

Attachment 5
PPBEP COMMUNITY GRANT FINAL RESEARCH REPORT FORM

Agreement No.:	FY2023-09		
Grantee Name:	University of West Florida		
Grantee Address:	11000 University Parkway, Pensacola FL 32514		
Grantee's Representative:	Dr. Amanda Croteau	Telephone No.:	(850) 474-2151
Project Title:	Why settle here? The effect of water quality, water column placement, and substrate on epibenthic settlement in Perdido Bay		
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 lmcdonald@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.

Various stakeholder groups have expressed concerns about water quality within Perdido Bay and possible declines in encrusting organisms including barnacles and bivalves. This project examined the settlement and colonization of fresh substrates at seven locations across the estuarine gradient, including three in the open bay and four in protected bayous with reduced wave action. Settlement arrays were designed and deployed to examine the influence of water column location (where depth allowed), substrate type, and time. Ambient water conditions were measured and samples were taken for nutrient, chlorophyll, and total suspended sediment concentrations. A sedimentation tube was deployed at each array location. Arrays were deployed and sampled monthly (Jan-Jun). Sampling location and month were more important than depth or substrate type to community composition. Initial colonization was primarily barnacles and tube dwelling mobile organisms (polychaetes, amphipods, etc.), but pulses of additional species (e.g., oysters, mussels, nudibranchs, etc.) were noted. Observed differences between surface and bottom treatments were primarily related to predator numbers (greater on bottom). Oysters were not observed until the last month of sampling and only at the most southern and highest salinity location.

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

During stakeholder engagements (workshops and presentations), members of the community and local community science groups have voiced concerns that the water quality within Perdido Bay is toxic to planktonic life. They have observed a decline in encrusting organisms and bivalves, many of which have a planktonic larval stage that would be susceptible to unfavorable water conditions. In response to this concern, we conducted a water quality and zooplankton monitoring study of Perdido Bay funded by a 2022-2023 PPBEP Community Grant and the UWF Summer Undergraduate Research Program. Initial findings indicate that water quality and community composition vary by location, but within much of the bay, water quality would be satisfactory for an estuarine epibenthic community.

The next logical step in understanding the epibenthic or biofouling community in Perdido Bay was to conduct a settlement study. Our goals were to look at natural settlement and colonization along the estuarine gradient in both open bay and protected bayou locations to better understand larval dispersal and settlement in the system. We used five different substrate materials at each

location to determine if there was a preference for substrate, which could inform future restoration efforts. At locations with a depth greater than 1.5m, a surface and bottom array were deployed. Each month the arrays were pulled and substrates were taken to the lab for taxonomic identification and quantification of monthly settlement patterns. An additional set of substrates remained for the entire study period to examine long-term settlement and survival.

This data will be useful to investigate and address stakeholder concerns. It will also supplement the long-term Perdido Bay water quality dataset as much of the regular monitoring of this system has not been reinitiated after pausing during the COVID-19 pandemic. This study is also helpful in the context of habitat restoration. Current datasets are needed to create suitability models for restoration, especially current datasets that include benthic and water column data in addition to surface measurements. Understanding salinity, oxygen, sedimentation, and settlement dynamics of the system are critical for assessing the suitability of large-scale habitat restoration such as the oyster restoration efforts currently occurring in the Pensacola Bay system.

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

Field Methods

Array construction and deployment

Prior to construction, oyster shells were bleached, cleaned, soaked in freshwater and dried. All other materials were cut to standard sizes (as needed), soaked in freshwater, and then dried. Inside each crate two sets of 5 substrates (hardy backer board tile, oyster stringers, limestone, concrete paver, and wood) were secured with cable ties. The long-term survival set of substrates was indicated with a white cable tie. A Hobo conductivity sensor and either a Hobo dissolved oxygen sensor or MiniDOT dissolved oxygen sensor that was wrapped and outfitted with antifouling copper were secured to opposite sides of the crate array.

Arrays were deployed in January either as surface and bottom treatments (2 crates per site) or mid-water treatments at shallower locations (Figure 1; Table 1). An independent sediment tube was deployed at locations where the bottom was predominately sand and secured to its own anchor and float to help maintain its upright position. At locations where there was a large amount of fluid mud and where the potential for disturbance contamination was a concern, a smaller sedimentation tube was attached directly to the outside of the crate. Ambient conditions (temperature, dissolved oxygen, conductivity, salinity, pH, and turbidity) were measured with a YSI. A surface measurement was taken at all sites, and a bottom reading was recorded when depth exceeded 1m. Water clarity was measured with a Secchi disk. A surface grab water sample was taken for total Kjeldahl nitrogen, total phosphorus, dissolved nutrients, chlorophyll-a, color and TSS. 60ml of water was filtered in the field for dissolved nutrients and color analysis, and the filter was retained for chlorophyll analysis. Bottom samples are collected with a van Dorn at locations where there is a bottom treatment, and processed for dissolved nutrients, chlorophyll-a, color, and TSS. All samples were stored on ice and transported back to UWF for analysis.

Monthly Monitoring

All sites were revisited monthly on a roughly two-week rotation. Sedimentation tubes were collected prior to any disturbance (if independent) or capped prior to the array being removed from the water if attached. Pulled arrays were inspected for live mobile organisms (fish, crabs, gastropods, etc.) which were recorded and released. Short-term settlement substrates were removed from the crate and placed in individual containers with site water. Long-term survival materials were photographed during monthly monitoring, and removed during the final sampling event in June. Sensors were removed, downloaded, and reattached to the crate. Ambient conditions and water quality samples were taken (as described above).

Lab Methods

Array Processing

Substrate materials were processed within 24-48 hours of removal. Substrates are removed from containers and photographed before being gently rinsed with deionized water to remove sediment and photographed again. Mobile organisms (e.g., amphipods, polychaetas, crabs, shrimp, etc.) were collected from the transport container and rinse water. Organisms are recorded and given rough identification before preservation in ethanol, for later identification to the lowest possible taxonomic level. Substrates were analyzed on the microscope for the detection of very small fouling organisms (e.g., barnacles a few days post settlement), and a subset of the surface area was enumerated under the microscope. Rinsed images were later processed using Coral Point Count software to determine percent cover.

Water quality

Total Kjeldahl nitrogen and total phosphorus were processed by UWF's Wetlands Laboratory (Table 2). All other water quality samples were processed within the Caffrey lab using the following standard methods (Table 2).

Sediment

Sieve fractioned (#18, 35, 60, 170) materials were dried to a constant weight. Liquid samples were filtered using preweighed, ashed filters and dried to a constant weight. Dried samples were ashed and weighed. Total sedimentation and % organic content were calculated.

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.

Bayou Marcus was the lowest salinity location, with recorded salinity ranging from 0.45 to 6.07 PSU (Figure 2). The Smith site, located just inside Arnica Bay consistently had the highest salinity, ranging from 14.7 to 24.7. Bayou Marcus often had the lowest dissolved oxygen concentrations. Bayou locations generally had lower oxygen concentrations than bay sites, but a reading below 4 mg/L was only recorded once over the course of the study (Figure 3). Nutrient concentrations (especially nitrogen) were generally higher at the bayou sites than the open bay locations (Figures 4-7). Chlorophyll concentrations were variable, with highest values observed at the bay locations both north (Lillian Oaks) and south (Hoffman) of the Lillian Bridge (Figure 8). Color was variable, but generally increased over the study period, with the highest values observed in May at Weekly Bayou and Bayou Marcus (Figure 9). Bayou Marcus consistently had the highest sedimentation (Figure 10). Percent organic content of the sediment samples was higher at the bayou sites (Figure 11).

Monthly settlement changed over time, with colonization of substrates generally increasing over the study period (Table 3). All locations did have some barnacle settlement in month 1 (Jan-Feb) and settlement increased over the study. Mussels began to appear at array locations as early as month 1, but were more widespread and numerous in month 2 (Feb-Mar) onward. Mussel species were observed at all locations, but were a more dominant part of the Bayou Marcus community. Oyster settlement occurred during month 5 (May-June) at the Smith array. Oysters were present on both water column treatments, but a greater number were found on the bottom array substrates than the surface (65 and 14, respectively). Other fouling taxa such as calcareous tube worms, bryozoans and hydroids were observed throughout the study as well, with taxa and distribution changing over time.

Images processed in Coral Point Count were classified into large groups (e.g., bivalves), and subgroups (e.g., mussels and oysters), and results were compared using mvabund in R at both levels (group and subgroup). There were significant differences in bay and bayou communities by group (Table 4). This difference was largely driven by percentage of bare coverage (lack of fouling) (Figure 12), which was higher at bayous sites, and the abundance of barnacles which were lower in the bayous (Figure 13) for both the group and subgroup analysis. Additional subgroup drivers were nerite eggs (higher in bayous) Figure 16. Although, not significant drivers, mussels were more abundant in bayou sites (Figure 14) and oysters were more abundant in bay sites (Figure 15).

Communities also differed by individual location, mirroring patterns observed with bare and barnacle distribution differences between bay and bayou site groups for both the group and subgroup analysis (Table 4, Figures 17-18). At the site level, bivalves were also a significant group with more observed on either end of the salinity gradient (Figure 19). When separated into subgroup, mussels were more prevalent in low salinity sites (Figure 22), while oysters were only observed at the highest salinity bay site (Smith) (Figure 23). Statistically there were some trends in other fouling organisms like sediment tube dwelling organisms and bryozoans, however these trends should be treated with some caution as their identification could be difficult, and when

present at the same point with other organisms (such as barnacles or bivalves) were not recorded (Figures 20, 21, 25, 26, 27). Calcareous worm tubes were notably higher in abundance at locations with higher salinities (Figure 24). Eggs were a driver for the group analysis, but this was primarily being driven by the high number of nerite eggs within one of the fresher bayou sites (Heron Bayou) (Figures 22 and 27).

Although the amount of bare substrate was not a driver for significant community differences (i.e., the amount of fowling on substrates was similar) (Table 4, Figure 28), there were some notable differences in the composition of the fowling between substrates types (oyster, rock, tile, paver, tile). In the group analysis this was driven by barnacles (fewer on rock) (Figure 29) and tube forming organisms (more on rock, fewer on oysters and wood) (Figure 30) (Table 4). When analyzed by subgroup, calcareous tubes were also a driver, with fewest found on wood and oysters (Figure 31). Sediment tubes from various organisms were also a subgroup driver with rock having the most noted (Figure 32), but as stated before these structures were difficult to discern in some photos (especially on darker substrates) and if the tube was on a barnacle, bivalve, etc. it was marked as that rather than a sediment tube.

February fell out in the group and subgroup analysis as having a greater percentage of bare substrate than all other months (Table 4, Figure 33). Monthly differences indicate that barnacles and bivalves increased over the study (Table 4), although there was a dip in barnacle abundance during May (possibly related to high rainfall) (Figures 34-35). Oysters were a subgroup driver and did not appear until the last sampling month, June (Figure 37). Hydroids and bryozoans increased in the spring and summer depending on category (Figures 36, 40, 41). Calcareous tubeworms generally increased into the spring and summer (Figure 38), while sediment tubes had a peak in the late winter to early spring (Figure 39). Nerite eggs were a weakly significant subgroup driver that increased in the later months of the study (Figure 42). Macroalgae, which was only noted in April (Figure 43) was also a weakly significant driver in the subgroup analysis.

Mobile organisms found in association with arrays included crustaceans (e.g., mud crabs, stone crabs, blue crabs, penaeid shrimp, grass shrimp, amphipods, isopods), fish (primarily Skilletfish, and various species of gobies and blennies), nudibranchs, flatworms, polychaetes, chitons, and gastropods (including rock snails, an oyster predator). These taxa did vary by location, but many were nearly ubiquitous. Many species also deposited eggs on the arrays (e.g., nerites, Skilletfish, gobies, blennies, etc.) which were noted.

Subgroups that were significant drivers for both month and substrate were compared with boxplots of each of these variables. The bare subgroup generally showed the same trend for all substrate over time as noted above in the monthly analysis (initial drop after February then relatively stable) (Figure 44). Barnacles generally increased over time with a dip in May for all substrates, pavers often had the highest percent coverages and the coverage on tiles tended to increase over time (except during the dip noted for all substrates in May) (Figure 45). Calcareous tube worms increased over time most often on pavers, but rocks and tiles had peaks toward the end of the study, while wood and oyster always had lower percent coverages (Figure 46). Sediment tubes were most abundant on rocks in all month, but tiles also showed some higher percent coverages. March had the highest percent coverages of sediment tubes for all substrates (Figure 47).

Long-term substrate treatments were placed in the array during the initial deployment and were not pulled for analysis until the last month of study (June). Analysis of the long-term substrates at each location showed some similar trends to the short-term substrates, but generally there were fewer differences overall (Table 5). When comparing bay to bayou sites, the bare, barnacles, and tunicates groups and subgroups and oyster subgroup were significant drivers. There tended to be more bare substrate in bayous (Figure 48) and more barnacles in open bay sites (Figure 49). Tunicates were only noted in open bay sites as was the case with oysters (Figures 49-50). When comparing the long-term substrates by location Heron Bayou and Sea Spray (a bayou site) had more bare surfaces, while Bayou Marcus and Weekly Bayou were more similar to the bay sites (Figure 51). Sea Spray and Weekly Bayou had the lowest percent coverages of barnacles while Smith (an open bay site with the highest salinity of all sites) had the greatest barnacle coverage (Figure 52). Heron Bayou and Hoffman (a bay site) (Figure 54) had the least tube coverage, but as noted previously the tube data (especially sediment tubes should) should be treated with caution. Similar to the short-term substrates, calcareous worm tubes had the highest percent coverages in the more southernly, saltier locations (Figure 59) while sediment tubes were more abundant at fresher sites (Figure 60). Hydroids and bryozoans were a driver but there were no clear patterns (Figures 55 and 61), similar to tubes, this group was not always readily visible in photos and often grew on barnacles (when this occurred, the point was marked as a barnacle). No bivalves were noted at Hoffman, Sea Spray, or Weekly Bayou with more generally being noted at Bayou Marcus, Heron Bayou, and Smith (Figure 53). Tunicates and oysters were only noted at Smith (Figures 56 and 58). Mussels were generally in greater presence at fresher sites, especially within bayous (Figure 57). When analyzing the long-term substrate by substrate type there was not a significant community difference at the group level (Table 5). There was a significant difference at the subgroup level, but it was driven entirely by the lace bryozoan subgroup (Figure 62). These bryozoans often grow on other encrusting organisms and were given a lower ranking (i.e., lace bryozoan grown on a barnacle would be marked as a barnacle in the coral point count analysis). Thus, the lower percent coverages on some substrate may not reflect a lack of lace bryozoan presence.

Observed differences in mobile organisms by substrate were mixed. If an organism was present at an array it was often found on multiple substrates. Tile and rock substrates were slightly less colonized, than the other substrates. Some mobile taxa had a higher affinity for substrates (e.g., polychaetes on wood, gobies and blennies on oysters).

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

We observed colonization of substrates by organisms (mobile and encrusting) at all sites within the first month of study. Monthly colonization increased in species diversity and coverage over time. Differences observed were attributed to location rather than substrate preferences. Mussels were observed at all locations, and were important components of the fouling community especially at our fresher locations. Oysters were observed during the final month of sampling, which was also the beginning of a typical oyster spawning season. Further study of settlement through the summer and fall will be needed to fully assess natural oyster recruitment in these areas.

Mortality events were an initial concern given stakeholder observations of previous loss. That was not observed during our study. While some loss occurred (dead barnacles, mussels, and oysters still attached to substrates), it was a few individuals at a time, not an array wide loss that would be indicative of an oxygen, temperature, or salinity stress event. These minor observed losses are more likely attributed to predation, or at most short-term stress losses with rapid recolonization and survival.

Wood, the preferred restoration material of local oystermen, did seem to be an effective substrate for settlement. While this study was not able to assess the long-term viability of these materials, it does indicate that further comparison studies of woody natural materials may be warranted.

During our study winter sea-level and tides and storms were challenge. Winter water levels were much shallower than anticipated leading to difficulties finding locations with depth differences. Storms throughout the study impacted not only the gear (causing anchor lines to weaken or substrates to shift location), but moved sediment and increased sedimentation on the substrates. Future restoration efforts will need to seriously consider reef placement, strength, and height in relation to these factors.

REFERENCES: Please list references cited throughout this report. Additionally, if there are key references that PPBEP needs copies of to fully understand your methods and overall research, please attach references as individual PDFs.

SUCSESSES AND CHALLENGES: Describe the significant successes and challenges the organization experienced related to the funded grant.

We experienced initial delays with purchasing and getting array materials due to backordered items. These delays combined with poor December weather pushed our deployment date back to January, instead of the initial plan of late October – early November. Due to the deployment delays sampling continued into June, and sample processing and data analysis was difficult to complete in the necessary timeline.

There were also unexpected issues with array placement due to seasonal low water levels, the intensity of winter storms limiting location due to the prevailing winds and wave action, and some public interference with placed arrays. Multiple arrays initially failed and had to be relocated.


Quantifying sedimentation in the system was also more difficult than expected. Our bay locations with sand dominated sediments were able to be deployed as planned, but it took a bit of trial and error to get a sedimentation sample in the bayous where sediments were fine, organic, and easily disturbed.

LESSONS LEARNED: 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.

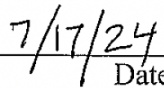
Our results cannot explain losses observed by residents, but do indicate that there is larval supply in the system for recolonization of areas that have been impacted, and for any new hard substrates that may be added. While oysters are not open or managed in either state's waters, there is oyster recruitment occurring in at least some regions of the bay, which is a positive indication that additions of suitable substrate placed in these regions may have natural recruitment. A longer term study that covered the full oyster spawning season, and captured sedimentation rates for all seasons is recommended.

Our volunteer dock owners were wonderful and helped us overcome both site limitations (finding neighbors with deeper docks) and keeping us informed if there were any issues or concerns following storms. There is a lot of support in the Perdido area for ongoing monitoring of their system, and enthusiasm to participate.

This report is submitted in accordance with the reporting requirements of Agreement No. FY2023-09 and accurately reflects the activities associated with the project.



Signature of Grantee's Representative



Date

Amanda Croteau, Research Assistant Professor
Print Name and Title