Dorosoma petenense*), and White Mullet (*Mugil curema*). A total of 28 species were observed within the Pond Creek and Blackwater River system, with 751 individuals captured overall. This system exhibited the highest catch numbers of the four, but this was likely due to high abundance of small fish (e.g., shiners species in the genus *Notropis*). Lower catch numbers were observed on 1/08/2025 because only three relatively short transects were completed due to elevated salinity in Pond Creek. Similarly, only three transects were completed on 04/11/2025 due to high salinity in the area. Catch numbers, however, were high on this date due to a high abundance of Coastal Shiners (*Notropis petersoni*) in one or more transects. The species with the highest overall abundance was Bluegill (n = 107 individuals) followed by Coastal Shiners (n = 94 individuals). Eight taxa were unique to the Pond Creek and Blackwater River systems: Bluespotted Sunfish (*Enneacanthus gloriosus*), a Crappie (*Pomoxis* sp.), Eastern Mosquitofish (*Gambusia holbrooki*), Golden Shiner (*Notemigonus crysoleucas*), Green sunfish (*Lepomis cyanellu*), Pirate Perch (*Aphredoderus sayanus*), Pygmy Killifish (*Leptolucania ommata*), and Russetfin Topminnow (*Fundulus escambiae*). A total of 30 species were observed within the Yellow River system and 390 fish were captured overall. Highest catch numbers were observed on sampling date 02/28/2025 when the most transects for this system were completed (n = 13). The lowest catch numbers were observed on sampling date 04/16/2025 because only one transect was completed due to equipment malfunction. The species with the highest overall abundance was the Weed Shiner (*Notropis texanus*) with 68 individuals captured, followed by Largemouth Bass (*Micropterus salmoides*) with 39 individuals captured. Two taxa were unique to the Yellow River system: Spotted Bass (*Micropterus punctulatus*) and a juvenile shiner species (Leuciscidae).

Habitat Surveys: The primary habitat types identified along the river edge were flooded hardwood (>50% of coverage overall), submerged aquatic vegetation (SAV), flooded brush, sandy bottom (sand), and emergent vegetation. The dominant habitat type across habitats among sampled transects was flooded hardwood, except in Pond Creek where SAV was also dominant (Figures 7-10). In addition to flooded hardwood and SAV, unvegetated sand bottom was a prominent habitat type in the Upper Escambia River. SAV taxa identified in each system included Bacopa (*Bacopa* sp.), Spatterdock (*Nuphar* sp.), Taro (*Colocasia* sp.), Pipeworts (*Eriocaulon* sp.), Frog's bit (*Limnobium spongia*), Eurasian Milfoil (*Myriophyllum spicatum*), Widgeon grass (*Ruppia maritima*), Pondweed (*Potamogeton* sp.), Bladderwort (*Utricularia* sp.), Swampweed (*Hygrophila* sp.), and Eel grass (*Vallisneria* sp.). The most frequently encountered species of SAV found in all systems were Taro and Spatterdock with the spring run of Boiling Creek on Yellow River and the upper transects of Pond Creek exhibiting greater presence (coverage) and diversity of SAV species. The Perdido and Styx River sites were dominated by flooded hardwood edge habitat with over 80% coverage at most sites and some SAV presence, generally less than 20% coverage (Figure 7). Two transects, UP_T3 (sampled December 13, 2024) and UP_T6 (sampled March 21, 2025), overlapped in sampling area and exhibited a seasonal increase in SAV coverage. Escambia River was the only system where sites exhibited significant unvegetated sand bottom coverage, primarily at the sites furthest upstream that were sampled on January 9th, 2025 (Figure 8). Sites on Escambia River were also dominated by flooded hardwood habitat. There were several overlapping transects in this system: UESC_T7 (sampled January 9, 2025) and ESC_T8 (March 26, 2025), ESC_T2 (February 26, 2025) and ESC_T11 (March 26, 2025), and ESC_T4 (February 26, 2025) and ESC_T12 (March 26, 2025). Little to no change in habitat coverage was observed at these sampling sites across sampling dates. This system exhibited the greatest coverage of SAV within transects. Out of the eight transects in Pond Creek, four exhibited SAV coverage >20-40%. All six Blackwater River transects exhibited SAV coverage with three transects exceeding 90% SAV coverage. There was no overlap between transects at the Pond Creek and Blackwater sites across sampling dates. Overall, sites sampled at Yellow River were dominated by flooded hardwood edge habitat with high presence of SAV only at Boiling Creek, a spring run tributary of the Yellow River (YR_BC_T1) characterized by shallow depths (roughly 6 ft or less) and high water clarity. There were several overlapping transects between sampling days in the Yellow River system: YR_T6 (January 15, 2025) and YR_T9 (February 28, 2025), and combined YR_T4 and YR_T5 (January 15, 2025) and YR_T8 (February 28, 2025). No habitat coverage change was observed across sampling months in overlapping transects, all of which exhibited 100% flooded hardwood habitat coverage. Overall, each system scored the same on the habitat quality rubric. A perfect score of 5 indicates presence and a high level (greater than 50% coverage) of structural habitat, presence and a high level of SAV habitat, and little presence of human shoreline development. The majority of transects sampled across systems contained a high level of coverage for structured habitat, primarily flooded hardwood edge habitat, SAV presence, and a low degree of shoreline development. Details on scoring for each habitat are available in Table 14.

Snook Tagging: An exploratory field trip was made with FWC scientists on December 17, 2024 to investigate potential electrofishing field sites that could serve as Snook habitat. Sites visited on this trip included Bayou Chico, Bayou Texar, and Trout Bayou. All three of these sites exhibited high conductivity with salinities higher than 6 ppt with the exception of upper Bayou Texar (1.34ppt but was only 2 ft or less in depth in the middle of the channel, creating navigational issues preventative of effective electrofishing). Only one snook was tagged in the Perdido watershed. The project team contacted a local angler and expert on fishing in the area, Chris Vecsey, who accompanied the team to a known snook location, Soldier Creek (site of the previous two Alabama state record Snook). The single fish was caught by hook and line and measured 45.7 cm total length (Figure 11). No other snook were captured by the research team within the Perdido and Pensacola Bays study area and no snook were captured using electrofishing in the study area during the project.

Abiotic Data Collection: Here, we report notable aspects and trends in the abiotic data collected for each system during the study period as well as how air temperature, river discharge, and daily fluctuations in tides could influence our fish community data collection. Depths across sampling sites are plotted for ease of visualization for stations where stratification occurred (Figure 17).

Stratification was observed at four sites during the sampling period in the Perdido and Styx rivers: at site SR_S3 (01/29/2025, depth 20 ft), at PR_S1 (01/29/2025, depth 6.7 ft), at UP_S4 (03/21/2025, depth 8 ft), and at UP_S5 (03/21/2025, depth 11.8 ft) (Table 8). The lowest temperature for the system was also recorded at PR_S1 (12.7ºC) at mid-depth, approximately 3 ft. Air temperature for this date ranged from 9-21ºC with 14ºC for the average (Figure 18). The highest salinity and lowest dissolved oxygen values were recorded at UP_S5 at the bottom of the water column at 8.48 ppt and 4.07 mg/L. Minimum salinity of 0.02 ppt occurred across sites and sampling days in the system measured throughout the water column. The maximum water temperature of 18.8ºC occurred at SR_S10 (03/21/2025) measured at the surface. Air temperature for this date ranged from 4-11ºC with an average 11ºC (Figure 18 , Table 8). In reference to river discharge, sampling days occurred during flow levels close to average flow levels (Figure 19). The hydrographs for the Perdido and Styx Rivers were less variable compared to the other systems. Peak flow of 18,000-20,000 cfs occurred in April for the Perdido and Styx Rivers where flow during the majority of the sampling period was near 264 cfs (Figure D). Stratification was observed at one site along the Escambia River, ESC_S4 (02/26/2025, depth 4 ft). The highest salinity value was also observed at this site at 0.09 ppt at the bottom of the water column (Table 8). The lowest salinity values observed for this system were 0.04ppt and were found at multiple sides throughout the water column. The lowest water temperature was observed at UESC_S1 (01/09/2025, depth 8 ft) at mid-depth measuring 8.3ºC with air temp on this date ranging from -1-11ºC with an average 5ºC. At the same site, the lowest DO value was observed at 6.26 mg/L in the bottom water. Sampling occurred during periods of high discharge (10000-23000 cfs) which could affect catch. In periods of high water, additional structurally complex habitat is made available to fish within flooded hardwood areas which may increase the possibility of escape during electrofishing. The Pond Creek and Blackwater River system exhibited the highest frequency of stratification in sites with 12 total stratified sites, six in Pond Creek and six in Blackwater River (Table 8). The lowest salinity value was observed as 0.01ppt at multiple sites in surface water measurements. The highest salinity value of 21.42 ppt was observed at BWR_S2 (01/08/2025, depth 28.8) at mid depth, approximately 14 ft. The lowest water temperature was recorded at 9.6ºC at PC_S3 (01/08/2025, depth 1.8 ft) with air temperatures for this sampling date ranging from -2-9ºC with an average of 3ºC. The highest water temperature was observed as 22ºC in the bottom waters of PC_S14 (04/11/2025, depth 9 ft) which also exhibited the lowest DO value of 2.1 mg/L in the bottom waters. Air temperature for this site ranged from 12- 24ºC, with an average of 18ºC. In reference to discharge, no specific data was available for Pond Creek. For the Blackwater River, sampling days occurred during high water events and lower flow events. The hydrograph for Blackwater River was more variable than other systems, fluctuating from 100-1100 cfs. However, the station used to gather discharge data for Blackwater River is much further upstream than the area sampled during this study so it is likely that discharge conditions in the study area varied from these data, but the upstream station can still provide insight into overall patterns of change in flow along the river downstream.No sites exhibited stratification in the Yellow River system. The lowest observed salinity value was recorded as 0 ppt at YR_S11 (04/16/2025, depth 3.8 ft) at the surface. The highest salinity value was 0.03 ppt recorded at YR_S4 (01/15/2025, depth 14.9 ft) at mid-water depth, approximately 7 ft. The lowest water temperature was recorded as 9.1ºC at two separate sites on sampling date 01/15/2025: YR_S1 (depth 4.8 ft) at the surface, and YR_S2 (depth 9.6 ft) at mid-water depth, approximately 4 ft. The air temperatures for this date ranged from 8-24ºC with an average of 15ºC. The highest water temperature of 20ºC was recorded at YR_S13 (04/16/2025, depth 17 ft) at mid-water depth, approximately 8 ft. Air temperature for this date ranged from 12-24ºC with an average of 18ºC. The lowest DO measurement of 7.84 mg/L was recorded at YR_S7 (02/28/2025, depth 9.4 ft) in the bottom waters. Of all the systems, this system exhibited the highest minimum DO value likely due to less stratification overall. Yellow River exhibited a highly variable hydrograph. Discharge fluctuated frequently from less than 500 cfs to more than 3000 cfs. However, the discharge level for Yellow River was of lower magnitude than the other systems. High and low tide conditions for each system for each sampling day are reported in Table 9.

eDNA Sampling: A total of 126 species were detected in the eDNA samples across all sites and replicates (Table 10). This total reflects some genetic variation detected that could only be identified at the family level but could indicate additional species. A total of 24 species were detected in both the electrofishing transects and the eDNA samples, 21 species were detected only by electrofishing, and 84 species were detected solely through eDNA (Table 11). We were able to positively detect Snook in all three replicates taken at Soldier Creek, our "Snook positive" site. Snook were not detected in any of the other sites. Several outliers were detected, highlighted in red in Table10. These outliers indicate non-native species, some of which are highly unlikely to occur in the sampled habitats. Two deep-sea species, deep-sea smelt (Bathylagidae (Family)) and Lanternfishes (*Stenobrachius* sp.), three Pacific species associated with southern California (Jack silverside (*Atherinopsis californiensis*), Shiner perch (*Cymatogaster aggregata*), and Pacific sardine (*Sardinops sagax*), and two non-native tropical species (a cichlid (*Apistogramma hippolytae*) and a Swordtail (*Xiphophorus* sp.)) were detected.

Social Media Campaign: Both accounts were launched February 4th, 2025. A total of five posts were made on Facebook and seven posts were made on Instagram with most activity occurring during the sampling period from February to April. The posts that received the most views, interactions, and greatest reach on both platforms was on March 7th, 2025 with 1120 views on Facebook and 2163 views on Instagram as of June 2025. Excluding these high-views posts, average views, interactions, and reach for Facebook posts was 126, 4, and 44 respectively and for Instagram posts 274, 9, and 139 respectively (Table 12, Figure 20 and 21). Instagram demonstrated overall higher engagement than Facebook posts. The "#hookedasnook" was only used in reference to the project and Snook catches in the study area in our own social media posts. On Facebook, one other use of "#hookedasnook" was found for Sanibel, FL. On Instagram, four uses of "#hookedasnook" were found for areas of South Florida and three uses by a single account non-related to Common Snook catches. At the time the social media accounts were launched, the project team also launched a project email, hookedasnook@gmail.com, to encourage members of the public to contact us with Snook sightings. The project team also provided emails of Dr. Martin and Zoey Hendrickson. Most sightings traffic was directed to Dr. Martin's and Ms. Hendrickson's emails rather than the project email or social media direct messaging. However, we did receive several sightings from messages sent via Instagram though these sightings occurred outside of the project study area in Alabama.

Online Survey: The online survey received zero responses from the public.

Analysis of FWC Data: To more accurately compare historical electrofishing catch data provided by FWC with the recent catch data conducted for this project, we limited our comparisons to data collected within the same systems and seasonal time frame (winter to spring). This restricted the FWC dataset to transects conducted in April of 2017 for Perdido River, and November through December of 2016 to 2023 for Escambia River and Yellow River. FWC conducted transects covering 1.7 river miles in the Perdido River system, 7 river miles in the Escambia River system, and 6.3 river miles in the Yellow River system.

The FWC electrofishing efforts resulted in a species detection rate (i.e. the number of species collected per river mile [RM] sampled) of 13.6 RM^{-1} for the Perdido River system, 14.3 RM^{-1} for the Escambia River system, and 6.4 RM^{-1} for the Yellow River system. This can be compared with the species detection rate for this project of 34.3 RM^{-1} for the Perdido River system, 18.9 RM^{-1} for the Escambia River system, and 37.4 RM^{-1} for the Yellow River system. When comparing species list agreement between the FWC dataset and ours, the FWC species list for the Perdido River system was in 87% agreement with ours (i.e., 87% of the species listed by FWC were also collected by us during this project), and our species list was in 60% of agreement with theirs (i.e. 60% of the species we collected were also listed in the FWC dataset), with differences in species lists described in Table 13. For the Escambia River system, the FWC species list was in 46% agreement with ours and our species list was in 91% agreement with FWC's, with species discrepancies listed in Table 13. For the Yellow River system, the FWC species list was in 52% agreement with ours and our species list was in 76% agreement with FWC's, species discrepancies are listed in Table 13. Species discrepancies in the Escambia and Yellow River systems are partially explained by the FWC vessel's abilities to sample in higher salinities, as suggested by the presence of at least 17 species that are predominantly located in brackish saltmarsh environments as opposed to the more tidal freshwater and forest habitats that we were restricted to for our sampling efforts.

DISCUSSION AND CONCLUSION: Present, interpret, and discuss the results, project outcomes, future research needs, and how this research connects back to the CCMP.

The information generated from this project helps to fill data gaps for seasonal sampling in the Perdido, Escambia, and Yellow Rivers and supports monitoring efforts in data-poor systems such as the Styx River and Pond Creek where regular sampling has not occurred. Filling data gaps is crucial for supporting restoration and conservation efforts for native fish communities and their associated habitats within the Pensacola and Perdido Bay watersheds, areas known for a high level of biodiversity (Elkins 2019) (CCMP Goal 6.1 and 6.2).

Electrofishing: We acknowledge limitations of collection gear including a depth limit on electrofishing effectiveness of ~6 ft, species- and size-dependent variations in effectiveness, and lower effectiveness in areas with higher flow. Therefore, we experienced lower electrofishing effectiveness in areas with higher river currents, deeper depths, and flooded edge habitats that allow for fish escapes. Electrofishing can confirm definitive presence of fish but cannot verify the absence of unobserved species from the overall community. Addressing these caveats is essential for contextualizing and qualifying our data, results, and conclusions. Overall, we observed similar species richness and abundance across systems. We succeeded in supplementing state monitoring efforts by FWC as shown by comparing species detected within our transects to those detected in FWC's data and finding species discrepancies. This shows potential evidence for differences in fish communities seasonally within each system and supports justification for increased monitoring efforts and allocation of resources to continue and increase regular sampling.

Habitat Surveys: The habitat surveys revealed distinct patterns in aquatic vegetation and substrate composition across the five river systems studied. Flooded hardwood dominated edge habitats in most systems, comprising over 50% of total coverage, which reflects the forested riparian character typical of these southeastern U.S. waterways (Warren & Burr, 1994). However, the distribution of SAV varied considerably between systems and appears to be a factor influencing fish community abundance. Our data support the need for increased monitoring of edge habitat for SAV presence and surveys to monitor the quality and provision of essential fish habitat in the rivers flowing into the Pensacola and Perdido Bay system.

Data from the surveys suggest an association between SAV presence and fish abundance. The Pond Creek/Blackwater River system, which exhibited the highest SAV coverage with several transects exceeding 90%, supported nearly twice the fish abundance (n = 751 individuals) compared to the Escambia River (n = 419 individuals), despite having seven fewer sampling transects. This pattern suggests that SAV density may be more important than overall habitat area in supporting fish populations (Bolduc et al., 2020; Miller et al., 2018). Research has consistently demonstrated that SAV provides critical habitat structure, with fish biomass increasing significantly with SAV patch size (Looby et al., 2021; Giacomazzo et al., 2023).

The numbers and types of different SAV species identified, including native taxa like Spatterdock and Taro alongside non-native species such as Eurasian Milfoil, indicates varying ecological conditions across systems. The spring-fed Boiling Creek tributary of the Yellow River demonstrated how specific hydrological conditions (shallow depths, high clarity) can create localized SAV hotspots even within systems otherwise dominated by woody structure. Spring-fed systems are characterized by more stable water temperatures and volumes, making them an increasingly important habitat for aquatic vegetation and fish (Lusardi et al., 2021).

The results highlight the need for monitoring programs to specifically track SAV coverage and composition, as changes in aquatic vegetation could significantly impact fish community structure (Smokorowski and Pratt, 2007). SAV serves as an important nursery habitat for juvenile fish and provides refuge for smaller species from predators (Lazzari and Stone, 2006), while also functioning as a key indicator of water quality and ecosystem health (Kovalenko et al., 2018). This habitat can also facilitate the movement of novel native species, such as Snook, into a new geographic area. Given that the southeastern U.S. represents a global hotspot for aquatic biodiversity (Burr and Mayden, 1992) but faces significant conservation challenges (De Freese, 1991) including novel natives and invasive species (Searcy et al., 2023), understanding and protecting SAV habitats becomes even more critical for maintaining healthy fish communities in these systems.

Abiotic Data Collection: This study documented spatial and temporal variability in environmental conditions across the Pensacola and Perdido Bay watershed river systems, providing critical baseline information that supports CCMP Goal 6 objectives through provision of environmental data supporting biological monitoring initiatives. The findings reveal distinct environmental gradients among systems, consistent with established research demonstrating that abiotic factors such as temperature, salinity, and dissolved oxygen may influence fish community structure and assemblage composition in estuarine and riverine systems (Gebrekiros, 2016). The Pond Creek and Blackwater River system exhibited the highest frequency of stratification (12 sites) and greatest salinity range (0.01-21.42 ppt), while the Yellow River showed no stratification and maintained consistently low salinity levels. These abiotic variations, particularly water temperature ranges (8.3-22°C) and dissolved oxygen levels (2.1-7.84 mg/L), likely create diverse habitat conditions that influence fish community structure (Gebrekiros, 2016). Furthermore, the documented salinity gradients are particularly important for the life histories of estuarine fish species (Bacheler et al., 2009).

Future research should focus on quantifying the relationships between these abiotic parameters and fish community metrics, building upon ongoing research in the Pensacola and Perdido Bays watersheds, which could also include eDNA sampling approaches as well as the establishment of monitoring frameworks for long-term environmental assessment. This comprehensive abiotic characterization provides the foundational environmental context necessary to inform effective fisheries monitoring and conservation planning in what are recognized as some of Florida's most productive estuarine ecosystems. The study directly advances CCMP Objective 6.2 by facilitating regular monitoring of estuarine aquatic species assemblages and supporting evidence-based management decisions for fish and wildlife conservation across the Pensacola and Perdido Bay watersheds.

eDNA Sampling: The results generated from eDNA sampling greatly enhanced species detection in all sampled systems. The deep-sea fish and Pacific species detection is likely an error in the Primer used for the metabarcoding or the result of contamination by human products or non-native fishing bait . The two tropical non-natives could be the result of error or could be the result of aquarium dumping. There are known populations of Swordtail/Platyfish in the Panhandle that are being monitored by FWC and other entities. The Cichlid was only detected in the Upper Escambia River but this sampling location is not far from a boat ramp and park accessible to the public, so aquarium dumping cannot be ruled out. The Swordtail was detected at three separate and geographically distant locations (though these locations are connected by the Pensacola and Perdido Bays): Lower Escambia, Lower Yellow River, and Lower Pond Creek. There are key considerations to take into account for eDNA sampling. In open water systems, several factors can complicate eDNA applications. DNA degradation and dilution in large water bodies may reduce detection sensitivity, particularly for species present in low densities. The method provides presence/absence and relative abundance data rather than absolute population estimates, and results can be influenced by water currents, mixing patterns, and seasonal variations in fish behavior (Fonseca, 2018). Depending on environmental conditions, DNA may degrade quickly or persist and be transported long distances, retained for prolonged periods, or resuspended into the water column at later times (Darling, 2021). eDNA can also be inadvertently transported by humans and wildlife. Studies show that eDNA detection distance can vary from a few kilometers in small streams to more than 100 kilometers in large rivers, where eDNA behaves like fine particulate organic matter in the water column (Pont et al., 2018). eDNA could enter the environment from sources other than living animals, such as sewage and wastewater, feces from predatory animals, or dead animals (Rees et al., 2014). This means that DNA from fish consumed by birds or other predators can appear in water bodies where the live fish never existed. As such, results should be interpreted cautiously regarding population abundance; integration with traditional survey methods may be necessary for comprehensive fisheries management decisions.

Community Engagement In-person and OnlineU: During the study period the research team implemented the Instagram and Facebook social media pages, the online survey, and attendance of in-person community outreach events to engage and educate the public concerning project efforts and topics involving local fish community diversity and drivers of ecological changes in the PPBEP area. Of these efforts, we experienced the greatest success with in-person outreach and relatively low online engagement. For this and future projects, in-person outreach events will continue to be included and prioritized in conjunction with strategic efforts to increase online engagement by working with PPBEP outreach personnel and additional community groups.

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<u>SUCCESSES AND CHALLENGES:</u> Describe the significant successes and challenges the organization experienced related to the funded grant.

Several significant successes were achieved throughout this project. The research team successfully sampled 6.54 total river miles and documented 55 fish species through electrofishing efforts, providing valuable baseline data on overwintering fish community composition in the river systems sampled. The integration of multiple sampling methods proved particularly effective, with eDNA analysis detecting 126 total species and confirming the presence of 24 species also captured through electrofishing. Notable achievements included the successful tagging of a Snook specimen and the positive eDNA detection of Snook at the known Soldier Creek location, validating both methodologies. The comprehensive habitat assessments revealed strong relationships between submerged aquatic vegetation (SAV) presence and higher fish abundance, particularly evident in the Pond Creek/Blackwater River system where 751 individuals were captured despite fewer transects than other systems.

However, the study also encountered several significant challenges that influenced data collection and interpretation. Electrofishing effectiveness was limited by depth constraints (≤ 6 ft), variable species-specific capture rates, and reduced efficiency in high-flow areas. Environmental conditions frequently disrupted sampling efforts, with elevated salinity levels restricting gear efficiency in certain areas of Pond Creek. Moreover, equipment malfunctions reduced transect completion on multiple occasions. The eDNA results, while extensive, included probable analytical errors such as detection of deep-sea species and Pacific coast fish highly unlikely to occur in the study area, highlighting the method's susceptibility to contamination or database inaccuracies. Additionally, the targeted Snook sampling proved challenging, with only a single individual captured despite exploratory efforts across multiple potential habitats, underscoring the difficulty of detecting presence of species in low densities using traditional sampling methods.

The research team also encountered challenges distributing and receiving responses for the online survey. The low response rate observed for the online survey was reflective of difficulties in survey distribution. Originally the project team planned to distribute the online survey at outreach events and through social media using a QR code and website links. However, this distribution method was changed out of concern over how the data could be analyzed. In order for the survey data to be meaningful, a known population pool needed to be identified and surveyed. Therefore, we relied on the PPBEP newsletter for distribution among their community members and reached out to various angler and recreational groups within the community for further distribution. Only one angling group replied and was unable to distribute the survey during the study period due to issues with their servers. However, creation of an IRB approved survey represents a success in this project. The survey produced by the research team can be deployed again, even repeatedly over different time periods. In this way, we successfully created a tool for sampling community knowledge even though distribution did not go as planned.

During the project period the research team attended a total of four community outreach events: a talk given to the Alabama Coastal Fishermen's Association April 3rd, 2025, the Dauphin Island Sea Lab annual Discovery Day held on Saturday April 5, 2025, Earth Day Pensacola held at Bayview Park in Pensacola, FL on Saturday April 19, 2025, and the Delta Woods and Waters Expo held at the 5 Rivers Delta Resource Center in Spanish Fort, AL on Saturday April 26, 2025. At each of these events the research team set up a table with a fish matching activity highlighting common species encountered during electrofishing sampling to educate the public and increase knowledge of local freshwater and estuarine species occurring in the PPBEP area. The project team also set up a life-sized, 3-D plastic Snook model to encourage conversations about shifting baselines and changes to local fish communities. Stickers were created to help encourage reporting of Common Snook sightings in the area with the project preferred hashtag, and with the project email and social media handle printed on the bottom (Figure 22). We also prominently displayed the PPBEP logo on our table to highlight the work being done by PPBEP, their support for this research, and their role in the community. We received very positive responses and feedback from community members both in person at outreach events and from social media. Overall outreach efforts were successful in broad engagement for community members of all ages towards educating the public concerning local fish species, as well as environmental and anthropogenic drivers of potential change in local fish communities (CCMP Goal 6.1)

<u>LESSONS LEARNED:</u> Describe what the organization learned based upon the results, successes, and challenges reported. Address programmatic, evaluative, or organizational changes that will be made based upon these lessons learned.

For future projects, a better strategy for engaging the public through social media would be to supply additional, ready-to-post content to PPBEP staff to post on their well-established platforms. The time necessary to gain an effective following for meaningful engagement is not conducive to the eight-month project period. The accounts established by the research team during this project period are useful and can remain active beyond the project period but for any future short-term projects, boosting public awareness and engagement with project efforts via the PPBEP's social media pages would be a more effective method rather than attempting to operate independent accounts.

For the online survey, the project team plans to re-distribute the online survey at a later date following a more widespread search for eligible populations to sample and more numerous distribution options. The survey in its current form can be re-deployed following a simple request for IRB re-approval (i.e., an augmentation to study period). Creation of this survey during the project allows for the use of the survey beyond the project period so that information on community knowledge can continue to be gathered.

Overall, the findings from this project underscore the importance of sustained and adaptive fisheries monitoring in dynamic estuarine environments like Pensacola and Perdido Bays (PPB). Moving forward, a key priority should be to expand the temporal scope of fisheries independent monitoring with comprehensive spatial and temporal sampling, particularly during the late fall through early spring period when range-expanding subtropical and tropical species are most likely to be overwintering in thermal refugia created by these riverine systems. Long-term, year-round monitoring will help capture seasonal transitions, phenological shifts, and interannual variability in fish assemblages—patterns that are likely to become increasingly important as environmental change continues to alter regional temperature regimes and hydrology.

Expanding the spatial resolution of sampling to include underrepresented habitats, such as tidal creeks, marsh edges, and backwater embayments, would improve understanding of nursery habitat usage and potential refugia for both native and novel species. For example, sampling in upper reaches of estuaries and the marine environments can document the connectivity of these different systems, with implications for allocation of conservation and restoration efforts. Additional research should be made to integrate complementary sampling methods, including environmental DNA to detect rare or cryptic species, otolith microchemistry to track natal origins and movement histories, and acoustic telemetry to monitor residency and habitat use of key species across seasons. These tools, when paired with traditional sampling, offer a more complete and nuanced picture of community dynamics in coastal rivers.

Furthermore, linking biological observations with continuous environmental data, such as salinity, temperature, dissolved oxygen, and freshwater inflow, will improve our ability to identify the key drivers of species turnover, shifts in assemblage composition, and potential thresholds of ecological change. Collaboration with water quality monitoring programs and hydrological modelers can enhance predictive capacity for future changes in species distributions and habitat suitability under various climate and restoration scenarios. Moreover, the role of SAV in influencing the broader ecological structure and function, as well as how environmental conditions influence SAV presence, is deserving of future study.

To support management and outreach efforts, it will also be important to synthesize findings into accessible formats, such as species range maps, seasonal abundance charts, and community composition indices, which can inform fisheries agencies, resource managers, and the general public. Finally, ongoing partnerships with local organizations, universities, and citizen science initiatives can expand data collection, foster public engagement, and ensure that future monitoring efforts are both scalable and sustainable. By building on the strong foundation established through this project, we can better detect and respond to ecological changes in PPB and contribute to a broader understanding of the ecology of coastal rivers and the influence of anthropogenic stressors across northern Gulf of Mexico estuaries.

This report is submitted in accordance with the reporting requirements of Agreement No. XXXX and accurately reflects the activities associated with the project.

Charles W. Martin

Signature of Grantee's Representative

06/12/2025

Date

Charles W. Martin, PhD

Print Name and Title