## *Results*

## *Question 1: Oyster restoration response across Pensacola, St. Andrew, and Apalachicola bays*

The best fitting GLM model (Table 2; Appendix 2) suggests that oyster restoration response over time in each bay were different but none of the predicted responses suggest a positive response in counts of live oysters after restoration. For live spat in Apalachicola and St. Andrew bays, we found the coefficient of the slope describing trends in live oyster spat per quadrat over period did not differ from zero (p=0.96 and p=0.23) but the slope coefficient did differ from zero for Pensacola (p=0.0006) and this slope coefficient was estimated to be negative (beta coefficient = -0.39) and highly uncertain (SE = 0.11, 95% CI = -0.61-0.17). Predicted live oyster spat (marginal means) per quadrat for Apalachicola was 14.08 live spat (95% CI 5.29-37.45), Pensacola was 0.70 live spat (95% CI 0.16-3.14), and St. Andrew Bay predicted live oyster spat was 226 (95% CI 13.79-3703.89).

A similar pattern was observed for seed size oysters across bays with the slope coefficient not differing from zero for Apalachicola Bay (p=0.99) and St. Andrew Bay (p=0.68) but the slope coefficient did differ from zero for Pensacola Bay (p=0.02) and the slope suggested a decline in seed size oysters over time (beta = -0.34, SE = 0.14, 95% CI = -0.61 - -0.06). Counts of legal-size oysters were near zero in all bays (Figure 5) creating model convergence issues limiting further analyses of legal-size oysters (Appendix 2).

## *Question 2 how do oyster spat temporal trends vary among separate restoration projects in Apalachicola Bay?*

To examine trends in Apalachicola Bay oyster spat by project, we created a new variable (SP) which combined the site and project name. This allowed us to fit models to the data which nested site within project and allowed period to vary across project (Appendix 2). fit eight different models to the data (Table 2). To simplify nesting of site within project in our model structure (which would allow period to vary by site across project). Of the eight models fit to the data (Table 2, Appendix 2), the model which included terms for Period, Project, a nested period by SP term, and an interaction term between Period and Project while controlling for effort was the best fitting (Table 2). For three of the projects, GEBF05007, NRDA-4044, and NFWF-2021 the coefficient of the slope of live oyster spat counts over time (Period) did not differ from zero (p = 0.51, p= 0.51, p= 0.09) and for the NFWF-1 project the slope did differ from zero (p<0.0001) and this trend was negative (slope coefficient beta = -0.64, SE = 0.15, 95% CI = -0.94 - -0.35). These results demonstrate that none of the restoration projects in Apalachicola Bay have had the desired positive response over time to restoration.

We then predicted the marginal means of oyster spat from a single ¼-m2 quadrat in the last period of sampling for comparison purposes between each project using the best fitting model from Table 3. For the projects that used limestone rock, predicted live oyster spat for GEBF-5077 in period 12 was 15.73 live spat per quadrat (95% CI 8.45-29.27) and for project FWC-2021 in period 15 we predicted 119.03 (95% CI 30.88-458.82). For the projects that used shell cultch, for NRDA-4044 in period 13 we predicted 5.14 live oysters (95% CI 3.06-8.63), and for NFWF-1 we predicted in period 9 there were 5.39 live oyster spat (95% CI 1.20-24.26).

*Question 3 are oyster spat counts in Apalachicola Bay associated with freshwater discharge?*

We added coefficients describing different river discharge metrics to the best fitting model comparing live spat counts across project and time in Apalachicola Bay to see whether including river discharge information would improve mode fit (Table 3). These river discharge metrics include the number of days river discharge was below 12,000 CFS, days below 12,000 CFS lagged by 1 period, number of days river discharge was below 6,000 CFS and days below 6,000 CFS lagged by 1 period. Including these river discharge metrics did not improve model fit (Table 3) suggesting that the observed lack of positive response in live oyster spat was not influenced by river discharge metrics assessed.

*Question 4: Is cultch biomass related to the number of live oysters?*

Efforts to predict cultch biomass had little success (Appendix 2). Diagnostic assessments of model fitting to cultch biomass data suggested most models were overparameterized (Appendix 2). The best fitting model (lowest AICc and highest model weight) was for a model that did not include oyster spat as a parameter (Table 5). Simple plots of mean cultch weight (kg, x-axis) and total live spat (y-axis) per quadrat suggests that for the two studies monitored immediately following cultching (NFWF-1 and NFWF-2021) show that as the number of live spat increases, so does cultch biomass, but only for one or two periods (Figures 13 and 14) before the number of spat collapses and retracts toward the origin, even for the same biomass of cultch (Figure 14).

*Question 5: How does cultch material persist?*

The best fitting model comparing trends in oyster cultch biomass over Period across all three bays included an interaction term between Period and Bay suggesting a different response on oyster cultch biomass over time in each Bay. St. Andrew and Pensacola bays only received a single cultching treatment. For St. Andrew Bay the slope did not differ from zero (p=0.23) suggesting a non-significant trend in cultch biomass over time. In Pensacola Bay the slope did differ from zero (p=0.02) and the sign of the slope coefficient was negative (beta = 0.03) suggesting a negative trend in cultch biomass over time. Because Apalachicola Bay received multiple cultching treatments, we examined trends in cultch biomass in this bay using models similar to Question 2 which allowed for unique responses by Project over time. The FWC-2021 project did not have a significant slope parameter (p=0.44), but the other three projects the slope parameter did differ from zero (GEBF-5007 p=0.02; NFWF-1 p<0.0001; NRDA-4044 p=0.0002) and the slope was positive for GEBF-5007 but negative for NFWF-1 and NRDA-4044 (Appendix 2).

We then predicted the marginal means of oyster cultch biomass from a single ¼-m2 quadrat in the last period of sampling for each project for comparison purposes between projects. Predicted oyster cultch biomass for the NFWF-2021 project was 8.58 kg per ¼-m2 quadrat (Period 15, 95% CI 4.03-18.30); GEBF-5077 was 4.29 kg per ¼-m2 quadrat (Period 12, 95% CI 2.94-6.27); the NFWF-1 was 0.97 kg per ¼-m2 quadrat (Period 9, 95% CI .47-2.02); and NRDA 4044 predicted cultch biomass was 1.45 kg per ¼-m2 quadrat (Period 13, 95% CI 1.01-2.09).

***Appendix 2***

## *Question 1: Oyster restoration response across Pensacola, St. Andrew, and Apalachicola bays*

From the GLM models, the dispersion parameter from the negative binomial distribution ("nbinom2" family formulation) was <1 for all models, suggesting over-dispersion. The best fitting model for oyster spat (lowest AICcC value, highest AICcC weight) was the Period + Bay + (Period | Site) + Period:Bay + offset(log(Num\_quads)) (Table 2). Because site is uniquely coded, this model allows different responses by site over time in each bay. No autocorrelation in residuals was detected (K-S test p= 0.40; D-W test p = 0.18). The significant interaction term suggests that each bay's temporal patterns in oyster counts are unique. Apalachicola Bay live spat counts per quadrat declined (beta of the slope = -0.004, SE = 0.07, 95% CI = -0.15-0.14) and this trend was not significantly different from zero (p = 0.96). Pensacola and St. Andrew bays show uncertain trends in oyster spat counts. Pensacola coefficient values for the slope of oyster spat counts over time were larger than Apalachicola (beta = -0.39, SE = 0.11, 95% CI = -0.61-0.17) and this slope coefficient did differ from zero (p=0.0006). For St. Andrew Bay, the slope coefficient was highly uncertain (beta = 0.21, SE = 0.18, 95% CI = -0.14-0.57) and this slope did not differ from zero (p=0.23).

## *Question 2 how do oyster spat temporal trends vary among separate restoration projects in Apalachicola Bay?*

To examine trends in Apalachicola Bay oyster spat by project, we fit eight different models to the data (Table 2). To simplify nesting of site within project in our model structure (which would allow period to vary by site across project), we created a new variable (SP) which combined the site and project name. Creating the variable SP allows different responses by site over time in each project. The best fitting model for oyster spat (lowest AICcC value, highest AICcC weight) was the Period + Project + (Period | SP) + Period:Project + offset(log(Num\_quads)) (Table 3). Autocorrelation in residual results were mixed as the K-S test was not significant (K-S test p= 0.21) but the Durbin-Watson test was (D-W test p = 0.03) likely due to different numbers of sites sampled with each project. No adjustment was made. The trend in live oyster spat counts per quadrat over time did not differ from zero for projects GEBF-5007 (slope coefficient beta = -0.06, SE = 0.10, 95% CI = -0.26-0.13, p = 0.51), NRDA-4044 (slope coefficient beta = 0.04, SE = 0.07, 95% CI = -0.09-0.18, p = 0.51) or NFWF-2021 (slope coefficient beta = -1.04, SE = 0.60, 95% CI = -2.24-0.15, p = 0.09). For project NFWF-1, the trend in live oyster spat per quadrat was significantly different from zero (p<0.0001) and this trend was negative (slope coefficient beta = -0.64, SE = 0.15, 95% CI = -0.94 - -0.35.

*Question 3 are oyster spat counts in Apalachicola Bay associated with freshwater discharge?*

We then fit four additional models (Table 4) that compared the best fit model from Question 2 (Table 3), to models with terms describing the number of days river discharge was below 12,000 CFS, days below 12,000 CFS lagged by 1 period, number of days river discharge was below 6,000 CFS and days below 6,000 CFS lagged by 1 period (Table 3). Including these river discharge metrics did not improve model fit (Table 3).

*Question 4: Is cultch biomass related to the number of live oysters?*

Four models all had similar AICc values (within three AICc units) and the model with the highest weight (0.38) was the most complicated model Roundwt ~ (1 | SP) + Spat\_sum + Period + Project + (0 + Period | SP) + Period:Project + offset(log(Num\_quads)) which also allowed for a unique negative binomial dispersion parameter. Diagnostic assessments of model fitting for these models suggests that several may be overparameterized. We examined nine simpler models to assess whether including the number of live spat did not improve model fit (Table 5). For these simpler models, model fit was not improved by including oyster spat counts as a main effect (across all projects) or as an interaction term for each project (Table 5). The lowest AICc and highest model weight was for a model that did not include information on oyster spat (Table 5). This suggests live oyster spat did not influence oyster cultch biomass.

*Question 5: How does cultch material persist?*

We plotted the weight per quadrat (kg) by bay and project over period to assess patterns (Figure 12). For Pensacola and St. Andrew, the cultch material used for project NRDA-4044 was limestone or granite (Table 1). Plotting the biomass of this material per quadrat over time (Figure 13) demonstrated a widespread in the amount of material collected over time but no strong indication of an increase or decline. Because Apalachicola Bay is the only system where multiple materials (rock and shell) have been used for different projects, we were able to examine Apalachicola Bay for more insight into cultch persistence by project (Figures 13-14). We fit the same GLM models described previously first to compare all bays (Question 1) and to compare projects within Apalachicola Bay (Question 2).

In comparing the persistence of cultch material across the three bays, the Roundwt ~ Period + Bay + (Period | Site) + Period:Bay + offset(log(Num\_quads)) model did not converge with either the default or the BFGS optimizer. From an AICcC perspective a simpler models the Roundwt ~ Period + Bay + (1 | Site) + Period:Bay + offset(log(Num\_quads)) was the top model (lowest AICc value and AICc Weight = 0.56; Table 5.

Apalachicola Bay cultch biomass per quadrat had a positive slope (beta of the slope = 0.04, SE = 0.02, 95% CI = 0.008-0.07) and this trend was significantly different from zero (p = 0.02). Pensacola beta values for the slope of oyster spat counts over time were negative (beta = -0.03, SE = 0.03, 95% CI = -0.08-0.03) and this slope did differ from zero (p=0.02). For St. Andrew Bay, the slope was highly uncertain (beta = -0.07, SE = 0.05, 95% CI = -0.20-0.05) and this slope did not differ from zero (p=0.23). We then predicted the marginal means of oyster biomass from a single ¼-m2 quadrat in Period 15 for comparison purposes between each bay. Predicted live oyster spat for Apalachicola was 3.76 kg cultch per quadrat (95% CI 2.54-5.56), Pensacola was 1.71 kg cultch per quadrat (95% CI 0.99-2.94), and St. Andrew Bay predicted cultch per quadrat was 1.34 kg (95% CI 0.46-37-3.85).

The same general GLM models fit to the counts of live oyster spat with Apalachicola Bay (Question2) were then fit to the four projects for Apalachicola Bay. The top 3 models (delta AICC< 3) were the Roundwt ~ Period + Bay + (Period | Site) + Period:Bay + offset(log(Num\_quads)) followed by the Round\_wt ~ Period + Project + (Period | Site) + Period:Project + offset(log(Num\_quads)) and Round\_wt ~ (1 | SP) + Period + Project + (0 + Period | SP) + Period:Project + offset(log(Num\_quads)) models. AICc weights were 0.45, 0.24, and 0.23 respectively.

The significant interaction term suggests that each project's temporal patterns in oyster cultch biomass are unique. The FWC-2021 project cultch biomass per quadrat had a positive slope over time (beta of the slope = 0.09, SE = 0.11, 95% CI = -0.14-0.31) and this trend was not significantly different from zero (p = 0.44). The GEBF-5007 project beta values for the slope of oyster spat counts over time were positive (beta = 0.05, SE = 0.02, 95% CI = 0.01-0.09) and this slope did differ from zero (p=0.02). For the NFWF-1 project, the slope was negative (beta = -0.14, SE = 0.02, 95% CI = -0.19- -0.09) and this slope did differ from zero (p<0.0001). Finally, for the NRDA-4044 project the slope was negative (beta = -0.05, SE = 0.01, 95% CI = -0.07- -0.02) and this slope did differ from zero (p=0.0002).