# ANNUAL REPORT TO TRIUMPH GULF COAST INC.

**Project #69:** Apalachicola Bay System Initiative (ABSI)
**Awardee:** Florida State University
**Reporting Period:** March 15, 2021-March 14, 2022

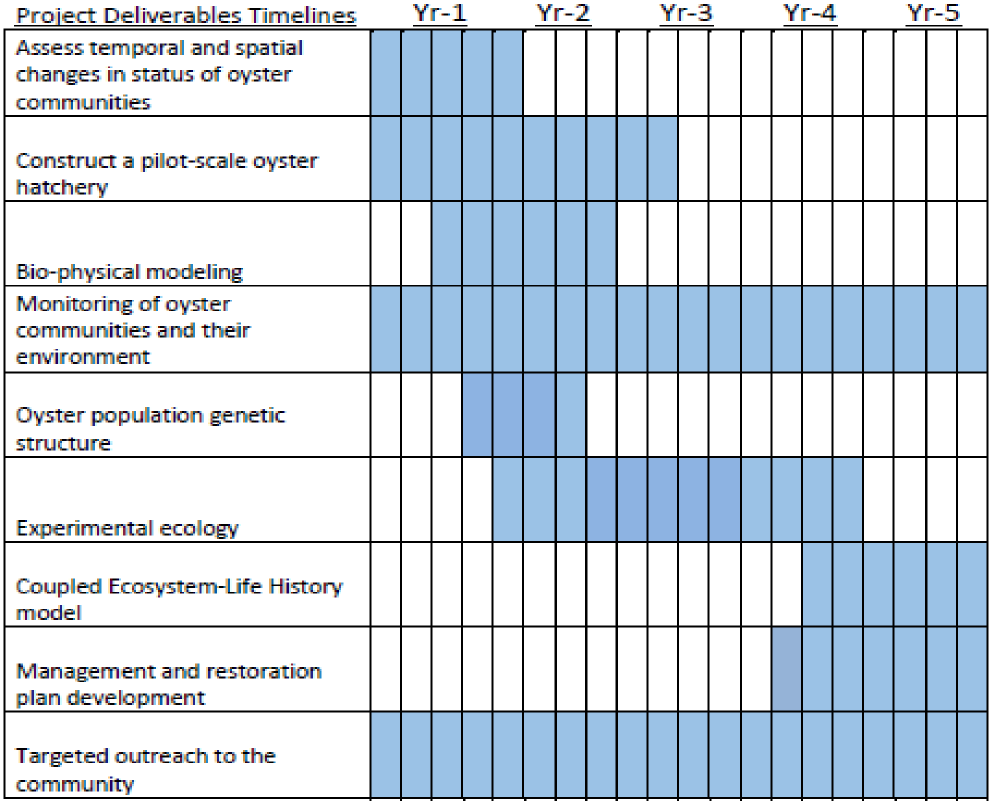
**ABSI LEADERSHIP TEAM**
Dr. Sandra Brooke (Principle Investigator)
D. Joel Trexler (Co-Principle Investigator February 2021)
Dr. Gary Ostrander (Professor, College of Medicine)

**Submitted:** March 31, 2022

# EXECUTIVE SUMMARY

The Apalachicola Bay System Initiative comprises a number of objectives and associated deliverables, each of which had a timeline for completion over the duration of the award. These deliverables are listed with their respective timelines in the table below (Table 1). Some of the deliverables comprise multiple parts; for example, Experimental Ecology includes a number of research studies, but others are very specific, such as the population genetic study. This report presents accomplishments for the third year of this large multi-disciplinary effort. There is also a section on other items that are not directly associated with the specific objectives.

***Table 1:*** *Initial timeline for project deliverables.*



| Project Deliverables Timelines | Yr-1 | | | | Yr-2 | | | | Yr-3 | | | | Yr-4 | | | | Yr-5 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Assess temporal and spatial changes in status of oyster communities | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | | | | | | | | | | | | | | | |
| Construct a pilot-scale oyster hatchery | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | | | | | | | | | | |
| Bio-physical modeling | | | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | | | | | | | | | | | | |
| Monitoring of oyster communities and their environment | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 |
| Oyster population genetic structure | | | | 🟦 | 🟦 | 🟦 | 🟦 | | | | | | | | | | | | | |
| Experimental ecology | | | | | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | | | | |
| Coupled Ecosystem-Life History model | | | | | | | | | | | | | | | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 |
| Management and restoration plan development | | | | | | | | | | | | | | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 |
| Targeted outreach to the community | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 | 🟦 |

Project accomplishments were severely impacted by Covid restrictions in 2020, causing the project to fall behind schedule. In 2021, field and lab activities were allowed to resume, with safety precautions, at a functional capacity and significant progress was made on the project objectives.

## Status of project deliverables

### 1. Assess temporal and spatial changes in oyster communities in Franklin County

There are two components to this objective, which was initiated in the first year of the project. The first was to create a database of literature on the ABSI ecosystem and the second was to analyze historical data to identify ecosystem change over time, with particular focus on oyster populations. ABSI has collected over 400 documents (peer reviewed manuscripts and technical reports). These documents are contained in a searchable database, which will continue to be augmented as the project progresses. Documents include historical and contemporary sources for data on oyster reef distribution, reef associated fish and invertebrate communities, oyster ecology and biology, and environmental conditions within Apalachicola Bay and adjacent waterways. This information has provided a baseline from which to evaluate changes observed during ABSI, and help generate target metrics for future restoration and management decisions. The database contains documents that are not in the public domain, so cannot simply be linked to the ABSI website. We are developing a user-friendly web-interface that makes the citations and associated documents available where appropriate, while protecting proprietary information.

Web-based products (in development) will show spatial and temporal patterns in environmental conditions using long term environmental data from multiple sources. This will be available soon through our website. Another product in development is a story map of historical through contemporary bathymetry and oyster habitat distribution, showing changes in available habitat over time.

## 2. Construct a pilot-scale oyster hatchery

The interim hatchery was operational during 2021 and four spawning events (tree in the spring and one in the fall) were accomplished. Two of these resulted in successful settlement of juveniles on oyster shell; these were used for restoration experiments and graduate student research. The other two spawns were impacted by environmental problems (wide temperature fluctuations and poor water quality) and the larvae did not reach settlement.

Construction of the permanent hatchery was hampered by supply chain problems but progressed significantly in 2021 and the facility is expected to be operational in summer 2022. The permanent hatchery is housed in a 50 x 70 ft metal building (to avoid some of the temperature problems encountered with the greenhouse), and has an algal culture room, a brood-stock conditioning room, spawning area with spawning racks, six larval culture tanks, and setting systems for spat-on-shell and single set oysters. The spring spawns will be conducted in the interim hatchery, with a transition to the new facility for the fall spawn(s).

## 3. Bio-physical modeling

This objective is comprised of two models: fresh-water flow and hydrodynamics. These models will be combined to create the final bio-physical model of the System.

Fresh-water flow dynamics is being addressed through a consultancy contract with Dr. Steve Leitman with the following objectives: 1) Develop a set of metrics to define optimal management of the watershed with regards to sustainable ecological productivity of both the river and estuarine aquatic resources; 2) Examine potential modifications to the current Water Control Manual operations, taking into account the metrics developed in objective 1; 3) Test current and proposed revised operations against alternative climate scenarios with regard to changes in both the volume of water being delivered to the river and estuary and the timing of rainfall events; 4) Encourage an adaptive management approach based on the outputs from the objectives above.

Hydrodynamic modeling of the ABSI system is being conducted by Dr. Steven Morey, a physical oceanographer at Florida Agricultural and Mechanical University (FAMU), and his post-doctoral researcher Dr. Xu Chen. Specific objectives of this work are: 1) Configure a hydrodynamic model for the lower Apalachicola River, Apalachicola Bay and the surrounding coastal and inner shelf regions (including Cape San Blas through Cedar Key, FL) based on the latest bathymetric and topographic data; 2) Run hindcast and future climate and management scenario simulations, incorporating flow inputs from Dr. Leitman's model; 3) Perform analyses of the simulations to characterize the variability of hydrographic properties throughout Apalachicola Bay; 4) Using a numerical particle tracking approach to simulate oyster larvae, conduct and analyze larval transport simulations to quantify factors such as larval recruitment, retention and inter-estuarine exchange.

These two projects were significantly delayed due to Covid restrictions and personnel issues but have made good progress in 2021 and most of the objectives have been accomplished. Further details on these modeling efforts can be found in sections 2.2 and 2.3.

## 4. Monitoring of oyster communities and their environment

Intertidal oyster populations have received relatively little research and monitoring attention in the ABSI region. Consequently, we do not have a good understanding of the temporal and spatial dynamics of oyster populations in intertidal habitats or their contribution to overall larval supply. The ABSI intertidal monitoring included a series of intertidal locations throughout Franklin County and continued throughout 2021 collecting monthly information on disease and reproductive status and recruitment. Collections were made twice annually for oyster density, size distribution and condition index. This work is labor intensive and is limited by tides, and the ABSI program has shifted field work focus to sub-tidal surveys and restoration experiments. However, inter-tidal monitoring will continue using high-resolution drone surveys to monitor oyster clump dynamics. Further details of inter-tidal research can be found in sections 4.1 and 4.2

The FWC oyster team surveys specific subtidal reef areas monthly using SCUBA and obtains density samples from these sites twice annually. This effort generates a valuable dataset that shows trends over time at the same sites, but does not provide a broad view of the oyster population status across the Bay. In October 2020, ABSI partnered with a former Apalachicola oysterman to survey subtidal areas throughout Apalachicola Bay using small oyster tongs. While this sampling does not provide density data comparable to the SCUBA surveys, it is supplementary information that has broader spatial coverage and can be collected quickly with fewer weather limitations than diving. In 2021, the tong sampling was repeated, with slight data collection modifications and is presented in section 4.3

The ANERR has five YSI Exo2 data sondes deployed in Apalachicola Bay; these instruments collect *in situ* data on temperature (ºC), salinity, conductivity (mS), dissolved oxygen (%, mg/L) pH, turbidity (NTU). To provide a broader spatial understanding of environmental conditions, ABSI deployed additional instruments of the same type in West Pass, Sikes Cut, the Miles, Indian Lagoon, the Apalachicola River mouth and St George Sound. Three of these instruments were lost in 2021; one in the the Miles was destroyed by a shrimp trawler and the two near the passes are missing, despite repeated efforts to locate them.

## 5. Oyster population genetic structure

This component of the ABSI is intended to help identify distributions of oyster sub-populations within Franklin County and the wider Florida Panhandle. Sub-populations may have characteristics that enhance survival under particular environmental conditions and thus could be used as different genetic lines of broodstock for restoration and aquaculture. It is important to understand local population structure so that genetic integrity (and any associated adaptation) can be maintained. Analysis of population distribution will also help ground-truth connectivity predictions generated by the bio-physical model. This project is almost complete and indicates some genetic structuring along the study region. Details of study can be found in section 3.1

### 6. Experimental ecology

This category includes a broad range of projects that are designed to help understand the ABSI system, with a view to identifying and addressing specific ecological problems. These include projects focused on oyster biology and ecology and broader Apalachicola Bay System ecology. These projects are listed below:

- High resolution sonar mapping of oyster areas (section 2.1)
- Habitat suitability model for eastern oysters in Apalachicola Bay (section 2.4)
- Oyster disease and other stressors (section 3.2)
- Oyster stress responses and physiological tolerances (section 3.3)
- Effect of salinity on juvenile oysters (section 3.4)
- Stress responses of oyster early life histories (section 3.5)
- Impacts of oyster populations on community development (section 4.5)
- Oyster and scallop restoration (sections 5.1 and 5.2)
- Apalachicola Bay food web and sediments (section 6.1)
- Influence of oysters on function and change in coastal ecosystems (section 6.2)
- Pollutant distribution in Apalachicola Bay (section 6.3)

### 7. Coupled ecosystem life-history model

Three models are being developed by ABSI (river flow, bio-physical and habitat suitability) and an oyster population model is being developed by our collaborator Dr. Ed Camp (University of Florida). Aspects of these models will be incorporated into the others as appropriate, and one or more decision support tools will be created from the combined modeling effort. The specific methods to combine the models and create the decision support tools will be developed in the coming year.

### 8. Development of a Management and Restoration Plan

This task was originally scheduled for the final year but has already been initiated through the CAB. A draft framework for the Plan was approved by the CAB in November 2021 and is available through the ABSI website (https://marinelab.fsu.edu/absi/cab/documents/). The ABSI CAB will continue to work on the Plan in 2022, in conjunction with other stakeholders.

### 9. Targeted outreach to the community

Community support is critical to the success of ABSI, so despite the continued restrictions caused by Covid-19, ABSI’s engagement with the public and local stakeholders was a major component of our efforts in 2021. These included (but are not limited to) the continuation of the Community Advisory Board, and associated sub-committees, creation of a bi-monthly ABSI newsletter, presentations to local commission meetings, partnerships with local organizations, and an expanded website that houses research data and educational materials. Section XX lists the many ABSI community engagement and outreach events conducted in 2021.

**Table of Contents**

# APALACHICOLA BAY SYSTEM INITIATIVE (ABSI) ANNUAL REPORT 2021-2022

## 1. Introduction

The Apalachicola Bay System Initiative was awarded in March 2019 and has now completed the third year of the study. This report summarizes the work being done under ABSI funding, with contributions and collaborations from numerous partners. The scientific projects are organized under the broad categories of habitat and environment, oyster biology, oyster ecology, restoration and system ecology. Many of these projects are incomplete and results presented are preliminary. Some studies began in 2019 or 2020 and were described in earlier annual reports. Previous information is not repeated, and this report contains only updates.

Community engagement is a critical component of the ABSI and the team has been active over the past year, conducting several activities in addition to the Community Advisory Board meetings, which have continued using the Zoom platform.

This past year was the first operational year for the interim research hatchery and despite some environmental and logistical challenges, two successful spawns let to the production of spat-on-shell for restoration experiments. The permanent facility is expected to be operational this year and the first spawning event in planned for the fall.

The ABSI team has expanded with the addition of new faculty, technicians, hatchery interns and graduate students. New projects have grown from the ABSI effort and new partnerships have formed. We expect to continue building on our ABSI foundation, and with the continuing decline of Covid, we hope to begin in person outreach activities in 2022.

## 2. Habitat and environment

### *2.1. Subtidal mapping*

Availability of suitable substrate is a critical factor in oyster population development and persistence. The most recent available bathymetric maps of the Bay are from more than 15 years ago (Twitchell et al 2006), and the status of oyster populations has changed considerably since then. In January 2021, Dr. Grizzle (University of New Hampshire) began multibeam and side-scan sonar mapping of the major historic oyster reefs in the Bay. They mapped ~14,000 acres of potential oyster habitat, using track-lines 80-160 m apart. These data will provide an overview of bottom topography and substrate type but do not capture small scale heterogeneity. In spring 2021, ABSI contracted the National Oceans Applications Research Center (NOARC) to conduct surveys over smaller areas of known or suspected oyster habitat using an autonomous survey vessel (ASV). This system collected simultaneous dual frequency sonar and bathymetry data and with a draft of 20 cm, can survey very shallow areas. The initial surveys were low frequency (25 cm resolution) over 6.5 km$^2$ (1600 acres) to identify extent and placement of hard-bottom, with high resolution surveys over 1.62 km$^2$ (400 acres) within the initial areas. One of the primary goals was to survey the ABSI restoration experiments (Section 5.1) just after they were deployed, with a follow up survey one year later to assess change. The conditions in Apalachicola Bay (strong currents and winds) were challenging for the vehicle and caused repeated malfunctions. The low-resolution maps were completed in the summer of 2021 (Fig. 1A, B) but the vehicle failed irreparably and the high-resolution surveys could not be accomplished.

Sonar maps from the NOARC surveys (Fig. 1 C, D) clearly showed the planted areas in the western Bay, but these were less well defined in the eastern Bay. This is possibly because the background substrate is has more hard material or that the planted material in the eastern Bay has become dispersed or buried in the high current areas. The sonar images can be used to place sampling in context; for example, some of the planted areas have extremely heterogeneous material distribution, which could lead to high spatial variance in oyster recruitment and survival. Information on persistence of substrate can inform design of restoration efforts; some materials may deteriorate faster than others, and thin layers of material may be susceptible to burial. Sonar can been used to show temporal changes in cultch placement over the duration of restoration efforts (Brucker et al 2021). The primary focus of this effort was to track changes in the ABSI restoration experiment, and some of the other cultched areas in the Bay that have previous sonar data (e.g. FLDEP Restore Act and NRDA projects). Unfortunately, the highest resolution data could not be collected because the ASV irreparably malfunctioned but sonar surveys will be conducted in the future on these and any new restoration sites.

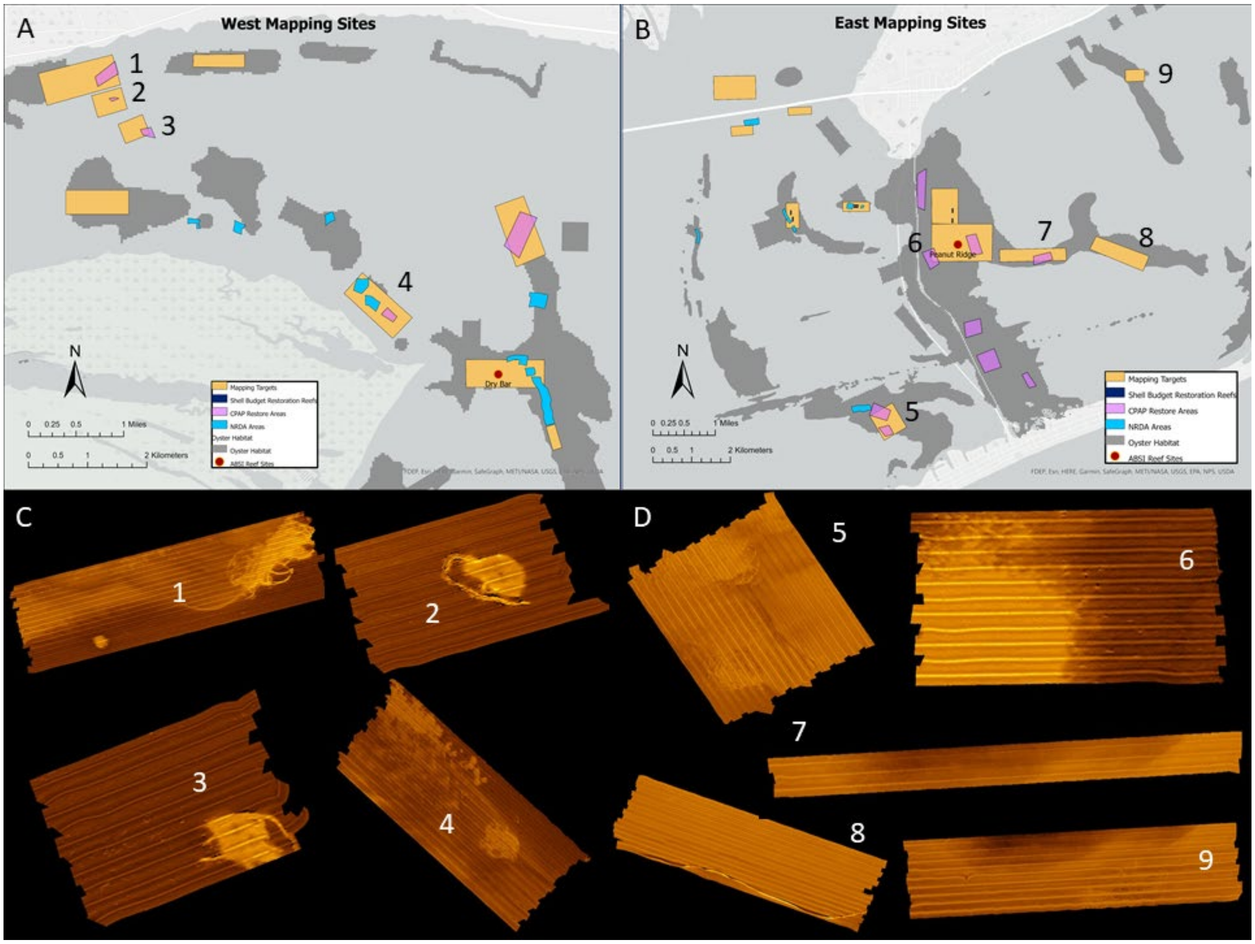


**Figure 1.** Mapping targets (tan boxes) for A) western and B) eastern areas of Apalachicola Bay. Grey areas are historical natural reefs, pink and blue areas were cultched in 2015 and 2017 respectively. Plates C and D are side scan sonar images of the mapped areas corresponding to the numbered boxes in A and B respectively.

### *2.2. Fresh-water flow dynamics update (Dr. Steve Leitman, Consultant)*

**Introduction and rationale:** This portion of the ABSI project includes fresh-water inflow from the Apalachicola-Chattahoochee-Flint (ACF) watershed into the Apalachicola estuary. The rationale for including this project component into the ABSI program was:
1) The project was conceived when Florida was in the midst of a U.S. Supreme Court lawsuit which was based the position that the State of Georgia was withholding/consuming water which resulted in harm to the Apalachicola estuary and 2) Fresh-water inflow from the ACF basin plays a major role in defining the salinity regime of the estuary and it was believed that salinity played a major role in the collapse of oyster population.

When the project was initiated, the U.S. Army Corps of Engineers had also just adopted a new Water Control Manual for managing the Federal storage reservoirs in the ACF basin. The Water Control Manual defines how the reservoirs in the basin should be operated as a system. Providing fresh-water inflow to the Apalachicola estuary was not considered a project purpose by the Corps in developing the reservoir management plan for the watershed because the ACF basin and the Gulf Intercoastal Waterway are considered as two separate projects by the Corps. Consequently, the preferred alternative for managing the Federal storage reservoirs in the Manual did not consider its impact on the Apalachicola estuary. Ultimately, the State of Florida lost the Supreme Court case because they could not prove Georgia had caused the harm to the estuary.

Under ABSI, fresh-water inflow is being addressed through using an existing river basin model (i.e., the ACF STELLA model). Under the ABSI project with regards to fresh-water inflow the focus of using this model has been to investigate 1) the hydrologic basis of the Supreme Court lawsuit, 2) how well the recently adopted Water Control Manual functioned under climate conditions other than that experienced historically and 3) what changes could be made to the Water Control Manual to enhance the sustainability of the Apalachicola estuary and its oyster industry.

The hydrologic basis of the Florida lawsuit was addressed through developing a presentation which was first provided to the Governor's Environmental Advisor and then to the Citizens Advisory Board. This presentation concluded that Florida's justification of the lawsuit from a hydrologic perspective was weak. Specific criticisms which were included in this evaluation were that the volume of consumptive demands used by Florida in evaluating impacts was significantly overstated for irrigation water usages' effects on flow in the Flint River and basing their position on the number of days historical flows were below 6,000 cfs leaving Jim Woodruff Dam between 1939 and 2016 was ill conceived. This is because of the amount of physical changes in the watershed above the Dam in that time frame were considerable. Evaluating this same issue using a river basin model with consistent management and consumption above the Dam show that this differences between pre-dam and post-dam conditions were mostly the result of climate, not Georgia's actions.

**Methods.** The Water Control Manual was developed using an unimpaired flow set which only included the historic climate in the basin from 1939 to 2012. Climate scientists, however, anticipate that in the future there will be a more extreme climate events both in terms of drought and flood. Therefore, the Manual was essentially designed to determine how best to manage the watershed in the past, not the future. To address this issue, I collaborated with Dr. Manuela Bruner, NCAR and Dr. Ebrahim Ahmadisharaf, FSU/FAMU Engineering School to develop 100 different stationary realizations of the historic climate with different magnitudes, frequencies, and durations of flood and drought events using a program called PRSim. These realizations maintain the same volume of water which was delivered in the historical data, but altered how this water would be delivered. It was decided to use this approach instead of the down-scaled global climate model data because the major stressor on the estuary is the occurrence of extreme events, not the average volume of flow entering the estuary.

**Results.** We are in the final stages of developing a manuscript for publication on the climate work. Specific problems associated with Water Control Manual's preferred alternative when evaluated under alternative climatic scenarios include draining the storage pool where Metro Atlanta gets their water supply (i.e., Lake Lanier) and significantly increasing the occurrence of low-flow events into the estuary in terms of duration and frequency of extreme events. These analyses have indicated that there is sufficient storage capacity for additional flow management to potentially improve river flows into the estuary. Metrics are being developed to define flow regimes that provide optimal benefit to the oyster populations in Apalachicola Bay.

**Future work**. Utilizing the river basin model as input into the estuarine salinity and ecological model models has proven to be more challenging because the river basin model already existed when this project was initiated and the estuarine model was being developed as part of the project. Therefore, linking of the two models has been asynchronous and my effort has been focused on expanding the capacity of the model to handle alternative climate scenarios and in defining metrics which can be used to distinguish between acceptable and unacceptable inflow to the estuary. Now that the estuarine model is operative, current and future work will be on running management/climate scenarios to define salinity profiles in the estuary.

### 2.3. *Bio-physical model of the Apalachicola Bay System (Dr. Steven Morey, Faculty and Dr. Xu Chen, Post-Doc, Florida A&M University)*

**Introduction and Rationale.** The goal of this study is to develop an estuarine and coastal hydrodynamic model of Apalachicola Bay and the surrounding coastal and shelf waters to provide a better understanding of the bay's hydrodynamics and response to differing atmospheric forcing and fresh-water flows. Future scenario simulations are run in collaboration with other ABSI investigators providing predictions of fresh-water flow variability under different management and climate scenarios. A coupled oyster larvae modeling component will provide predictions of factors that impact oyster larval recruitment, retention, and inter-estuarine exchange. Model output will be analyzed to develop derived products aimed at informing restoration activities.

**Methods.** The hydrodynamic model is based on the Finite Volume Coastal Ocean Model (FVCOM), an unstructured mesh model that is widely applied for realistic coastal simulations including flooding and drying of nearshore regions. The unconstructed mesh grids for the Apalachicola Bay simulations are generated based on high-resolution bathymetry from NOAA with modification from collaborators. The mesh is configured with high resolution near features such as coastlines, oyster habits, ship channels, and steep bathymetry slopes. The model resolution is 30 m near the coasts and bathymetric features of interest with fresh-water input from multiple distributary sources. Distribution of the Apalachicola River flow among the distributaries is estimated from a further refined mesh FVCOM simulation that extends up the rivers (developed by this project team and run by collaborators Ken Jones and Jiahua Zhou). The simulation is nested within the Navy Research Laboratory HYCOM Gulf of Mexico nowcast/forecast system to provide initial conditions and boundary conditions with tides. Atmospheric forcing is derived from the Climate Forecast System Reanalysis (CFSR) with wind fields corrected using observations within the bay.

**Results.** To date, realistic model hindcasts have been run for four different years representative of years with anomalously high river discharge (1998), low river discharge (2011-2012), and climatologically average (2019). Data from ANERR and NOAA/NOS observations have been used to assess the simulations, with several iterations of the model being run with modifications to improve the veracity of the simulation. Figure 2 shows an example of the model's tidal response at Apalachicola compared to the NOAA/NOS water elevation measurements. Figure 3 shows a comparison between model temperature and observations at the ANERR Cat Point station.

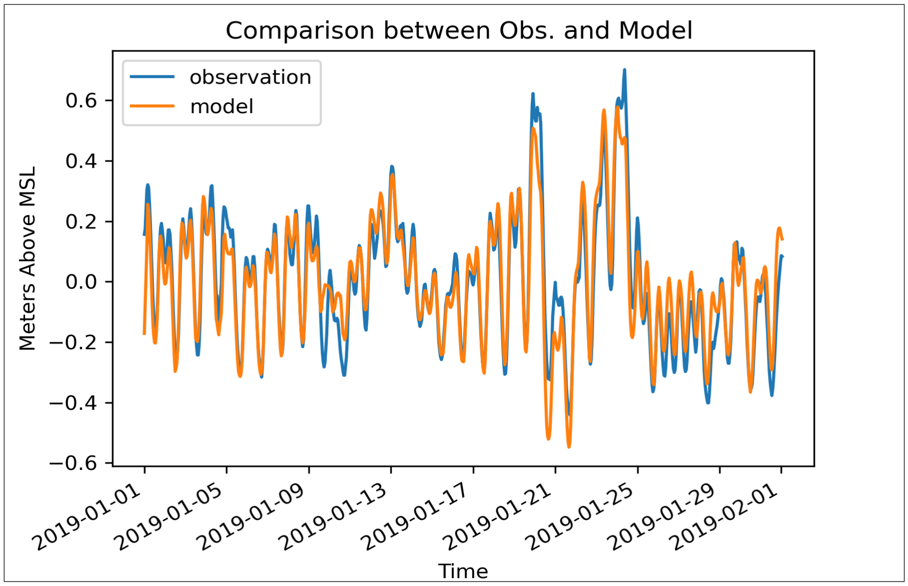


**Figure 2.** Time series of water elevation from the model (orange line and NOAA NOS observations at Apalachicola (blue line) for January 2019

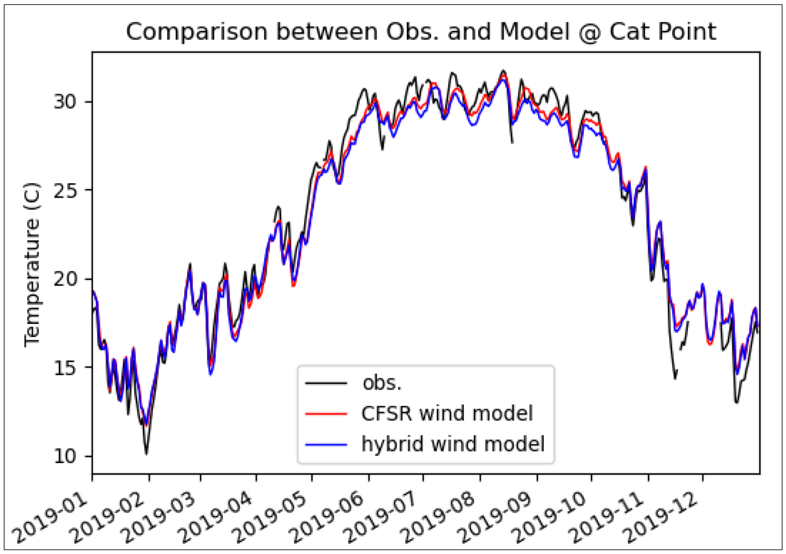


**Figure 3**. Time series of water temperature from the model (red and blue lines represent different wind forcing) and ANERR observations at Cat Point (black line) for 2019.

Figure 4 presents an example of the comparison between the model simulations of salinity and observations at two ANERR stations. These comparisons are also made at the Pilots Cove station and for all available stations for the simulation years for model assessment and tuning. In these plots, time series from two different simulations are shown: one forced by the CFSR winds, and the other forced with a wind product incorporating more realistic high-frequency variability measured at the NOAA NOS station at Apalachicola. The experiments have led to a new understanding of the importance of high-frequency (diurnal) wind variability for mixing of the high and low salinity waters within the estuary, particularly near the Dry Bar station. Further work is being done to incorporate additional wind observations in coordination with ANERR.

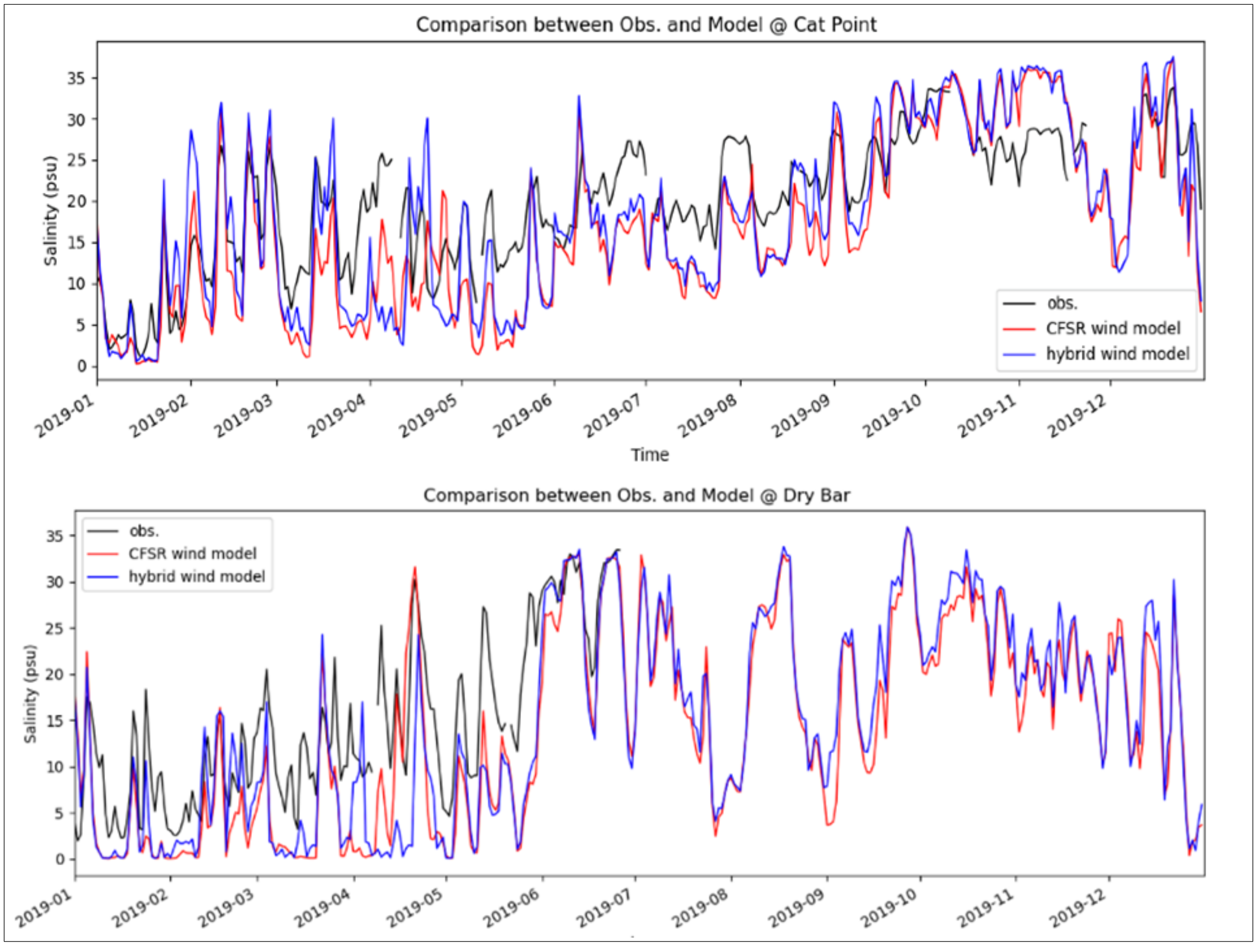


**Figure 4.** Time series of salinity from the model (red lines represent CFSR wind forcing and blue line represents simulation with corrected wind field) and ANERR observations (black lines) at Cat Point (top) and Dry Bar (bottom)

**Future work.** The simulations are being used to develop derived products to inform restoration efforts and for use in other studies. These derived products, some developed with other ABSI collaborators, can also be used as prediction tools (e.g., statistical relations between river flow and salinity response, mapped products of environmental variables and incorporation into habitat suitability modeling, and estimates of larval recruitment) complementing the hydrodynamic model output. Additional modeling for time periods of low and high river discharge will provide model output for developing these derived products and for analyzing different future scenarios. An example of a model-derived product, maps showing median salinity values across the bay for different river flow regimes, is presented in Figure 5. The next major stage of this work will be incorporating individual-based modeling to simulate oyster larvae transport and recruitment.

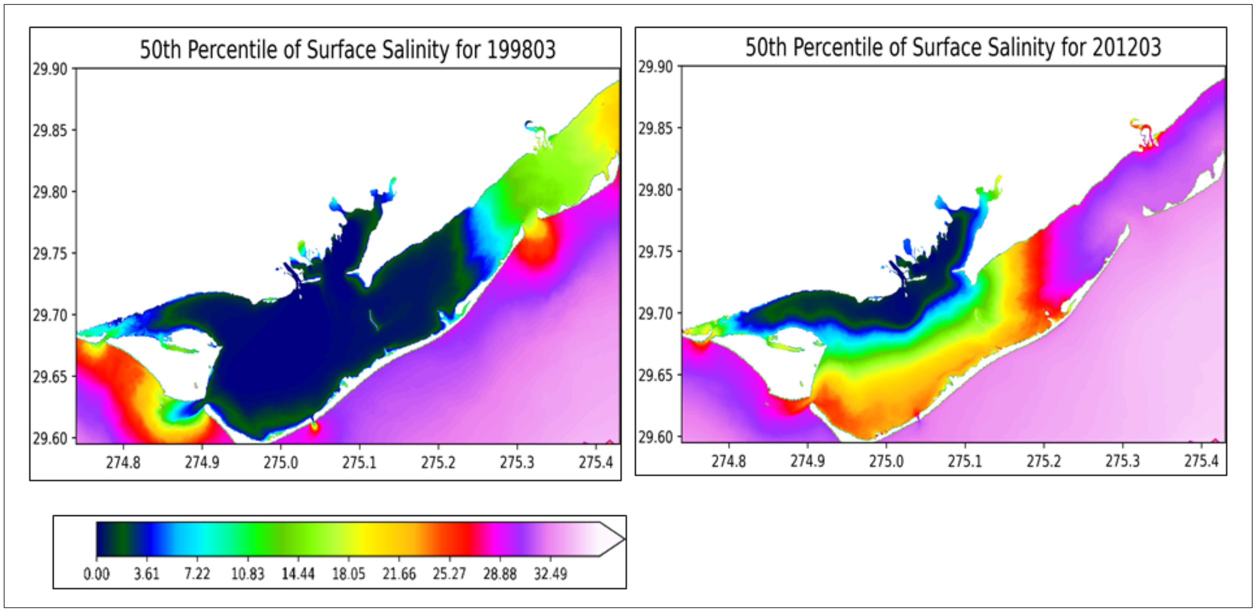


**Figure 5.** Maps of median salinity for March 1998 (left – a month with anomalously high river discharge) and March 2012 (right – a month with anomalously low river discharge)

### *2.4. Predictive habitat suitability model (Adam Alfasso, Ph.D. student)*

**Introduction and rationale.** Estuarine ecosystems and the biota that inhabit them are particularly vulnerable to changes in salinity, which can impact rates of disease, predation, and growth (Petes et al., 2012; Visser et al., 2002). The combination with anthropogenic impacts such as habitat destruction and overharvesting of commercially lucrative species have left many estuarine systems around the world severely degraded, in some cases loosing 90% of historical habitats (Vitousek et al., 1997). In the case of the Eastern Oyster (*crassostrea virginica),* a collapse in the historically productive oyster fishery in Apalachicola Bay, FL led to significant underemployment of fisheries workers, with NOAA officially declaring a fisheries disaster in 2013 (USDC 2013).

This region has seen decades of agency and academic research (Camp et al., 2015; Coen and Luckenbach, 2000; Fisch & Pine, 2016; Pine III et al., 2015; Seavey et al. 2011) to ascertain the cause of and possible solutions to the current state of the fishery, with several of the efforts still ongoing. This research includes creations of population dynamics and stock assessment models, reconstruction of historical fishery data, river discharge and salinity models, and management action simulations. However, many of these are focused on the extrapolating past fluctuations to explain what is currently happening in the system, and what may have caused the fishery to collapse so rapidly. There are few looking quantitatively at what may happen to this system in a future scenario of temperature increases and sea level change, although there have been general efforts in other estuaries (Eierman & Hare 2013, Altieri & Gedan 2015, Hewitt et al. 2016). In a future of elevated temperatures, precipitation patterns will likely reduce riverine input into estuaries, while at the same time allowing greater incursions of saline ocean water (Graeff et al. 2013, Hegerl et al. 2014). These changes have the potential to shift the sessile benthic community structure of nearshore reefs from coral and sponge dominated to algae dominated, the survivability of oyster reefs, and the extents of marshland as sea levels rise causes inundation along coastal regions.

To effectively protect a system, you must attempt to understand the system as whole. The objective of my research is to construct a series of spatially explicit models that describe and evaluate the effects of changing environmental conditions on the suitability of the estuarine habitat of Apalachicola Bay for the eastern oyster. These would include predictive habitat suitability and distribution models describing the current state of oysters in the bay, larval and planktonic distribution models to incorporate biological variables, a model incorporating anthropogenic needs to inform restoration efforts, and a suitability model incorporating predicted changes in the bay's hydrodynamics based on the 2016 IPCC recommendations (Parris et al. 2012, Passeri et al. 2016).

The overarching research objective is to quantify effects of changing environmental variables on distribution of the eastern oyster, and their implications for future oyster restoration. Specific objectives are:

1. **1.** Create a spatially explicit predictive habitat suitability model for the eastern oyster in Apalachicola Bay.
    a. Evaluate individual effects of environmental variables on model performance.
    b. Create biologically derived variables for evaluation and inclusion into distribution model.
2. **2.** Incorporate anthropogenic considerations into models to inform restoration and management efforts of the eastern oyster.
3. **3.** Integrate future predicted hydrodynamics into distribution models to describe (evaluate) the potential changes in oyster survivability and distribution.

Research Hypotheses:
H1: Oyster habitat suitability in Apalachicola can be accurately modelled using a presence-only modelling approach.
H2: The incorporation of biological variables will significantly improve model performance over environmental variable-only models.
H3: Predicted shifts in hydrographic conditions caused by climate change can be integrated into models to predict future suitable habitats and oyster survivability.

**Methods.** The main modelling technique I will be using is called Maximum Entropy Modelling (MAXENT). This is a machine learning technique that uses presence-only data and georeferenced raster ASCII's of environmental variables to calculate habitat suitability (Phillips et al, 2017), as well as evaluate the importance of each variable to the model. MAXENT has been shown to be a robust modelling framework that produces results consistent with traditional presence/absence techniques (Bridge et al, 2012; Rengstorf et al 2013). MAXENT will allow me to incorporate biological components, such as larval supply and nutrient concentration, two derivatives from forthcoming models. These two models will be created using a variety of toolboxes in ArcGIS Pro 2.9 (Marine Geospatial Ecology, Benthic Terrain Modeler), in combination with our collected oyster density data, the outputs of the ABSI hydrographic modelling team, and published oyster life-cycle metrics.

**Results**. At the time of this report, presence data is in the form of sampling via tonging is progressing, with over 150 new sites added from the last survey, with a full summer season of sampling being planned. Data collation of ANNER, FIMS, FWC and ABSI nutrient data, sensor data, reef locations (historical, planted, and research) are complete, and continuing to be updated as new data is released. The hydrographic modelling team has recently presented their high-resolution watershed model for the bay, from which many of the environmental variables are being derived from. With this data now available, work can begin to move forward on the MAXENT, larval distribution, and nutrient(chl a) distribution models. It is my aim to have several robust model outputs ready for ground truthing by the Fall, and the focus of most of my efforts this coming year.

**Future work**

- Continue Presence/Absence sampling
- Begin MAXENT model formulation for habitat suitability
- Begin Larval distribution modelling for current brood reef sites
- Fill in data gaps for substrate types in East Bay, Indian Lagoon
- Ground truth model outputs
- Planning for possible planting of experimental sites based on suitability indices

## 3. Oyster biology

### *3.1. Genetic structure (Dr. Amy Baco-Taylor, Faculty Dr. Nicole Morgan Post-doc)*

**Introduction and rationale**. Understanding the stock structure of the eastern oyster, *Crassostrea virginica*, is critical for management of these economically valuable species. Within the Gulf of Mexico, The Texas Parks and Wildlife Dept (TWPD) has determined that there are at least 3 stocks, with the northern Gulf of Mexico stock being distinct from the Southern Texas stock and both from the southern Florida Stock (TWPD, pers. comm., Anderson et al 2014). However, the location of the transition zone between the southern Florida and Northern Gulf stocks is unclear, and only a single population was sampled within Apalachicola Bay for this work. To further understand the stock structure of populations within the Apalachicola Bay, and to identify the transition zone between these stocks, we will obtain population genetic data from samples collected by ABSI from 7-9 sites. These sites will encompass locations known to host the southern Florida population, sites known to harbor the Northern Gulf population, several sites within the Apalachicola Bay, and an array of sites between these areas. This work will address the question of which of the two stocks the Apalachicola Bay sites are most well connected to, or if they fall on the boundary between them. It will also provide data on connectivity among sites within the Bay and reveal any subpopulation structure or unique genetic strains within the Bay. The results of this study will therefore inform management of the populations within Apalachicola Bay. For example, if the Apalachicola Bay sites are most closely related to the southern Florida stock, restoration efforts should use oysters from southern Florida to replenish harvested sites. If there is a mix of stocks in the Bay, then different source populations will need to be used based on the observed patterns.

**Methods**. Oysters were sampled using hand tongs under an FWC Special Activity License (SAL) and maintained at -80°C until the oysters could be dissected. Approximately 1 – 2 g of adductor muscle was dissected from each frozen oyster and DNA was extracted using the DNeasy Blood and Tissue kit. Modifications to the prescribed method included overnight lysis with proteinase K and a reduction of the recommended final elution buffer volume to 150µl (instead of 200µl). The remaining oyster tissue was preserved in 100% ethanol and the shells were dried and stored.

Ten loci were amplified using primer sequences. One locus, RUCV01, amplified for two different size classes in most individuals, thus eleven total loci were amplified for 223 individuals at ten different locations in the Gulf of Mexico (Fig. 6). Polymerase chain reaction (PCR) products were pooled in sets of two and sent to the University of Arizona Genetics Center for genotyping using an Agilent 3730 Analyzer with ROX 500 ladder.

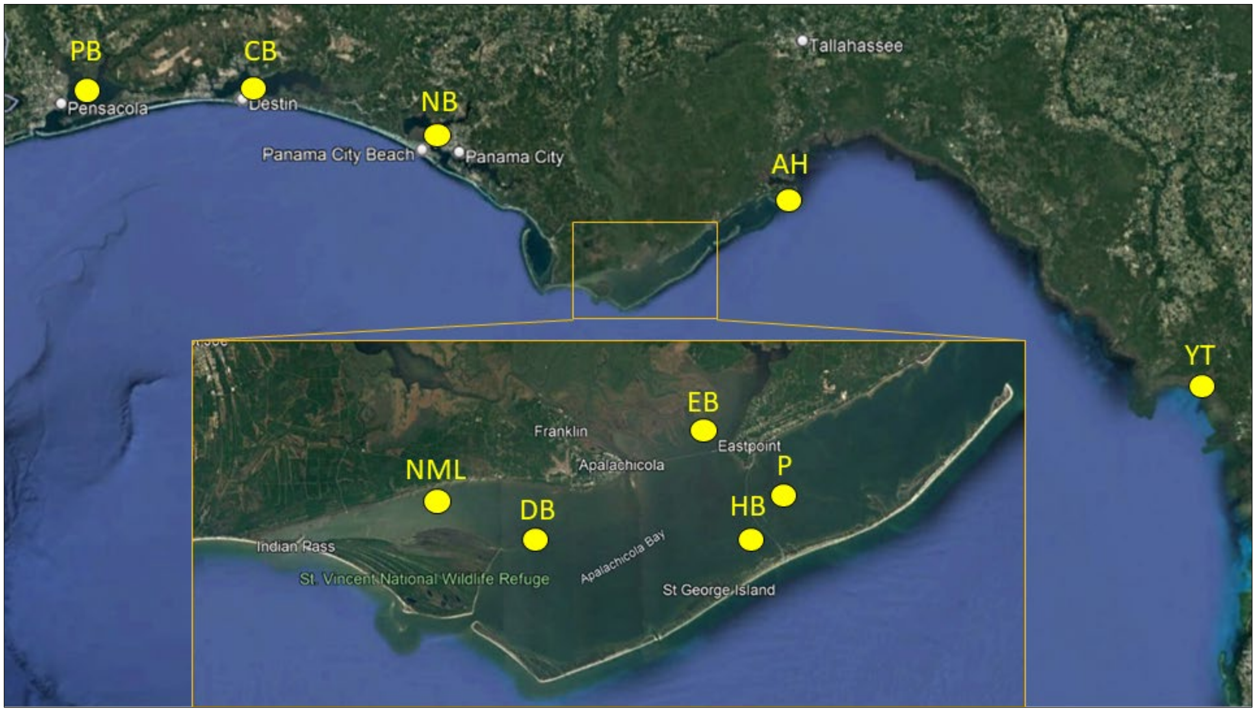


**Figure 6.** Map of oyster populations sampled in the Gulf of Mexico. PB = Pensacola Bay, CB = Choctawatchee Bay, NB = St. Andrews Bay North, NML = Nine Mile Lump, DB = Dry Bar, EB = East Bay, P = Platform, HB = Hotel Bar, AH, Alligator Harbor, YT = Yankee Town.

Fragment length was analyzed using the R package 'Fragman' with default scoring settings for peak calls (Covarrubias-Pazaran et al., 2016) in R version 4.1.0 (R Core Team, 2021). All calls were then checked by eye for stutter peaks or amplification of more than two alleles. Null allele frequencies and genotyping error were analyzed using MICROCHECKER. Stutter peaks were identified for two loci (RUCV 74 and RUCV51) and those loci were fully called by eye to limit the effect of stutter peaks. Linkage disequilibrium was analyzed using Genepop 4.2 online (Raymond and Rousset, 1995; Rousset, 2008).

To test the ability of these loci to find population differentiation, a power analysis was run. First, effective population size ($N_e$) was estimated using the molecular coancestry method from NeEstimator v2 (Do et al., 2014). This method was used as it is less biased by population substructure, age structure, or small sample sizes (Luikart et al., 2010). Results from NeEstimator had a wide range per population of effective population size, so power analysis, using POWSIM v4.1 (Ryman and Palm, 2006), was run twice: once with an $N_e$ of 20 and another time with an $N_e$ of 40, with 1000 replications for both runs. Summary statistics for populations and loci were calculated using the R packages 'adegenet' (Jombart, 2008), 'PopGenReport' (Adamack and Gruber, 2014), and the 'ShannonGen' function (Zahl, 1977; Konopiński, 2020). Tests for per-locus, per-population Hardy-Weinberg Equilibrium (HWE) were run using 'pegas' (Paradis, 2010). Population pairwise $G'_{ST}$ values and per-locus $G'_{ST}$ values were calculated with 'mmod' (Winter, 2012). $G'_{ST}$ is preferable to $F_{ST}$ values for highly variable loci as $F_{ST}$ is unlikely to ever reach its maximum value of 1.0 for more than two alleles with high variability, while $G'_{ST}$ is standardized by the maximal genetic variation of the locus (Hedrick, 2005), which allows $G'_{ST}$ to reach 1.0 in differentiated populations. Population differentiation by locations was analyzed using Analysis of Molecular Variance (AMOVA) in the package 'poppr' (Kamvar et al., 2014). Discriminant analysis (DAPC) and admixture were analyzed in 'adegenet' by site. These methods are better suited than STRUCTURE for this dataset as it is not affected by deviations from HWE or by linkage disequilibrium (Jombart et al., 2010).

**Results.** All eleven loci were highly variable (12 – 70 alleles per locus), especially RUCV 61 with 70 alleles. This locus also has the second widest range of allele sizes (326 – 504), with the Cvi2g14 having the largest range (135 – 357) and the second largest number of alleles found (66). Observed heterozygosity ($H_o$) was generally much lower than expected heterozygosity ($H_e$) for all loci (range $H_e$-$H_o$ = 0.20 – 0.69). Allelic diversity was also high by site (Table 1), and the number of private alleles ranged widely between locations (3 – 26), though the values did not show a spatial trend (Table 1). The lowest number of private alleles occurred at Alligator Harbor (AH), and the highest number at both Platform (P) in Apalachicola Bay and Yankeetown (YT) the southern-most location. Null alleles were present in most locations at most loci therefore none were removed so that further analyses could still be completed with the understanding that null alleles have the tendency to inflate F-statistics, leading to a positive bias in population differentiation. There was no evidence of linkage disequilibrium between loci; RUCV74 and RUCV94 showed evidence of association at five locations while all other significant pairings occurred at only 1 – 2 populations.

Power analysis showed the 11 loci have a strong ability to detect genetic differentiation at a p-value <0.05 ($\chi^2$=1.000, Fisher's exact test = 1.000). Globally, no locus was in Hardy-Weinberg Equilibrium, which is also likely at least partially related to the presence of null alleles in most loci. Most populations were in HWE for only three or four loci, and St Andrews Bay North (NB) and Hotel Bar (HB) were only in HWE for one locus.

**Table 1**. Summary statistics by site. N = number of samples, $F_{IS}$ = Wright’s inbreeding coefficient, Ho = observed heterozygosity, He = expected heterozygosity

| Site | N | No. of Alleles | Private Alleles | $F_{IS}$ | Zahl's Diversity | $H_o$ | $H_e$ |
|---|---|---|---|---|---|---|---|
| PB | 26 | 193 | 20 | 0.46 | 2.72 | 0.49 | 0.88 |
| CB | 25 | 194 | 14 | 0.42 | 2.70 | 0.50 | 0.87 |
| NB | 21 | 168 | 5 | 0.34 | 2.64 | 0.58 | 0.88 |
| P | 23 | 193 | 26 | 0.43 | 2.87 | 0.52 | 0.92 |
| NML | 26 | 214 | 17 | 0.43 | 2.84 | 0.50 | 0.89 |
| DB | 20 | 165 | 13 | 0.37 | 2.70 | 0.56 | 0.90 |
| EB | 12 | 123 | 9 | 0.41 | 2.54 | 0.51 | 0.90 |
| HB | 25 | 185 | 12 | 0.42 | 2.69 | 0.51 | 0.88 |
| AH | 18 | 144 | 3 | 0.37 | 2.72 | 0.57 | 0.91 |
| YT | 27 | 206 | 26 | 0.43 | 2.81 | 0.50 | 0.90 |

Global value for genetic distance ($G'_{ST}$) was 0.16 ($p = 0.01$), suggesting low to moderate, but significant global genetic differentiation. Pairwise $G'_{ST}$ values were also generally low to moderate, (Range: 0.05 – 0.30, Median = 0.15) with only three comparisons having a $G'_{ST}$ over 0.25 (Table 2). AMOVA showed significantly greater variation than expected between populations and between samples within populations (Figure 7, $p=0.01$ for both comparisons). Variation between samples within sites was 37.48% of the covariance with variation between sites comprising 0.76% of the variation. Variation within samples (heterozygosity) was again lower than expected ($p=0.01$) but comprised 61.76% of the covariance of the AMOVA test

**Table 2**. Pairwise measures of genetic distance ($G'_{ST}$) between sites. Values greater than 0.25 are highlighted in green

| Location | CB | NB | P | NML | DB | EB | HB | AH | YT |
|---|---|---|---|---|---|---|---|---|---|
| PB | 0.05 | 0.11 | 0.20 | 0.12 | 0.30 | 0.20 | 0.17 | 0.26 | 0.18 |
| CB | | 0.05 | 0.21 | 0.13 | 0.28 | 0.18 | 0.10 | 0.22 | 0.14 |
| NB | | | 0.14 | 0.08 | 0.21 | 0.15 | 0.06 | 0.21 | 0.12 |
| P | | | | 0.12 | 0.15 | 0.14 | 0.22 | 0.16 | 0.14 |
| NML | | | | | 0.18 | 0.14 | 0.13 | 0.13 | 0.14 |
| DB | | | | | | 0.24 | 0.12 | 0.22 | 0.17 |
| EB | | | | | | | 0.21 | 0.20 | 0.18 |
| HB | | | | | | | | 0.15 | 0.10 |
| AH | | | | | | | | | 0.16 |

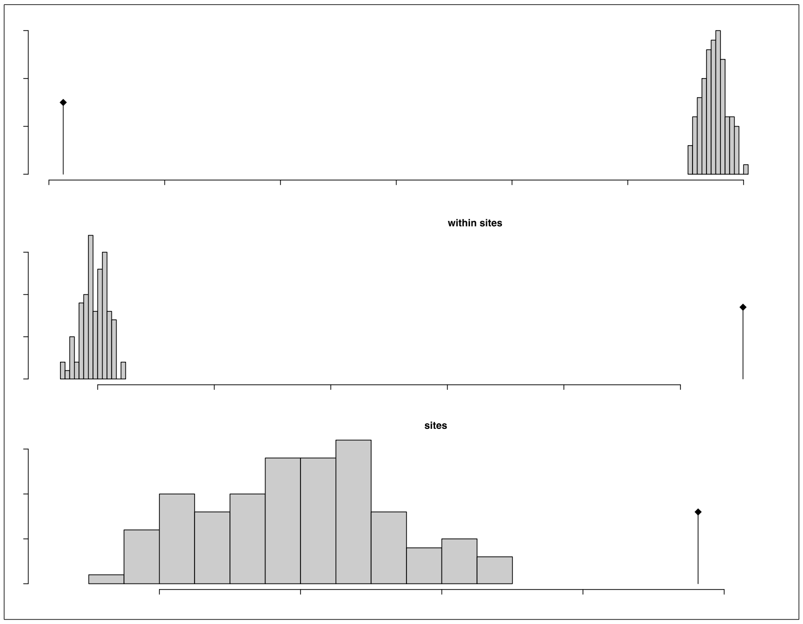


**Figure 7**. AMOVA histograms of expected genetic variation within oysters (top), within sites (middle), and between sites (bottom). Levels of variation are shown by the black bar with diamond.

Discriminate analysis by site showed Pensacola Bay (PB) and Dry Bar (DB) to be separated from the remaining populations, and there does not appear to be a longitudinal relationship between the closely clustered populations (Fig. 8) As PB is the westernmost site, it is more expected for that population to be differentiated than DB, which is centrally located and close to Nine Mile Lump (NML) and East Bay (EB) (Fig. 6). East Bay (EB) is more differentiated from the central locations than expected, but is more inshore than DB or NML, and could be affected by genetic exchange.

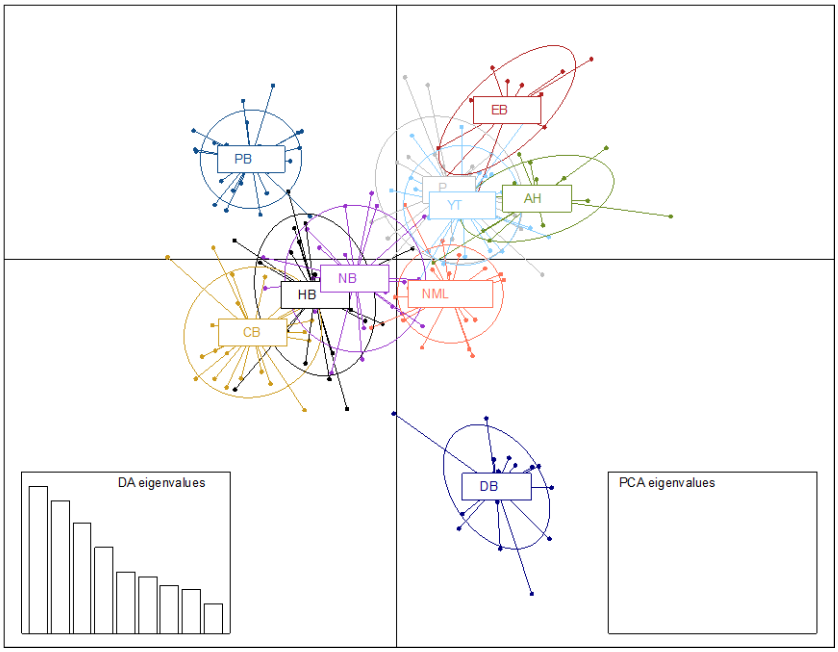


**Figure 8.** Discriminant analysis scatterplot showing genetic relationship between sites.

No difference was found in shell morphologies or weights between sites to account for the differences seen in DAPC. Admixture analysis by site shows much lower admixture than would be expected for Choctawhatchee Bay (CB), P, NML, and EB (Fig. 9) compared to the overlap in the DAPC scatter plot (Fig. 8). North Bay (NB) and Hotel Bar (HB) showed considerable admixture between the two populations, despite being separated by more than 100 km. AH and YT also showed high admixture though the populations are nearly 180 km apart. No evidence of isolation by distance (IBD) was found through Mantel tests. However, the more sensitive RDA test did show evidence of IBD along the longitudinal distribution of the oyster populations.

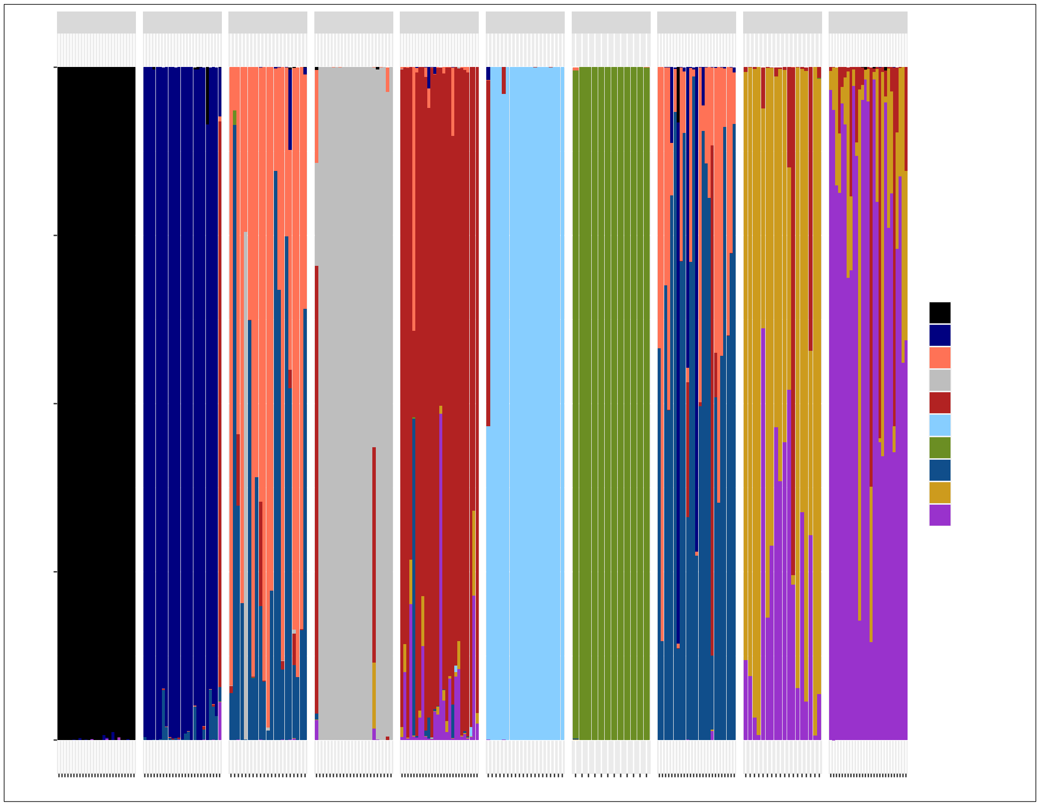


**Figure 9**. Admixture comparison plot to show likelihood of genetic exchange between populations. Each bar is one oyster.

### *3.2. Disease and other stressors (Dr. Tara Stewart Merril, Assistant Research Faculty)*

Dr. Stewart Merrill began research on oyster pathogens and other ‘enemies’ in January 2022. The principal objective of her work is to identify the lethal and sublethal effects of disease (and additional enemy stressors) on oysters, as well as the extent to which these forces inhibit oyster population growth. A thorough understanding of how disease negatively impacts oysters—and how patterns of infection are shaped by environmental conditions—will allow us to model and potentially mitigate disease into the future. Below is a summary of Dr. Stewart Merrill’s ongoing progress and future directions for the 2022 calendar year:

***3.2.1 Identifying the impacts of disease on oysters*****:** Using datasets collected by the Florida Fish and Wildlife Conservation Commission (FWC), the Apalachicola National Estuarine Research Reserve (ANERR), and the Apalachicola Bay System Initiative (ABSI), Dr. Stewart

Merrill is currently developing statistical models that broadly explore: **1)** how Perkinsus marinus (the pathogen that causes Dermo disease) and shell pests (Cliona, Diplothyra, and Polydora) interact to influence individual oyster condition; **2)** how natural variation in the abundance of these enemies corresponds with oyster demographic processes, including recruitment and mortality; and **3)** how environmental factors (e.g., temperature and salinity) are associated with the abundance and impacts of enemies. Preliminary analyses indicate that oysters experience reduced condition in areas that have high "enemy pressure" (a score that combines abundance of *Perkinsus marinus* and the three shell pests; Fig. 10).

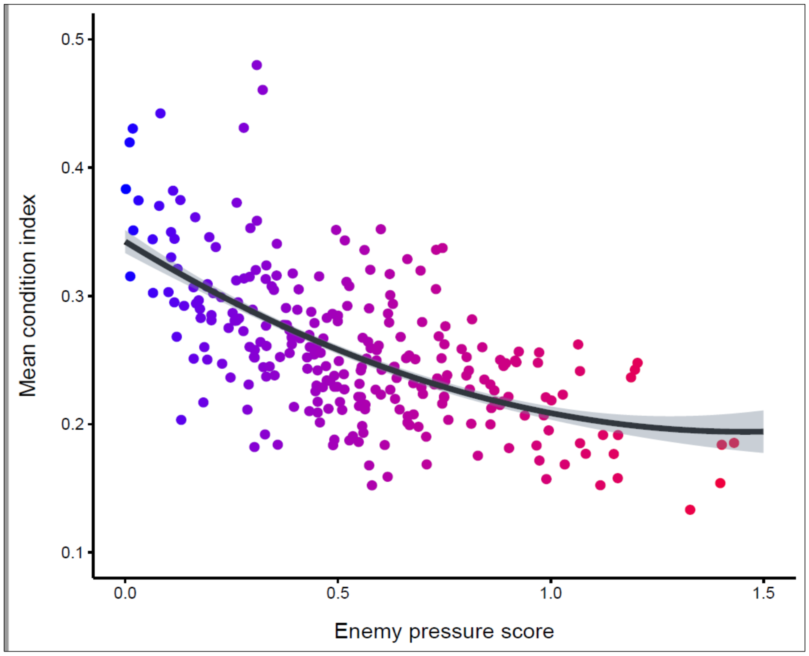


**Figure 10.** Relationship between enemy pressure score and mean condition index of oysters in Apalachicola Bay

***3.2.2. Understanding disease thresholds in the Apalachicola Bay*:** In Northeastern regions of the United States, *Perkinsus marinus* has been responsible for myriad mass die-offs of oysters. However, this pathogen appears to be less deadly in some populations of the Gulf of Mexico. Research is critically needed to assess the conditions under which the outcome of *P. marinus* infection shifts from asymptomatic to lethal. Dr. Stewart Merrill is working to prepare a manuscript that leverages existing data (described above) to compare infection patterns and transmission processes in the Apalachicola Bay to those previously described in the Northeast. The results of this study will provide new directions for investigating disease thresholds in Apalachicola oysters, while granting insight into whether mass die-offs may be expected under future environmental scenarios.

***3.3.3. Exploring consequences of disease for Apalachicola Bay*:** Oysters are well-known for their ability to filter suspended particles and maintain water clarity. Pathogenic infection, however, may alter the capacity of oysters to filter water, which can have ramifications for other species in the ecosystem (e.g., organisms that depend on clear water for access to light). In many invertebrate species, reduced foraging and suspension feeding are known symptoms of infection, which motivates study of similar responses in oysters. In Fall 2022, Dr. Stewart Merrill will confront these ideas with new laboratory experiments to identify how disease can have cascading effects on an ecosystem through modifying host traits.

### *3.3. Stress responses and physiological tolerances (Emily Fuqua, Ph. D. student)*

**Introduction and rationale.** Impacts of anthropogenic activities, such as fresh-water management, fishing, and climate change, are rapidly and irrevocably changing coastal ecosystems. These environmental changes are driving alterations in the physiology of coastal organisms which ultimately scales to changes in population and community dynamics. However, management and conservation strategies that do not include plans for environmental change and the physiological consequences will not be effective as the environment continues to change. So, the purpose of this research is to identify and characterize the effects of two main environmental stressors, temperature and salinity, on the physiology and energetics of *Crassostrea virginica*. While temperature and salinity have been studied extensively separately (Davis 1958, Lannig et al. 2006, McCarty et al. 2020, Griffiths et al. 2021), how these stressors interact to frame the energetics of this species is not well understood but has implications for critical population rates such as mortality and reproduction (Heilmayer et al. 2008, Jones et al. 2019).

As estuarine animals, oysters experience a wide range of abiotic stressors (Fig. 11), but they are most often exposed to multiple stressors concurrently (Heilmayer et al. 2008, Jones et al. 2019).

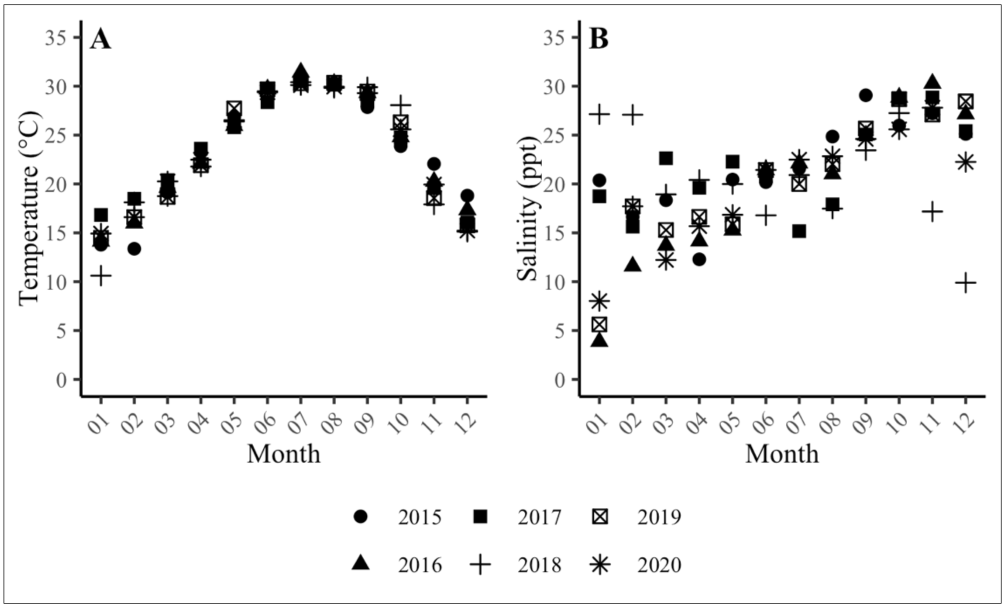


**Figure 11.** Apalachicola Bay A) Average monthly temperature and B) salinity across six years (2015-2020). Data downloaded from NOAA NERRS Cat Point Station in Apalachicola Bay, FL

In Apalachicola Bay, water temperatures range from 2 to 33ºC, and salinity ranges from 0 to 35 ppt depending largely on location in the Bay (NOAA NERRS, 2021). So, oysters experience a large range of temperatures seasonally, but salinity is more dependent on factors such as proximity to the river, and local water currents. With different salinity regimes present in Apalachicola Bay, it is possible oysters from different reefs may exhibit differences in salinity tolerance due to the salinity regime they experience. This difference in salinity tolerance has been noted in other estuaries, such as Delaware Bay (Eierman and Hare 2013). Additionally, while adult oysters need to tolerate the full range of temperature and salinity, larvae are only present during the summer season, and so are not exposed to as large of a range in water parameters (Menzel 1951, Aranda et al. 2014). Indeed, organisms with complex life cycles are known to exhibit ontogenetic shifts in tolerance (Rybovich et al. 2016). Older and larger individuals are more tolerant than larval stages and smaller size classes (Rybovich et al. 2016). So, mortality and growth rates will depend on the environmental conditions as well as life stage of the animal. This work will identify how a range of ecologically relevant temperatures and salinities will interact to change the physiology, growth, and survival of larval, juvenile, and adult oysters.

Research objectives

**O1**: To identify ontogenetic shifts in tolerance that may impact oyster performance.

**O2**: To characterize how growth, survival, and energetics of oysters change due to two main environmental stressors, both individually and in tandem.

**O2A**: To determine optimal culture conditions for larval, juvenile, and adult life stages of the eastern oyster.

**O2B**: To provide information useful for the guidance of restoration efforts of the eastern oyster.

**O3**: To provide life-stage specific impacts of common environmental stressors on the growth, survival, and energetics of oysters.

**O3A**: To provide information useful for the near and far future management and conservation of the eastern oyster.

Research Hypotheses

**H1:** Adult oysters from high salinity reefs will have a different salinity tolerance from adult oysters from low salinity reefs.

**H2**: Larval oysters will display a narrower tolerance to single environmental stressors than later life stages.

**H3**: Young adult (10 - 20 mm) and adult oysters (>40 mm) will display a wide tolerance to single environmental stressors.

**H4**: Combining suboptimal salinity and temperature conditions will have significant impacts on the growth, survival, and metabolism of all life stages.

This work will begin to identify (i) how the interactions of important abiotic stressors shape the energetic balance of the eastern oyster and (ii) the life-stage specific consequences of lethal and sub-lethal stress and the interactions of stressors on growth, survival, and the physiology of oysters. This work will help to understand how complexity in the environment shape the physiology of organisms with complex life cycles. Additionally, this work can be applied in aquaculture, restoration, conservation, and management of *C. virginica* in the Apalachicola Bay, FL area. These data will identify optimal conditions for oyster culture and the consequences of exposure to suboptimal conditions, and so this work can inform oyster growers in this area to help maximize their yield. Also, these results can guide restoration efforts to reefs where out-planted oysters have the best chance to thrive. This work will also give managers and conservationists an idea of how suboptimal conditions and extreme events (i.e., storms that severely lower salinity, heat waves, cold snaps, etc.) will impact the health of these animals.

**Methods.** To complete research objectives, temperature tolerance, salinity tolerance, and interaction experiments will be carried out for three stages of the eastern oyster: larvae, small adult (10 – 20 mm), and larger adult (>40 mm). Briefly, the tolerance experiments consist of exposing oysters a large, but ecologically relevant, range of temperatures or salinities and measuring performance. These experiments determine the responses of oysters to single stressors. In young and older adults, survival, growth, and oxygen consumption are measured, and metabolomics will be used to provide a detailed and fine-scaled picture of oyster stress response. In larval experiments, growth rate and survival are measured. The interaction experiments have a 3x3 factorial design with three levels of temperature and three levels of salinity. The same responses are measured, and this experiment will investigate the stress response of oysters to both stressors concurrently.

**Results and future work.** Larval experiments began in summer of 2021 and continued through early fall during the ABSI Oyster Hatchery spawning season. Methodology for keeping larvae in small culture was developed, and preliminary results of how stressors interact on oyster larvae were obtained. Larval work will be continued during the summer of 2022 once the ABSI hatchery begins spawning season. Salinity tolerance experiments on large (>40 mm), adult oysters began in January 2022, are ongoing, and are expected to be completed March 2022. The remaining adult experiments (temperature and interactions) will begin after the wild adult spawning season in 2022 ends, as reproduction and gametogenesis processes could interfere with energetic results. Juvenile experiments will begin in fall 2022 when first generation oysters of appropriate size can be obtained from the ABSI Oyster Hatchery.

### *3.4. Effect of salinity on juvenile oysters (Donaven Baughman, M.Sc. Student)*

**Introduction and rationale**. The goal of this project is to examine how sub-lethal changes in salinity regimes in Apalachicola Bay impact oyster growth and vulnerability to predators hypothesized to have contributed to the oyster population collapse. Results from this project will clarify how fluctuations in bay salinity influence oyster growth, reef accretion, and mortality rates, information that will be critical for the adaptive management of oyster populations as conditions in the Bay change.

**Methods.** A pilot study was conducted from October to December 2021 to raise recently settled juveniles from the FSU experimental hatchery under low (23-26 psu), medium (28-30 psu), and high (32-35 psu) salinity regimes.

**Results**. During preliminary experiments, oyster reared under low and high salinity regimes experienced substantially more mortality than oysters reared at moderate salinity regimes (Fig. 12). Furthermore, oysters exposed to waterborne chemical cues from predatory oyster drills showed evidence of inducing morphological defenses against predation by growing thicker shells. However, our ability to detect whether induction of shell defenses differed between salinity regimes was hampered by the high mortality rates in high salinity treatments.

**Future work.** After completion of the pilot study, the full experiment is planned to begin in May 2022. Alongside the experiment, field surveys will begin at sites across Apalachicola Bay to regularly record oyster and predatory drill abundance at sites capturing a gradient of salinity regimes. Surveys and experiments are scheduled to end in September 2022 with results presented in a draft manuscript prepared for submission by December 2022.

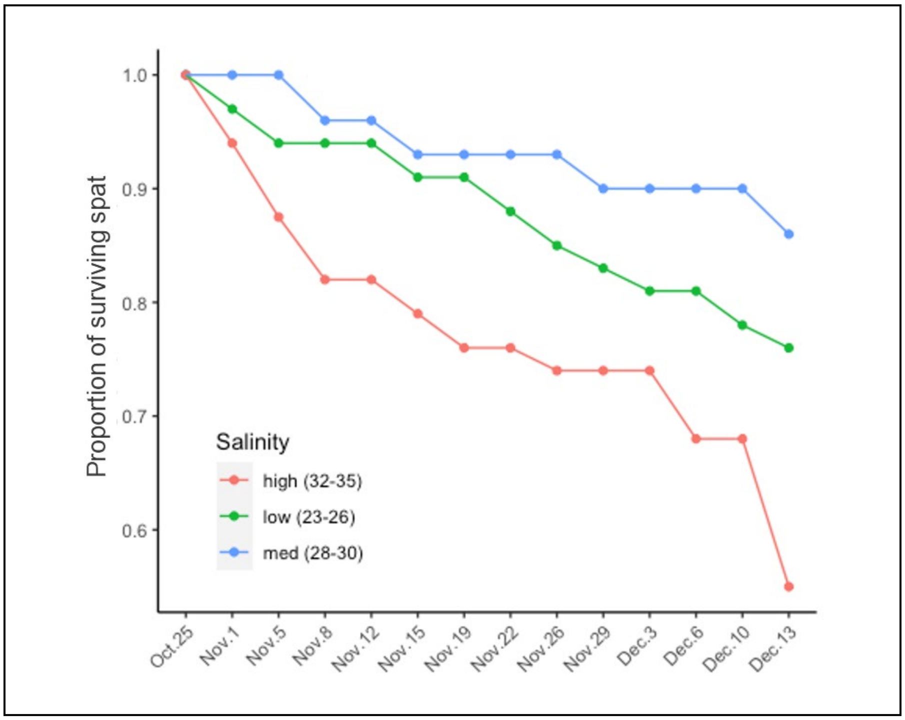


**Figure 12.** Survival of oyster juveniles reared under high, medium and low salinity regimes

### *3.5. Stress responses of oyster early life-stages (Michael Wintermantel, PhD student)*

**Introduction.** Michael Wintermantel is one of the newest members of the ABSI team, but is rapidly familiarizing himself with Apalachicola Bay and oyster biology. He is currently developing a number of research ideas and will likely have one or more experiments up and running by summer. Michael's research interests focus on how larval oysters respond to stress. His research goals focus on elucidating potential causes of larval oyster mortality, and/or additive sublethal effects which may influence their survival and settlement when combined with other factors.

## 4. Oyster ecology

### *4.1. Intertidal monitoring (ABSI Core Team)*

Compared with subtidal oysters there is relatively little research done on intertidal oysters in the ABSI region and very little publicly available data on intertidal oysters. This knowledge gap has been noted by Grizzle et al. (2015, 2018) and the Oyster Integrated Mapping and Monitoring Program. Most of the research from the ABSI region has focused upon subtidal oysters as they comprise most of the commercial harvest, however, omitting intertidal oysters when assessing the local oyster population provides an incomplete understanding of oyster status. Consequently, we do not have a good understanding of oyster populations in intertidal habitats or their contribution to overall larval supply.

It is estimated that intertidal oysters cover a total area of 94 ha and have a mean live oyster density of 406 oysters/m$^2$ (Grizzle et al. 2018). In comparison, subtidal oysters were sampled by FWC in 2016 and covered approximately 1,600 to 4,000 ha with a mean live oyster density of 17 oysters/m$^2$. This comparison shows that although subtidal oysters cover substantially more bottom area within the region than intertidal oysters do, intertidal oysters are clearly contributing to overall larval supply and are potentially in better condition than subtidal reefs. Intertidal reefs in the ABSI region are also primarily natural reefs while there are few remaining natural subtidal reefs, which have been cultched multiple times with a range of materials. Additionally, it should be noted that in Grizzle (2018) the study area terminated at East Cove and did not extend further to the east, where there is substantial intertidal oyster habitat near the Carrabelle River mouth and within Alligator Harbor.

The ABSI intertidal oyster monitoring continue built upon the initial assessment of intertidal oyster reef condition made by Grizzle et al. (2018) and examined intertidal oysters over a wider spatial and temporal scale. ABSI intertidal oyster monitoring will quantified spatiotemporal variations in oyster density and compared key oyster metrics such as length-weight relationships, condition index, size structure, and disease prevalence across space and time throughout the ABSI region.

**Methods**. Intertidal oyster reef monitoring concluded in November 2021. As in 2020 four areas were repeatedly sampled for oyster size, reproductive condition, disease, and recruitment: Alligator Harbor (AH), Carrabelle River (CR), East Cove (EC), and Indian Lagoon (IL) (Fig. 13). Five sites at each area were sampled for a total of 20 sites. A total of 32 sampling trips were completed, representing wide spatial-temporal coverage. Spat traps (3 per reef, 5 140 spat traps were deployed in conjunction with sampling efforts to estimate oyster recruitment. In the fall of 2020 and the spring and fall of 2021, all 20 intertidal reef sites were sampled for oyster density, as well as the standard monthly condition, reproduction and disease sampling.

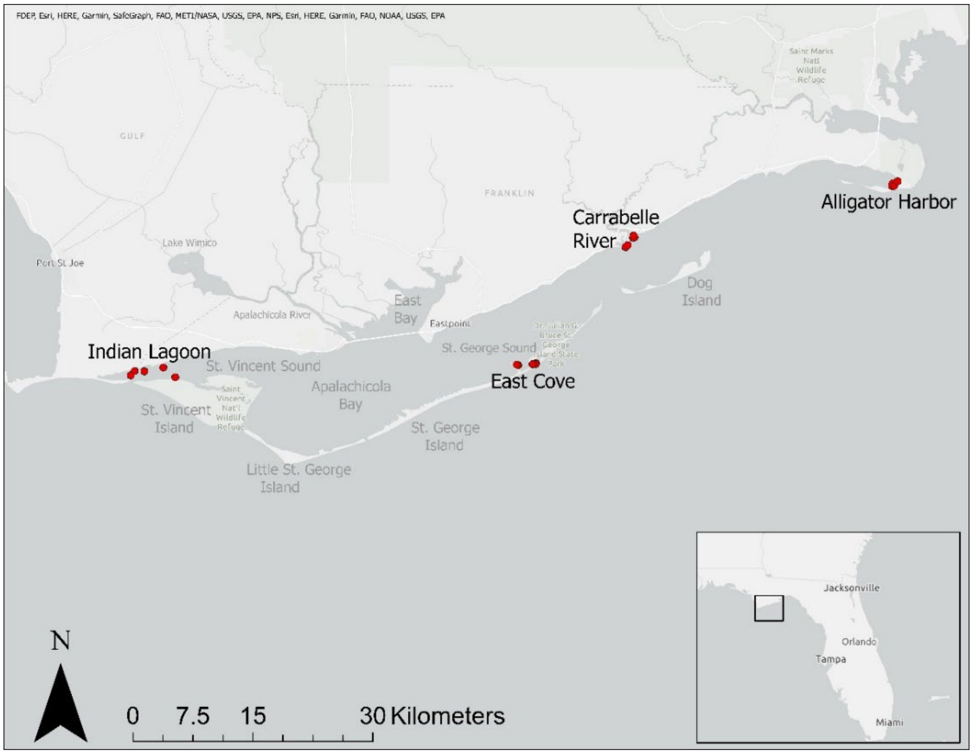


**Figure 13.** Intertidal oyster monitoring sites (Indian Lagoon, East Cove, Carrabelle River, Alligator Harbor).

**Results**

Oyster density. Intertidal density sampling revealed that oyster density and the proportion of live spat, live oysters, and dead shell varies among our sites. Grizzle (2018) observed that oyster density increased from the west to the east, but this analysis did not include sites further east than East Cove. Density sampling over four time periods and four sites indicate that overall density and size class distributions change temporally and seasonally with no clear trends (Tables 2-5, Fig. 14).

**Table 2.** January – March 2020 mean density (0.25-m$^2$) counts per site for spat (≤25mm), live oysters (>25mm), dead oysters and the total live. SE = standard error of the mean.

| Site | Spat (SE) | Oyster (SE) | Dead (SE) | Total Live (SE) |
|---|---|---|---|---|
| IL | 2.52 (0.87) | 2.72 (1.08) | 1.20 (0.45) | 5.24 (1.78) |
| EC | 50.05 (11.54) | 38.90 (10.04) | 26.65 (7.28) | 88.95 (21.22) |
| CR | 53.38 (12.20) | 37.96 (9.53) | 30.13 (8.27) | 91.33 (21.43) |
| AH | 22.17 (3.46) | 43.67 (6.03) | 10.33 (1.86) | 65.83 (8.80) |

**Table 3.** November-December 2020 mean density (0.25-m$^2$) counts per site for spat (≤25mm), live oysters (>25mm), dead oysters and the total live. SE = standard error of the mean.

| Site | Spat (SE) | Oyster (SE) | Dead (SE) | Total Live (SE) |
|---|---|---|---|---|
| IL | 3.75 (1.09) | 3.83 (1.33) | 2.04 (0.61) | 7.58 (2.26) |
| EC | 79.38 (17.52) | 41.75 (9.60) | 48.92 (12.95) | 121.13 (26.75) |
| CR | 40.88 (9.21) | 13.5 (3.65) | 11.08 (2.98) | 54.38 (12.48) |
| AH | 10.18 (2.18) | 22.05 (6.53) | 9.09 (2.64) | 32.23 (8.54) |

**Table 4.** May-June 2021 mean density (0.25-m$^2$) counts per site for spat (≤25mm), live oysters (>25mm), dead oysters and the total live. SE = standard error of the mean.

| Site | Spat (SE) | Oyster (SE) | Dead (SE) | Total Live (SE) |
|---|---|---|---|---|
| IL | 0.13 (0.09) | 2.17 (1.05) | 0.25 (0.17) | 2.29 (1.07) |
| EC | 18.42 (5.08) | 31.0 (9.0) | 11.95 (3.45) | 49.42 (13.61) |
| CR | 4.40 (1.53) | 12.04 (3.70) | 2.24 (0.90) | 16.44 (4.62) |
| AH | 12.72 (2.75) | 30.0 (6.15) | 8.96 (2.50) | 42.68 (8.73) |

**Table 5.** November-December 2021 mean density (0.25-m$^2$) counts per site for spat (≤25mm), live oysters (>25mm), dead oysters and the total live. SE = standard error of the mean.

| Site | Spat (SE) | Oyster (SE) | Dead (SE) | Total Live (SE) |
|---|---|---|---|---|
| IL | 11.68 (3.68) | 4.32 (1.16) | 1.92 (0.61) | 7.58 (2.26) |
| EC | 46.28 (8.00) | 24.08 (3.14) | 16.68 (2.96) | 121.13 (26.75) |
| CR | 38.76 (6.50) | 22.40 (4.78) | 24.08 (4.38) | 54.38 (12.48) |
| AH | 18.1 (4.3) | 15.68 (6.51) | 6.72 (2.53) | 32.23 (8.54) |

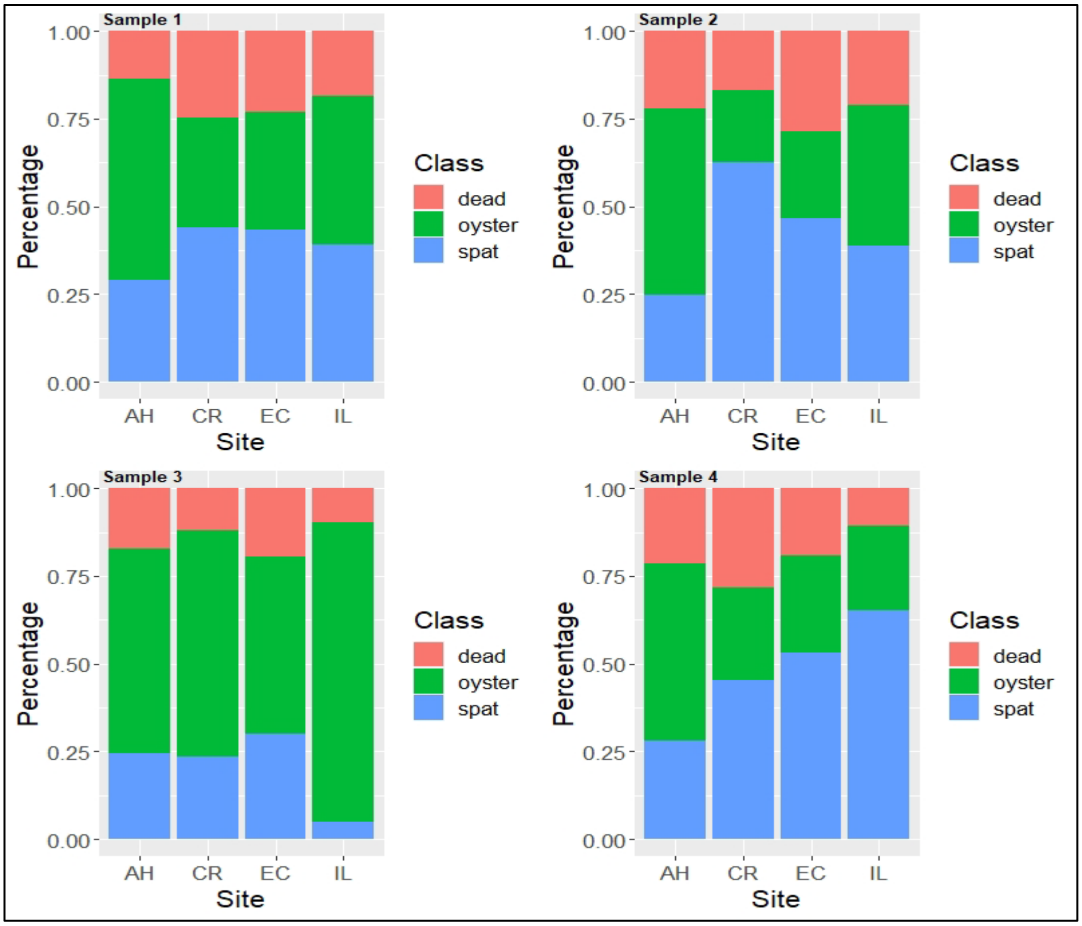


**Figure 14.** Percentage of live spat (<25 mm), live oysters (>25 mm), and dead oysters from density sampling by site. Sample 1 = Jan-Mar 2020, Sample 2 = Nov-Dec 2020, Sample 3 = May-Jun 2021, Sample 4 = Nov-Dec 2021.

A two-way ANOVA was performed on each category (spat, live oysters and dead oysters), using site and sampling period as factors. The spat showed no difference among time periods ($F = 2.65$, $p = 0.11$), but there was a statistically significant difference among sites ($F = 7.71$, $p < 0.05$). A Holm-Sidak post-hoc comparison test identified differences between East Cove and Indian Lagoon ($t = 4.43$, $p < 0.05$) and East Cove and Alligator Harbor ($t = 3.29$, $p < 0.05$), were driving the overall significance. The same outcomes were found for live oysters (>25 mm), with no significant differences among sampling periods ($F = 2.45$, $p = 0.13$) but significant differences among sites ($F = 10.91$, $p<0.05$), driven by differences between Indian Lagoon and East Cove ($t = 5.40$, $p <0.05$), Alligator Harbor ($t = 4.33$, $p <0.05$) and Carrabelle River ($t = 3.21$, $p <0.05$). Dead oysters also showed no significant difference among sampling periods ($F = 1.17$, $p = 0.37$) but significant differences among sites ($F = 4.36$, $p <0.05$), driven by differences between Indian Lagoon and East Cove ($t = 3.43$, $p <0.05$). These results support Grizzle (2018) observations that the overall density of oysters was higher in East Cove than Indian Lagoon, but important populations also occur further east in Carrabelle and Alligator Harbor.

Condition and morphometrics. Five oysters were selected from each intertidal reef (20 total sites) per month and processed for condition and morphology data. Each oyster had standardized height, length, and width measurements taken to the nearest millimeter using vernier calipers (Fig. 15). Oysters were then opened, drained, and blotted with a towel prior to obtaining a total weight. Afterwards oyster tissue was completely removed from the shell and total tissue and shell weights were separately recorded. Shell and tissue were then placed in a drying oven at 50°C for a period of 72 hours to remove excess water mass and weights of dry tissue and dry shell were then recorded. Condition index for intertidal oysters was calculated as meat dry weight/shell dry weight x 100 (Table 3)

Height-weight relationships. Height-weight relationships were visualized using loess regression curves (Fig. 16). Due to the complexity of statistically comparing loess curves we used generalized linear models of log transformed heights and (wet) weights with site as an interaction term. The general linear model results show that the slope for height and weight relationships for Indian Lagoon, Carrabelle River, and Alligator harbor are not significantly dissimilar but the slope for East Cove oysters is dissimilar from the rest. When examining the height-weight relationships (Fig. 16) and the density data it seems that the sites on the edge of the system (Indian Lagoon and Alligator Harbor), and more likely to be influenced by the marine environment are larger and lower in density, while the opposite is true for Carrabelle River and East Cove which are closer in proximity to fresh-water influence from the Apalachicola River and Carrabelle River.

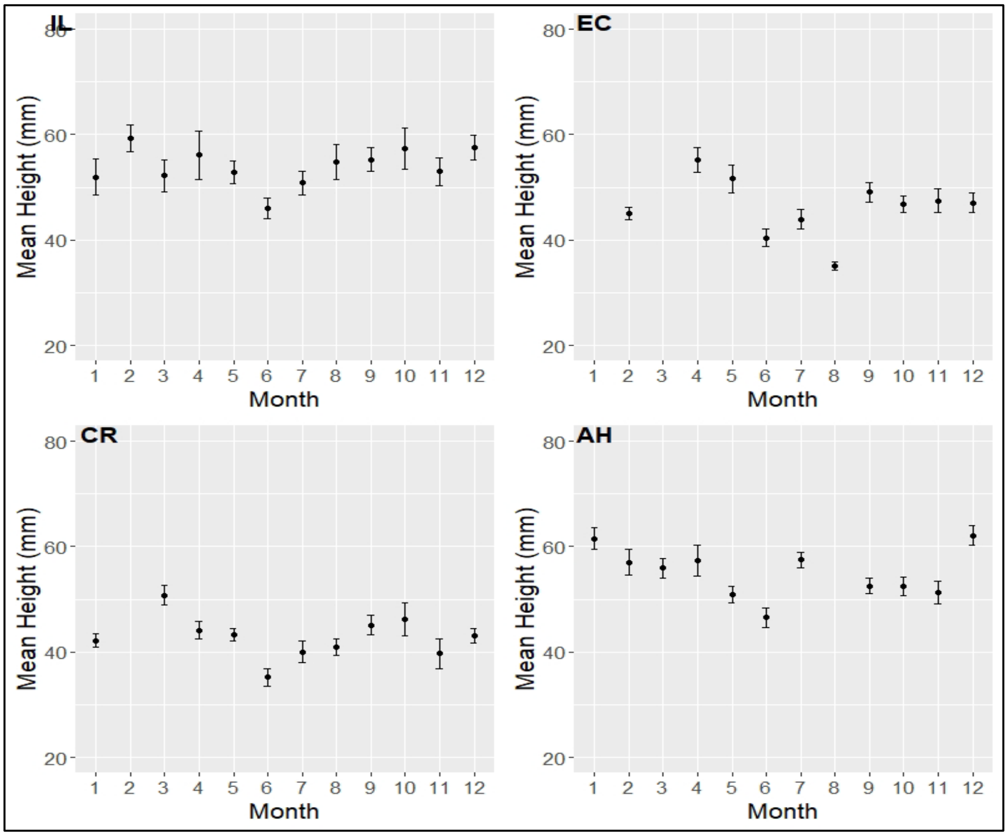


**Figure 15.** Mean oyster heights from intertidal sampling by month and across sites (IL = Indian Lagoon, EC= East Cove, CR = Carrabelle River, AH = Alligator Harbor).

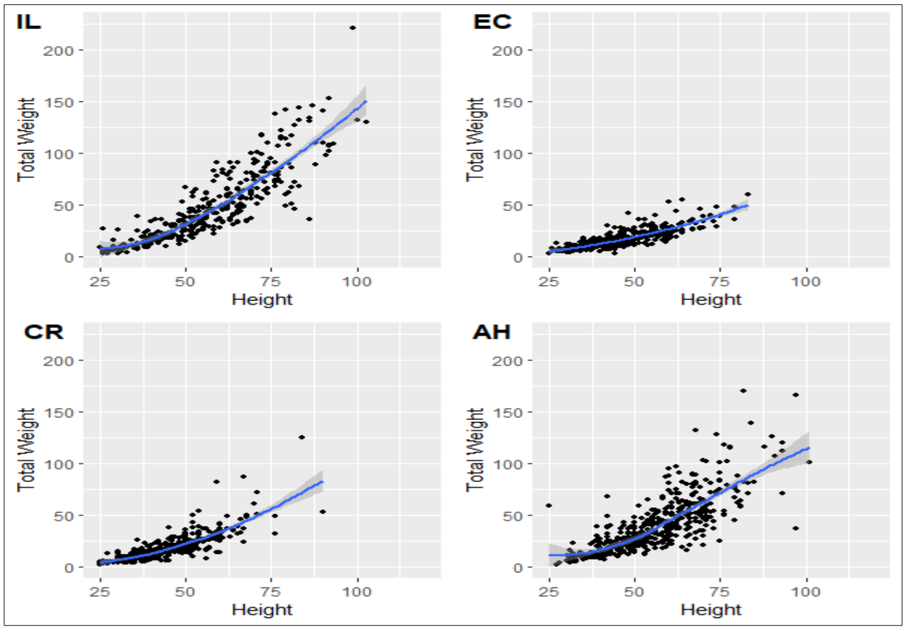


**Figure 16.** Loess regression curves of height and weight relationships amongst sampling areas.

**Table 3.** Summary of intertidal oyster metrics. Minimum, maximum, and mean values with standard error for height, total weight, shell weight (wet/dry), and meat weight (wet/dry).

| | Height (mm) | Total weight (g) | Shell weight (g) (wet) | Shell weight (g) (dry) | Meat weight (g) (wet) | Meat weight (g) (dry) |
|---|---|---|---|---|---|---|
| Indian Lagoon | | | | | | |
| Min/max | 21/103 | 2.55/221.4 | 2.09/188.0 | 1.82/179.6 | 0.39/45.32 | 0.05/7.78 |
| Mean (SE) | 56.30<br>(0.88) | 47.03<br>(1.80) | 40.1<br>(1.55) | 37.7<br>(1.48) | 6.90<br>(0.30) | 1.23<br>(0.05) |
| East Cove | | | | | | |
| Min/max | 3/83 | 2.6/60.25 | 1.86/48.79 | 1.79/45.6 | 0.2/11.46 | 0.01/3.06 |
| Mean (SE) | 42.14<br>(0.77) | 16.22<br>(0.49) | 13.27<br>(0.41) | 12.20<br>(0.37) | 2.95<br>(0.10) | 0.49<br>(0.02) |
| Carrabelle River | | | | | | |
| Min/max | 13/90 | 0.45/124.96 | 0.38/97.28 | 0.3/89.76 | 0.07/27.68 | 0.01/5.6 |
| Mean (SE) | 43.30<br>(0.59) | 17.34<br>(0.70) | 14.36<br>(0.57) | 13.18<br>(0.54) | 2.98<br>(0.14) | 0.51<br>(0.03) |
| Alligator Harbor | | | | | | |
| Min/max | 18/101 | 2.26/169.82 | 1.81/154.3 | 1.68/149.88 | 0.3/26.14 | 0.02/5.04 |
| Mean (SE) | 55.33<br>(0.65) | 39.33<br>(1.25) | 33.28<br>(1.10) | 31.22<br>(1.06) | 6.05<br>(0.18) | 1.05<br>(0.03) |

### *4.2. Spatial and temporal patterns of intertidal oyster reefs using remote sensing techniques (Jenny Bueno, MSc student)*

**Introduction and rationale.** The eastern oyster (*Crassostrea virginica*, Gmelin 1871) is a fisheries species that has been declining in various regions with its more recent collapse of 2012 in the Apalachicola Bay of Florida. Various factors have contributed to its decline including low fresh-water inputs and overharvesting of the population (Camp et al., 2015). Since its decline, the bay was suspended of further harvesting until 2025 (FWC, 68B-27, F.A.C.), with various efforts implemented to better manage and research the bay. Current efforts are mainly focused on subtidal oyster reefs due to its extensive bottom coverage. However, there are also intertidal areas of this ecosystem, and little is known on its ecological function and patterns driving its persistence within the bay. Filling the knowledge gap of its ecological function is necessary for effective ecosystem-based management of this fishery species. To fill this knowledge gap, this research will focus on mapping the intertidal oyster reefs to understand temporal and spatial patterns in the Apalachicola Bay region of Florida.

Current methods of monitoring the intertidal oyster reefs involve on-the-ground quadrat sampling, which is time- and cost-intensive, destructive to the reef, (Espriella et al., 2020) and provides only a small snapshot of the larger landscape extent. Satellite imagery analysis is an alternative to this method and has been implemented in this region by Grizzle et al., 2018. However, this approach has coarse resolution, with insufficient detail for monitoring efforts (Espriella et al., 2020). Unoccupied aerial systems (UAS), more commonly called drones, have recently become a powerful research tool in coastal and marine environments (Joyce et al., 2019). They have capabilities and flexibility of capturing high-resolution imagery in conditions where satellite imagery is inadequate (Joyce et al., 2019). Combining these technological advances and research ventures can provide a holistic insight to the landscape dynamics. Additionally, mapping is one of many integral parts of a better management framework outlined by Beck et al., 2011.

Research objectives:
**O1**: Create high resolution maps of the intertidal oyster reefs
**O2**: Use the high-resolution maps to analyze intertidal oyster spatial patterns
**Q3**: Analyze temporal and spatial change of oyster abundance in the intertidal

Mapping the intertidal will provide digital maps to better understand the broad-scale dynamics of the intertidal to assist with better management, conservation, and future restoration. Additionally, this research can provide a foundation for continued monitoring with innovative tools.

**Methods.** To complete the research objectives outlined, intertidal oyster reefs will be mapped using a UAS and an RTK-GPS system. The UAS will capture high-resolution imagery with high overlap to recreate the exposed intertidal reefs. Ground control points are placed strategically within the bounds of the UAS flight. The RTK-GPS system collects high-accuracy location information of GCPs to geo-reference the final products. The imagery and coordinate location of the GCPs are processed in a photogrammetric software to create orthomosaics, or high-resolution georeferenced mosaics, and digital elevation models (DEMs), or a digital representation of elevation data. The maps are then analyzed in ArcGIS Pro, a geographic information system software to extract surface parameters such as average elevation of reefs, surface area, and number of oyster clusters per reef. Spatial statistics of these parameters will be explored in R software.

**Results and future work**. Data collection began in December of 2021 due to the longer low tides of the winter. The areas of interest include Alligator Harbor, East Cove, Indian Lagoon, and Carrabelle river area where intertidal oysters have been identified by Grizzle et al., 2018. As of March 2022, East Cove and Alligator Harbor have been mapped. Processing of East Cove is complete (Fig. 17). Indian lagoon and Carrabelle will be collected in the summer of 2022 during low tides, which will complete research objective 1. Analysis of cluster presence for East Cove is ongoing (Fig. 18), and analysis will be completed for all other sites to fulfill objective 2. Objective 3 will be completed concurrently with objective 2 by looking at oyster cluster change through time.

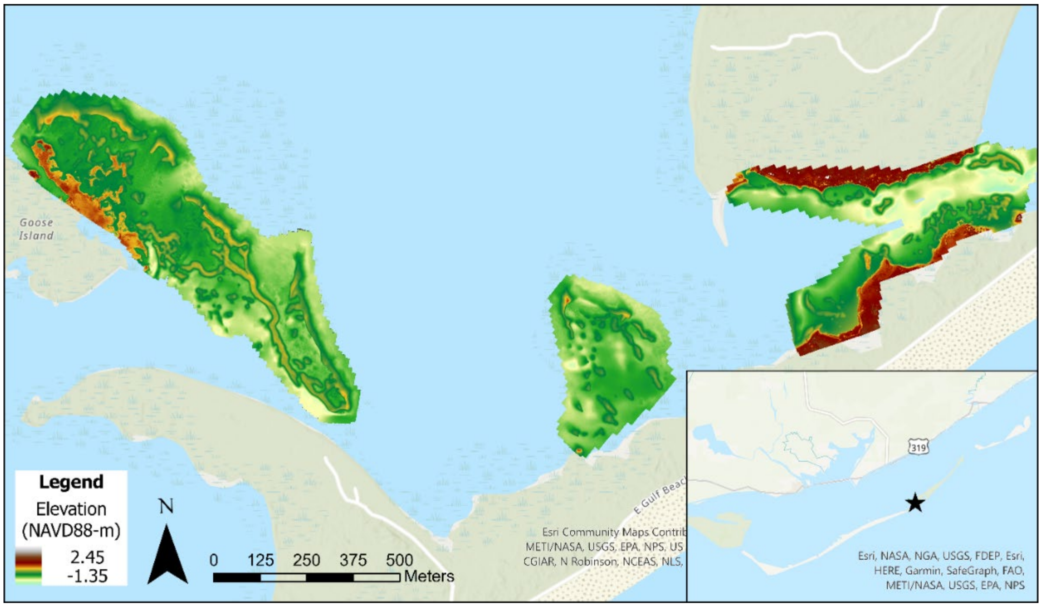


**Figure 17:** *E*levation range of intertidal oyster reefs - collected at East Cove on St George Island

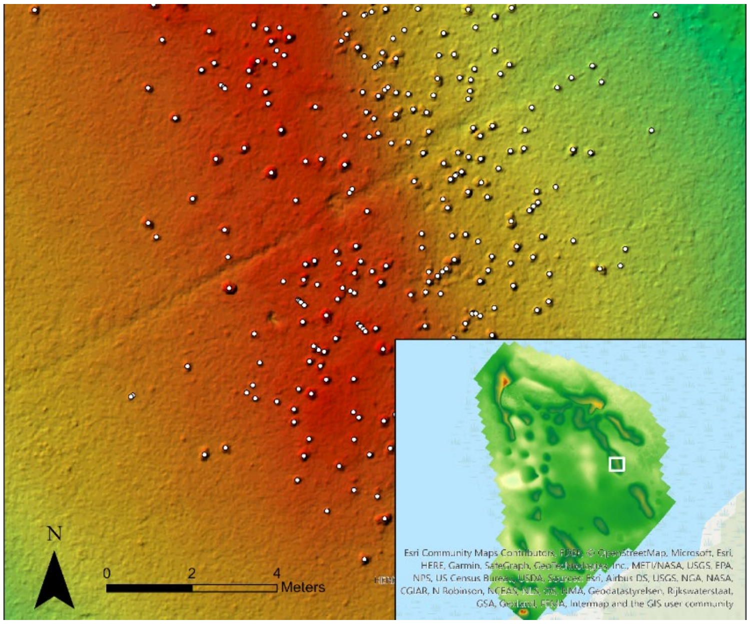


**Figure 18:** This figure illustrates the oyster cluster presence, the white points, extracted from the digital elevation models collected at East Cove on St. George Island

### *4.3. Subtidal monitoring (ABSI Core Team)*

**Introduction and rationale.** The objectives of these surveys are to (1) prioritize areas for more detailed mapping efforts, (2) locate sites for oyster reef restoration experiments, (3) refine the current understanding of the extent of oyster reefs in Apalachicola Bay, and (4) detect patterns in live oyster density and size distribution.

**Methods.** The first subtidal surveys were conducted from late fall 2020 to early spring 2021 and consisted of 132 sites (Fig. 19). At each station, six replicate single tong samples are taken from the bow, middle and stern of both sides of the vessel. The following parameters were recorded for each tong sample: volumes of total material, shell (non-living), live oysters and rock; numbers of spat (<25 mm), sub-legal oysters (25-75 mm), market-sized oysters (> 75mm), and boxes (dead, articulated shells). In addition, history of cultch planting and type of cultch (shell, limestone, fossil shell) planted were recorded.

The second surveys occurred in the fall of 2022 and consisted of 117 sites (Fig. 20). These comprised 82 known sites from the first survey, and 35 unknowns. Sites were selected using two shapefiles, created in ArcGIS Pro, which had “known” and “unknown” site designations. The “known” locations are places where live oysters were present in the first round of tonging, or areas that are Restore/NRDA sites, or were identified via side scan as potential oyster substrate. The “unknown” locations are areas where no sidescan data is available and no tonging monitoring has been conducted to better understand the FWC subtidal natural and planted classified areas. The data used in ArcGIS Pro include low and high resolution sidescan raster data collected from NOARC in 2021, DEP sidescan data, all ABSI subtidal monitoring tonging data, and the FWC bottom classification data from 2007. The data was loaded into ArcGIS Pro to determine what areas have no data and 35 unknown sites were selected throughout these areas in order to provide ample spatial coverage. Tong samples for the second round of subtidal sampling were collected in the same manner as the first round of sampling, however, the height of the first 100 oysters was measured (rather than assigning a size class) and remaining oysters were counted.

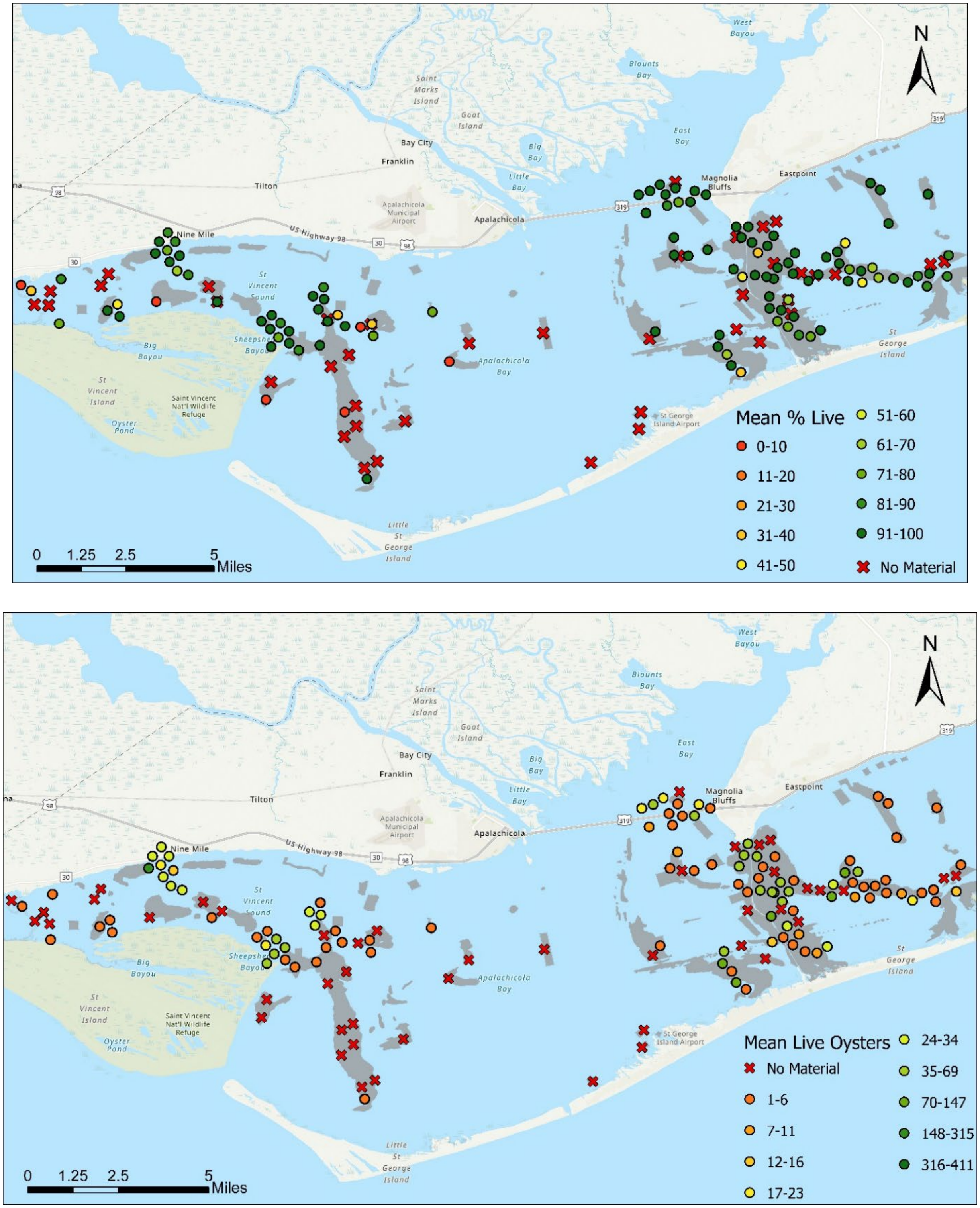


**Figure 19.** Subtidal tonging locations from year one. Top panel shows the percentage of live oysters at each sampling site and the bottom panel shows the mean count of live oysters.

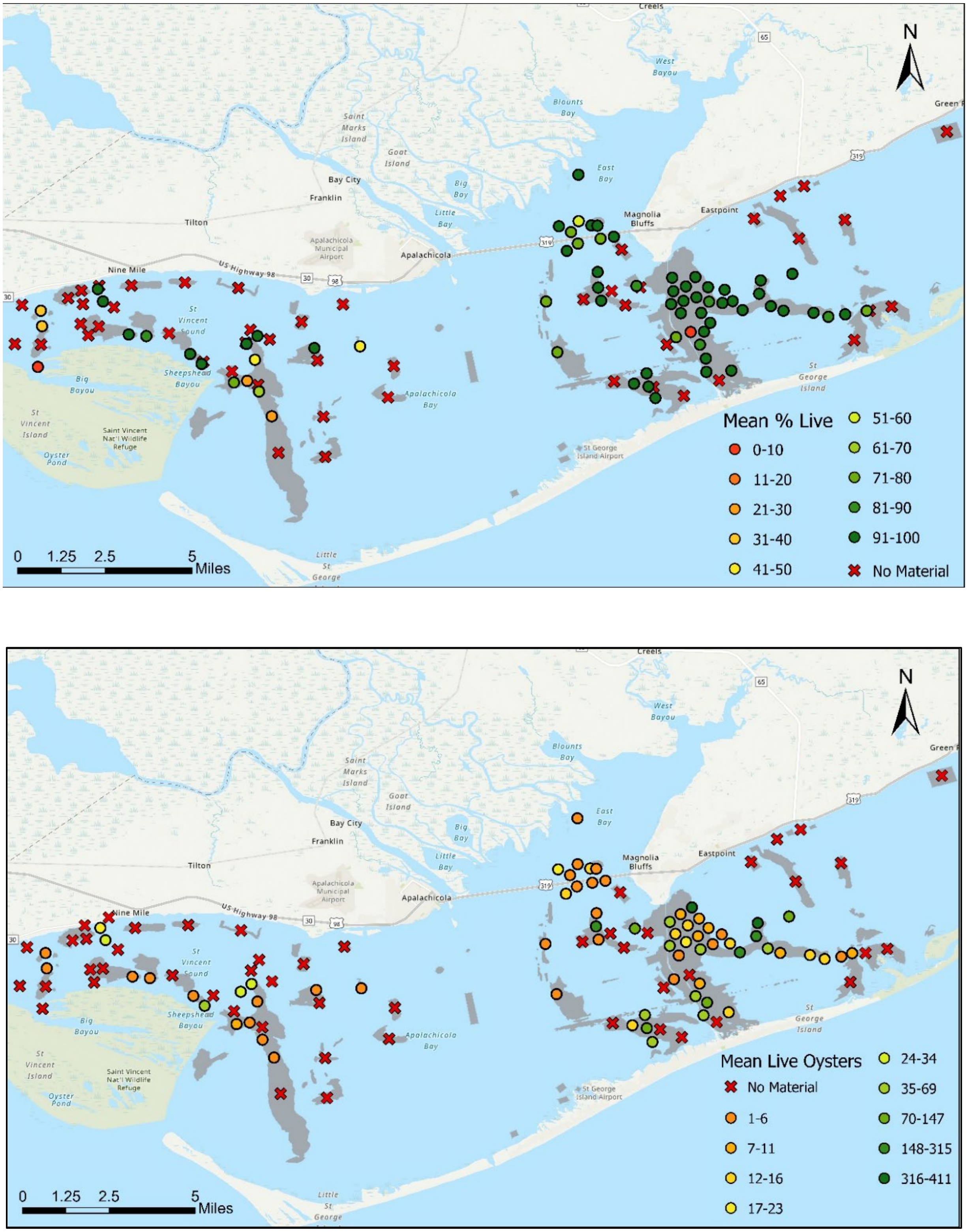


**Figure 20.** Subtidal tonging locations from year two. Top panel shows the percentage of live oysters at each sampling site and the bottom panel shows the total count of live and dead oysters.

**Results.** These subtidal surveys indicate that the current distribution of oyster populations in Apalachicola Bay is spatially patchy and sparse. There are very few areas that support market sized oysters, and those areas with significant numbers of live oysters were generally those that were recently planted (2017-2019) with limestone, particularly in the eastern part of the bay. Both years of the survey yielded results with similar trends. There were several areas with no oysters to the west and more areas with oysters present clustered in the east. However, these clustered sites with oysters present typically had few oysters. Areas with a large portion of live oysters and a large number of oysters were scarce, but primarily scattered on the eastern portion of the study area

### *4.4. Intertidal and subtidal recruitment (ABSI Core Team)*

**Methods** Intertidal spat traps (1 per reef, 5 reefs per site) were placed adjacent to each reef site and then collected and replaced (~ monthly) during each intertidal sampling event. Initially intertidal spat traps were placed in very shallow water and spat recruitment seemed to be minimal to nonexistent. In June of 2021 intertidal spat traps were placed in deeper waters adjacent to each reef site and spat recruitment results appeared to become reliable and useful. A total of 120 spat traps with two stringers of six shells each were collected from June to December 2021. Top and bottom shells from each stringer were omitted from spat counts to account for predation. The spat on the 8 middle shells per trap were added, and the average number of spat per site per month are shown in figure 21. Spat traps were also placed in 26 locations throughout Apalachicola Bay and St. George Sound from September 2021 to January 2022 for a total of 130 subtidal spat traps.

**Results.** Intertidal spat recruitment varied across space and time (Fig. 21). The Carrabelle River and East Cove sites, which are closest in proximity to one another, seemed to follow similar trends. Spat counts at these sites were low from June to August and then peaked in September and October. Spat recruitment at Alligator harbor and Indian Lagoon followed similar recruitment patterns that were dissimilar to Carrabelle River and East Cove. These sites had consistently lower recruitment rates and peak recruitment rates happened at different times. It is also worth noting that these lower recruitment rates seen at Indian Lagoon and Alligator Harbor coincide with the density data which shows these sites have lower oyster densities than East Cove and Carrabelle River (Fig. 21 and Table 4). Recruitment on subtidal spat traps was highly variable and exhibited no easily discernable trends (Fig. 22). Some spat traps received various amounts of spat throughout the sampling period while others that are relatively close by, received none in the same time span.

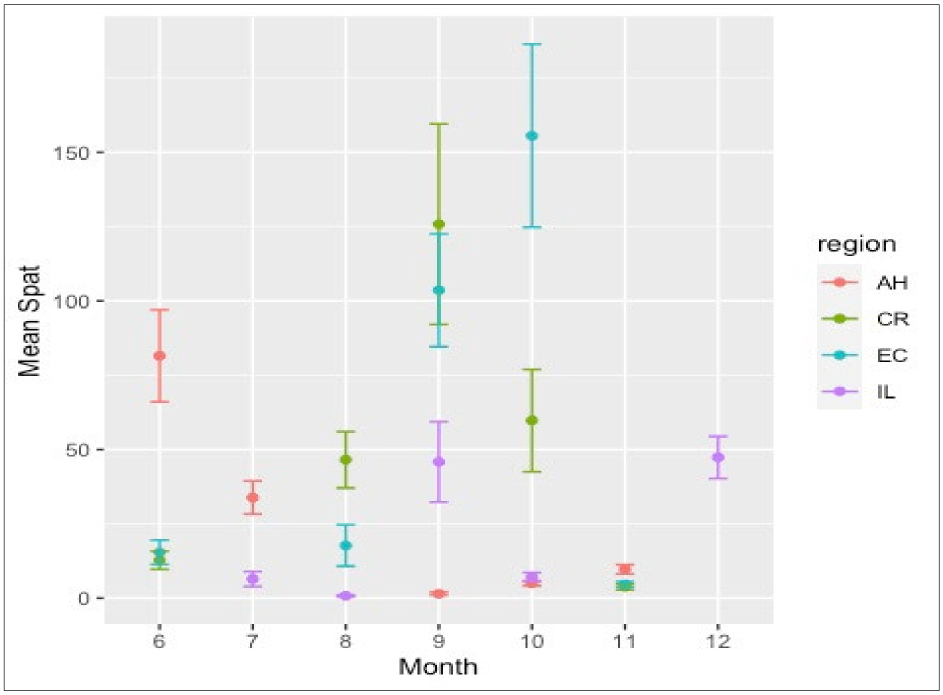


**Figure 21.** Mean monthly spat collected on spat traps (n=5 per site; 120 total) at each intertidal sampling area with standard error bars. Months 6 – 12 = June-December 2020

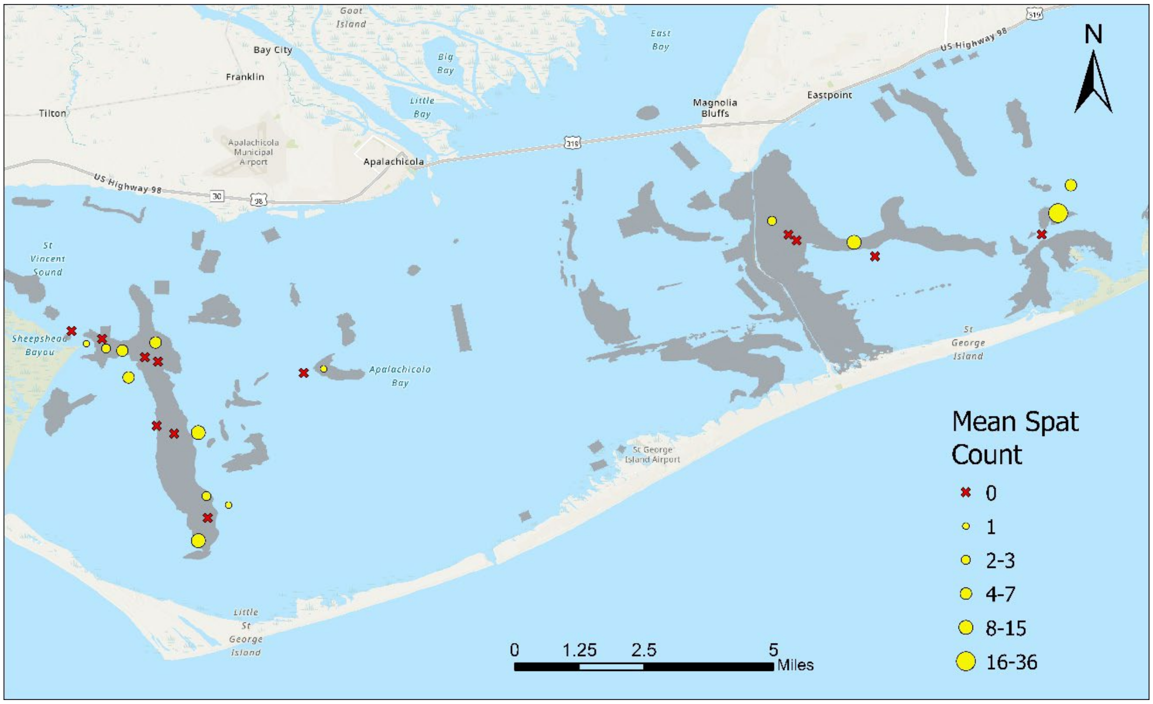


**Figure 22.** Mean spat abundance observed on subtidal spat traps (Sep 2021 – January 2022).

### *4.5. Impacts of oyster populations on community development (Dr. Andrew Shantz, Assistant Research Faculty)*

**Introduction and rationale**. Oysters are the foundation species in Apalachicola Bay but are only part of this productive and valuable ecosystem. In addition to oysters, the estuary houses numerous economically important species and is critical nursery habitat for an array of commercially important fishes harvested throughout the Gulf of Mexico. Effectively restoring the lost ecosystem goods and services provided by Apalachicola Bay will require understanding how the broader fish and invertebrate communities in the bay have been impacted by the recent oyster population decline and ensuring that these species are also responding to restoration efforts. The goals of this project are: 1) To utilize existing data to assess how the decline of oyster populations in Apalachicola Bay have impacted the broader ecological community in and around the bay, particularly commercially and recreationally important species (Fig. 23); and 2) Identify how restoration efforts are impacting community development and habitat use by these species throughout the bay.

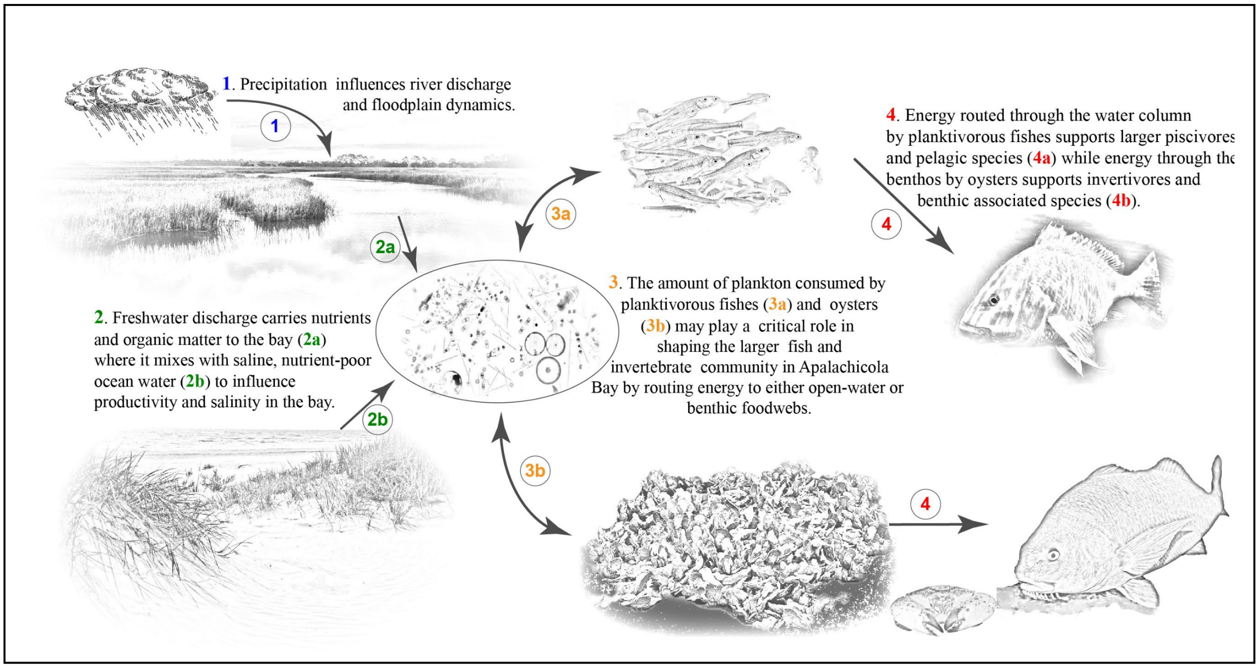


**Figure 23:** Conceptual diagram illustrating a potential pathway through which oysters in Apalachicola Bay mediate energy flow to influence productivity in pelagic and benthic foodwebs.

**Methods and results.** Part 1 of this project was initiated the Summer of 2021. Existing data on fish and invertebrate communities, fisheries landings, and environmental conditions was compiled throughout the winter of 2021 and initial analyses begun. Preliminary data suggest that prior to their collapse, oyster populations exhibited significant top-down control of phytoplankton within Apalachicola Bay (Fig. 24A). In doing so, oysters channeled large amounts of energy from the primary productivity in the water column to the benthos, where it supported extensive, productive benthic foodwebs in the bay. However, with the collapse of oyster population, this productivity has shifted towards midwater and pelagic species. As a result, the region has experienced a shift from supporting highly productive benthic fisheries species to more midwater and open water species (Fig. 24B).

**Future work.** Analyses for Part 1 of this project are being completed, with plans for submission of a peer reviewed manuscript for publication during Year 4. Part 2 of this project began in March 2022. This portion of the research will focus on understanding the recovery potential of the broader fish and invertebrate community in Apalachicola Bay. Sampling trays filled with culch, and artificial reef structures are being deployed at sites across the bay spanning a gradient of environmental conditions. Trays will be censused at regular three-month intervals to assess oyster recruitment and to record community composition and succession of associated species. During each quarterly census, reef structures will be surveyed for recruitment and photogrammetric calculations of total volume to quantify reef accretion rates at each site. Deployments are being paired with *in situ* temperature, DO, and salinity loggers to record local conditions. Data will be analyzed to understand how environmental conditions influence the recovery and colonization of sites across the bay. Combined with ABSI fisheries independent surveys, this data will help understand how environmental characteristics influence habitat use and recovery of associated oyster reef communities and identify the most promising sites for successful future shelling and restoration efforts.

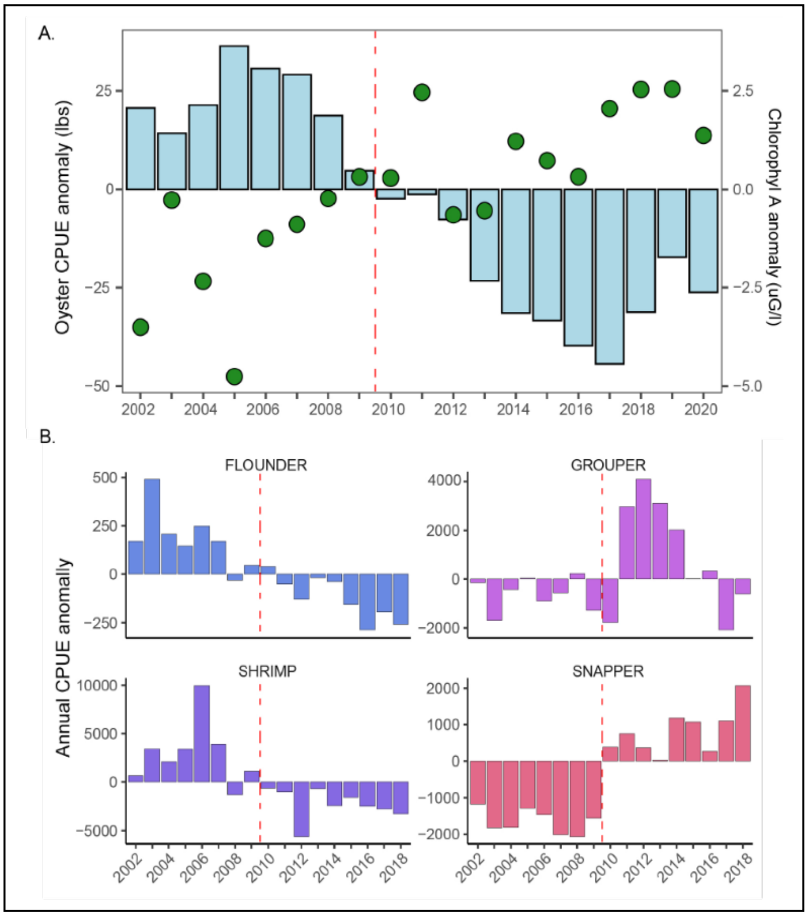


**Figure 24: A.** Change in annual oyster catch per unit effort (CPUE, blue bars) and Chlorophyl A concentrations (green circles) from 2002 to 2020 in Apalachicola Bay. Oyster CPUE is an indicator of the oyster population status, and the decline of oysters explains the greatest amount of variation in Chlorophyl abundance ($r^2 = 0.57$) in the Bay.

**B.** The corresponding shift in CPUE for other commercially important species in Franklin County. As oyster CPUE drops below the 20 year average (dashed red line) CPUE of species dependent on benthic foodwebs (e.g., flounder and shrimp) show declines while piscivores and species dependent on open-water food sources (Grouper and Snapper) increase. Note, decline in Grouper CPUE in 2015 occurs with changes in Red Grouper fishing regulations

## 5. Restoration

### *5.1. Oyster restoration experiments (ABSI Core Team)*

**Introduction and rationale**. The 2012 collapse of the Apalachicola oyster fishery has been relatively well studied and it has become clear that the collapse was caused by a combination of reasons, each exhibiting varying levels of influence and perhaps acting synergistically. After the collapse, millions of dollars in restoration funding was released from the Fishery Disaster fund, and Deepwater Horizon oil spill funding. These projects included deployment of cultch and post-deployment monitoring. All the projects met their construction objectives, but the oysters did not recover. All of these studies used a similar traditional approach of placing a thin layer of material over a large area. Studies in the Chesapeake Bay (Colden et al 2017) showed that 0.3 m was the minimum height to allow oysters to survive, rather than being buried by sediment. It has been noted by several studies pertaining to the 2012 collapse that a more thorough understanding of oyster recruitment and survivorship within the Apalachicola Bay System is needed to better equip oyster restoration efforts and management decisions. The restoration experiment was designed to 1) investigate the efficacy and persistence of different materials 2) Assess recruitment and survival of oysters on the elevated reef structures, and 3) assess the benefits of deploying hatchery spat on shell to the reefs to enhance recruitment.

**Methods.** In April of 2021 a total of thirty experimental reefs were created in Apalachicola Bay. Fifteen were placed at Dry Bar and another 15 at Peanut Ridge (Fig. 24). Each reef (100 $m^2$) was built with 50 cubic yards of material, which created a reef height of approximately 0.5 meters. Materials included natural shell, which is a traditional clutching substrate but is very light and rather expensive, small limestone rocks (~ 8 x 4 cm), which are made from similar material to natural shell but are cheaper, easier to obtain and heavier than shell, and larger limestone rock (~ 18 cm diameter) which should be more stable and provide interstitial spaces for reef associated animals to inhabit. Reef sites were created by employing local oysterman to transfer and deploy material within the boundaries of each reef site. In June of 2021 ABSI placed two vexar cages at each of the experimental reef sites (n=60). One cage was filled with oyster shells seeded with spat from the ABSI hatchery. Each of these cages had 50 shells, with ~ 150 spat in total. A second cage was placed adjacent to the first and filled with blank oyster shells (no spat). The amount of shell used in the blank cages was the same as for the spat seeded cages (~2.8kg). Cages were placed in the center of each reef site and anchored with a five-pound weight.

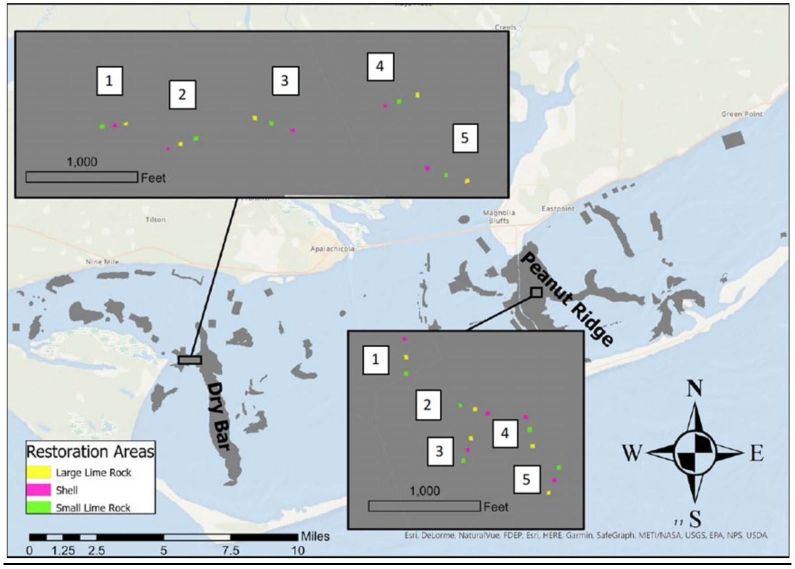


**Figure 24.** Experimental reef sites at Dry Bar and Peanut Ridge. Three materials were used with five replicate reefs at each site. Reef height was approximately 0.5 m

**Results.** Each cage which was placed in June was removed in September and again in December (2021). Once removed each cage was returned the FSU marine lab and total spat counts were completed for each cage and cages were returned to the sites within 48 hours. While spat counts were being completed, cages were kept inundated in seawater holding tanks. Of the thirty cages placed at Dry Bar 28 were recovered in September and December while 11 of 30 were recovered from Peanut Ridge in September and only 4 in December. Future iterations of this experiment will implement a more substantial anchoring system to secure cages to the reef sites.

Cages from Dry Bar provided much more useful data than did Peanut Ridge due to the loss of so many cages from Peanut Ridge (Fig. 25). In blank cages at Dry Bar, it appears that natural recruitment occurred in similar fashion for all three substrate types with natural shell performing better than the others. In Dry Bar cages with spat it seems that the seeded spat initially died off but recovered. All three treatment types followed similar trends but, in this case, small lime rock showed the most recruitment while shell had the least.

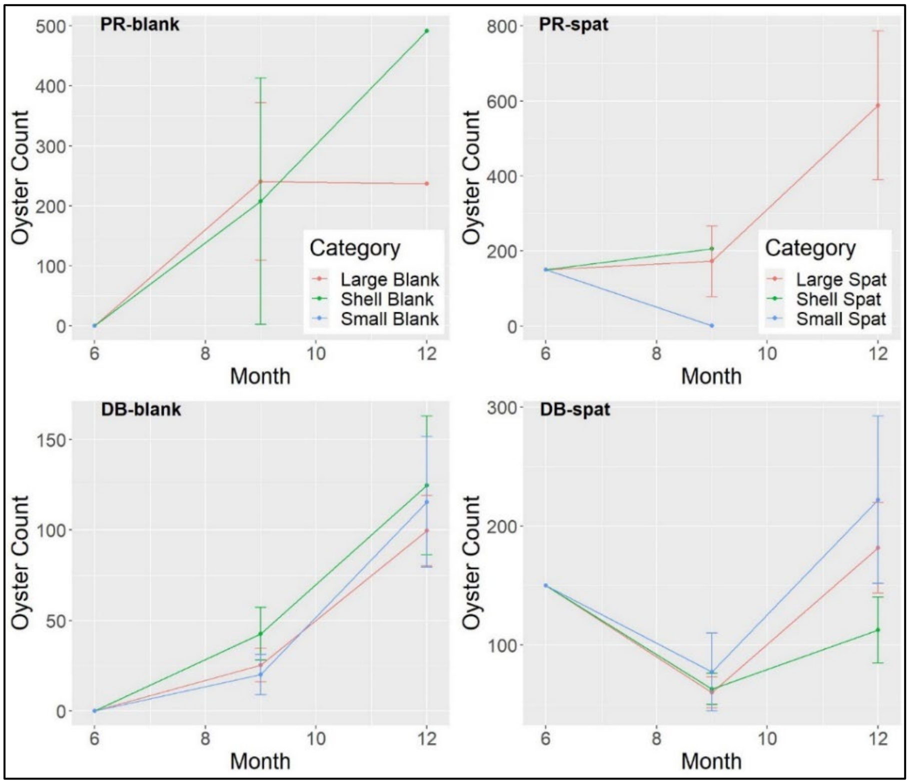


**Figure 25.** Spat count results from cages placed on experimental reefs. Top panels show results for Peanut Ridge with blank cages on the left and spat filled cages on the right. Bottom panels display the same information for Dry Bar sites.

### *5.2. Improving Restoration Success in the Bay Scallop (Morgan Hawkins, M. Sc. Student)*

**Introduction and rationale.** Bay scallops (*Argopecten irradians*) are commercially and ecologically important bivalves that are equipped with 40+ light detecting eyes, swim freely, and grow to reach market size in 10-12 months. In the 1950s, the bay scallop fishery was popular, as fishermen in Florida harvested an average of 250,000 pounds of scallop meat per year (NOAA Commercial Fisheries Landings). Over time, populations began to decline due to poor water quality, loss of seagrass habitat, and overharvesting. In 1994, Florida legislators banned commercial harvest of bay scallops indefinitely. Since then, bay scallops have only been available for recreational harvest, which increased the popularity of "scalloping", the practice of collecting scallops by hand while snorkeling in seagrass meadows. In 2018, revenue from this sport exceeded 1.8 million dollars in Steinhatchee, with both locals and tourists from 16 states participating (Granneman *et al*. 2021). Take limits and shortened scalloping seasons have been imposed to limit overharvesting. However, even with management, the fishery has suggested to be unsustainable in Steinhatchee, and further investigations should be conducted to assess scallop populations in other harvest zones (Granneman *et al.* 2021). The alarming decline of bay scallops suggests there are insufficient numbers of reproductive adults to replenish depleted populations. Therefore, aquaculture is becoming a focus of many restoration efforts that aim to supplement natural populations with hatchery-grown scallops. Bay scallops are collected from the wild, spawned in a hatchery, and raised until a certain growth milestone is reached. The desired-sized scallops then begin the grow-out process in cages in the wild. This transition commonly results in high mortality, losing up to 90% of hatchery-raised scallops before reaching their reproductive stage with no identifiable cause (Arnold *et al.* 2005, Clyde and Mackenzie 2009, Seyoum *et al.* 2003). This results in a waste of time and money for restoration efforts that have already incurred high labor and hatchery operation costs. To keep costs low and feasible, it is important to understand why this mortality occurs, and how to limit it. Surprisingly, there is relatively little information regarding the performance or biological differences between wild and hatchery raised bay scallops. Understanding the biological differences between wild and hatchery raised scallops as well as studying the optimal deployment size will increase efficiency of restoration efforts.

Research objectives

**O1**: Identify if there is difference in the survivability and growth rate of juvenile hatchery raised bay scallops compared to juvenile wild bay scallops.

**O2**: Investigate the differences/similarities in performance of respiration, condition index, gonadal index and shell breaking strength between wild and hatchery raised bay scallops.

**O2A**: To better understand the costs and benefits of using hatchery raised bay scallops in restoration efforts.

**O3**: Identify the optimal size for the release of hatchery raised bay scallops to maximize survival when transferred to grow-out cages.

**O3A**: To improve on-going restoration efforts of the bay scallop, as well as saving time and money.

Research Hypotheses

**H1:** Hatchery raised bay scallops will display a stunted growth rate and higher mortalities when first transferred to the field compared to wild bay scallops.

**H2**: Surviving hatchery raised bay scallops will perform equally compared to wild bay scallops in performance of respiration, condition index, gonadal index, and shell breaking strength

**H3**: The optimal release size of hatchery raised bay scallops for restoration efforts is 5mm.

This work will begin to answer questions (i) do hatchery raised and wild bay scallops differ in growth, survivability, or performance (ii) at what size do hatchery raised bay scallops display the highest survival and performance. This research will directly benefit current restoration and local aquaculture. By using a multidisciplinary approach combining conservation biology, ecology, physiology, and aquaculture, this research has the potential to identify current weaknesses in restoration techniques as well as determine the best practices to improve restoration success. Human interference may be the only way to prevent severe population depletion and failure of the recreational fishery. This study is vital to establish cost-effective and efficient restoration practices for this economically and ecologically valuable fisheries species. Also, restoring scallop populations will renew the public's participation in 'scalloping', supporting local economies and fostering a connection to nature.

**Methods.** To complete research objectives 1 and 2, during peak scallop spat season, 50 spat traps will be deployed and monitored following FWC's standard methods in St. George Sound, close the FSUCML. Ideally, after a few months the wild spat will reach 5-10mm and be transported to FSUCML. They will be sorted, placed in mesh bags, and housed in flow-through tanks overnight. Depending on the sizes collected, hatchery spat relatively the same size will be selected to undergo the same process. Hatchery spat will be sourced from bay scallops originating from St. George Sound and spawned multiple times in FSUCML's experimental hatchery following FSUCML's protocols to ensure multiple size classes of spat. On the day of deployment, bay scallops will be placed in a seawater filled cooler and boated out to sites in St. George Sound. Bay scallops will be placed in cages that are placed in pairs at a site, with red zip ties for wild bay scallops and blue zip ties for hatchery raised bay scallops. Every three weeks, spat will be measured, mortalities quantified, and cages exchanged to account for fouling. Once >85% of individuals reach 20mm, cages will be upgraded to a 15mm mesh, following FWC methodology. This experiment will conclude when bay scallops reach adult size of 50mm or until survival has reached 20% of the original stocking density.

Live bay scallops will be retrieved and placed in labeled mesh bags in a cooler of seawater for transport to FSUCML. Dependent upon survival, a number of randomly chosen bay scallops from each bag will undergo respirometry assays. Then, extracted tissue will be used for condition indexing by weighing the total wet weight of the tissue, gonad, and adductor muscle. Once complete, all flesh will be placed in the oven to dry and weighed again for a dry weight. The bay scallops' shell will then be placed in a tensile strength test machine to quantify how much force is needed to break the shell.

To investigate the optimal release size for hatchery raised bay scallops, treatments will consist of hatchery spat at sizes 5mm, 7mm, and 10mm sourced by FSUCML's experimental hatchery. Sorted bags with specific sizes of hatchery raised bay scallops will be placed in a cooler of seawater and boated to sites in St. George Sound. At each site, one bag of each size will be unloaded into cages identical to those described previously. Cages will be upgraded once >85% of scallop spat reach 20mm. Three cages will be placed together at each site, color-coated with zip ties corresponding to the initial size class of scallop. Every three weeks, spat will be measured, mortalities quantified, and cages exchanged to account for fouling. The experiment will continue until conditions are met as described previously.

In the summer of 2021, I was able to build a connection with the Eastern Shore Laboratory of the Virginia Institute of Marine Science. This connection helped ensure I had the tools and knowledge to make cultivating scallops a reality. In February, I was able to visit Eastern Shores' bay scallop hatchery where they raise millions of bay scallops every month for restoration. The techniques I learned from this training has enforced my confidence and experience to cultivate bay scallops in FSUCML's experimental hatchery to complete this study.

Spat traps have been deployed to conduct preliminary studies in 2021. This will help understand the feasibility of collecting wild bay scallops using spat traps. Results from this short study will be used to decide if spat traps are the most viable option, or if collection by hand is more efficient. An SAL permit for the spawning and grow-out of the bay scallop has been approved by the Florida Fish and Wildlife Conservation Commission. During summer 2022, dive trips will take place in St. George Sound to collect adult wild scallops to use for the fall. The first spawn is set to occur in September 2022. Multiple spawns will occur during fall 2022 to assist with this research and possibly FWC restoration efforts. After the fall spawning season, methods as described above will begin and continue into spring/summer 2023. Spawning season will then occur once more in fall 2023, and methods once more in spring/summer 2024.

## 6. System ecology

### *6.1. Apalachicola Bay food web and sediments: 1994 vs. 2020 (Dr. Jeff Chanton, faculty)*

**Introduction and rationale**. The purpose of this portion of the study is to investigate changes to the Apalachicola Bay food web and carbon cycle with respect to a previous study conducted in the mid to late 1990's (Chanton and Lewis, 2002). The earlier study examined and quantified the relative importance of terrestrial versus marine carbon inputs to the bay. The goal of the current study is to test the hypothesis that the bay has shifted to rely more upon marine inputs relative to terrestrial carbon inputs due to waning fresh-water delivery to the bay. Both studies rely upon variations in $^{13}$C and $^{34}$S values of the various pools examined. The values differ according to the relative inputs of marine and terrestrial carbon, with marine inputs being enriched in $^{13}$C and $^{34}$S while terrestrial inputs are depleted in $^{13}$C and $^{34}$S. For sediments, we use $^{13}$C only as $^{34}$S is influenced heavily by sulfate reduction which occurs in anerobic sediments.

**Methods.** Our plan was to collect fauna samples at sites similar to those utilized in the earlier study and analyze them for $^{13}$C, $^{15}$N and $^{34}$S. In addition we planned to evaluate sediment samples once across the bay on several transects on a N-S axis originating in East Bay, two-mile west of Apalachicola and across the dry bar to compare to the earlier study values.

**Results – sediments.** We have collected 31 sediment samples, processed them by acid treatment to remove carbonates and conducted isotopic analysis. Isotopic results indicate no shift in $^{13}$C that would be indicative of a decline in terrestrial inputs to the bay. If anything, the Bay appears to have shifted to a more terrestrial-like signature as sedimentary organic carbon values are more depleted in the 2020's than they were in the 1990's (Fig. 26 and 27).

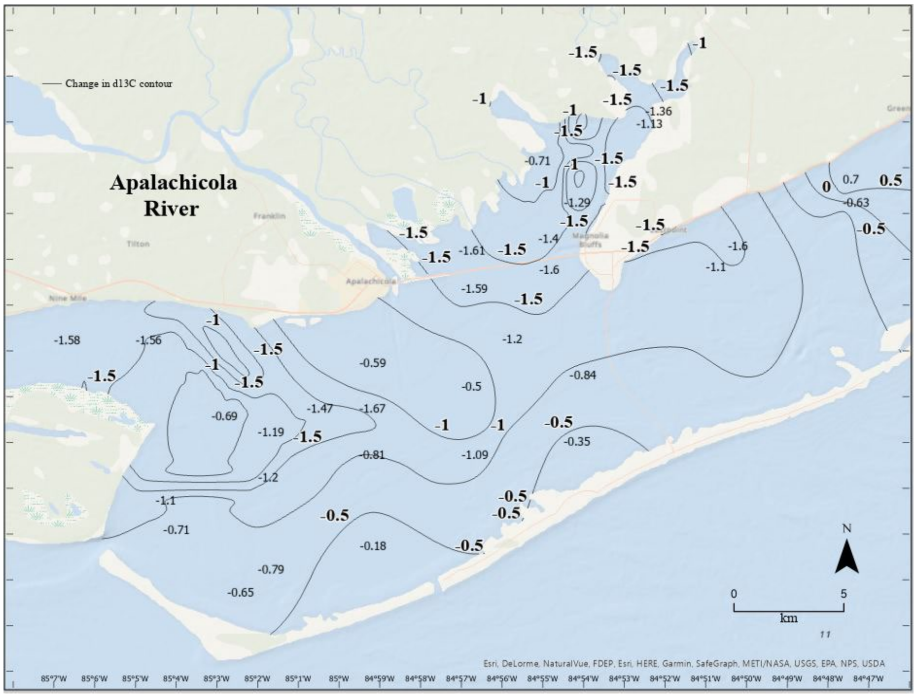


**Figure 26.** Difference in $^{13}C$ isotopic composition between the samples collected in 2021 and samples collected in the early 1990's. Negative values indicate $^{13}C$ depletion (indicating a more terrestrial nature) in the 2021 sample set relative to the earlier data

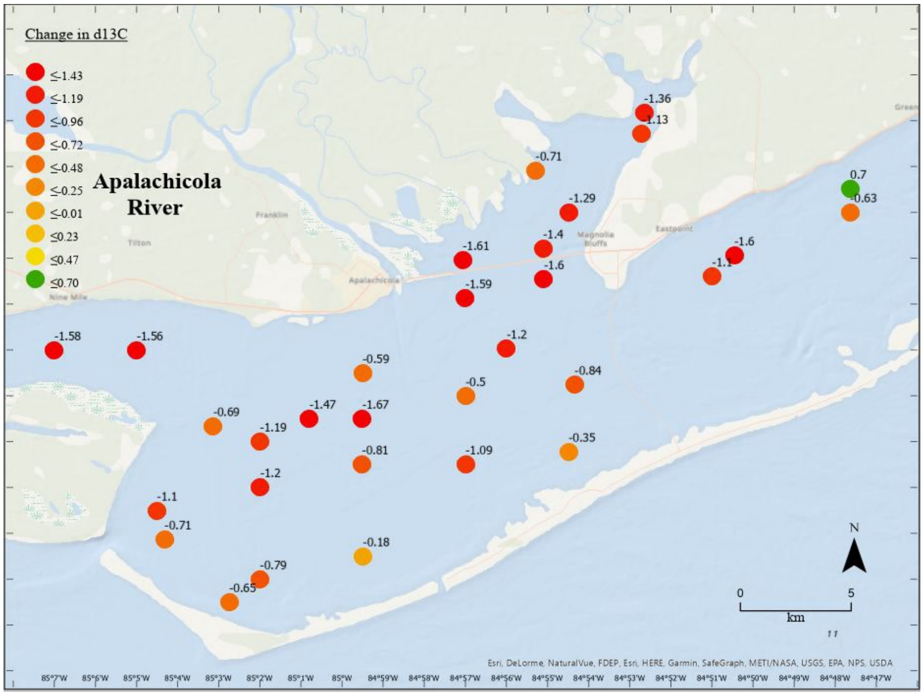


**Figure 27.** Same results as figure 1 but portrayed as color differences. Negative values indicate $^{13}C$ depletion (indicating a more terrestrial nature) in the 2021 sample set relative to earlier data.

In addition, the carbon content of the sediments appears to have increased over this same time period (Fig. 28). This could be due to increasing deposition of terrestrial organic matter to the bay from the river, or to the erosion of the fresh-water marshes in upper East Bay. Sediment samples were treated with 10% HCl to remove calcium carbonate so results are carbonate-free.

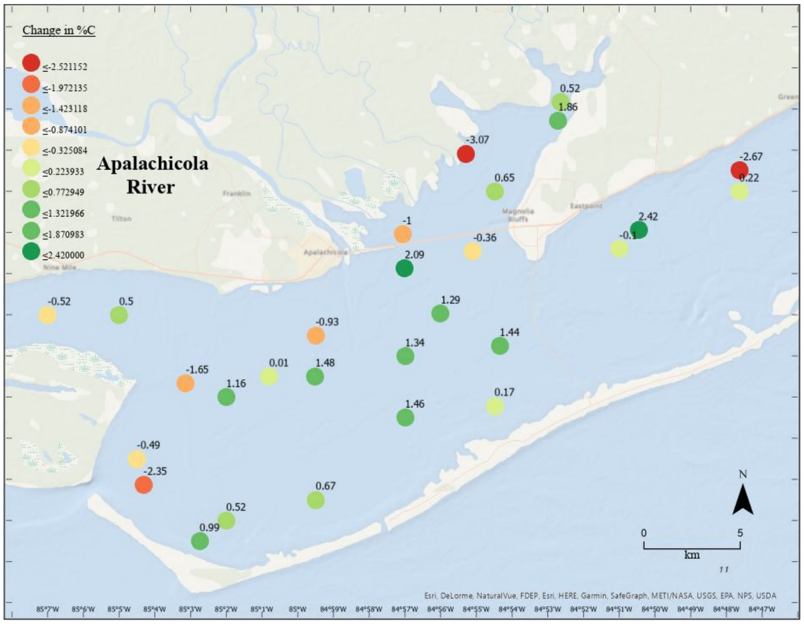


**Figure 28.** Difference in % carbon composition of sediments between the samples collected in 2021 and samples collected in the early 1990's. Positive values indicate an increase in carbon quantity in sediments in the 2021 sample set relative to the earlier sample set. Note that near the marshes of East Bay, samples had less carbon, possibly due to marsh erosion, which could have contributed carbon to the sediments of the Bay in addition to carbon from river deposition.

**Results - fish Samples.** A total of 51 fish individuals were collected from December 2020 to May 2021 to compare with the fish collected in 1992/93/94 in Apalachicola Bay (Table 4). Most of the fish were taken from Dry Bar, with the dominant species being *Micropogonias undulatus* (Croaker). Stable carbon and nitrogen ($\delta^{13}$C, $\delta^{15}$N) and sulfur ($\delta^{34}$S) were analyzed for each individual fish. Fish taken from East Bay were more depleted in $\delta^{13}$C than those taken from Cat Point and Dry Bar (Fig. 29). Significant differences were identified in $\delta^{13}$C values between fish taken in East Bay and Dry Bar (Tukey Q statistic = 3.473, $P = 0.046$). However, no significant differences were found between East Bay and Cat Point (Tukey Q statistic = 3.408, $P = 0.051$, and Cat Point and Dry Bar (Tukey Q statistic = 1.236, $P = 0.65$). Nitrogen isotope ($\delta^{15}$N) values were highest for individuals taken from East Bay (Fig. 29). Significant differences were only observed in $\delta^{15}$N values between Cat Point and East Bay (Tukey Q statistic = 3.964, $P = 0.020$). Fish taken in Cat Point had higher $\delta^{34}$S values while those taken in East Bay had the lowest $\delta^{34}$S values (Fig. 29). Significant differences in $\delta^{34}$S isotope values were observed between Cat Point and East Bay (Tukey Q statistic = 4.398, $P = 0.009$), and Dry Bar and East Bay (Tukey Q statistic = 4.905, $P = 0.003$). However, no significant differences were observed between Cat Point and Dry Bar (Tukey Q statistic = 1.173, $P = 0.673$).

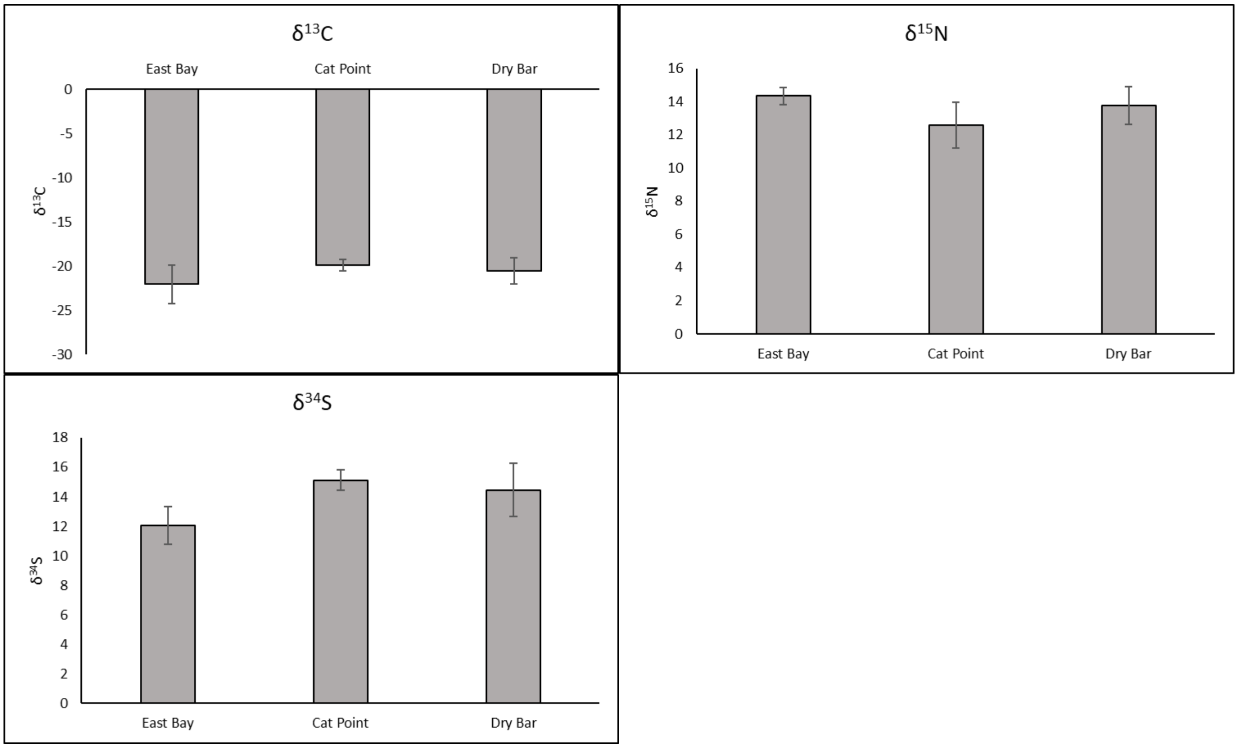


**Figure 29.** Average $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values +/- SD for fish taken in East Bay, Cat Point and Dry Bar in 2020/21.

When comparing the 2020/2021 Apalachicola fish to the fish taken in 1992/93/94, the fish taken from Cat Point and Dry Bar were combined due to their similar isotope data. We also categorized fish species into habitat type, pelagic (living throughout the water column) and demersal (living primarily near the bottom). Demersal and pelagic fish taken in 2020/2021 in Cat Point/ Dry Bar were more depleted in $\delta^{13}C$ than those taken in 92/93/94 (Fig. 30). Significant differences were observed between the two time periods for demersal ($F_{1,47} = 7.957$; $P = 0.007$) and pelagic fish ($F_{1,58} = 4.673$; P = 0.0348). The demersal and pelagic fish taken in 2020/2021 in Cat Point/Dry Bar were also found to be higher in $\delta^{34}S$ values than those taken in 1992/93/94 (Fig. 30). Significant differences were also observed between the two time periods for demersal ($F_{1,47} = 9.117$; $P = 0.004$) and pelagic fish ($F_{1,55} = 14.112$; $P = 0.0004$).

No pelagic fish were taken from East Bay in 2020/2021 (Fig. 30). ANOVA test revealed no significant difference between the demersal fish of East Bay between 2020/2021 and 1992/93/94 for $\delta^{13}C$ ($F_{1,27} = 0.0729$; $P = 0.789$) and $\delta^{34}S$ values ($F_{1,25} = 1.8629$; $P = 0.1844$).

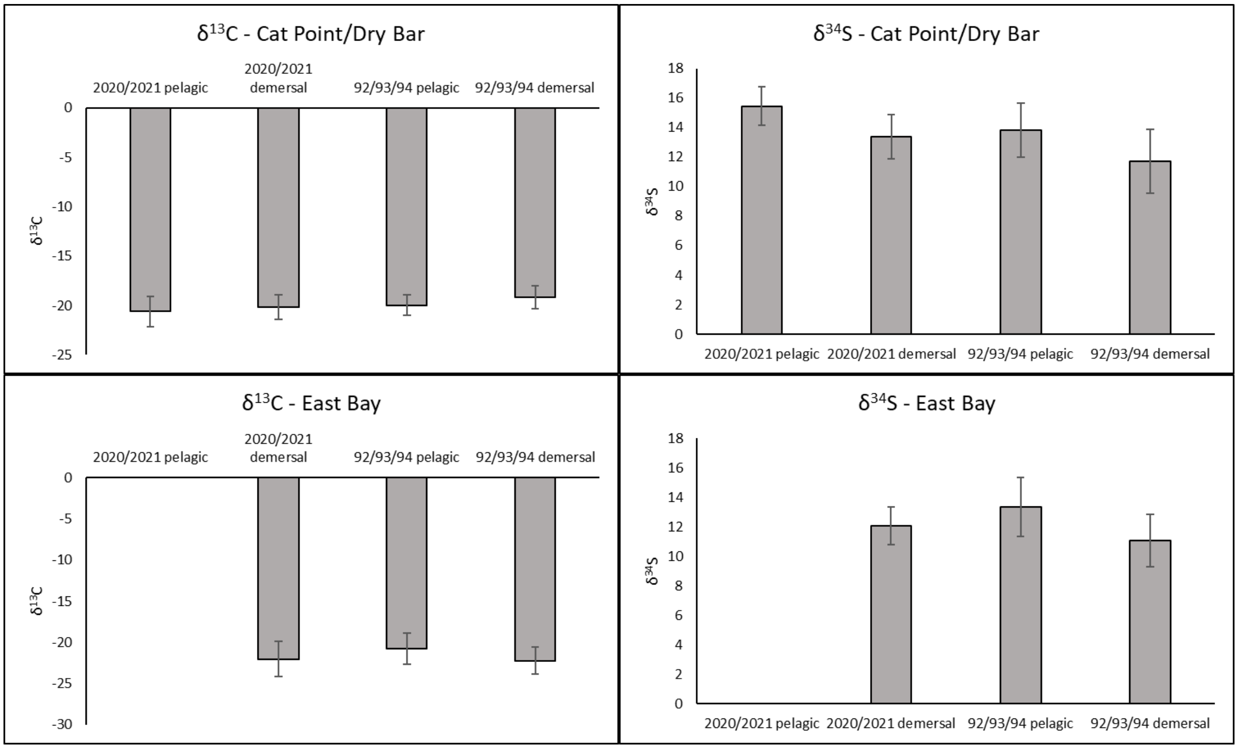


**Figure 30.** Average $\delta^{13}C$ and $\delta^{34}S$ values of pelagic and demersal fish taken in 2020/21 and 1992/93/94 from Cat Point/Dry Bar (top graphs) and East Bay (bottom graphs). No pelagic fish were taken from East Bay in 2020/21.

The goal of this study was to test the hypothesis that the bay has shifted to rely more upon marine inputs relative to terrestrial carbon inputs due to waning fresh-water delivery to the bay. The isotopic analysis for both sediments and fish do not offer any evidence to support the hypothesis that the Bay has shifted towards more marine inputs.

Table 4. Summary of fish taken in Apalachicola Bay in 2020/21 and carbon, nitrogen and sulfur isotope values. Site codes: DB = Dry Bar, CP = Cat Point, EB = East Bay

| Site | Species (common name) | Habitat | N | $\delta^{13}$C Ave | SD | $\delta^{15}$N Ave | SD | %C Ave | SD | %N Ave | SD | $\delta^{34}$S Ave | SD | %S Ave | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DB | *Micropogonias undulatus* (Croaker) | demersal | 14 | -20.5 | 0.9 | 13.8 | 0.7 | 45.7 | 1.1 | 13.7 | 0.3 | 13.3 | 1.4 | 1.4 | 0.1 |
| DB | *Anchoa mitchilli* (Anchovy) | pelagic | 9 | -21.8 | 1.5 | 14.5 | 0.3 | 44.7 | 3.2 | 13.5 | 0.8 | 16.3 | 1.0 | 1.3 | 0.1 |
| DB | *Bairdiella chrysoura* (Silver perch) | pelagic | 5 | -19.9 | 2.0 | 14.7 | 1.1 | 46.6 | 0.7 | 13.6 | 0.6 | 14.1 | 1.6 | 1.7 | 0.1 |
| DB | *Ariopsis felis* (Hardhead catfish) | demersal | 4 | -19.7 | 1.5 | 12.8 | 0.8 | 45.9 | 0.7 | 13.0 | 0.5 | 13.8 | 2.2 | 1.5 | 0.1 |
| DB | *Brevoortia patronus* (Gulf menhaden) | pelagic | 4 | -20.5 | 0.4 | 11.6 | 0.3 | 44.1 | 0.9 | 13.3 | 0.1 | 15.9 | 0.4 | 0.9 | 0.0 |
| DB | *Bagre marinus* (Gafftopsail catfish) | pelagic | 1 | -19.8 |  | 13.8 |  | 45.2 |  | 13.9 |  | 14.1 |  | 1.4 |  |
| DB | *Menticirrhus americanus* (Southern kingfish) | demersal | 1 | -17.6 |  | 14.0 |  | 45.4 |  | 12.8 |  | 13.7 |  | 1.7 |  |
| CP | *Brevoortia patronus* (Gulf menhaden) | pelagic | 4 | -19.9 | 0.7 | 12.0 | 0.3 | 44.6 | 0.7 | 13.4 | 0.7 | 15.4 | 0.5 | 1.4 | 0.0 |
| CP | *Alosa alabamae* (Alabama shad) | pelagic | 1 | -31.6 |  | 15.7 |  | 47.3 |  | 13.2 |  | 3.7 |  | 1.5 |  |
| CP | *Bagre marinus* (Gafftopsail catfish) | pelagic | 1 | -19.7 |  | 15.0 |  | 43.4 |  | 13.6 |  | 14.1 |  | 1.3 |  |
| EB | *Leiostomus xanthurus* (Spot) | demersal | 4 | -22.2 | 1.0 | 14.5 | 0.6 | 46.2 | 4.8 | 12.6 | 2.4 | 12.5 | 1.5 | 1.2 | 0.3 |
| EB | *Ariopsis felis* (Hardhead catfish) | demersal | 3 | -20.6 | 2.3 | 14.2 | 0.6 | 43.1 | 2.6 | 13.2 | 0.6 | 11.5 | 0.8 | 1.6 | 0.1 |
| EB | *Micropogonias undulatus* (Croaker) | demersal | 1 | -25.6 |  | 14.2 |  | 44.6 |  | 13.5 |  |  |  |  |  |

### ***6.2. Influence of oysters on function and change in coastal ecosystems (Dr. Josh Breithaupt, Assistant Research Faculty)***

The following projects were conducted by Dr. Josh Breithaupt's lab in 2021. Research in this lab focuses on carbon, nutrients, and sediment dynamics, and how these can be used to understand function and change in coastal ecosystems that may affect, or be affected by, the regional oyster population. Sedimentary organic matter (SOM) in Apalachicola Bay is derived from primary and secondary production in the water column of the Bay and estuary, and terrestrial detritus from uplands and wetlands. In the past half-century Apalachicola Bay has seen several major changes to factors that contribute to SOM including a catastrophic decline in the oyster population, reduced and impaired riverine interaction with the Apalachicola River floodplain, and encroachment of barrier island salt marshes by mangroves. Projects in Dr Breithaupt's lab seek to quantify temporal changes in the quantity and quality of SOM in Apalachicola Bay and to investigate connections between organic matter abundance, microbial activity, and substrate suitability.

### *6.2.1. Investigating changing benthic sediment characteristics in Apalachicola Bay*

**Introduction and rationale**. The purpose of this investigation is to characterize benthic sediment throughout Apalachicola Bay and to determine if changes have occurred since 1963 and the mid-1990s when similar studies were previously published. Bay sediment characteristics are influenced both by source inputs that may occur via riverine or marine deposition, and by trophic processes that intercept or rework organic matter before or after it reaches the bottom. Therefore, spatial and temporal changes to the organic and mineral constituents of the Bay sediments are a measure of both changing sources and changing processes within the Bay. Two potentially important regional changes being investigated are: 1) changes to flooding and transport of floodplain-derived detritus and sediments to the Bay, and 2) changes to the system-wide oyster population and a resulting decrease in the metabolic processing and sequestration of organic matter.

**Methods.** Sediment samples have been collected from the bottom of the Bay and analyzed for content of organic matter, calcium carbonate, organic carbon, total nitrogen, grain size, and stable isotopic ratios of carbon and nitrogen which can be used to trace the terrestrial or marine origin of the organic matter.

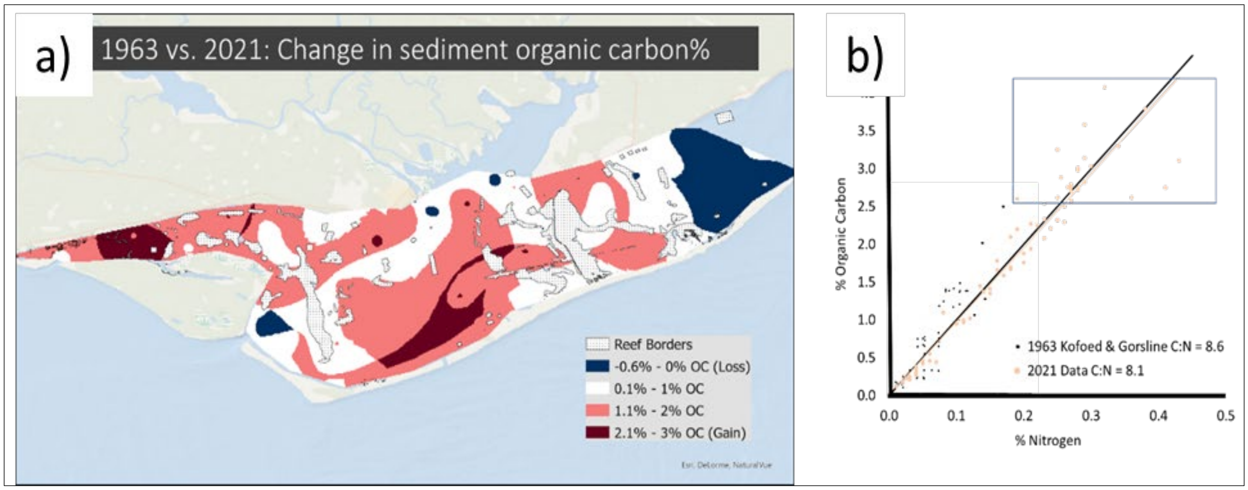


**Figure 31 a)** Map depicting changes in the organic carbon content of sediment in Apalachicola Bay and St. George Sound and b) scatter plot comparing carbon and nitrogen of 1963 samples (black dots) and present-day samples (orange dots). The heatmap indicates that organic carbon has increased, and the overlapping data in the scatterplot indicate that the source has not changed

**Results.** Our findings indicate that organic carbon has increased in over 95% of the Bay, sometimes by up to 1-2%. (Fig 31). While these percentage changes may seem small, they represent a substantial increase when scaled to the area of the Bay.

**Future work** Additional work is being conducted to measure and map changes to mean sediment grain size. After presenting findings at the February 2022 ANERR Science Symposium, the manuscript for this study is now in preparation with the goal of submission this Fall. Kevin Engelbert, the research technician responsible for most of the sample collection and data analysis will enter graduate school as a Masters student during the Fall semester 2022. His Thesis research will build on this project by conducting more rigorous investigations of the timing and mechanisms of these changes.

### *6.2.2. Oyster Shell Dissolution Dynamics in Apalachicola Bay Region*

**Introduction and rationale**. Microbial respiration of organic matter (OM) is a known mechanism for dissolution of calcium carbonate. Evidence of increasing abundance of OM in intertidal and subtidal sediments raises the possibility that some environments of the Apalachicola Bay region are less suitable for oyster shell (and therefore for recruitment as well) than they may have been in the past. One of the uncertainties about oyster reef communities and restoration efforts in the bay is the durability and fate of oyster shell. Microbial respiration of organic matter may contribute to loss of shell substrate and contribute to deleterious conditions for spate settlement and shell development. This project has begun with a proof-of-concept study to investigate how soils with different concentrations of OM contribute to changes in oyster shell mass over time.

**Methods.** Substrates being used include intertidal oyster reef sediment (2% OM), intertidal reef sediment amended with varying masses of mangrove leaves (93% OM), subtidal muds (14% OM), wetland soils (20% OM), and a control treatment consisting only of seawater (no OM). Shells of known mass were introduced to mesocosms with each sediment type and subjected to one of two flooding regimes: either permanently flooded (to mimic anaerobic conditions) or intermittently flooded (to mimic tidal conditions and occurrence of periodic aerobic conditions to stimulate microbial activity). Mesocosm pH is being monitored weekly and shells are being collected, cleaned, dried, and weighed to detect mass changes every three months.

**Results.** This project is being run primarily by an undergraduate student (Anna Jacoby) through FSU's Undergraduate Research Opportunity Program (UROP) and she will present her preliminary results (Fig. 32) at the Undergraduate Research Symposium in April 2022.

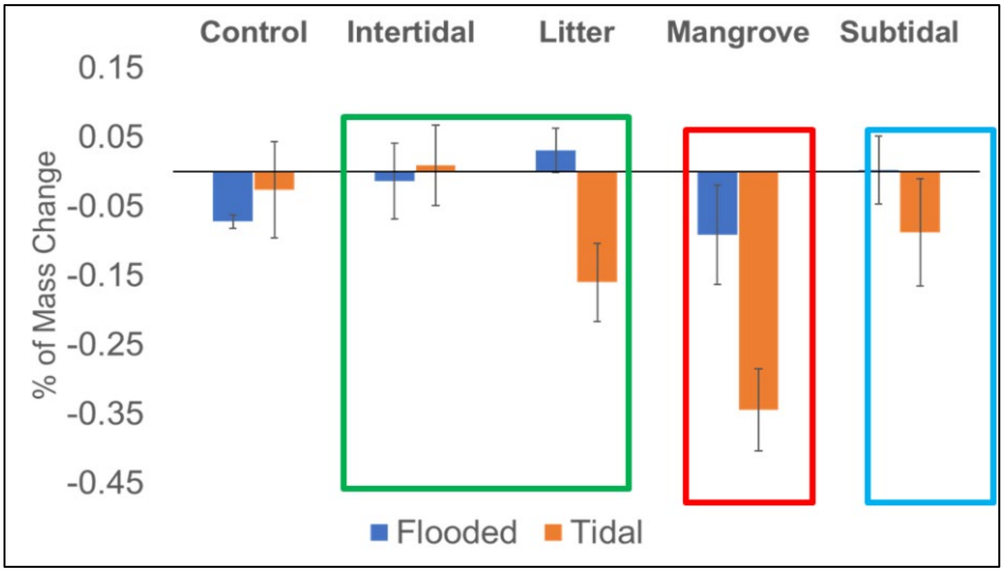


**Figure 32.** Mean change of shell mass after 90 days. Green box indicates that mangrove leaves with tidal treatment increase dissolution of shell in intertidal reef sediments. Red box shows that oyster shells are dissolving fastest in mesocosms with mangrove soil. Blue box shows that there is limited dissolution of shells in subtidal mud when exposed to aerobic conditions.

**Future work**. The project will continue at least through December, 2022 to allow for longer observations of changes in shell mass. We expect to begin writing a manuscript for this study starting at that time. Additionally, the results of this simple experiment are informing research plans for secondary studies that will include more rigorous examination of dissolved inorganic carbon (DIC) and alkalinity dynamics.

### *6.2.3. Coastal carbon dynamics occurring because of mangrove replacement of regional tidal marshes*

**Introduction and rationale.** Dr. Breithaupt has begun work with collaborators at the Apalachicola NERR, the University of South Florida, the University of Florida, and Auburn university to document temporal differences in sediment accumulation as well as retention of C, N, and P in the wetlands on the barrier islands. Specific attention is being given to climate-driven habitat shifts from saltmarsh to mangroves (Fig. 33 A – E). Results of this work will be important for quantifying the carbon and nutrient stocks and sequestration rates over time.

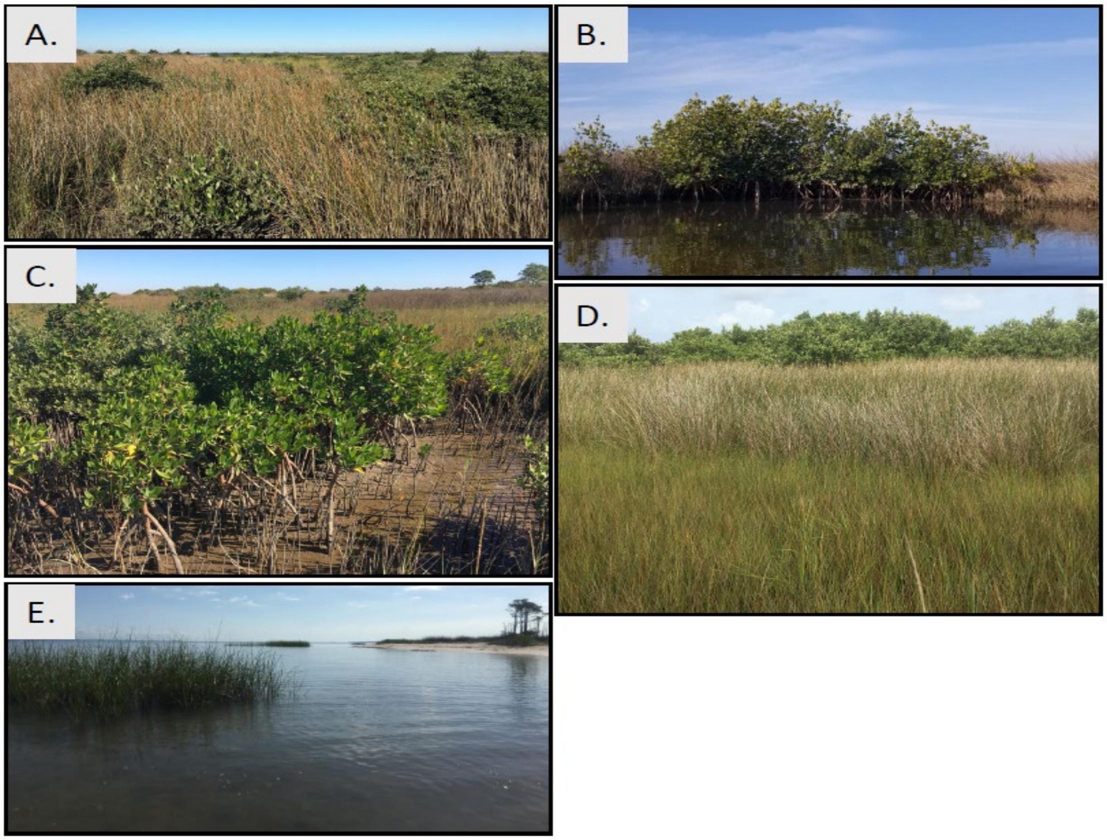


**Figure 33.** Regional photos of neighboring vegetation types. Panel A shows *A. germinans* replacement of *J. roemerianus* on Dog Island; Panel B shows *R. mangle* replacement of *J. roemerianus* at Pilot’s Cove. Panel C shows *A. germinans* and *R. mangl*e adjacent to *S. alterniflora* on Dog Island. Panel D shows *S. alterniflora* in the foreground, *J. roemerianus* in the middle, and *A. germinans* in the background at Pilot’s Cove. Panel E shows patches of *S. alterniflora* being inundated and isolated by sea-level rise at Unit 4.

Coastal wetlands have interacted with oyster reefs for long periods of time in various climates. Some of these interactions are direct, such as when mangrove or marsh vegetation overtakes and replaces intertidal reefs (a so-called greening effect), and also through production and export of carbon and nutrients by wetlands to adjacent intertidal and sub-tidal reefs. Mangroves generally have higher productivity than saltmarshes, although it should be noted that there are caveats to this that depend on a number of important climate factors. However, given the trends of increasing temperatures, increasing sea-level rise and increasing presence of mangroves at the cost of local saltmarshes, there is much that is unknown about the way these shifts will affect shoreline stability and export of sediment, carbon, nutrients as well as alkalinity and pH products to the Bay and thus potentially effecting oyster populations.

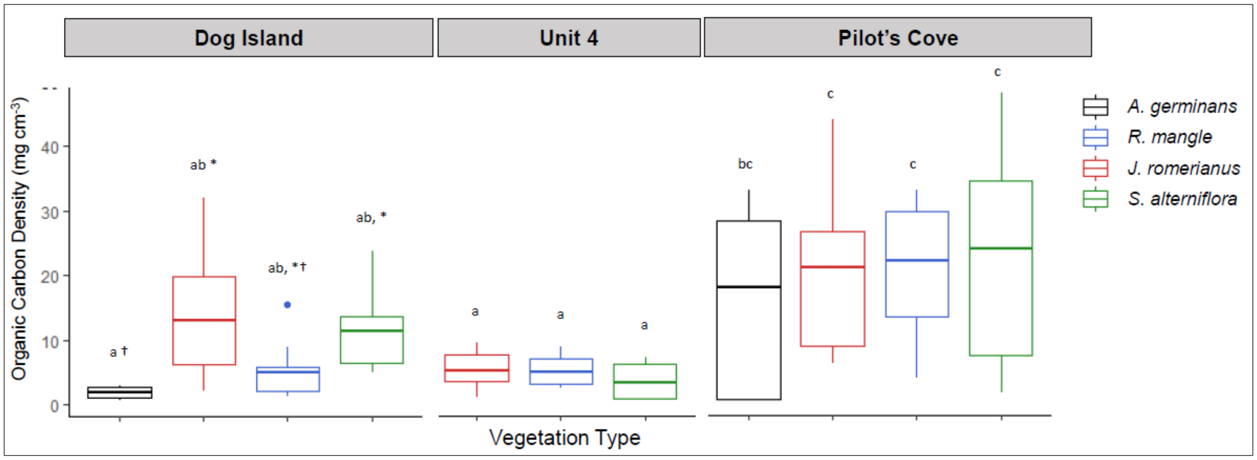


**Fig. 34**: Boxplot soil organic carbon density underlying each vegetation type, as indicated by color, at each site. Lowercase letters indicate significant differences across sites, while asterisks and crosses indicate statistical differences within site.

**Results.** The first paper from this study, led by Dr. Breithaupt's Post-doc Dr. Havalend Steinmuller, is currently in revision with Frontiers in Forests and Global Change. The paper is a first step in the investigation of changing carbon dynamics in coastal wetlands, and finds that there is presently no discernible change in soil carbon storage following mangrove replacement of regional saltmarshes across the region (Fig. 34).

**Future work.** Additional work investigating the potential for enhanced oxidation of wetland soils by mangroves and the role of oxidized iron in helping to retain more soil carbon is being investigated by a PhD student (Prakhin Assavapanuvat) who Dr. Breithaupt is working with at the University of Florida in Dr. Thomas Bianchi's lab. Additional projects include a UROP project investigating root biomass of mangroves and saltmarshes, and a collaboration with Dr. Derrick Vaughn, a postdoc working in the lab of Dr. Robert Spencer in the EOAS Department at FSU, whose work is utilizing FT-ICR-MS to characterize dissolved organic matter (DOM) of mangroves and saltmarshes at the molecular level; this work will be important for fingerprinting the DOM being generated by different wetland types to facilitate understanding of potential changes in trophic pathways.

### 6.2.4. *Vulnerability of regional wetlands to sea-level rise and changing sediment delivery from Apalachicola River*

**Introduction and rationale.** Partnering with the ANERR in their monitoring of surface sediment accretion and elevation change in fresh-water and saline wetlands (Fig. 35), this project compares decadal timescale records of sediment accumulation and vertical accretion to identify how these coastlines have responded to changing riverine hydrology and sea-level rise. Wetlands filter water by collecting and retaining nutrients, sediments, and pollutants and preventing them from entering the Bay. However, there is evidence that regional sea-level rise is accelerating and that extended areas of wetland shoreline are being lost, which suggests the vulnerability of a vital ecosystem important to Apalachicola Bay and its estuary.

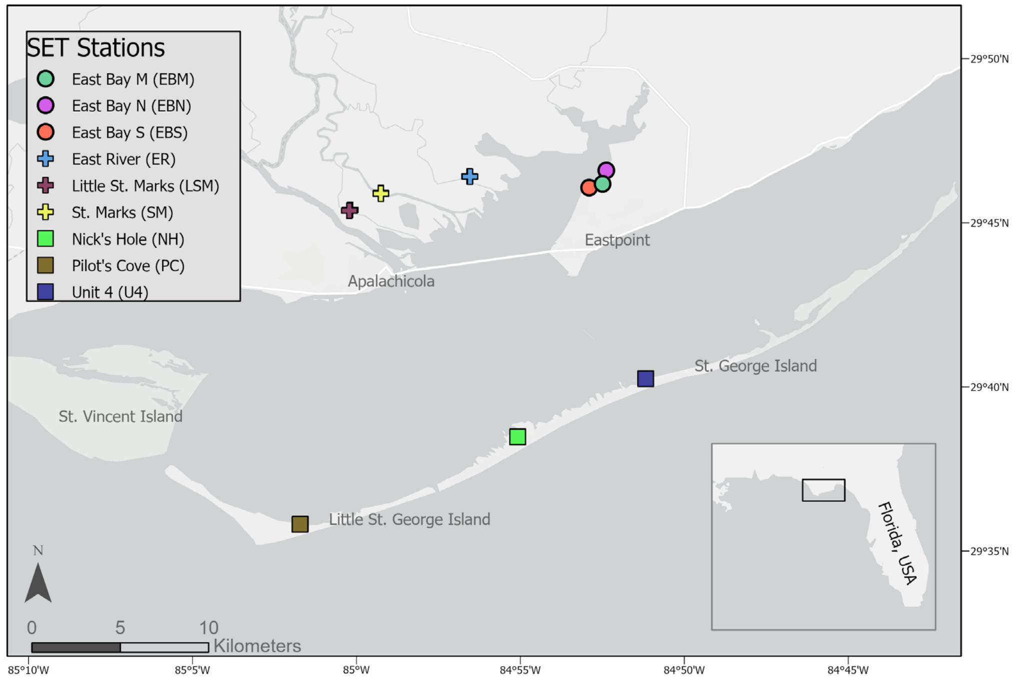


**Figure 35.** Location of surface elevation table (SET) stations in Apalachicola Bay. Circles indicate stations at bay sites, while crosses show stations at riverine sites, and squares are stations on barrier islands. Color corresponds to station names. Two SETs are located at each station

**Results.** A manuscript led by Dr. Havalend Steinmuller, Dr. Breithaupt's Post-doc, is currently being reviewed by co-authors and will be submitted to Estuaries and Coasts by March 16th. General findings of this study are that vertical accretion at the riverine sites is controlled by soil OM content; in contrast, the negative correlation between soil OM content and the rate of vertical accretion at the bay sites demonstrates that these sites rely on inorganic sediment from uplands and the river for vertical accretion. In the past decade, corresponding to the time period of our surface elevation observations, regional relative sea level rise has occurred at a rate of $11.46 \pm 0.57$ mm $y^{-1}$ (2010-2021). All the sites in this study display elevation deficits, where the rate of surface elevation change measured by SETs is less than the rate of sea-level rise (Fig. 36). Over the course of the study, two SETs at barrier islands demonstrated negative rates of elevation change; though these rates were quite different in terms of magnitude, both SETs were entirely drowned over the course of the study.

**Future work.** *Plans for coming year:* Dr. Breithaupt's lab will continue monitoring these stations with ANERR in coming years. This work is providing a foundation for future research efforts to use radiometric dating to provide a longer timescale context for rates of wetland surface change relative to regional changes in sea level.

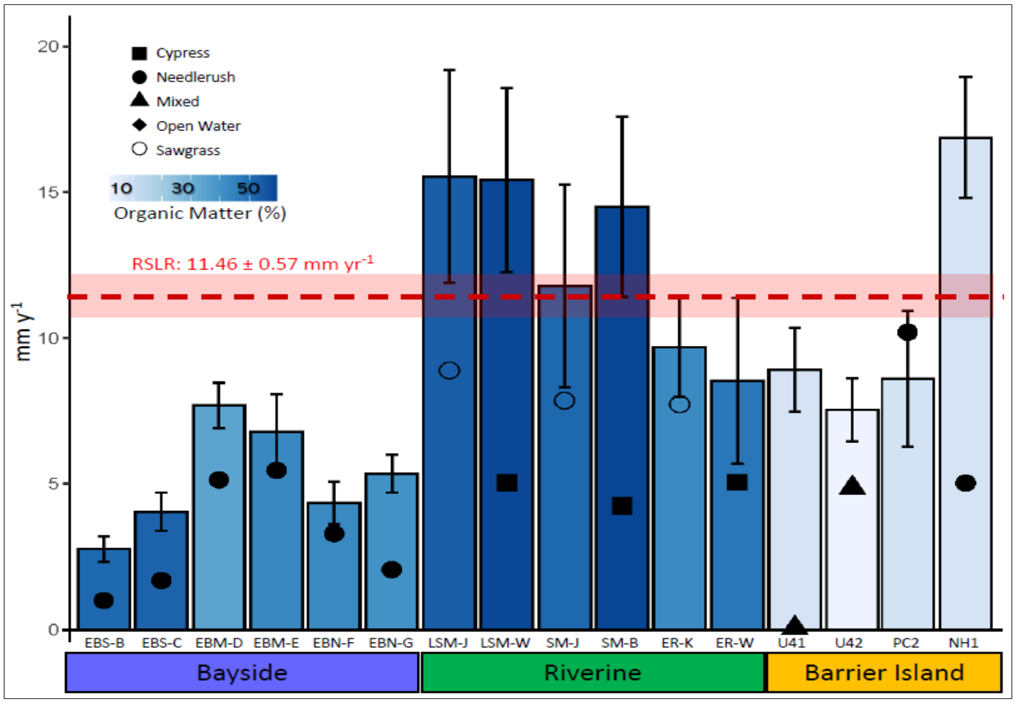


**Figure 36.** Average accretion rates (mm $y^{-1}$) at each site (bars) colored by organic matter content and average rates of elevation change per SET (mm $y^{-1}$, points). Shape of points indicate dominant vegetation type at each SET. Horizontal dotted red line and shading represents the calculated rate of relative sea-level rise between 2010-2021 ± standard error.

### *6.3. Apalachicola Bay environmental evolution and pollutant status (Dr. Michael Martinez-Colon Faculty and Adebayo Solanke PhD student, FAMU)*

**Introduction and rationale.** The overall project scope is to develop and implement a low-cost and high- impact tool for determining historical changes of coastal ecosystem health. Benthic foraminifera (BF) are an excellent bioindicator proxy. The known relationships between key taxa of BF communities and sediment quality enables the assessment of environmental health and status changes. The excellent preservation potential of intact and fossil foraminiferal shells in sediments, unlike macrofauna, allows us to reconstruct the historical evolution (19th – 20th centuries) of marine environments, thus providing invaluable information on environmental health changes ("deterioration" vs. "restoration"). With heavy agricultural activity in the ACF watershed, stakeholders expressed concern over potential pollution and negative impacts from the use of pesticides and herbicides in agricultural operations. This project will also assess levels of common pesticides in sediment cores in Apalachicola Bay.

This new ecological baseline data will identify how coastal environments have changed through time and will identify time periods of high ecological risk which will in turn benefit future decision-making policies for monitoring assessments. The PI has engaged the Stakeholders at the

Apalachicola National Estuarine Research Reserve (ANERR) not only on the design of this pilot project but also, they will be involved in the evaluation of the results to determine the feasibility for future system-wide implementation, thus facilitating the expansion of this assessment approach into other coastal and estuarine ecosystems within the NERR System.

Study objectives are: 1) Provide information on the levels of heavy metals and pesticides from sediment cores and surface samples; 2) Assess fossil BF assemblages from all sediment cores; and 3) Determine the radiometric age (Pb-210 & Cs-137) of all the core sediments. The need for using fossil BF to establish reference conditions will help ANERR stakeholders assess pre-polluted and/or pre-management conditions.

**Methods.** A total of 12 surface sediments samples were collected at Apalachicola Bay using a stainless-steel bottom dredge sampler. In addition, a sediment push core was collected. All sediments samples were analyzed for grain size to determine mud fraction (< 63 μm) and total organic matter (combusted at 550 °C for 4 hours) and carbonate content (combusted at 1000 °C for one hour). For the sediment core only, each sample was analyzed using Pb/Cs radiometric dating in addition to applying a CRS (Constant Rate of Supply) model to determine the age of the sediments. For heavy metals extractions, all sediment samples were analyzed for Ni, Cr, Cd, Cu, Co, Pb, Se, Ti, Fe, As, Hg, and Zn. The sequential extraction method followed was that of Tessier et al. (1979). All samples were subsequently analyzed using PerkinElmer Optima 8000 inductively coupled plasma-optical emission spectrometer (ICP-OES). Arsenic was the only metal to be "below detection limit".

**Results.** Only two metals (Cd and Se) based on their spatial distribution seem to be coming from the Apalachicola River. The rest of the metals (Cr, Co, Ti, Cu, Ni, Cd, Pb, Zn, Hg) seem to have a more local source with two "depocenters" one being south of the city of Apalachicola and a second towards the center of the bay (Fig. 37) which coincide as well with the distribution of total organic matter and mud content (Fig. 38). Regardless of the source, none of the metals are currently having an effect on the biota since these do not exceed ERL (Effect Range Low) values.

From a historical perspective, over the past 114 years the temporal changes of individual heavy metal (Cr, Cu, Ni, Cd, Pb, and Zn) concentrations are having no effect on the biota (low ERL values). However, it is uncertain if the other metals have affected the biota. With respect to Se, it is the only metal found in the core sediments at "extremely" to "severely" enriched levels. The Pollution Load Index on the other hand, which considers the cumulative effects of all the metals, showed that Apalachicola Bay is still experiencing "progressive deterioration" (Fig. 39).

**Future work**. All surface and core samples are currently being extracted for pesticides (Aldrin, benzene hexachloride, Dieldrin, Endosulfans, Endrin, Heptachlor, Methoxychlor, and DDT) and being processed for benthic foraminiferal analysis.

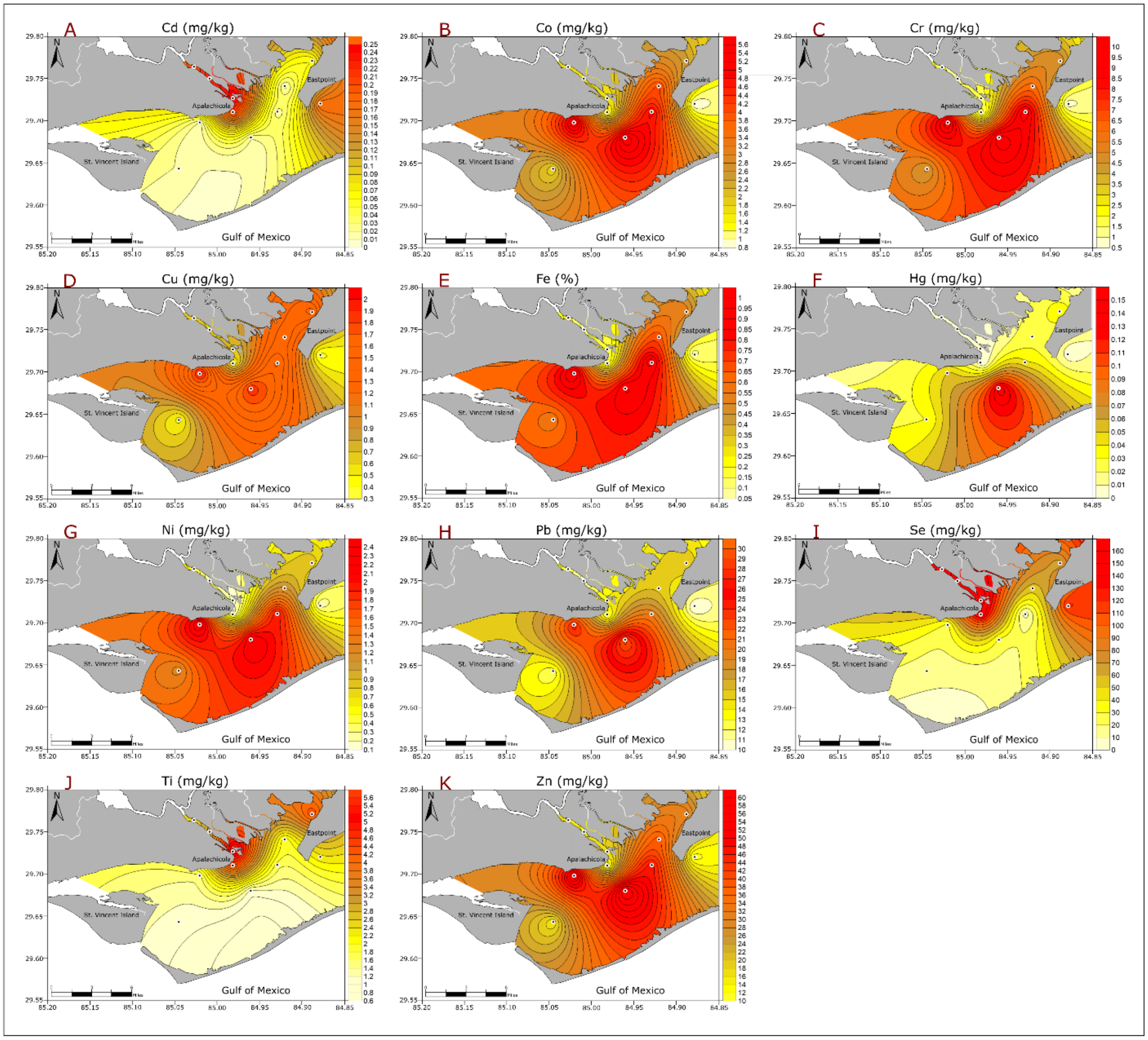


**Figure 37.** Heat maps showing concentrations of heavy metals in Apalachicola Bay. Panels are as follows: A) Cadmium, B) Cobalt, C) Cromium, D) Copper, E) Iron, F) Mercury, G) Nickel, H) Lead, I) Selenium, J) Titanium, K) Zinc.

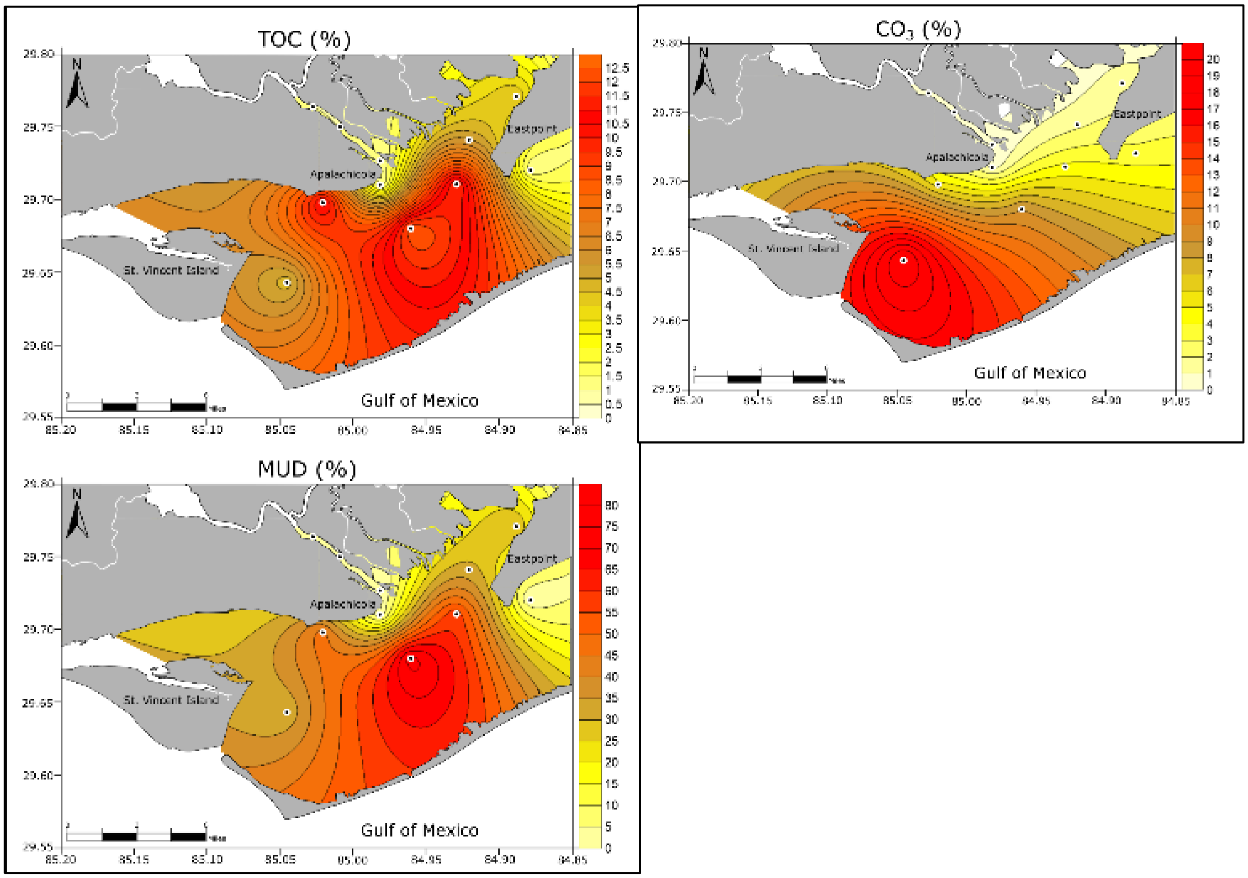


**Figure 38.** Heat maps showing distribution of total organic carbon (TOC), mud, and Carbonate ($CO_3$)

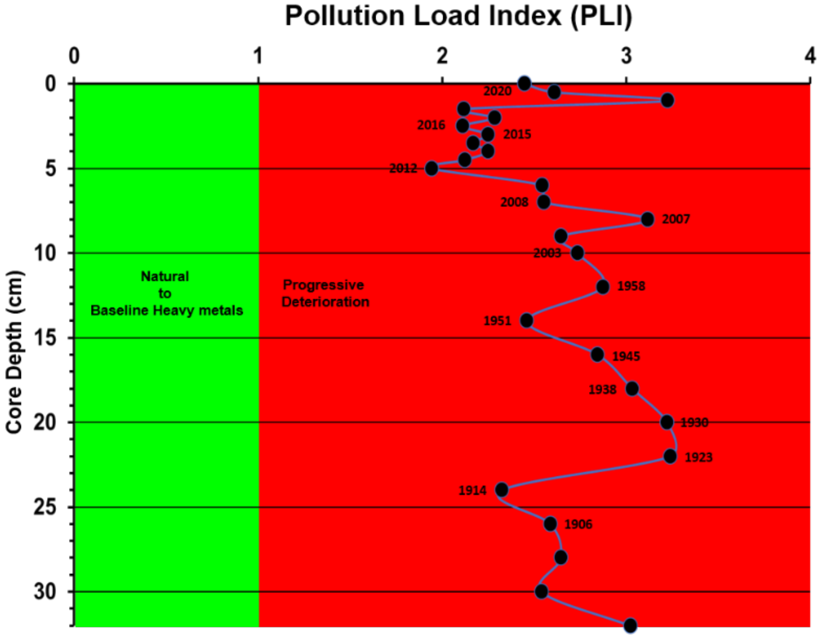


**Figure 39.** Pollution load index over time/core depth

## 7. Research hatchery

Spring 2021 was the first operational season for the ABSI interim hatchery. During 2021, the hatchery produced a total of four spawns, two of which resulted in spat-on-shell for oyster restoration experiments and graduate student research. The two other spawns produced larvae, but cultures did not survive to metamorphosis due to environmental and logistical problems with the interim hatchery, which have since been resolved. Larvae from the 2021 season were all settled on oyster shell, which is suitable for restoration experiments, but future research objectives will require individually set oysters. So, single-set silos were constructed in 2021, but water flow was inadequate for spat. The silo design was refined and will be tested in 2022. Additionally in 2021, guidelines for broodstock care, larval culture, and spat setting were developed and refined. From the 2021 season, key areas were identified to increase future production, including water quality and temperature consistency, feeding strategies, larval grading, and evaluation of competence in pediveliger larvae, discussed in detail below. In 2022, guidelines from 2021 will be adjusted for implementation in the new hatchery facility. A summary of hatchery accomplishments for 2021 and goals for 2022 are outlined below.

### *7.1 Hatchery accomplishments in 2021*

**Collection and spawning of wild oysters.** In 2021, the first hatchery goal was to spawn wild oysters (*Crassostrea virginica*) from Apalachicola Bay using thermal cues in a hatchery setting. Adult oysters (200 total) were collected from Peanut Ridge and Platform Reefs on 3/4, 4/7, 5/6 and 6/7 , cleaned via freshwater rinse and hand-scrubbed with stainless steel brushes, and held in the CML brood stock tanks at 21ºC prior to spawning. The oysters were fed 3% algal concentrate per gram estimated wet weight per day (Reeds Mariculture, Shellfish Diet 1800®). In all spawns, a spawning rack which circulates water throughout 48 small plastic tanks, was used, which allowed for isolation of individuals and their gametes. To provide thermal cues, water temperature in the spawning rack was gradualy increased and decreased between 22 and 27°C every 30 minutes to stimulate volitional gamete release. For the first spawn, the males naturally spawned in response to thermal cycling, but no females responded to thermal cues. So, 1 female was strip spawned (a process through which gametes are removed directly from the animals), but all successive spawns were successfully conducted using thermal cues. So, the spawning rack provided a reproduceable, non-lethal spawning method of wild oysters in 2021.

**Gamete fertilization.** Once gametes were collected from adult oysters, female gametes cleaned by being rinsed through a 53 µm sieve into a 5-gal bucket of clean water. Male gametes were added in 50 mL solution increments and gently mixed in the bucket. Once 15 minutes elapsed, fertilization was quantified, and more sperm was added in the same manner until fertilization was above 90%. Fertilization rates were based on the presence/absence of nuclei and polar bodies and cellular division of eggs. Fertilization rates ranged from 90 to 100% in 2021. Once fertilized, larvae were transferred into 946L tanks at an initial maximum stocking density of 10 larvae/mL.

**Larval culture.** Larvae remained in 946 L tanks for approximately 14 days or until they were viable to settle. Larvae were fed algal concentrate twice daily following suggested feeding rates from Reed Mariculture diet feeding chart and Rikard and Walton (2012). While larvae were under 120 µm, larval tanks were drained down every second day, graded based on size, and restocked into tanks at stocking densites appropriate for new size classes. Grading consisted of pouring larvae gently throught sieves into a 5-gal bucket of clean water, then doing so again with the next sequential sieve. Between 2-4 sieves were used for a given drain-down. When restocking larvae, all stocking densities were derived from Rikard and Walton (2012). When larvae reached 120 µm in size, tanks were drained down daily to evaulate larvae for settling competency. If competency had not been reached, larvae were re-stocked into tanks at an appropriate stocking density. If larvae were deemed competent, stocking procedure was followed (see Larval Settlement for details). Larval survival varied widely from 0% (the unsuccessful spawns) to 19.4% in June 2021. Development time varied slightly for successful spawns most likely due to temperature differences. In May, larvae were set after 16 days in larval tanks, and in June, larvae were set on days 15 and 16 of development.

**Larval settlement.** Developing larvae through settlement was a primary goal of the 2021 season as much of ABSI research is dependent on the production of oyster juveniles (spat). Larvae were deemed competent to settle once the majority had eye spots developed and some had developed a foot. Once competent, larvae were transferred from larval tanks to settlement tanks. Settling tanks were prepared with cleaned bags of shell, aeration, and heaters if needed. Target settlement was 5-6 spat per shell in 150 bags. Once larvae were stocked in the settlement tanks, the tank was kept static for 24 hours to allow larvae to settle and metamorphose into juveniles. Then, the tank was provided flow-through raw seawater filtered to 50 µm to remove sediment. . While the juveniles were provided with a natural diet (i.e. the raw seawater), feed was supplemented with Shellfish Diet 1800® to ensure high food concentration according to Reed Mariculture recommendations. Two spring spawns produced roughly 20,000 spat on shell for the restoration experiments, and the remaining spat were placed in biodegradable bags and deployed adjacent to the restoration experiments. Although spat production was sufficient for the 2021 project objectives, increasing production rates is a primary goal of the 2022 season. Increasing production and survival will be accomplished by reducing larval mortality during grading, increasing feeding rates to reduce food competition, and by ensuring the majority of larvae have developed a fully mobile foot before setting (see Hatchery Goals for 2022 for details).

**Construction of a small-scale single set system.** Single set systems will diversify the ability of the hatchery to support ABSI researchers that require single oysters rather than multiple spat settled on shell as previously described. In the fall of 2021, a small system was constructed and was successful in initial settlement of spat on crushed oyster shell. However, these single set tanks did not provide sufficient water flow to remove debris and maintain spat in suspension, and so animals from this system were stunted in growth. These observations informed the development of a new single set tank which has higher flow rates and increased space for setting. In the 2022 season ABSI hatchery staff will use this new system to produce *C. virginica* individuals. The design of the system will be amended as necessary.

**Assessment of larval feeding rates.** Since the interim hatchery had not established a live algal culture system, the adults and larvae were fed an algal concentrate (Reeds Mariculture Shellfish Diet 1800®). Recommended feeding rates among literature sources, hatchery staff experience and the Reed Mariculture feeding guidelines were highly inconsistent. During 2021, higher larval survival correlated with higher feeding rates, but variable environmental conditions may have confounded the results. In light of hatchery observations, optimizing feeding and larval survival will continue to be an important objective in 2022.

**Internship program.** The ABSI grant has funding allocated for a hatchery internship program that was intended to target local residents to increase workforce capacity in the aquaculture industry. In 2021 the internship program was a small pilot program that was funded outside of ABSI and supported two FSU graduates from the Biological Sciences department. These students had a strong scientific and laboratory skills and did not need foundational science training. The students worked with hatchery staff for 8 weeks and learned a wide range of oyster hatchery techniques. The funding for the student program will continue in 2022, and we are also working with the ANERR and the Forgotten Coast Conservation Corp to integrate local young people (the OysterCorps) into the internship with the original ABSI goal of increasing desirable skills of local residents. The internship program focuses on cultivating practical aquaculture knowledge and skills that are applicable in most aquaculture systems and on introducing interns to aquaculture as a tool for conservation and restoration of estuarine species. Interns learn the practical basis of different aquaculture systems (e.g. static, recirculating, and flow-through systems) as well as the biology and chemistry behind the husbandry necessary for culturing animals. This internship also gives interns opportunities for professional networking in the aquaculture industry.

### *7.2 Hatchery Goals for 2022*

The hatchery was successful in 2021 in producing larvae and spat for ABSI objectives while developing protocols and working around infrastructure challenges. However, improvements to a variety of procedures have been identified to increase and diversify production spat this upcoming season. In 2022, initial spawns will be performed in the temporary hatchery system, with a planned transition during the summer to the new hatchery facility (Fig. 40). Protocols will be adjusted for the larger hatchery and improved to increase overall production, and the new hatchery will host the fall spawn for 2022. A summary of the goals for this year are outlined below

**Figure 40.** Images of the new ABSI research hatchery showing open tank area with larval culture and setting tanks (left) and culture room for microalgae (right).

**Optimize larval feeding rates.** A primary focus in 2021 was evaluating the algal density required for optimal development using Reed Mariculture Shellfish Diet. Initially, feeding rates were prescribed according to the feeding guidelines outlined by Reed Mariculture for Reed Mariculture Shellfish Diet 1800®. This feeding rate was then augmented depending on larval gut contents.

This methodology resulted in inconsistent feeding rates, as evaluating gut contents is subjective and gut contents varied between individual larvae. However, feeding and survival data collected throughout the 2021 season showed that higher survival rates occurred in tandem with increased algal feed. Prior to production spawns in 2022, a feeding trial with a small scale culture system will be completed to assess effects of algal density and type on larval survival. l. Then, feeding methods will be updated to inform future feeding rates.

**Test *Isochrysis* algae concentrate for feeding of early larva.** The hatchery team will evaluate the effectiveness of feeding alternative algal species to early-stage larvae, which are unable to ingest medium to large algae species. This will be evaluated by using Reed Mariculture Iso 1800®. This concentrate is comprised only of *Isochrysis* algae (5 – 6 microns) and does not contain the larger cells that are in the Mariculture 1800® concentrate (4 – 20 microns). By using the Iso concentrate, the larvae will be provided with a higher and more consistent concentration of the optimal algal cell size, without wasting food and potentially increasing bacterial load in the culture tanks from unconsumed algae. Initial tests will be conducted as a part of the aforementioned feeding trial to inform 2022 feeding protocols.

**Improve on feeding method to ensure consistent algal density within larval tanks.** In 2021, all feeding was done in twice daily batches, which results in daily fluctuations of food concentration. In 2022, batch feeding will be used initially to reach the necessary algal density (50,000-15,000 cells/mL), but continuous supply will be provided (via a metered pumping system) to maintain algal density throughout the day. This approach will maintain optimal food concentrations and reduce stress due to food limitation.

**Alleviate larval tank temperature fluctuations during periods of low temperature.** In 2021, periods of cold weather during larval culture decreased water temperatures in the tanks beyond the capacity of the heaters to maintain the optimal temperature of 28°C (82°F). Temperatures dropped significantly overnight but warmed during the day, creating wide temperature fluctuations, and causing significant larval mortality. In 2022, broodstock will be collected while water temperatures are below the spawning threshold 22°C and maintained in the broodstock tanks at 21°C until the ambient temperatures are sufficiently high that the tank heaters can maintain consistent optimal temperatures.

**Improve larval grading methods.** Larvae are graded and restocked according to size to reduce food competition between size classes. In 2021, ungraded larvae were collected and graded sequentially from smallest (60um) to largest (220um) sieve size. This was accomplished by placing a sieve atop a 5-gallon bucket, pouring 2L pitchers of ungraded larva into the sieve while continually rinsing with a 1L squirt bottle. Larvae larger the sieve were transferred to a sieve one size larger and graded accordingly. Each size class of larvae was graded until all larvae had been sorted by size class. Hatchery staff hope to decrease handling stress and time out of the water for larvae in the coming 2022 season. So, grading will be done on a wet table using stacked, interlocking, sieves to grade all size classes at once. Additionally, a catch screen will be used to prevent loss due to human error. This goal for the 2022 season is to save time, increase survival, and allow for more accurate grading between size classes.

**Assess pediveliger larvae for settlement cues.** In the 2021 season the main indicator used to assess pediveliger larva for settlement was the development of an eye spot. This indicator required that larvae were in the setting tank for a minimum of 48 hours to settle. There are alternative strategies used by other facilities and can reduce larval time in setting tanks and increase settlement rates. In 2022 additional cues will be used to evaluate larval competence to metamorphose such as foot development and onset of settlement behavior.

## 8. Outreach and Education

### *8.1 Targeted Outreach to the Community*

As the effects of the COVID-19 pandemic carried on through 2021, ABSI outreach and engagement efforts continued to adapt to align with current health and safety protocols throughout the Tallahassee and Florida panhandle region. ABSI's engagement with the public survived and thrived through various endeavors such as (but not limited to) the continuation of the Community Advisory Board and its subsequent subcommittees of Outreach and Education and the Successor Group, increased mailings of the ABSI newsletter, participation with local organizations to host in-person and virtual events, education initiatives, and an updated website.

### *8.1.1 Community Advisory Board.*

The Community Advisory Board (CAB), led by *Florida Conflict Resolution Consortium* (FCRC) Consensus Center facilitator Jeff Blair has continued to flourish. The over-arching objective of the CAB is to develop and agree on overall ABSI goals, objectives, and timelines; to seek consensus on actions and options informed by science for restoring the health of the Apalachicola Bay ecosystem; and agree on an overall management and restoration plan for the Apalachicola Bay system. The 21 CAB members represent local stakeholders, including watermen, local, state, and federal government officials and business owners, seafood and recreational fishing industry workers, and environmental groups. Due to time constraints some of the original CAB members have stepped down and been replaced with others from similar stakeholder organizations. Current CAB members are listed below.

***Agency personnel***: Mike Allen - University of Florida/IFAS Nature Coast Biological Station, Jim Estes - Florida Fish and Wildlife Commission (FWC) Division of Marine Fisheries, Jenna Harper - Apalachicola National Estuarine Research Reserve, Katie Konchar - FWC Aquatic Habitat Conservation and Restoration, Erik Lovestrand - Florida Sea Grant, Extension Director for Franklin County, Alex Reed - Florida Department of the Environment, Office of Resilience and Coastal Protection, Portia Sapp - Florida Department of Agriculture and Consumer Services Division of Aquaculture Paul Thurman - Northwest Florida Water Management District
***Local government***: Anita Grove - Apalachicola City Commission, Bert Boldt - Franklin County Commission
***Local business***: Gayle Johnson – Apalachicola Oyster Company, Chuck Marks - Acentria Insurance, Mike O'Connell - Saint George Island Civic Club, 2025 Vision, Steve Rash - Water Street Seafood
***Non-governmental organizations***: Georgia Ackerman - Apalachicola Riverkeeper, Chad Hanson - PEW Charitable Trusts, Fisheries Science and Policy, Frank Gidus - CCA Florida, Habitat and Environmental Restoration, Chadwick Taylor - Riparian County Stakeholder Coalition
***Watermen:*** Shannon Hartsfield - Waterman, SMART group director, Roger Mathis - Waterman
TJ Ward - Buddy Ward and Sons Seafood

The ABSI CAB web page contains detailed information on the CAB membership (https://marinelab.fsu.edu/absi/people/community-advisory-board/

**CAB Meetings**

All meetings since May 22, 2020 have been held virtually via Zoom. Documents from each meeting have been posted on CAB website, including meeting agendas, presentations, summaries, and video recordings (https://marinelab.fsu.edu/absi/cab/documents/)

- ***April 21, 2021 presentations:*** 1) ABSI Research Update (S Brooke, FSU) 2) Estuarine Metrics (S Brooke, FSU)
- ***June 16, 2021 presentations***: 1) ABSI Research Update (S Brooke, FSU), 2) ABSI Pollution Study (A Solanke, M Martínez-Colón)
- ***August 18, 2021 presentations:*** 1) ABSI Research Update (S Brooke, FSU), 2) Apalachicola Bay NFWF 1.0 Restoration Update (M Davis, FWC), 3) FDACS Apalachicola Bay Water Quality Sampling (C Jones, FDACS), 4) Creating an Oyster Fisheries and Habitat Management Plan for the Pensacola Bay System (A Birch, The Nature Conservancy, L Geselbracht, The Nature Conservancy & ABSI Science Advisory Board)
- ***October 19, 2021 presentations***: *Focus on approving Draft Framework Plan for the Apalachicola Bay System*
- ***November 16, 2021 presentations***: 1) ABSI Research Update (S Brooke, FSU)
- ***January 26, 2022 presentations***: 1) CAB Phase IV Workplan and Collaborative Modeling Presentation (J Blair, FCRC Consensus Center & ABSI CAB Facilitator), 2) Ecological Model Presentation (E Camp, UF), 3) River Flow Model Presentation (S Leitman, FSU), 4) Hydrodynamic Model Presentation (S Morey & X Chen, FAMU, A Alfasso, FSU), 5) Riverine Model Presentation ( X Chen, FAMU, J Zhou, UF)

### *8.1.2 Outreach and Education Subcommittee*

The Outreach and Education Subcommittee was developed in August 2020 and has helped spotlight ABSI news and research within the local community.

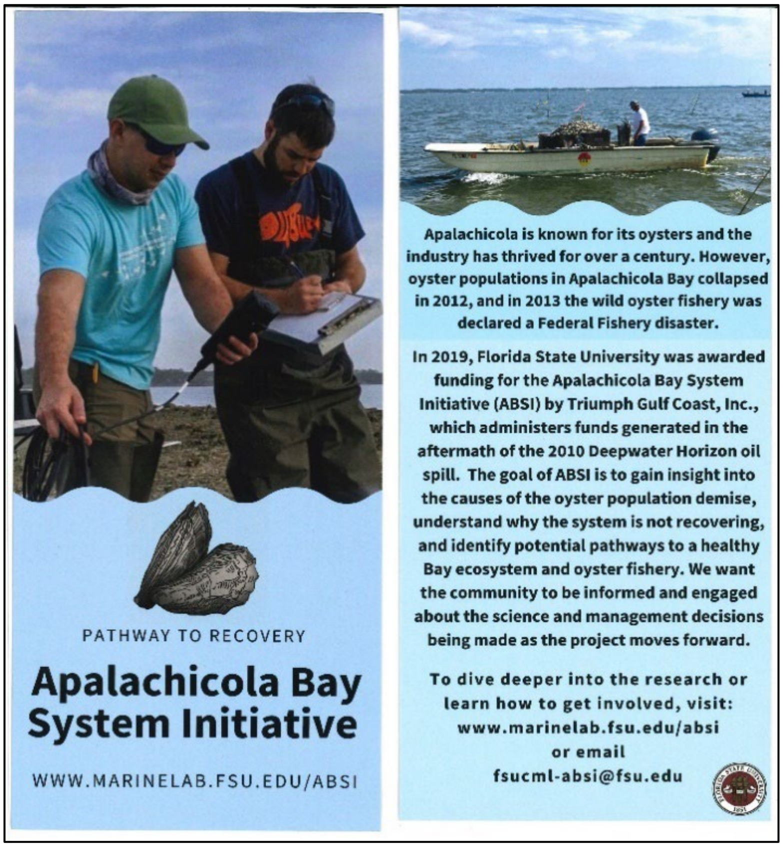
**Members of the Outreach and Community Engagement Subcommittee.** Chad Hanson (Chair), The Pew Charitable Trusts; Georgia Ackerman, Apalachicola Riverkeeper; Anita Grove, Apalachicola City Commissioner, Michael O'Connell, St. George Island Civic Club, 2025 Vision. Sandra Brooke, FSUCML, Maddie Mahood, FSUCML (Maddie left in January 2022. Rachel Walsh is current ABSI Outreach and Education Specialist).

**Subcommittee Meeting Dates**. April 8, 2021; May 20, 2021; July 1, 2021; September 2, 2021; November 11, 2021; January 31, 2022; March 21, 2022. (Agendas/Minutes found here: https://marinelab.fsu.edu/absi/cab/cab-subcoms/)

**Initiatives developed by this committee***:*

Development and distribution of a bi-monthly ABSI Newsletter (via email). Following each Community Advisory Board meeting, a newsletter is created summarizing the progress of theCAB, ABSI research updates, and upcoming events and education opportunities. The ABSI Newsletter email list currently has 432 subscribers, a 37% increase from March 2021. Previous issues can be found here: https://marinelab.fsu.edu/absi/commengage/newsletterarchive/

- Development of a media distribution plan for the ABSI newsletter and additional updates
    - Every ABSI update and newsletter are posted on Florida State University Coastal and Marine Laboratory's website and social media outlets: Facebook (@FSUCML), Twitter (@FSUMarineLab) and Instagram (@fsumarinelab)
    - Strengthened relationships with Michael Allen, Oyster Radio; Petra Shuff, Wakulla Chamberof Commerce; and Lisa Munson, Carrabelle Chamber of Commerce. Each of these organizations share the ABSI Newsletter on their respective Facebook pages
    - Subcommittee members share with their respective organizations' social media pages and newsletters, including Apalachicola National Estuarine Research Reserve, Apalachicola Riverkeeper, Apalachicola City Commission, Franklin County Commission, Wakulla Citizens group, Focus on Franklin County, as well their individual social media accounts.
- Development of virtual and in-person public workshops and outreach events throughout the community (see ***Public Outreach (In-Person and Virtual)***)
- Implementation of "Op-Eds" written by CAB members into local newsletters and newspapers, including *The Wakulla Times*, *The Apalachicola Times*, *The Star* (Port St. Joe, FL), and the St. George Island Civic Club Newsletter (*The Islander*)
- Development of ABSI rack cards (Fig. 41). These 4'' x 9'' cards include general information and contact information for ABSI. The cards have been distributed during outreach events and throughout the local community including, but not limited to:
    - Educational Facilities: FSUCML, ANERR
    - Visitor Centers: Apalachicola City Visitor Center, Carrabelle Visitor Center, Eastpoint Visitor Center, St. George Island Visitor Center
    - Libraries: Apalachicola Margaret Key Library, Franklin County Public Library – Eastpoint Branch and Carrabelle Branch
    - Local Businesses and Organizations: Oyster City Brewing Company, Eastpoint Beer Company, The Beach Pit (St. George Island), The St. George Island Civic Club, Eastpoint Civic Association
    - Local Government: Franklin Co. Commission and Apalachicola Bay Chamber of Commerce
- Participation in virtual education lectures and workshops (see ***ABSI Website/Online Education***)
- In early 2022, the Outreach Subcommittee began planning for the process of soliciting feedback from the public on the Framework for the Draft Apalachicola Bay System Ecosystem-Based Adaptive Management and Restoration Plan (The Plan). This effort is intended to help inform and educate the public on the CAB's work and to gather input that will aid in future development and prioritization of the Plan's strategies. The process will likely include a questionnaire and, if public health considerations permit, in-person strategies for reaching key stakeholders.



PATHWAY TO RECOVERY

**Apalachicola Bay System Initiative**

WWW.MARINELAB.FSU.EDU/ABSI

**Apalachicola is known for its oysters and the industry has thrived for over a century. However, oyster populations in Apalachicola Bay collapsed in 2012, and in 2013 the wild oyster fishery was declared a Federal Fishery disaster.**

**In 2019, Florida State University was awarded funding for the Apalachicola Bay System Initiative (ABSI) by Triumph Gulf Coast, Inc., which administers funds generated in the aftermath of the 2010 Deepwater Horizon oil spill. The goal of ABSI is to gain insight into the causes of the oyster population demise, understand why the system is not recovering, and identify potential pathways to a healthy Bay ecosystem and oyster fishery. We want the community to be informed and engaged about the science and management decisions being made as the project moves forward.**

**To dive deeper into the research or learn how to get involved, visit: www.marinelab.fsu.edu/absi or email fsucml-absi@fsu.edu**

**Figure 41.** Rack cards, developed to inform the local community about the Apalachicola Bay System Initiative, and provide contact information and resources for additional information. These have been widely distributed throughout Franklin County.

### *8.1.3 Successor Group Subcommittee*

Following the November 12th CAB meeting, a Successor Group Subcommittee was formed to ensure the continuation of the work of the ABSI CAB. The purpose of this Subcommittee is to develop a strategy to form a permanent, representative stakeholder successorgroup to advocate for the adoption and implementation of the restoration plan.

**Members of the Successor Group Subcommittee***:* Georgia Ackerman (Co-chair), Apalachicola Riverkeeper; Shannon Hartsfield (Co-chair), waterman; Jim Estes, FWC; Anita Grove, Apalachicola City Commission; Chad Hanson, PEW Charitable Trusts; Ricky Jones, Franklin County Commission, District 1; Steve Rash, Water Street Seafood; Chadwick Taylor, Riparian County Stakeholder Coalition.

**Subcommittee Meeting Dates.** February 2, 2021; February 23, 2021 (Agendas/Minutes found here:https://marinelab.fsu.edu/absi/cab/cab-subcoms/ )

### *8.1.4 Oystermen's workshops*

After a successful first Oystermen's Workshop on December 2, 2020, the ABSI held two more Workshops in 2021 (April 15 and July 14) in an effort to continue communicating ABSI goals and soliciting input from local watermen and seafood workers. These meetings encourage open discussion about management and restoration approaches and options. The April 15th and July 14th workshops were held at the Apalachicola National Estuarine Research Reserve (ANERR).

To abide by COVID-19 safety guidelines, only the watermen were invited to attend in-person, along with the ABSI project leads and facilitator, Jeff Blair. The members of the Community Advisory Board and public were invited to view the meeting via Zoom.

***April 15, 2021 Presentations***: ABSI Restoration Experiments (S Brooke, FSU)
Summary: At the April 15, 2021 Oystermen's Workshop the Apalachicola Bay System Initiative (ABSI) Community Advisory Board (CAB) conducted the second in a series of Oystermen workshops for the purpose of seeking oystermen's feedback on a variety of possible management approaches as well as ultimately on the Draft Apalachicola Bay System Ecosystem-Based Adaptive Management and Restoration Plan. During the workshop, the oystermen were provided an overview of the Project Workplan and Schedule; received an update and provided feedback on an ABSI restoration experiment; received an update and provided feedback on a FWC restoration project; and, provided feedback and input on a suite of possible management approaches.
(https://marinelab.fsu.edu/media/4835/absi_oystermens_workshop_ii_april_15-_2021_facilitators_summary_report.pdf )

**Oystermen:** Rickey Banks, Ronnie Gilbert, Shannon Hartsfield,* Brett Lolley, Roger Mathis,* Coy Shiver, Wayne Williams
**ABSI Representation**: Sandra Brooke, ABSI Principal Investigator; Maddie Mahood, ABSI Outreach and Education, Anita Grove, Apalachicola City Commission
**FCRC Consensus Center:** Jeff Blair

***July 14, 2021 Presentations***: ABSI Research Update (S Brooke, FSU)
Summary: At the July 14, 2021 Oystermen's Workshop the ABSI CAB conducted the third in a series of Oystermen's Workshops for the purpose of seeking their feedback on a variety of possible management approaches as well as ultimately on the Draft Apalachicola Bay System Ecosystem-Based Adaptive Management and Restoration Plan. During the workshop, the oystermen were provided an overview of the Project Workplan and Schedule; received an update and provided feedback on ABSI restoration experiments; provided feedback and input on a suite of possible management approaches; and discussed possible enforcement approaches with FWC Law Enforcement officers.
(https://marinelab.fsu.edu/media/4967/absi_oystermens_workshop_iii_july_14_2021_facilitators_summary_report.pdf )

**Oystermen:** Shannon Hartsfield, Roger Mathis, Coy Shiver, Wayne Williams
**ABSI Representation**: Sandra Brooke, ABSI Principal Investigator; Joel Trexler, ABSI Co-Principal Investigator, Maddie Mahood, ABSI Outreach and Education, Anita Grove*, Apalachicola City Commission
**FWC Law Enforcement**: Lt. Steven Cook, Lt. Randy McDonald
**FCRC Consensus Center:** Jeff Blair
*Members of the Community Advisory Board

### *8.1.5 Public outreach (In-person and virtual)*

Amidst ever-changing COVID-19 health and safety protocols, the ABSI team felt it was important to continue to present research updates to governmental and non-governmental organizations throughout the Apalachicola region. The ABSI was fortunate to coordinate both in-person and virtual meetings to provide ample opportunity for local community members to participate. (https://marinelab.fsu.edu/absi/commengage/aboutoyster/absi-events/ )

*Franklin County Commission Meeting – March 16, 2021*

- Sandra Brooke presented an update on the progress of ABSI to the Franklin County Commission meeting. The presentation was well received and ABSI agreed to present an update at Commission meetings every couple of months. Agenda and minutes: https://www.franklincountyflorida.com/wp-content/uploads/2021/06/AgendaPacket-Franklin-County-Board-of-County-Commissioners-Regular-Meeting-March-16-2021-9.00-AM.pdf

*Carrabelle Riverfront Festival – June 12, 2021*

- Members of the ABSI team hosted a booth alongside the FSUCML at the 30th Annual Carrabelle Riverfront Festival in Carrabelle, Florida. Members of the community stopped to talk about ABSI, the status of oysters in the Bay, and how they can get involved in ABSI. The team was thrilled to participate in the first "in-person" event in over a year! (Fig. 42)

*ANERR SciCafé: Apalachicola Bay System Initiative – September 22, 2021*

- Sandra Brooke provided an overview of ABSI and its current restoration experiments in partnership with ANERR's Virtual SciCafé series. This series was held at Eastpoint Beer Company. Attendees (53) represented a broad cross-section ofstakeholder groups and local citizens (fishermen, business owners, NGOs, state agencies, etc.)

*Franklin County Commission Meeting – November 2, 2021* ***at 9 am***

- Sandra Brooke provided an update on the progress of ABSI to the Franklin County Commissioners.

*Apalachicola City Commission Meeting – November 2, 2021* ***at 6 pm***

- Sandra Brooke presented an update on the progress of ABSI to the Apalachicola City Commission. The presentation was well received and solidified the commitment of the Apalachicola City Commission to continue to support the ABSI initiatives

*Florida Seafood Festival – November 5th and 6th*

- After a year hiatus due to COVID-19, the ABSI project was excited to participate in the Florida Seafood Festival in Apalachicola, Florida. Armed with GIS maps, spat traps, and a rotating digital presentation, the ABSI team communicated research updates and future goals to members of the public.

*Eastpoint Civic Association – November 8, 2021*

- After many postponements due to the rise in COVID-19 cases in the area, Sandra Brooke presented to the Eastpoint Civic Association for the first time. She provided a general overview of the ABSI project as well as its current research initiatives. This meeting was held at the Eastpoint Fire Station.

**Figure 42.** FSUCML-ABSI outreach displays at Carrabelle Riverfront Festival (left) and Apalachicola Seafood festival (right)

### *8.2 ABSI website/online education*

The ABSI team has worked to improve the availability of information on the ABSI website. Information on research progress, Community Advisory Board meetings and documents, ABSI leadership and staff, and educational materials are present and updated on a regular basis. In April 2021, the ABSI website received a new look to become more vibrant and user-friendly. The homepage now features drone video of ABSI research sites in the Apalachicola Bay System, as well as a Calendar of Events updated with CAB meetings and public workshops.

On July 29, 2021, ABSI presented an online Zoom lesson on the importance of oysters and the Apalachicola Bay ecosystem as well as the goals of ABSI in partnership with Camp STEMtastic, a summer camp program sponsored by the Division of Education at Thomas University. The summary on their **website** reads, "Camp STEMtastic was designed in collaboration with the Division of Education faculty and local area educators from the Thomasville City and Thomas County school systems to offer students the opportunity to explore the exciting fields of Science, Technology, Engineering, and Math. Each summer, campers have the opportunity to engage in activities and field trips centered around a specific theme related to these STEM areas."

The lesson was broken into four parts:

1) General introduction to ABSI and an overview of oyster biology and ecosystems
2) Oyster filtration and a live walk-through of the FSUCML hatchery
3) A live presentation of dissecting an oyster cluster to identify the various animal species living inside (we counted 18!) (Fig. 43)
4) An overview of ABSI restoration initiatives and the importance of stewardship and a final Q&A with our scientists

The lesson was led by Maddie Mahood, Outreach and Education Assistant, and co-lead by Chris Matechik, ABSI Lead Research Assistant; Alek Valles, ABSI Research Technician; Morgan Hawkins, ABSI Hatchery Technician; and Benton Jaco, ABSI Hatchery Technician. There were 12 students in virtual attendance, and the ABSI team received glowing reviews from the STEMtastic staff and faculty.

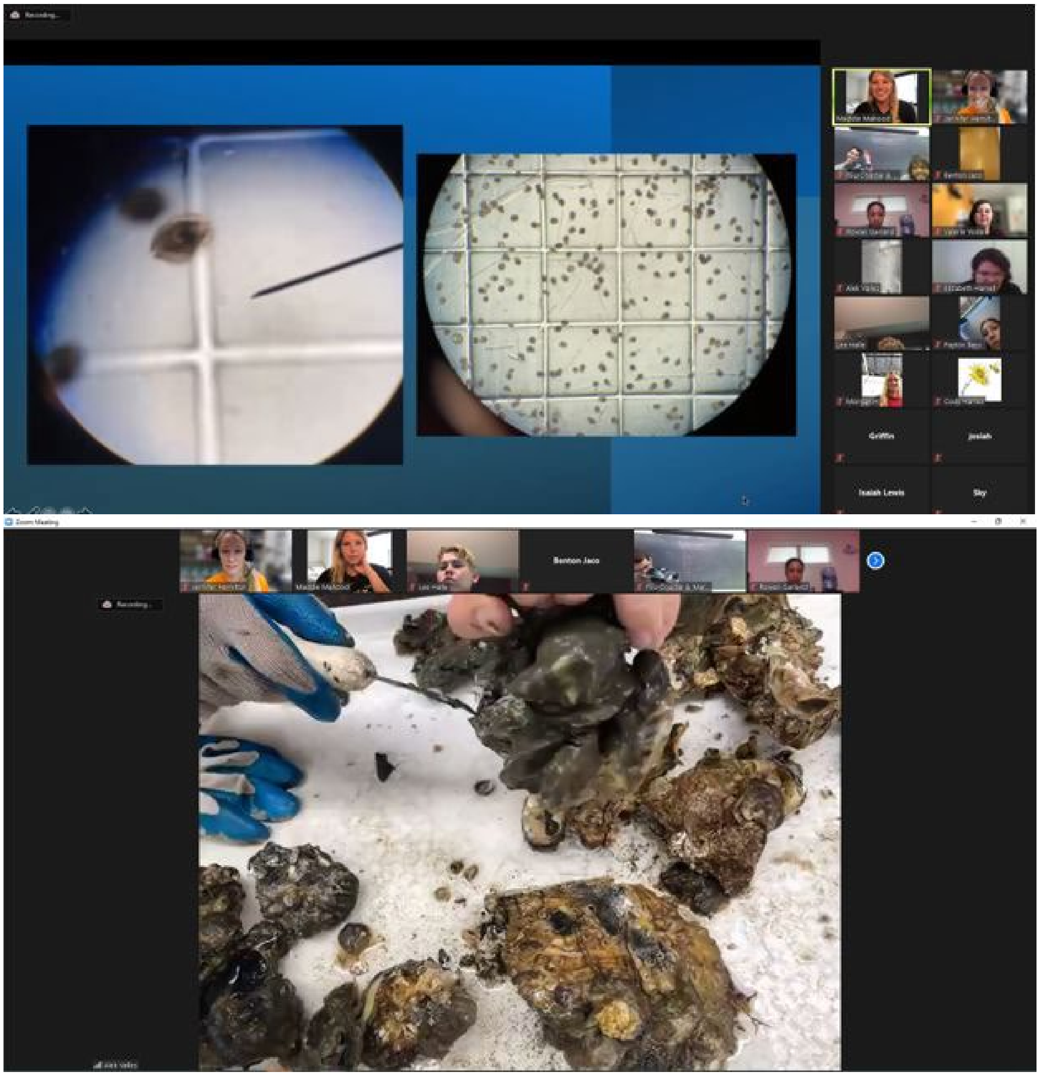


**Figure 43.** Camp Stemtastic framegrab of demonstration of oyster hatchery larvae (top) and oyster clump dissection (bottom)

ABSI also had a unique opportunity this year to work with high school junior, Emily White, from Peachtree City, Georgia. After several visits to the area with her family, Emily developed a strong interest in the Apalachicola Bay ecosystem and, in 2020, began to research variables that might be causing the decline of the Bay's oyster populations. After months of research, she developed her own mix of oyster reef restoration material. In 2021, she reached out to the ABSI team to create a plan to come to the Florida State University Coastal and Marine Laboratory (FSUCML) to test her experimental artificial oyster habitats (Fig. 44). In September, she set 54 oyster habitat experiments in the Bay to test which experimental mix attracted the most spat. After 49 days, she returned to the lab to evaluate the results, and is preparing to return in spring 2022 to deploy a new set of experiments. She worked with members of the ABSI team including (but not limited to) Chris Matechik, ABSI Lead Research Assistant, and ABSI Research Technicians, Barry Walton, Elana Hutner, Alek Valles, and Kevin Engelbert. In her words, "It was really cool to me to talk to and work with these people who do [oyster research] for a living…and also to be treated like an equal, a colleague." Read Emily's full story here**:** https://marinelab.fsu.edu/absi/news/emily-white_absi/

**Figure 44**. Emily White working with ABSI staff and technicians on her research project

For the 2022 – 2023 year, ABSI has a strong focus on increasing our education efforts within the community and we are currently in partnership with the Apalachicola National Estuarine Research Reserve (ANERR) to develop education programming for Franklin County students focused on ecology and restoration of the Apalachicola Bay System. The programming will be designed to complement and fill gaps in ANERR's existing education programming and build upon knowledge the students have gained through participation in ANERR's programs at lower grade levels.

### *8.3 Local News Coverage*

The ABSI project was featured in many news articles and interviews this year, particularly in the summer months to cover the first deployment of the ABSI restoration material experiments. Below is a partial list of articles and news segments from March 2021 – March 2022.

- **WCTV.tv** – April 2021
- **WFSU Public Media** – June 2021
- **The Islander Newsletter** – July 2021
- **The Florida Channel** – July 2021
- **Oyster Radio** – July 2021
- **The Wakulla Times** – July 2021
- **WFSU Public Media** – August 2021
- **Pew Charitable Trusts Blog** – August 2021
- **Apalachicola Riverkeeper** – November 2021
- **Guy Harvey Magazine** – December 2021

### *8.4 Shell Recycling Program – O.Y.S.T.E.R*

Staff at the Apalachicola National Estuarine Research Reserve (ANERR) and the Conservation Corps of the Forgotten and Emerald Coasts are in the process of developing an OysterCorps Pilot Program for oyster recycling in Franklin, Gulf and Bay counties with the Northwest Florida Water Management District and The Nature Conservancy's GulfCorps Program. ABSI and the FSUCML are partners in this program and will provide hatchery internships in 2022 for the young people of the OysterCorps.

## 9. Literature Cited

Adamack, A. T., and Gruber, B. (2014). PopGenReport: simplifying basic population genetic analyses in R. *Methods in Ecology and Evolution* 5, 384–387. doi:10.1111/2041-210X.12158

Altieri AH, Gedan KB (2015) Climate change and dead zones. Glob Change Biol 21:1395–1406.

Aranda, D. A., M. E. Díaz, F. L. Reynoso, T. Brulé, J. Montero, and E. B. Cárdenas. 2014. Reproductive Strategies of the Eastern Oyster *Crassostrea virginica* (Gmelin 1791) in Tropical Lagoons of the Mexican Gulf of Mexico. Journal of Shellfish Research 33:145–152.

Arnold, W., N. J. Blake, M. M. Harrison, D. C. Marelli, M. L. Parker, S. C. Peters, and D. E. Sweat. 2005. Restoration of Bay Scallop (*Argopecten irradians* (Lamarck)) Populations in Florida Coastal Waters: Planting Techniques and the Growth, Mortality, and Reproductive Development of Planted Scallops. Journal of Shellfish Research 24:4 883-904

Beck, M. W., Brumbaugh, R. D., Airoldi, L., Carranza, A., Coen, L. D., Crawford, C., Defeo, O., Edgar, G. J., Hancock, B., Kay, M. C., Lenihan, H. S., Luckenbach, M. W., Toropova, C. L., Zhang, G., & Guo, X. (2011). Oyster reefs at risk and recommendations for conservation, restoration, and management. *BioScience*, *61*(2), 107–116. https://doi.org/10.1525/bio.2011.61.2.5

Bridge T, Beaman R, Done T, Webster J (2012) Predicting the location and spatial extent of submerged coral reef habitat in the Great Barrier Reef World Heritage Area, Australia. PLoS One 7:e48203.

Camp, E. V., Pine, W. E., Havens, K., Kane, A. S., Walters, C. J., Irani, T., Lindsey, A. B., & Glenn Morris, J. (2015). Collapse of a historic oyster fishery: Diagnosing causes and identifying paths toward increased resilience. *Ecology and Society*, *20*(3). https://doi.org/10.5751/ES-07821-200345

Camp E, Pine III W, Havens K, Kane A, Walters C, Irani T, Lindsey A, Morris J (2015) Collapse of a historic oyster fishery: diagnosing causes and identifying paths toward increased resilience. Ecol Soc 20.

Chanton, J., & Lewis, F. G. (2002). Examination of coupling between primary and secondary production in a river-dominated estuary: Apalachicola Bay, Florida, USA. *Limnology and Oceanography*, *47*(3), 683-697.

Clyde, L., and J. R. Mackenzie. 2009. Small-scale Commercial Culturing of Northern Bay Scallops, *Argopecten irradians irradians*, in Atlantic United States and Canada. Marine Fisheries Review 71:3 46 – 49

Coen LD, Luckenbach MW (2000) Developing success criteria and goals for evaluating oyster reef restoration: Ecological function or resource exploitation? Ecol Eng 15:323–343.

Combs, E. M., Belgrad, B. A., and Smee, D. L. (2019). Comparison of Nursery Methods to Strengthen Oysters for Aquaculture. *Gulf and Caribbean Research*, SC17–SC21. doi:10.18785/gcr.3001.09.

Covarrubias-Pazaran, G., Diaz-Garcia, L., Schlautman, B., Salazar, W., and Zalapa, J. (2016). Fragman: An R package for fragment analysis. *BMC Genetics* 17, 1–8.

Davis, H. C. 1958. Survival and growth of clam and oyster larvae at different salinities. The Biological Bulletin 114:296–307Eierman LE, Hare MP (2013) Survival of oyster larvae in different salinities depends on source population within an estuary. J Exp Mar Biol Ecol 449:61–68.

Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J., and Ovenden, J. R. (2014). NeEstimator v2: Re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Molecular Ecology Resources* 14, 209–214. doi:10.1111/1755-0998.12157.

Eierman, L. E., and M. P. Hare. 2013. Survival of oyster larvae in different salinities depends on source population within an estuary. Journal of Experimental Marine Biology and Ecology 449:61–68.

Espriella, M. C., Lecours, V., Frederick, P. C., Camp, E. V., & Wilkinson, B. (2020). Quantifying intertidal habitat relative coverage in a Florida estuary using UAS imagery and GEOBIA. *Remote Sensing*, *12*(4). https://doi.org/10.3390/rs12040677

Fisch NC, Pine WE (2016) A Complex Relationship between Fresh-water Discharge and Oyster Fishery Catch Per Unit Effort in Apalachicola Bay, Florida: an Evaluation from 1960 to 2013. J Shellfish Res 35:809–825.

Graeff T, Baroni G, Bronstert A, Brunk C, Martínez I, Oswald S (2013) Modelling changing hydrology in a coastal area under the influence of climatic change until 2100. 15:EGU2013-12559.

Granneman, J., C. Baxely, M. Bollinger, A. Heil, M. LaGanke, E. Levine, W. Pearson, E. Pudlak, and K. Williams. 2021. Evaluating the Impact of Recreational Harvest and Management Strategies for Bay Scallops *Argopecten irradians concentricus* in a Florida Gulf Coast Management Zone. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science 13:413–432

Griffiths, J. S., K. M. Johnson, K. A. Sirovy, M. S. Yeats, F. T. C. Pan, J. F. La Peyre, and M. W. Kelly. 2021. Transgenerational plasticity and the capacity to adapt to low salinity in the eastern oyster, *Crassostrea virginica*. Proceedings of the Royal Society B: Biological Sciences 288:20203118

Grizzle, R., Ward, K., Geselbracht, L., & Birch, A. (2018). Distribution and Condition of Intertidal Eastern Oyster (Crassostrea virginica) Reefs in Apalachicola Bay Florida Based on High-Resolution Satellite Imagery. *Journal of Shellfish Research*, *37*(5), 1027–1038. https://doi.org/10.2983/035.037.0514

Hajovsky, P., Beseres Pollack, J., and Anderson, J. (2021). Morphological Assessment of the Eastern Oyster Crassostrea virginica throughout the Gulf of Mexico. *Marine and Coastal Fisheries* 13, 309–319. doi:10.1002/mcf2.10156

Hedrick, P. W. (2005). A standardized genetic differentiation measure. *Evolution* 59, 1633–1638. doi:10.1111/j.0014-3820.2005.tb01814.x.

Heilmayer, O., J. Digialleonardo, L. Qian, and G. Roesijadi. 2008. Stress tolerance of a subtropical *Crassostrea virginica* population to the combined effects of temperature and salinity. Estuarine, Coastal and Shelf Science 79:179–185.

Hegerl GC, Black E, Allan RP, Ingram WJ, Polson D, Trenberth KE, Chadwick RS, Arkin PA, Sarojini BB, Becker A, Dai A, Durack PJ, Easterling D, Fowler HJ, Kendon EJ, Huffman GJ, Liu C, Marsh R, New M, Osborn TJ, Skliris N, Stott PA, Vidale P-L, Wijffels SE, Wilcox LJ, Willett KM, Zhang X (2014) Challenges in Quantifying Changes in the Global Water Cycle. Bull Am Meteorol Soc 96:1097–1115.

Hewitt JE, Ellis JI, Thrush SF (2016) Multiple stressors, nonlinear effects and the implications of climate change impacts on marine coastal ecosystems. Glob Change Biol 22:2665–2675.

Joyce, K. E., Duce, S., Leahy, S. M., Leon, J., & Maier, S. W. (2019). Principles and practice of acquiring drone-based image data in marine environments. *Marine and Fresh-water Research*, *70*(7), 952. https://doi.org/10.1071/MF17380

Jombart, T. (2008). adegenet: an R package for the multivariate analysis of genetic markers. *Bioinformatics* 24, 1403–1405. doi:10.1093/bioinformatics/btn129.

Jombart, T., Devillard, S., and Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics* 11. doi:10.1186/1471-2156-11-94.

Jones, H. R., K. M. Johnson, and M. W. Kelly. 2019. Synergistic Effects of Temperature and Salinity on the Gene Expression and Physiology of *Crassostrea virginica*. Integrative and Comparative Biology 59:306–319

Kamvar, Z. N., Tabima, J. F., and Grunwald, N. J. (2014). Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* 2, e281. doi:10.7717/peerj.281.

Konopiński, M. K. (2020). Shannon diversity index: A call to replace the original Shannon's formula with unbiased estimator in the population genetics studies. *PeerJ* 2020. doi:10.7717/peerj.9391.

Lannig, G., J. F. Flores, and I. M. Sokolova. 2006. Temperature-dependent stress response in oysters, *Crassostrea virginica*: Pollution reduces temperature tolerance in oysters. Aquatic Toxicology 79:278–287.

Legendre, P., Fortin, M. J., and Borcard, D. (2015). Should the Mantel test be used in spatial analysis? *Methods in Ecology and Evolution* 6, 1239–1247. doi:10.1111/2041-210X.12425.

Luikart, G., Ryman, N., Tallmon, D. A., Schwartz, M. K., and Allendorf, F. W. (2010). Estimation of census and effective population sizes: The increasing usefulness of DNA-based approaches. *Conservation Genetics* 11, 355–373. doi:10.1007/s10592-010-0050-7.

McCarty, A. J., K. McFarland, J. Small, S. K. Allen, and L. V. Plough. 2020. Heritability of acute low salinity survival in the Eastern oyster (*Crassostrea virginica*). Aquaculture 529:735649.

Menzel, R. W. 1951. Early Sexual Development and Growth of the American Oyster in Louisiana Waters. Science 113:719–721.

NOAA Commercial Fisheries Landings. **https://www.fisheries.noaa.gov/foss/f?p=215:200:5382813302095:Mail:NO**

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., et al. (2020). Package "vegan" Title Community Ecology Package Version 2.5-7Parris AS, Bromirski P, Burkett V, Cayan DR, Culver ME, Hall J, Horton RM, Knuuti K, Moss RH, Obeysekera J (2012) Global sea level rise scenarios for the United States National Climate Assessment.

Paradis, E. (2010). pegas: an R package for population genetics with an integrated–modular approach. *Bioinformatics* 26, 419–420. doi:10.1093/bioinformatics/btp696

Passeri DL, Hagen SC, Plant NG, Bilskie MV, Medeiros SC, Alizad K (2016) Tidal hydrodynamics under future sea level rise and coastal morphology in the Northern Gulf of Mexico. Earths Future 4:159–176.

Petes LE, Brown AJ, Knight CR (2012) Impacts of upstream drought and water withdrawals on the health and survival of downstream estuarine oyster populations. Ecol Evol 2:1712–1724.

Phillips SJ, Anderson RP, Dudík M, Schapire RE, Blair ME (2017) Opening the black box: an open-source release of Maxent. Ecography 40:887–893.

Pine III W, Walters C, Camp E, Bouchillon R, Ahrens R, Sturmer L, Berrigan M (2015) The curious case of eastern oyster <em>Crassostrea virginica</em> stock status in Apalachicola Bay, Florida. Ecol Soc 20.

R Core Team (2018). R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria* 3.5. doi:https://www.R-project.org/.

Raymond, M., and Rousset, F. (1995). GENEPOP (Version-1.2) - Population-genetics software for exact tests and ecumenicism. *Journal of Heredity* 86, 248–249. Available at: %3CGo

Rengstorf AM, Yesson C, Brown C, Grehan AJ (2013) High-resolution habitat suitability modelling can improve conservation of vulnerable marine ecosystems in the deep sea. J Biogeogr 40:1702–1714..

Rousset, F. (2008). Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources* 8, 103–106. doi:10.1111/j.1471-8286.2007.01931.x.

Rybovich, M., M. K. L. Peyre, S. G. Hall, and J. F. L. Peyre. 2016. Increased Temperatures Combined with Lowered Salinities Differentially Impact Oyster Size Class Growth and Mortality. Journal of Shellfish Research 35:101–113.

Ryman, N., and Palm, S. (2006). POWSIM: A computer program for assessing statistical power when testing for genetic differentiation. *Molecular Ecology Notes* 6, 600–602. doi:10.1111/j.1471-8286.2006.01378.x.

Seavey JR, Pine WE, Frederick P, Sturmer L, Berrigan M (2011) Decadal changes in oyster reefs in the Big Bend of Florida's Gulf Coast. Ecosphere 2:1–14.

Seyoum, S., T. M. Bert, A. Wilbur, W. A. Arnold, and C. Crawford. 2003. Development, Evaluation, and Application, of a Mitochondrial DNA Genetic Tag for the Bay Scallop, *Argopecten irradians*. Journal of Shellfish Research 22:1 111-117

Visser JM, Sasser CE, Chabreck RH, Linscombe RG (2002) The impact of a severe drought on the vegetation of a subtropical estuary. Estuaries 25:1184–1195.

Vitousek PM, Mooney HA, Lubchenco J, Melillo JM (1997) Human Domination of Earth's Ecosystems. Science 277:494–499.

Winter, D. J. (2012). MMOD: An R library for the calculation of population differentiation statistics. *Molecular Ecology Resources* 12, 1158–1160. doi:10.1111/j.1755-0998.2012.03174.x.

Zahl, S. (1977). Jackknifing An Index of Diversity. *Ecology* 58, 907–913.