ANNUAL REPORT TO TRIUMPH GULF COAST INC.

Project #69: Apalachicola Bay System Initiative (ABSI)
Awardee: Florida State University
Reporting Period: March 15, 2022-March 15, 2023

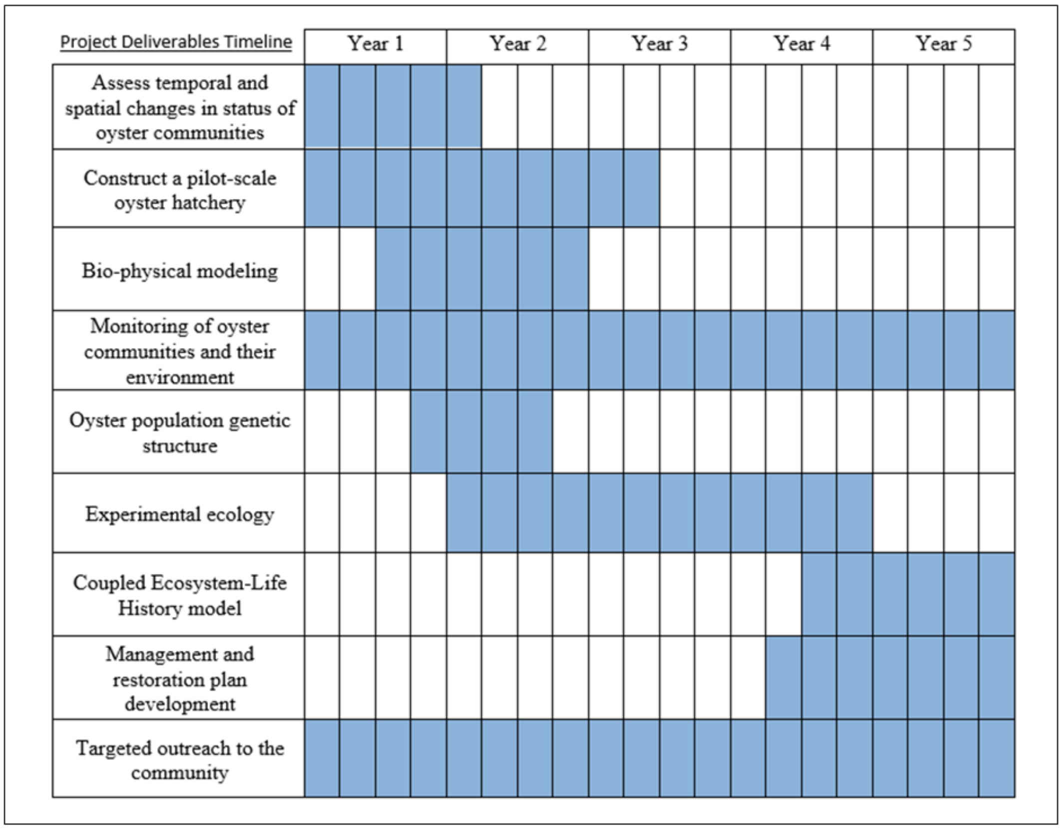
Submitted by ABSI LEADERSHIP TEAM
Dr. Sandra Brooke (Principal Investigator)
Dr. Joel Trexler (Co-Principal Investigator)
April 10, 2023

Report Contributors

FSUCML Faculty: Dr. Josh Breithaupt, Dr. Tara Stewart Merrill, Dr. Andrew Shantz
FSUCML Post-doc: Fabio Caltabellotta
Collaborators: Dr. Amy Baco, Dr. Xu Chen, Dr. Steve Leitman, Dr. Steve. Morey, Dr. Michael Martinez-Colon, Dr. Nicole Morgan,
Graduate Students: Adam Alfasso, Donaven Baughman, Jenny Bueno, Emily Fuqua, Morgan Hawkins, Barry Walton, Michael Wintermantel,
ABSI Technicians: Rand Romas, Lillian Bradshaw, Adin Domen, Jazzy Jones, Cassandra Zimmer, Shannon Murphy
Hatchery Team: Emily Fuqua, Morgan Hawkins, Louis Lockhart, Bailey Kilroy, Landon Millender, Hayden Mock, Reid Tilley
Outreach Team: Jared Fuqua, Madeline Mahood.

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. Some of the deliverables comprise multiple parts; for example, Experimental Ecology includes multiple research studies, but others are very specific, such as the population genetic study. This report presents accomplishments for the fourth year of this large multi-disciplinary effort. There is also a section on other items that are not directly associated with the specific objectives.



Status of project deliverables

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 is available on the ABSI website (https://marinelab.fsu.edu/absi/research/absi-literature-database/). This database 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.

1. Construct a pilot-scale ABSI Research Hatchery

Construction of the permanent hatchery building was completed in spring 2022, but internal construction (plumbing, electrical work, insulation etc.) was ongoing until recently (spring 2023). The hatchery is now fully operational and is housed in a 50 x 70 ft insulated metal building, 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 purpose of the ABSI hatchery is to produce shellfish larvae and juveniles for restoration and research only. Details of the hatchery operations can be found in section 7

2. Bio-physical modeling

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

Fresh-water flow models were accomplished 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 and Dr. Xu Chen from Florida Agricultural and Mechanical University (FAMU). 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 have made good progress in 2022 and the project is nearing completion; however some complications were discovered during the hydrodynamic modeling process that has delayed complete resolution of the freshwater flow dynamics into Apalachicola Bay. In summary, some of the flows into the Apalachicola River appear to be diverting west into Lake Wimico, reducing the estimated flows into Apalachicola Bay. The effects of this diversion are more pronounced during extreme climate events (droughts and floods). The modelers are making progress on resolving this critical and previously undocumented issue. Further details on these modeling efforts can be found in sections 2.2 and 2.3

3. Monitoring of oyster communities and their environment

Intertidal monitoring of four oyster reef areas throughout Franklin County (Indian Lagoon, East Cover, Carrabelle River and Alligator Harbor) continued throughout 2022 and into 2023, collecting monthly information on disease and condition. Additional studies include assessing the use of high-resolution drone surveys to monitor intertidal habitats. Further details of intertidal 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, Shannon Hartsfield, to survey subtidal areas throughout Apalachicola Bay using small oyster tongs. Comparisons of the tonging sampling data and SCUBA sampling have shown comparable data can be collected with both approaches. Tong data has broader spatial coverage than the SCUBA surveys and can be collected quickly with fewer weather limitations than diving. In 2021, 2022 and 2023, the tong sampling was repeated, with slight data collection modifications and is presented in section 4.3.

The Apalachicola Estuarine Research Reserve (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 and 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 Miles was destroyed by a shrimp trawler and the three near the passes are missing, despite repeated efforts to locate them. The instrument that was previously deployed at the mouth of the Apalachicola River was destroyed during a storm event in 2022. Within the past year, two Manta Sub 3 Eureka Water Probes were deployed, which replaced lost instruments in the Miles and St. George Sound. To provide a broader spatial perspective, the FWC fisheries independent monitoring program monthly water quality data were interpolated to generate maps of annual average water quality parameters (temperature, salinity, pH, oxygen). Details can be found in section 2.1

4. 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 complete and indicates some genetic structuring along the study region. Details of study can be found in section 3.1

5. 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 and developing effective restoration approaches. These include projects focused on oyster biology and ecology and broader Apalachicola Bay System ecology. Some of these projects are complete and were documented in the 2021-2022 ABSI report, available on the FSU ABSI website. According to the project timeline, this objective was due to be completed by the end of Y4; however, several new projects have been initiated and progress on others was delayed due to the Covid-19 pandemic. The ongoing and new projects are listed below:

- Pathogen transmission and disease impacts on oysters (section 3.2)
- Oyster stress responses and physiological tolerances (section 3.3)
- Effect of salinity on juvenile oysters (section 3.4)

- Responses of oyster early life-stages to pesticide exposure (section 3.5)
- Impacts of oyster populations on community development (section 4.4)
- Fish communities associated with oyster habitats (section 4.5)
- Oyster and scallop restoration (section 5)
- Ecosystem ecology (section 6)

6. Coupled ecosystem life-history model

Three models are being developed by ABSI 1) freshwater flow (section 2.2), 2) bio-physical (section 2.3) and habitat suitability (section 2.4). Aspects of these models will be incorporated into the others as appropriate. An oyster population model developed by our collaborator Dr. Ed Camp (University of Florida) is being used to model management strategies as requested by the Community Advisory Board. Dr. Fabio Caltabellotta (ABSI Postdoc) is developing a decision support tool that can be used through cell phones and computers. This tool uses Dr. Camps model to provide a user friendly platform to assess the effects of different fishery management strategies on oyster populations (section 6.3)

7. Development of a Management and Restoration Plan for the Apalachicola Bay System

This task was originally scheduled for the final year but was initiated in 2019 with the establishment of the Community Advisory Board (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/). This objective will terminate in November 2023 with the completion of the Management and Restoration Plan, which will contain a series of options that have the consensus approval of the CAB. Development of the final document will proceed through bi-monthly CAB meetings. These are open to the public and are held at the Apalachicola National Estuarine Research Reserve. Meetings are recorded and all information and presentations are available on the ABSI website (https://marinelab.fsu.edu/absi/cab/). The final Plan will also be posted on the ABSI website. More information on the CAB can be found in section 8.1

8. Targeted outreach to the community

Community support is critical to the success of ABSI, and with Covid-19 restrictions diminished, ABSI's engagement with the public and local stakeholders increased in 2022. In-person events included (but were not limited to) the continuation of the CAB meetings and CAB-associated sub-committees, presentations at city and county commission meetings, an oystermens workshop and a public meeting (both in conjunction with FWC), and attendance at local festivals. Digital outreach included regular posts on the FSUCML Facebook page, creation of a bi-monthly ABSI newsletter, and an expanded website that houses more research data and educational materials. Section 8 details the many ABSI community engagement and outreach events conducted in 2022.

Table of Contents

APALACHICOLA BAY SYSTEM INITIATIVE (ABSI) ANNUAL REPORT 2022-2023

1. Introduction

The Apalachicola Bay System Initiative was awarded in March 2019 and has now completed the fourth 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. Some studies began in 2019 or 2020 and were described in earlier annual reports. Previous information is not repeated unless it pertains to project updates. Information on projects completed prior to 2022 were omitted. Projects that have been initiated within the last year are documented with preliminary results where available.

The permanent ABSI Research and Restoration Hatchery became operational in the fall of 2022; however, there were a number of problems with water quality, which were resolved using additional funding from FSU to clean intake lines and install sophisticated water handling and monitoring systems. In late fall the hatchery had a successful bay scallop spawn that resulted in several million spat. Some of these were used for restoration research and others were donated to the FWC scallop restoration program. The hatchery had a successful oyster spawn in early April and these continue to do well. The hatchery internship program was very successful, with two of the interns becoming full time hatchery technicians.

Community engagement is a critical component of the ABSI and the team has been active over the past year, conducting many activities in addition to the CAB meetings, which have been conducted in person over the last year but were still broadcast over the zoom platform and recorded. Information from the CAB is available to the public through the ABSI website to ensure transparency.

2. Habitat and environment

2.1 Apalachicola Bay water quality and hydrology (Barry Walton, Ph.D. Student, FSU)

Introduction. Understanding system-wide water quality and hydrology is an ongoing ABSI objective. Long-term historic data can be useful to provide context and a baseline from which to assess system change. The Apalachicola National Estuarine Research Reserve (ANERR) water quality instruments generate long-term time-series data from several fixed locations in the Bay. While these data are valuable, they are limited spatially. This section presents water quality data collected monthly by the Florida Wildlife Research Institute (FWRI) long term Fishery Independent Monitoring (FIM) program between 2005 and 2022. These point-data (collected over time from a different location during each sampling event) have been interpolated to augment the ANERR instrument data.

Methods. The FWRI FIM program collects fish samples and associated environmental data at 70 stations within Apalachicola Bay. Water quality data is collected using a YSI (Xylem Brand) multiparameter handheld instrument at the surface and the bottom of each station. For the purpose of this analysis, bottom data (>1m) only was used. Water quality data was visualized using a process called Ordinary Kriging. This is a common interpolation method that estimates the value of an unknown point by averaging known values of neighboring points. Water-quality data were also analyzed using an Optimized Hotspot Analysis which identifies areas of significantly higher (hot spots) or lower (cold spots) values compared with neighboring values. The software 'R

Studio' was used for data cleaning and spatial analyses were performed using ArcGIS Pro 3.1

Results and discussion. The Ordinary Kriging and Optimized Hotspot Analysis of Apalachicola Bay illustrates high salinity values along the northeastern and southwestern areas of the Bay. Moderate salinity values (13.2-24.5) can be seen along the central portion of the Bay, an area directly influenced by the Apalachicola River (Fig. 1A). Optimized Hotspot Analysis values illustrate 90% to 95% confidence in salinity within Apalachicola Bay from 2005-2022 (Fig. 1B). Temperature throughout the bay from 2005 to 2022 was highly variable and was found to range from 13.9 to 29.9ºC (Fig. 2A). The Apalachicola River and East Bay showed the highest cold spot confidence levels with respect to overall temperature (Fig. 2B). Analysis of dissolved oxygen (DO_2) illustrates significant variability within the Bay, with the highest DO_2 values being present within the southwest portion. Similarly, to the salinity data, the statistically significant spatial clusters of dissolved oxygen were highest along the boundary of Apalachicola Bay and lowest in areas with high freshwater influences, specifically the Apalachicola River and East Bay (Fig. 3A, B). The pH was found to range between 6.7 and 8.6 throughout Apalachicola Bay. At Peanut Ridge and Dry Bar, which are the focus of ABSI restoration experiments, the pH ranged from 7.62 to 8.2, with the lower pH observed closest to the Apalachicola River (Fig. 4A). Optimized Hotspot Analysis illustrated high confidence intervals of both cold and hot spots along the Apalachicola River and East Bay (Fig. 4B).

The kriging and hotspot analysis of salinity shows clear and expected patterns of fresh, brackish and marine water (Fig. 1a, B). The freshest water is close to the river mouth and East Bay, intermediate salinities occur over most of the Bay and extend east into St George Sound. The far eastern part of the Sound has marine salinities, being furthest from the river mouth and influenced by Gulf water flowing through east pass. While this kind of analysis can provide a useful overview of environmental conditions, the data outputs should be interpreted with caution and understanding of the system and data used. For example, these maps show data interpolated over one year, but several environmental factors change predictably in time and space and interpolation can mask these trends with variability. For example, temperature changes seasonally as well as periodically in response to extreme hot or cold events, but the kriging data (Fig 2A, B) does not reflect this because spatial trends are masked by temporal (seasonal) variability. Oxygen levels are generally higher in freshwater than marine systems, which is reflected in the kriging map (Fig. 3A), although there is variation within this generalization. High levels of organic material combined with reduced flow can lower oxygen levels in freshwater systems. Conversely, systems such as coral reefs and seagrass beds that have abundant photosynthetic organisms may have high oxygen levels. The Hotspot Analysis (Fig. 3B) of dissolved oxygen shows areas of 'cold spots' around the Apalachicola River and East Bay, indicating lower than average oxygen levels. This is probably due to the persistently high organic content of Apalachicola River water. The patterns of pH values mimic those of salinity (Fig. 4a, B); lower pH (6.5-8.0) occur in freshwater, whereas ocean water generally has pH of 8.0 or higher.

Future work will include subsampling the data by season or time period to determine the optimal temporal resolution of the kriging process. The ANERR water quality instrument data may also be included in these analyses to increase temporal data although over a limited spatial scale.

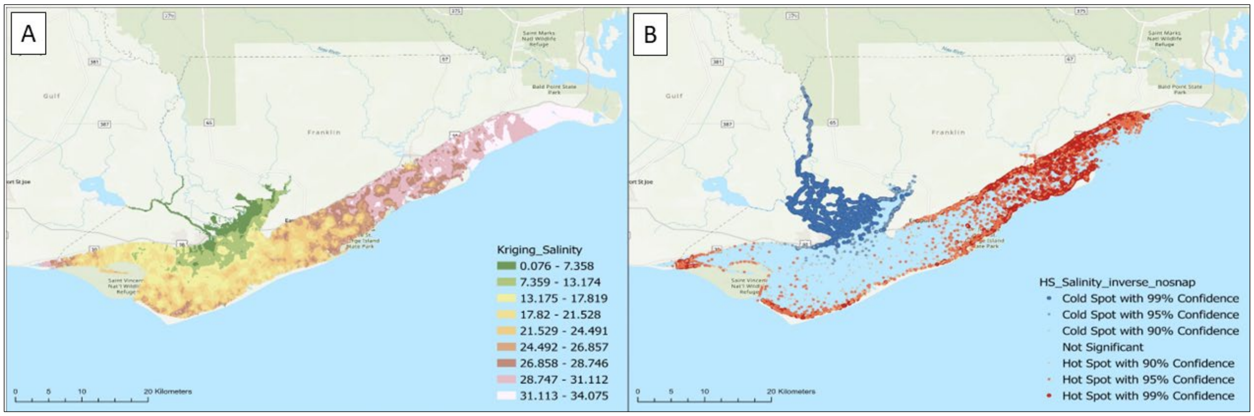


Figure 1 A) Ordinary Kriging and B) Optimized Hotspot Analysis of salinity in Apalachicola Bay from 2005 – 2022.

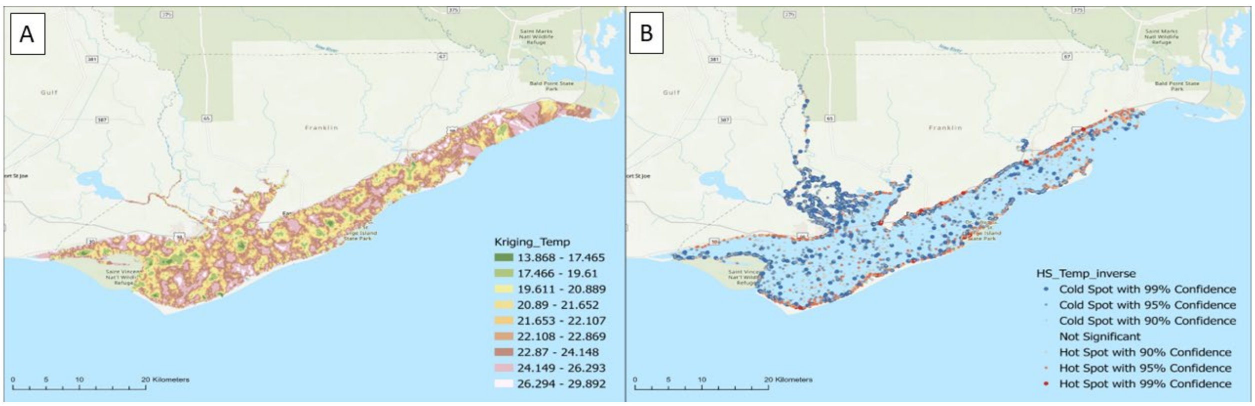


Figure 2. A) Ordinary Kriging and B) Optimized Hotspot Analysis of temperature (C°) in Apalachicola Bay from 2005 – 2022.

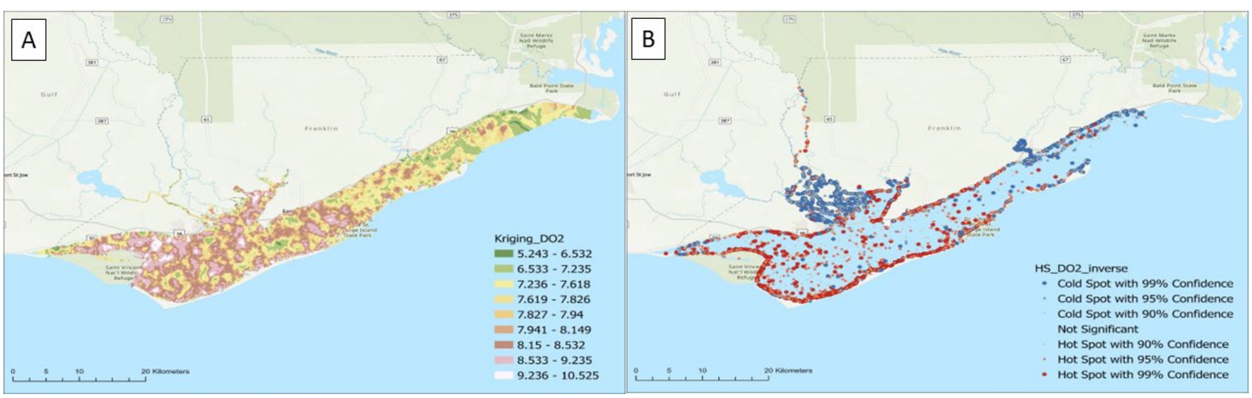


Figure 3. A) Ordinary Kriging and B) Optimized Hotspot Analysis of dissolved oxygen (DO_2) in Apalachicola Bay from 2005 – 2022.

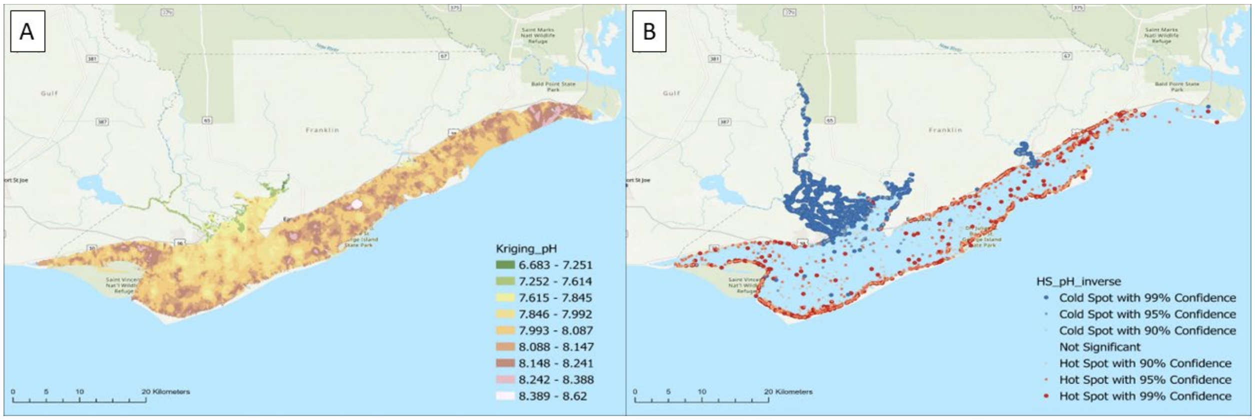


Figure 4. A) Ordinary Kriging and B) Optimized Hotspot Analysis of pH in Apalachicola Bay from 2005 – 2022.

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

Introduction. This portion of the ABSI project includes analysis of fresh-water inflow from the Apalachicola-Chattahoochee-Flint (ACF) watershed into Apalachicola Bay. 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 Bay and 2) Fresh-water inflow from the ACF basin plays a major role in defining the salinity regime of the Bay and it was hypothesized that high salinity played a role in the collapse of oyster populations. When ABSI was initiated, the U.S. Army Corps of Engineers had recently 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 Bay was not considered by the Corps in developing the reservoir management plan for the watershed because they consider the ACF basin and the Gulf Intercoastal Waterway as two separate projects. Consequently, the preferred alternative for managing the Federal storage reservoirs in the Manual did not consider its impact on Apalachicola Bay. Ultimately, the State of Florida lost the Supreme Court case because they could not prove Georgia had caused harm to the estuary.

Under ABSI, the ACF STELLA river basin model has been used 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) potential changes to the Water Control Manual that would enhance the sustainability of the Apalachicola Bay oyster populations. A report on the hydrologic basis of the Supreme Court was made to the CAB in 2021-2022 and it was concluded that the hydrologic basis for the lawsuit was weak.

Methods. The Water Control Manual was developed using an unimpaired flow set that 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, Dr. Letiman, Dr. Manuela Bruner, (Institute for Atmospheric and Climate Science, Switzerland) and Dr. Ebrahim Ahmadisharaf, (FSU/FAMU) collaborated to develop 100 different stationary realizations of the historic climate with different magnitudes, frequencies, durations and timing of flood and drought events using a program called PRSim. These realizations maintain the same volume of water that 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.

Work has also focused on developing metrics to define acceptable flows to the estuary and investigating the capacity of the reservoir system to provide pulses during low-flow events and then testing the effects of these pulses in the estuarine model. These analyses have indicated that there is sufficient storage capacity for additional flow management to potentially improve river flows into the estuary. Before ABSI, a model was developed to test the capacity of the storage reservoirs to protect tupelo trees by providing water pulses to the Apalachicola River floodplain during dry periods. This tupelo model was used to assess potential benefits of water pulses to oyster populations through salinity reduction during low flow years. Specific metrics have been developed to help define flow regimes that will provide optimal benefit to the oyster populations in Apalachicola Bay.

Results and discussion. A manuscript detailing the results of this work entitled *Multi-reservoir system response to alternative stochastically simulated stationary hydrologic scenarios: An evaluation for the Apalachicola-Chattahoochee-Flint (ACF) Basin* has been submitted to the journal 'Earth Futures' for publication. In summation, specific problems associated with Water Control Manual's preferred alternative when evaluated under alternative climatic scenarios include draining the storage pool (i.e. Lake Lanier) that supplies Metro Atlanta in extreme events (Fig. 5) and significantly increasing the duration and frequency of low-flow events into the Bay (Fig. 6).

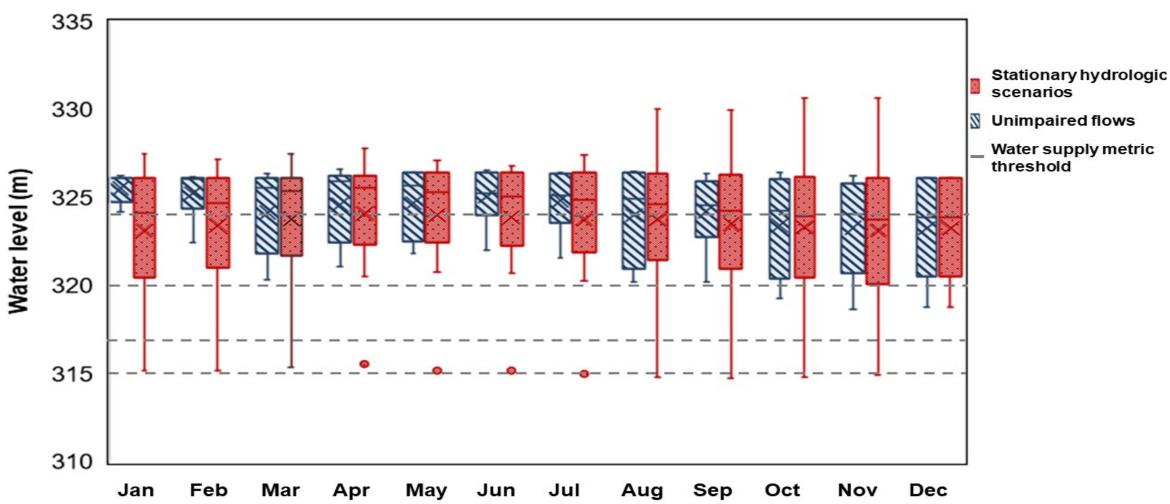


Figure 5. Water levels at Lake Lanier under observed historical (unimpaired) flows and simulated (stationary hydrologic) scenarios using the Army Corps of Engineers Water Control Manual

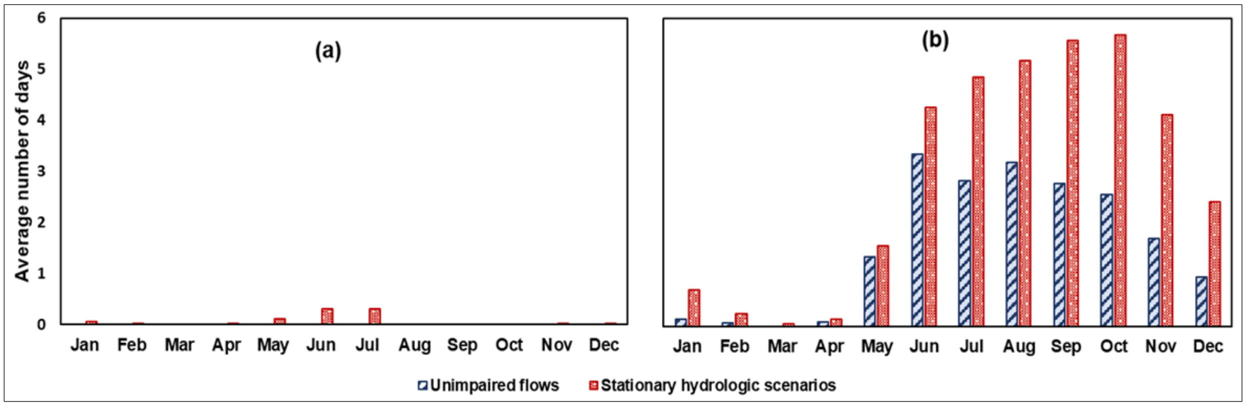


Figure 6: Average number of days/month that Jim Woodruff outflows were at or below the: (a) emergency drought threshold (141.6 m^3/s); and (b) drought threshold (169.9 m^3/s). Graphs compare historically observed (unimpaired) flows and modeled (stationary hydrologic) scenarios

Tupelo trees support a small but lucrative honey industry that is centered around the Apalachicola floodplain. The tupelo trees need floodplain inundation during their growing season but in recent years there have been increasing periods of low flow (Fig. 7), which impacts the trees and thereby the honey industry. The tupelo model shows that there is sufficient storage capacity in the combined reservoir system to allow the release of pulses of water to protect the tupelo trees in the floodplain (Fig. 8). The model ran pulses of water released after 40, 50 and 60 days of low flow (i.e. with no floodplain inundation). The optimal frequency and duration are still unclear. Preliminary trials using this model have shown some potential benefits to releasing pulses of water into the Bay during oyster spawning season to maintain optimal salinities during this vulnerable period.

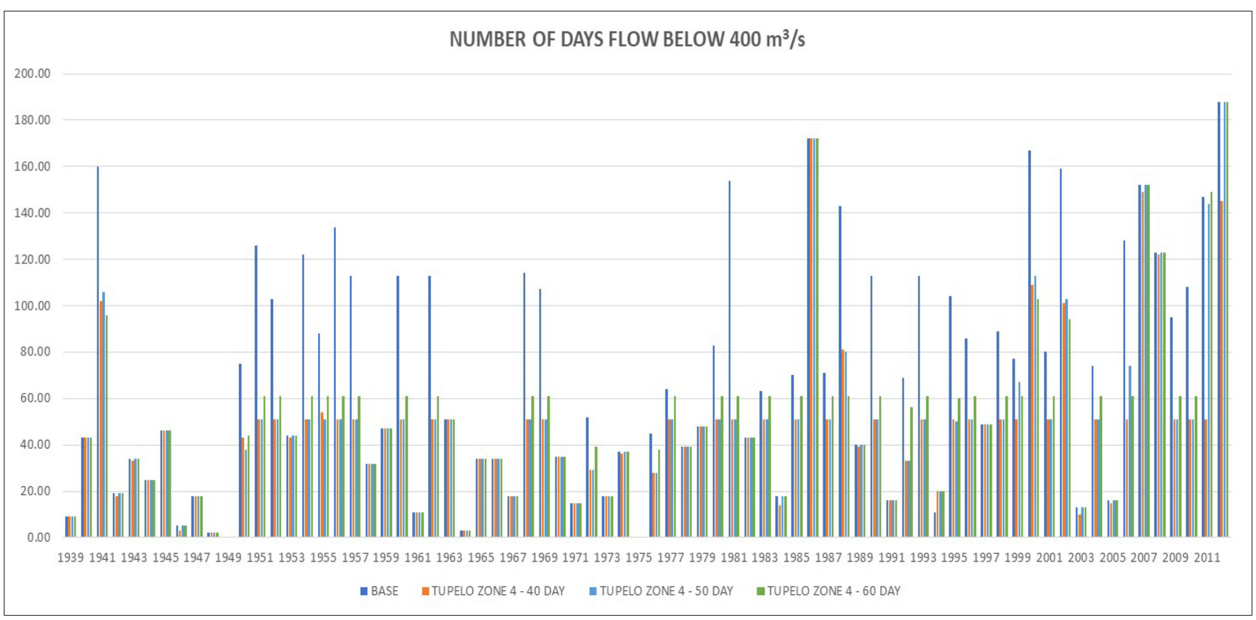


Figure 7. Maximum number of consecutive days flows did not flood riverbank levees of the 'Tupelo zone 4' of Apalachicola River.

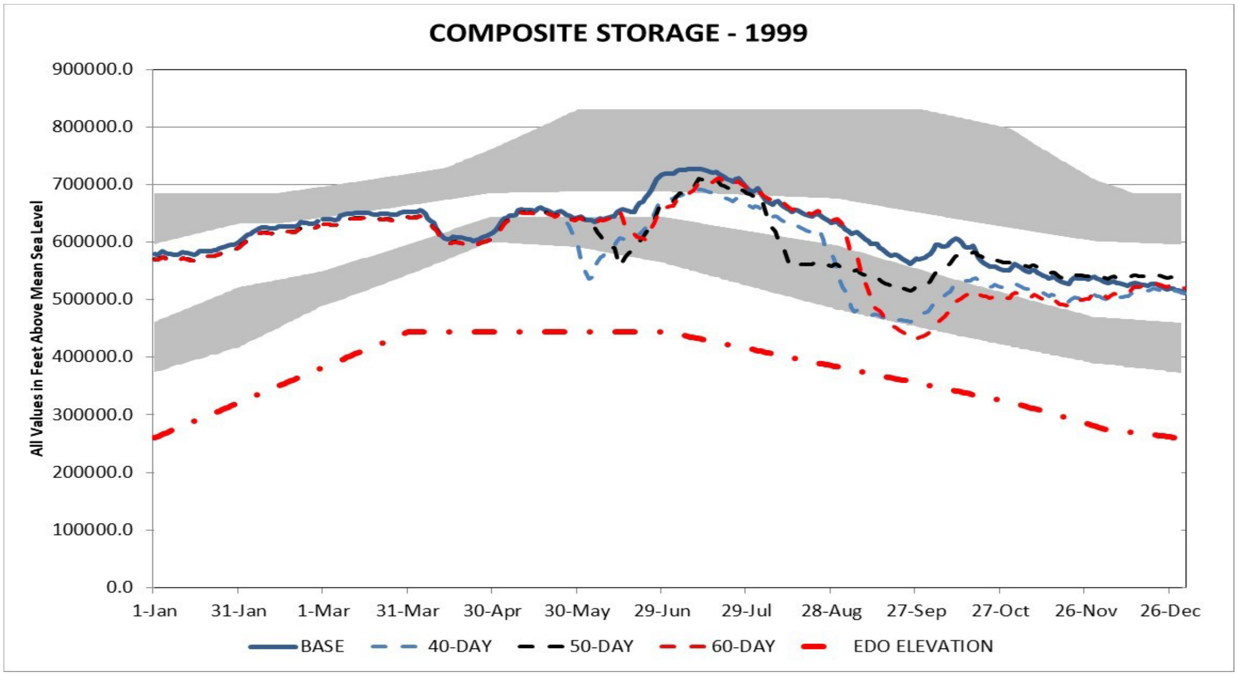


Figure 8. Effects on composite storage in Lake Lanier, West Point Lake and W.F. George Lake from adding pulses to inundate the Apalachicola River's floodplain.

Utilizing the river basin model as input into the estuarine salinity and ecological models has proven 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. River basin work has 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 operational, 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, and Dr. Xu Chen, Florida A&M University)

Introduction. 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. A hydrodynamic model is developed 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 freshwater 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.

An individual-based model (IBM) simulates oyster larvae as a set of Lagrangian particles, each representing a group of larvae traveling together. This model is configured for this application using the FVCOM I-State Configuration Model (FISCM) and driven by the results of the hydrodynamic simulations. Larvae are advected from their release locations in the 3-dimensional velocity field with mortality parameterized based on the ambient salinity. At present, the mortality is considered zero when salinity is in the range $6<S<27$, and the mortality rate is 0.95/7 days when the salinity is outside of this range. This parameterization is being refined based on information gathered from other ABSI studies. Larvae are advected for 20 days and can settle if they encounter reef locations during their last 5 days. The results are analyzed to provide information on the overall survival of larvae, locations spawning successfully recruited larvae, and locations receiving larvae. The IBM and analyses of results are performed for the different hydrodynamic model scenarios.

Results and discussion. 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. The IBM has been run for each of these time periods. Though the hydrodynamic simulation is still being refined for the anomalously dry period (2011-2012) based on recent knowledge of river flow diversions through the intracoastal waterway and local evaporation, the normal and anomalously wet period simulations compare well to observations.

Results from analyses of the IBM runs for the anomalously wet (1998) and climatologically normal (2019) years demonstrate how the inhospitable low salinity conditions can affect larval survival across the bay (Fig. 9 and 10). The simulations predict that substantially more larvae survive to settlement during the hydrologically "normal" 2019 simulation than during the anomalously wet 1998 simulation due to the IBM formulation of larval mortality at extreme low salinity. A greater fraction of larvae survives to settlement during the spring release simulation than the autumn release simulation in both years. Spatial patterns of settlement, as well as release locations producing larvae that survive at greater rates, differ among simulation time periods. Notably, successful settlement in the eastern part of the bay is more probable during the spring time period than during the autumn.

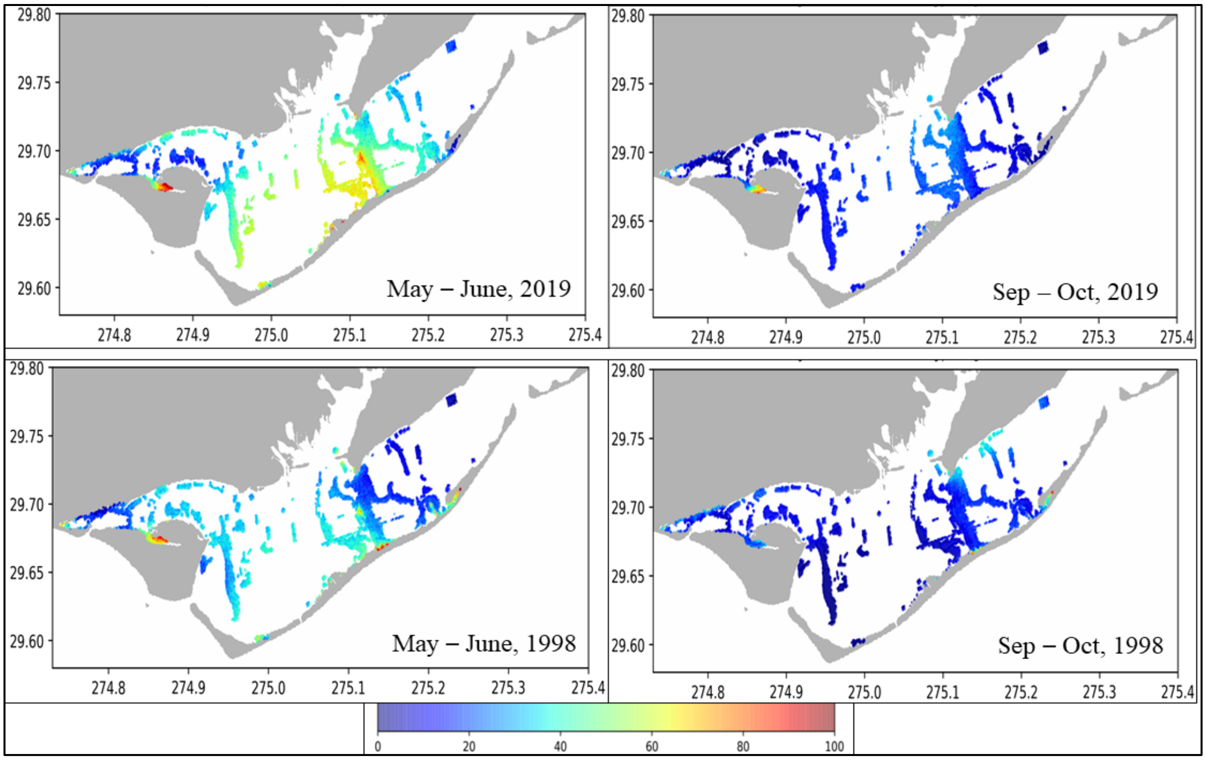


Figure 9. Larval release locations showing percent larval survival over a 20-day period for the spring (left) and autumn (right) in 2019 (top) and 1998 (bottom). Survival is color coded according to the scale bar with blue = 0% and red = 100%.

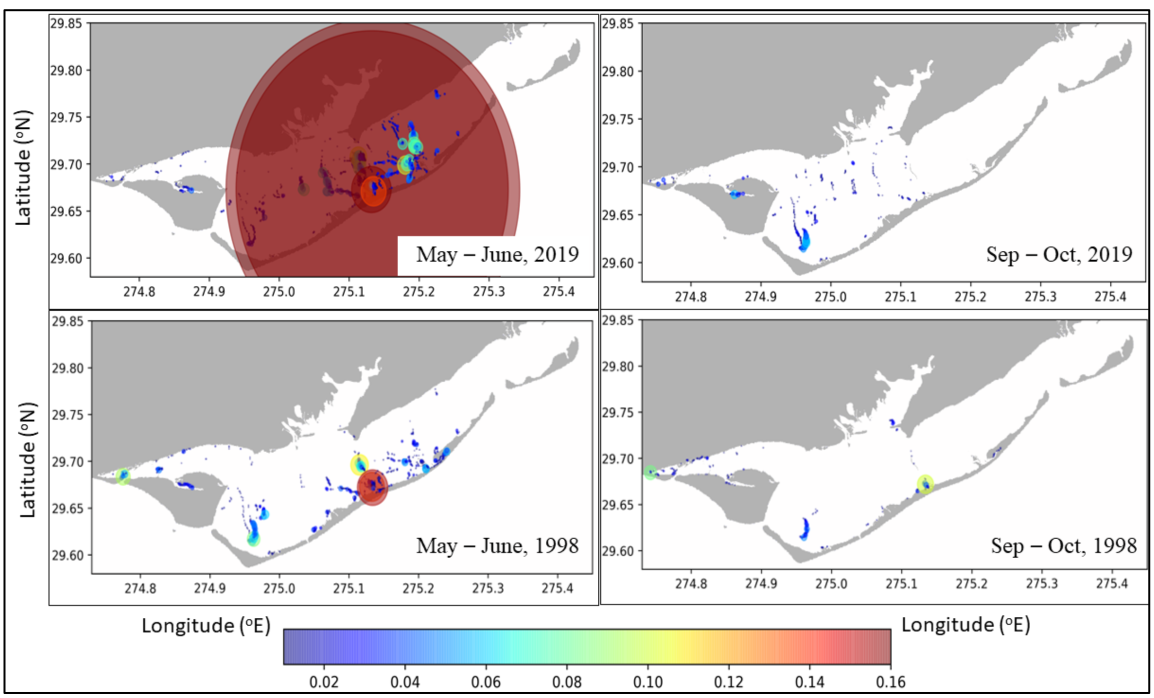


Figure 10. Percent larvae settling on different locations. Spring (left) and autumn (right) in and normal year, 2019 (top) and a wet year, 1998 (bottom). Circle size indicates relative importance of settling location. Settlement is coded according to the scale bar with blue = 0%; red = 100%.

Future work will include refining the dry period hydrodynamic simulations to better simulate the observed high salinity in the bay. Recent developments have revealed that a substantial amount of water from the Apalachicola River is diverted westward in the intracoastal waterway, which can strongly affect the freshwater delivery to the bay during low-flow times. This requires that the flow measured at the gauge upstream of this diversion needs to be adjusted downward to account for the diversion. Additionally, the dry years also exhibit strong evaporation that contributes to freshwater loss, particularly during the dry spring season, requiring this to be included in the simulation. Vertical stratification also affects the simulated salinity at the measurement locations and, hence, adjustments are being made to the model's vertical mixing parameterization. Following these refinements to the simulation, the IBM will be run for the dry period to assess the impacts of the high salinity on the larval survival.

The mortality function in the IBM will be modified to include a more realistic transition of the mortality rate between optimal and sub-optimal salinity ranges. Results from the hydrodynamic model are being made available to other ABSI investigators for use in other projects, such as the development of habitat suitability models. Funding for this ABSI project has ended and all future work relies on securing funding from additional sources.

2.4 Predictive habitat suitability modeling (Adam Alfasso, Ph.D. student)

Introduction. While Apalachicola Bay has seen decades of effort by agencies and academic researchers to ascertain the cause of and possible solutions to the current state of the fishery (Camp et al., 2015; Coen & Luckenbach, 2000; Fisch & Pine, 2016; Pine et al., 2015; Seavey et al. 2011), a quantitative assessment of the system in a future scenario of temperature increases and sea level change is lacking. Simulations of future climate scenarios have been conducted in several estuarine systems (Eierman & Hare 2013, Altieri & Gedan 2015, Hewitt et al. 2016), and have proven useful when considering the restoration and management of degraded systems. In a future of elevated temperatures, precipitation patterns are expected to become variable with more extreme droughts reducing riverine input into estuaries and allowing greater incursions of saline ocean water (Graeff et al. 2013, Hegerl et al. 2014) and more frequent rain events generating very low salinity conditions. These changes have the potential to impact estuarine communities and change coastal ecosystems as sea-level rise causes inundation along coastal regions.

This research will construct a series of spatially explicit models that describe and evaluate the effects of changing environmental conditions on habitat suitability in Apalachicola Bay for the eastern oyster (*Crassostrea virginica*). The models will include predictive habitat suitability and distribution models describing the current state of oysters in the Bay, oyster larval distribution models that incorporate biological variables, a model incorporating anthropogenic needs to inform restoration efforts, and a suitability model incorporating predicted changes in Bay hydrodynamics based on the 2016 IPCC recommendations (Parris et al. 2012, Passeri et al. 2016).

The overarching goal of this research is to quantify effects of changing environmental variables on distribution of the eastern oyster, and their implications for future oyster restoration.

Objectives.

1. To create a spatially explicit predictive habitat suitability model for the eastern oyster in Apalachicola Bay.
    a. To evaluate individual effects of environmental variables on model performance.
    b. To create biologically derived variables for evaluation and inclusion into distribution model.

2. To incorporate anthropogenic considerations into models to inform restoration and management efforts of the eastern oyster.
3. To 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 comprehensive ensemble-based modelling approach.
H2: Incorporation of biological variables will 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. Habitat model construction has been delayed by problems with the hydrodynamic model (section 2.3) discrepancies between modelled and observed environmental data. At this time, the model variability is too high at critical ecological thresholds to justify using for product creation. While this aims to be rectified in the near future, efforts have instead been focused on the creation of alternative data layers for testing the ensemble model process by using datasets collated from multiple sources (ANNER, FIMS, ABSI, National Oceanographic and Atmospheric Administration [NOAA]), which are then interpolated at a 100-m resolution within the bay. These alternative datasets will allow progress on model formulation processes and evaluation. When the high-resolution environmental datasets are within tolerable limits, they can then be integrated seamlessly to replace the lower resolution data.

Due to an increase in data density and quality, the modelling technique used for this research has changed from using a single approach (MAXENT) to an ensemble-based modelling approach. Ensemble modelling involves combining predictions from multiple habitat suitability models into a single predicted variable, generally an averaged layer of predictions weighed by the Area Under the Curve metric, although other evaluation metrics can be used (Kaky et al. 2020). This weighting approach is used to compare model performances so poorly fit models will be penalized before being included in the averaging, giving them less power in the final aggregated model. The ensemble (combined) model theoretically should produce more accurate and robust predictions than any single model could alone (Marmion et al. 2009). It specifically has been advocated for as a better alternative to single models for future climate projections, (Araujo & New 2007), as a lower mean yield error is expected from the combination of techniques. An ensemble of six models is being used: Generalized Linear models (GLM), Generalized Additive models (GAMS), Multi-variate Adaptive Spline (MARS), Maximum Entropy (MAXENT), Boosted Regression Tree (BRT), and Artificial Neural Networks (ANN).

These models will still use the same derived environmental and biological data layers as the singular MAXENT model and can incorporate future climate change scenarios (Passeri et. al 2016) using the package 'biomod2' in the R statistical analysis program (Thuiller et al. 2021). Restoration and management scenarios of Objective 2 will be conducted using ArcGIS Pro 2.9 (Marine Geospatial Ecology, Benthic Terrain Modeler, Spatial Analyst toolboxes).

Agency data and ABSI tong sampling from 2023 will be used to augment existing oyster presence/absence data in Apalachicola Bay to support model development and groundtruthing outcomes. The Ensemble models will be formulated for habitat suitability using data from the hydrodynamic models (Section 2.3) for wet, dry, and 'normal' years and from the in interpolated

FWC FIMS data (section 2.1). Finally, the model outputs will be ground-truthed for accuracy.

3. Oyster biology

3.1. Population genetic structure of the eastern oyster along the Florida Gulf Coast (Dr. Amy Baco, Dr. Nicole Morgan, FSU)

Introduction. 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, population genetic data was obtained from samples collected by ABSI from 10 sites. These sites 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 provides data on connectivity among sites within the Bay, and subpopulation structure within the Bay. The results of this study will therefore inform management of the oyster populations within Apalachicola Bay.

Methods. Oysters were sampled by hand 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. Oyster shells were measured for fan, cup, and dry weight to compare shell morphologies between populations as environmental effects can cause differences in oyster shells (Combs et al., 2019; Hajovsky et al., 2021). Ten loci were amplified using polymerase chain reaction (PCR) with primer sequences and protocols from previous work on this species (Wang and Guo 2007). One locus, (RUCV01) amplified twice (a large and small size class) in most individuals, thus eleven total loci were amplified for 223 individuals at ten different locations in the Gulf of Mexico (Fig. 11). PCR products were sent to the University of Arizona Genetics Center for genotyping.

Microsatellite Analysis. 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). Null allele frequencies and genotyping error were analyzed using Micro-checker (van Oosterhout et al 2004). Linkage disequilibrium was analyzed using Genepop 4.2 online (Raymond and Rousset, 1995; Rousset, 2008). A power analysis was run to test the ability of these loci to find population differentiation. First, effective population size (N_e) was estimated using the molecular coancestry method from NeEstimator v2 (Do et al., 2014). Results showed a wide range per population of effective population size, so power analysis, using POWSIM v4.1 was run twice: once with an N_e of 20 and another time with an N_e of 40, with 1000 replicates for both runs.

Summary statistics for populations and loci were calculated using a number of statistical packages (Zahl 1977, Jombart 2008, Adamack and Gruber 2014, Konopinksi 2020). Tests for per-locus, per-population Hardy-Weinberg Equilibrium (HWE) were run (Paradis, 2010) and population pairwise genetic distance (G'_{ST}) values were calculated (Winter, 2012). Population differentiation by location was analyzed using Analysis of Molecular Variance (AMOVA).

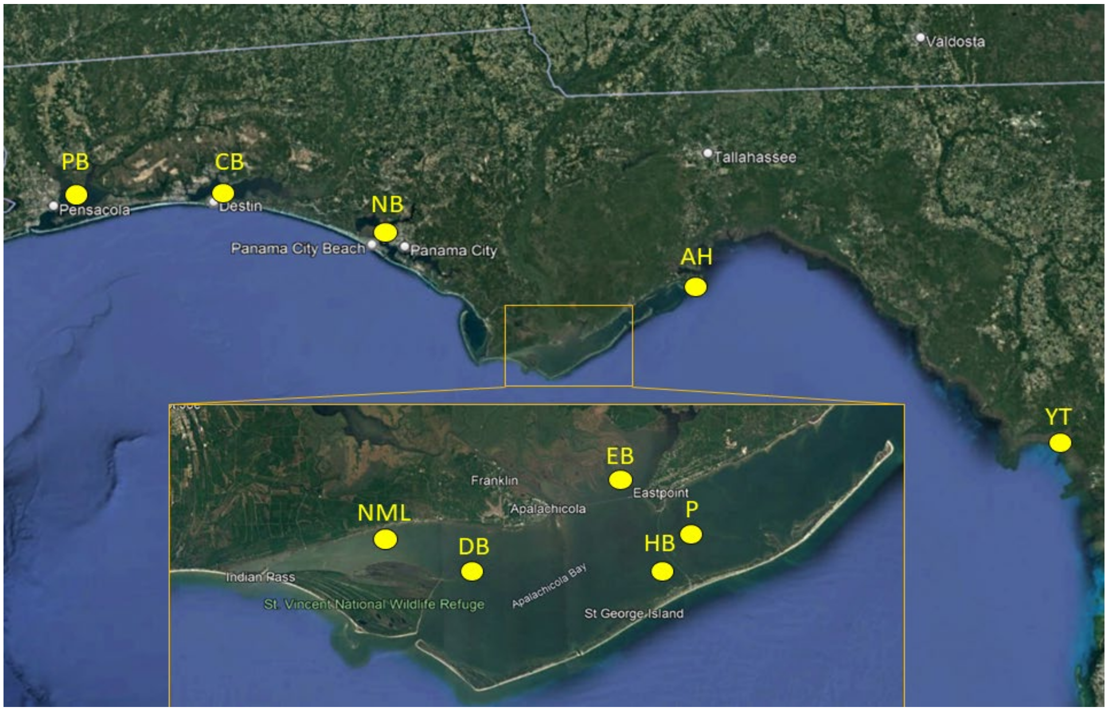


Figure 11. Map of oyster populations sampled in the Gulf of Mexico. PB = Pensacola Bay, CB = Choctawatchee Bay, NB = St. Andrews Bay North, NML = Nine Mile Lump, DB = Dry Bar, EB = East Bay, P = Platform, HB = Hotel Bar, AH, Alligator Harbor, YT = Yankee Town.

Results and discussion: All eleven loci were highly variable (12 – 70 alleles per locus), especially RUCV 61 with 70 alleles. Observed heterozygosity (H_o) was generally much lower than expected heterozygosity (H_e) for all loci. Allelic diversity was also high by site (Table 2), and the number of private alleles (those found only in one population) ranged widely between locations (3 – 26), though the values did not show a spatial trend (Table 2). The lowest number of private alleles occurred at Alligator Harbor (AH), which is a more central location, and the highest number of private alleles occurred at both Platform (P) in Apalachicola Bay, and Yankeetown (YT) the southeastern-most location. Power analysis showed the 11 loci have a strong ability to detect genetic differentiation at p-value <0.05 (χ^2=1.000, Fisher's exact test = 1.000). H_o was much lower than H_e for all locations, which is likely somewhat influenced by the presence of null (non-functional) alleles but could also be due to inbreeding. No locus was in Hardy-Weinberg Equilibrium across all populations, which is also likely at least partially related to the presence of null alleles in most loci. F_{IS} values are high across all sites (Table 2).

Table 2. Summary statistics by site

Pop	N	Number 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

Genetic differentiation (G'_{ST}) across all sites was low to moderate, 0.16 but significant ($p = 0.01$). Pairwise G'_{ST} values were also generally low to moderate, with only three comparisons having a G'_{ST} over 0.25 (Table 3). The AMOVA showed significantly greater variation than expected between populations and between samples within populations ($p=0.01$ for both comparisons).

Table 3. Pairwise G'_{ST} values show genetic distance between populations. Values greater than 0.25 indicate moderate differentiation and 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

The PB and DB sites are separated from the remaining populations as shown in the scatterplot (Fig. 12). PB is the westernmost site, which can explain the population separation. However, DB and EB are more differentiated from the other Apalachicola Bay locations than expected. Admixture analysis by site shows much lower mixing than would be expected for CB, P, NML, and EB (Fig. 13) compared to the overlap in the scatter plot. NB and HB showed considerable mixing between the two populations, despite being separated by more than 100 km, and AH and YT also showed high mixing though the populations are nearly 180 km apart. The relationship between distance and mixing among these populations is apparently complex.

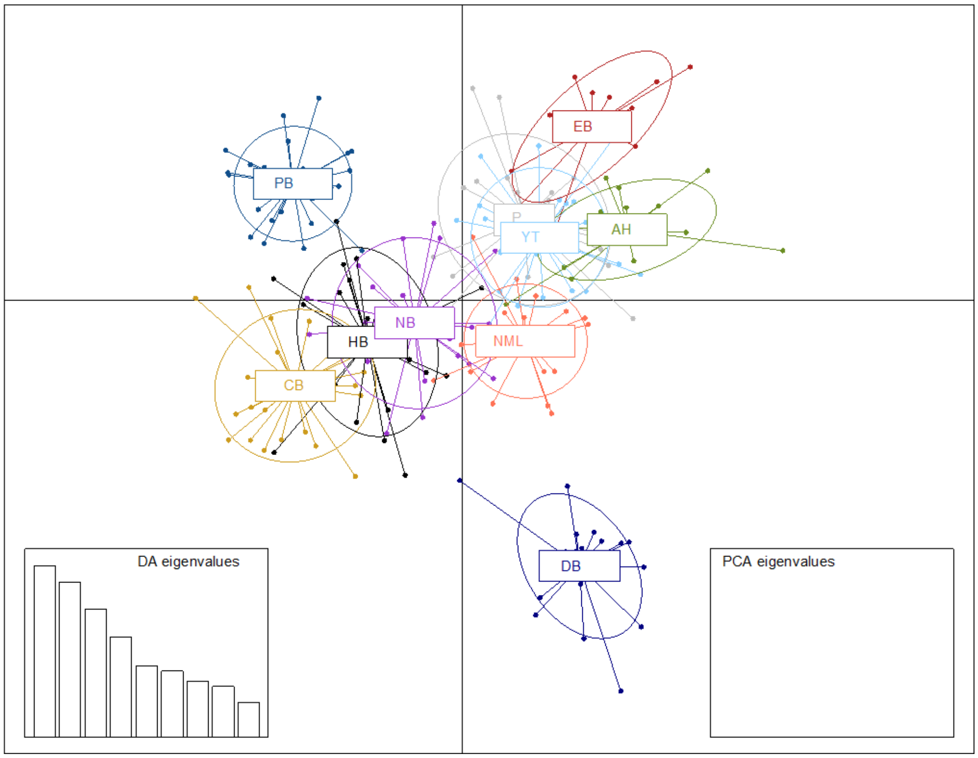


Figure 12. Scatterplot showing genetic relationship between sites. Each point is a sample, and the population name is in the center of the 95% confidence interval denoted by the oval.

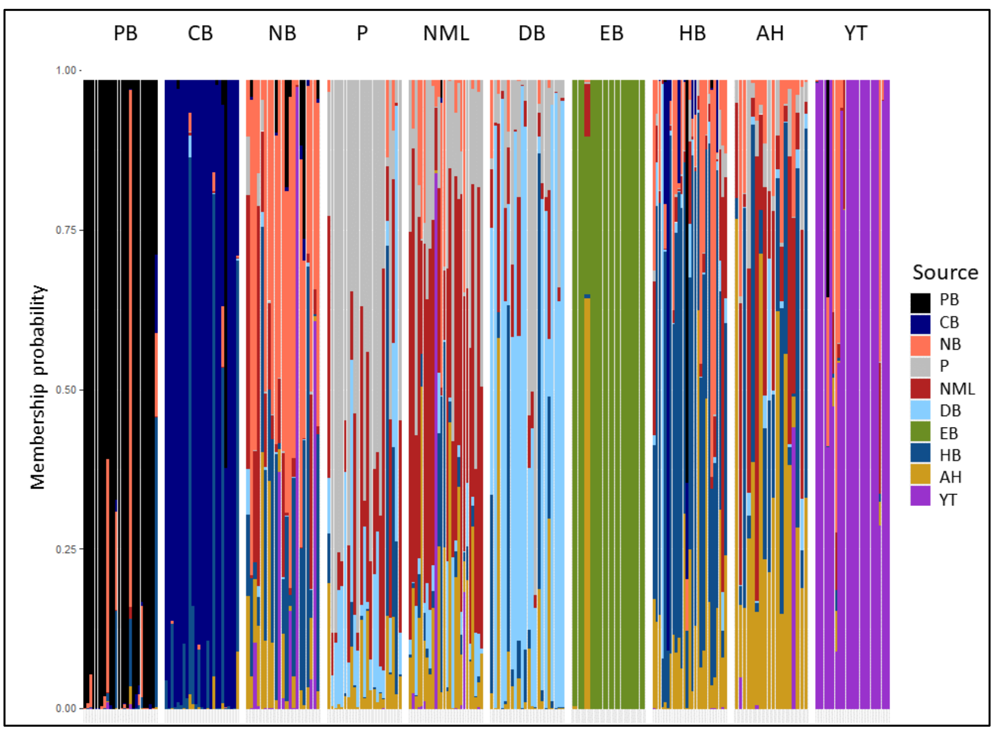


Figure 13. Admixture comparison plot to show likelihood of genetic exchange between populations. Collection sites are shown on columns, and the legend shows likely larval source populations. Each bar within the columns represents a single oyster.

Early studies of oysters in the Gulf of Mexico using various genetic markers indicated at least 3 populations were present. In the western Gulf two populations overlap in the region of Corpus Christi and Aransas Bay (e.g. Anderson et al 2014, TWPD, pers. comm.). In the Eastern Gulf of Mexico sampling has been sparser with most studies only including Cedar Key and one location in Florida (Apalachicola Bay), along with Grand Isle, Louisiana. Depending on the markers used, the affinity of these populations to the populations on the Atlantic coast of Florida and to each other tends to vary, as does their level of connectivity to the populations in the western Gulf (e.g. Varney et al 2009, TWPD, pers. comm.). In the current study, the finer-scale geographic sampling and the use of microsatellite markers together provide higher resolution data to improve our understanding of the connectivity of Apalachicola Bay populations to the broader Gulf.

A surprising amount of genetic variation and genetic structure were found among the populations of this study despite the surveyed area covering only ~4-5 degrees of longitude, with the Apalachicola Bay in particular having a high degree of fine scale differentiation. Pairwise G'_{ST} values ranged from 0.05 to 0.30 (Table 3), indicating little to moderate genetic structure between sites. F_{IS} values were also surprisingly high, ranging from 0.34-0.46, suggesting moderate levels of self-recruitment at all sites. F-statistics may be inflated by using microsatellite loci with null alleles (Chapuis and Estoup 2007), as were used in this study. However, since null alleles and low levels of heterozygosity are common in bivalves and in *Crassostrea* (reviewed in Galindo-Sanchez et al 2008), this caveat is hard to avoid. High F_{IS} values may also be caused by the Wahlund effect, but since samples were taken over such a small area at each site, it is unlikely multiple populations were sampled at any location. The low levels of admixture indicated in the plot (Fig. 13) also support high levels of self-recruitment. The scatterplot (Fig. 12) analyses are less affected by null alleles than are F-statistics (Jombart et al 2010), and therefore are likely the least biased of the results presented here. Most of the sampled sites fall into a core group on the plot with two major outliers, PB and DB.

Sample site YT was included as one of the northernmost known locations of the previously described southern Florida stock (e.g. Varney et al 2009, TWPD, pers. comm.). Pairwise G'_{ST} values of all other sites in comparison to this site ranged from 0.10-0.18 (Table 2), indicating some genetic differentiation of the other sites from YT, but probably not enough to consider them different genetic stocks. HB and NB had the lowest pairwise G'_{ST} values when compared with YT, but AH showed the highest level of admixture with YT (and vice versa), with a small amount of NML admixture also occurring at YT. In the scatterplots, YT was most similar to P and AH (Fig. 12). Among the Apalachicola Bay sites, only NML had individuals that could potentially be assigned to the YT population (Fig. 13). At the opposite end of the geographic range, the westernmost sampled site, PB, showed little similarity and little mixing with any of the other sampled sites. Four of the nine pairwise comparisons between PB and the other sites (including three Apalachicola Bay populations), show G'_{ST} values ≥ 0.2 (Table 3). These results suggest there is infrequent genetic exchange between PB and most of the Apalachicola Bay sites

Two sites within Apalachicola Bay, EB and DB, also showed surprisingly little genetic exchange with other sites, or with each other. DB stands out as an outlier in the DAPC plots and had 5 out of 9 of its pairwise comparisons to other sites show G'_{ST} values ≥ 0.2. EB had 4 out of 9 with values ≥ 0.2 and fell on the periphery of the core group in the plot, most similar to YT and P. Each site only showed 1-2 individuals that could potentially be assigned to other populations in the admixture plots (Fig. 13). Thus these sites appear to have some degree of isolation compared to each other and to the other Apalachicola Bay sites. Site P within Apalachicola Bay shows an intermediate amount of connectivity. Although it shows little admixture in the plot (Fig. 13), with a small amount of potential exchange with site NB and NML, in the scatterplot it overlaps strongly with YT, and the only pairwise comparisons that showed G'_{ST} values $\geq$ 0.2 were with PB, CB and HB (Table 2). In contrast, the other Apalachicola Bay sites, NML and HB show more connectivity to other locations. NML had 0 pairwise G'_{ST} comparisons >0.2 (Table 3), falls in a central location in the scatterplot (Fig. 12), and shows some admixture with several sites (Fig. 13). HB shows strong admixture with NB, (and vice versa), shows strong overlap in the scatterplot with NB (Fig. 12), and only had G'_{ST} values greater than 0.2 in comparison to EB and P.

Some of the caveats of using loci with null alleles can be reduced by reanalyzing the data without the null allele loci. However this also reduces the resolving power of the data to be able to distinguish genetic structure. The current dataset was reanalyzed with the only 4 loci that had no null alleles and also with the 6 loci with the least evidence of null alleles (not shown). The overall conclusions from these analyses are largely consistent with the use of the full dataset. In the results with fewer loci, the evidence for isolation of PB and EB from the other populations is stronger, while the isolation of DB depends on the number of loci used, (with 6 loci indicating more isolation than 4). HB and NML are consistently well connected, and P shows intermediate connectivity compared to the other Apalachicola Bay sites.

These results suggest that Apalachicola Bay harbors multiple populations of oysters that are generally more similar to populations to the east of the Bay than to PB. These populations experience a notable amount of self-recruitment, and DB and EB in particular do not have high levels of exchange with other populations. For protecting and restoring the Apalachicola Bay populations the most effective method would be to preserve a portion of each population, rather than expecting rapid repopulation from outside sources. Similarly, restoration efforts should incorporate local populations to help preserve local genetic diversity. Some of the genetic patterns may indicate adaptation to local environments within the Bay. Finer-scale sampling within the Bay and surrounding areas using higher resolution genetic markers would yield further insights into connectivity patterns as well as local adaptations due to selective pressures.

3.2 Pathogen transmission and disease impacts on oysters (Dr. Tara Stewart Merrill, FSUCML)

Introduction. Oysters can be infected by a diverse array of parasites and pathogens, some of which can cause problematic diseases. Of notable interest to both oyster fisheries and restoration programs is the pathogen, *Perkinsus marinus*, a protozoan that causes “Dermo disease”. Dermo disease is present in oysters along the Eastern seaboard and across the Gulf of Mexico (GoM). While Dermo epidemics are known to cause largescale oyster die-offs in the Northeastern United States (e.g., the Chesapeake Bay), considerably less is known about the impacts of Dermo on oyster individuals and populations in the Gulf of Mexico. It is generally thought that Dermo disease is less virulent (causes less harm) in the Gulf of Mexico. This assumption warrants testing to determine if and why local infections are less harmful to oysters, and whether the outcome of infection might change under future environmental scenarios.

The overarching goal of this project is to compare patterns of *Perkinsus marinus* infection between Apalachicola oysters (with empirical data) and Northeastern oysters (with data from the primary literature). To accomplish this goal, the Stewart Merrill lab is evaluating transmission-related metrics in sub-tidal and intertidal Apalachicola oysters and investigating relationships among infection and oyster health parameters (body size, condition, mortality).

Methods. This project relies on three data sources: 1) existing data collected by the Florida Fish and Wildlife Conservation Commission (FWC) on subtidal oysters; 2) new data collected on intertidal oysters; 3) existing data from the primary literature. In brief, the FWC collected *Perkinsus marinus* infection data from 3,157 oysters from 12 subtidal sites between 2016 and 2019. The FWC dataset contains body sizes (shell heights in *mm*) for each oyster, presence and intensity of *P. marinus* infection, and associated datasets provide estimates of environmental parameters (temperature, salinity, dissolved oxygen) and body condition of proximal oysters at the time of each collection. In 2022, ABSI and the Stewart Merrill lab collected similar data (oyster body size and presence and intensity of infection) from 658 oysters from four intertidal sites. Additionally, we estimated condition for each oyster that was also assayed for infection to investigate whether individual body condition was associated with disease severity. With these data, a series of general and generalized linear mixed models were constructed to explore questions on *P. marinus* transmission and impacts (see results). We use data from the primary literature to compare our results to general trends observed in the Northeastern United States.

Results and discussion. A classic pattern in disease ecology is an increase in probability of infection with body size (because older, larger individuals have accumulated greater exposure to parasites over time). We observed this same pattern in Apalachicola oysters: larger oysters are more likely to be infected by *P. marinus* and have a 50% probability of infection at approximately 60 *mm* shell height. Interestingly, we found that the intensity of infection (the within-host pathogen load) did not exhibit a relationship with body size, suggesting that *P. marinus* infections are not simply growing as their hosts grow. Rather, within-host replication of *P. marinus* may be controlled by environmental conditions and/or host condition, and we are currently investigating these two possibilities with further analyses. We also found that Dermo disease in Apalachicola Bay differs substantially from Northeastern populations in its temporal dynamics (Fig. 14). While oysters in the Northeast tend to experience annual cycles in infection presence and severity (with Dermo disease peaking in the late summer months and declining in the winter), Dermo prevalence and intensity are fairly constant over the annual cycle in Apalachicola Bay, with over half of the oyster population infected at any given time. This constant and high level of prevalence suggests that transmission is occurring continuously, which may be facilitated by warmer temperatures in the Gulf of Mexico (a hypothesis we are currently pursuing with additional analyses). When transmission is continuous, it can be challenging to detect disease-caused mortality (because mortality is likely also occurring continuously). Nonetheless, we have found some associations between the abundance of Dermo within site and the proportion of the population that is dead at that site. In collaboration with the ABSI project leads, we are building survivorship curves for Apalachicola oysters and will use these curves to more robustly examine whether Dermo disease is impacting oyster survival. We have not detected major differences in patterns of infection between subtidal and intertidal habitats.

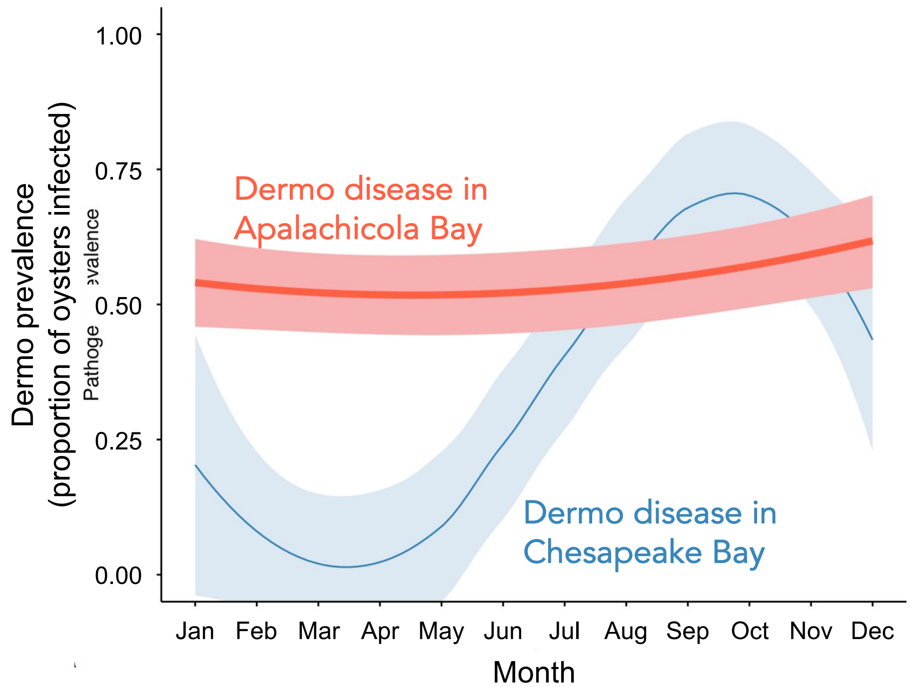


Figure 14. Prevalence (proportion of oysters infected) of Dermo disease over the annual cycle in Apalachicola Bay (red line) compared to Chesapeake Bay (blue line). Prevalence in Apalachicola Bay is fairly constant over time, suggesting continuous transmission processes.

The Stewart Merrill Lab is conducting two experiments in the near future that will refine our understanding of the patterns described above. In Spring 2023, David DuBose (Stewart Merrill Lab research technician) will lead a six-week experiment with 114 intertidal oysters examining how Dermo presence and intensity affects oyster feeding rate, growth, condition, and survival. The results of this experiment will illustrate how oyster vital rates are affected as *P. marinus* replicates. Moreover, the study will offer insight into how ecosystem services offered by oysters may be impacted by Dermo disease (e.g., if infection reduces feeding rate, there may be downstream consequences for water filtration). In Summer 2023, Grace Westphal (Stewart Merrill Lab PhD student) will conduct an experiment evaluating effects of temperature on the oyster-*P. marinus* interaction. By generating thermal-performance curves for oyster and *P. marinus* traits, she will build models evaluating how disease outcomes may shift over a range of predicted future temperatures. For this work, Grace will be piloting a novel technique for tracking Dermo infections in individual oysters.

3.3 Oyster stress responses and physiological tolerances (Emily Fuqua, Ph.D. student, FSU)

Introduction. In Apalachicola Bay, eastern oysters (*Crassostrea virginica*) are exposed to highly dynamic and variable environments, both in space and over time. This environmental variation in water quality seems to be driven mainly by changes in salinity, DO, and pH in space and temperature and salinity fluctuations over seasons (Table 2, Figure 1). These drivers in environmental variation will influence oyster population dynamics throughout Apalachicola Bay via their physiological responses to these parameters, and it is critical to understand oyster tolerance and sublethal stress response to single and multi-stressor environments. Additionally, ontogenetic shifts in stress response or differences in reef-specific responses can influence population dynamics. Larval stages of oysters are known to be more sensitive to environmental conditions (Davis 1958, Davis and Calabrese 1964), and size of oysters can affect their tolerance to hypoxia, temperature, and salinity (Rybovich et al. 2016). Moreover, reefs in different locations respond differently to changes in salinity (Eierman and Hare 2016; Figure 2B). This work investigates how two of the main drivers of environmental variation, salinity and temperature, differentially affect oyster life stages in single and multi-stressor environments. The main aims of this work are to guide successful restoration efforts of *C. virginica* in Apalachicola Bay and to provide critical information about the population dynamics of the eastern oyster for management and conservation.

Objectives.

1. Characterize environmental variation in water quality in Apalachicola Bay to identify main drivers and key physical water parameters that may influence oyster population dynamics.
2. To identify ontogenetic shifts in tolerance that may impact oyster performance, and to provide life-stage specific data on the growth, survival, and energetics of oysters.
3. To determine consequences to growth and survival of single and multi-stressor systems for oyster life stages.

Methods. To begin addressing objective 1, data from ANERRS water quality loggers consisting of 5-year continuous times series of 6 sites in Apalachicola Bay were analyzed using univariate and multivariate approaches (NOAA NERRS, 2021). Generalized additive models (GAMs) were used to characterize seasonal trends in water quality parameters separately, and Principal Components Analysis (PCA) was used to determine which parameters drive environmental variation in Apalachicola Bay. Objectives 2 and 3 are being addressed using experimental approaches. Three life stages of oysters, larvae, spat (20-25mm), and adult (>40mm) were exposed to ecologically relevant gradients of temperature and salinity, and assessed for performance in growth, survival, and energetic responses (measured via respirometry and metabolomics). Interactive stressor effects are measured through a 3x3 factorial experiment and use the same metric as the single-stressor experiments. Data from experiments were analyzed using generalized linear models (GLM).

Results and discussion. Results from GAMs and PCA indicate that the salinity, DO, and pH drive principal component 1 (PC1), and temperature and salinity drive principal component 2 (PC2, Fig. 15, Table 4). Results indicate that the spatial variation in salinity, DO, and pH and the seasonal changes in salinity and temperature are the main drivers of water quality variation in Apalachicola Bay.

Table 4. PCA principal components (PC) with associated standard deviation, proportion variance explained, and cumulative variance explained

	PC1	**PC2**	**PC3**	**PC4**	**PC5**
Standard Deviation	1.597	1.078	0.941	0.518	0.369
Proportion Variance	0.51	0.232	0.177	0.054	0.027
Cumulative Explained Variance	0.51	0.74	0.92	0.97	1.00

An ontogenetic shift in oyster tolerance to salinity was found, where larvae were least tolerant to salinity and displayed optimal survival in moderate salinities (16 ppt). Adult oysters survived salinities 12-36 ppt, but only 75% survived at 4 ppt (Fig. 16A). Increases in salinity significantly increased energy expenditure in adult oysters (G_{-1}=-431.23, P<<0.001; Fig. 16B), and considering survival, these results potentially indicate a higher optimal salinity than larvae. Moreover, adult oysters from different reefs did not differ in survival across salinities but displayed a significant difference in energy expenditure (G_2=-114.97, P=0.025; Fig. 16). This indicates adult oyster tolerance is similar across reefs, but salinity could drive sublethal and reef-specific changes in growth or reproduction, which can influence population dynamics.

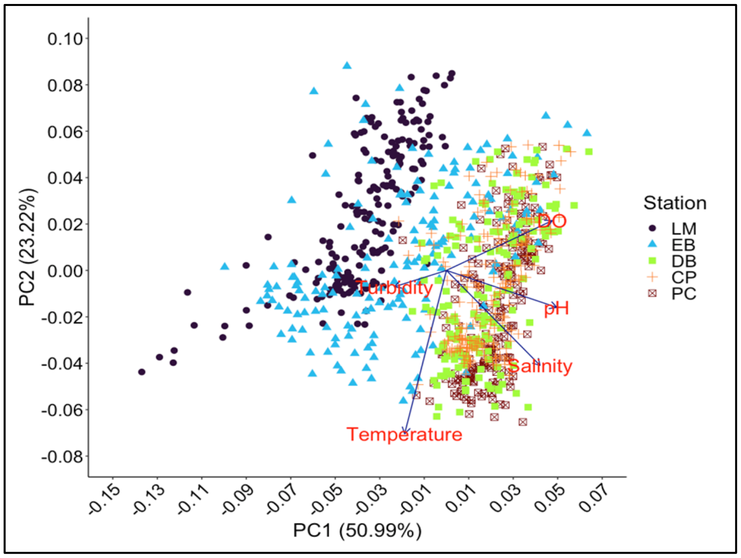


Figure 15. Ordination plot of PC1and PC2 with associated variable loadings (blue arrows) and labels (in red). Sites are indicated by different color and shaped points. Site legend: LM - Little Marks, EB - East Bay, DB - Dry Bar, CP – Cat Point, PC – Pilots Cove.

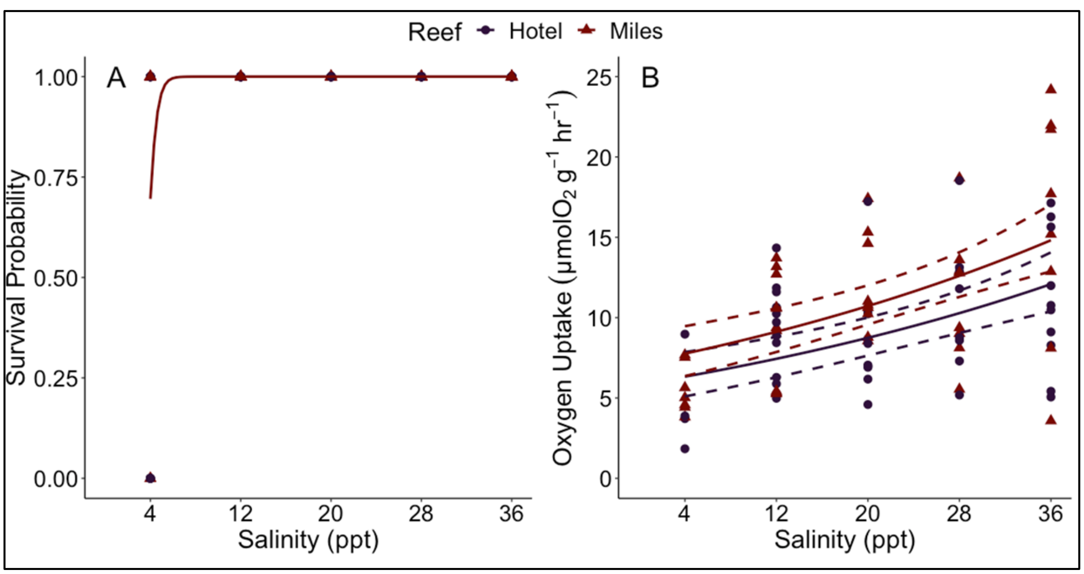


Figure 16. Survival probability (A) and oxygen uptake (B) of adult oysters from different reefs

across salinity. In A, points represent individuals that lived (p=1) or died (p=0), and lines represent the best fit binomial GLM. In B, points represent individual's respiration by reef. Solid lines representing the best fit GLM, and the dotted lines represent 95% confidence intervals.
The last experiments for this project will be finished in 2023-24. Moving forward, planned research includes investigating larval and parental carry-over effects. Carry-over effects from larval or parental environment have the potential to influence oyster performance to environmental stress, causing changes in their stress response, survival, and growth. Future research will begin assessing if Apalachicola Bay oysters are significantly impacted by these carry-over effects.

3.4 Effect of salinity on juvenile oysters (Donaven Baughman, Ph.D. Student, FSU)

Introduction. The goal of this project is to examine how sub-optimal salinity regimes in Apalachicola Bay impact oyster growth and vulnerability to predators, hypothesized to have contributed to the oyster population collapse. Gastropod oyster predators are more common in Apalachicola Bay at higher salinities (Kimbro et al. 2017, Pusack et al. 2018), and sub-optimal salinities impose physiological stress on oysters (Lavaud et al. 2017, Casas et al. 2018). Therefore, juvenile oysters may experience higher predation risk, while simultaneously possessing a lower ability to defend from predation as a result of altered physiological performance in sub-optimal conditions. Results from this project will clarify how fluctuations in bay salinity influence oyster growth and predation defenses, information that is critical for the adaptive management of oyster populations as conditions in the Bay change.

Methods. Recently settled oysters (~10mm) were reared in the presence or absence of actively feeding predators (*Stramonita haemastoma* – southern oyster drill) and in one of three salinity regimes (low: 8-15 ppt; medium: 18-25 ppt; high: 28-35 ppt) in 52L experimental aquaria systems at the FSUCML from August to November 2022. Juvenile oysters were sourced from a commercial shellfish supplier and adult *S. haemastoma* were collected from an intertidal oyster reef in Apalachicola Bay. Adult oysters were placed into the sumps of all aquaria systems, and an equal biomass of *S. haemastoma* was added to the sump of cue-present systems and allowed to consume the adult oyster. Consumed adult oysters were removed and replaced at a maximum of twice per week. Individual oysters were tagged, weighed, and photographed for growth analysis. Oysters were fed ad libitum with Reed Mariculture 1800 shellfish diet (2x manufacturer's instructions) to provide an abundance of food for the growing juveniles. Growth was determined as the difference in total oyster wet-weight between initial and final measurements. Shells were dried and weighed at the end of the experiment to determine shell weight of each oyster.

Results and discussion. Oysters reared under low salinity regimes experienced substantially more growth than oysters reared in medium and high salinity regimes (Fig. 17). Furthermore, oysters exposed to waterborne chemical cues from predatory oyster drills showed evidence of inducing morphological defenses against predation by growing heavier shells (Fig. 17).

To uncover the mechanism behind reductions in growth in medium and high salinity regimes, similar experiments will be conducted in fall 2023 in which measurements of algal concentration in the experimental systems over time will be collected. These measurements of algal density will reveal the impacts of salinity regime and predation risk on filtration rate of juvenile oysters. Results of these experiments will determine the effects of salinity and predation risk on the rate at which oysters obtain energy, which ultimately impacts their ability to grow and defend from predation. Additionally, field experiments will be conducted beginning in summer

2023 in which juvenile oysters will be grown to adult size in the presence or absence of waterborne cues from predatory gastropods at subtidal and intertidal locations in Apalachicola Bay. Results of the field experiment will provide an understanding of how predators influence the growth and allocation of oysters, and if the effects of predators on oysters varies spatially within the Bay.

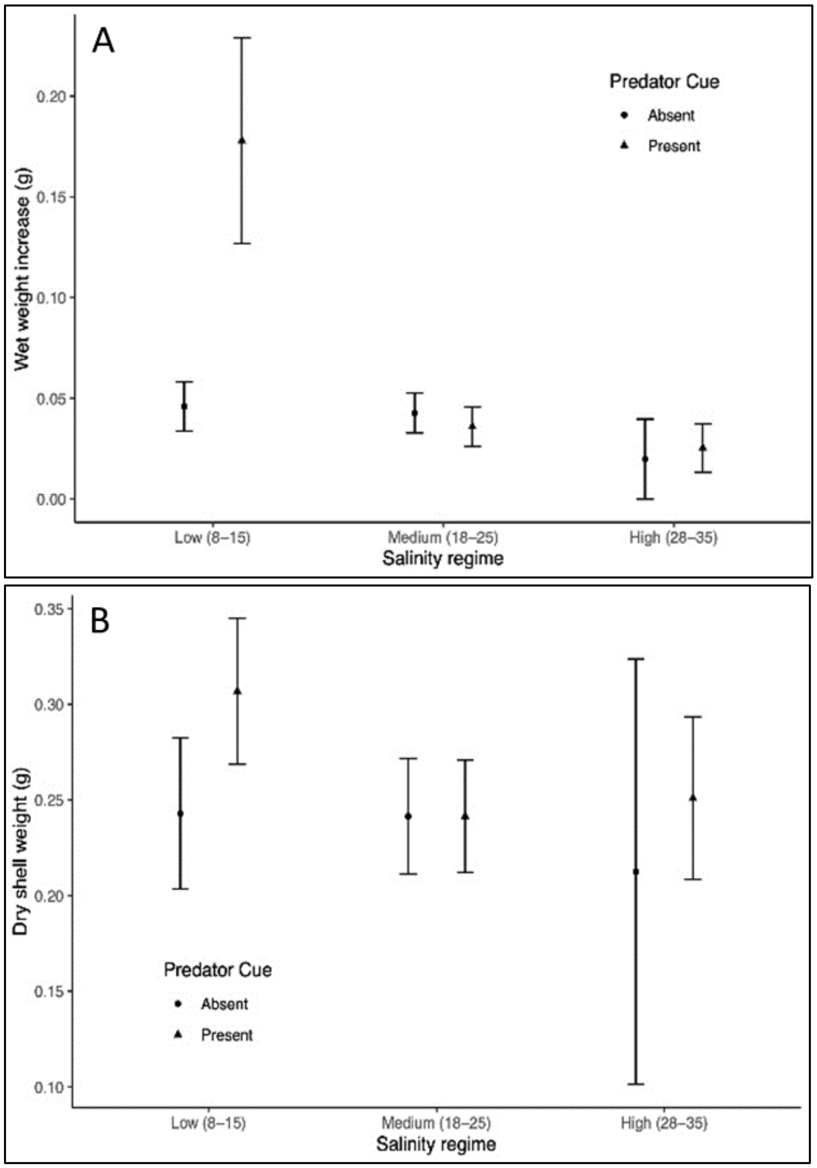


Figure 17. Growth (increase in wet weight) and dry shell weight of oysters grown A) in the presence and B) absence of actively feeding predator (*S. haemastoma*) cues and in low (8-15 ppt), medium (18-25), or high (28-35) salinity regimes.

3.5 Responses of oyster early life-stages to pesticide exposure (Michael Wintermantel, Ph.D. student, FSU)

Introduction. This research is focused on investigating potential issues in oyster development arising from runoff of popular herbicides. While little information exists to support or reject claims regarding pesticide toxicity in oysters, preliminary investigation of the scientific literature has revealed some areas of potential interest (Bringer et al 2021, Bouilly et al 2003, Huong et al 2013). Based on existing research, there is little support for claims that adult oyster mortality is due to acute toxicity from herbicides (Bouilly et al 2003, Tanguy et al 2005); however, many acute exposure experiments performed on larvae show that common herbicides can cause deformities or abnormalities during larval development (Bringer et al 2021, Huong et al 2013). It has also been shown that some herbicides can cause genetic issues (Bouilly et al 2003, Barranger et al 2014), or cause changes in gene regulation (Tanguy et al 2005, Rondon et al 2017).

Methods. This project aims to test a number of the most relevant herbicides for the area surrounding Apalachicola Bay, focusing on acute and chronic exposure regimes at environmentally realistic concentrations. It also seeks to create a concentration profile for pesticide pollution in Apalachicola Bay. This project is beginning its investigations with a focus on the herbicide atrazine. This herbicide is commonly used in the farming of corn and can last for years in water (U.S. Environmental Protection Agency 2003, Vonberg et al 2014). Atrazine causes male frogs, reptiles, and fish to develop female sex organs (Hayes et al 2011, Hoskins et al 2018, Hayes et al 2011), and is approved for use in Florida.

Only one experiment has examined the effects of atrazine on oyster larvae (Hayes et al 2011). This experiment showed an EC50 of >30 mg/L atrazine for the development of abnormalities in oyster larvae; however, the authors failed to properly monitor levels of atrazine present in the oyster component of their experiment. Atrazine concentrations were only measured in the mysid shrimp and sheepshead minnow experimental components. Depending on whether a solvent was used, atrazine levels were 50-70% of the nominal value (with solvent), or 20-27% of the nominal value (without solvent). As there is no information given about solvent use for the oyster exposure treatments and given the relative insolubility of atrazine (NIOSH 2019), it cannot be confirmed that oysters were exposed to appreciable levels of herbicide in this experiment. The potential lack of exposure could entirely explain the absence of a measured effect. The authors also failed to perform any examinations of chronic exposure on oyster larvae, which is a necessary test for any chemical which has a half-life greater than a year in water (U.S. Environmental Protection Agency 2003). Given the limitations in methodology in this experiment, the impact atrazine may have on oyster larvae remains unclear.

Given the lack of information about atrazine's effects on oysters, and its popularity in corn farming (one of the top 4 crops grown in North Florida), atrazine is an ideal starting point for this research. Once the experimental methodologies have been refined, this research will be expended to investigate several other key herbicides including glyphosate, 2,4-D, hexazinone, and other herbicides identified to be relevant to North Florida’s prominent agricultural and forestry industries (Dittmar et al 2022, Minogue 2022). Initial results will be available within the ABSI funding period but the work will be completed and funded outside ABSI.

4. Oyster ecology

4.1 Intertidal monitoring (ABSI Core Team[1])

Introduction. 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 (OIMMP). Most of the research from the ABSI region has focused on

[1] ABSI Core team is led by Dr. Brooke and includes ABSI technicians and Brooke graduate students

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 and the contribution of intertidal oysters to the overall system.

Intertidal oyster habitat in the ABSI region cover an estimated area of 94 ha and have a mean live oyster density of 406 oysters/m^2 (Grizzle *et al*. 2018). This is a relatively small area compared with sub-tidal reef estimates that range from 1,600 to 4,000 ha (Radabaugh et al 2019). This comparison shows that although subtidal oysters cover substantially more bottom area within the region, intertidal oysters are contributing to ecological and ecosystem services 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, as many of these 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 from 2019 to 2021 continued to build upon the initial assessment of intertidal oyster reefs made by Grizzle et al. (2018) and quantified spatiotemporal variations in oyster density and size structure, condition index, and disease prevalence across space and time throughout the ABSI region. In 2022, ABSI focused on sub-tidal work and reduced effort in the intertidal by omitting the bi-annual density assessments. Previous work was documented in the 2019, 2020 and 2021 ABSI annual reports available through the ABSI website (https://marinelab.fsu.edu/absi/about-absi/#annual-reports). Monthly sampling for condition index and disease continued.

Methods. Monthly intertidal oyster reef monitoring has been conducted from December 2019 to March 2023. Collection was paused from March to May 2022 due to staff limitations and recommenced in June 2022. This report includes monthly data from June 2022 to February 2023 (March 2023 sample data has not yet been processed). During this time, four sites were repeatedly sampled: Alligator Harbor (AH), Carrabelle River (CR), East Cove (EC), and Indian Lagoon (IL) (Fig. 18). Five reefs at each of the four study sites were sampled for a total of 20 reefs per sampling period, with five oysters per reef collected for analysis. Clusters were chosen by haphazardly tossing a 0.25 m^2 quadrat and the first five oysters larger than 35 mm were collected for analysis. Metrics included shell height (mm), total oyster weight (g), shell wet and dry weight (g), tissue wet and dry weight (g). Condition index was calculated and disease level (reported separately in section 3.2) was assessed for each animal.

Shell height measurements were taken to the nearest millimeter using vernier calipers. Oysters were shucked, blotted dry and weighed (total wet weight) and tissue and shell wet weights were recorded separately, then placed in a drying oven at 50°C for 72 hours or until a constant weight was reached. Condition index was calculated as tissue dry weight/shell dry weight x 100 (Walne and Mann 1975). This method was used as it is simple and provides a strong correlation to more complex biochemical indices such as percent carbohydrate, carbohydrate: nitrogen ratio, carbon: nitrogen ratio and percent organic content (Ranier and Mann 1992). This method is also used by the FWRI subtidal oyster monitoring program, which will provide a useful comparison between the two habitat types.

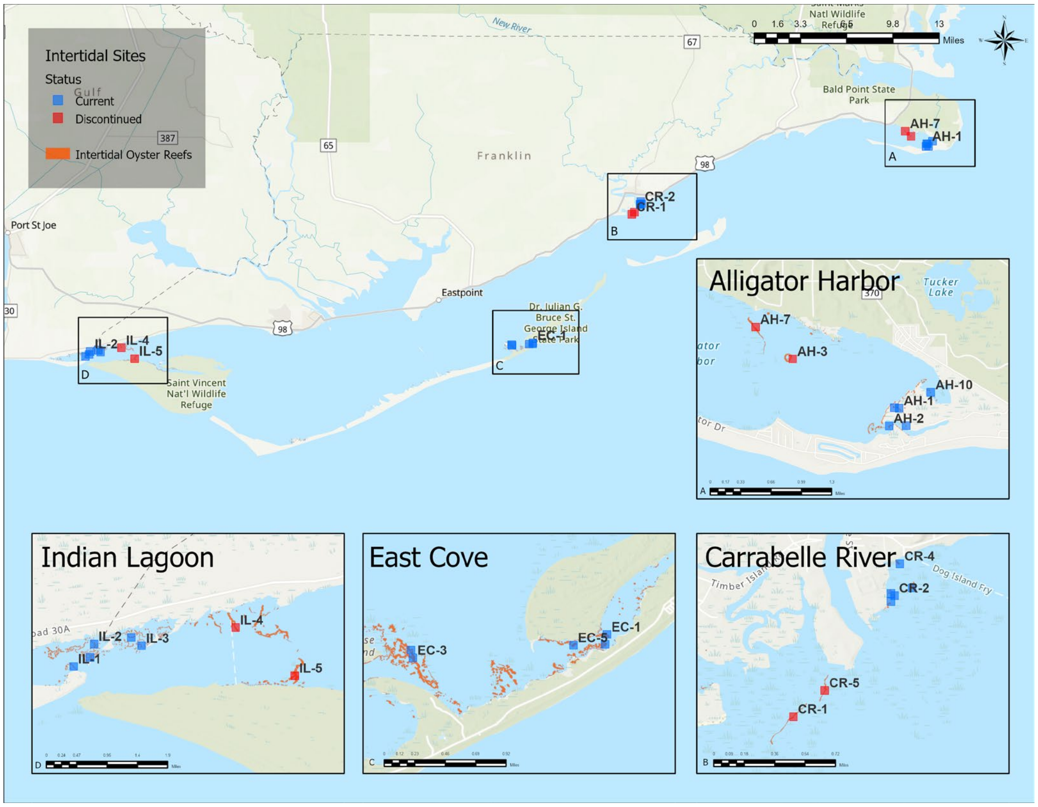


Figure 18. Intertidal study sites showing current (blue) and discontinued (red) oyster monitoring sites: Indian Lagoon, East Cove, Carrabelle River, Alligator Harbor.

Results and discussion. Oyster morphometrics are summarized by site in table 5. Mean shell height (Fig. 19) and condition indices (Fig. 20) were plotted by sample month for each study site. There are no seasonal signals in shell height as collections targeted larger animals to obtain sufficient tissue for condition index and disease analysis. The condition index data do not show consistent patterns across sites or any clear relationship with time of year. A reduction in condition was expected during the stressful hot summer months but this trend was not observed. Loess regression curves (Fig. 21) show the relationship between oyster height to total (wet) weight for each study site. The height-weight relationships for East Cove and Carrabelle River are similar, with very few oysters larger than 75 mm. Although the non-random sampling is an artefact in these data, larger animals were targeted during collections so if present, would probably have been sampled. Indian Lagoon and to a lesser extent, Alligator Harbor, have larger oysters, with higher weights at equivalent shell height than the other sites, although these trends are not strongly reflected by the condition index values. The 2021-2022 ABSI report (https://marinelab.fsu.edu/absi/about-absi/#annual-reports) showed a significantly higher number of large oysters at Indian Lagoon than for the other sites, supporting the larger sizes shown in figure 21. Additional analysis is needed to assess the statistical significance of the relationships between the oyster metrics across time space. Future work will include a resumption of the bi-annual community sampling in 2023 and 2024, continued collection of samples for condition index, disease and additional samples for reproduction. *In situ* salinity/temperature dataloggers will be deployed at each site to provide environmental data to correlate with biological data.

Table 5. Minimum, maximum, and mean values with standard error (SE) for height, total weight, shell weight (wet/dry), and tissue weight (wet/dry). Site names are Indian Lagoon (IL), East Cove (EC), Carrabelle River (CR) and Alligator Harbor (AH).

	Height (mm)	Total weight (g wet)	Shell weight (g wet)	Shell weight (g dry)	Tissue weight (g wet)	Tissue weight (g dry)
IL						
Min/max	33/119	7.6/334.9	6.5/310.3	6.1/293.8	1.2/33.3	0.2/18.8
Mean (SE)	59.23	51.77	44.17	41.70	7.60	2.85
	(0.95)	(2.69)	(2.44)	(2.32)	(0.30)	(0.12)
EC						
Min/max	26/82	3.72/78.63	3.05/62.06	2.77/59.32	0.52/19.11	0.07/3.96
Mean (SE)	49.91	21.77	17.69	16.48	4.08	1.63
	(0.58)	(0.61)	(0.51)	(0.47)	(0.12)	(0.07)
CR						
Min/max	23/76	3.74/57.57	3.23/51.22	2.99/47.24	0.50/10.51	0.01/4.05
Mean (SE)	48.32	21.67	17.83	16.46	3.84	1.67
	(0.61)	(0.65)	(0.54)	(0.50)	(0.13)	(0.07)
AH						
Min/max	36/114	11.12/167.41	9.61/151.28	8.89/147.18	1.51/16.77	0.26/5.95
Mean (SE)	63.32	45.83	38.78	36.90	7.05	2.33
	(0.63)	(1.20)	(1.09)	(1.08)	(0.15)	(0.07)

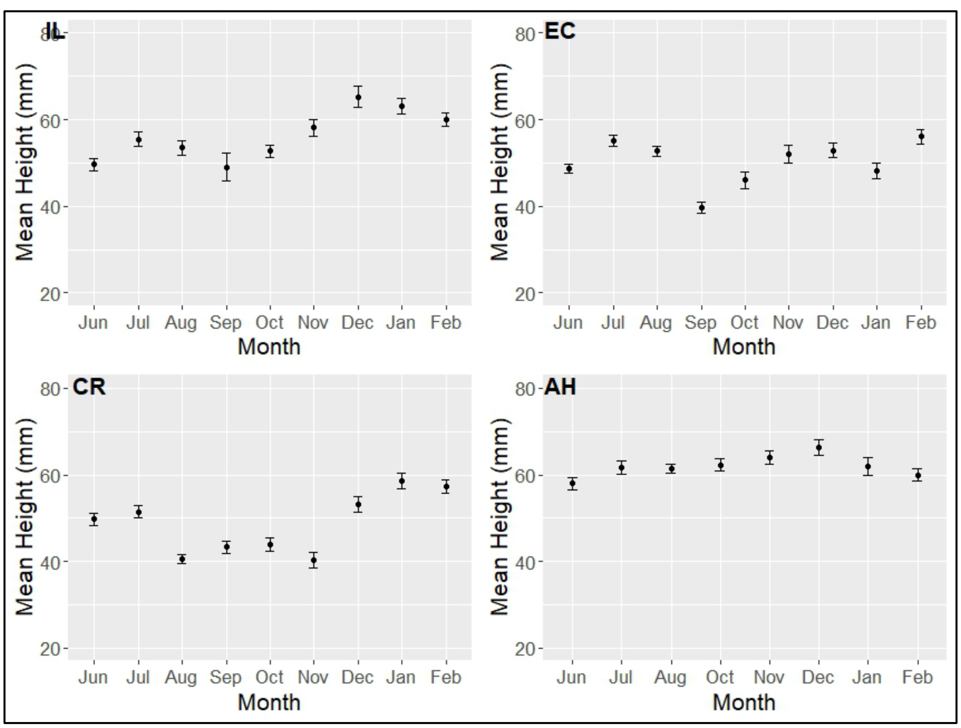


Figure 19. Mean oyster heights from intertidal sampling by month and across sites: IL = Indian Lagoon, EC= East Cove, CR = Carrabelle River, AH = Alligator Harbor. Months represent June 2022 – February 2023. AH was not sampled in September

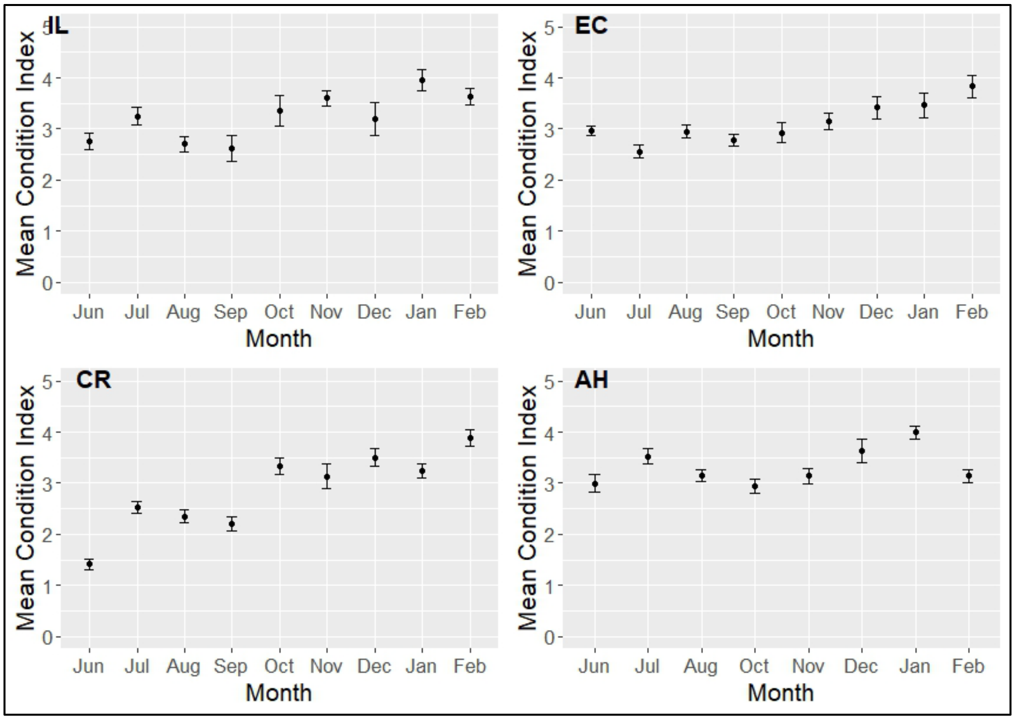


Figure 20. Mean oyster condition index from intertidal sampling by month and across sites: IL = Indian Lagoon, EC= East Cove, CR = Carrabelle River, AH = Alligator Harbor. Months represent June 2022 – February 2023. AH was not sampled in September

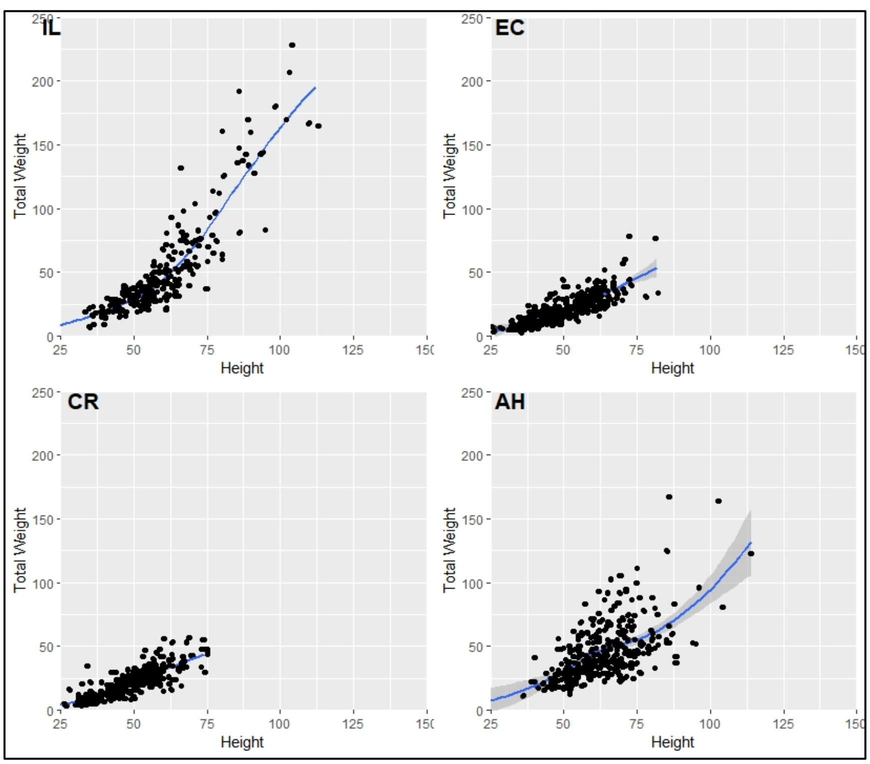


Figure 21. Loess regression curves of height (mm) and total weight (g) relationships amongst sampling sites from June 2022 to February 2023

4.2 Spatial and temporal patterns of intertidal oyster reefs using remote sensing techniques (Jenny Bueno, Ph.D. candidate, FSU)

Introduction. Current methods of monitoring the intertidal oyster reefs in the ABS 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. Analysis of satellite imagery is an alternative to this approach and has been implemented in this region by Grizzle et al., (2018). However, publicly available satellite images are low resolution with insufficient detail for monitoring (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). Drones have capabilities and the 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).

The overarching goal of this research is to create high resolution digital maps of intertidal oyster habitats to better understand their broad-scale dynamics that can inform management, conservation, and future restoration. Additionally, this research can provide a foundation for continued monitoring with innovative tools.

Objectives.

1. Create high resolution maps of the intertidal oyster reefs and sample clusters within the mapped intertidal areas
2. Use the high-resolution maps to analyze intertidal oyster spatial patterns
3. Analyze temporal and spatial change of oyster abundance in the intertidal

Methods. There are five intertidal areas with high density of oyster reefs across the Apalachicola Bay, Florida (Grizzle et al., 2018). Of those five areas, two were chosen as the main sites for the scope and timeframe for this research. Data collection began in December of 2021 during low tides for maximum reef exposure. The East Cove site was strategically split into four sections for optimal launching and retrieval of the drone. The Alligator Harbor site was also split into three sections. To complete the research objectives outlined, intertidal oyster reefs were mapped using a drone and an RTK-GPS (1-2cm positional accuracy) system. At each site, the drone was launched to collect high-resolution and high overlapping imagery at a 40-meter altitude. Additionally, ground control points (GCPs) were placed strategically within the bounds of the drone flight. The RTK-GPS system was then used to collect accurate horizontal and vertical positions of the GCPs. The imagery and locations of the GCPs were processed in a photogrammetric software using structure from motion techniques. The products include orthomosaics, or high-resolution georeferenced mosaics, and digital elevation models (DEMs), or a digital representation of elevation data (Fig. 22).

Since the last report, ground sampling was added as another objective to validate drone products. In the winter of 2022 one section per site was sampled to collect oyster clusters. This was done by randomly collecting oyster clusters and recording the overall cluster mass, volume, and measurements and the live, boxes, and shell heights within the clusters (Fig. 23). At one of the sites, presence and absence data was also collected to validate the accuracy of cluster detection. The orthomosaics and DEMs were 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.

Results and discussion. As of March 2023, objective 1 is complete for East Cove and Alligator Harbor, shown in figure 1. Additionally, the cluster data collection has been processed. Results are shown in figure 2, illustrating the relationship between cluster size and quantity of live oysters. Analysis of spatial patterns of cluster presence for East Cove and Alligator Harbor is ongoing to fulfill objective 2 and a manuscript is in preparation using these data. Objective 3 will be completed concurrently with objective 2 by looking at oyster cluster change through time.

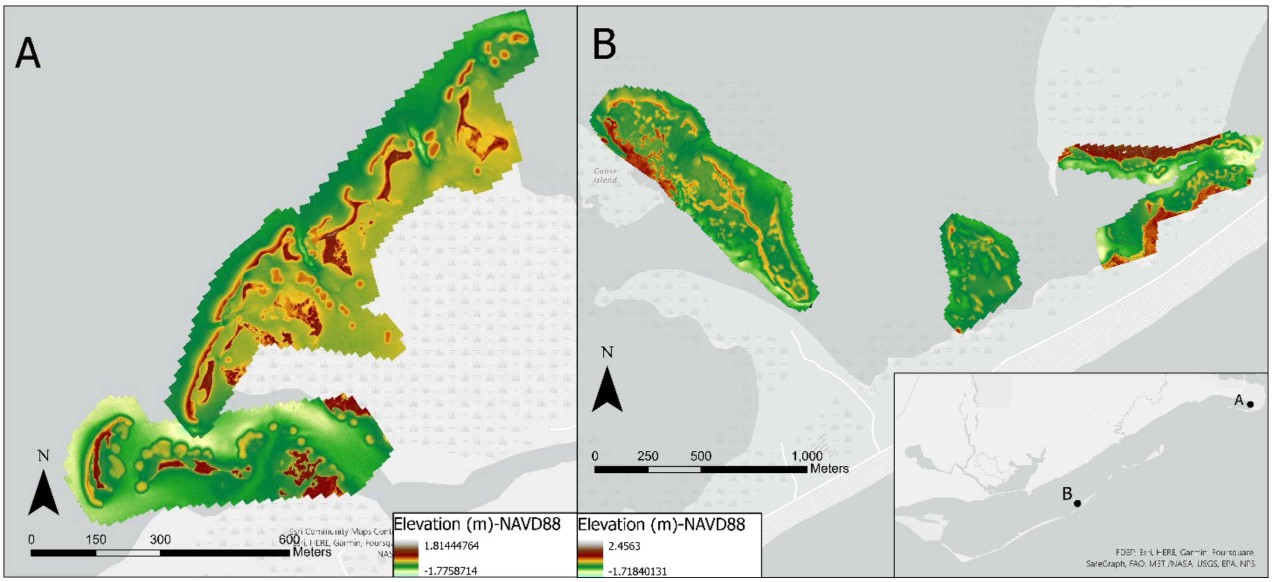


Figure 22: Digital elevation models, shown in NAVD88 vertical datum in meters, of Alligator Harbor (panel A) and East Cove (panel B), within Apalachicola Bay, Florida.

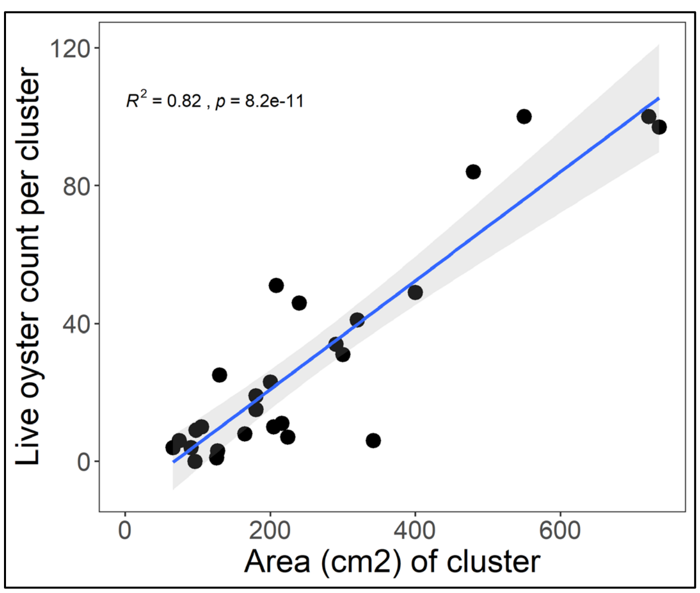


Figure 23: Regression model showing the relationship between cluster size on the x-axis versus the count of live oysters per cluster on the y-axis.

4.3 Subtidal oyster monitoring (ABSI Core Team)

Introduction. Sub-tidal monitoring has traditionally been done using SCUBA, but this approach is weather dependent, requires specific skills and expensive equipment, and is potentially hazardous given the low visibility and strong currents in Apalachicola Bay. Recent monitoring has also focused on specific areas that were replanted under grant funding and therefore do not provide a broad spatial perspective of the status of sub-tidal oyster populations.

Objectives

1. Expand the current understanding of the extent and status of oyster habitat and populations
2. Detect spatial patterns in oyster abundance and size distribution
3. Identify sites for oyster reef restoration experiments

Methods. The first subtidal surveys were conducted from late fall 2020 to early spring 2021 and consisted of 132 sites (Reported in the 2021 ABSI Annual Report). The initial objective of this sampling survey was to acquire an understanding of the status of oyster habitat and populations in the Bay. Target sites were driven by local knowledge and were not scientifically randomized or structured. Despite these limitations, the survey provided useful data that would have been challenging to acquire using SCUBA in the same timeframe. At each station, six replicate single tong samples were taken from the bow, middle and stern of both sides (port and starboard) of the vessel. The following parameters were recorded for each tong sample: volumes and mass of total material, material type (shell, rock, other); numbers of spat (<25 mm), sub-legal oysters (25-75 mm), market-sized oysters (> 75mm), and boxes (dead, articulated shells). Predators were identified and counted. 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 2021 to early spring 2022 and consisted of 117 sites (Reported in the 2022 ABSI Annual Report). 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, were identified through side-scan sonar mapping as potential oyster substrate or are areas that were part of the FDEP restoration projects (funded by the RESTORE Act and Natural Resource Damage Assessment). The mapping data used included side-scan sonar collected by the National Oceans and Applications Research Center in 2021 and FDEP side-scan data from their RESTORE project. The "unknown" locations are areas of historical oyster habitat (according to FWC maps) but where no contemporary data was available. Tonging samples for the second round of subtidal sampling were collected in the same manner as the first, however, the height of the first 100 oysters was measured to generate a size-frequency distribution of the population. Remaining oysters and boxes were counted.

The third round of tonging surveys were conducted from January to March 2023 and comprised 227 locations throughout Apalachicola Bay (Fig. 24). Areas of similar habitat type were identified and a power analysis was conducted using samples from the previous two years to determine adequate sampling effort for each substrate type and region. This approach provided a statistically supportable assessment of substrate type and quantity, and oyster abundance and size distribution throughout the Bay. Tonging samples for round three subtidal sampling were collected the same way as round two, but additional information collection included the size classification (spat, sub-legal, market) of boxes. This allows more insight into the mortality occurring in different size classes.

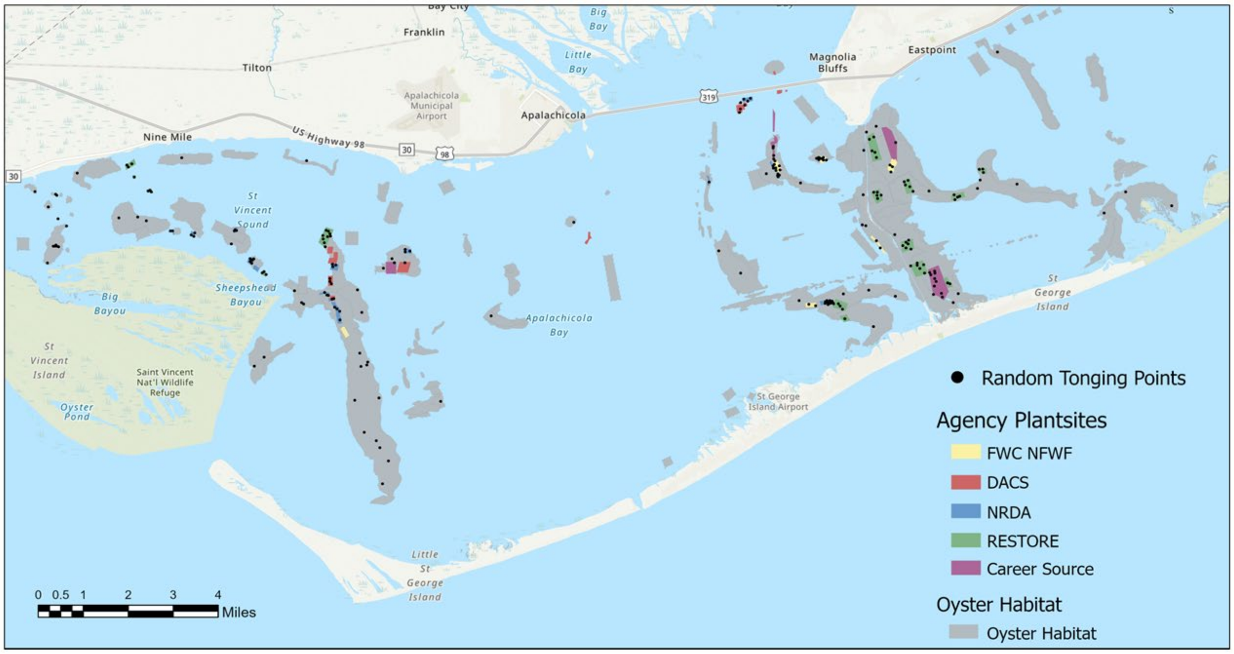


Figure 24. Subtidal tonging locations from year three showing agency planted sites (colored polygons) and tonging point locations (black dots. These were collected in a nested random design, with higher intensity sampling on restoration projects Tonging has been completed and data processing is ongoing.

Results and discussion. The 2023 tonging data is currently undergoing data entry and quality checks, but results of previous surveys show that the distribution of oyster populations in Apalachicola Bay is spatially heterogeneous and very few areas supported market sized oysters as recently as spring 2022 (Fig. 25). These data show much higher recruitment (spat), more sub-legal, and marginally more market sized oysters in the eastern versus western sections of the Bay. Anecdotal information from before the 2012 fishery collapse estimated that approximately 25 market sized oysters would be a common expectation for a single tong sample. None of the 117 sites sampled were close to reaching this threshold. Larger oysters were generally found in areas that were recently planted (2017-2019) with limestone, particularly in the eastern part of the bay. Historical oyster habitat and areas planted with shell or fossil shell had few oysters, and many no longer have stable material to support oyster recruitment and growth. These observations are supported by the most recent subtidal tong sampling.

Future work includes a fourth round of sampling in fall 2023-spring 2024, and spatial analysis of all data to identify statistical differences between regions and substrate type and to assess changes since the oyster fishery was closed in December 2020. Environmental data from ANERR instruments, the FIM Kriging analysis and the hydrodynamic model will be used as factors in the analyses to identify potential environmental drivers of the observed oyster distributions.

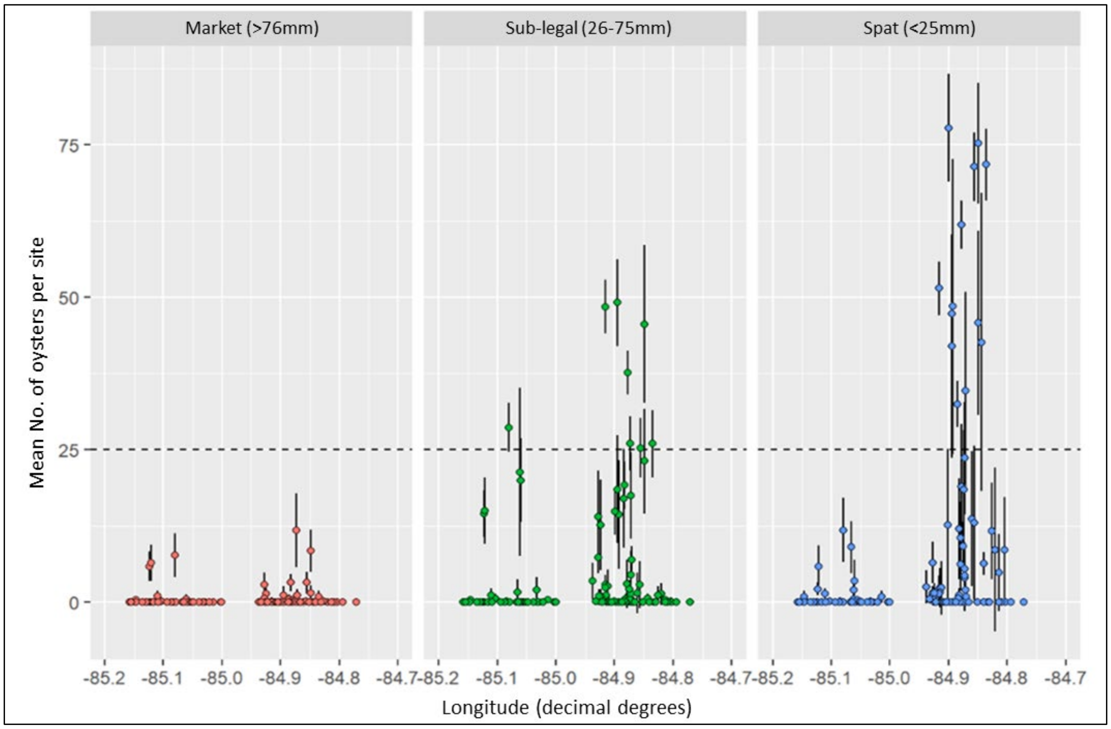


Figure 25. Distribution of oyster size classes (market, sub-legal and spat) found during 2021-2022 tonging survey. The X-axis shows longitude so data is presented from west to east across the Bay

4.4 Impacts of oyster populations on community development (Dr. Andrew Shantz, Courtesy Research Faculty, FSUCML)

Introduction. Oysters are the foundation species in Apalachicola Bay but are only part of this productive and valuable ecosystem. In addition to oysters, the estuary supports 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.

Objectives

1. To utilize existing data to assess how the decline of oyster populations in Apalachicola Bay have impacted the broader ecological community, particularly commercially and recreationally important species
2. Identify how restoration efforts are impacting community development and habitat use by these species throughout the bay.

Part 1 of this project was initiated the Summer of 2021 and reported in the ABSI 2022 Annual Report. Part 2 began in March 2022. This portion of the research will focus on understanding the recovery potential of the Apalachicola Bay fish and invertebrate communities.

Methods. Part 2 of this project began in March 2022 with the deployment (under the Florida DEP scientific exemption) of two types of restoration modules: reefballs and layer cakes (Fig. 26) at six study sites: three on Dry Bar and three on the eastern bars (Fig. 27), spanning a gradient of environmental conditions. These units are complex and difficult to assess using traditional approaches, so benthic community development will be monitored using photogrammetry. Prior to deployment, each unit was labeled and approximately 100 overlapping high-resolution images were taken to cover from every aspect and angle. These images were used to create three-dimensional (3-D) models of the units using Agisoft Professional software. One unit per site will be removed quarterly, images will be taken and 3-D models constructed. Changes in total volume will be calculated to quantify reef accretion rates at each site.

To understand the recovery potential of the broader fish and invertebrate community, sampling trays filled with shell, were deployed next to the restoration modules. Trays will be recovered at three-month intervals to assess community composition and succession of associated species and data analysis is underway. Deployments are being paired with in situ temperature, dissolved oxygen, and salinity dataloggers 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 monitoring surveys, these 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 restoration efforts.

To date, one sampling event has been completed with photographs of the reefball units and community tray collections completed but not yet analyzed. The second round of sampling will occur this spring, with the remainder of the sampling completed by early 2024.

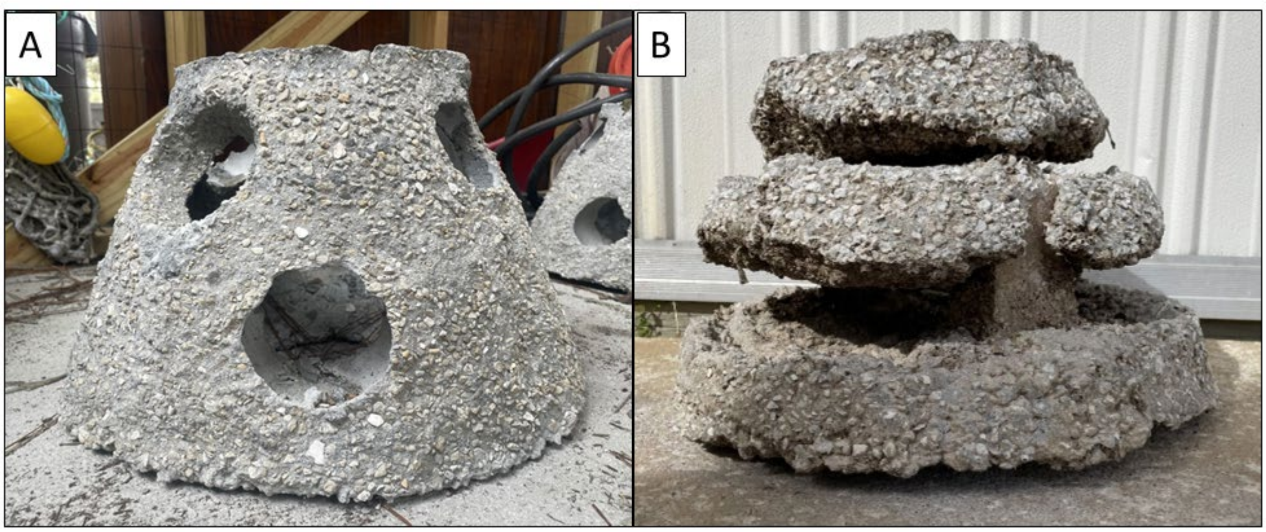


Figure 26. Restoration structures prior to deployment. A) Oyster Reef Ball, B) Layer Cake. Units were deployed in groups of four of each type at three sites in the west bay and three in the east

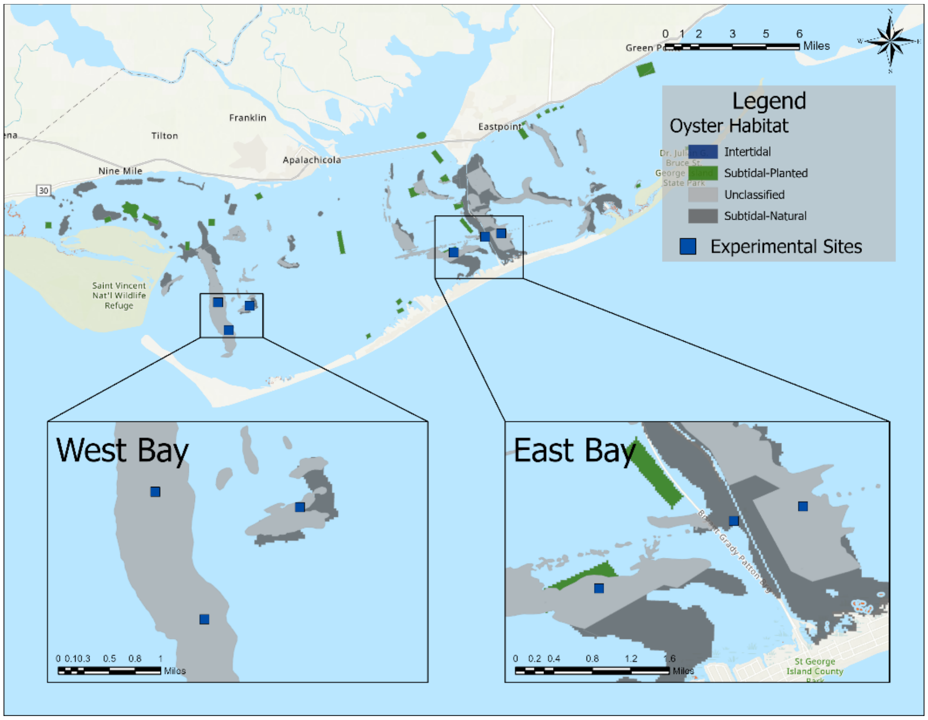


Figure 27. Subtidal community development (reefballs, layer cakes and community cages) experimental sites.

4.5 Fish communities associated with oyster habitats (Barry Walton, PhD student, FSU)

Introduction. Oysters create biogenic habitat that can provide food, spawning substrate, and refuge from predation for numerous species of fish that are either residents, facultative residents, or transients of oyster habitat (Tolley & Volety 2005). As oyster restoration efforts continue to rebuild the depleted oyster population, it is important that we seek to gain a better understanding of the fish communities that rely upon the oyster habitats. This research will describe the existing community structure and functional diversity of fish species (teleost and elasmobranch) found in oyster habitats and compare oyster habitat community metrics to the communities within seagrass and mudflat habitats. Environmental DNA from water samples paired with traditional fishing methods (longline, gill net, seine net) will be used to assess the presence of fish species associated with the three habitat types, and fish muscle tissue will be sampled for stable isotope analysis to investigate food web dynamics. These two projects will provide information on which species are using each habitat, the degree of species overlap, the level of functional redundancy and the structure of food webs across three different habitats in the Apalachicola Bay System.

Another objective will focus specifically upon two species of predatory fish; Red Drum (*Sciaenops ocellatus*) and Spotted Seatrout (*Cynoscion nebulosus*). Both species primarily use oyster habitat for foraging (Moulten et al. 2017), but Red Drum larvae have been shown to select oyster habitat for recruitment and have reduced mortality when oyster habitat is available (Tolley and Volety 2005). These species are important recreational fishing and food fish, which makes understanding their habitat use, foraging ecology, and health important for management. ***This objective is partially funded by the FWC Forage Fish Research Program Fellowship awarded to Barry Walton.***

To investigate the trophic ecology of these two species, fatty acid profiles and stable isotope analysis were used to determine how these species are partitioning resources as they often simultaneously share the same space. Fatty acid profiles and stable isotope (C-13 and N-15) data were collected from 15 Red Drum, 15 Spotted Seatrout and ~15 of each of the following prey species known to locally cooccur in the diet of both species : Pinfish (*Lagodon rhomboides*), Atlantic Croaker (*Micropogonias undulatus*), Pigfish (*Orthopristis chrysoptera*), Spot (*Leiostomus xanthurus*), Silver Perch (*Bairdiella chrysoura*), Blue Crab (*Callinectes sapidus*), White Shrimp (*Litopenaeus setiferus*), Brown Shrimp (*Farfantepenaeus aztecus*). Prey species were selected by using gut content data for Red Drum and Spotted Seatrout provided by the Florida Wildlife Research Institute. Preliminary results from fatty acid profiles are provided below and stable isotope data are currently being analyzed.

Methods. Fish were collected in September and October 2022 (Fig. 28) by accompanying FWC finfish independent monitoring (FIM) surveys (3 trips) and fishing independently. Rod and reel were used for predators and some forage fish, seine nets for forage fish, and cast nets/dip nets for shrimp and crabs. Sampling and fish handling were in accordance with SAL-2413 and IACUC # 202200000014.

A total of 330 samples (165 stable isotope and 165 fatty acid) were taken from the 11 species in the study (Table 6). For Stable isotope analysis, muscle tissue samples were taken in the field via biopsy punch and stored at -80ºC. Samples were lyophilized for 48 hours and then ground into powder using a ball mill grinder and liquid nitrogen. Samples (400-700 μg) were placed into tin capsules and shipped to the University of New Mexico Center for analysis of C-13 and N-15 isotopes. After punch cores were removed, the carcasses were stored at -20ºC for fatty acid analysis. Fish were partially thawed and homogenized using a commercial food processer. A 5g subsample of the homogenized fish, shrimp, or crab was extracted and lyophilized for 48 hours, ground into powder using a ball mill grinder and liquid nitrogen. A subsample (0.5g) was shipped (on dry ice) to Lipid Analytical Laboratories (Ontario, Canada) for fatty acid analysis

Figure 28. Map of the Apalachicola Bay System with black circles indicating collection stations.

Table 6. Summary of samples showing mean length (mm) ± standard error (SE) for each species. (CNEB = spotted seatrout, SOCE = red drum, FARF = brown shrimp, LSET = white shrimp, CSAP = blue crab, MCEP = striped mullet, BCHR = silver perch, LXAN = spot, LRHO = pinfish, MUND = Atlantic croaker, OCHR = pigfish)

Species	N	Length (mm)	SE
CNEB	15	368.33	21.73
SOCE	15	416.0	26.65
FARF	15	98.73	3.90
LSET	15	89.53	1.42
CSAP	15	86.27	6.34
MCEP	15	144.73	4.31
BCHR	15	122.07	4.34
LXAN	15	141.4	3.20
LRHO	15	126.4	5.27
MUND	16	154.63	3.55
OCHR	14	138.15	6.40

Results and discussion. Environmental DNA methods are being developed in conjunction with scientists at the University of West Florida and test samples will be analyzed in early April. The fatty acid samples have been returned and preliminary results show some distinct grouping of species based on their fatty acid profiles (Fig. 29). Brown shrimp, white shrimp, and blue crab are clustered together, with more variation seen in blue crab whereas the two shrimp species are tightly packed and distinct. Many of the forage fish species (Spot, Silver Perch, Atlantic Croaker, Pinfish) cluster together, but Striped Mullet is an exception, possibly due to its more herbivorous diet. Red drum and spotted seatrout cluster distinctly from their prey species. The next steps in this research are to conduct a statistical analysis of the fatty acid data to identify species that have significantly different profiles from each other, and to incorporate the stable isotope data to assess trophic structure of the target species and determine the diets of Red Drum and Spotted Seatrout in Apalachicola Bay. Environmental DNA data collection and analysis will commence this summer.

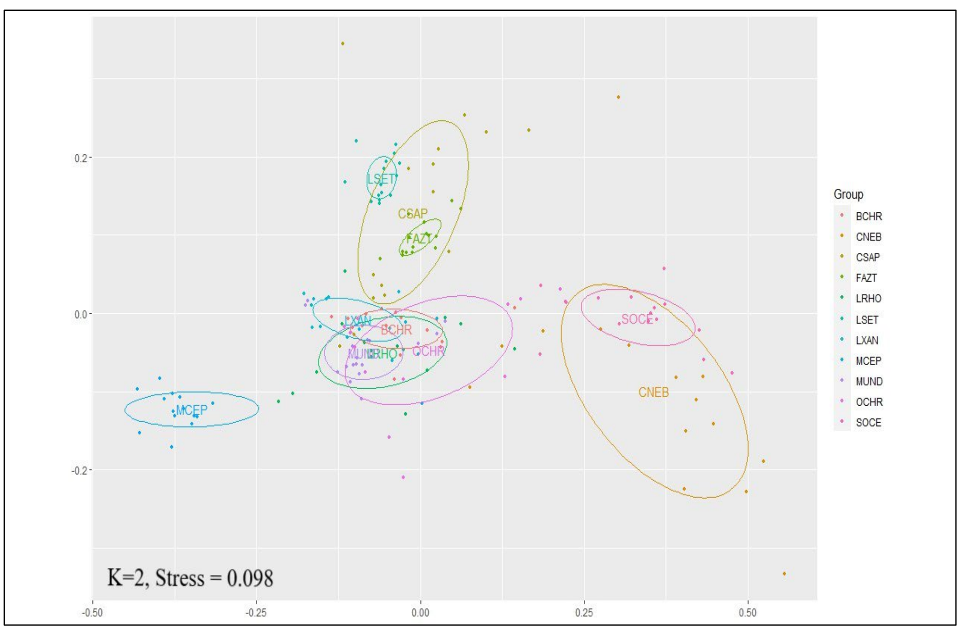


Figure 29. Non metric multi-dimensional scaling plot of fatty acid profiles among species. (CNEB = spotted seatrout, SOCE = red drum, FAZT = brown shrimp, LSET = white shrimp, CSAP = blue crab, MCEP = striped mullet, BCHR = silver perch, LXAN = spot, LRHO = pinfish, MUND = Atlantic croaker, OCHR = pigfish)

5. Restoration

5.1 Oyster restoration experiments (ABSI Core Team)

Introduction. 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 were 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. 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 subsequently enhance recruitment.

Methods. Thirty experimental reefs were created in Apalachicola Bay in early summer (May 26 – June 24) of 2021; fifteen were placed on northern Dry Bar and another 15 at Peanut Ridge in the eastern Bay (Fig. 30). From the tonging surveys, the eastern bars generally have more oysters than the west, despite similarities between restoration materials used and timing of material deployment. The environmental conditions differ between these areas; the southern end of Dry Bar has generally high salinity (> 25) as it is close to West Pass, which is a large opening to the Gulf of Mexico marine waters. The northern section of Dry Bar however, can have low to moderate (10-25) salinities depending on river outflow. Peanut Ridge has high to moderate salinities (15-25) and generally much higher current speeds and wind driven waves than Dry Bar. These two locations were selected to assess the success of different materials under different abiotic conditions. Each reef (100 m^2) was built to a height of approximately 0.5 meters. Three materials were used: natural shell, which is a traditional cultching material but is unstable in strong currents and not available in large quantities, small limerock (~8 cm diameter), which similar in chemical composition to natural shell but heavier and easier to obtain, and larger limerock (~18 cm diameter) which is stable and provides 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.

Monitoring of the restoration reefs has been conducted four times to date. The initial post-deployment restoration was done using SCUBA diving in the fall (September 21- October 27) of 2021. Divers collected five bagged samples from each reef (five replicate reefs of three materials at two sites) using a 0.25m^2 quadrat placed haphazardly on the substrate. Divers collected the material that was on the immediate surface within the quadrat, placing the material (substrate and oysters) inside mesh bag. Samples were stored in coolers and transported to the marine lab where they were processed before being re-deployed in the field. Divers were used for the initial monitoring as this approach is used by FWC and FDEP for their restoration project surveys. However, diving requires specific skills, expensive equipment and is limited by potentially hazardous climatic conditions such as strong currents, low visibility and rough seas. The second cycle of monitoring was done in spring (April 18 -May 4) 2022 using tongs to enable comparison of the restoration sites with the broader tonging surveys. Methods were the same as those used for the subtidal tonging surveys (section 4.3) and resulted in more efficient data collection than using divers. The next two monitoring events were conducted in late summer of 2022 to compare data collected by diving (July 22 – August 3) and tonging (August 24 – September 2) and to determine the feasibility of replacing dive surveys with tonging, which has fewer environmental and personnel limitations.

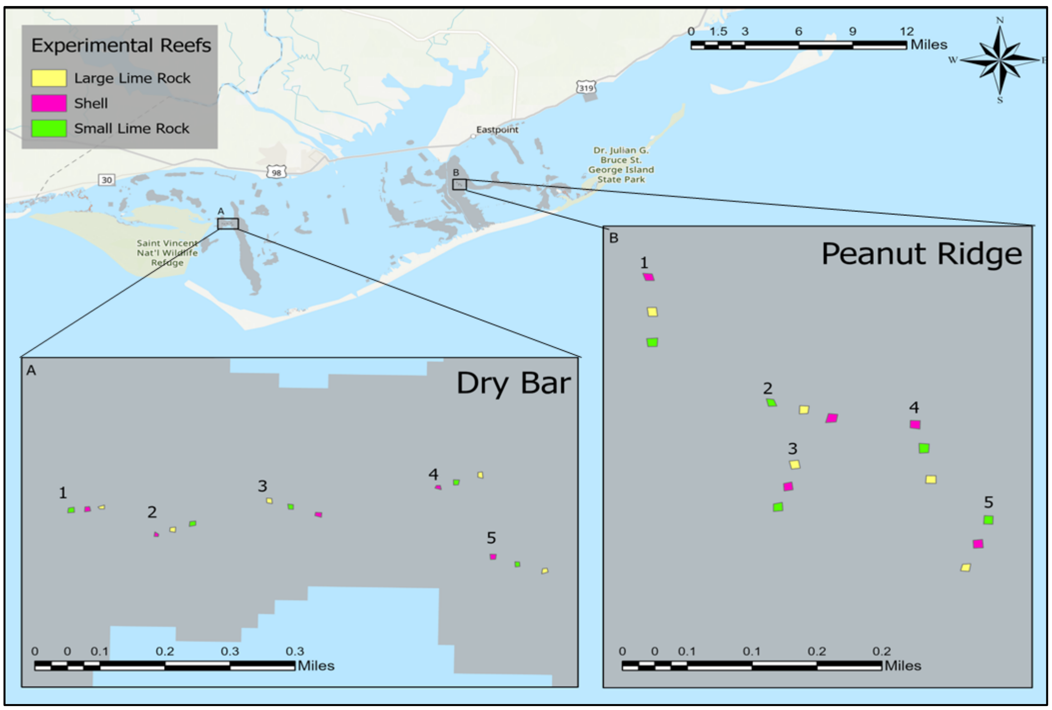


Figure 30. Experimental reef sites at Dry Bar and Peanut Ridge. Three materials (large limerock, shell and small limerock) were used with five replicate reefs at each site.

Results and discussion. The spring and summer surveys showed similar trends so only data from the summer is presented. The western Dry Bar reefs showed overall lower performance (fewer total oysters and smaller average shell height) than the eastern Peanut Ridge reefs, and some differences in materials were also observed. A Chi-squared (χ^2) analysis showed that settlement differed between reef materials ($\chi^2 = 24.54$, $p < 0.001$) and region ($\chi^2 = 9.74$, $p = 0.001$). On the Dry Bar sites, oyster settlement over 14-months was significantly higher on small limerock reefs than shell ($p = 0.01$) or large limerock ($p = 0.006$); Figure 1A). On Peanut Ridge shell had significantly lower settlement than small limerock ($p = 0.003$) and large limerock ($p = 0.037$) but settlement did not differ between the two limestone sizes in this part of the bay ($p = 0.49$; Fig. 31A). Average oyster survival rates on the experimental reefs ranged from 21 ± 7.7% to 73 ± 8.1% and were very dependent on location in the Bay ($\chi^2 = 61.45$, $p < 0.001$) and an interaction with cultch material ($\chi^2 = 6.19$, $p = 0.05$). On Peanut Ridge, average survival rates were 72% on large limerock 61% on small limerock and 50.6% on shell reefs but due to the high variance these differences were not statistically significant. In contrast, oyster survival on Dry Bar was similar across all materials and significantly lower than Peanut Ridge (Fig. 31B).

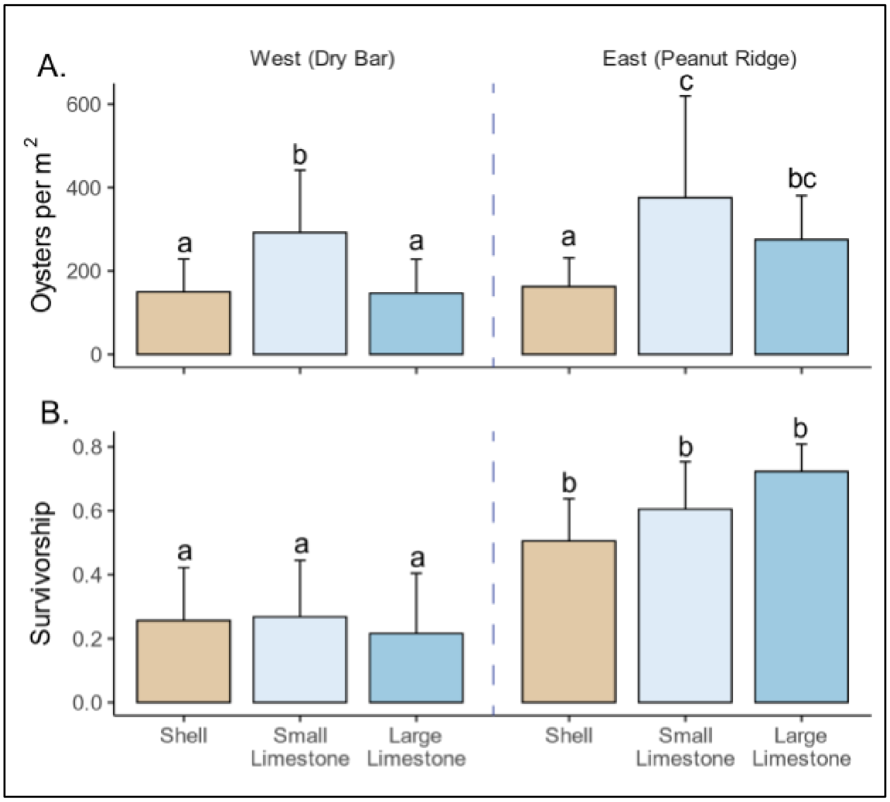


Figure 31. Results of dive surveys (summer 2022) showing A) average oyster settlement and B) percent survivorship for each reef material, by region. Different letter assignments represent significant differences between the reefs.

Comparing live oyster shell heights across reefs showed differences in the size structure of oyster populations (Fig. 32). On Peanut ridge, shell height of live oysters for the 0.25 quantile (i.e. 25% of the population is below the 0.25 quantile height) was no different between shell and small limerock reefs but was >8 mm larger on large limerock reefs. For the 0.5 quantile, oysters were ~9 mm larger on large limerock than shell, which were ~6 mm larger than on small limerock. This pattern was consistent and slightly stronger for the 0.75 and 0.95 quantiles. On Dry Bar, the oysters in the 0.25 quantile were approximately half the size of Peanut Ridge oysters on small limerock and shell reefs and were even smaller on large limerock. Differences between sites were less apparent across the 0.5, 0.75, 0.95 quantiles, with oysters on Dry Bar approximately ~2-4 mm smaller than in the same treatments on Peanut Ridge (Fig. 32). The total number of oysters and their size structure found on individual experimental reefs is shown in figure 33. The diver collections on Dry Bar (Fig. 33A) clearly show the lower abundances and sizes of oysters than collections taken from Peanut Ridge (Fig. 33B). This trend holds true for the collections made by tonging (Fig. 33 C, D), but oyster abundance was generally higher, particularly at the Dry Bar sites (Fig. 33C)

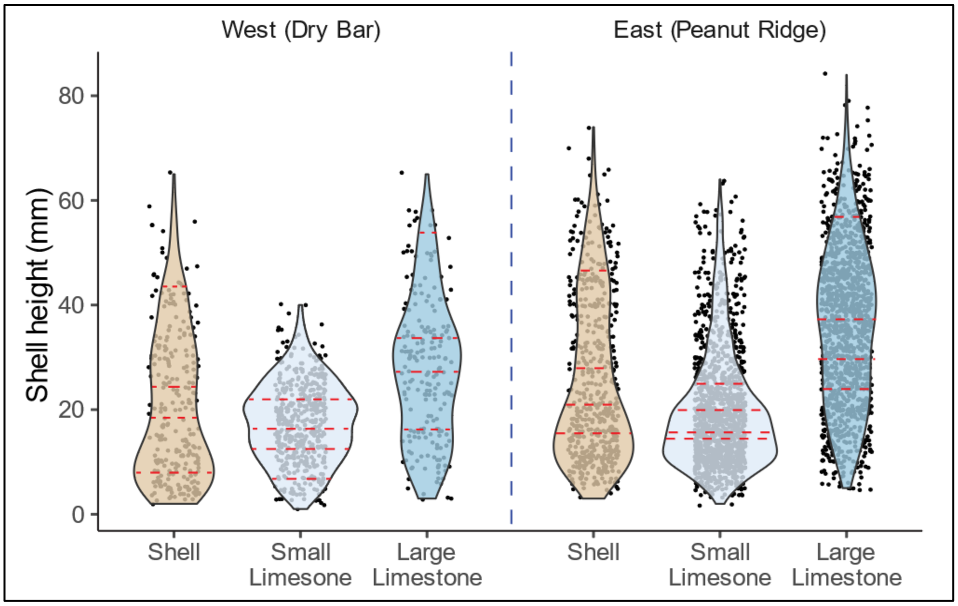


Figure 32. Violin plot of shell heights showing size structure of oysters collected by divers by reef material and location. Red dotted lines show the 0.25, 0.50, 0.75 and 0.95 quartiles.

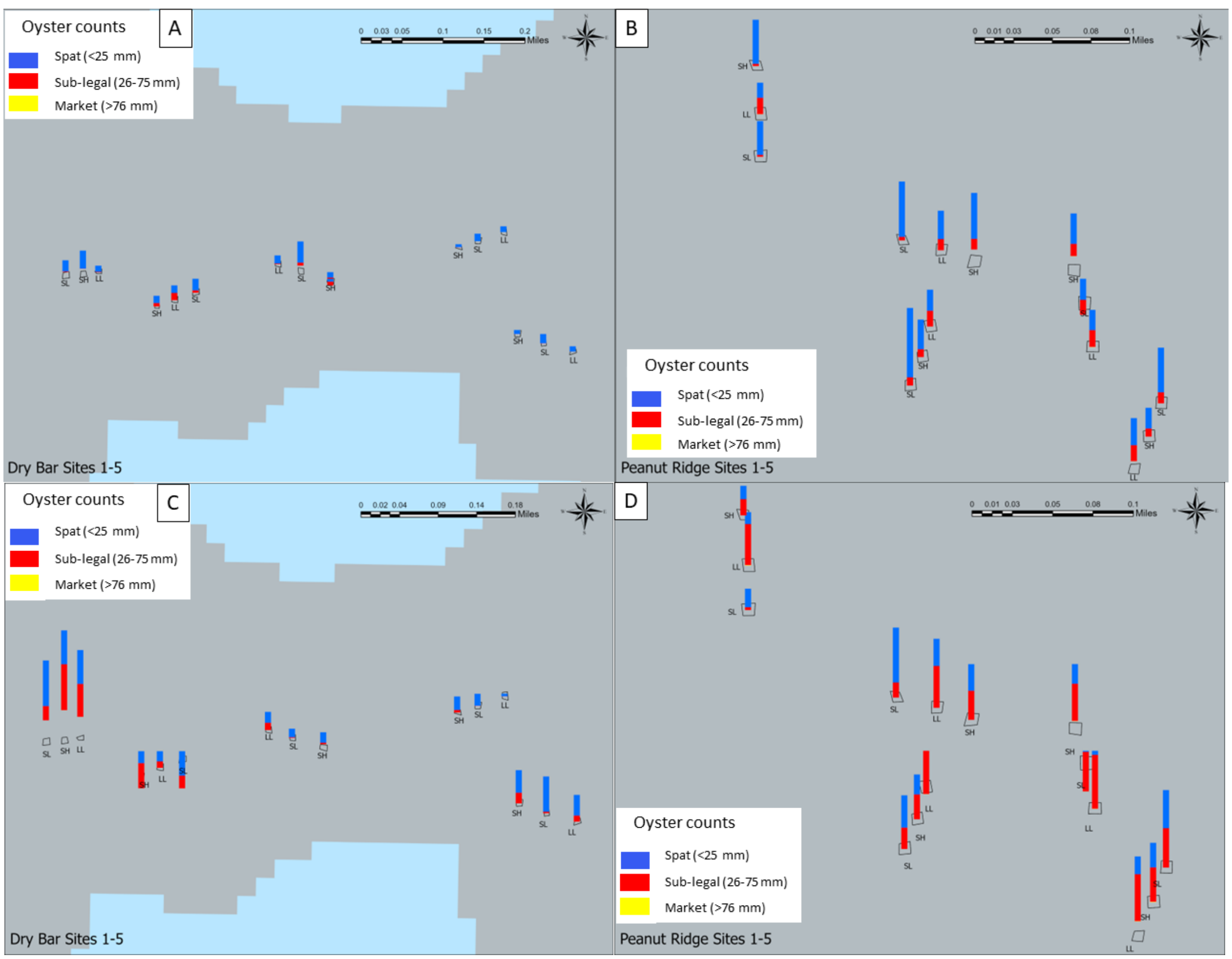


Figure 33. Average counts of spat, sub-legal and market oysters collected by divers at A) Dry Bar and B) Peanut Ridge and by Tonging at C) Dry Bar and D) Peanut Ridge restoration reefs.

The diver and tong surveys were done in the same timeframe to compare the methods. Paired Mann-Whitney Rank Sum tests (data failed normality for the t-test) were used to compare the weight of material and number of live oysters collected using the different techniques. The tests showed significantly more material ($T = 1135.0$, $p = 0.001$) and live oysters ($T = 1084.0$, $p = 0.0130$, Fig. 34) were collected by tonging than diving. The difference in live oysters was most likely a function of the larger amounts of material collected rather than any real differences

Two-way ANOVAs were conducted to compare site and treatments for weight and live oysters using each method. The analyses revealed no significant difference in weights collected using tongs between sites ($F = 1.82$, $p = 0.19$) but the amount of material collected varied significantly among treatments ($F = 10.99$, $p < 0.001$), specifically between large limerock and shell ($t = 4.26$, $p<0.001$). The number of live oysters showed no significant differences between site ($F = 2.29$, $p = 0.14$) or treatment ($F = 1.88$, $p = 0.17$) for the tong sampling.

For the dive collections significant differences were observed for both site ($F = 5.08$, $p = 0.03$) and treatment ($F = 30.95$, $p <0.001$), with more material collected at Dry Bar, particularly the large limerock and other treatments. The number of live oysters in the dive collections also showed significant differences by site ($F = 47.1$, $p < 0.001$), which was driven by differences in the limerock treatment between sites. There were also treatment effects ($F = 7.70$, $p = 0.003$), which were driven by differences between limerock and shell treatments at Peanut Ridge.

The weight differences observed between materials was most likely simply due to the properties of the materials as shell is much less dense than limerock. The site differences in the dive surveys however, may have been due to operator artefacts as several different divers were used during these surveys and some collections were larger than others. The tonging was done by one individual using tongs with a consistent ‘gape’, so the collections were more consistent.

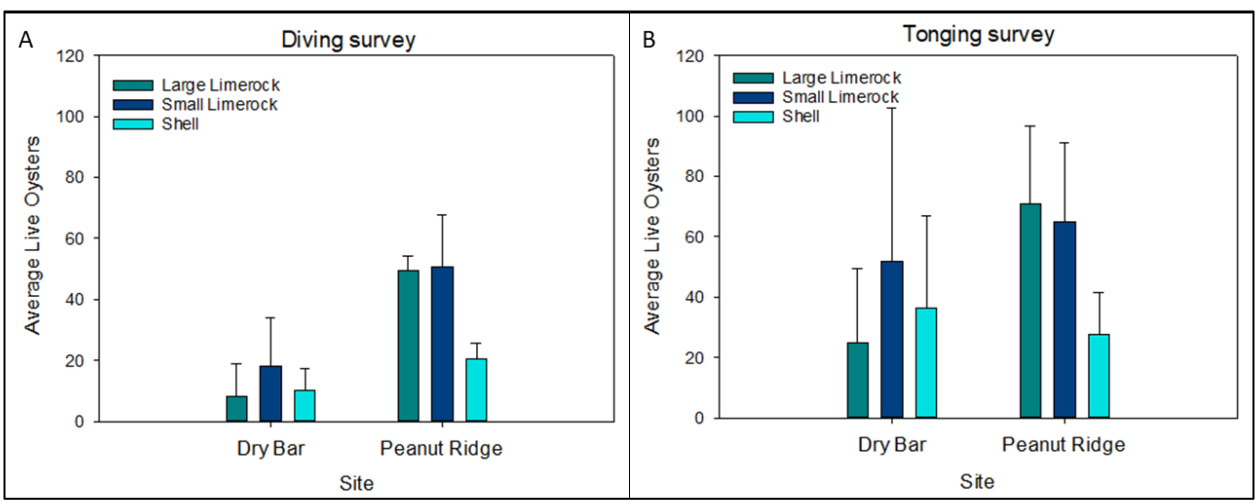


Figure 34. Comparison of average live oysters (all size classes) using diver surveys and tong surveys conducted in late summer 2022.

The data so far indicate that although the total number of live oysters did not differ between treatments, large limerock is performing slightly better in terms of oyster size structure, with more reaching a larger size than on the small limerock. Shell also performs well, but on Peanut Ridge where currents are particularly strong, the material was rapidly dispersed and much of it was lost or buried. Creating a foundation of stable material seems beneficial in these severely degraded habitats. The small limerock was relatively stable but tended to become compacted and although had high spat settlement, did not support larger oyster sizes.

These experimental reefs will be monitored bi-annually (Spring and Fall) for the remainder of ABSI to assess their performance. A successful restoration effort will support multiple cohorts of oysters and generate the oyster population recruitment, growth and mortality, and development of structural complexity necessary to restore ecological and ecosystem services.

Given the success of the larger limerock, in terms of superior stability and oyster size structure, another experiment is planned for April-May 2023. This will comprise four treatments: 1) large limerock (15-20 cm diameter), 38 cm high; 2) large limerock, 30 cm high plus 8 cm of shell; 3) concrete (12-18 cm diameter), 38 cm high; 4) concrete 30 cm high plus 8 cm shell. The four treatments will have four replicates, each with a reef footprint of 16 x 8 m and will be deployed by oyster fishers. This experiment will test the performance of limerock vs concrete, which is less expensive, is readily available and avoids the environmental impact of mining. The addition of shell will test the cost-benefit and efficacy of enhancing the stable rock foundation with a layer of natural recruitment substrate.

5.2 Survival and growth of oyster juveniles on restoration experiments (Dr. Andrew Shantz Courtesy Faculty, Dr. Sandra Brooke FSUCML, ABSI Core Team)

Introduction. Understanding of growth rates throughout different life stages is essential for informed management strategies in marine systems (Pukett et al. 2012, Pine et al. 2015, Lowe et al. 2017). Modeling baseline oyster growth and survivability from juvenile to adult life stages is challenging on natural reefs; however, this monitoring is applicable to a wide range of bivalve studies including effects of parasites and pathogens (Paynter et al, 2010, Paynter et al. 1991), response to varying environmental conditions (Christensen et al.1998), aquaculture practices (Bodenstein et al. 2021), habitat suitability (Zarnoch et al. 2012), and others. Existing studies that pre-date the bay's collapse in 2012 model oyster growth rate using historical and experimental data, however, the current oyster growth rate and survival following their 2012 population collapse is unknown.

This study will contribute to current ABSI research that aims to assess survival and growth potential within Apalachicola Bay, specifically at the experimental restoration sites, to create a predictive curve, accounting for restoration site location, water quality and flow dynamics.

Methods. In September 2022, a single Vexar ® aquaculture cage (75 x 50 x 15 cm) was sand augered into each of the five ABSI experimental shell reefs at Dry Bar and Peanut Ridge (section 5.1; Fig. 35). Cages were oriented horizontally using five-pound weights, with floats to prevent burial of the cages. Oyster juvelins (~10 mm) were adhered to 70 x 40 cm PVC sheets using cyanoacrylate glue with a 10 x10 cm allocated initial growth space for a total of 28 juveniles per cage. Individual oyster height was measured monthly. This initial experiment ended in December of 2022 due to high mortality and gear failure.

The second round of this experiment was conducted in January 2023 and is currently ongoing. During this deployment, two Vexar ® cages were placed within 5 experimental reef sites (n=20). In one cage, 28 numbered oysters were adhered to the PVC sheets as described above. In the second cage at each site, 50 numbered oysters were placed inside the Vexar ® cage. Cages were once again oriented on their sides using weighs and floats. Oyster height has been measured biweekly, dependent upon weather.

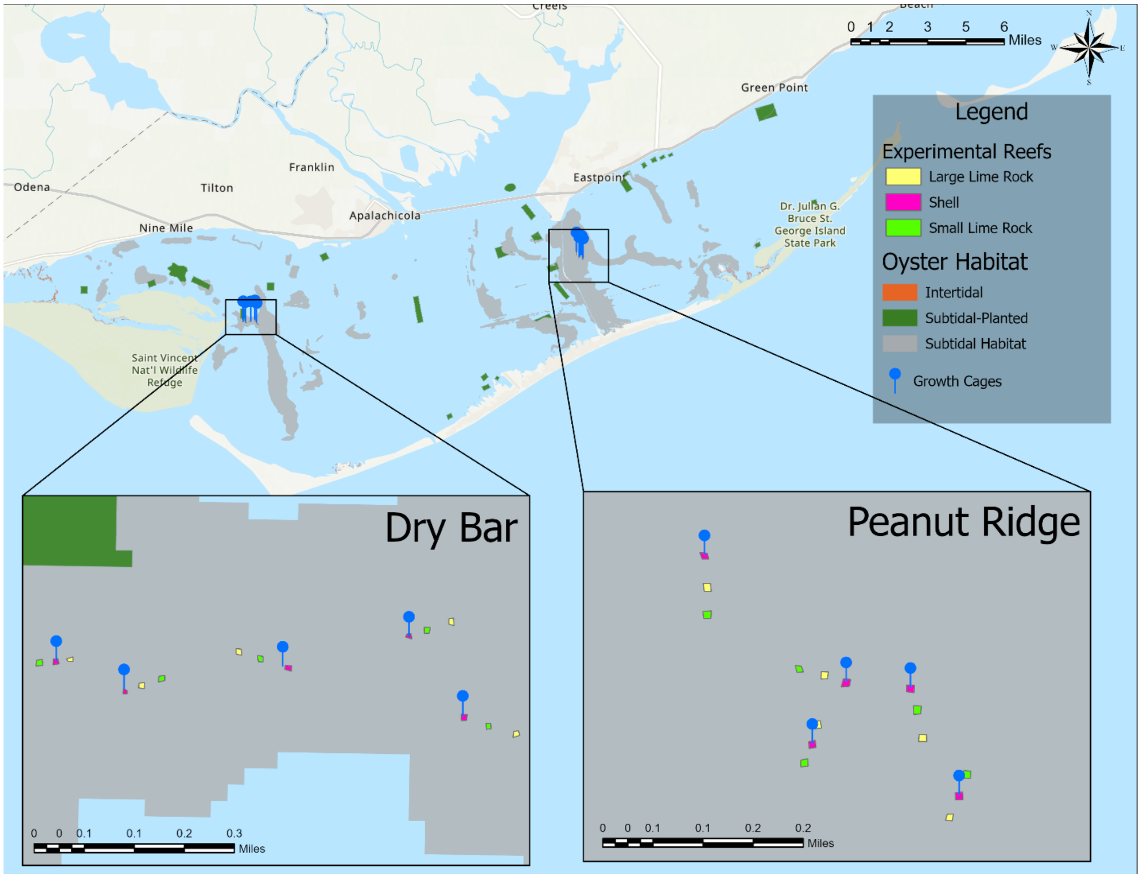


Figure 35. Growth cage locations on experimental shell reefs at Dry Bar and Peanut Ridge.

After completion of the current deployment, the next experiment will use ABSI Hatchery 'spat on shell' instead of individual juveniles. While current deployments use juveniles approximately 10 mm in height, hatchery raised spat can be utilized at approximately 4-6 mm. This future experiment will also include flow dynamics at each site, using bi-weekly current profiler measurements. Results from this project are intended to be used in modeling the recovery potential of oysters in Apalachicola Bay in from the current post-collapse state.

5.3 Improving restoration success in the bay scallop (Morgan Hawkins, Ph.D student, FSU)

Introduction. 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, they have only been available for recreational harvest, or "scalloping", where scallops are collected 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 Steinhatchee fishery is possibly unsustainable, and further investigations should be conducted to assess scallop populations in other harvest zones (Granneman *et al.* 2021). The scallop decline suggests there may not be sufficient numbers of reproductive adults to replenish depleted populations and aquaculture is becoming a focus of many restoration efforts that aim to supplement natural populations with hatchery-grown scallops. For restoration programs, bay scallops are collected from the wild, spawned in a hatchery, and raised until a certain growth milestone is reached. These hatchery-reared scallops are transferred to grow-out cages maintained in natural habitat. Unfortunately, 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. 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.

Objectives

1. Identify if there are differences in the survivability and growth rate of juvenile hatchery raised bay scallops compared to juvenile wild bay scallops.
2. Investigate the differences/similarities in performance of respiration, condition index, gonadal index and shell breaking strength between wild and hatchery raised bay scallops.
3. To better understand the costs and benefits of using hatchery raised bay scallops in restoration efforts.
4. Identify the optimal size for the release of hatchery raised bay scallops to maximize survival when transferred to grow-out cages.
5. To better understand the costs and benefits of using hatchery raised bay scallops in restoration efforts and improve on-going restoration efforts.

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 efforts and will contribute to our understanding of wild scallop ecology. 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 will help inform 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.

Through a collaboration with the Eastern Shore Laboratory of the Virginia Institute of Marine Science, Morgan received training and technical guidance that enabled her to successfully cultivate bay scallops in the ABSI hatchery.

Methods. To complete research objectives 1 and 2, during peak scallop spat season, 50 spat traps will be deployed and monitored in St. George Sound, following the FWC standard methods. When the wild spat reach 5-10mm, they will be transported to FSUCML, where they will be sorted, placed in mesh bags, and housed in flow-through tanks overnight. Depending on the sizes collected, hatchery spat in the same size category will be selected to undergo the same process. Hatchery spat will be sourced from adults (broodstock) from St. George Sound and spawned multiple times in the ABSI research hatchery to ensure multiple size classes. On the day of deployment, scallop spat will be placed in coolers and transported to sites in St. George Sound. Scallops will be placed in cages that are placed in pairs at a site. Every three weeks, spat will be measured, mortalities quantified, and cages exchanged to account for fouling. When >85% of individuals reach 20 mm, cages will be upgraded to a 15 mm mesh, following FWC methodology. This experiment will conclude when bay scallops reach adult size of 50 mm or until survival has reached 20% of the original stocking density.

Live 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.

Spat traps were deployed in late 2022 to 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. During summer 2023, dive trips will take place in St. George Sound to collect adult wild scallops to use for the fall. Multiple spawns will occur during fall 2023 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 2024.

6. System ecology

6.1. Historical changes in Apalachicola Bay ecosystems (Dr. Josh Breithaupt, Faculty, FSUCML)

The following projects were conducted by Dr. Josh Breithaupt's lab in the period from March 2022 – March 2023. Research in this lab focuses on carbon, nutrients, and sediment dynamics. This information can be used to understand function and change in coastal ecosystems that may affect, or be affected by, the regional oyster population. Projects in Dr Breithaupt's lab seek to quantify temporal changes in the quantity and quality of sediment organic matter (SOM) in intertidal and subtidal ecosystem of the Apalachicola Bay region.

6.1.1. Organic enrichment of benthic sediments in Apalachicola Bay, St. Vincent Sound, and St. George Sound. There are two stages of this investigation that form two chapters of an M.S. Thesis project by Kevin Engelbert. The first stage is a historical comparison of present-day surface sediment characteristics with historical data published approximately 30 and 60 years ago (Fig. 36; Kofoed and Gorsline, 1963; Chanton and Lewis 1994). The second stage focuses on stratigraphic change in radiometrically-dated sediment cores.

1a. Organic Enrichment Stage 1: Introduction. The purpose of this investigation is to characterize benthic sediment throughout Apalachicola Bay and determine if changes in organic content of surface sediments have occurred in the past half-century. 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.

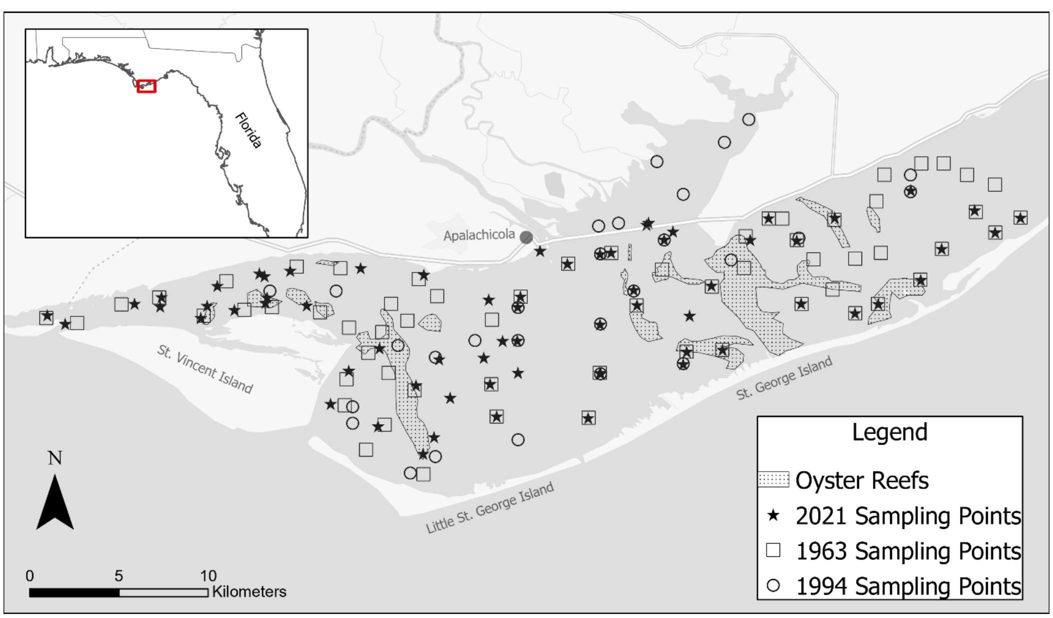


Figure 36. Map of surface sediment collections from 1963, 1994, and 2021 (this study).

1a. Methods. Surface sediment samples have been collected from the bottom of the Bay in the same locations as the historical studies (Fig. 36) 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. Heat maps have been constructed to show areas of high and low concentration of these constituents at the different time points, and separate maps have been created to identify the spatial changes occurring for each broad timestep.

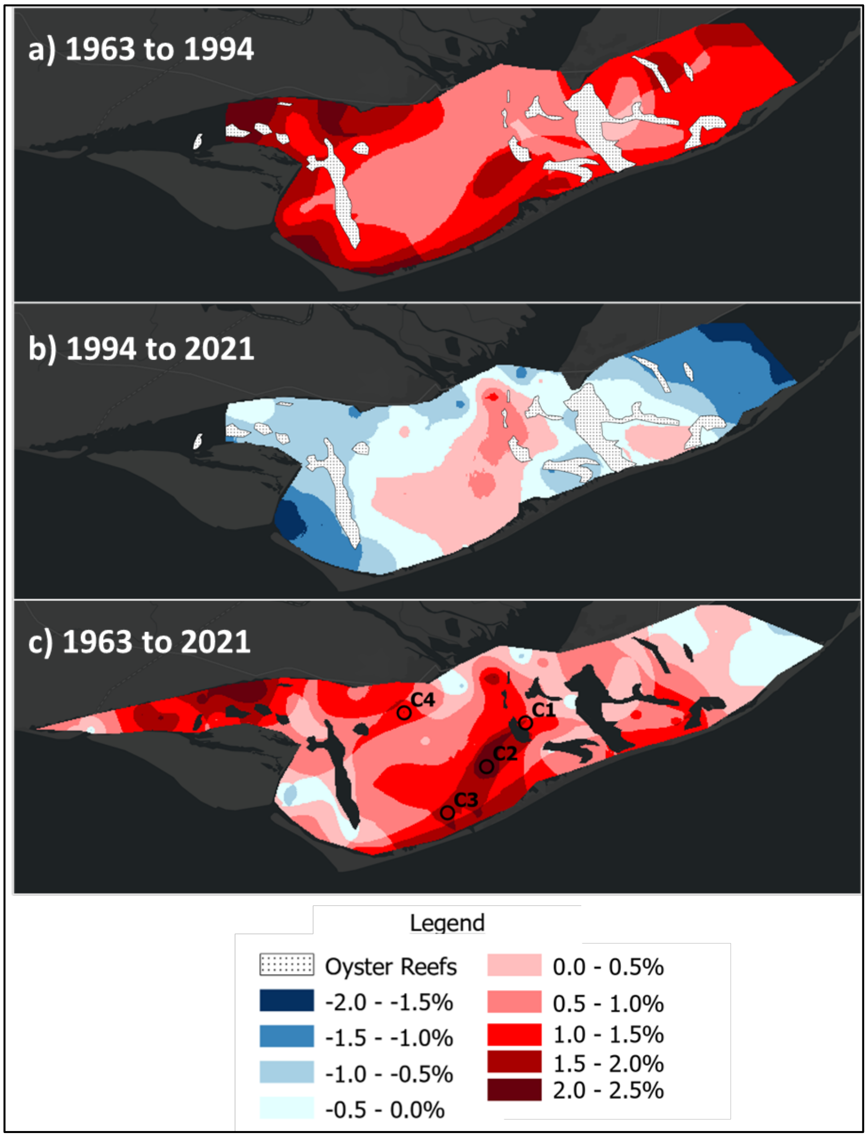


Figure 37. Heat maps identifying regions and amount of change in sediment organic carbon content in three time periods: a) 1963 – 1994, b) 1994 – 2021, and c) 1963 – 2021. Labelled open circles in Panel C represent coring locations C1 – C4.

1a. Results and discussion. Findings indicate that the majority of the Bay saw an increase in organic carbon between 1963 and 1994, with numerous regions seeing increases of greater than 1 – 1.5% (Fig. 37a). From 1994 to 2021 this region-wide trend changed, with increases of up to

1.5% occurring in only isolated locations in the central portion of the Bay and on the southeastern edge of the Cat Point bar (Fig. 37b). Additionally, during this period most of the benthic sediments in the region saw substantial decreases. However, the increases that occurred in between 1963 and 1994 were greater than the ensuing decreases that occurred between 1994 and 2021, so that the net trend was that over 95% of the Bay saw an increase in sediment OC, sometimes by up to 1-2% for the whole period (Fig. 37c). This work is currently in preparation by graduate student Kevin Engelbert as lead-author, with an intended submission date of fall 2023.

1b. Organic Enrichment Stage 2. Introduction. The advantage of the previous study is that it makes comparisons of surface sediments at known points in time. However, there are limitations to a comparison of data collected using different methods and there is uncertainty about the net results of those observations when only three broadly separate time points are known.

1b. Methods. To further examine the question of organic enrichment of the Bay including the timing and causes of the changes, a second project is underway using sediment cores that have been collected in areas identified as representing the greatest amount of change between 1963 and 1994. These cores have been sectioned in 1 cm intervals and are being radiometrically dated with ^{210}Pb. In addition to use of nutrient ratios and stable isotopes, these samples are being analyzed for a suite of lignin phenols to determine if downcore changes can be attributed to source or degradation changes.

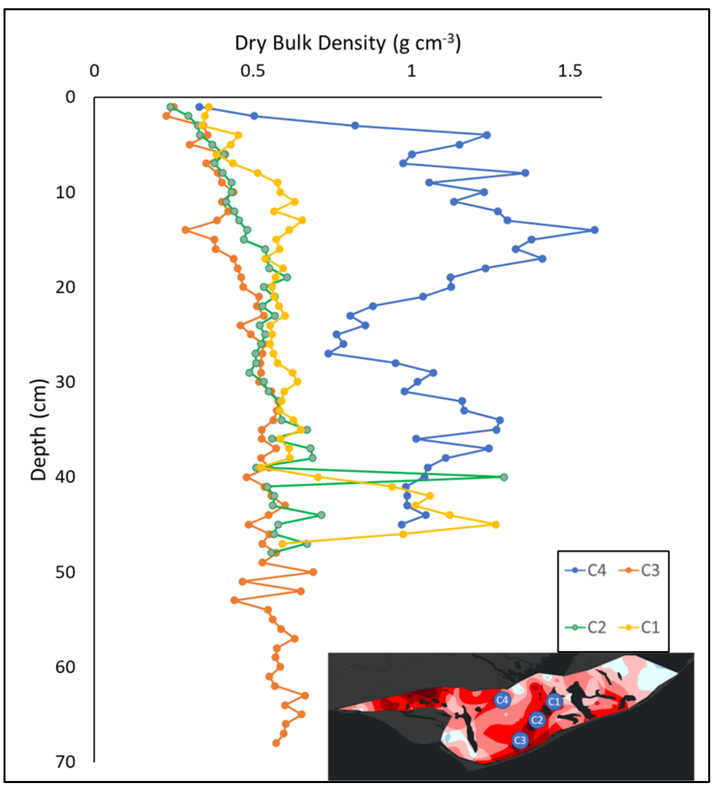


Figure 38. Dry bulk density with depth of sediment cores collected from Apalachicola Bay. Inset: map of surface sediment collections in 1963, 1994, and 2021 (this study).

1b. Results and discussion. Results of this work are in process, but figure 38 identifies the stratigraphy of the dry bulk density of the sediments from these four cores. Cores 1 – 3 are highly similar, with relatively low surface bulk densities that increase very slightly with depth, with some notable exceptions between 40-50 cm deep. These increases likely reflect an increase in grain size; dating of these cores will be useful to identify the timing of these events to determine likely factors for these changes. Alternatively, C4 in the northern portion of the bay has consistently higher bulk densities and a substantial amount of down-core variability. The dating tools will be useful for determining if this core has been highly mixed. This work is ongoing. We anticipate dating of these cores being complete by end of 2023. The biomarker and lignin phenols analysis will be analyzed in summer 2023.

6.1.2. Investigating stratigraphy and sediment composition of intertidal oyster reefs

Introduction. The decline, collapse, and current closure of the subtidal oyster fishery in Apalachicola Bay is well documented. However, there are numerous inter-tidal oyster reefs in the region, and there is much less understood about the condition of these reefs, including whether they are in decline compared to historical conditions and whether restoration efforts are needed. Inter-tidal reefs provide multiple important ecological functions and ecosystem services important to the natural environments in this region and the people who utilize and depend on them. The objective of this pilot study was to begin collecting data about the sedimentary composition of intertidal reefs in Apalachicola Bay to understand their variability and make comparisons to other regions where intertidal reef monitoring and restoration have occurred.

Methods. As a pilot project, a single 55 cm sediment core was taken from an intertidal reef in East Cove on Little St. George Island. The core was sectioned in 1-cm depth intervals. Large (> 2 cm in size) and small shell (>2mm – 2 cm) was removed from each interval and dry mass recorded. Sediment was sieved to identify coarse sand (>63 microns) and fines. Measurements were made of sediment dry bulk density, organic matter content, calcium carbonate content, total carbon, total nitrogen, and total phosphorus. Measurements from the top 10 cm were compared with measurements from live, dead, and restored intertidal reefs in Mosquito Lagoon using data from Chambers et al. (2018).

Results and discussion. Figure 39 shows the results by core depth of different geochemical analyses. Sediment dry bulk density was consistent from top to bottom of the core and averaged 0.33 ± 0.06 (SD) g cm^{-3}. Interestingly, shell mass (> 2 mm) decreased steadily from the surface to the bottom of the core. We can only speculate about whether the increase at the surface represents an increase in supply of shell hash over time, or whether the decrease downcore may represent post-depositional loss such as dissolution. Sediment organic matter (SOM) was overall quite low with a mean value of 2.9 ± 1.5%; this is highly similar to the average SOM content of benthic sediments. However, there are several depth intervals where SOM values increase substantially, over 5%. Total nitrogen was low and varied substantially with a mean of 66 ± 39 mg g^{-1}. Total phosphorus values were generally quite low, with mean value of 0.10 mg g-1, however the middle depths of the core between 20 – 30 cm had elevated values closer to 2 mg g^{-1}.

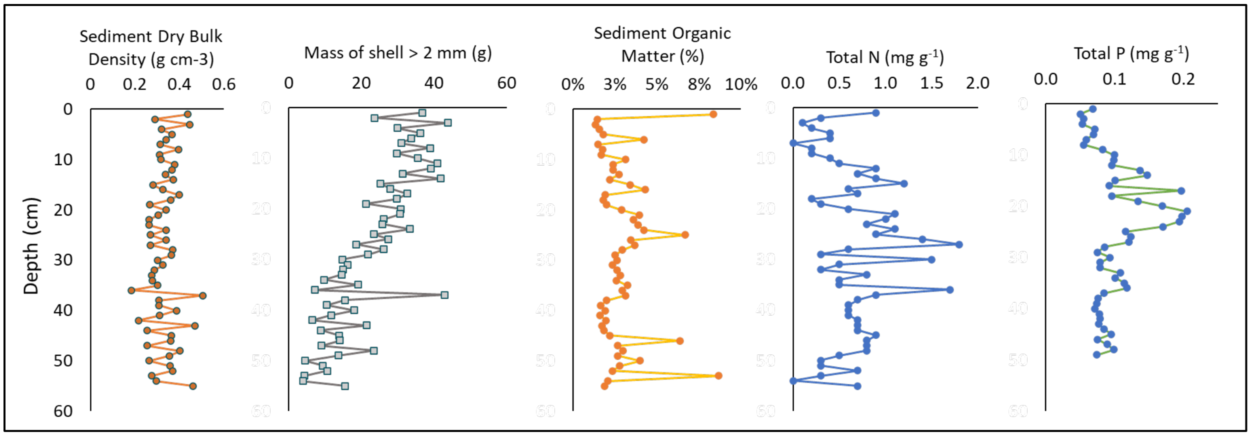


Figure 39. Core depth profiles of sediment dry bulk density, shell mass, sediment organic matter, total N, and total P.

These values are very low for all variables. When compared with values from reefs in Mosquito Lagoon, the biogeochemical indicators of this reef in Apalachicola Bay has even less organic matter than a "dead" reef in Mosquito Lagoon (Fig. 40).

This work is only in the beginning stages. This sediment core is also in the process of being radiometrically dated and will provide some historical context for the timing of changes within the core. Additionally, extensive fieldwork is planned for summer 2023 to add spatial replication to this study. We will be limiting our analysis to the top 15 cm for purposes of comparison with the biogeochemical restoration measures collected by Chambers et al. (2018) in Mosquito Lagoon. We will be collecting replicate short cores on multiple reefs of intertidal reef locations throughout Franklin and Wakulla Counties with the objective of understanding what the baseline conditions of sediment biogeochemistry are regionally. Additionally we will attempt to relate surface sediment organic matter concentrations to abundance and spatial density of oyster clusters using data collected by the ABSI core teams. Ultimately the objective of this work is to understand the condition of these reefs to inform plans for conducting restoration of intertidal oyster reefs.

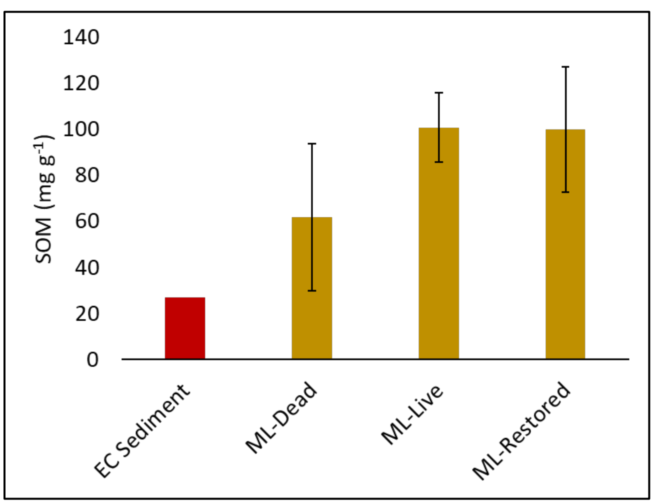


Figure 40. Comparison of average sediment organic matter content (SOM; mg g^{-1}) in the surface 10 cm in East Cove (EC) in Apalachicola Bay (Red bar) compared to mean SOM content in equivalent surface depths for Dead, Live, and Restored reefs in Mosquito Lagoon.

Publications:

The following two ABSI-related papers were published by Dr. Breithaupt's lab in 2022.

1. Steinmuller, Havalend E, Joshua L Breithaupt, Kevin M Engelbert, Prakhin Assavapanuvat, Thomas S Bianchi, and Raymond D Ward. 2022. "Coastal Wetland Soil Carbon Storage at Mangrove Range Limits in Apalachicola Bay, FL : Observations and Expectations." *Frontiers in Forests and Global Change* 5 (July): 1–14. https://doi.org/10.3389/ffgc.2022.852910.

Abstract: Globally, mangrove range limits are expanding, often at the cost of adjacent coastal ecosystems including saltmarshes, potentially leading to a change in ecosystem services such as organic carbon (OC) sequestration. Studies in the southeastern US have focused almost exclusively on Avicennia germinans range expansion, the most cold-tolerant mangroves in North America. The Apalachicola Bay region of north Florida represents the northern range limit of mangroves in the Gulf of Mexico, and uniquely also includes Rhizophora mangle. The objective of this research was to quantify soil OC density beneath both mangrove species and compare results to the soils beneath two contiguous native tidal saltmarsh species: Juncus roemerianus and Spartina alterniflora in a barrier island setting. Dominant plant taxa were not a significant predictor of soil OC density, highlighting the relative importance of site-specific environmental attributes as controls on soil properties. Soil profile δ13C compositions included a range of values reflective of C3 and C4 plant inputs, suggesting that shifts in plant taxa, both from marsh to mangroves and between marsh species, have been occurring at all sites in this study. These findings support much of the literature on mangrove encroachment, which indicates mangrove soil OC concentrations, densities, or stocks are less than or equal to that of co-located tidal marsh habitats. Through a systematic review, the potential of several proposed explanatory variables (climate, environmental setting, plant physiology and productivity, and duration of encroachment) were identified to evaluate how soil OC density in mangrove habitats might increase over time, which is critical to forecasting how continued mangrove expansion might affect blue C storage as these habitats evolve.

2. Steinmuller, Havalend E, Ethan Bourque, Samantha B Lucas, Kevin M Engelbert, Jason Garwood, and Joshua L Breithaupt. 2022. "Comparing Vertical Change in Riverine , Bayside and Barrier Island Wetland Soils in Response to Acute and Chronic Disturbance in Apalachicola Bay , FL." *Estuaries and Coasts*. https://doi.org/10.1007/s12237-022-01131-4.

Abstract: Coastal wetlands experience acute and chronic disturbances which can affect rates of surface elevation change and vertical accretion of surface sediments. Disturbance can either amplify or impair the ability of wetlands to maintain their position within the tidal frame, with implications for their long-term persistence. Using an 8-year dataset collected from coupled surface elevation table-marker horizon (SET-MH) stations spanning riverine, bayside, and barrier island settings in the Apalachicola Bay region of north Florida, USA, this study investigated decadal-scale surface elevation change and vertical accretion rates to assess wetland vulnerability to acute (Hurricane Michael) and chronic (relative sea-level rise; RSLR) disturbance in different geomorphic settings. All sites had long-term accretion rates that exceeded rates of surface elevation change (pre-Michael), indicating that surface accretion was not a good indicator of changes in surface elevation for any of these coastal geomorphic settings. Hurricane Michael increased surface elevation change rates at bayside and riverine sites; barrier island sites consistently displayed the lowest surface elevation change rates, which did not differ between pre- and post-Michael periods. Accretion rates were greatest in the riverine sites, which were

characterized by highly organic soils. Barrier island and bayside sites demonstrated elevation and accretion deficits relative to the rate of RSLR for Apalachicola Bay between 2010 and 2022, indicating high vulnerability of these sites to chronic increases in sea level. These estimates of marsh resilience relied exclusively on rates of vertical change and neglecting to account for lateral erosion failed to predict that each of the three barrier island sites experienced rapid loss of the seaward SET-MH stations during the observation period. These results provide evidence of different vertical change responses among coastal wetlands of three geomorphic settings exposed to hurricanes and RSLR in the same region and suggest different timelines for long-term persistence of these sites.

6.2 The evolution of heavy metals and organochlorine pesticides in Apalachicola Bay (Dr. Michael Martinez-Colon (Faculty) and Solanke Adebayo (PhD Student), FAMU)

Introduction. 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 (19^{th} – 20^{th} centuries) of marine environments, thus providing invaluable information on environmental health changes (“deterioration” vs. “restoration”). This 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 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. The ultimate goal of this project is to facilitate the expansion of this assessment approach into other coastal and estuarine ecosystems within the National Estuarine Research Reserve System.

Objectives

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
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. Sediment sample sites were selected to encompass a diversity of depositional environments. Samples were collected from the mouth of the river as it extends to the marine environment. 12 surface sediment samples were collected at Apalachicola Bay using a stainless-steel bottom dredge sampler and transferred into clean Ziplock plastic bags using a plastic spoon for metal analysis. In contrast, parts of the sediment samples for pesticide analysis were wrapped in aluminum foil twice and sealed in plastic bags. In addition, a sediment push core (34 cm ad sliced between 0.5-2cm intervals) was collected at Apalachicola Bay. The core liner was acid washed before sampling and capped immediately after collection. Both sediment and core samples collected were stored below 4ºC until sample analysis. Extreme care was taken to avoid sample contamination during sample collection.

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 metal 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".

For the pesticide analysis, all samples were placed in an amber glass bottle. All subsamples were freeze dried before analysis for organochlorine pesticides (OCPs). These pesticides are commonly used for high agricultural production to destroy fungi, weeds, insects, bacteria, and other pests, thereby increasing pollution of the environment-water, air, and soil. The OCPs are a group of chlorinated compounds used as pesticides. OCPs are organic compounds known for persistence, toxicity, lipophilicity, slow degradation, long-range transport, and bioaccumulation. In this project, the following OCPs were analyzed a-Lindane, b-Lindane, d-Lindane, g-Lindane, Heptachlor, Aldrin, Heptachlor Epoxide, Endosulfan I, Dieldrin, Endrin, Endosulfan II, Endrin Aldehyde, Endosulfan Sulfate, 4,4'-DDE (Dichlorodiphenyldichloroethylene), 4,4'-DDT, Methoxychlor and 4,4-DDD (Dichlorodiphenyldichloroethane). Sediment and surface samples were analyzed according to Zhao et al. (2019). Sediment samples (2g) were homogenized with previously baked (at 660ºC) 10g anhydrous sodium sulphate Na_2SO_4 with mortar and pestle. Soxhlet extraction was done with 250 mL dichloromethane:hexane (1:1 v/v) for 16 hours. Before the extraction, the mixture was spiked with 0.3 ng/µL 13C12-labelled PCB 111 to monitor recovery. The extracts were rotary evaporated to 10 mL at 38ºC, and active copper powder was added to remove sulphur.

The extracts were cleaned using a multilayer silica gel column that was packed from bottom to top with 2 g silver nitrate silica gel, 1 g activated silica gel, 3 g basic silica gel, 1 g activated silica gel, 4 g 44% acidic silica gel, 4 g 22% acidic silica gel, 1 g activated silica gel, and 2 g high anhydrous sodium sulfate. The column was eluted with 110 mL hexane. Subsequently, the column was eluted with 50 mL dichloromethane:hexane (1:1 v/v). A basic aluminum column cleaned and separated the concentrated elute from 110 mL hexane. While the concentrated eluate from 50 mL dichloromethane:hexane was cleaned up and separated by a florisil column. Each fraction was added, and the eluate was concentrated to about 20 µL under a gentle stream of ultra-pure nitrogen, solvent exchanged to hexane in a mini-vial, followed by instrumental analysis. Acenapthlene-d10 and Chrysene-d12 were used as internal standards. Target analytes were identified and quantified using gas chromatography-mass spectrophotometry (Agilent GC-MS Triple Quad 7000c). The GC column DB-5MS (0.25 mm x 60 m x 0.25 µm film thickness) and the electron ionization (EI) mode were used. The column oven temperature was from 40ºC (1 min) to 300ºC at a rate of 9ºC/min, held for 5.10 minutes, and the injector temperature of 250ºC. The GC-MS was operated on selected ion monitoring (SIM) mode, and analytes were monitored using the two most intense ions of the molecular ion clusters. Quality control standards were analyzed for every five samples to monitor instrument stability.

Results and discussion. Based on their spatial distribution, only two metals (Cd and Se) appear to be coming from the Apalachicola River (Fig. 41A and I). The rest of the metals (Cr, Co, Ti, Cu, Ni, Cd, Pb, Zn, Hg; Fig 41) seem to have a more local source with two “depocenters” one being

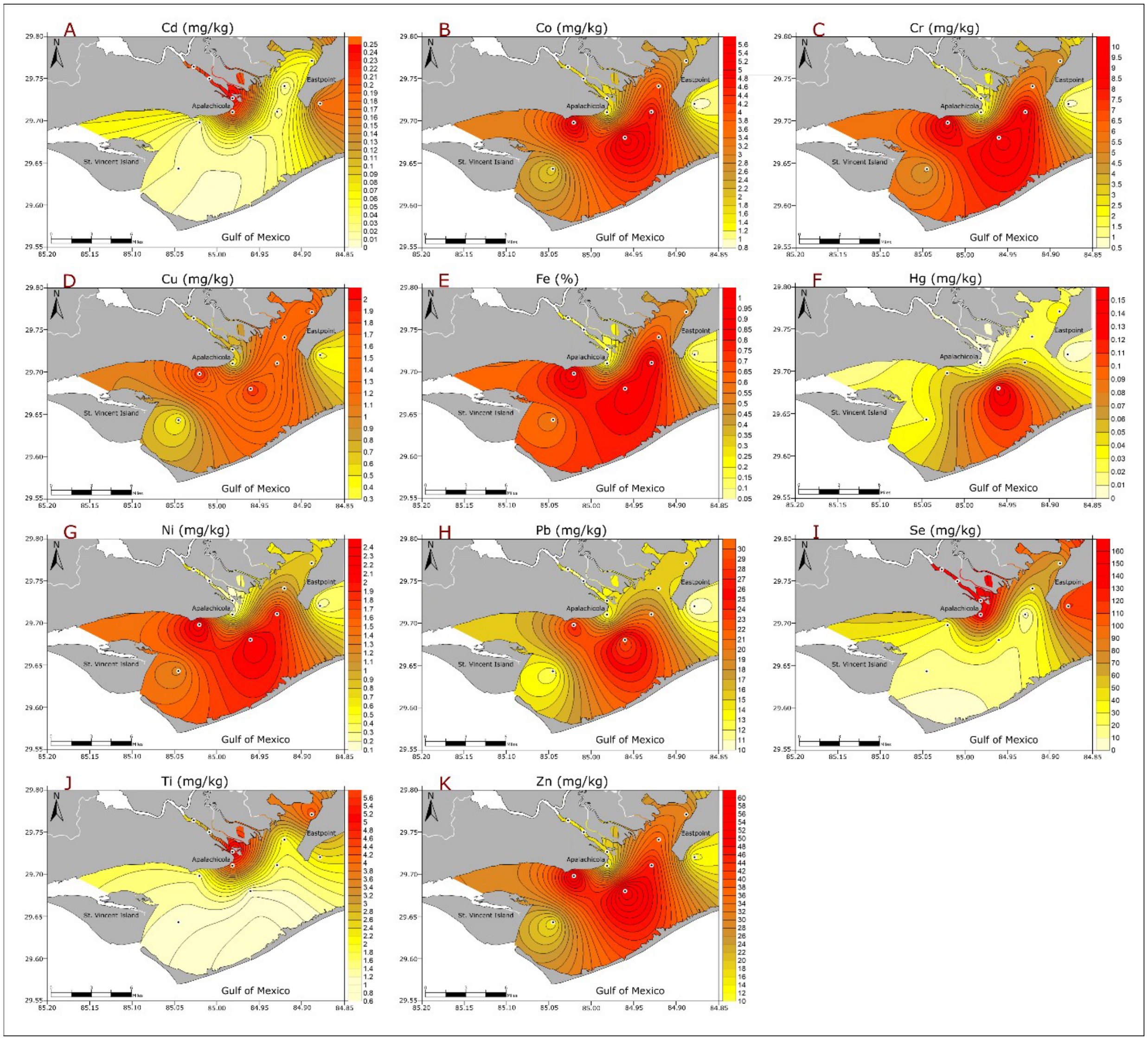


Figure 41. Heat maps showing concentrations of heavy metals in Apalachicola Bay. Panels are as follows: A) Cadmium, B) Cobalt, C) Cromium, D) Copper, E) Iron, F) Mercury, G) Nickel, H) Lead, I) Selenium, J) Titanium, K) Zinc.

south of the city of Apalachicola and a second towards the center of the bay which coincide as well with the distribution of total organic matter and mud content (Fig. 42). 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 had an effect on the biota. In regard to Se, it is the only metal to have been found in the core sediments to be “extremely” to “severely” enriched. 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. 43).

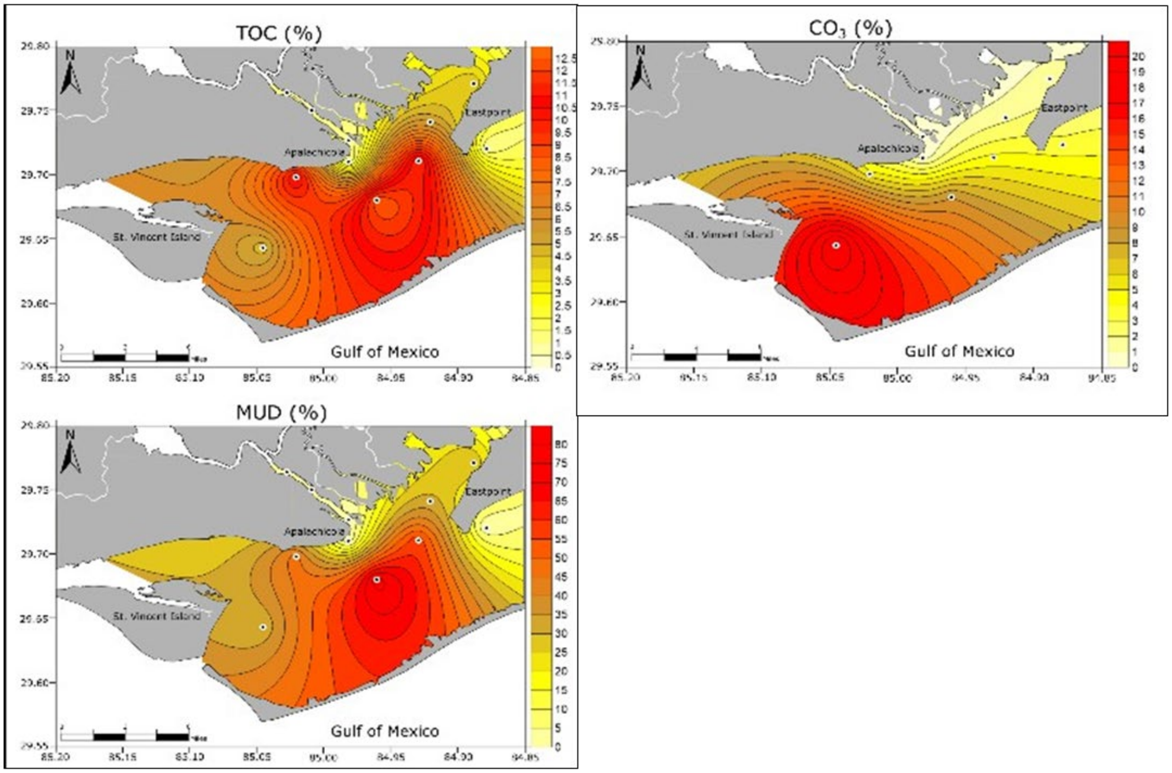


Figure 42. Heat maps showing distribution of total organic carbon (TOC), mud, and carbonate (CO_3)

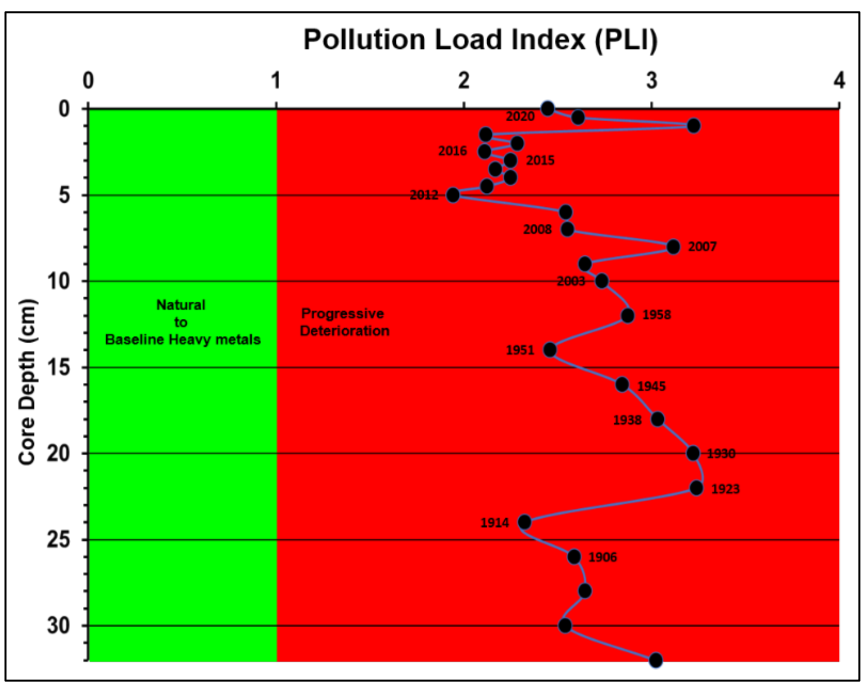


Figure 43. Pollution load index over time/core depth

The data indicate that the levels of the OCPS in the sediment samples analyzed were below the detection limit. Although OCPs persist in the environment, it takes time to degrade from days to years. DDT and some OCPs were banned in the US over forty years ago. In 1988, US Environmental Protection Agency (EPA) banned all chlordane usage. OCPs' half-life analyzed in this study is between 1 day to 15 years; this may have contributed to the non-detection of these pesticides in the sediment samples analyzed. Furthermore, it could be that OCPs were not used in essential quantities in any potential source- agricultural, silvicultural, urban, and suburban areas nearby. There could be no or minimal input of the OCPs from any potential source areas into the study area (Apalachicola Bay). Also, the specific site where the surface and core samples were collected did not get any input, even if there was input from upstream.

6.3. ABSI Oyster management decision-support tool (Fabio Prior Caltabellotta, Postdoctoral fellow, FSUCML)

Introduction. The Eastern Oyster (*Crassostrea virginica*) is an important fishery component for the economy of Apalachicola Bay, Florida, and has accounted in the past for about 10% of the U.S. oyster harvest. Unfortunately, the oyster fishery collapsed in 2012, leading to major losses to the oystermen's livelihoods and the local economy. The Apalachicola oyster fishery was closed on August 1, 2020, for five years, resulting in frustration within the oyster fishery community. There's an urge for solutions that would allow oyster reefs to recover while meeting oystermen's needs are needed. This project aims to develop a decision-support tool to enable the oyster harvesting community and other stakeholders to explore and evaluate the relative effect of proposed management strategies to ensure a healthy oyster population in Apalachicola Bay and simultaneously allow reasonable access to use this resource.

Methods. The main components of this decision-support tool are 1) user interface, where the user can find information about the general description of the ABSI project and the development team involved in building this tool, a control panel that the user will be able to manipulate some of the oyster population model inputs (e.g., total effort per month and amount of shell added annually to the restored areas), and a performance metrics panel generated by user inputs that were specified in the control panel to evaluate the desired outcomes (e.g., numbers of bag per day); and 2) server, which contains the oyster population model that has been developed by Dr. Ed Camp (University of Florida). This is an age-structured biological operating model coupled with a dynamic-effort model linked to habitat and socio-economic models to provide full transparency when selecting optimal policies.

Results and discussion. The decision-support tool is under construction, and a preliminary version will be available to test at the Community Advisory Board (CAB) meeting scheduled for April 12, 2023. The decision-support tool will be hosted on the R Shiny app website (https://www.shinyapps.io/). Future CAB meetings, oystermen's workshops and community workshops will be used to improve the decision-support tool, including the user interface framework and performance metrics outputs desired, based on a greater understanding among diverse and potentially adverse participants with respect to management actions.

7. ABSI Research and Restoration Hatchery (ABSI Hatchery Team)

7.1. Hatchery accomplishments in 2022-2023.

Construction on the permanent facility was completed in summer 2022, and facilities were finished throughout the fall of 2022, including the building of the algae culture room and flow-through and recirculating tank systems for the larval rearing area, broodstock conditioning room, and juvenile setting systems. To facilitate year-round larval culture, the building was insulated, and temperature control units were installed in 2023 to decrease temperature fluctuations in the larval and juvenile culture areas. Preliminary hatchery work used commercial algal paste, which has been successfully used elsewhere, but live algal food has more flexibility for custom feeding for different species and life stages, and is less likely to promote bacterial growth. The algal stock and grow out rooms are a critical part of the hatchery and became fully functional in September 2022. The facility is currently producing 7 different species of microalgae (Table 7). Successful production of live algae also improved feeding techniques for larvae, which was a hatchery goal from 2021.

Table 7: Microalgal species with type successfully cultured at production densities and volumes for shellfish feed in 2022.

Algal Species	**Algal Type**
Isochrysis galbana	flagellate
T-isochrysis lutea	flagellate
Tetraselmis chuii	flagellate
Pavlova pinguis	flagellate
Chaetoceros muelleri	diatom
Chaetoceros neogracile	diatom
Thalassiosira weissflogii	diatom

In addition to the hatchery building, ABSI has access to an open-water lease in Alligator Harbor (29.92049 N, 84.41014 W; section 19-AQ-939). This lease allows the project to grow out spat on shell and single juveniles prior to their deployment for restoration or use in research. Grow-out of shellfish in a hatchery setting is inefficient as it requires cultured algal feed and significant staff time for maintenance, as well as a great deal of space. Hatchery staff developed sampling protocols for monitoring health and growth of animals maintained on the lease. A second lease site in Oyster Bay is currently being established as well to facilitate grow-out and will be operational in 2023.

Much of the 2022 production season was dedicated to building necessary culture systems in the new facility as described above. The completion of the facility allowed for successful culture to settlement of local bay scallops, *Argopecten irradians*, which are challenging to culture as the juveniles require more space and food than oysters and are more delicate. The oyster spawns were less successful than desired (Table 8), primarily due to water related issues at the FSUCML that coincided with the oyster spawns and with temperature fluctuations and bacterial blooms in the larval tanks. The facility was incomplete at this time and the problems could not be resolved, despite the best efforts and hard work of the hatchery team. To resolve the water quality problems, FSU invested significant funds to install a sophisticated filtration system and cleaned and repaired the seawater intake system. Temperature fluctuations have been addressed with insulation and temperature control systems in the facility, and the experimental use of probiotics, together with the use of live food appears to have resolved the bacterial problems.

In 2022, hatchery staff also optimized non-lethal volitional spawning techniques for both *C. virginica* and *A. irradians* using a combination of thermal cycling and naturally acquired hormonal cues. For *C. virginica*, volitional parental contribution in spawns increased from 50% in 2021 to 90-100% in 2022 using the optimized methods. In *A. irradians*, parental spawning contribution was 75% for the first attempt and 90 - 100% for the consecutive spawns.

Hatchery staff also optimized larval handling and drain down methodologies by adopting new grading methods and the use of a flow-through water table. Using these methods, larvae recovered faster from handling stress and displayed fewer stress signs, overall, which accomplished a main goal set in 2021.

The hatchery had a successful volitional oyster spawn at the beginning of April and currently has over 100 million oyster larvae, which appear healthy and will hopefully produce a significant batch of spat-on-shell for restoration experiments.

Table 8: Summary of spawns attempted in 2022, including date of spawn, number of larvae produced, species used, overall survival, new methodology attempted, and outcomes

Spawn date	Species	No. Larvae	Survival	Comment	Outcomes
May 3, 2022	*C. virginica*	22,200,000	0%		High abnormality and mortality, stunted growth, raised to eye-spot development, but no metamorphosis
Aug. 29, 2022	*C. virginica*	835,500	0%	New facility	Bacterial infection
Sep. 9, 2022	*C. virginica*	54,000	0%	New facility	Bacterial infection
Sep. 15, 2022	*C. virginica*	1,615,800	0%	New facility	Extreme temperature fluctuations
Sep. 20, 2022	*A. irradians*	8,450,000	0.12%	First attempt at *A. irradians* culture	Successful to setting
Nov. 2, 2022	*A. irradians*	10,047,000	24.8%	Probiotics and live algae with paste supplement	Successful to setting
Dec. 8, 2022	*A. irradians*	644,000	Culled	N/A	Low larval counts, hatchery at max setting capacity

Hatchery post-production grow-out in 2022. Hatchery grow-out accomplishments were facilitated by the establishment of the open-water lease in Alligator Harbor. Although no *C. virginica* spat were produced in the FSUCML Hatchery, two diploid crops of oysters originating from local broodstock lines were acquired from Bay Shellfish Company for grow-out. The first crop totaled 33,000 oysters at 6 mm and was planted in August 2022. This crop currently averages 78 mm in shell height. The second crop of 10,000 oysters at 6 mm was planted in November 2022, and the current average shell height is 53 mm. Mortality has been negligible in both crops. Oysters have been used to supply ABSI researchers for experiments, and animals will be used in restoration research in 2023 (see "**Planned research for 2023**").

Additionally, 500,000 *A. irradians* spat have been successfully grown out to multiple sizes ranging from 1mm - 10mm using hatchery-based nursery techniques. Methodologies involve using high-flow wet tables to house spat and feeding live algae. Wash-downs occur daily. Using these methods, the survival of juvenile scallops is estimated around 20%. This survival does not factor in juveniles euthanized to allow for the majority to continue growing with proper spacing. *A. irradians* spat is supplying researchers with animals for restoration experiments. Additionally, an estimated 50,000 *A. irradians* spat were donated to FWC for their scallop restoration program.

Research in 2023 was slowed due to the amount of work needed to make the new facility operational (setting up algal systems, installing insulation and finalizing electrical systems for temperature control). However, in an effort to resolve bacterial problems, research focused on increasing larval survival and growth through the use of *Sanolife® MIC* commercial probiotics, combined with the use of live algae rather than dead algal concentrate.

7.2. Use of probiotics in bay scallop larval culture

Introduction. The main aim of this pilot study was to alleviate previous blooms of harmful bacterial in larval tanks through the use of probiotics, to increase growth and survival of larvae to competency. This pilot study evaluated the use of probiotics under different food regimes on the survival and growth of bay scallop (*A. irradians*) larvae. Prior to the completion of the algal culture system, the hatchery was using Reed Mariculture 1800 Shellfish Diet. Using dead paste may have contributed to the bacterial blooms that caused problems in 2022. Using probiotics is common in shrimp aquaculture so the hatchery team conducted a pilot experiment to investigate the potential benefits of using a commercially available probiotic (*Sanolife MIC*) in combination with dead algal paste and live microalgal food.

Methods Four larval tanks (1400 L) were filled with 1-µm filtered seawater 24 hours prior to stocking with larvae. Half the tanks were treated with Sanolife MIC probiotic (which is used in commercial shrimp farms) at a concentration of 1.4×10^{10} cell forming units (CFUs) per ml. The tanks were stocked with 800,000 two-day old larvae per tank into one of four treatments 1) No probiotic, dead algal paste (ND); 2) No probiotic, live microalgae (NL); 3) Sanolife probiotic, dead algal paste (PD); 4) Sanolife probiotic, live microalgae (PL).

Results and discussion. At the end of the trial, the highest survival was observed in the probiotic treatments, but the treatment with live algae and no probiotic was doing equally well until the last time point. The lowest survival was observed in the treatment without probiotic using dead algal paste (Fig. 43). The growth results (Fig. 44) also showed poorest performance in the treatment fed dead algal paste without probiotics (ND). The probiotics fed algal paste (PD) had high survival but fewer larvae in the larger size classes than the no probiotic fed live algae (NL). This outcome could be interpreted as probiotics reducing bacterial mortality but dead algal paste is not as good a food source as the live algae. The best growth results were observed when probiotics were used in conjunction with live algal food. Overall, survival increased with the use of probiotics and live feed supported faster growth (Fig. 44) However, further investigation is necessary to understand if the effect is statistically significant since this pilot project was unreplicated (due to logistical limitations). The hatchery team plan to expand this work using a fully replicated design for *C. virginica* and *A. irradians* to evaluate methodology for incorporation into hatchery protocols

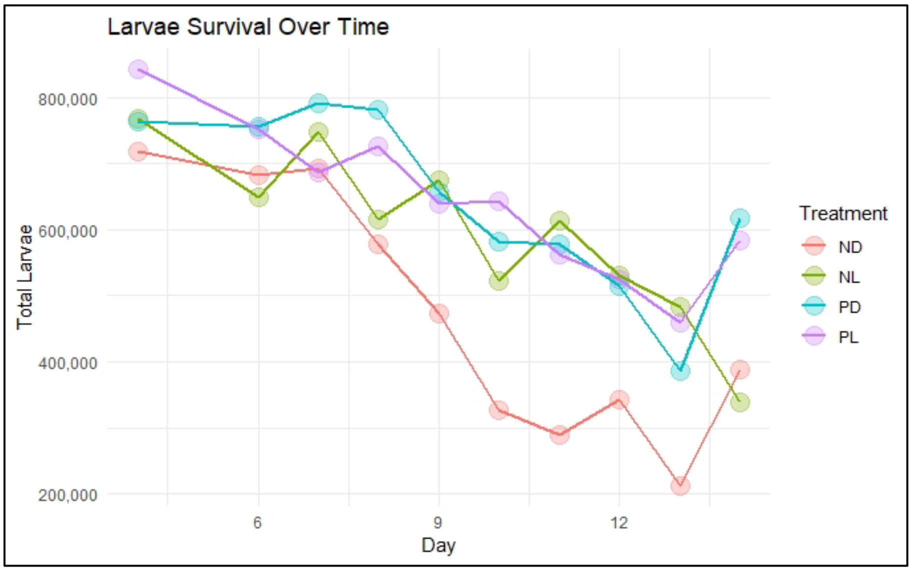


Figure 43. Counts of live bay scallop larvae over 14 days of exposure to four treatments. ND: no probiotic, dead algal paste; NL: no probiotic, live microalgae; PD: probiotic, dead algal paste; PL: probiotic, live microalgae.

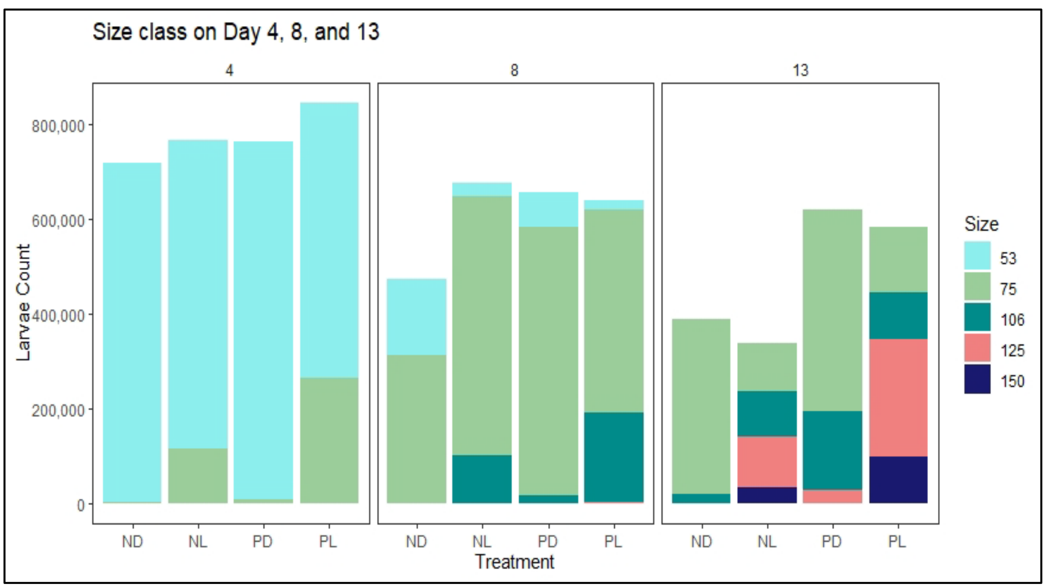


Figure 44. Size classes of bay scallop larvae for each treatment on days 4, 8, and 14. Treatments are as follows. ND: no probiotic, dead algal paste; NL: no probiotic, live microalgae; PD: probiotic, dead algal paste; PL: probiotic, live microalgae. Larval size is in micrometers.

7.3. Hatchery plans for 2023-2024.

The focus of hatchery research will be to improve hatchery operations and produce spat on shell for restoration research. This includes survival and growth trials of hatchery spat in the field and development of optimal restoration approaches using hatchery reared shellfish. The following describes research that has been initiated recently or will begin in the near future

7.3.1. Effects of temperature on oyster conditioning

Objective: Determine optimal temperature for conditioning *Crassostrea virginica* broodstock from north Florida.

This work will test the role of temperature in conditioning in broodstock oysters, *C. virginica* for use in the ABSI shellfish restoration and research hatchery. The main aim of this research is to support out-of-season broodstock conditioning for obtaining larvae and juveniles during times that wild broodstock are not spawning. This approach has been used in the northeast region to assess in situ survival of hatchery spat without the confounding effects of wild spat set. It will also provide a wider time window for research on oyster early life histories. Animals for this project have been collected, and the project will be finished by June 2023.

7.3.2. Effects of probiotic on shellfish larval survival and growth

Objectives: 1). Repeat the trial experiment described in section 7.2, to assess effectiveness of using probiotics with larval culture for oysters and bay scallops; 2) assess effects of probiotics on spat settlement and survival; 3) identify the microbial community present in hatchery water using 16S rRNA sequencing and verify species composition of *Sanolife MIC*

This work investigates whether adding probiotics to increase the abundance of beneficial bacteria in larviculture shows a difference in growth, survival, and setting success in shellfish larvae. This research hopes to identify a cost effective way for hatcheries to increase survival and production of commonly cultured shellfish species. Increasing production yields allows the FSUCML shellfish restoration and research hatchery to optimize cost and time spent rearing shellfish.

7.3.3. Effects of live oyster presence and alternative substrate on oyster recruitment

Objective. 1) Compare oyster spat settlement on natural shell vs limestone; 2) determine whether the presence of adult hatchery oysters (vs no oysters) changes the dynamics of oyster spat settlement on natural shell and limestone; 3) Monitor spat settlement during the spawning period.
This work will support development of restoration techniques for the eastern oyster, *Crassostrea virginica*, using hatchery produced animals, or potentially unwanted adults from oyster farms. This project will provide a comparison of the proposed substrates to be used in restoration efforts in Apalachicola Bay and will determine whether the presence of live adult oysters will help increase recruitment on restoration substrates. Animals for this experiment are from the August 2022 crop of oysters and were deployed in March 2023. The experiment will be finished at the end of the natural spawning season, October 2023.

7.3.4. Use of cultured oysters in restoration strategies

Objectives. 1) Assess survival, growth, and wild spat recruitment on hatchery animals across an environmental gradient in Apalachicola Bay; 2) Evaluate the degradation and longevity of alternative mesh materials for restoration across sites in Apalachicola Bay; 3) Determine the feasibility and effectiveness of using hatchery animals for restoration.

This research aims to support and expand restoration research for the eastern oyster, *C. virginica*, in Apalachicola Bay by assessing the application of cultured oysters in population enhancement across a range of environmental conditions. This project will also explore the use and efficacy of different mesh materials, biodegradable cultch mesh and poultry wire, in restoration techniques. Animals for this project are from the 2022 oyster crop and will be deployed in April 2023 for a year-long study of recruitment and survival.

7.3.5. Phytoplankton identification and seasonality on and oyster lease in Alligator Harbor

Objectives 1) Monitor phytoplankton species composition throughout the year; 2) Determine changes in phytoplankton species abundance seasonally; 3) Monitor environmental conditions that may affect phytoplankton blooms

This research works to identify and infer abundances of phytoplankton on an aquaculture lease used for shellfish grow-out. In collaboration with the NOAA Harmful Algal Bloom Monitoring and Reference Branch, this work will help the ABSI shellfish hatchery identify the native species of phytoplankton in our area, as well as better understand the species composition of the bay throughout the year. This information assists the hatchery in understanding the local shellfish diet options and provides a warning system for the aquaculture lease for harmful algal blooms.

7.4. Hatchery Internship program

The ABSI grant has funding allocated for a hatchery internship program that is intended to target local residents to increase workforce capacity in the aquaculture industry. In 2022, ABSI collaborated with 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. In 2022, four interns were trained through this program, which 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 learned 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. In Spring 2023, two of the interns were hired as technicians by the ABSI hatchery. This program will continue throughout the remainder of the ABSI grant, and hopefully beyond.

7.5. Pilot shell recycling program

Shell recycling programs are often focused on obtaining shell from restaurants, but with the expansion of oyster aquaculture, the mortalities that unfortunately occur in this industry are a potential untapped shell resource. The ABSI hatchery (with approval from Franklin County) have established two shell recycling bins at the boat ramp near the Alligator Harbor oyster leases. This pilot project will assess the cost-effectiveness of providing oyster farmers with a repository for their shell, which is often simply dumped by the farmers who have no use for it. If this proves successful, ABSI will work with Franklin Promise recycling program to include the Apalachicola oyster farmers. Farmed shell, although intermittently available, may provide significantly more material and be less time consuming than restaurant sourced shell.

8. Targeted outreach to the community

ABSI outreach increased significantly in 2022, with the lifting of Covid-19 quarantines and lockdowns. From the continuation of the Community Advisory Board and its subsequent subcommittees of Outreach and Education and the Successor Group, an increase in subscriptions and mailings of the bimonthly ABSI newsletter, an influx of participation in events with local organizations throughout the Tallahassee and Florida panhandle region, and new additions to ABSI's website including FAQs and Volunteer Opportunities, ABSI's engagement with the local community thrived.

8.1. Community Advisory Board

The Community Advisory Board (CAB), facilitated by Jeff Blair (Facilitated Solutions), has continued to flourish. The overarching 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 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 commitment issues and/or retirement, a few Board members stepped down and were replaced with members of similar stakeholder organizations.

8.1.1. Community Advisory Board Membership

Agency personnel: Mike Allen - University of Florida/IFAS Nature Coast Biological Station, Director; Jenna Harper - Apalachicola National Estuarine Research Reserve, Reserve Manager; Becca Hatchell - Florida Fish & Wildlife Conservation Commission, Marine & Estuarine Habitat Conservation & Restoration Biologist; Erik Lovestrand - Florida Sea Grant, Extension Director for Franklin County; Alex Reed - Florida Department of the Environment, Director of Office of Resilience and Coastal Protection; Devin Resko - Florida Fish and Wildlife Commission Marine Fisheries Management, Disaster Relief Coordinator; Portia Sapp - Florida Department of Agriculture and Consumer Services Division of Aquaculture, Director; Paul Thurman - Northwest Florida Water Management District, Environmental Scientist

Local government: Anita Grove - Apalachicola City Commissioner; Ottice D. Amison – Franklin County Commissioner (District 4)

Local business: Gayle Johnson – Indian Lagoon Oyster Company, Director of Operations; Chuck Marks - Acentria Insurance, Vice President (*ret.*); Steve Rash - Water Street Seafood, Owner

Non-governmental organizations: Georgia Ackerman - Apalachicola Riverkeeper, Executive Director; Chad Hanson – The Pew Charitable Trusts, Fisheries Science and Policy Analyst

Non-profit organizations: Frank Gidus - CCA Florida, Director of Habitat & Environmental Restoration; Chadwick Taylor - Riparian County Stakeholder Coalition; Katie Konchar – The Nature Conservancy

Watermen: David Barber – Barber Seafood, Owner; Shannon Hartsfield - Seafood Management Assistant Resource Recovery Team (SMARRT), Chair; TJ Ward - Buddy Ward & Sons Seafood

The ABSI CAB web page contains detailed information on the CAB membership (https://marinelab.fsu.edu/absi/people/cab_members/)

8.1.2. CAB Meetings

All meetings since March 30, 2022 have been held in person at the Apalachicola National Estuarine Research Reserve (ANERR) with the option to attend virtually via Zoom. Documents from each meeting have been posted on the ABSI CAB web page. These include agendas, presentations, reports, and videos (https://marinelab.fsu.edu/absi/cab/documents/)

March 30, 2022 presentations:

1) CAB Phase IV Workplan Update March 30, 2022 (J Blair, Facilitated Solutions, LLC)
2) ABSI Science Update March 30, 2022 (S Brooke, FSU)
3) Apalachicola Bay Oyster Management Options (D Resko, FWC)
4) Management Strategies and Actions (J Blair, Facilitated Solutions, LLC)

May 25, 2022 presentations:

1) ABSI Science Update (S Brooke, FSU)
2) Alabama Oyster Management and Oyster Reef Restoration Strategy (J Herrman, DNCR)
3) OysterFutures: A Collaborative Process for Developing Oyster Management Recommendations in Maryland (M Wilberg, University of Maryland Center for Environmental Science)

July 27, 2022 presentations:

1) ABSI Phase IIII; Meeting IV – Meeting Objectives (J Blair, Facilitated Solutions, LLC)
2) Apalachicola Bay Oyster Restoration Phase II – FWC (D Resko, FWC)
3) Fishery Dependent and Fishery Independent Data (E Camp, UF)
4) Results from DEP and FWC Led Restoration Projects (B Pine, UF)
5) ABSI Tonging Data (S Brooke, FSU)
6) ABSI CAB Meeting – Assessment and Simulation Models (E Camp and N Fisch, UF)

October 18, 2022 presentations:

1) ABSI Work Plan and Updates (J Blair, Facilitated Solutions, LLC)
2) ABSI Science Update 10-18-22 (S Brooke, FSU)
3) FWC and NFWF Restoration Update (D Resko, FWC)
4) ABSI Model Simulation Results (E Camp, UF)

November 30, 2022 presentations:

1) CAB Workplan (J Blair, Facilitated Solutions, LLC)
2) ABSI Science Update (S Brooke, FSU)
3) Modeled Scenarios (E Camp, UF)

February 1, 2023 presentations:

1) CAB Workplan (J Blair, Facilitated Solutions, LLC)
2) ABSI Science Update (S Brooke, FSU)
3) FWC and NFWF Restoration Update (D Resko, FWC)
4) CAB Outreach and Messaging Strategy (C Hanson, The Pew Charitable Trusts)

3) Modeling Scenarios (E Camp, UF)

Three CAB sub-committees were generated to focus on specific aspects of community engagement that were not specifically being addressed through the CAB proper. These were the Outreach and Education Subcommittee, the Successor Group Subcommittee and the Restoration Funding Working Group. The structure of the CAB, the subcommittees and the ABSI science team are shown in figure 45.

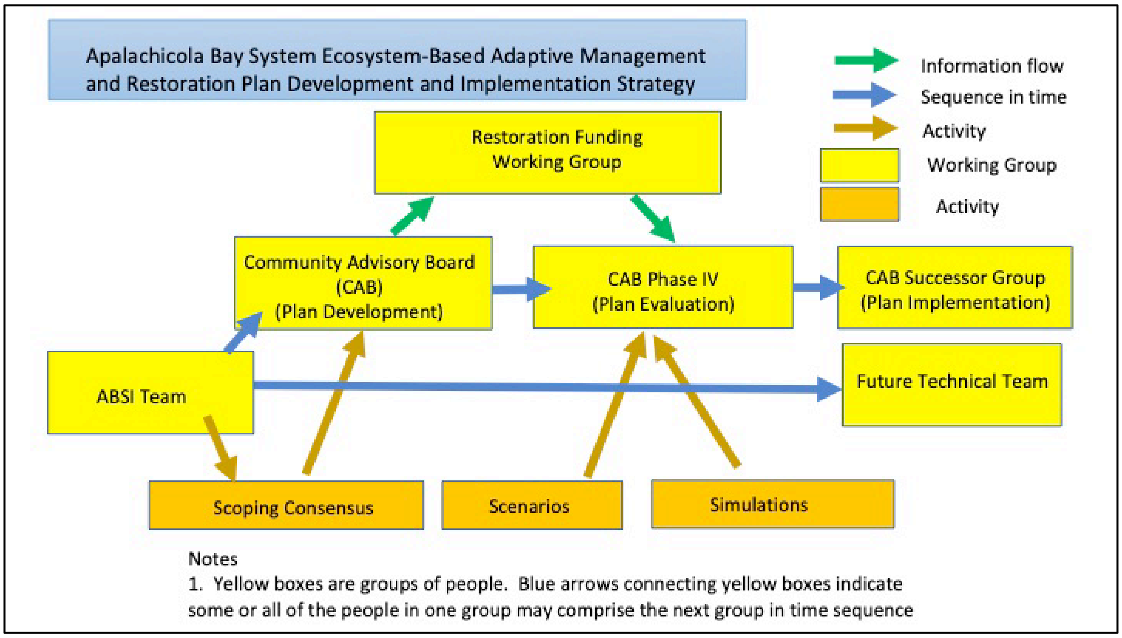


Figure 45. Schematic showing structure, timing and information flow of the community advisory board and subcommittees, and the ABSI science team

8.1.3. Outreach and Education Subcommittee (Madelin Mahood)

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 Education Subcommittee:

FSU: Dr. Sandra Brooke, Post-Doctoral Researcher Dr. Betsy Mansfield, and Outreach and Education Coordinators Jared Fuqua and Maddie Mahood

ABSI CAB: Chad Hanson (Chair), The Pew Charitable Trusts; Georgia Ackerman, Apalachicola Riverkeeper; Anita Grove, Apalachicola City Commissioner; and Devin Resko, Florida Fish and Wildlife Conservation Commission

***Subcommittee meeting dates*:**

March 21, 2022; April 13, 2022; May 11, 2022; June 29, 2022; August 17, 2022; November 2, 2022; December 5, 2022; January 18, 2023; and February 22, 2023.

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 the CAB, ABSI research updates, and upcoming events and education opportunities. The ABSI Newsletter email list currently has 521 subscribers, a **20.6%** increase from March 2022. Over the six newsletters sent between April 2022 and February 2023, the open rate per email averaged **58%,** well above the industry average (studies conducted by MailChimp and Constant Contact) of 20-21%. 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 (including an Op-Ed that was written and distributed in Sept. 2022):
    - 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 and Heather Bryan of Wakulla Chamber of Commerce; and Lisa Munson, Carrabelle Chamber of Commerce. Each of these organizations shares 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 ABSI and FWC "Frequently Asked Questions." The "Frequently Asked Questions" webpage was developed to provide current information on ABSI and FWC projects. As of March 2023, only the ABSI FAQs are being updated by the ABSI team.
    - This development also created the social media campaign "FAQ Mondays." Every Monday, one of ABSI's Frequently Asked Questions is highlighted and shared across the FSUCML social media pages.
- Development of in-person public workshops and participation in outreach events throughout the community (see ***Public Outreach***)
- Creation of CAB Summary Plan – a brief document that describes CAB Draft Framework Plan" https://marinelab.fsu.edu/media/5580/absi_cab_plan_framework_adopted_16_nov-2021_and_revised_27-july-2022__1-feb-2023.pdf) that is optimized for public consumption. *This document is currently a work in progress.*
- Development of messaging strategy – the subcommittee met with members of FSU's School of Communications Department to strategize new outreach opportunities and messaging strategies. A "Strategy Worksheet" was developed and highlighted the target audience (all members of the local community of Franklin County, specifically those of Eastpoint and Apalachicola), as well as new ideas on how to best reach and talk with the community. *This document is currently a work in progress.*

8.1.4. Successor Group Subcommittee (Dr. Joel Trexler)

Following an extensive development process involving 19 meetings, the CAB adopted a framework document for an ecosystem-based adaptive management and restoration plan in November 2021 for Apalachicola Bay. Since completion of the framework, the CAB has held community and stakeholder workshops to gather input on the plan. Additionally, the CAB has been evaluating fisheries scenario modeling with the goal of finalizing the restoration and management plan by the end of 2023. Upon adoption of the final plan, the CAB is set to end its work and be replaced by a *"**Successor Group**"* of community members and stakeholders. The yet unnamed Successor Group (e.g., Apalachicola Bay Restoration Community Advisory Board) will replace the CAB in January 2024 and will provide oversight of implementation of the recommended restoration and management strategies, including review of the results of late-developing restoration experiments, environmental monitoring, and restoration planning with continued stakeholder and community engagement.

The final plan for restoration and adaptive management of Apalachicola Bay will be submitted to its primary user, the Florida Fish & Wildlife Commission (FWC), as well as other State agencies including the Department of Environmental Protection and Department of Agriculture & Consumer Services. FWC will be the primary entity conducting and monitoring restoration as well as developing, adaptively modifying and enforcing management policies. The CAB's Successor Group will provide direct feedback to the FWC on such issues as possible adjustments to restoration methods, results of monitoring of restored sites as well as need for adaptive changes in management.

The Successor Group will be a permanent, representative long-term stakeholder group that will advocate for the adoption and implementation of the restoration plan long into the future. The Successor Group will ensure continuity between the ABSI CAB members and the agencies responsible for oyster restoration and adaptive management. The Successor Group will actively engage with state programs to encourage the adoption of ABSI's long-term monitoring guidelines and metrics for assessing water quality, oyster abundance and demographics, and to regularly review and update these guidelines and metrics to maintain a healthy and sustainable oyster harvest and ecosystem. The Successor Group will encourage agencies to prioritize the Plan's recommendations for investing more funding in the management and restoration of oyster resources. Additionally, the Successor Group will help seek other potential funding opportunities to properly implement the Plan.

Members of the Successor Group Subcommittee

ABSI CAB: Anita Grove (Co-chair), Apalachicola City Commission; Shannon Hartsfield (Co-chair), Seafood Management Assistant Resource Recovery Team (SMARRT); Chad Hanson, PEW Charitable Trusts; Steve Rash, Water Street Seafood; Chadwick Taylor, Riparian County Stakeholder Coalition.

Subcommittee meeting dates:

December 08, 2022 (Agendas/Minutes found here: https://marinelab.fsu.edu/absi/cab/cab-subcoms/)

8.1.5. Restoration Partners Funding Working Group (Dr. Joel Trexler, FSUCML)

The Restoration Partners Funding Working Group was included in the original proposal of the Apalachicola Bay System Initiative (ABSI) that was funded by Triumph Gulf Coast. The role of this Working Group is to locate financial resources necessary to implement actions prioritized by the Community Advisory Board (CAB) in the Apalachicola Bay System Ecosystem-Based Adaptive Management and Restoration Plan. The goal of the Plan is to develop community supported options for the restoration of oyster reefs and their associated ecological and ecosystem services, and for a sustainably managed oyster fishery in Apalachicola Bay. The $20M National Fish and Wildlife Foundation grant was awarded to FWC for research in Apalachicola Bay and

Suwannee Sound, and for deployment of restoration materials ($17M) in Apalachicola Bay. This Working Group will coordinate closely with FWC to seek future funding to continue the necessary restoration in the Bay.

The Working Group is charged with creatting a comprehensive funding approach for the Apalachicola Bay System Ecosystem-Based Adaptive Management and Restoration Plan implementation including a comprehensive analysis for future grant funding for strategies, including support for sustainable monitoring deriving from the Plan.

1. Evaluate potential funding sources (both public and private) for implementation of specific management and priority restoration strategies encompassed within the Management and Restoration Plan.
2. Match these potential funding sources and opportunities to specific prioritized strategies in the Management and Restoration Plan.
3. Create timelines for securing funding needed to address each of the prioritized strategies.
4. Provide advice and assistance to the CAB/Successor Group on contact persons and application requirements of each public and private funding entity.

The writing of proposals does not fall under the purview of the Working Group. However, it is likely that a small subset of members may be actively involved in such efforts.

Members of the Restoration Partners Funding Working Group

The committee has 12 members representing NGOs (TNC, Pew Trust, Apalachicola River Keepers), state agencies (FWC, FDACS, DEP, NWWMD), and universities (FSU, UF).

Subcommittee meeting dates:

The meetings on January 24 and March 22, 2022, finalized the committee charge and priorities from the Apalachicola Bay System Ecosystem-Based Adaptive Management and Restoration Plan. Committee activities were then put on hold while the Florida Fish and Wildlife Conservation Commission (FWC) developed their plan for use of NFWF funds to create a restoration program in Apalachicola Bay. The committee next met on November 7, 2022, to prepare a proposal for support of the CAB's Successor Group.

8.1.6. Oystermen and community workshops

This year, the ABSI took a slightly different approach and held both an Oystermen's Workshop (specifically designed for oystermen and watermen) and a Community Workshop (open to anyone from the public) on October 18th and 19th 2022. ABSI felt it was important to continue to hold Oystermen's Workshops to gather input from local watermen and seafood workers who have generations of experience making their livelihood off the Bay. ABSI also uses these meetings to be as transparent as possible about ABSI management and restoration discussions. ABSI felt it was equally as important to hold a Community Workshop to invite locals to learn about ABSI one-on-one from our researchers and scientists.

The Oystermen's Workshop on October 18th was held at the Apalachicola National Estuarine Research Reserve (ANERR). Only oystermen and watermen were invited to attend, as well as the ABSI project leads, presenters, and facilitator, Jeff Blair. The members of the Community Advisory Board and the public were invited to view the meeting via Zoom.

The Community Workshop on October 19th was held at the Eastpoint Firehouse. Anyone was welcome to attend, and this event was in-person only, with no virtual option.

October 18, 2022 Oystermen's Workshop presentations:

1) ABSI Science Update (S Brooke, FSUCML)
2) FWC and NFWF Restoration Update (D Resko, FWC)

Summary: At the 18 October 2022 Oystermen's Workshop, the Apalachicola Bay System Initiative (ABSI) Community Advisory Board (CAB) conducted the first in a series of Community Workshops planned for Phases IV and V of the ABSI project. The Oystermen's Workshop was convened for the purpose of seeking their feedback on restoration experiments and projects and on a variety of possible management scenarios for modeling using the Fisheries (Socioecological) Model developed by Ed Camp of the University of Florida. The Workshop was conducted at the Apalachicola National Estuarine Research Reserve for invited oystermen, and virtually for all other participants. 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; received an update and provided feedback on the FWC NFWF funded restoration project; and received an overview and provided feedback and input on a suite of possible management scenarios for modeling.

Oystermen: Jonny Chambers, Ronnie Gilbert, Abe Harstfield, Shannon Hartsfield*, Brett Lolley, Matt Polous, and Wayne Williams.

ABSI Representation: Sandra Brooke, ABSI Principal Investigator; Maddie Mahood and Jared Fuqua ABSI Outreach and Education, Anita Grove*, Apalachicola City Commission, Joel Trexler, ABSI Principal Co-Investigator and Director of FSUCML, and W. Ross Ellington, ABSI Partner

Facilitated Solutions, LLC: Jeff Blair

October 19, 2022 Community Workshop Presentations:

1) ABSI Science Update (S Brooke, FSU)
2) FWC and NFWF Restoration Update (D Resko, FWC)

Summary: At the 19 October 2022 Community Workshop the Apalachicola Bay System Initiative (ABSI) Community Advisory Board (CAB) conducted the second in a series of Community Workshops planned for Phases IV and V of the ABSI project. The Community Workshops was convened for the purpose of seeking public feedback on restoration experiments and projects, and on a variety of possible management scenarios for modeling using the Fisheries (Socioecological) Model developed by Ed Camp of the University of Florida. The Workshop was conducted at the Eastpoint Firehouse.

During the Workshop the Community: were provided an overview of the Project Workplan and Schedule; received an update and provided feedback on ABSI restoration experiments; received an update and provided feedback on the FWC NFWF funded restoration project; and received an overview and provided feedback and input on a suite of possible management scenarios for modeling. Thirteen Community Members participated, with an additional 8 others from FSU, FWC, and members of the CAB.

ABSI Representation: Sandra Brooke, ABSI Principal Investigator; Jared Fuqua ABSI Outreach and Education, Joel Trexler, ABSI Principal Co-Investigator and Director of FSUCML, and W. Ross Ellington, ABSI Partner

Facilitated Solutions, LLC: Jeff Blair

8.2 Public outreach and engagement

During 2022, many COVID-19 health and safety protocols were lifted making it more feasible for the ABSI team to engage in a variety of public outreach events. As a result, over the last year, we have engaged with over 20,000 individuals through a variety of events.

8.2.1. Targeted outreach events

ABSI has continued to implement targeted outreach events and presentations given by Dr. Brooke around the local community. These include research updates, presentations and "Q&A" opportunities at the county and city commissions, local organizations, and more:

- Franklin County Commission (June 7 and November 1, 2022)
- Apalachicola City Commission (May 3, 2022)
- ANERR SciCáfe Series (July 28, 2022)
- St. George Island Civic Club (March 17, 2022)
- ABSI Oystermen's Workshop (October 18, 2022)
- ABSI Community Workshop (October 19, 2022)

Community Workshop at Eastpoint Firehouse

8.2.2. FSUCML Open House

Held on April 22nd, 2022, this event was an opportunity for those in our local community to visit the lab and hear firsthand about the research being conducted by ABSI. Over 1,500 people attended and interacted with the FSUCML research professors, graduate students, and facilities. We had several presentations throughout the property as well as educational activities to get people engaged and interested in learning more about Apalachicola Bay and its ecosystem.

Dr. Sandra Brooke at FSUCML Open House

8.2.3. School Groups

ABSI has greatly increased its outreach within public schools and homeschool collectives in Franklin, Wakulla, and Leon Counties. These programs were tailored to best fit the age of the children participating. We have created inclusive cross-curriculum educational resources and experiences for students of all ages to help address some of the environmental issues that Apalachicola Bay faces. Our hope is to promote the science of the Bay and the importance of a healthy ecosystem through fun and engaging programs. Schools have had the option of either coming to the lab or bringing us to their organizations in person or virtually. Since April of last year, we have reached 13 different school groups in-person and several more over virtual platforms. In total, we have engaged with more than 600

One of the many school groups visiting the FSUCML.

students (ages 5 – 21). That number is expected to continue to rise as schools resume regular activities after COVID-19 restrictions.

8.2.4. Library outreach events

These events were held at the Apalachicola, Eastpoint, and Carrabelle public libraries. They were educational events tailored towards children and families to help expand their knowledge of the Bay. The more intimate setting allowed for research staff to have more in-depth conversations with community members about ABSI. We reached over 70 people throughout these events.

- 6/1/2022 Apalachicola Library
- 6/15/2022 Apalachicola Library
- 6/21/2022 Eastpoint Library
- 6/21/2022 Carrabelle Library

A father and son try their hand at tonging at an ABSI library event.

8.2.5. Festivals

These events were larger scale in which the goal was to reach as many people as possible. We brought a variety of materials (posters, lab equipment, oysters, and more) to showcase the full breadth of ABSI. The events were held throughout the Big Bend area. We have attended or are planning to attend several festivals in Franklin County, Wakulla County, and Leon County. In the past year, we have reached more than 15,000 people through these opportunities and expect that number to continue to rise in the coming months. We have an additional seven festival events scheduled for the spring of 2023.

- 10/15/2022: University of Florida Open House
- 10/22/2022: 11th Annual Tallahassee Science
- 11/4/2022 - 11/5/2022: Apalachicola Seafood
- 11/12/2022 – Sopchoppy Oyster and Mullet
- 2/25/2023 – Tallahassee Magnet Lab Open House

ABSI set up at the Sopchoppy Oyster and Mullet Festival

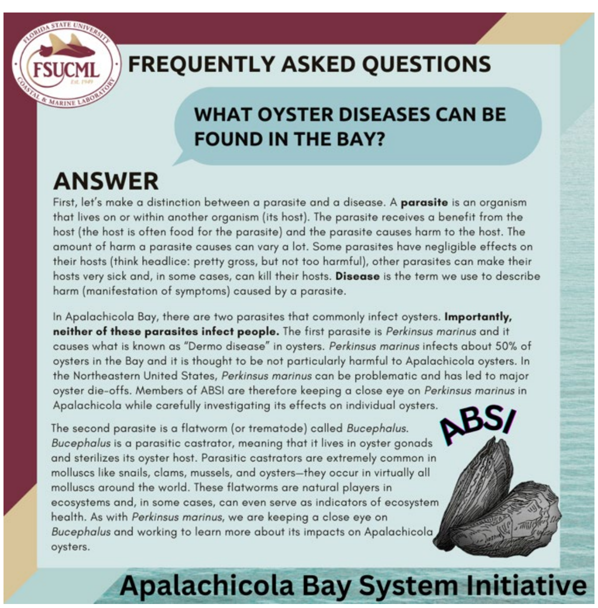
8.2.6. Free Friday FSUCML tours

These tours take place at the FSUCML and are open to the public any Friday that the lab is open. During these tours, individuals receive a detailed look at the current research being conducted by our staff, a large part of which is ABSI. This includes an overview of the Bay area and the issues that it faces. As part of the tour, individuals also get the chance to walk through our shellfish research hatchery and see oysters up close. These tours provide the perfect setting for individuals to get a glimpse of what the ABSI team does daily while providing them an opportunity to ask any questions they may have about the Bay or our role in its recovery. Since the fall of 2022, we have had over 300 people take part in the tours.

One of our Friday tour groups learns about the hatchery.

8.2.7. ABSI social media

To continue engagement with the community, the ABSI team decided to increase its social media efforts. In the fall of 2022, ABSI started "FAQ" Mondays, where each Monday, a new "FAQ" from ABSI is published and shared across FSUCML social media accounts. Additionally, since January 2023, ABSI increased its social media presence (via FSUCML social media accounts - Facebook, Twitter, Instagram) with weekly updates. Using a new template to distinguish ABSI from regular FSUCML postings, the team strives to post 2-3 times a week showcasing the day-to-day activities of ABSI. Recent updates include - monitoring of oyster predators in subtidal reefs, cleaning of restoration replicates, and volunteer opportunities. Examples of an "FAQ" post and "ABSI Update" post are below:



8.2.8. ABSI website (https://marinelab.fsu.edu/absi/)

The ABSI team has continued 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. Recent additions to the website include separate pages for research personnel and graduate students, highlights on student research, and the "Frequently Asked Questions" page.

8.2.9. Local news coverage

The ABSI project continues to be featured in local news, however, this year most news was shared across social media platforms rather than formal blogs and paper mediums. Below is a list of articles and news segments from March 2022 – March 2023, but it is not exhaustive.

Apalachicola Riverkeeper - February 2023
Oyster Radio - November 2022
The Wakulla News (Newsletter) - October 2022
The Wakulla News (Status of Apalachicola Bay Oysters) - October 2022

9. Literature Cited

Adamack, A T, B Gruber (2014). PopGenReport: simplifying basic population genetic analyses in R. Methods in Ecology and Evolution 5: 384–387

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

Anderson JD, WJ Karel, CE Mace, BL Bartram, MP Hare (2014) Spatial genetic features of eastern oysters (*Crassostrea virginica* Gmelin) in the Gulf of Mexico: northward movement of a secondary contact zone. Ecology and Evolution 2014; 4(9): 1671–1685.

Araujo M, M New (2007) Ensemble forecasting of species distributions. Trends in Ecology and Evolution 22:42–47.

Arnold W, NJ Blake, MM Harrison, DC Marelli, et al. (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.

Barranger A, F Akcha, J Rouxel, et al. (2014) Study of genetic damage in the Japanese oyster induced by an environmentally-relevant exposure to diuron: evidence of vertical transmission of DNA damage. Aquatic toxicology 146: 93-104.

Beck MW, RD Brumbaugh, L Airoldi, L et al. (2011). Oyster Reefs at Risk and Recommendations for Conservation, Restoration, and Management. BioScience, 61(2): 107–116.

Bouilly K, A Leitão, H McCombie, S Lapegue (2003) Impact of atrazine on aneuploidy in Pacific oysters, *Crassostrea gigas*. Environmental Toxicology and Chemistry: An International Journal 22(1): 219-223.

Bodenstein S, WC Walton, TD Steury (2021). Effect of farming practices on growth and mortality rates in triploid and diploid eastern oysters *Crassostrea virginica*. Aquaculture Environment Interactions. 13: 33–40.

Bringer A, H Thomas, G Prunier, et al. (2021) Toxicity and risk assessment of six widely used pesticides on embryo-larval development of the Pacific oyster, *Crassostrea gigas*. Science of The Total Environment 779: 146343.

Camp EV, WE Pine III, K Havens, et al. (2015). Collapse of a historic oyster fishery: Diagnosing causes and identifying paths toward increased resilience. Ecology and Society, 20(3):45.

Casas SM, R Lavaud, MK La Peyre, et al. (2018) Quantifying salinity and season effects on eastern oyster clearance and oxygen consumption rates. Marine Biology. 165: 90

Chambers, LG, SA Gaspar, CJ Pilato, et al. (2018). How Well Do Restored Intertidal Oyster Reefs Support Key Biogeochemical Properties in a Coastal Lagoon? Estuaries and Coasts 41(3): 784–99

Chanton J, FG Lewis (2002). Examination of Coupling between Primary and Secondary Production in a River-Dominated Estuary: Apalachicola Bay, Florida, U.S.A. Limnology and Oceanography 47(3): 683–97

Chapuis MP, A Estoup (2007) Microsatellite null alleles and estimation of population differentiation. Molecular Biology and Evolution 24: 621–631.

Christensen JD. United States National Ocean Service. Strategic Environmental Assessments Division. (1998). Potential Impacts of Reduced Freshwater Inflow on Apalachicola Bay, FL Oyster (*Crassostrea virginica*) Populations: Coupling Hydrologic and Biological Models. NOAA/NOS Strategic Environmental Assessments Division.

Clyde L, 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? Ecological Engineering 15:323–343.

Colden AM, RJ Latour, RN Lipcius (2017) Reef height drives threshold dynamics of restored oyster reefs. Mar Ecol Prog Ser 582:1-13.

Combs EM, BA Belgrad, DL Smee (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, L Diaz-Garcia, B Schlautman, et al. (2016). Fragman: An R package for fragment analysis. BMC Genetics 17: 1–8.

Davis HC (1958) Survival and growth of clam and oyster larvae at different salinities. The Biological Bulletin 114:296–307.

Davis HC, A Calabrese (1964) Combined effects of temperature and salinity on development of eggs and growth of larvae of *M. mercenaria* and C. *virginica*. Fishery Bulletin 63:13.

Dittmar PJ, S Agehara, NS. Dufault (2022) Vegetable Production Handbook of Florida, 2022–2023 Edition. UF IFAS Extension.

Eierman LE, Hare MP (2013) Survival of oyster larvae in different salinities depends on source population within an estuary. Journal Experimental Marine Biology and Ecology 449:61–68.

Eierman LE, MP Hare (2016) Reef-Specific Patterns of Gene Expression Plasticity in Eastern Oysters (*Crassostrea virginica*). Journal of Heredity 107:90–100.

Espriella MC, VC Lecours, P Frederick, et al. (2020). Quantifying Intertidal Habitat Relative Coverage in a Florida Estuary Using UAS Imagery and GEOBIA. Remote Sensing 12(4): 677.

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

Galindo-Sánchez CE, PM Gaffney, CI Pérez-Rostro, et al. (2008) Assessment of Genetic Diversity of the Eastern Oyster *Crassostrea virginica* in Veracruz, Mexico Using Microsatellite Markers. Journal of Shellfish Research, 27(4): 721-727

Granneman J, C Baxley, M Bollinger, et al. (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.
Graeff T, G Baroni, A Bronstert, et al (2013) Modelling changing hydrology in a coastal area under the influence of climatic change until 2100. 15:EGU2013-12559.
Grizzle R, K Ward, L Geselbracht, A Birch (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
Hajovsky P, PJ Beseres, J Pollack, J Anderson (2021). Morphological Assessment of the Eastern Oyster *Crassostrea virginica* throughout the Gulf of Mexico. Marine and Coastal Fisheries 13: 309–319
Havalend E, JL Breithaupt, KM Engelbert, et al. (2022) Coastal Wetland Soil Carbon Storage at Mangrove Range Limits in Apalachicola Bay , FL: Observations and Expectations. Frontiers in Forests and Global Change 5: 1–14.
Hayes TB, V Khoury, A Narayan, et al. (2010) Atrazine induces complete feminization and chemical castration in male African clawed frogs (Xenopus laevis). Proceedings of the National Academy of Sciences 107(10): 4612-4617.
Hayes TB, LL Anderson, VR Beasley, et al (2011) "Demasculinization and feminization of male gonads by atrazine: consistent effects across vertebrate classes. The Journal of steroid biochemistry and molecular biology 127(1-2): 64-73.
Hegerl GC, E Black, RP Allan et al. (2014) Challenges in Quantifying Changes in the Global Water Cycle. Bulletin American Meteorology Society 96:1097–1115.
Hewitt JE, JI Ellis, SF Thrush (2016) Multiple stressors, nonlinear effects and the implications of climate change impacts on marine coastal ecosystems. Global Change Biology 22:2665–2675.
Hoskins TD, MD Boone (2018) Atrazine feminizes sex ratio in Blanchard's cricket frogs (*Acris blanchardi*) at concentrations as low as 0.1 µg/L. Environmental toxicology and chemistry 37(2): 427-435.
Interim Reregistration Eligibility Decision - Atrazine; U.S. Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office, 2003
Jombart T (2008). adegenet: an R package for the multivariate analysis of genetic markers. Bioinformatics 24: 1403–1405.
Jombart T, S Devillard, F Balloux (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. BMC Genetics 11.
Joyce KE, S Duce, SM Leahy, et al. (2019). Principles and practice of acquiring drone-based image data in marine environments. Marine and Freshwater Research 70(7): 952–963.
Kaky E, V Nolan, A Alatawi, F Gilbert (2020) A comparison between Ensemble and MaxEnt species distribution modelling approaches for conservation: A case study with Egyptian medicinal plants. Ecological Informatics 60:101150.
Kimbro DL, WJ Wilson, H Tillotson, Et al. (2017) Local and regional stressors interact to drive a salinization-induced outbreak of predators on oyster reefs. Ecosphere. 8:11.
Kofoed, JW, DS Gorsline (1963) Sedimentary Environments in Apalachicola Bay and Vicinity, Florida. Journal of Sedimentary Petrology 33 (i): 205–23
Konopiński MK (2020). Shannon diversity index: A call to replace the original Shannon’s formula with unbiased estimator in the population genetics studies. PeerJ doi:10.7717/peerj.9391.
Lavaud R, MK La Peyre, SM et al (2017) Integrating the effects of salinity on the physiology of the eastern oyster, *Crassostrea virginica*, in the northern Gulf of Mexico through a Dynamic

Energy Budget model. Ecological Modelling. 363.
Lowe M., T Sehlinger, TM Soniat, MK Peyre (2018). Corrigendum to “interactive effects of water temperature and salinity on growth and mortality of eastern oysters, *Crassostrea virginica*: A meta-analysis using 40 years of monitoring data”. Journal of Shellfish Research, 37(5):1167.
Mai H, B Morin, P Pardon, et al. (2013) Environmental concentrations of irgarol, diuron and S-metolachlor induce deleterious effects on gametes and embryos of the Pacific oyster, *Crassostrea gigas*. Marine Environmental Research 89: 1-8.
Marmion M, M Parviainen, M Luoto et al. (2009) Evaluation of consensus methods in predictive species distribution modelling. Diversity Distribution 15:59–69.
Minogue P (2022) Forestry herbicide update and trends in silvicultural vegetation management." May 2022 FL AFC, University of Florida.
Moulton DL, MA Dance, JA Williams, et al. (2019) Habitat Partitioning and Seasonal Movement of Red Drum and Spotted Seatrout. Estuaries and Coasts 40: 905–916
NIOSH Pocket Guide to Chemical Hazards (2019) Atrazine". National Institute for Occupational Safety and Health (NIOSH). https://www.cdc.gov/niosh/npg/npgd0043.html
NOAA National Estuarine Research Reserve System (NERRS). System-wide Monitoring Program. Data accessed from the NOAA NERRS Centralized Data Management Office website: http://www.nerrsdata.org;
NOAA Commercial Fisheries Landings. https://www.fisheries.noaa.gov/
Paradis E. (2010). pegas: an R package for population genetics with an integrated–modular approach. Bioinformatics 26, 419–420
Parris AS, P Bromirski, V Burkett et al. (2012) Global sea level rise scenarios for the United States National Climate Assessment.
Passeri DL, SC Hagen, NG Plant et al. (2016) Tidal hydrodynamics under future sea level rise and coastal morphology in the Northern Gulf of Mexico. Earths Future 4:159–176.
Paynter KT. EM Burreson (1991) Effects Of *Perkinsus marinus* Infection In The Eastern Oyster, *Crassostrea virginica*: II. Disease Development and Impact On Growth Rate at Different Salinities. Journal of Shellfish Research, 10(2): 425-431.
Paynter KT, V Politano, HA Lane, et al. (2010) Growth rates and prevalence of *Perkinsus marinus* in restored oyster populations in Maryland. Journal of Shellfish Research 29(2): 309–317.
Pine III WE, CJ Walters, EV Camp, et al. (2015). The curious case of eastern oyster *Crassostrea virginica* stock status in Apalachicola Bay, Florida. Ecology and Society, 20(3)
Puckett BJ, DB Eggleston (2012) Oyster demographics in a network of no-take reserves: Recruitment, growth, survival, and density dependence. Marine and Coastal Fisheries 4(1): 605–627
Pusack TJ, DL Kimbro, JW White, CD Stallings (2018) Predation on Oysters Is Inhibited by Intense or Chronically Mild, Low Salinity Events. Limnology and Oceanography. 64.
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/.
Radabaugh KR, RP Moyer, SP Geiger SP (2019). Oyster integrated mapping and monitoring program report for the state of Florida. St. Petersburg, FL: Fish and Wildlife Research Institute, Florida Fish and Wildlife Conservation Commission. FWRI Technical Report 2
Raymond M, F Rousset (1995). GENEPOP (Version-1.2). Population-genetics software for exact tests and ecumenicism. Journal of Heredity 86: 248–249
Rainier JS. RL Mann (1992) A comparison of methods for calculating condition index in eastern oysters *Crassostrea virginica* (Gmelin, 1791). Journal of Shellfish Research 11(1): 55-58.

Rondon R, C Grunau, M Fallet, et al. (2017) Effects of a parental exposure to diuron on Pacific oyster spat methylome. Environmental Epigenetics 3(1)

Rousset F (2008) Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Molecular Ecology Resources 8: 103–106.

Rybovich M, MK La Peyre, SG Hall, JF La 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, S Palm (2006) POWSIM: A computer program for assessing statistical power when testing for genetic differentiation. Molecular Ecology Notes 6: 600–602.

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

Seyoum S, TM Bert, A Wilbur et al. (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

Steinmuller, HE, E Bourque, SB Lucas, et al. (2022). Comparing Vertical Change in Riverine , Bayside , and Barrier Island Wetland Soils in Response to Acute and Chronic Disturbance in Apalachicola Bay , FL. Estuaries and Coasts https://doi.org/10.1007/s12237-022-01131-4.

Tanguy A, I Boutet, J Laroche, D Moraga (2005) Molecular identification and expression study of differentially regulated genes in the Pacific oyster *Crassostrea gigas* in response to pesticide exposure. The FEBS Journal 272(2): 390-403

Thuiller W, D Georges, M Gueguen et al. (2021) Biomod2: Ensemble Platform for Species Distribution Modeling.

Tolley SG, AK Volety (2005) The role of oysters in habitat use of oyster reefs by resident fishes and decapod crustaceans. Journal of Shellfish Research, 24(4): 1007-1012.

Van Oosterhout C, WF Hutchinson, DPM Wills, P Shipley (2004) Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4, 5353–5538.

Varney RL, CE Galindo-Sánchez, P Cruz, PM Gaffney (2009) Population Genetics of the Eastern Oyster Crassostrea virginica (Gmelin, 1791) in the Gulf of Mexico," Journal of Shellfish Research, 28(4): 855-864

Vonberg D, J Vanderborght, N Cremer, et al. (2014) 20 years of long-term atrazine monitoring in a shallow aquifer in western Germany." Water Research 50: 294-306.

Walne PR. R Mann (1975) Growth and Biochemical Composition in *Ostrea edulis* and *Crassostrea gigas*. In: Proceedings of the Ninth European Marine Biology Symposium, H. Barnes (Ed.), pp. 587-607. Aberdeen University Press

Wang Y, X Guo (2007) Development and Characterization of EST-SSR Markers in the Eastern Oyster *Crassostrea virginica*. Marine Biotechnology 9:500–511

Ward GS, L Ballantine (1985) Acute and chronic toxicity of atrazine to estuarine fauna. Estuaries 8: 22-27.

Winter DJ (2012) MMOD: An R library for the calculation of population differentiation statistics. Molecular Ecology Resources 12, 1158–1160

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

Zarnoch CB, MP Schreibman (2012). Growth and Reproduction of Eastern Oysters, *Crassostrea virginica*, in a New York City Estuary: Implications for Restoration. Urban Habitats, 7:1541-7115