ANNUAL REPORT TO TRIUMPH GULF COAST INC.

Project #69: Apalachicola Bay System Initiative (ABSI)
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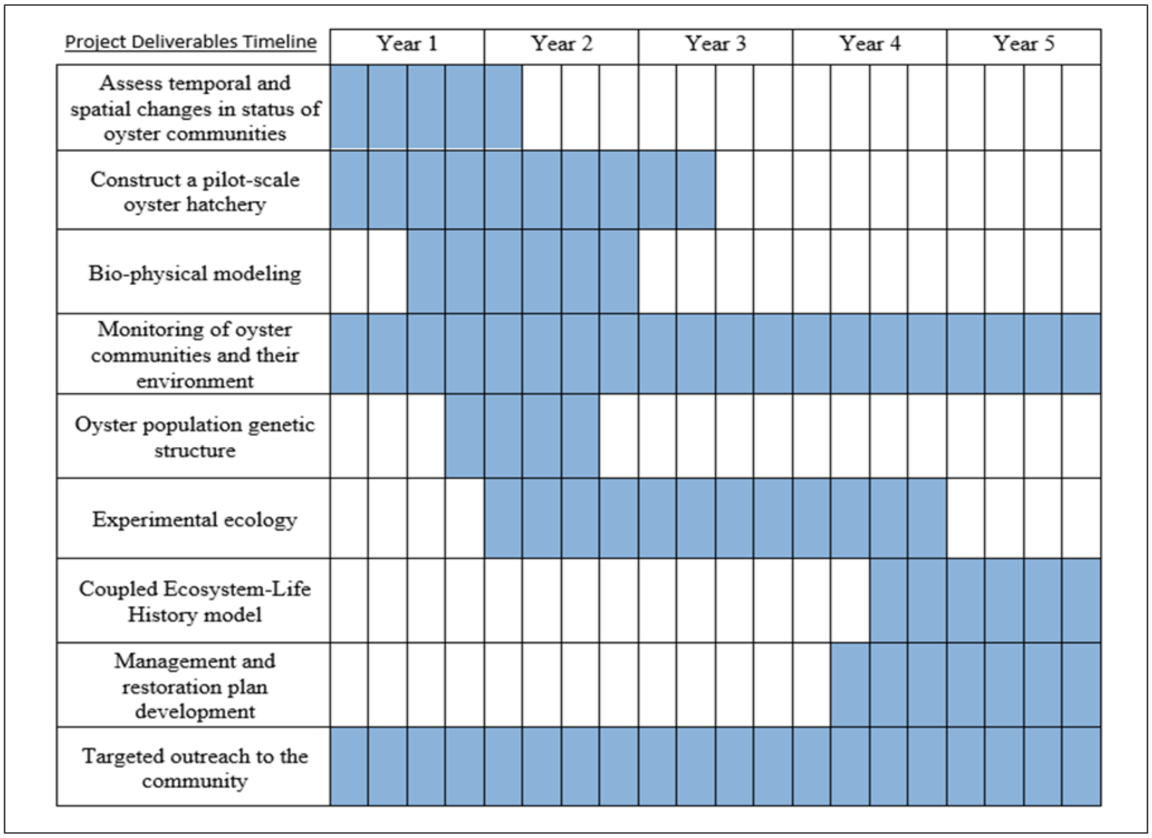
EXECUTIVE SUMMARY

The Apalachicola Bay System Initiative (ABSI) comprises multiple objectives and associated deliverables, each of which had a timeline for completion. 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 fifth year of this large multi-disciplinary effort. There is also a section on other items that are not directly associated with the specific objectives.



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

Status of project deliverables

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

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.

2. Construct a pilot-scale ABSI Research Hatchery: Complete

Construction of the permanent hatchery was completed in spring 2023 and is fully operational. The hatchery 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, a series of 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, not for commercial enterprise.

3. Bio-physical modeling: Complete

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.

4. Monitoring of oyster communities and their environment: Ongoing

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. Intertidal monitoring of four oyster reef areas throughout Franklin County (Indian Lagoon, East Cover, Carrabelle River and Alligator Harbor) collected monthly information on disease and condition as well as environmental data. Additional studies include assessing the use of high-resolution drone surveys to monitor intertidal habitats. This work has been completed and a manuscript published in the journal of 'Remote Sensing in Ecology and Conservation'.

In October 2020, ABSI partnered with a former Apalachicola oysterman, Shannon Hartsfield, to survey subtidal areas throughout Apalachicola Bay using small oyster tongs. Bay-wide monitoring of subtidal habitat has been conducted annually by tong sampling since 2020.

5. Oyster population genetic structure: Complete

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 the study are in the 2022-2023 Annual Report.

6. Experimental Ecology: Ongoing

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 so this objective will continue to the end of the project. The ongoing and new projects are described in detail in the report.

7. Coupled ecosystem life-history model: Ongoing

Four models have been developed by ABSI 1) freshwater flow, 2) bio-physical 3) larval dispersal and 4) habitat suitability. The first two models are complete and are currently being incorporated into the larval dispersal and habitat suitability models. An oyster population model developed by our collaborator Dr. Ed Camp (University of Florida) was used to model management strategies as requested by the Community Advisory Board. Dr. Fabio Caltabellotta (ABSI Postdoc) developed 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. This tool will be adapted to enable access through the FSU website.

8. Develop a Management and Restoration Plan for the Apalachicola Bay System: Complete

This task was initiated in 2019 with the establishment of the Community Advisory Board (CAB). Meetings were held bi-monthly at the Apalachicola National Estuarine Research Reserve and were open to the public. Meetings were recorded and all information and presentations are available on the ABSI website (https://marinelab.fsu.edu/absi/cab/). The Plan contains a series of options for management and restoration of the Apalachicola Bay System, that have the consensus approval of the CAB. This objective was completed in January 2024 with the production of the Management and Restoration Plan, which can be accessed through the ABSI website.

9. Targeted outreach to the community: Ongoing

Community support is critical to the success of ABSI, and after Covid-19 restrictions diminished, ABSI's engagement with the public and local stakeholders increased. 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, oystermen's workshops, public meetings (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.

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APALACHICOLA BAY SYSTEM INITIATIVE (ABSI) ANNUAL REPORT 2023-2024

1. Introduction

The Apalachicola Bay System Initiative was awarded in March 2019 and has now completed the fifth 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 were completed in previous years and described in earlier annual reports, available through the ABSI website. Previous information is not repeated unless it pertains to project updates.

The ABSI Research and Restoration Hatchery continued to support research into oyster restoration techniques as well as student research projects on oyster physiology and bay scallop culture and restoration techniques. The microalgal production facility has been expanded to allow maximum production capacity of several algal strains that are critical food sources for larvae.

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 were conducted in person but were also broadcast over the zoom platform and recorded. Information from the CAB and community meetings and the final Management and Restoration Plan are available through the ABSI website (https://marinelab.fsu.edu/absi/).

2. Habitat and environment

2.1 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 was 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.

Methods. A hydrodynamic model was 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 were generated based on high-resolution bathymetry from NOAA with modification from collaborators. The model resolution is 30 m near the coasts and bathymetric features of interest, with freshwater input from multiple 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) was created to simulate oyster larvae as a set of Lagrangian particles, each representing a group of larvae traveling together. This model was configured for this application using the FVCOM I-State Configuration Model (FISCM) and driven by the results of the hydrodynamic simulations. Larvae were advected from their release locations in the 3-dimensional velocity field with mortality parameterized based on the ambient salinity. The mortality was considered zero when salinity was in the range $6<S<27$, and the mortality rate is 0.95/7 days when the salinity is outside of this range. Larvae were advected for 20 days and allowed to settle if they encountered reef locations during their last 5 days. The results were 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 were performed for the different hydrodynamic model scenarios.

Results and discussion. Model hindcasts were run for three periods 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 were used to assess the simulations, with several iterations of the model being run with modifications to improve the veracity of the simulation. The normal and anomalously wet period simulations compared well to *in situ* observations but the simulation for the anomalously dry period (2011-2012) showed a consistently lower salinity than was observed *in situ*. The model was refined by incorporating information on river flow diversions through the intracoastal waterway and local evaporation.

Results from analyses of the IBM runs demonstrate how inhospitable salinity conditions can affect larval survival across the bay. A greater fraction of larvae survives to settlement during the spring release simulation than the autumn release simulation in all 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 period than during the autumn. This project is now complete, but the models are being refined and incorporated into a more extensive analysis of larval dispersal and habitat suitability models.

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

Introduction: Significant effort has been expended to ascertain the cause of and find possible solutions to restore oyster populations in Apalachicola Bay (Camp et al., 2015; Coen & Luckenbach, 2000; Fisch & Pine, 2016; Pine et al., 2015; Seavey et al. 2011); however, a quantitative assessment of the system under future climate scenarios is lacking. Simulations 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 increasingly 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 a coupled biophysical oyster larval distribution model (Arnold et al. 2017) and subsequent reef connectivity analyses (Balbar et al. 2024; Hansen et al. 2024), predictive habitat suitability and distribution models (HSM) describing the current state of oysters in the Bay, 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 the effects of changing environmental variables on the 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 integrate future predicted hydrodynamics into distribution models to describe (evaluate) the potential changes in oyster survivability and distribution.
3. To use output models to evaluate and inform restoration and management scenarios of the eastern oyster.

Hypotheses.

1: Oyster habitat suitability in Apalachicola can be accurately modelled using a comprehensive ensemble-based modelling approach.

2: Incorporation of biophysical variables will improve model performance over environmental variable-only models.
3: Predicted shifts in hydrographic conditions caused by climate change can be integrated into models to predict future suitable habitats and oyster survivability.

Methods. The issues of variability in the high-resolution hydrodynamic model have been addressed to a degree that model formulations can progress. Larval dispersal models using the corrected hydrodynamic models, as well as locally derived salinity tolerance curves and growth/mortality functions are in the process of being evaluated, while larval connectivity analyses are being conducted on 'normal' year datasets. Preliminary analyses on seasonal changes in reef connectivity will be presented at the upcoming Benthic Ecology Meeting 2024. Finalized data layers of larval retention, larval dispersal distance, and reef connectivity are projected to be ready for inclusion into the HSM framework this upcoming summer season. Additionally, the completion of a bay wide multibeam sonar bathymetry and substrate analysis by Grizzle et al 2022 (Grizzle et al., 2022. FWC Contract #19286) has resulted in the addition of as a secondary objective for larval connectivity analyses, evaluating changes in connectivity due to loss of substrate between 2007 and 2022 datasets.

The main framework for HSM formulation remains 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 biophysical 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 3 will be conducted using ArcGIS Pro 2.9 and R (v4.1.2; R Core Team 2021, Marine Geospatial Ecology, Benthic Terrain Modeler, Spatial Analyst toolboxes).

Continued Agency data and ABSI tong sampling efforts will be used in conjunction with existing oyster presence/absence data in Apalachicola Bay for model development and ground truthing 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 interpolated FWC FIMS data (section 2.1). Finally, the model outputs will be ground-truthed for accuracy.

3. Oyster biology

3.1 Parasite transmission and disease impacts on Apalachicola oysters (Dr. Tara Stewart Merrill, Grace Westphal, David DuBose, and ABSI Core Team)

Introduction: Infectious diseases represent an important threat to fisheries and marine restoration programs around the world (Lafferty et al. 2015). In Apalachicola Bay, the oyster parasite, *Perkinsus marinus* (which causes "Dermo disease"), is of notable interest. This parasite has been implicated in largescale oyster die-offs in the Northeastern United States (e.g., Chesapeake Bay; Burreson & Calvo 1996) and considerable effort has been invested in understanding its transmission dynamics and impacts in the Northeast region (Villalba et al. 2004). Yet, how *P. marinus* affects individual oysters and populations in the Gulf of Mexico remains unclear (Westphal et al. in prep). The Stewart Merrill lab has combined monitoring, experiments, and analyses of large datasets to address a series of core questions on this parasite, including: ***1)*** What are the general patterns of infection in Apalachicola Bay and can their similarities and deviations from Northeastern patterns reveal important information about the biology of this disease? ***2)*** What are the lethal and non-lethal effects of *P. marinus* on Apalachicola oysters, and how might these effects scale up to impact the Apalachicola oyster population? ***3)*** What are the biotic and abiotic drivers of disease-induced die-offs?

Objectives

From March 2022–March 2023, our work addressed four objectives:

1. ***Intertidal monitoring***: We monitored the presence and intensity of *P. marinus* infection from intertidal reefs in Apalachicola Bay to establish baseline data on patterns of parasitism and to complement existing subtidal disease monitoring efforts.
2. ***Literature review and large-scale analysis*****:** We conducted a comprehensive literature review on *P. marinus* and analyzed three data streams to synthesize knowledge on Dermo disease, identify differences in infection patterns and virulence between the Gulf and Northeastern regions, and outline critical needs for the Apalachicola Bay system.
3. ***Experimental assessment of effects of infection*****:** We ran a longitudinal experiment investigating the within-host growth dynamics of *P. marinus* and its effects on oyster survival and performance.
4. ***Pilot study on new experimental methods*****:** We ran a pilot study to determine whether oysters could be experimentally cleared of pre-existing *P. marinus* infections.

Methods

Intertidal monitoring: To assess patterns of infection in Apalachicola oysters, four intertidal locations (Alligator Harbor, Carabelle River, East Cove, and Indian Lagoon) were visited monthly for one year (June 2022 – July 2023). At each intertidal location, five oysters were collected from each of five sub-sites, then transported to the FSUCML. Presence and intensity (severity) of *P. marinus* infection was assessed using standard histological methods (Mackin 1962), and we collected additional data from each oyster, including body size (shell length, width, and height) and condition (a ratio of dry to wet weight).

Literature review and large-scale analysis: We conducted a comprehensive literature survey in the ISI Web of Science (WOS), selecting any articles that contained words referring to both the oyster host (i.e., oyster or Crassostrea virginica or eastern oyster or American oyster) and the parasite (i.e., Dermo or *Perkinsus* or *Labyrinthomyxa*). These search criteria captured both current and historical names that have been applied to eastern oysters and P. marinus. We recorded the country (U.S. or Mexico), coast (Atlantic or Gulf of Mexico), and state(s) in which each study took place then generated a spatial map of the published literature. The goal of the map is to highlight any regional biases in our knowledge of this disease. With these published studies, we asked how regional differences in Dermo disease and disease-induced die-offs may stem from difference in host-parasite interactions, ecological communities, and abiotic factors (e.g., temperature and salinity).

To bolster the literature survey, we combined and analyzed three large-scale datasets that monitored *P. marinus* in eastern oysters: 1) Florida Fish and Wildlife Conservation Commission subtidal monitoring in Apalachicola Bay; 2) Maryland Department of Natural Resources subtidal monitoring in Chesapeake Bay; and 3) ABSI intertidal monitoring in Apalachicola Bay (reported herein). With these datasets, we examined regional (Apalachicola versus Chesapeake) and habitat-associated (subtidal versus intertidal) differences in fundamental patterns of infection and explored other potential biotic and abiotic drivers of infection (e.g., co-infection, temperature, salinity, and dissolved oxygen). To explore our questions, we performed a series of binomial and ordinal logistic regressions using a Bayesian framework that accounted for spatial and temporal co-variance of observational units.

Experimental assessment of effects of infection: To quantify the lethal and non-lethal effects of *P. marinus* on Apalachicola oysters, we collected 114 intertidal oysters known to vary in natural rates of infection presence and severity (based on intertidal monitoring data) and monitored them in standardized conditions for six weeks. Oysters were held individually in 2.8L microcosms in a recirculating seawater system. Seawater was maintained at approximately 26° Celsius to encourage growth and development of infections (Chu & Volety 1997; Burreson & Calvo 1996). Throughout the study, oysters were checked daily for survival and any dead oysters were immediately processed to estimate the presence and severity of *P. marinus* infection. Each week, a sub-sample of the experimental population was assessed for feeding rate, then sacrificed to estimate body condition and *P. marinus* presence and severity. With these data we could then explore whether infections became more severe over time (as is expected at the elevated temperature used in this study; Chu et al. 1996), whether more severe infections are associated with greater probabilities of mortality, and whether infection severity resulted in reductions to body condition or feeding rate. The study was completed in 2023 and data analyses are ongoing.

***Pilot study on new experimental methods*:** While our longitudinal study (above) leveraged natural variation in infection rates to ask questions about the impacts of disease, future work will benefit from experimentally dosing oysters with *P. marinus* in the lab. Experimental infections allow for tight control over which oysters are exposed to the parasite, as well as the timing and dose of exposure. While this can be achieved with hatchery-reared oysters (known to be uninfected) there is value to performing experiments on wild-caught oysters that have been reared in natural Apalachicola conditions (i.e., oysters that reflect the genetic and phenotypic diversity of the bay). We therefore need an experimental method that can remove pre-existing infections from wild-caught oysters.

In summer 2023, we piloted such a method. Prior work on *P. marinus* demonstrated that the common disinfectant, N-halamine, could kill *P. marinus* stages *ex vivo* (outside of the oyster host; Delaney et al. 2003). We therefore asked whether exposing infected oysters to N-halamine could kill the parasite *in vivo* (inside of the oyster host) and whether oysters survived the treatment. Wild-caught oysters from Apalachicola intertidal sites were held individually in 1L seawater tanks and exposed to 25 mg/L of N-halamine (experimental treatment; N = 10) or without the treatment (control N = 9). After a 24-hour exposure period to both treatments, oysters were sacrificed and underwent routine histology to quantify the presence and severity of *P. marinus*.

Results and Discussion: These separate projects have provided a series of interconnected results that help us to better understand the spread of *P. marinus* in Apalachicola Bay, as well as its potential impacts on local eastern oysters.

Our ***intertidal monitoring*** has revealed distinct differences in the infection patterns of subtidal versus intertidal Apalachicola oysters. Between June 2022 and July 2023, we collected 1350 intertidal oysters, of which 1338 (99.1%) were assessed for *P. marinus*. Of those oysters, 891 were infected with *P. marinus*, resulting in an average prevalence of infection of approximately 67%. The entry and cleaning of the intertidal data was recently completed, and analyses of the intertidal data have just begun. Some preliminary findings are that there is limited spatial variation in infection among the four intertidal locations (Fig. 1), and that intertidal populations appear to have similar temporal dynamics, with infections peaking in the early months of the year. This pattern (while still preliminary) stands in contrast to seasonal dynamics of subtidal Apalachicola infections, which are relatively constant over the annual cycle (Westphal et al. in prep). Another disparity between intertidal and subtidal populations is in the force of infection (or how the likelihood of infection changes as a function of oyster age). While subtidal oysters show a classic pattern of an increased probability of infection with body size (a proxy for age), intertidal oysters have generally stable and higher likelihoods of infection across all sizes (Fig. 2; Westphal et al. in prep). This suggests that intertidal oysters become infected at earlier size classes, leading to questions on whether differences in the intertidal environment, or in the oysters themselves, are leading to increased levels of exposure and/or susceptibility to infection.

The comprehensive ***literature review*** has provided insight into where and when efforts to understand Dermo disease have been concentrated. We found that interactions between *P. marinus* and eastern oysters have been well-studied in both the Northeast Atlantic and U.S. Gulf of Mexico regions, but that few studies have explicitly compared infections between these two regions (Fig. 3; Westphal et al. in prep). By conducting ***largescale analyses*** of datasets from both regions, we have begun to make such explicit comparisons. While oysters from the Northeast Atlantic are known to suffer significant population declines from *P. marinus*, we have little information regarding the magnitude of *P. marinus*-caused die-offs in Gulf of Mexico oysters. One significant barrier to estimating disease-induced mortality in Apalachicola is that infection prevalence is relatively constant over time (Westphal et al. in prep). Without large temporal fluctuations in disease (such as those that are characteristic of Northeastern systems), it is challenging to dissociate disease-caused mortality from background mortality in monitoring data (Westphal et al. in prep). In further comparisons of infection patterns between the regions, we found that the force of infection is slightly higher in the Chesapeake Bay than it is in Apalachicola Bay (Fig. 4; Westphal et al. in prep). In both populations, the probability of being infected with *P. marinus* increases with oyster body size (as expected). However, Chesapeake oysters have a 50% probability of infection at ~45 mm shell height, whereas Apalachicola oysters do not reach this same probability of infection until they are ~75 mm shell height. Whether these differences in infection patterns lead to differences in mortality at the population level remains to be determined.

Our ***experiment*** revealed a remarkable resilience of Apalachicola oysters to high ambient temperatures. Over the six-week study, only two oysters died (following some initial expected mortality that occurred during acclimation to the experimental conditions). These two oysters also had the most severe infections observed in the study. Interestingly, none of the other oysters in the experiment had severe infections and there was no indication that infections were growing and becoming more severe over the course of the study (Fig. 5; DuBose et al. in prep). This result contrasts with observational and experimental evidence from oysters examined in the Northeast Atlantic, where elevated temperatures tend to result in a rapid progression of *P. marinus* infection (Chu & Volety 1997; Burreson & Calvo 1996). Other preliminary results of this study are that the low severity infections observed had no detectable effects on oyster condition or feeding rate (DuBose et al. in prep). Whether Apalachicola oysters have high resistance to infection (i.e., are able keep their infections at low levels) remains an important possibility that can best be investigated with experimental infections in the lab. Our ***pilot study*** has revealed that oysters exposed to the N-halamine disinfectant survived the 24-hour treatment and had lower levels of infection than control oysters (Fig. 6). While this result was not statistically significant, increasing sample sizes should reveal whether this is a viable method for clearing oysters of their infections. If so, we can begin using this method to run controlled experimental infections. We hope that such experiments will establish whether regional differences in oyster infection patterns and impacts are best explained by oyster source population, parasite strain, or environmental conditions.

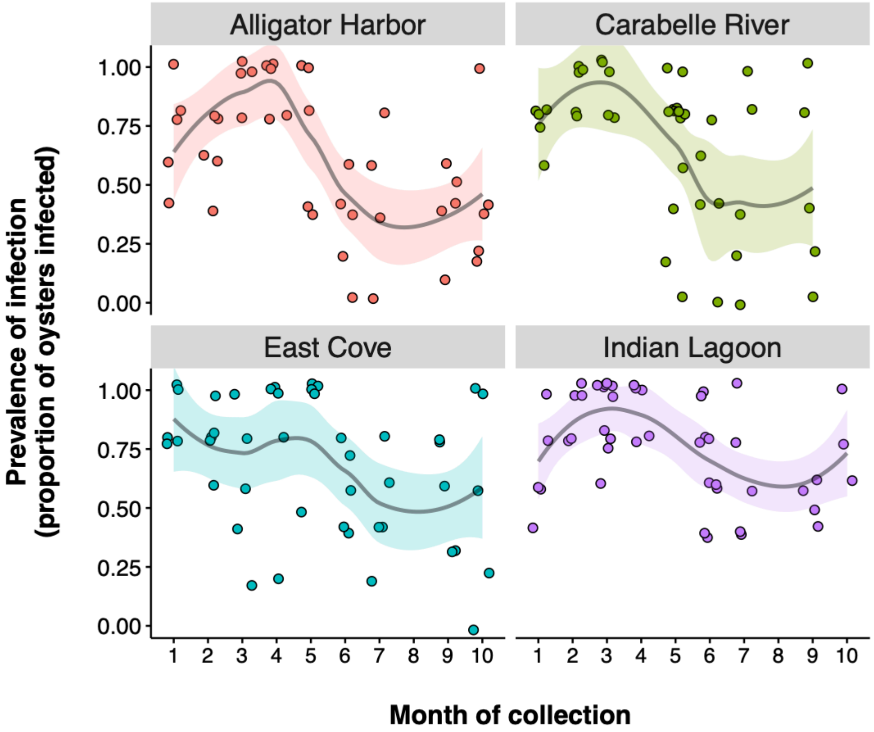

Figure 1. We have completed one year of regular disease monitoring in four intertidal sites (indicated with gray labels above each plot; note that data were not collected in November [month 11] or December [month 12] due to inclement weather). Analyses of these data are in their preliminary stages. Some important observations are that the prevalence of *Perkinsus marinus* (proportion of oysters infected) does not exhibit appreciable differences among the four sites, but does show a relatively similar temporal dynamic characterized by an increase in infection prevalence early in the year (in the spring months) followed by a decline in infections later in the year (in the late summer and early autumn). These trends are captured with smoothed conditional means (lines and standard error shading) and will be formally investigated using a Bayesian statistical approach. Each point represents the prevalence of infection observed at a sub-site on a given collection date. Some slight horizontal and vertical jitter has been added to better visualize overlapping points.

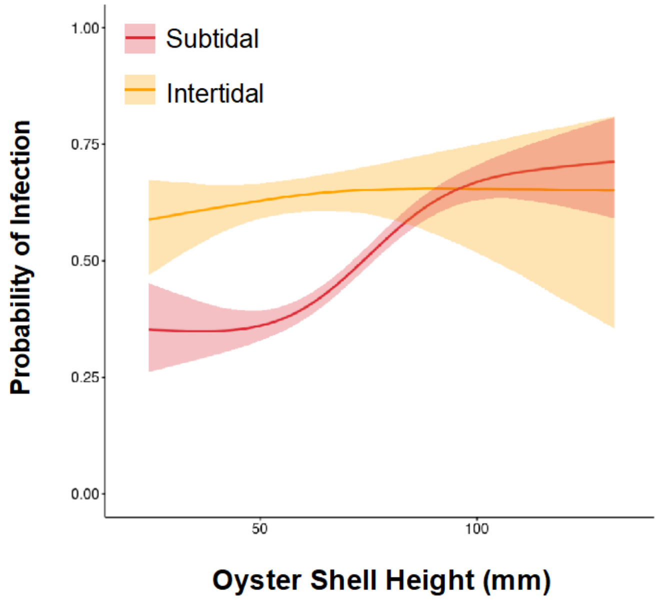

Figure 2. Comparing the force of infection (probability of infection as a function of body size) between Apalachicola subtidal and intertidal oysters reveals differences in patterns of transmission. More specifically, intertidal oysters become infected at earlier size classes suggesting that they experience higher levels of exposure, greater levels of susceptibility, or both, compared to subtidal oysters. We note that points are not included in this figure because the response variable data in the model are 0 (uninfected) or 1 (uninfected). The high sample sizes (N > 3,000 for subtidal and N > 1,300 for intertidal) of both datasets would result in clouds of points at 0 and 1 from which patterns are difficult to distinguish (see Figure 1 for raw intertidal data). Rather, this figure provides the probabilities of infection predicted from Bayesian logistic regressions examining infection status as a function of oyster shell height and can better demonstrate general trends. Figure in preparation for publication (Westphal et al. in prep).

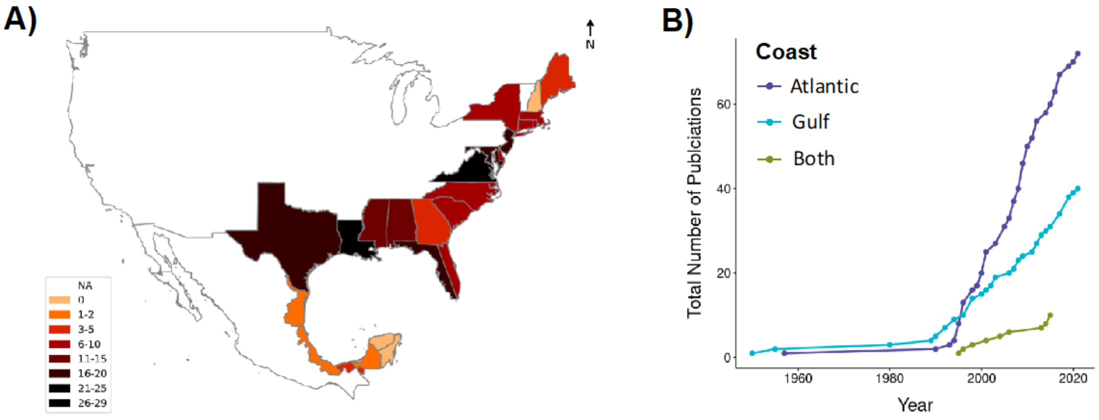

Figure 3. By surveying the literature, we generated a spatial map of current knowledge on oyster-*Perkinsus marinus* interactions. **A)** States in the US and Mexico are colored based on the number of published field studies on *P. marinus* since 1950, where darker colors refer to more publications (see key in lower left of plot). While there has been considerable effort to describe field infection patterns in both the US Gulf of Mexico and the Northeast Atlantic, **B)** few studies have simultaneously considered disease along both coasts to draw conclusions on the generalities of Dermo disease or any region-specific differences. For

instance, publications on Dermo disease in the Atlantic have risen dramatically since the 1990s (dark blue line) while studies on Dermo disease in the Gulf have risen moderately (light blue line). Yet, fewer than 20 studies have evaluated data from both coasts (green line). To address this gap, we are synthesizing the literature across the full range of eastern oysters and *P. marinus* to identify important avenues for future research. Figure in preparation for publication (Westphal et al. in prep).

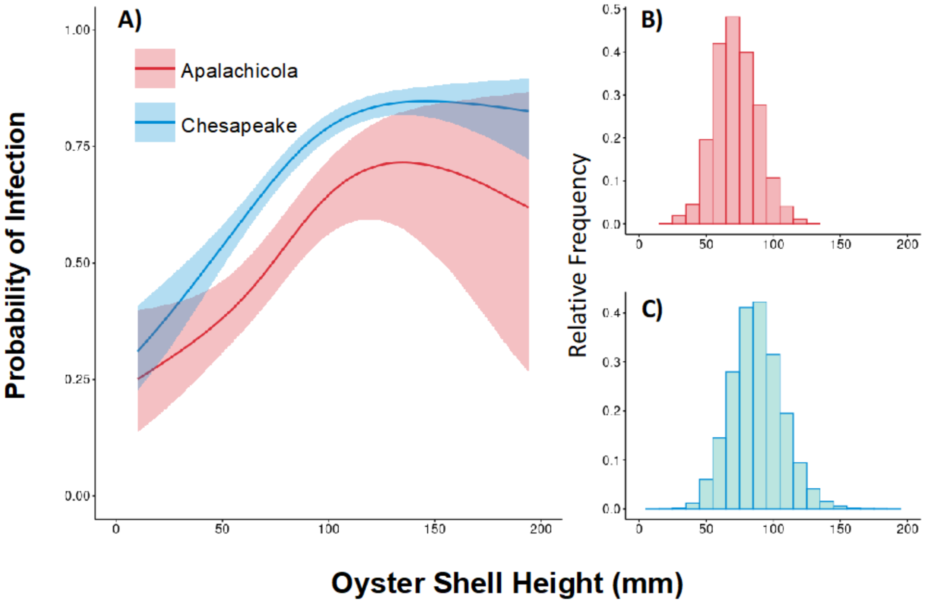

Figure 4. Combining publicly available datasets collected by the Florida Fish and Wildlife Conservation Commission (Apalachicola Bay data, red) and the Maryland Department of Natural Resources (Chesapeake Bay data, blue), we find differences in transmission. **A)** While oysters in both regions experience an increased likelihood of infection as they grow/age, probabilities of infection are slightly lower in Apalachicola oysters. As in Figure 2, we note that points are not included in panel **A** because the response variable data in the model are 0 (uninfected) or 1 (uninfected). The high sample sizes (N > 3,000 for Apalachicola and N > 30,000 for Chesapeake) of both datasets would result in clouds of points at 0 and 1 from which patterns are difficult to distinguish. Rather, panel A provides the probabilities of infection predicted from Bayesian logistic regressions examining infection status as a function of oyster shell height and can better demonstrate general trends. **B)** Distribution of shell heights from oysters collected from Apalachicola Bay (n = 3,160, $\bar{\mu}_A$ = 72.40mm, sd = 16.01 mm). **C)** Distribution of shell heights from oysters collected in the Chesapeake Bay (n = 34,686, $\bar{\mu}_C$ = 88.89, sd = 19.10 mm). On average, shell heights of processed oysters (those examined for infection) are larger and more variable in oysters collected from the Chesapeake Bay than in Apalachicola. Figure in preparation for publication (Westphal et al. in prep).

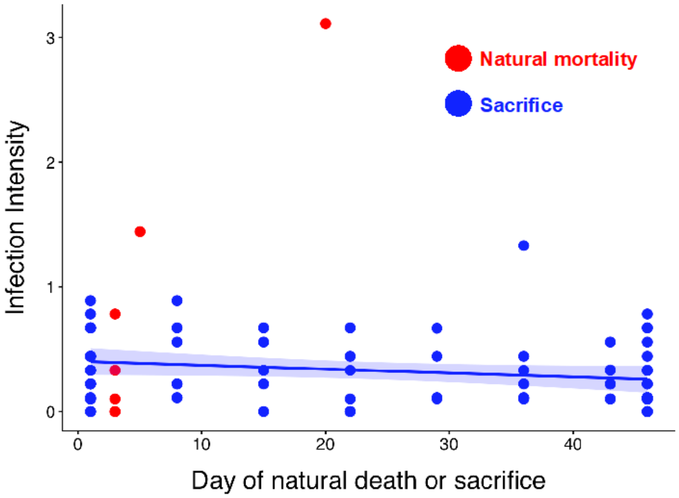

Figure 5. To experimentally assess the lethal and non-lethal effects of *Perkinsus marinus*, we held oysters that varied naturally in the presence and intensity (severity) of infection in constant, high temperature conditions (26°C) for over six weeks. We monitored oyster survival daily and dissected a subset of oysters at regular intervals to determine whether and how infections were growing. Oysters exhibited some early mortality while acclimating to experimental conditions, and two oysters died later during the study. The low sample size of dead oysters (red points) precludes analyzing effects of infection severity on mortality. All other oysters (blue points) were sacrificed and processed to determine the presence and intensity (severity) of infection. While other studies have found that *P. marinus* infections increase rapidly over time at high temperatures, we observed no change in the average level of infection severity over the course of our study. Apalachicola oyster defenses against *P. marinus* may be somewhat resilient to thermal stress. Figure in preparation for publication (DuBose et al. in prep).

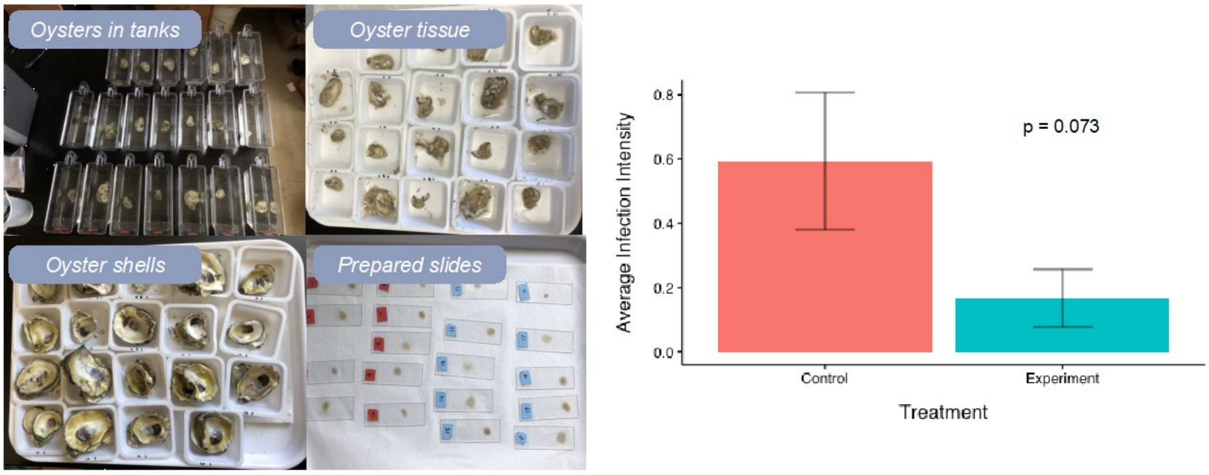

Figure 6. Our Young Scholars Program summer undergraduate, Miaohan Lin, performed a pilot study investigating whether the disinfectant, N-halamine, could clear oysters of their *Perkinsus marinus* infections. Oysters were exposed individually to N-halamine or a control in experimental tanks for 24 hours (see 'Oysters in tanks', top left image). Following the 24-hour period, oysters were shucked (see 'Oyster tissue' and 'Oyster shells' in the top right and bottom left images, respectively) then processed with standard histology (see 'Prepared slides', bottomright image). We observed lower infection intensities in oysters

exposed to N-Halamine (blue bar) compared to control oysters (red bar). This result was not statistically significant ($p = 0.073$) and future work will use greater replication alongside different doses and durations of N-Halamine exposure to determine whether this disinfectant can be effectively used to treat oysters for *P. marinus* infection.

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

Introduction: As estuarine animals, oysters experience a wide range of environmental conditions, particularly salinity. In Apalachicola Bay, salinity ranges from 0 to 35 ppt (NOAA NERRS 2023) and is highly dependent on factors such as proximity to the river, local water currents, and wind direction. With a planktonic larval phase at the mercy of water currents, oysters can be exposed to the entirety of this salinity range within the first two weeks of their lives, and so their salinity tolerance is critical to surviving the first stage of their life cycle and successful metamorphosis into spat (Davis 1958, Lawlor and Arellano 2020).

Additionally, in many marine species with complex life cycles, early and late life stages can be exposed to drastically different environments. This difference in environments can have pervasive effects on growth and survival in later life stages (Hettinger et al. 2012, Gobler and Talmage 2013). However, these 'carry-over effects' from early life stages stages are context-specific, and so, consequences are not easily predicted (Hettinger et al. 2012, 2013, Gobler and Talmage 2013, Fischer and Phillips 2014, Donelan et al. 2021). Carry-over effects from early life stages can be beneficial, increasing growth rates and survival, or disadvantageous, decreasing these parameters, depending on the later environment, and they have the potential to greatly impact the performance of organisms (Hettinger et al. 2012, 2013, Gobler and Talmage 2013, Fischer and Phillips 2014, Donelan et al. 2021).

The main aim of this work was to investigate both the short- and long-term effects of larval salinity environment on the growth, survival, and physiology of the eastern oyster by addressing the following research objectives:

Objectives:

1: Characterize larval tolerance to salinity and determine how salinity affects larval survival, growth, and development.

2: Investigate how salinity exposure during larval period affects post-settlement growth, survival, and physiology.

Methods. To test larval salinity tolerance, wild oysters were strip-spawned, and gametes were allowed to fertilize and develop for 24 hours. Then, larvae were counted and haphazardly split among ten 140L tanks. Each tank was randomly assigned to one of ten salinity treatments: 5, 8, 11, 14, 17, 20, 23, 26, 29, and 32 ppt. Salinity was adjusted over roughly 2-3 days to match the salinity treatment. Once established, salinity was kept at treatment salinities for 14 days. Each day, water was changed, and larvae were fed. Every other day, number of live larvae were estimated, and pictures of a random subsample of larvae were taken under a microscope for growth measurements.

To determine how larval salinity exposure affects post-settled oysters, a second spawn was conducted as the previous. Larval salinity treatments were 10, 12, 14, 16, 18, 20, 22, 24, 26, and 28 ppt. Larvae were taken through setting, grown in the hatchery until they reached 2-4 mm in size, and out-planted onto 2 field sites. Spat were out-planted for 10 weeks, and growth was measured throughout the field period. At the end of 10 weeks, spat were retrieved and sampled for physiological tests including salinity stress tests, metabolomics, and respirometry.

Results. Salinity has significant and immediate effects on oyster larvae. Increasing salinity significantly decreased larval survival ($G_{-1}=-1355$, $P = 0.009$; Fig. 7), and salinity also significantly affected growth rate of larvae ($G_{-2} = -0.00005432$, $P << 0.001$). Optimal salinity for growth is between 15 and 20 ppt, and growth rate slows as salinity increases and decreases away from this range. So, both salinity extremes pose obstacles for larvae. At low salinities, larvae do not grow as efficiently as the mid-range salinities, and at high salinity, survival and growth are both greatly reduced.

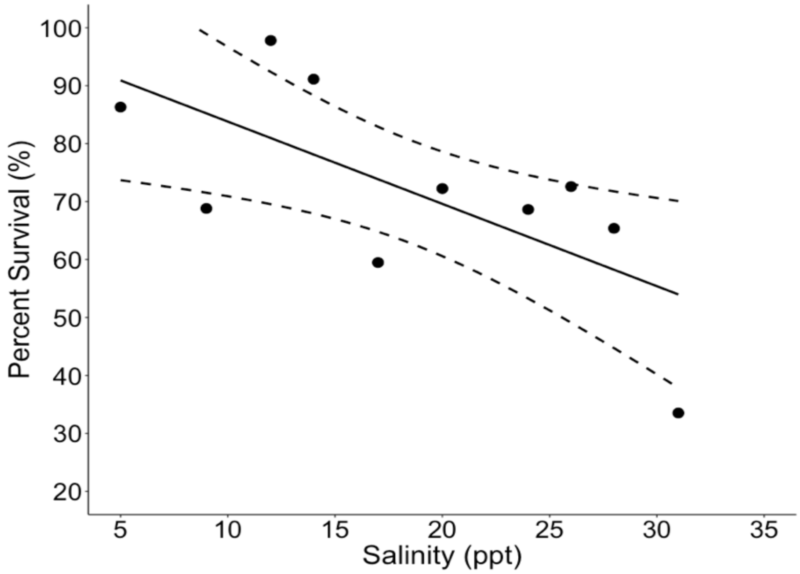

Figure 7: Larval survival across salinities (5-32 ppt). Points represent estimated treatment survivals. The solid line is the best-fit linear model, and the dotted lines represent the 95% confidence interval.

Larval salinity environment also affected early post-settled stages. Larvae grown in low salinities exhibited significantly increased growth rates immediately post-settlement (G-$_{2}$=-0.00021, P = 0.002; Fig. 8).

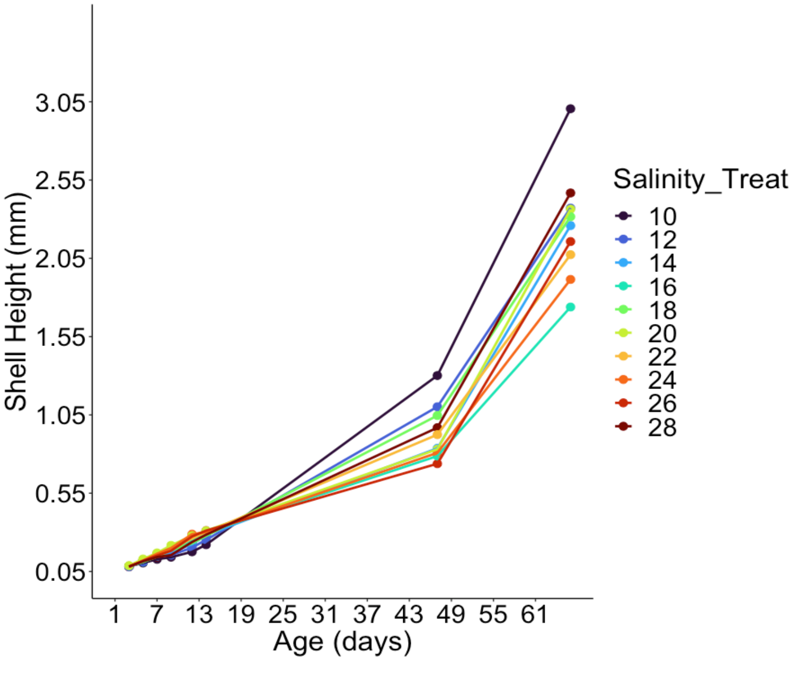

Figure 8: Time series of oyster growth (shell height in mm) grown in salinity treatments (10 – 28 ppt). Different colored lines indicate different larval salinity treatments.

After approximately 6 weeks, treatments no longer differed significantly in growth rate in the hatchery (G-$_1$=-0.000019, P = 0.73; Fig. 8), nor after being transplanted into the field (G-$_1$=-0.0008, P = 0.16). This implies that larvae exposed to suboptimal salinities, reducing growth in the larval stage, potentially undergo a period of compensatory growth immediately after metamorphosis.

Future work. In 2024-2025, experiments characterizing the effects of temperature and combined effects of temperature and salinity on oyster larvae will be conducted. Further work into carry-over effects will be explored, specifically parental carry-over effects. These effects are changes in offspring performance due to parental conditioning and can have context-specific effects on offspring growth and survival.

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

Introduction: The goal of this project is to provide data on the abundance of gastropod oyster predators (Oyster drill – *Stramonita haemastoma*; Florida crown conch – *Melongena corona*) on oyster reefs in Apalachicola Bay. Predator densities may fluctuate over time depending on changes in river flow and salinity regimes, and despite concern for gastropod predation on oysters in Apalachicola Bay during the fishery collapse (Camp *et al.* 2015), quantitative descriptions of *S. haemastoma* and *M. corona* abundance are sparse. Results from this project will inform managers and farmers of locations in Apalachicola Bay in which predators are abundant, helping to aid planning and implementation of restoration and aquaculture activities.

Methods: Predator abundance was monitored monthly from May to October 2023. At intertidal sites (Indian Lagoon, East Cove), three individual reefs separated by mudflats serve as replicates for the respective site (n = 3 reefs per site). At each replicate reef, a 50m transect tape was placed in the middle of the reef, equidistant from the waterline on both sides of the exposed reef at low tide. Researchers quantified abundance of predatory gastropods on the reef by haphazardly tossing a 0.25 m^2 quadrat over-the-shoulder every 5 m along the transect, for a total of twenty 0.25 m^2 quadrat tosses (5 m^2 total area) per replicate reef. In each quadrat, percent cover of intact oyster clusters, number of predatory gastropods (*S. haemastoma* or *M. corona*), and size of gastropods (shell length, mm) in quadrats was recorded. Water parameters (salinity, temperature, conductivity) were measured with a YSI water quality meter, and tidal height (+/- ft) was recorded. At subtidal locations (Peanut Ridge, Dry Bar), SCUBA divers collected 20 haphazard samples of benthic oyster substrate and associated fauna present within the 0.25 m^2 quadrat. Substrate and associated fauna were collected in mesh bags and processed on the boat to count the number and size (mm) of gastropod predators present in each of the 20 substrate collections. This process was repeated for three replicate subtidal reefs at each location, yielding a total of 60 substrate collections covering 5 m^2 of each replicate reef. Water parameters (salinity, temperature, conductivity) of each site were measured using a YSI water quality meter.

Results and Discussion: *S. haemastoma* were most abundant at Dry Bar with a density of 7.6 individuals per 5m^2 but were not abundant at Indian Lagoon (< 1 individual/5m^2), East Cove (0 individuals/5m^2) or Peanut Ridge (0 individuals/5m^2). *M. corona* were most abundant at East Cove, with a density of 9.5 individuals/5m^2, and were present at Dry Bar (< 1 individual/5m^2) and Indian Lagoon (2 individuals/5m^2) in low abundances. *M. corona* were not overserved at Peanut Ridge during the study period.

Results show that the density of gastropod oyster predators varies among oyster reefs in Apalachicola Bay (Fig. 9). Both species of predator were observed at Indian Lagoon and Dry Bar. At East Cove, *M. corona* was abundant, but *S. haemastoma* was not observed at this site. Neither species of predator was observed at Peanut Ridge. Dry Bar and Indian Lagoon are locations in the western portion of Apalachicola Bay that tend to maintain higher salinity levels than sites in the eastern portion of Apalachicola Bay (East Cove, Peanut Ridge) which have relatively lower salinity regimes. Higher salinity levels are favorable for *S. haemastoma* (Schechter 1943), which may help explain their presence at sites in western Apalachicola Bay but absence at sites in eastern Apalachicola Bay.

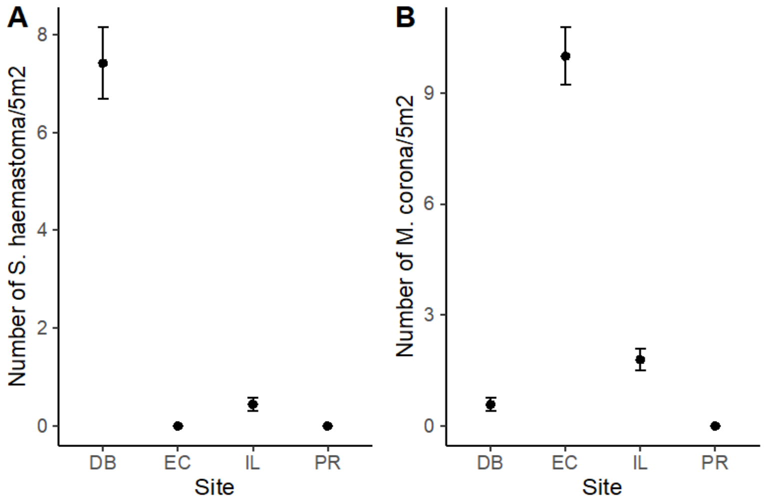

Figure 9: Mean number of **A)** *S. haemastoma* and **B)** *M. corona* per $5m^2$ in subtidal and intertidal habitats of Apalachicola Bay. "DB" and "PR" are subtidal sites, while "IL" and "EC" are intertidal sites. DB has the highest salinity on average, followed by IL and EC, while PR has the lowest salinity on average (D. Baughman, *unpublished data*). Error bars represent 95% confidence intervals.

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

Introduction: Synthetic herbicides are a form of pesticidal compound designed with the intent to kill unwanted photosynthetic organisms. These compounds can be extremely important in agriculture, forestry, and other industries centered around the cultivation of plants ("Pesticides Glossary" 2012). Herbicides eliminate "pest" plants which can otherwise take nutrients away from or competitively exclude more desirable species (Korav et al 2018). These compounds work by targeting a molecular binding site in plants, often blocking or interfering with the site in a manner that subsequently interrupts, disables, or amplifies the delicate chain reactions occurring in plant cells (Hall et al. 1999). The resulting alteration in cell functioning kills the plant. Because these molecules are designed to attach to binding sites in plant cells, they may coincidentally be capable of binding to unrelated but similarly-shaped binding sites in other non-target organisms (Hall et al 1999, Fakhouri et al 2010). For example, the herbicide atrazine appears to bind to growth hormone release receptors in mice brains, interrupting the usual production of growth hormone in these animals (Fakhouri et al 2010). This can cause developmental issues in exposed mice and their progeny (Fakhouri et al 2010). The lack of extreme specificity in herbicidal compounds makes them a potential danger for wildlife, even at extremely low concentrations (Mai et al 2013, Nikoloff et al 2013). Studies of non-target effects on oysters are extremely limited for most common herbicides, although some publications do show capacity for harm (Mai et al. 2013, Jamal 2023). The majority of these studies tend to focus on acute, short-term exposures; however, herbicides can remain in the environment for weeks or longer at low concentrations (Jamal 2023). This means that it is important to understand the chronic and sublethal effect of herbicides, especially on the sensitive early-life-history stages of these animals.

Objectives

1. To determine which herbicides are the most-likely candidates to cause harm to oysters: There are over 70 herbicides approved for use for the top four crops in North Florida and Georgia, and several more commonly used for forestry and ecosystem maintenance (Dittmar 2023, Shelton et al 2024, Prostko 2024). Investigating all of these would require an enormous collaboration of research and vast amounts of funding; however, I aim to identify some of the most likely problem candidates using monitoring and experimentation.

2. To determine the chronic effects of the most-likely problem candidates: The measurement of acute herbicide effects on oysters is not sufficient to determine whether herbicides could have a significant impact on oyster populations. Sub-lethal and chronic effects must be evaluated. I aim to evaluate these effects using long-term-exposure experiments.

Objective 1. Methods: To evaluate the most-likely candidates for harm, I am combining an herbicide monitoring study in Apalachicola Bay with acute stress exposure experiments to determine the most common herbicides in Apalachicola Bay, as well as the herbicides which have pronounced short-term impacts.Monitoring the herbicide concentrations in the Bay will be done using Polar Organic Chemical Integrative Samplers (POCIS). POCIS will be deployed at three sites for a duration of one month at a time. Three POCIS will be deployed at each site, but processed as a single data point. This is to collect as much herbicide as possible, allowing for accurate detection of even extremely low concentrations of herbicide in the water column. At the end of each month, the samplers will be processed and new POCIS will be deployed. This process will continue until each month of the year has been monitored once. POCIS work by absorbing polar and semipolar pesticides (including herbicides) directly from the water. They allow a time-weighted average of pesticide exposure to be estimated (Alvarez 2010). I will deploy three POCIS to each of three sites at varying distances from the mouth of the Apalachicola River. These sites represent different stages of herbicide dilution as they move from fresh to salt-water. The POCIS will be supplemented with water samples taken twice a week from the water column near where the POCIS are deployed, as well as samples taken before and after heavy rains pass through the region. Rainstorms will potentially increase runoff of pesticides from land and the chance for their detection in single samples. They also represent the most likely reasons for extreme herbicide exposure events.

I will extract samples according to the methods in Furlong 2001 and Alvarez 2010. These samples will be processed by the FSU College of Medicine UPLC- Mass Spectrometry Facility using the Pesticide Monitoring methods in Furlong 2001.

The acute exposure experiments involve placing twenty oyster D-stage-larvae in a 2 ml chamber of a chamber slide. An amount of pesticide is then added, with an amount of solvent (methanol) as needed for the expedient dissolution of the chosen pesticide in water. Uncontaminated seawater will then be added to create a total working volume of 2 ml. Treatment concentrations will vary by pesticide, but will be separated by magnitudes of 10. Each concentration will be based on environmental concentrations, and strive to model a low, medium, and high environmental concentration, as well as a concentration which exceeds environmental conditions to investigate if effects occur beyond environmental levels. Seawater and solvent controls will also be included. Each treatment will be replicated three times.

Once larvae are placed in pesticide water, they will be left for 24 hours without feeding. Once 24 hours have passed, they will be stained with neutral red for 30 minutes. Neutral red helps differentiate live oysters from dead. These will be examined under a scope. The larvae will be evaluated for deformities and mortality. Preliminary acute-exposure experiments have been run using the herbicide atrazine as the toxin; while these investigations are ongoing, other herbicides will soon be added to the evaluations, including common herbicides glyphosate, 2,4-D and more.

Water samples will be taken when creating dilutions of herbicide prior to adding the herbicide to the enclosures. These water samples will be extracted and evaluated for actual herbicide concentration according to the methods of Furlong 2001. The FSU College of Medicine will provide assistance with this process. The actual concentration will be used in the final evaluation to control for partial dissolution effects and non-experimental contamination of seawater with herbicides.

Objective 2. Methods: The chronic exposure experiments aim to expose larval oysters to herbicide for an entire week. For each of seven herbicide treatments, three replicates of 1000 larvae will be kept in a 1 L beaker, with water changes every two days. Pesticide will be added to a target concentration, and clean seawater added in order to reach a total volume of 1000 ml. Treatment concentrations will vary by pesticide, but will be separated by magnitudes of 10. Water changes will involve filtering larvae through a sieve of metal mesh, and rinsing the larvae into a clean container. Larvae will be fed live algae sourced from the FSUCML algae production facility. Feedings will occur twice per day. After larvae have been exposed for seven days, larvae will be placed in pesticide-free water and stained with neutral red dye. Evaluations will be made on deformity and mortality of oyster larvae, and compared across all treatments.

Water samples will be taken when creating dilutions of herbicide prior to adding the herbicide to the enclosures. These water samples will be extracted and evaluated for actual herbicide concentration according to the methods of Furlong 2001. The FSU College of Medicine will provide assistance with this process. The actual concentration will be used in the final evaluation to control for partial dissolution effects and non-experimental contamination of seawater with herbicides.

4. Oyster ecology

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

Introduction: The eastern oyster (*Crassostrea virginica*, Gmelin 1871) is an important fisheries species that has declined globally (Beck et al., 2011). One area with a more recent collapse in 2012 is Apalachicola Bay, Florida (Camp et al., 2015). This estuary previously provided up to 90% of Florida's oyst4rs and 10% of the USA (MacKenzie et al., 1997). Various factors have contributed to its decline including low freshwater inputs, depletion in shell, and continued overharvesting (Camp et al., 2015). Since its decline, the bay was suspended for further harvesting until 2025 (FWC Rule: 68B-27, F.A.C.), with various efforts implemented to better manage and research the bay. Current efforts are mainly focused on subtidal oyster reefs due to their extensive bottom coverage (Twichell et al., 2007). However, there are also intertidal oyster reefs in Apalachicola Bay (Grizzle et al., 2018), and little is known about their ecological function and patterns driving their persistence within the bay. Filling the knowledge gap of its ecological function is necessary for effective ecosystem-based management of this fishery species. To fill this knowledge gap, this research will focus on mapping the intertidal oyster reefs to understand temporal and spatial patterns in the Apalachicola Bay region of Florida.

Current methods of monitoring the intertidal oyster reefs involve on-the-ground quadrat sampling, which is time- and cost-intensive, destructive to the reef, (Baggett et al., 2015; Espriella et al., 2020) and provides only a small snapshot of the larger landscape extent. Satellite imagery analysis is an alternative to this method and has been implemented in this region by Grizzle et al., (2018). However, this approach has a coarse resolution, with insufficient detail for monitoring efforts (Espriella et al., 2020). Unoccupied aerial systems (UAS), more commonly called drones, have recently become a powerful research tool in coastal and marine environments (Joyce et al., 2019). 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).

Objectives

1: Create high-resolution orthomosaics and digital elevation models (DEMs) of the intertidal oyster reefs.

- **1a**: Extract clusters and respective sizes using the orthomosaics and DEMs.

2: Conduct ground sampling within areas mapped.

- **2a**: Assess cluster and non-cluster differences.
- **2b**: Assess the relationship between cluster size and quantity of live oysters.

3: Estimate the number of live oysters using the regression model from 2b to the extracted clusters from 1a.

4: 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 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 1-2cm 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 (Westoby et al., 2012). The products include orthomosaics, or high-resolution georeferenced mosaics, and digital elevation models (DEMs), or a digital representation of elevation data.

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 size, mass, volume, and the live, boxes, and shell heights within the clusters. A regression model was fitted to examine the relationship between cluster size and the number of live oysters. Additionally, at one site, presence and absence data were collected to validate the accuracy of cluster detection.

Using ArcGIS Pro, orthomosaics and DEMs were analyzed to extract oyster clusters per reef. This extraction was conducted for both sites to determine the total number of clusters and their size ranges per site and per reef. The regression line derived from ground sampling was utilized to estimate the number of live oysters for all clusters extracted from the maps. Oyster density per reef and per site was calculated based on the estimated number of live oysters. Statistical tests were then conducted to assess the significance of oyster density between sites and over time at one site.

Results: As of March 2024, all objectives have been completed and have been submitted to the journal of *Remote Sensing in Ecology and Conservation*. Orthomosaics and DEMs were collected at East Cove and Alligator Harbor (Fig. 10). The ground sampling showed that there were more live oysters within clusters than in areas with no clusters. Additionally, there was a significant positive relationship between live oysters and the size of clusters. This allowed us to use that model and estimate live oysters at East Cove and Alligator Harbor, which showed that East Cove has a higher density of live oysters per reef than Alligator Harbor. The statistical test showed that this difference was significant. A temporal evaluation of a section within East Cove between 2022 and 2023 showed a decrease in cluster count but the change in live oyster density was not significant change due to the high overlapping uncertainties.

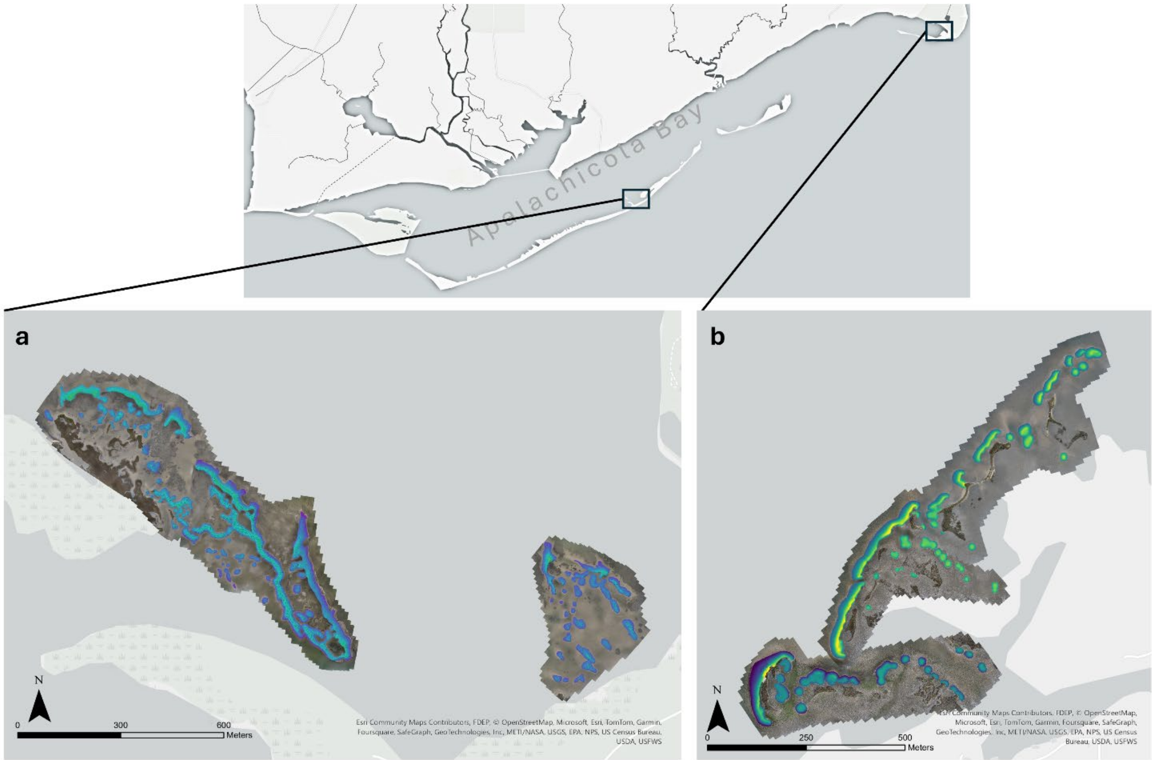

Figure 10: Orthomosaics with DEMs in color (a) East Cove and (b) Alligator Harbor, within Franklin County, Florida.

4.2 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 methods and data from the 2020-2021 and 2021-2022 tong surveys are detailed in the 2021 and 2022 Annual Reports respectively. The third round of tonging surveys were conducted from January to March 2023 and comprised 227 locations throughout Apalachicola Bay (Fig. 11). 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 with oysters measured then categorized into size classes (spat <25mm, sub-legal 25-76 mm, market >76 mm). Additional information collected included size classification of boxes, which provides more insight into the mortality occurring in different size classes.

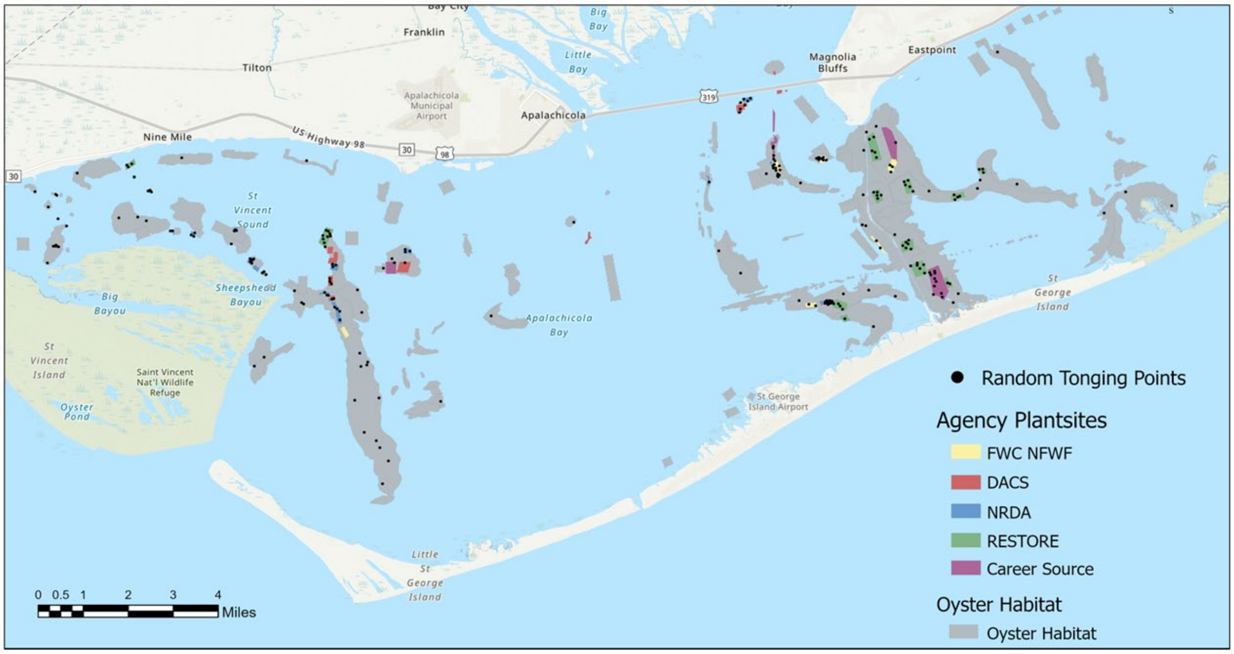

Figure 11. Subtidal tonging locations from 2023 showing agency planted sites (colored polygons) and tonging point locations (black dots. These were collected in a nested random design, with higher intensity sampling on restoration projects.

Results and discussion: The 2023 tonging data was undergoing data entry and quality checks at the writing of the 2022 annual report. Previous surveys showed that the distribution of oyster populations in Apalachicola Bay is spatially heterogeneous with more oysters of all size classes in the eastern side of the Bay. Very few areas supported market sized oysters and no site reached the 400 bag/acre threshold for a sustainable fishery (Fig. 12). The 2023 data show an overall increase in oyster abundance, and the eastern region remains considerably better than the west with and several sites reaching or exceeding the 400 bags/acre of market oysters. Larger oysters were generally found in areas that were planted with limestone (FDEP 2017, FWC 2021), but there were some indications of recovery in other locations (sites planted with shell and unplanted areas) particularly in the eastern part of the bay. Most of the harvest targeted the limestone sites, potentially relieving pressure on the smaller patchy areas. While these sites have fewer oysters, their total area is much greater than the limestone planted sites. If recovery is indeed occurring, these habitats could represent a much larger resource than the relatively small 'restored' sites.

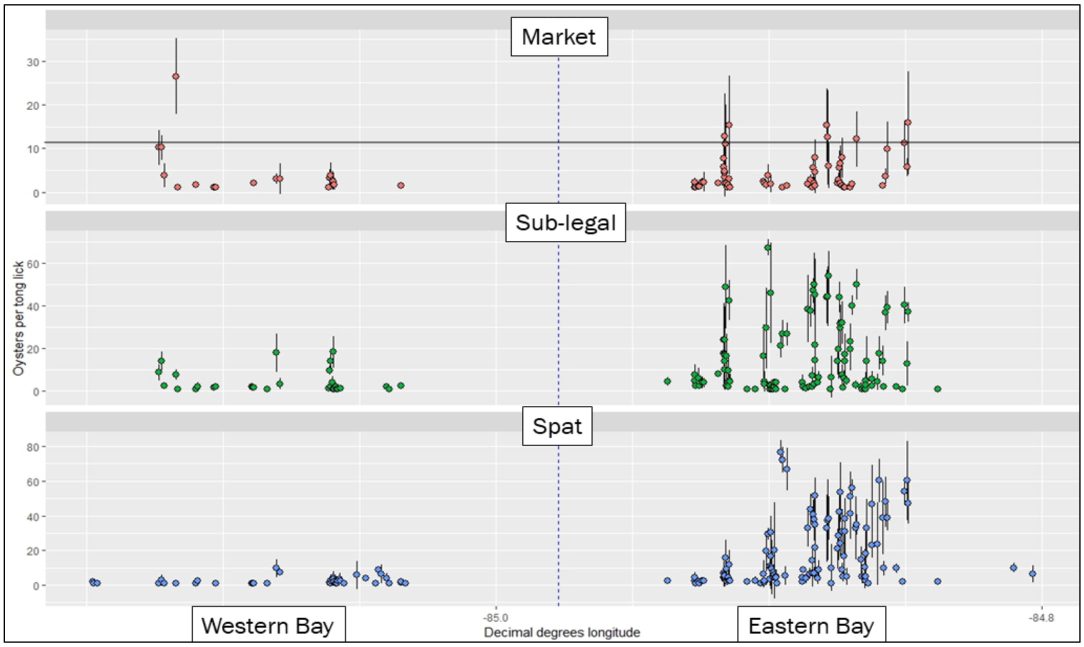

Figure 12. Distribution of oyster size classes (market, sub-legal and spat) found during 2022-2023 tonging survey. The X-axis shows longitude so data is presented from west to east across the Bay. Horizontal line represents an estimate of 400 bags/acre for market sized oysters.

The fourth tonging surveys were conducted from March to April of 2024 and consisted of 66 sites focused on FDEP and FWC restoration plant sites (Fig. 13). Mapping data from FDEP side scan was used to create accurate polygon shapes (ArcGIS Pro) of each reef and to assess amount of reef area (ReefMaster 2.0) within each site. Tonging samples for round four subtidal sampling were collected and the height of all oysters was measured and recorded except spat <10mm, which were counted. Data were also collected on size classification (spat, sub-legal, market) of boxes, as done in round three tonging collections.

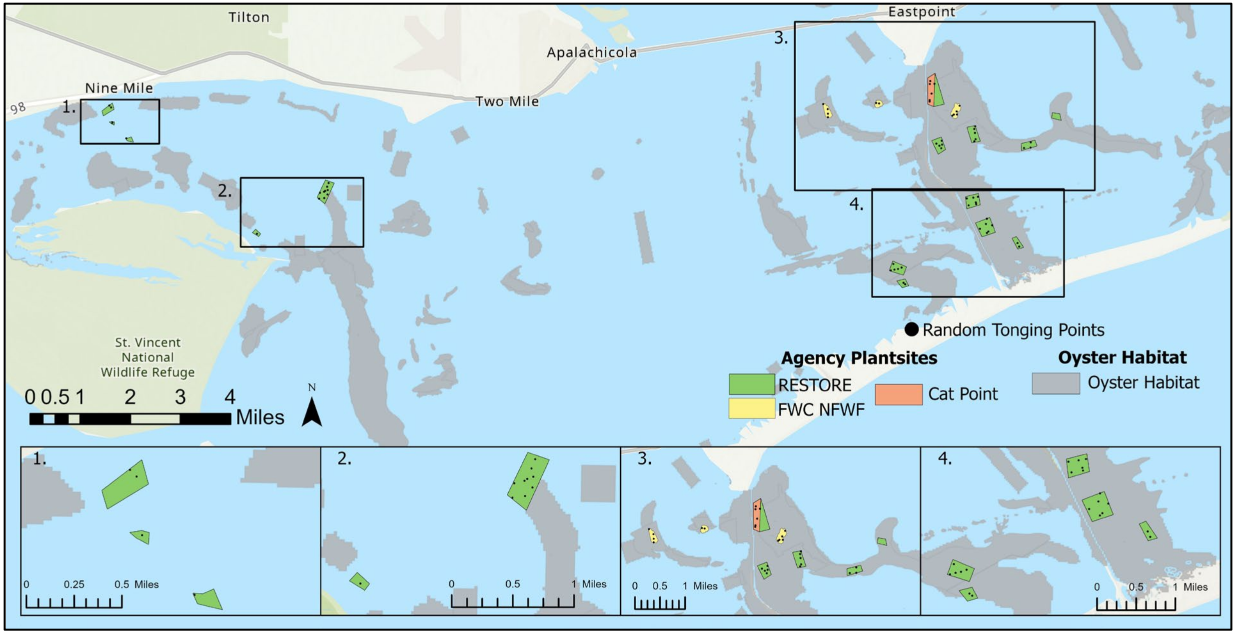

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Figure 13. Subtidal tonging locations from year four (2024) showing planted sites (colored polygons) and tonging point locations (black dots).

Results and discussion. The 2024 tonging data is currently undergoing data entry and quality checks, but preliminary observations indicate that oysters are growing well where there is sufficient substrate, but some of those areas seem to be diminishing. The sites deployed by FWC in 2021 generally support more oysters than the earlier FDEP deployments in 2016-2017, and the number of market sized oysters has increased overall since the fishery closure.

4.3 Oyster community development on high relief structures (Dr. Sandra Brooke and 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 numerous commercially important fishes. Effectively restoring the lost ecosystem goods and services provided by Apalachicola Bay will require understanding how different restoration approaches influence the development of oysters and associated reef communities.

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 high relief prefabricated restoration modules contribute to oyster population development in different parts of the Bay

Research on the first objective was reported in the ABSI 2022 Annual Report. Part 2 began in March 2022.

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. 14) at six study sites: three on Dry Bar and three on the eastern bars (Fig. 15), 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.

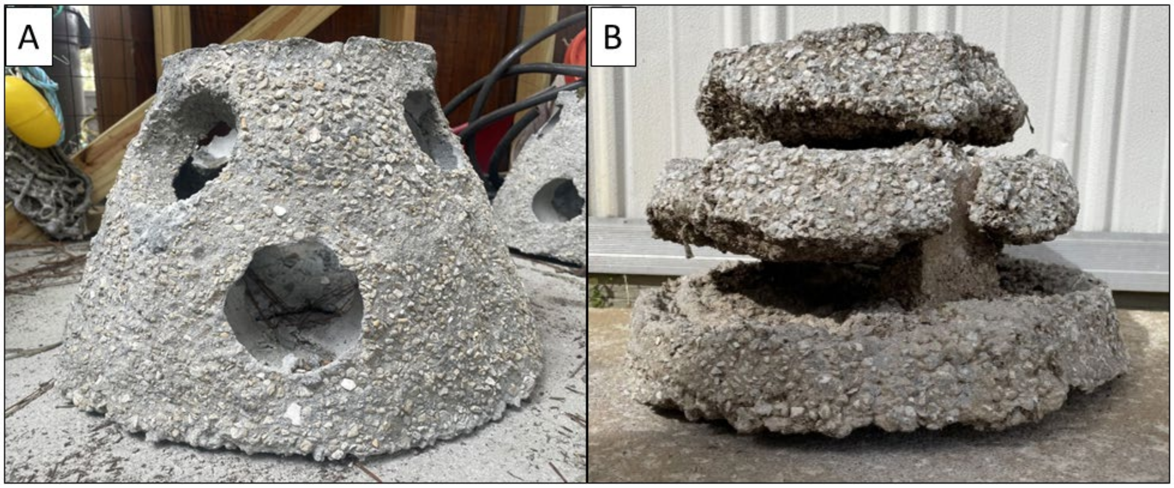


Figure 14. Restoration structures prior to deployment. A) Oyster Reef Ball, B) Layer Cake. Units were deployed in groups of four of each type at three sites in the west bay and three in the east

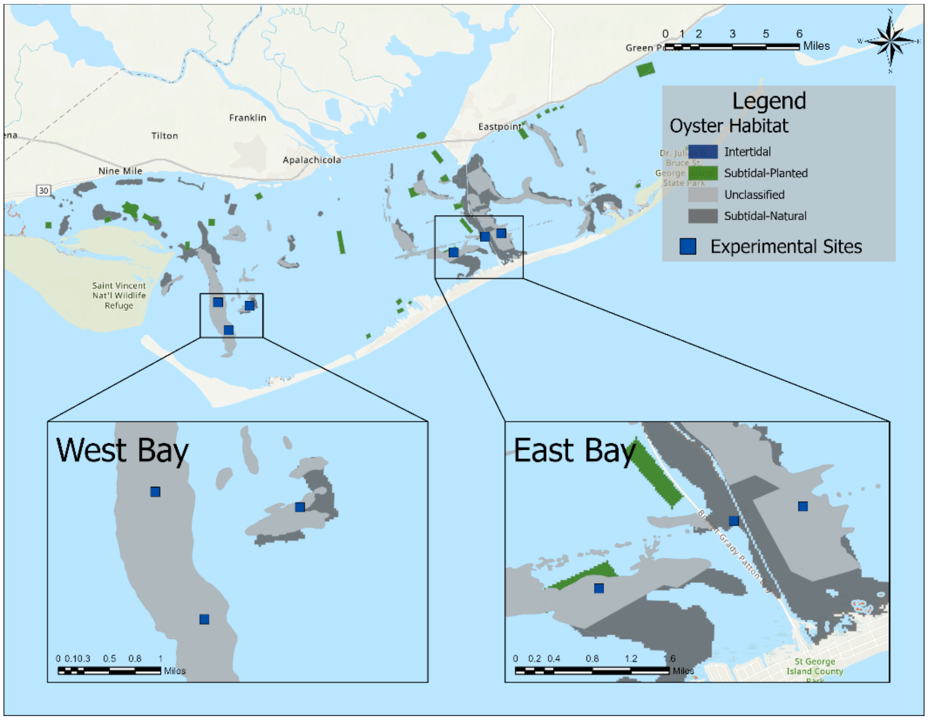


Figure 15. Subtidal high relief structures (reefballs and layer cakes) deployment sites.

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.

Objectives.

1) investigate the efficacy and persistence of different materials
2) Assess recruitment and survival of oysters on the elevated reef structures
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. 16). 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.

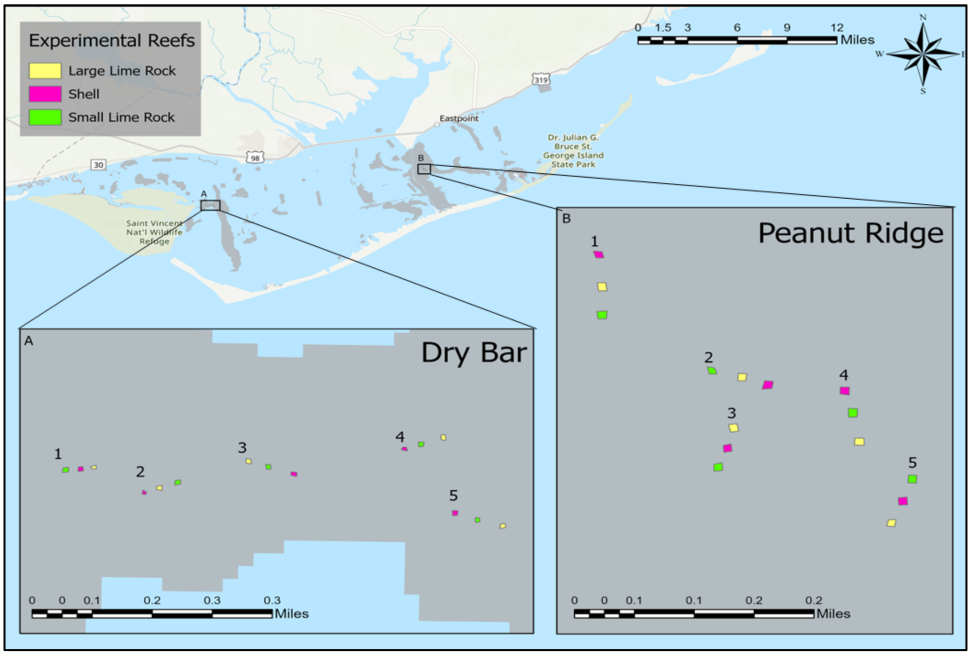


Figure 16. Experimental reef sites at Dry Bar and Peanut Ridge. Three materials (large limerock, shell and small limerock) were used with five replicate reefs at each site.

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); Fig. 17A). 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. 17A). 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. 17B).

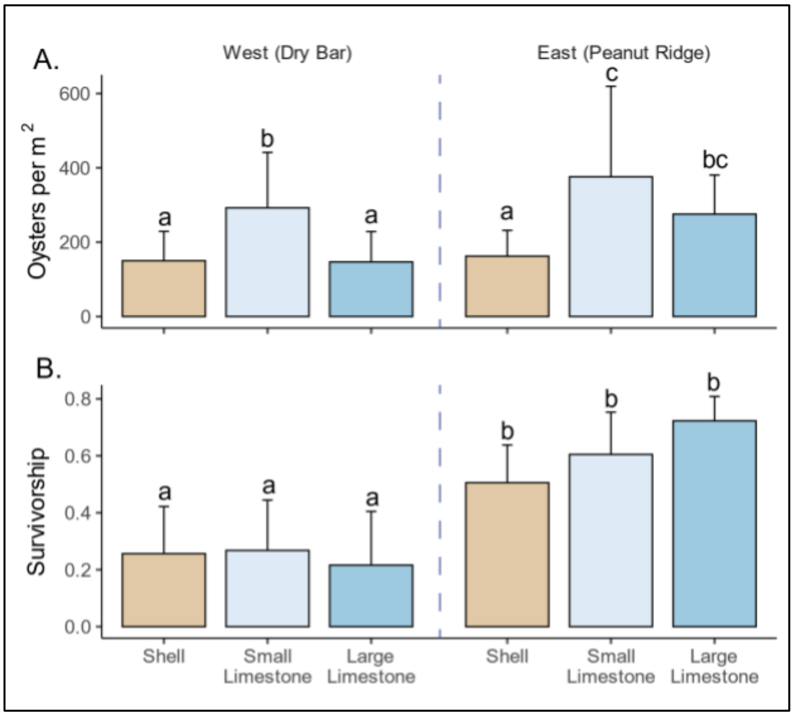


Figure 17. Results of dive surveys (summer 2022) showing A) average oyster settlement and B) percent survivorship for each reef material, by region. Different letter assignments represent significant differences between the reefs.

Comparing live oyster shell heights across reefs showed differences in the size structure of oyster populations (Fig. 18). 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. 18). The total number of oysters and their size structure found on individual experimental reefs is shown in figure 19. The diver collections on Dry Bar (Fig. 19A) clearly show the lower abundances and sizes of oysters than collections taken from Peanut Ridge (Fig. 19B). This trend holds true for the collections made by tonging (Fig. 19 C, D), but oyster abundance was generally higher, particularly at the Dry Bar sites (Fig. 19C)

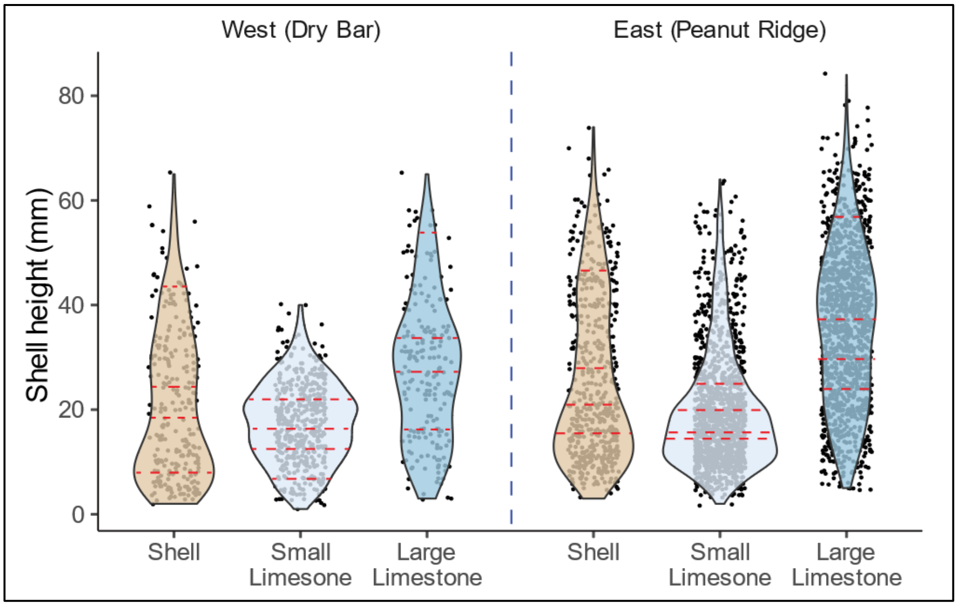


Figure 18. Violin plot of shell heights showing size structure of oysters collected by divers by reef material and location. Red dotted lines show the 0.25, 0.50, 0.75 and 0.95 quartiles.

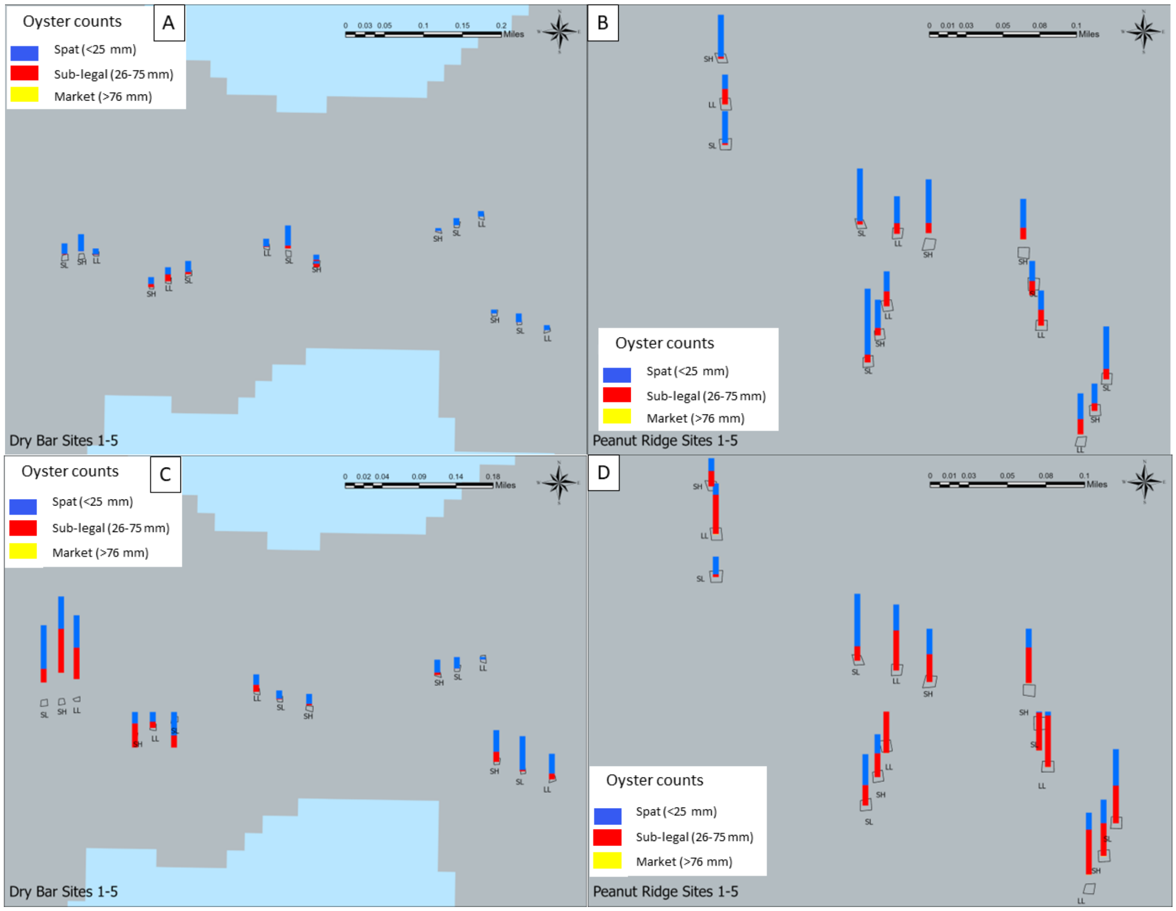


Figure 19. Average counts of spat, sub-legal and market oysters collected by divers at A) Dry Bar and B) Peanut Ridge and by Tonging at C) Dry Bar and D) Peanut Ridge restoration reefs.

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. 20) 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.

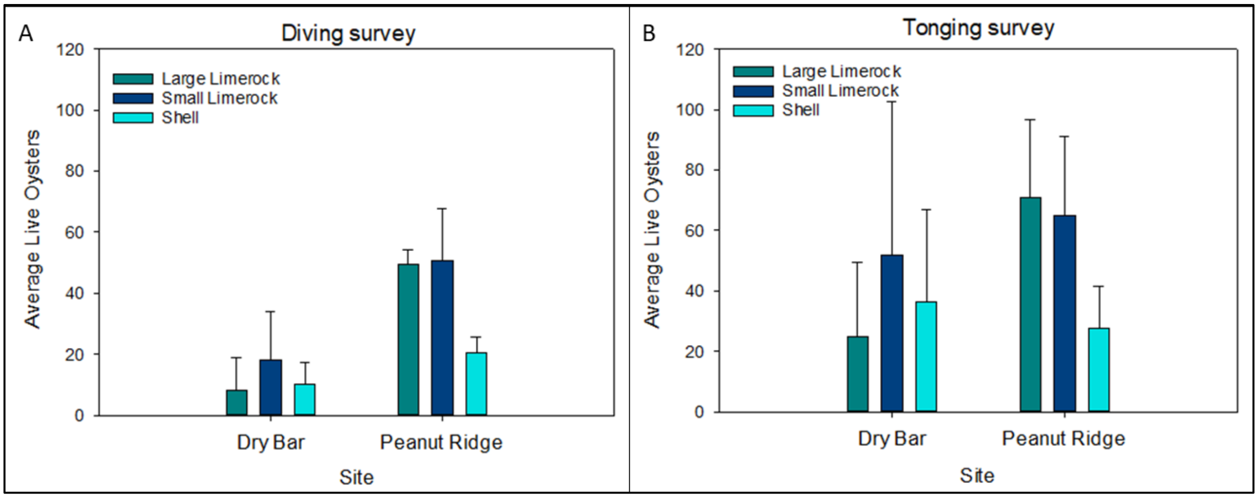


Figure 20. Comparison of average live oysters (all size classes) using diver surveys and tong surveys conducted in late summer 2022.

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 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, bay scallops have only been available for recreational harvest, which increased the popularity of "scalloping", the practice of collecting scallops by hand while snorkeling in seagrass meadows. In 2018, revenue from this sport exceeded 1.8 million dollars in Steinhatchee, with both locals and tourists from 16 states participating (Granneman *et al*. 2021). Take limits and shortened scalloping seasons have been imposed to limit overharvesting. However, even with management, the fishery has suggested to be unsustainable in Steinhatchee, and further investigations should be conducted to assess scallop populations in other harvest zones (Granneman *et al.* 2021). The alarming decline of bay scallops suggests there are insufficient numbers of reproductive adults to replenish depleted populations. Therefore, aquaculture is becoming a focus of many restoration efforts that aim to supplement natural populations with hatchery-grown scallops. Bay scallops are collected from the wild, spawned in a hatchery, and raised until a certain growth milestone is reached. The desired-sized scallops then begin the grow-out process in cages in the wild. This transition commonly results in high mortality, losing up to 90% of hatchery-raised scallops before reaching their reproductive stage with no identifiable cause (Arnold *et al.* 2005, Clyde and Mackenzie 2009, Seyoum *et al.* 2003). This results in a waste of time and money for restoration efforts that have already incurred high labor and hatchery operation costs. To keep costs low and feasible, it is important to understand why this mortality occurs, and how to limit it. Surprisingly, there is relatively little information regarding the performance or biological differences between wild and hatchery raised bay scallops. Understanding the biological differences between wild and hatchery raised scallops as well as studying the optimal deployment size and the best grow out techniques will increase efficiency of ongoing and future restoration efforts.

Objectives

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

2: Investigate the differences/similarities in condition index, gonadal index and shell breaking strength between wild and hatchery raised bay scallops.

3: Identify the optimal size (2-3mm or 6-7mm) for the release of hatchery raised bay scallops to maximize survival when transferred to grow-out cages.

4: Identify the best gear type for yielding 20mm bay scallops to be used in restoration from grow-out in a shallow Florida aquaculture lease.

Hypotheses:

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

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

3: The hatchery scallops raised to 6-7mm display less mortality upon release and a faster initial growth rate.

4: Lantern nets and bottom cages produce more 20mm spat compared to floating cages.

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

Methods: In 2023, all above objectives were addressed. To complete objectives 1 and 2, wild bay scallop spat was obtained from spat traps deployed on Nov. 21st, 2022 in Turkey point shoal. 34 spat traps were removed and processed on Feb. 14th. 2023, yielding 235 wild spat ranging from 3mm-14mm. These juveniles were housed at the FSUCML hatchery for 48hrs while being measured and paired with hatchery spat. Hatchery spat resulted from 30 wild bay scallop parents during a spawn on Nov. 2nd 2023 and the culture set on Nov. 17th, 2022. Their husbandry consisted of daily water changes, live algal feed ranging from 150K- 250K depending on age and were weaned off the live algae diet a few weeks before deployment. The largest juvenile scallops were handpicked from the tank for pairing. 126 wild spat were paired with hatchery spat of equal shell length. Each individual was measured for length, width, depth, and weighed with a microbalance. Hatchery spat and wild spat were placed in separate 1.5mm spat bags and then placed in a 18mm mesh aquaculture bag placed inside a bottom cage in Turkey point shoal (SAL- 23-2415-SR), a seagrass meadow known to house native bay scallops. The site was visited monthly for sampling and cleaning. The sampling consisted of pouring the contents of the bags onto a white tray with a ruler placed in the middle. Images of the scallops were taken with an iPhone 13 Pro Max and processed on Image J. Shell areas were measured to calculate gross growth rates, and survival was scored over time. Undesirable contents (dead shell, crabs, snails) were removed during the cleaning process. Monitoring the growth and survival over time addressed objective 1.

Once the paired scallops reached reproductive maturity in October 2023, they were returned to the lab on 10/09/2023 for condition indexing, gonadal indexing, adductor indexing, and shell breaking strength comparisons. Wet weights of shell, total tissue, gonad, and adductor muscle were recorded using a microbalance. Prior to breaking the shells of the scallops, pictures were taken with an iPhone 13 ProMax with a nearby ruler to measure final shell area using the image processing software, ImageJ. Using a tensile strength testing device, shell breaking strength was recorded for each individual. Then, samples were placed in a drying oven for 1 week, and all measurements were recorded for a dry weight. The following indexes were calculated as: Condition Index: Dry Total Tissue Weight / Dry Shell Weight ; Gonad Index: Dry Gonad Weight / Dry Total Tissue Weight; Adductor Index: Dry Adductor Weight / Dry Total Tissue Weight (Shriver et al. 2002). This series of data collection worked to address objective 2.

To address objective 3, hatchery bay scallops were obtained from the Nov. 2nd spawn. An estimated 5,000 2mm-3.3mm hatchery scallop spat were used for each replicate with a total of 6 replicates. Each spat bag was labeled A-F and housed in flow through tanks with no supplemental feed until deployment. Deployment size spat bags deployed inside 18mm plastic mesh at cage site on Turkey pt shoal on 2/16/23 in bottom cages. For the following treatment, size 6mm-7mm hatchery spat were used and the bags were filled in the same manner but with a lower stocking density due to larger size of scallops. Each bag was stocked with roughly 1200 scallops on 3/17/23 and held in flow through until deployment on 3/21/23. This site was visited monthly, and processing took place in the same effect as mentioned for objectives 1 and 2.

To address objective 4, hatchery bay scallops from the Nov. 2nd spawn were collected on 2/3/2023 ranging from 1mm – 3mm in size. Using a microbalance, 34g of mixed scallop spat were stocked into 18 bags. Each bag was randomly assigned to a gear type treatment of floating cages, bottom cages, or lantern nets. Bagged scallops were deployed to a shallow water aquaculture lease in Alligator harbor in their assigned gear type replicate. Every month, this site was visited, and bags were assessed in the same manner as described for objectives 1, 2, and 3.

Results:

Objective 1**:** All data has been collected and requires further processing using ImageJ.

Objective 2**:** Figure 21 below shows comparisons of hatchery and wild scallop juveniles for five condition metrics. In summary, hatchery raised bay scallops showed no difference in terms of adductor and condition index. Statistics will confirm or deny a significant difference in gonadal index and shell breaking strength. Treatment H represents hatchery origin scallops, and treatment W represents wild origin scallops.

Objective 3: All data has been collected and awaits further processing on ImageJ.

Objective 4*:* All data has been collected and awaits further processing on ImageJ.

ImageJ processing, statistics, and further refinements to presented graphs are a goal to be completed in 2024. A large collection of images was generated during these various projects and will need a large investment in time to complete all measurements. After completing measurements, plots, and statistics for all objectives, a complete discussion on how these results compare to other completed research will be included in the final report.

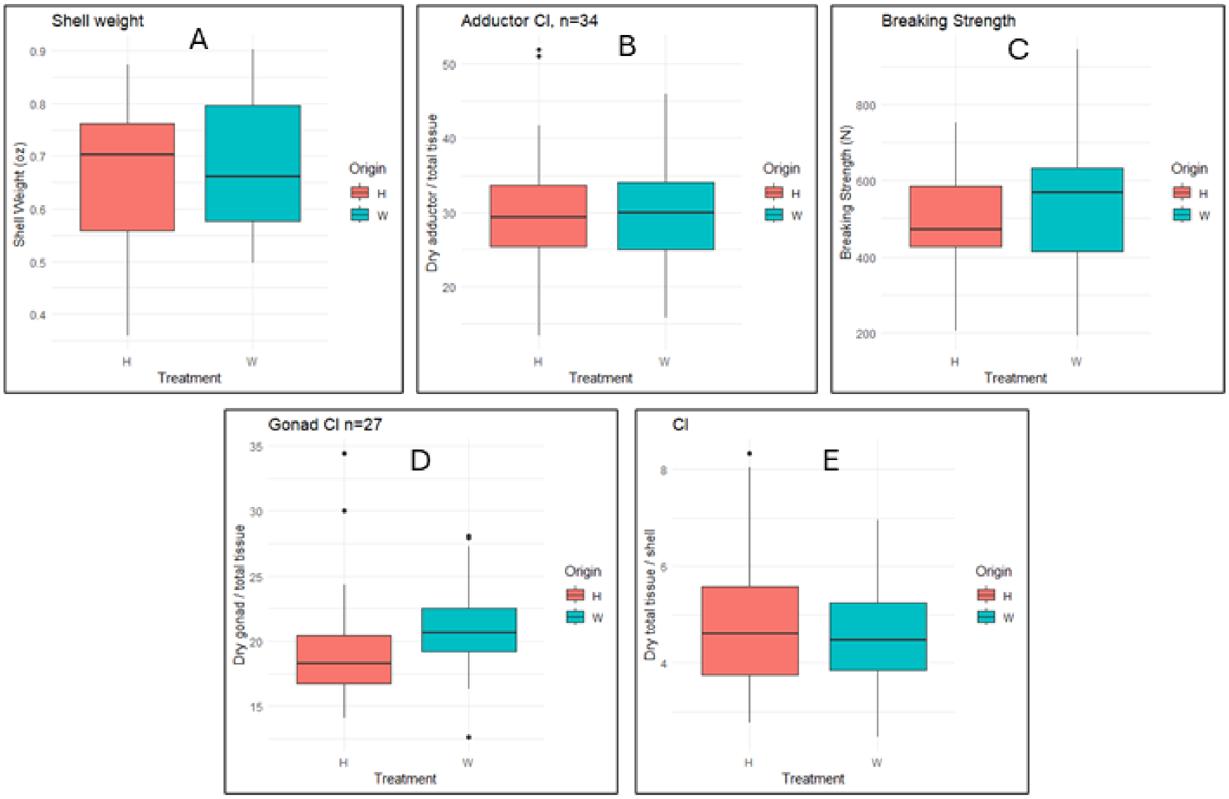


Figure 21. Condition metrics for wild (W) and hatchery (H) scallops: A) shell weight, B) Adductor tissue dry weight as a fraction of total tissue, C) shell breaking strength, D) Gonad dry weight as a fraction of total tissue, E) ratio of tissue dry weight to shell.

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 2023 – March 2024. 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. 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 the 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. 22; Kofoed and Gorsline, 1963; Chanton and Lewis 2002). The second stage focuses on stratigraphic change in four locations using radiometrically-dated sediment cores.

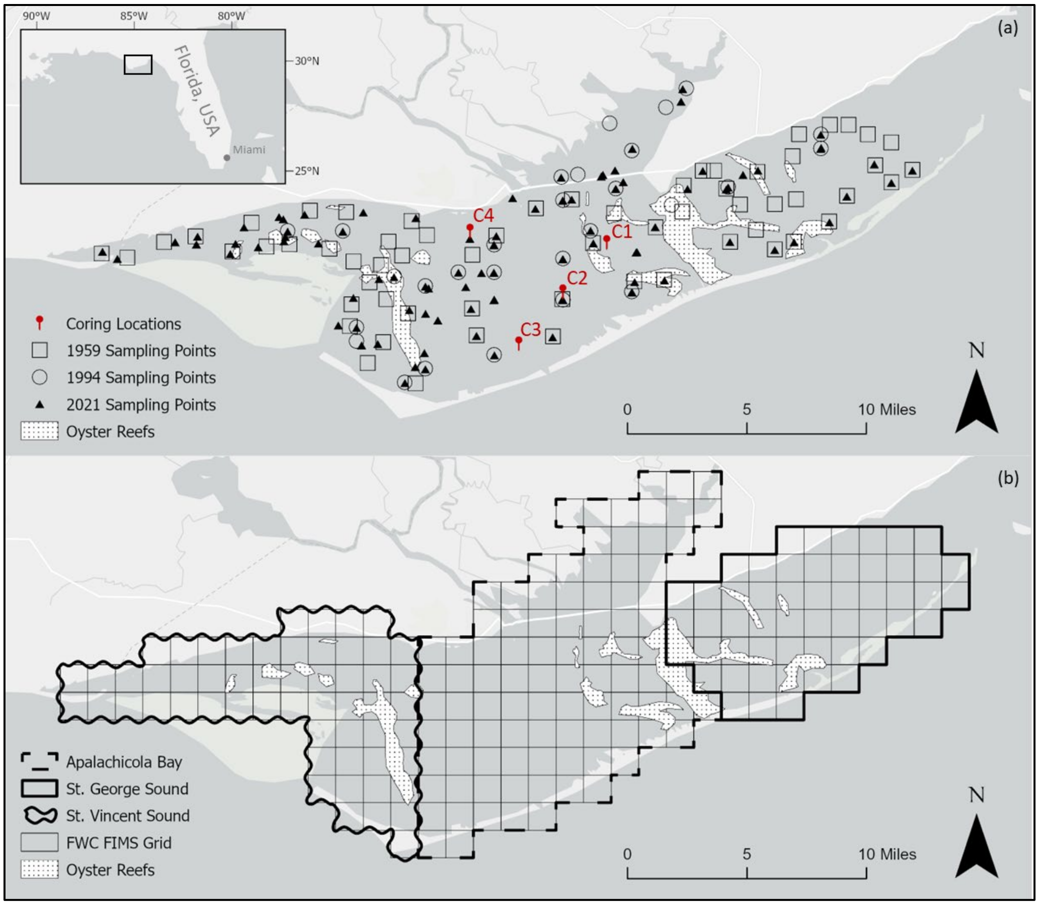


Figure 22. a) Locations of sediment collection in 1959 (open squares), 1994 (open circles), and 2021 (dark triangles) in the Apalachicola Bay region of Northwest Gulf of Mexico. The largest oyster reefs of the bay are outlined. Red points identify locations of the dated sediment cores 1 – 4 (C1 – C4) used in the second stage of this study. **b)** Application of the FWC FIMS spatial grid divided into three subregions for the comparisons, including St. Vincent Sound (west), Apalachicola Bay (central), and St. George Sound east).

6.1.1a. Organic Enrichment Stage 1:

Introduction. The investigation characterizes benthic sediment throughout Apalachicola Bay to determine if changes in surface sediment organic content has 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. Spatial and temporal changes to sediment organic and mineral constituents are a measure of changing sources and processes in the Bay. Two potentially important regional changes are: 1) floodplain-derived detritus and sediments to the Bay, and 2) system-wide oyster population and a resulting decrease in the metabolic processing and sequestration of organic matter.

Methods. Surface sediment samples have been collected from the bottom of the Bay in the same locations as the historical studies (Fig. 22) 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 show areas of high and low concentration of these constituents at the different time points, and separate maps have been created (Figs. 23, 24) to quantify rates of change occurring across the region for each time point

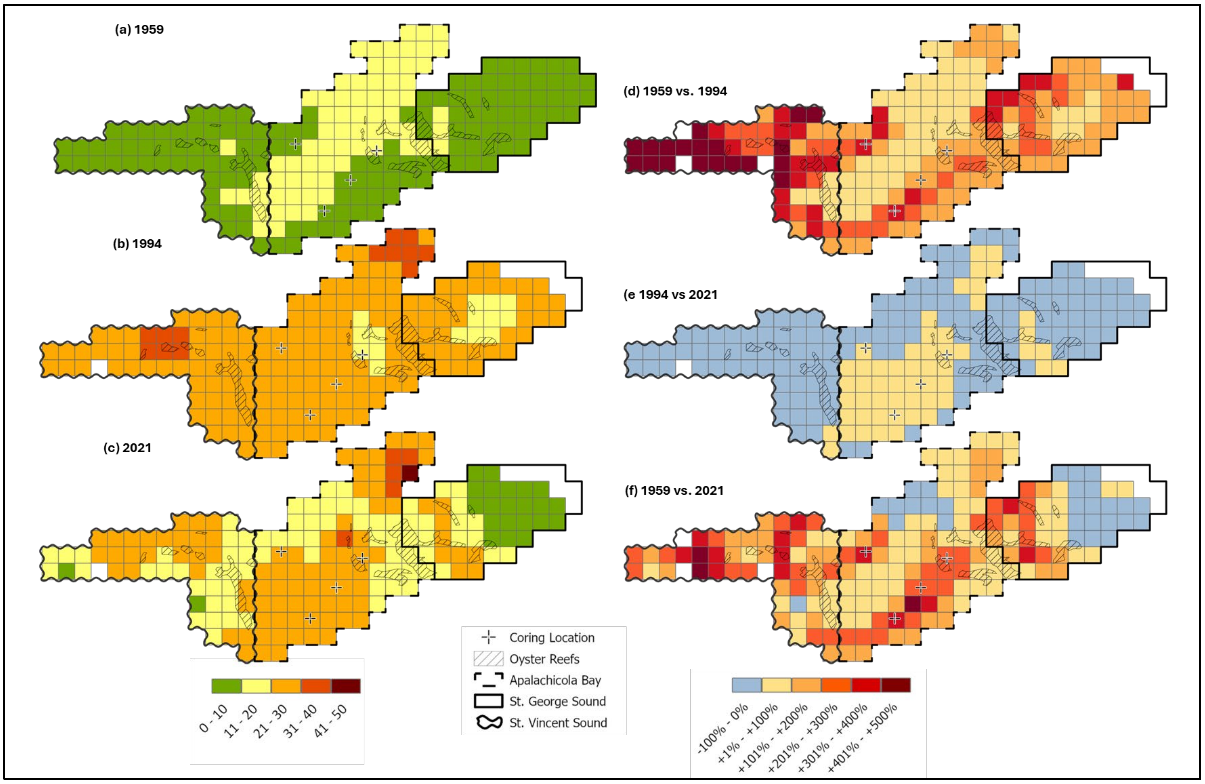


Figure 23. a-c) Heat maps identifying sediment organic carbon content (mg g^{-1}) in 1959, 1994, and 2021. **d-f)** Heat maps identifying percent change over the observed periods from 1959 to 1994, 1994 to 2021, and 1994 to 2021.

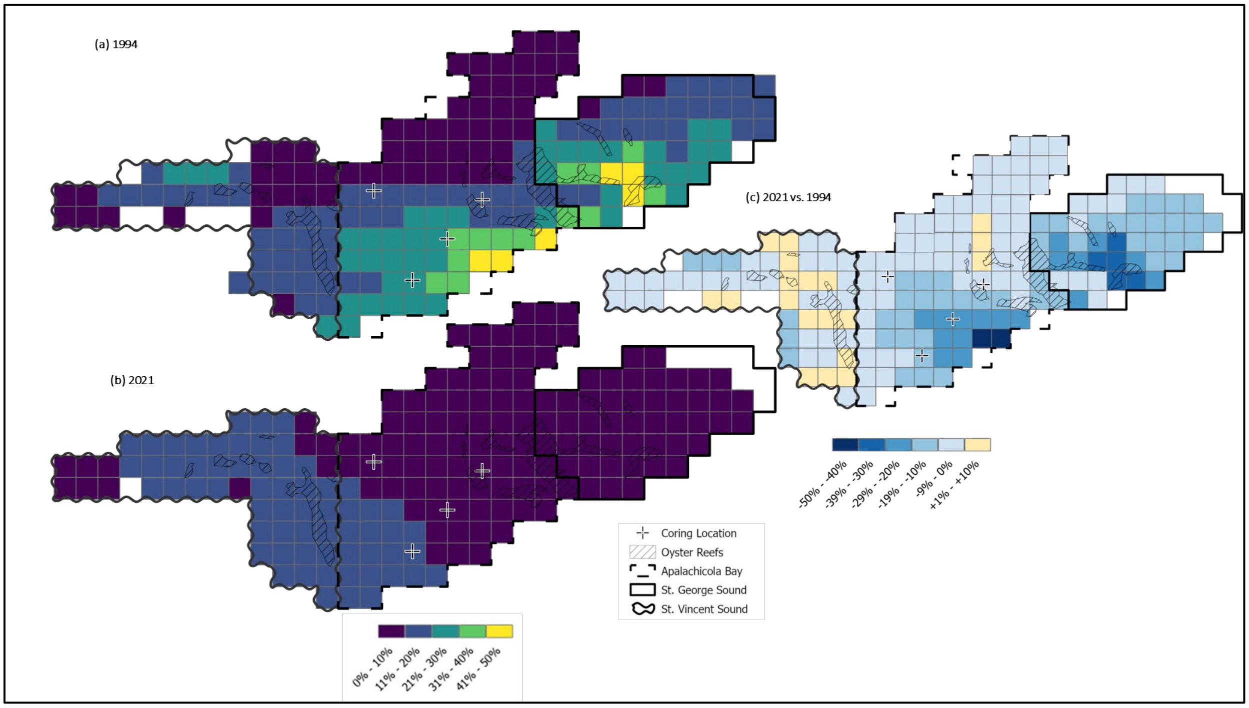


Figure 24. a, b) Heat maps identifying sediment calcium carbonate content ($CaCO_3$; %) in 1994 and 2021

(no $CaCO_3$ measurements were conducted in the 1959 study). **c)** Heat map identifying percent change in $CaCO_3$ from 1994 to 2021.

Table 1. Spatial and temporal comparisons of variables measured in Apalachicola Bay (Entire Region) and each of the three subregions (St. Vincent Sound, central Apalachicola Bay, and St. George Sound) in 1959, 1994, and/or 2021. Different trailing capital letters indicate significant differences ($p<0.05$) between years for a given variable by region; different trailing lowercase letters indicate significant differences between sub-regions within a year for a given variable.

Variable	Year	Entire Region	Subregions		
			St. Vincent Sound (Western)	*Apalachicola Bay (Central)*	*St. George Sound (Eastern)*
OC (%)	*1959*	0.66(0.92)†A	1.00(0.00)aA	1.25(0.63)aA	0.50(0.50)bA
	1994	2.38(1.03)B	2.88(0.80)aB	2.34(0.95)aB	2.00(0.99)aB
	2021	2.21(1.93)B	2.08(1.62)abB	2.61(1.74)aB	1.21(1.65)bAB
TN (%)	*1959*	0.07(0.05)†A	N/A	N/A	N/A
	1994	0.21(0.08)B	0.26(0.09)aA	0.22(0.06)aA	0.18(0.08)aA
	2021	0.22(0.19)B	0.22(0.16)abA	0.25(0.18)aA	0.13(0.18)bA
OC:TN	*1959*	12.70(6.55)†AB	N/A	N/A	N/A
	1994	13.10(1.34)A	13.13(0.50)aA	12.82(1.72)aA	13.15(0.91)aA
	2021	11.20(1.97)B	10.91(1.05)bB	12.05(2.58)aA	10.83 (1.02)bB
$CaCO_3$ (%)	*1959*	20.00(10.00)A	10.00(10.00)aA	10.00(10.00)aA	20.00(10.00)bA
	1994	N/A	N/A	N/A	N/A
	2021	9.48(5.30)B	9.68(5.93)aA	10.02(5.05)aA	7.16(6.18)aB
$\delta^{13}C$ (‰)	*1959*	N/A	N/A	N/A	N/A
	1994	-23.30(1.59)A	-22.26(0.84)bA	-23.85(2.00)aA	-23.05(0.68)abA
	2021	-24.00(1.72)B	-23.64(0.79)bB	-24.94(1.80)aB	-23.37(1.10)bA
$\delta^{15}N$ (‰)	*1959*	N/A	N/A	N/A	N/A
	1994	6.00(0.45)A	6.00(0.28)aA	6.00(0.41)aA	6.00(0.55)aA
	2021	5.33(0.92)B	5.38(0.40)aB	5.48(1.20)aA	5.05(0.53)aA

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 200% (Fig. 23a). From 1994 to 2021 this region-wide trend changed, with much of the region seeing a stabilization or decrease in sediment organic content; however, portions of the central bay and east bay continued to see increases of up to 100% (Fig. 23 d,e,f). Overall, the net trend was that over 95% of the Bay saw an increase in sediment OC for the whole period. Conversely, much of the region indicates a loss of $CaCO_3$ in the surface sediments from 1994 to 2021 (Fig. 24). While some of this may be due to dissolution, it is more likely that this represents a combination of decreasing oyster shell production and increasing burial of residual shell material by sediments.

6.1.1b. Organic Enrichment Stage 2:

Introduction. The second stage of this work focused on hot-spots of change identified in the first stage of the regional comparisons, and uses sediment cores to investigate the stratigraphic record of soil composition. The advantage of the first stage of this study is that it compares surface sediment composition at known points in time. However, there are limitations to a comparison of data collected by different investigators using different methods, leading to uncertainty about the net results of those observations when only three broadly separate time points are known.

Methods. To further examine the question of organic enrichment of the Bay including the timing and causes of the changes, four sediment cores were collected in areas identified as representing the greatest amount of change between 1963 and 1994 (Fig. 22a). These cores were sectioned in 1 cm intervals and radiometrically dated with ^{210}Pb (Fig. 25). Additionally, each core has been analyzed for nutrient content, stable isotopic composition, and a suite of lignin oxidation products (LOPs) to determine if downcore changes can be attributed to source or degradation changes.

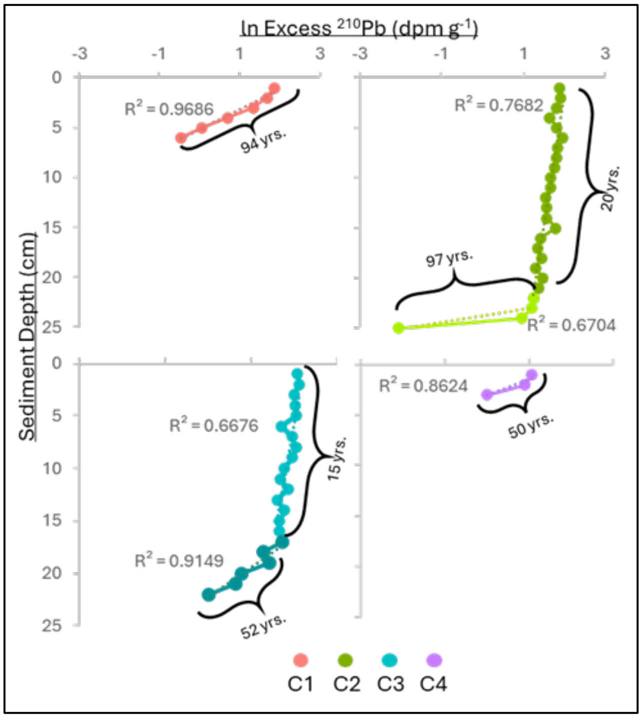


Figure 25. Natural log (ln) of excess ^{210}Pb activities (dpm g^{-1}) by sediment depth (cm) for cores 1 – 4, along with model-derived lengths of time for each profile segment.

Results and discussion. Figure 25 identifies an important spatial difference in the cores collected from the Bay. Cores 1 and 4, which were collected from outside the central Bay, represent very slow rates of sediment accumulation, whereas cores 2 and 3 both show very rapid rates of sediment accumulation in the last 15-20 years. This analysis is ongoing, but it indicates that sedimentation rates in the central Bay underwent a drastic change within the last decade and a half. Figure 26a supports the general findings of the stage 1 research that OC content in the Bay has increased in the past half century. Ratios of OC:TN have decreased in all four cores, most likely indicating post-depositional N loss and not a change in source material. Interestingly, the δ^{13}C values are mostly stable for cores 1-3 over the observed record, but there is a strong indication of greater contribution from marine-derived OC in core 4, which also saw a steady increase in total phosphorus and $CaCO_3$. Though core 4 has values considerably lower than those of cores 1-3, rates of change in core 4 are higher than the other cores. Lignin oxidation products have been collected for each of these cores and analysis is ongoing; these results will be crucial for differentiating the processes driving change in the organic matter abundance and composition of these cores.

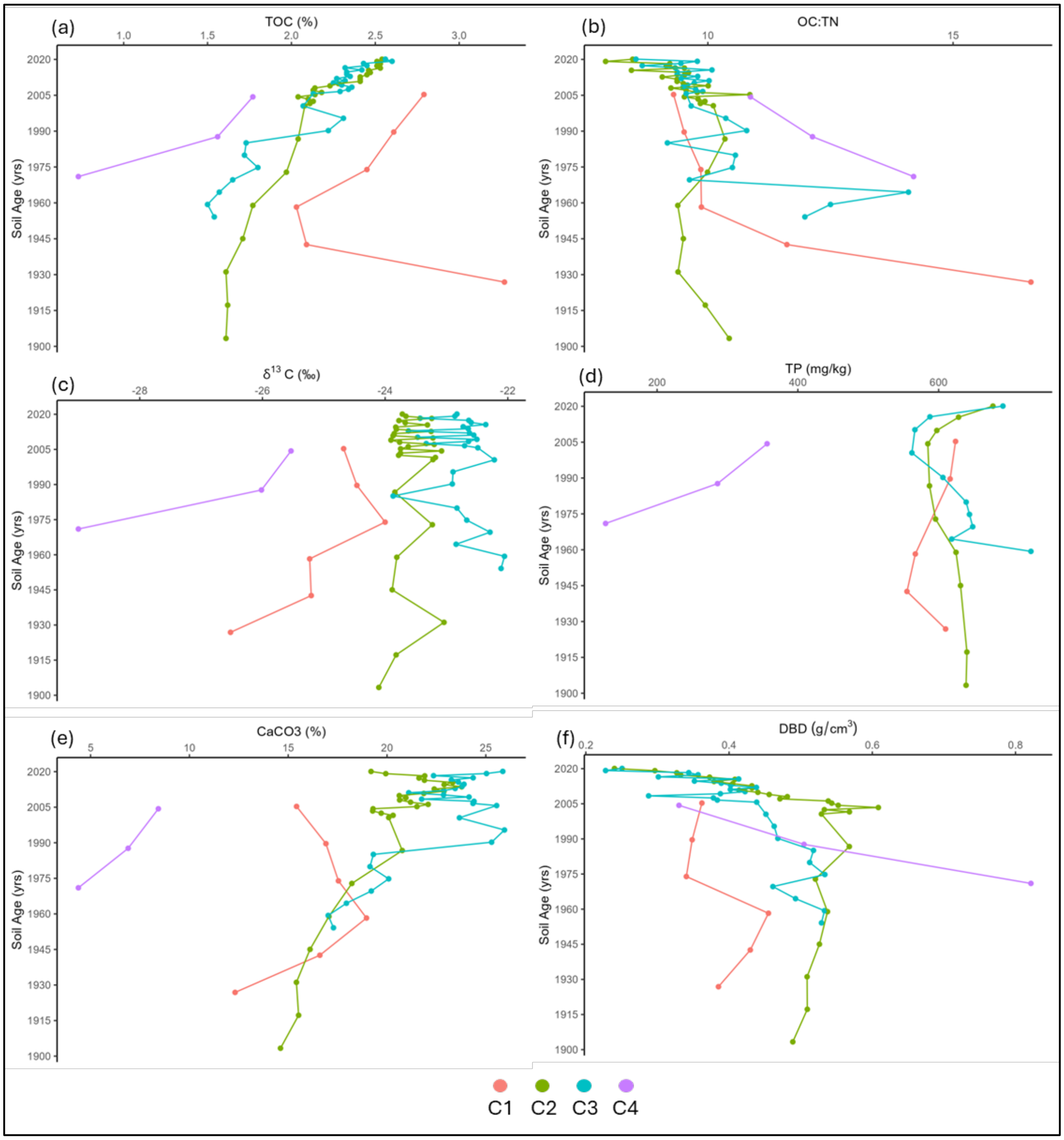


Figure 26. Stratigraphic profiles by age of cores 1 – 4 for **a)** total organic carbon (%), **b)** the elemental ratio of organic carbon to total nitrogen, **c)** stable isotopic composition of organic carbon (δ^{13}C), **d)** total phosphorus (mg kg^{-1}), **e)** calcium carbonate (%), and **f)** dry bulk density (g cm^{-3}).

Project Timeline: This manuscript is currently in preparation and the graduate student leading this project (Engelbert) is expected to defend his MS Thesis during the Summer 2024 semester.

6.2. Assessing Intertidal Oyster Reef Condition (Dr. Josh Breithaupt, Faculty, FSUCML)

***6.2.1.* 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 much less is known 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 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 study is to collect data about oyster cluster characteristics and reef sediment composition of intertidal reefs in Franklin County to understand their variability and make comparisons to other regions where intertidal reef monitoring and restoration has occurred. The research questions of this work are as follows: 1) What is the condition of intertidal oyster reefs across the region? 2) Do spatial density and size characteristics of oyster clusters affect sediment physical and chemical properties? 3) How does the condition of Franklin County reefs compare to the dead and restored intertidal reefs elsewhere in Florida? 4) Does the origin of organic matter (i.e., terrestrial detritus vs. marine phytoplankton) deposited on intertidal oyster reefs vary across the region?

Field Methods. Five major intertidal reef complexes were selected to be sampled across Franklin County (Fig. 27). Field sampling was conducted during June – August, 2023. Six reefs within each site were selected, 5 of which were already the focus of quarterly monitoring by the Apalachicola Bay System Initiative (ABSI), and one additional reef that was selected to increase the number of reef replicates within each site. Because ABSI does not sample Pilot's Cove, six reefs were haphazardly selected based on satellite images from Grizzle et al. (2018). Six 1 m^2 quadrats were deployed on each reef (n=36 quadrats per site; n=180 total quadrats). One quadrat was placed at the highest point of the reef and four additional quadrats were placed five to ten paces away in an X shape at approximately equivalent elevations. A sixth quadrat was tossed haphazardly on the reef. GPS coordinates were recorded for all quadrats. Within each quadrat, the cluster height and two perpendicular horizontal measurements (length and width) were recorded for up to ten clusters. Volume was calculated as length multiplied by width multiplied by height. This is a slight overestimation of cluster volume as they are not perfectly cubic but allows rough size estimation for comparisons. Burial depth was measured as the height from the bottom of the cluster to where it previously met the reef surface for up to 10 clusters per quadrat. When more than 10 clusters were present, the number of clusters up to 50 individuals was counted but not measured, in order to calculate average spatial density. This numerical limitation was due to the difficulty of counting clusters when the tide covered the reef, as it often did during the times of our field work. Sediment cores were collected at the four quadrats (n=24 cores per site) surrounding the center by driving a polycarbonate tube (7cm diameter, 15cm length) into the reef and extruding sediments into pre-weighed plastic bags in the field.

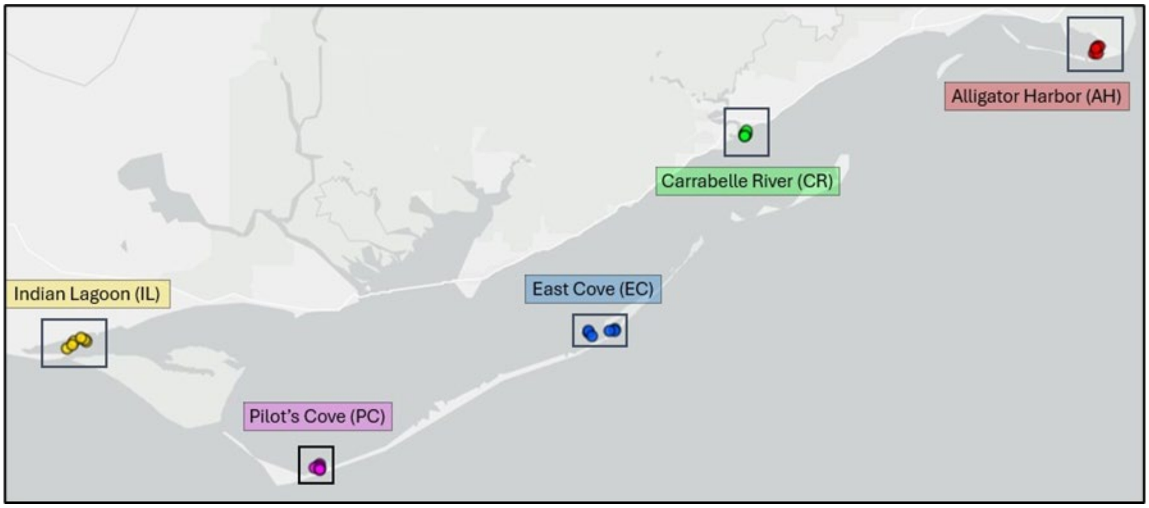


Figure 27. Location of five intertidal reef complexes (colored squares) and six reefs (circles within squares) across Franklin County that were sampled for this study.

Lab Methods. Upon return to the lab, all cores were freeze-dried then weighed for dry weight. Dry bulk density was calculated as dry mass divided by core volume. Material from each core was separated into four size classes. Large shell fragments (>2cm) were removed by hand and the remainder of the sample was mechanically sieved into three sequential size classes using a shaker: fine shell (>710µm), sands

(>63µm), and fine material (<63µm). Total dry mass of each size fraction was recorded.

Previous data collected during a preliminary study revealed that all size fractions greater than the fine sediments had negligible organic matter and radionuclide content, so for this project only the fine fraction was used for measuring organic carbon, nutrients, stable isotopes, and radionuclides. Organic matter content will be determined using loss-on-ignition by combusting samples in a muffle furnace at 550°C for 3 hours. Samples will then be analyzed for total phosphorus content via acid dissolution followed by colorimetric analysis (EPA 365.1) using a SmartChem 200 Wet Chemistry Analyzer. Organic carbon and total nitrogen will be measured at the University of Florida's Light Stable Isotope Mass Spec Lab.

The origin of organic material deposited on intertidal oyster reefs is being investigated through stable isotope analysis of sediment organic carbon and nitrogen. One factor that is thought to have contributed to the 2012 oyster population collapse was a series of severe droughts that reduced the flow of the Apalachicola River, potentially limiting organic material brought into the bay from the floodplain (Camp et al., 2015). Depending on the findings of this study, identifying reefs influenced predominantly by marine waters and the organics within them may help identify sites for restoration that will be more resistant to the adverse effects of droughts.

Results and discussion. Figure 28 shows the first set of results comparing oyster cluster characteristics on reefs in the region. The Pilot's Cove site has significantly more clusters per square meter than the other sites, but overall cluster volume, height, and burial depth is significantly lower (Fig. 28). Conversely, Alligator harbor has relatively lower spatial density of oyster clusters, but the clusters are larger, taller, and have the greatest burial depth (tied with the Carabelle River oysters). Burial depth of oysters could vary by two primary factors – rates of sediment accumulation or amount of movement or disturbance of the clusters. The burial depth of oyster clusters at Pilots Cove averages less than 1 cm, and when combined with the small size indicates that these oysters are less physically stable. This may be due to reworking and tumbling by wave action, but the small size and the isolated location of this site also suggest that human interference such as illegal oyster harvesting, may be a factor. The deeper burial and larger clusters sizes at Alligator Harbor suggest that there is less physical interference of these oysters, allowing them to grow and aggregate in greater cluster sizes than the other sites.

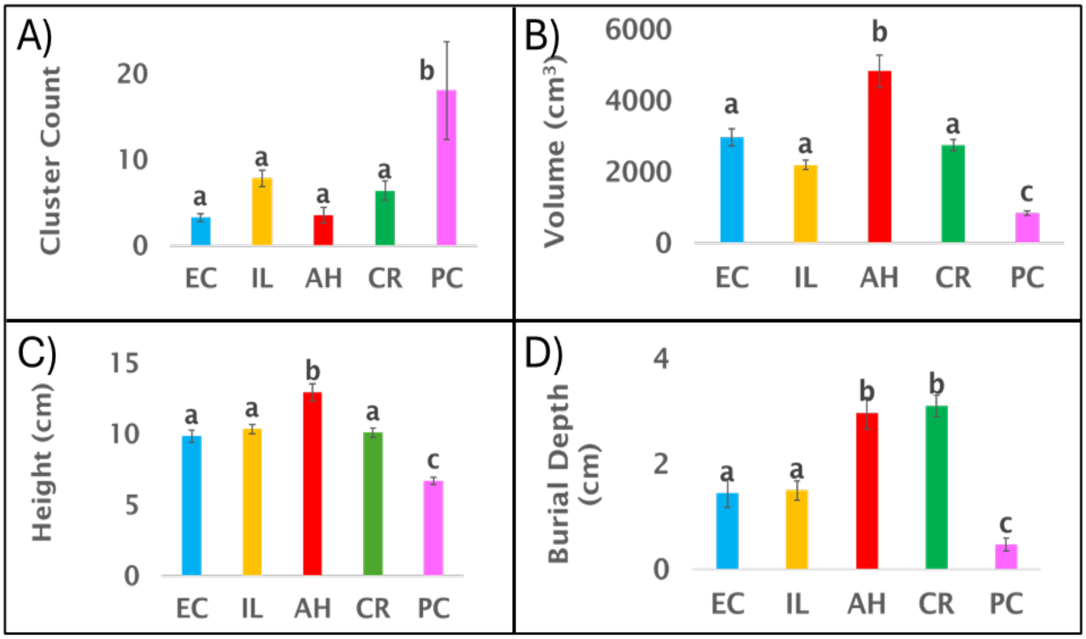


Figure 28. Mean (± 1 SE) values for oyster cluster characteristics: **A)** number of clusters per m^2, **B)** cluster volume (cm^3), **C)** total height (cm), and **D)** depth of burial in reef sediments (cm). Different lowercase letters within each panel indicate significant difference ($p < 0.05$).

Much of the work analyzing the sediment characteristics of these reefs is ongoing so results are relatively limited at this point. However, Figure 29 shows that reef sediment density is roughly 30% greater at the Carabelle River and Pilots Cove sites. Factors that influence sediment density include grain size (analyses ongoing), and disturbance or reworking of sediments. We hypothesize that a healthy reef with abundant, functional oysters will have lower sediment density facilitated by presence of infauna associated with abundant oyster bio-deposits in the sediments. However, more data are needed to fully evaluate this as initial results do not support this hypothesis. Carabelle River has high bulk density but also has some of the highest organic matter. It will be important to identify whether this organic matter is allochthonous material deposited on the reef by the river or whether it represents oyster bio-deposits, and we expect to test this using stable isotopes and nutrient ratios.

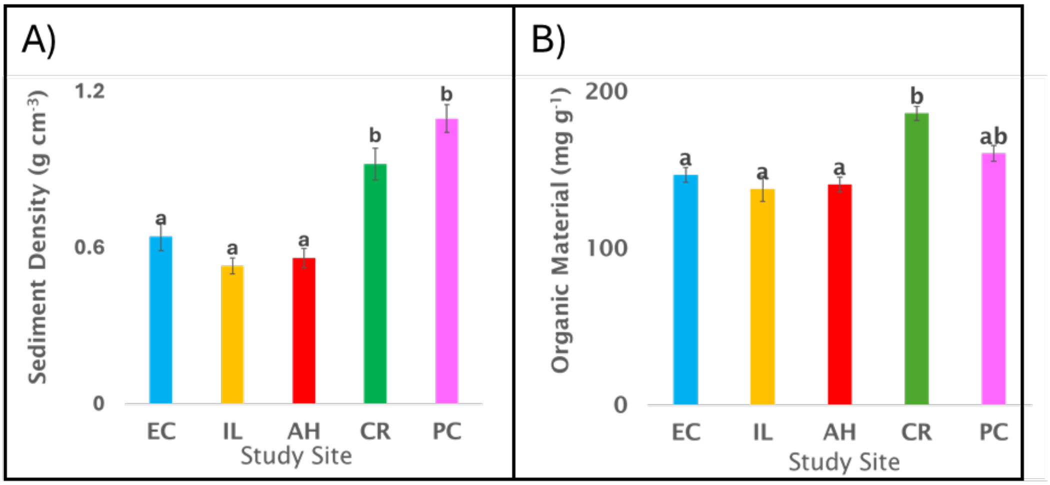


Figure 29. Mean (± 1 SE) values for reef sediment characteristics: **A)** bulk density (g cm^{-3}), and **B)** organic matter content (mg g^{-1}). Different lowercase letters in each panel indicate significant difference ($p < 0.05$).

Project Timeline: This project is being led by undergraduate student Erin Tilly as part of her Honors in the Major Thesis. We expect all field and lab analyses to be completed by end of summer 2024 and Erin will graduate from FSU in Spring 2025.

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

7.1. Hatchery accomplishments in 2023-2024.

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. Successful production of live algae also improved feeding techniques for larvae, which was a hatchery goal from 2021.

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.

7.1.1. 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 ABSI shellfish hatchery to optimize cost and time spent rearing shellfish.

7.1.2. 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.

7.1.3. 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.

8. Outreach and stakeholder engagement

ABSI outreach continued with gusto in 2023. The Community Advisory Board wrapped up with the finalization of its Apalachicola Bay System Ecosystem-Based Adaptive Restoration and Management Plan (Final version: https://marinelab.fsu.edu/media/5894/apalachicola-bay-system-ecosystem-based-adaptive-restoration-and-management-plan.pdf) and its Outreach and Education subcommittee continued working to engage and inform local stakeholders about the Plan. The CAB's Successor Group became a new organization, The Partnership for a Resilient Apalachicola Bay. The bi-monthly ABSI newsletter subscriptions continued to increase, and ABSI was represented at a variety of events throughout the Tallahassee and Florida panhandle region. Finally, new additions to ABSI's website including a "Key Points" document; an interactive "What We Do" tool, and a shell recycling program review and database continued to help foster engagement within the local community.

8.1 An interactive map of ABSI-related research (Dr. Tara Stewart Merrill, Dr. Sandra Brooke)

Introduction: ABSI encourages communication regarding the project's reearch and restoration efforts. To enhance communication, we designed and launched a new public interactive graphic. The 'ABSI Knowledge Network' shows the research projects conducted by ABSI (and collaborators) and displays how the projects link back to the oyster life cycle and the importance of oysters for Apalachicola Bay.

Methods: We first distilled the research being conducted by ABSI into four core areas that provided the basic framework for the figure: ***1)*** Oyster larval supply; ***2)*** Oyster spat settlement; ***3)*** Oyster post-settlement growth, survival, and reproduction; and ***4)*** The ecological and societal impacts of oysters. We then asked for information from all the scientists directly involved in ABSI research, as well as scientists at the FSUCML who were doing research related to oysters and the health of Apalachicola Bay. More specifically, we asked asked each scientist to provide: ***1)*** information on the name of their research project; ***2)*** which of the four core areas their research project linked to (and we allowed projects to link to more than one area); ***3)*** the name of the personnel involved in the research, and ***4)*** the key questions being investigated by the research project. Responses were then consolidated into documents and spreadsheets to populate the figure. Tara Stewart Merrill designed the draft figure with feedback from Sandra Brooke and ABSI faculty. Further design, production, and online publication was conducted by Kevin John, Eric Clark, and Sarita Finn of the FSU MagLab.

Results: The new interactive graphic was officially launched on the ABSI website in September 2023 at https://marinelab.fsu.edu/absi/what-we-do/. Users visiting the tool can hover over a core area to see which research projects are related to that area. Similarly, users can hover over a research project to see which core areas it connects to. If a user clicks on a research project, an information card pops up that specifies the scientists involved in the project and the key questions being investigated by the project. The graphic can be considered as a blueprint of our science and showcases the diversity of projects being conducted by ABSI. Figure 30 shows the structure of the graphic, which is interactive when viewed on the website.

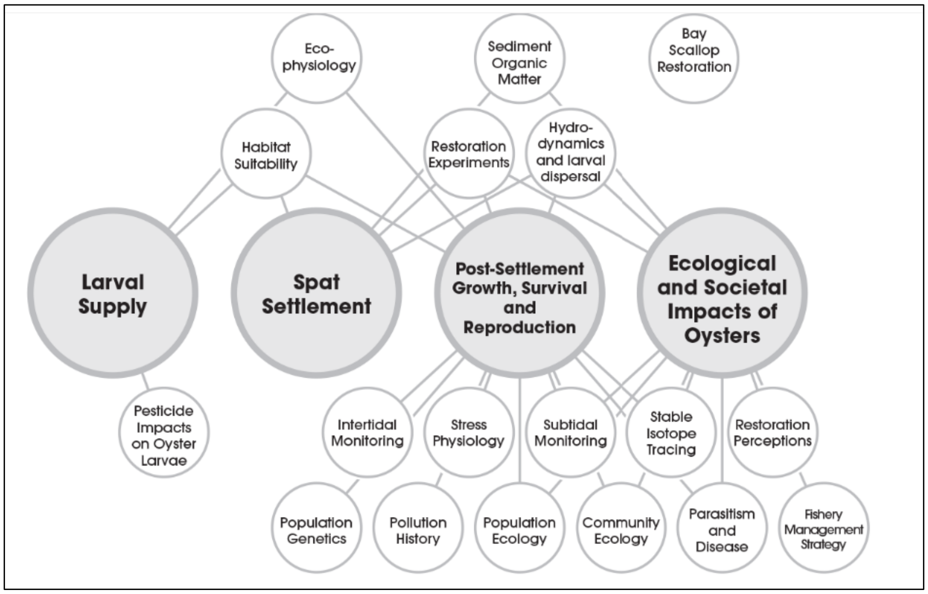


Figure 30. Graphic of the ABSI Knowledge Network. Large circles represent the four main research areas and small circles represent individual projects. In the interactive online version, connections are color coded to show linkages and background information is being developed for each project.

8.2 Community Advisory Board

The Community Advisory Board (CAB), facilitated by Jeff Blair (Facilitated Solutions), concluded on Wednesday, November 29th. The overarching objective of the CAB was 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, some Board members stepped down and were replaced with members from similar stakeholder organizations.

8.2.1 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; Grayson Shepard – Hang On Fishing Charters

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

8.2.2 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/)

April 12, 2023 presentations:
1) CAB Phase IV Workplan Update April 12, 2023 (J Blair, Facilitated Solutions, LLC)
2) Shiny App Overview (F Caltabellotta, FSU)
3) FWC and NFWF Restoration Update (D Resko, FWC)
4) ABSI Modeling Hydrographic Modeling Update (S Morey, FAMU; X Chen, FSU)

May 31, 2023 presentations:
1) CAB Phase IV Workplan Update May 31, 2023 (J Blair, Facilitated Solutions, LLC)
2) ABSI Science Update May 31, 2023 (S Brooke, FSU)
3) FWC and NFWF Restoration Phase II Update (D Resko, FWC)
4) Baysavers Group Presentation (D, May, Baysavers)

August 9, 2023 presentations:
1) CAB Phase IV Workplan Update August 9, 2023 (J Blair, Facilitated Solutions, LLC)
2) ABSI Science Update August 9, 2023 (S Brooke, FSU)

3) FWC and NFWF Restoration Phase II Update (D Resko, FWC)

September 27, 2023 presentations:
1) CAB Phase IV Workplan Update September 27, 2023 (J Blair, Facilitated Solutions, LLC)
2) ABSI “What We Do” Media Presentation (T Stewart Merrill, FSU)
3) FWC and NFWF Restoration Phase II Update (D Resko, FWC)

November 29, 2023 presentations (Last meeting of the CAB):
1) CAB Phase IV Workplan Update November 29, 2023 (J Blair, Facilitated Solutions, LLC)
2) FWC and NFWF Restoration Phase II Update (D Resko, FWC)
3) ABSI Science Update November 29, 2023 (S Brooke, FSU)

Three CAB subcommittees 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 31.

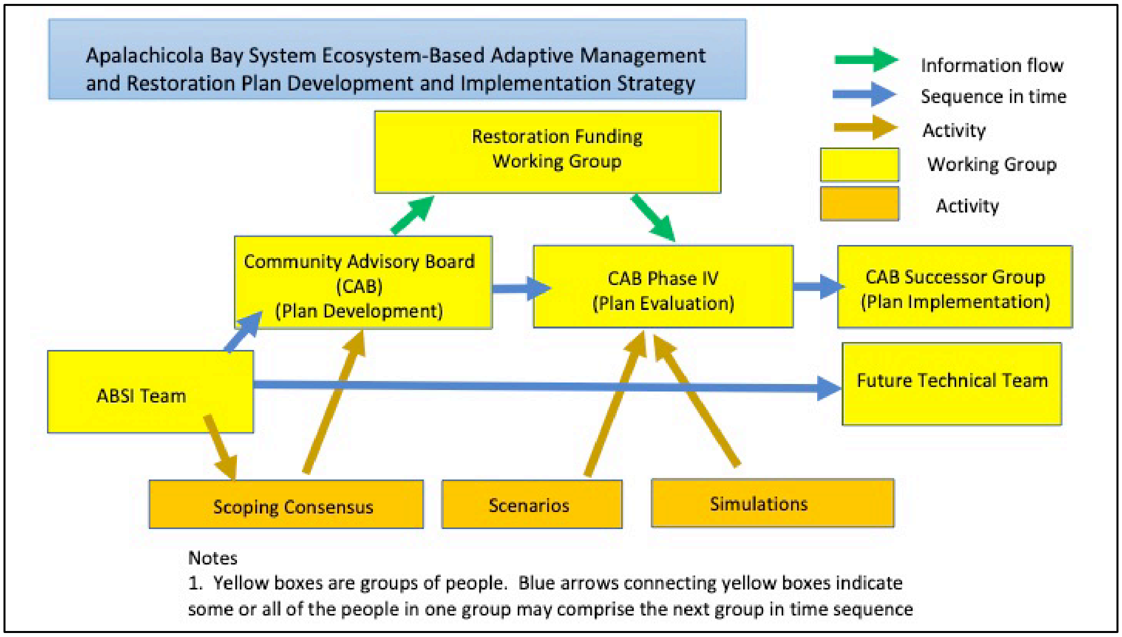


Figure 31. Schematic showing structure, timing and information flow of the community advisory board and subcommittees, and the ABSI science team

8.2.3 Outreach and Education Subcommittee (Madelein Mahood, FSUCML)

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

Membership:

FSU: Dr. Sandra Brooke, Post-Doctoral Fellow Dr. Betsy Mansfield, and Outreach and Education Coordinators Jared Fuqua and Maddie Mahood
ABSI CAB: Chad Hanson (Chair), The Pew Charitable Trusts; Georgia Ackerman and Cameron Baxley, Apalachicola Riverkeeper; Anita Grove, Apalachicola City Commissioner; and Devin Resko, Florida Fish and Wildlife Conservation Commission

***Subcommittee meeting dates*:**
March 22, 2023; April 28, 2023; July 11, 2023; September 6, 2023; October 16, 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 562 subscribers, a **7.9%** increase from March 2023. Over the three newsletters sent between April 2023 and November 2023, the open rate per email averaged **64%,** 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/
- Continuation of a media distribution plan for the ABSI newsletter and additional updates:
    - Every ABSI update and newsletter are posted on Florida State University Coastal and Marine Laboratory's website and social media outlets: Facebook (@FSUCML), Twitter (@FSUMarineLab), LinkedIn @FSUCML, 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 "Key Points" document – this is a one-page document that outlines the main goals of ABSI: https://marinelab.fsu.edu/absi/faqs-and-key-points/key-points/
- Development of in-person public workshops and participation in outreach events throughout the community (see ***Public Outreach***)
- Creation of "The Plan" PowerPoint presentation – an easily digestible presentation covering the main goals and strategies of the Restoration and Management Plan. https://marinelab.fsu.edu/media/5821/4-harper-absi-cab-plan-summary_24-october-2023.pdf
- Creation and distribution of the Plan Summary Report. This is an 8-page "glossy" document that outlines the Plan in an accessible and abbreviated manner. This Summary has been sent to every Community Advisory Board member, several members of FWC and DEP, the Franklin County Commissioners, and the Apalachicola City Commissioners, and has been distributed at various festivals and events in the greater Franklin County area. The Summary "Plan" can be viewed here: https://marinelab.fsu.edu/media/5901/the-plan-glossy-version.pdf
- Coordination and distribution of the Florida State University press release about the finalization of the Restoration and Management Plan. View the press release here: https://news.fsu.edu/news/university-news/2024/03/05/an-ecosystem-roadmap-apalachicola-bay-system-initiative-plan-is-guide-to-sustainable-fishery/

8.3 Public outreach and engagement

In the aftermath of the pandemic, 2023 had a wide range of different events including community workshops, public presentations, school groups, tours, field trips, and festivals. As a result, over the last year, ABSI engaged with over 23,800 individuals through a variety of events.

8.3.1 Targeted community outreach

After seeing the benefit of creating an open forum for local watermen and concerned citizens to engage with ABSI staff and researchers one-on-one, ABSI hosted three more Community Workshops on April 12, 2023, August 9, 2023, and October 24, 2023. All three workshops were held in person at the Apalachicola National Estuarine Research Reserve (ANERR). Dr. Sandra Brooke also presented to the Franklin County Commission on October 17, 2023 (postponed) and March 19, 2024 (forthcoming). Additionally, Dr. Brooke gave a targeted seminar about the progress of ABSI and hopes for future studies in the Bay during ANERR's SciCafé series on November 16, 2023.

April 12, 2023 Community Workshop:

Summary: At the April 12, 2023 Community Workshop Forum the Apalachicola Bay System Initiative (ABSI) Community Advisory Board (CAB) conducted the first of three Community Workshop Forums planned for Phase V (2023) of the ABSI project. The Community Workshop Forum was convened for the purpose of seeking public feedback on Restoration Approaches, Management Strategies, and ABSI Science including a computer and monitor showing a map of FSUCML's ABSI restoration experiments. The Workshop was conducted at the Apalachicola National Estuarine Research Reserve (ANERR) in Eastpoint, Florida.


April 12th Community Workshop

During the Workshop, Community participants were provided an overview of the ABSI Project Workplan and Schedule and were provided opportunities to move among the tables and ask questions, and provide feedback on ABSI restoration approaches, management strategies, and ABSI science. 20 citizens with no affiliation to ABSI, FSU, or the CAB attended.

ABSI Representation: Sandra Brooke, ABSI Principal Investigator; Jon Creamer, FWC; Jared Fuqua, ABSI Outreach and Education; Anita Grove*, Apalachicola City Commission; Shannon Hartsfield*, SMARRT Group; Betsy Mansfield, FSU Post-Doctoral Fellow; Devin Resko*, FWC; Grayson Shepard*, Hang on Charters Fishing; Joel Trexler, ABSI Principal Co-Investigator and Director of FSUCML; and W. Ross Ellington, ABSI Partner
Facilitated Solutions, LLC: Jeff Blair
*CAB member

August 9, 2023 Community Workshop Presentations:

Summary: On August 9, 2023, the Apalachicola Bay System Initiative (ABSI) Community Advisory Board (CAB) conducted the second of three Community Workshop Forums planned for Phase V (2023) of the ABSI project. The Community Workshop Forum was convened for the purpose of seeking public feedback on Restoration Approaches, Management Strategies, and ABSI Science including a computer and monitor showing a map of FSUCML's ABSI restoration experiments. The Workshop was conducted at the Apalachicola National Estuarine Research Reserve (ANERR) in Eastpoint, Florida.

During the Workshop, Community participants were provided an overview of the ABSI Project Workplan and Schedule and were provided opportunities to move among the tables ask questions and provide feedback on ABSI restoration approaches, management strategies, and ABSI science. 23 citizens with no affiliation to ABSI, FSU, or the CAB attended.

ABSI Representation: Georgia Ackerman*, Apalachicola Riverkeeper; Ottice D. Amison*, Franklin County Commission; Sandra Brooke, ABSI Principal Investigator; Jon Creamer, FWC; Jared Fuqua, ABSI

Outreach and Education; Anita Grove*, Apalachicola City Commission; Shannon Hartsfield*, SMARRT Group; Betsy Mansfield, FSU Post-Doctoral Fellow; Landen Millender, ABSI Hatchery Technician; Devin Resko*, FWC; Barry Walton, FSUCML Ph.D. Student; Charlie Wood, FWC LE.
Facilitated Solutions, LLC: Jeff Blair
*CAB member

October 24, 2023 Community Workshop Presentations:

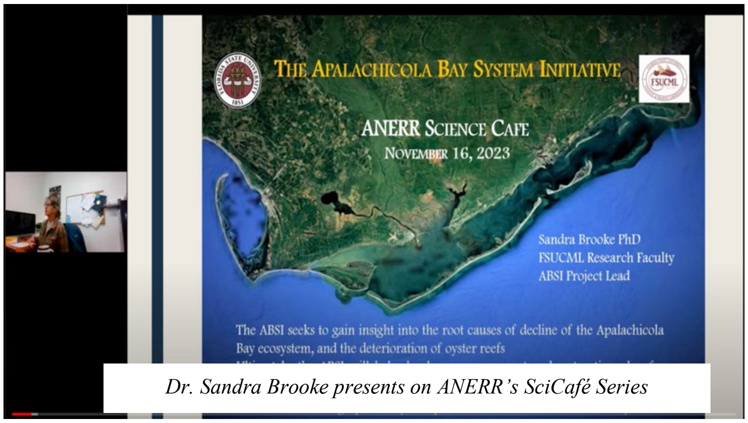
1) CAB Phase IV Workplan Update October 24, 2023 (J Blair, Facilitated Solutions, LLC)
2) ABSI Science Update (S Brooke, FSU)
3) The Apalachicola Bay System Restoration and Management Plan (J Harper, ANERR)
3) FWC and NFWF Restoration Phase II Update (D Resko, FWC)

Summary: At the October 24, 2023 Community Workshop Forum the Apalachicola Bay System Initiative (ABSI) Community Advisory Board (CAB) conducted the third of three Community Workshop Forums planned for Phase V (2023) of the ABSI project. The Community Workshop Forum was convened for the purpose of providing information on ABSI restoration experiments and seeking public feedback on the *CAB's Draft Report and Recommendations for the Apalachicola Bay System Ecosystem-Based Adaptive Management and Restoration Plan* (Plan). The Workshop was conducted at the Apalachicola National Estuarine Research Reserve (ANERR) in Eastpoint, Florida.

During the Workshop, Community participants were provided an overview of the ABSI Project Workplan and Schedule, an update on ABSI restoration experiments, and a summary of the CAB's Report and Recommendations for the Plan. Following the presentations participants were provided with opportunities to move among three information stations and ask questions and provide feedback on: 1) the CAB's recommendations for management and restoration strategies for the Plan; 2) ABSI science and restoration experiments; and 3) FWC fisheries management and restoration projects. 7 citizens with no affiliation to ABSI, FSU or the CAB attended.

ABSI Representation: Emily Albetta, FWC; Sandra Brooke, ABSI Principal Investigator; Jon Creamer, FWC; Jared Fuqua, ABSI Outreach and Education; Anita Grove*, Apalachicola City Commission; Jenna Harper*, ANERR; Gayle Johnson*, Indian Lagoon Oyster Company; Madelein Mahood, ABSI Outreach and Education; Betsy Mansfield, FSU Post-doctoral Fellow; Devin Resko*, FWC and W. Ross Ellington, ABSI Partner
Facilitated Solutions, LLC: Jeff Blair
*CAB Member

November 16, 2023 – ANERR SciCafé Series
Summary: Principal Investigator and FSU Marine Lab Faculty Dr. Sandra Brooke gives an update on the progress of the Apalachicola Bay System Initiative and the results of their restoration research and experiments.
Video Available Here:
https://youtu.be/71aCGZcz4eI?si=DGvsp_d1ofS-vYGC



THE APALACHICOLA BAY SYSTEM INITIATIVE

ANERR SCIENCE CAFE
NOVEMBER 16, 2023

Sandra Brooke PhD
FSUCML Research Faculty
ABSI Project Lead

The ABSI seeks to gain insight into the root causes of decline of the Apalachicola Bay ecosystem, and the deterioration of oyster reefs

Dr. Sandra Brooke presents on ANERR's SciCafé Series

8.3.2 School Groups

A school group looking for oysters in intertidal environment at the FSUCML.

ABSI has greatly increased its outreach within public schools and homeschool collectives in Franklin, Wakulla, and Leon Counties, as well as out-of-state groups from South Carolina and Georgia. 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. We hope to promote the science of the Bay and the importance of a healthy ecosystem through fun and engaging programs. Since April of last year, we have reached 52 different school and university groups. In total, we have engaged with more than 1,700 students (ages 5 – 24).

8.3.3 Festivals

These events are of a larger scale in which the goal is 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. In the past year, we have reached more than 23,000 people through these opportunities and expect that number to continue to rise in the coming months.

- 03/21/2023: FSU Day at the Capitol (Tallahassee)
- 03/22/2023: FIO Oceans Day at the Capitol (Tallahassee)
- 03/25/2023: Panacea Beer and Oyster Fest (Panacea)
- 04/01/2023: FAMU STEM Day (Tallahassee)
- 04/08/2023: Worm Gruntin' Festival (Sopchoppy)
- 04/22/2023: Carrabelle Riverfront Festival (Carrabelle)
- 05/05/2023: ANERR's Estuaries Day (Eastpoint)
- 05/06/2023: 11th Annual Autism OdysSea (Navarre Beach)
- 08/25/2023: Involvement Fair (Tallahassee)
- 09/30/2023: Blue Crab Festival (Panacea)
- 09/30/2023: Tallahassee Science Festival (Tallahassee)
- 10/21/2023: University of Florida's Open House (Cedar Key)
- 11/03/2023 – 11/04/2023: Florida Seafood Festival (Apalachicola)
- 11/11/2023: Sopchoppy Oyster and Mullet Festival (Sopchoppy)
- 02/01/2024: FSU Day at the Capitol (Tallahassee)
- 02/06/2024: FIO's Oceans Day at the Capitol (Tallahassee)
- 02/24/2024 MagLab Open House (FSU, Tallahassee)

FSUCML Outreach Coordinators Jared Fuqua and Maddie Mahood (middle) at the Apalachicola Seafood Festival.

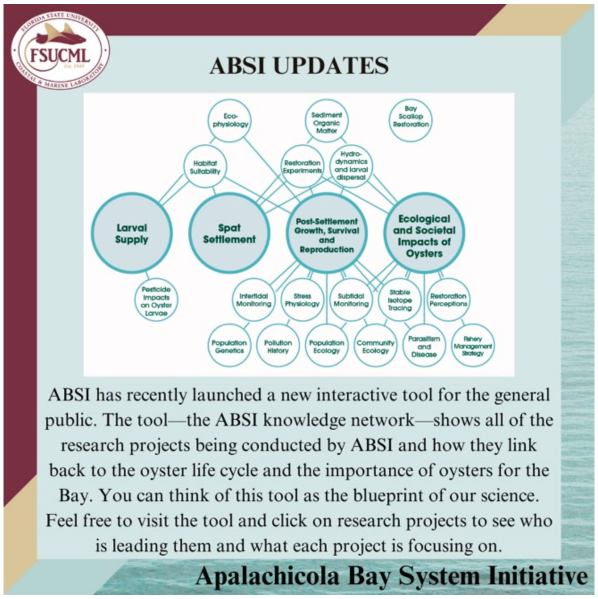
8.3.4 Free Friday FSUCML tours

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 April 2023, we had over 600 people take part in the tours.

Jared Fuqua speaks with a group in the ABSI shellfish hatchery.

8.3.5 ABSI Social Media

To continue engagement with the community, the ABSI team has continued to increase its social media efforts. ABSI continued with "FAQ" Mondays, where each Monday, a new "FAQ" from ABSI was published and shared across FSUCML social media accounts until October 2023. Additionally, ABSI continued to share weekly updates. Using this template to distinguish ABSI from regular FSUCML postings, the team strived to post 2-3 times a week showcasing the day-to-day activities of ABSI. Recent updates include – the "What We Do" interactive tool reveal and a call for people to donate shells at our shell bins. Examples of these posts are pictured above.



ABSI UPDATES

ABSI has recently launched a new interactive tool for the general public. The tool—the ABSI knowledge network—shows all of the research projects being conducted by ABSI and how they link back to the oyster life cycle and the importance of oysters for the Bay. You can think of this tool as the blueprint of our science. Feel free to visit the tool and click on research projects to see who is leading them and what each project is focusing on.

Apalachicola Bay System Initiative

ABSI UPDATES

ABSI Hatchery Technician Landen Millender emptying shell bins at Leonard's Landing near Alligator Harbor. Local farmers are donating oyster shell for restoration projects in Apalachicola Bay.

Apalachicola Bay System Initiative

8.3.6 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 include the "What We Do" Interactive Tool page (https://marinelab.fsu.edu/absi/what-we-do/), "Key Points" fact sheet (https://marinelab.fsu.edu/absi/faqs-and-key-points/key-points/), and oyster shell recycling review (https://marinelab.fsu.edu/absi/research/oyster-shell-recycling-review/).

8.3.7 Local News Coverage

The ABSI project continues to be featured in local news, however, as news is more commonly shared across social media pages, rather than formal blogs and paper mediums. Below is a list of articles and news segments from March 2023 – March 2024, but it is not exhaustive.

Garden and Gun Magazine – July 2023
OysterCatcher – July 2023
The Florida Channel – July 2023
Oyster Radio – September 2023
WCTV – October 2023
FSU Communications – December 2023
850 Business Magazine – December 2023
Friends of St. Vincent – February 2024
Florida State University News – March 2024
NewsWise – March 2024

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