Appendix – Analysis of oyster count data through winter 2021-2022

Part 1. Analysis of the repeated measures sites oyster live count data.

Background

Four stations (individual reef elements in the Lone Cabbage reef chain; LCO9C, LCO10A, LCO11B, and LCO12 [Figure 1]) have been sampled repeatedly over time before and after reef restoration. This was done to provide insight into the response in oyster counts following the restoration action of placing limestone boulders on the degraded reef. The prediction was that by placing durable substrate (limestone rock) Lone Cabbage reef would transition from an undesired, low oyster count reef to a more desired high oyster count reef. Sampling these “repeated measures” sites provides insight into trends in oyster counts at these four locations only and does not compare these responses to unrestored oyster bars. Three replicate transects have been sampled on each of these four stations in eight periods of time (Summer 2013, Winter 2014-2015, Summer 2015, Winter 2017-2018, Winter 2018-2019, Winter 2019-2020, Winter 2020-2021, Winter 2021-2022) at the exact same transect location (to instrument precision). This repeated sampling offers some statistical advantage in measuring a single treatment effect such as the addition of rock substrate.

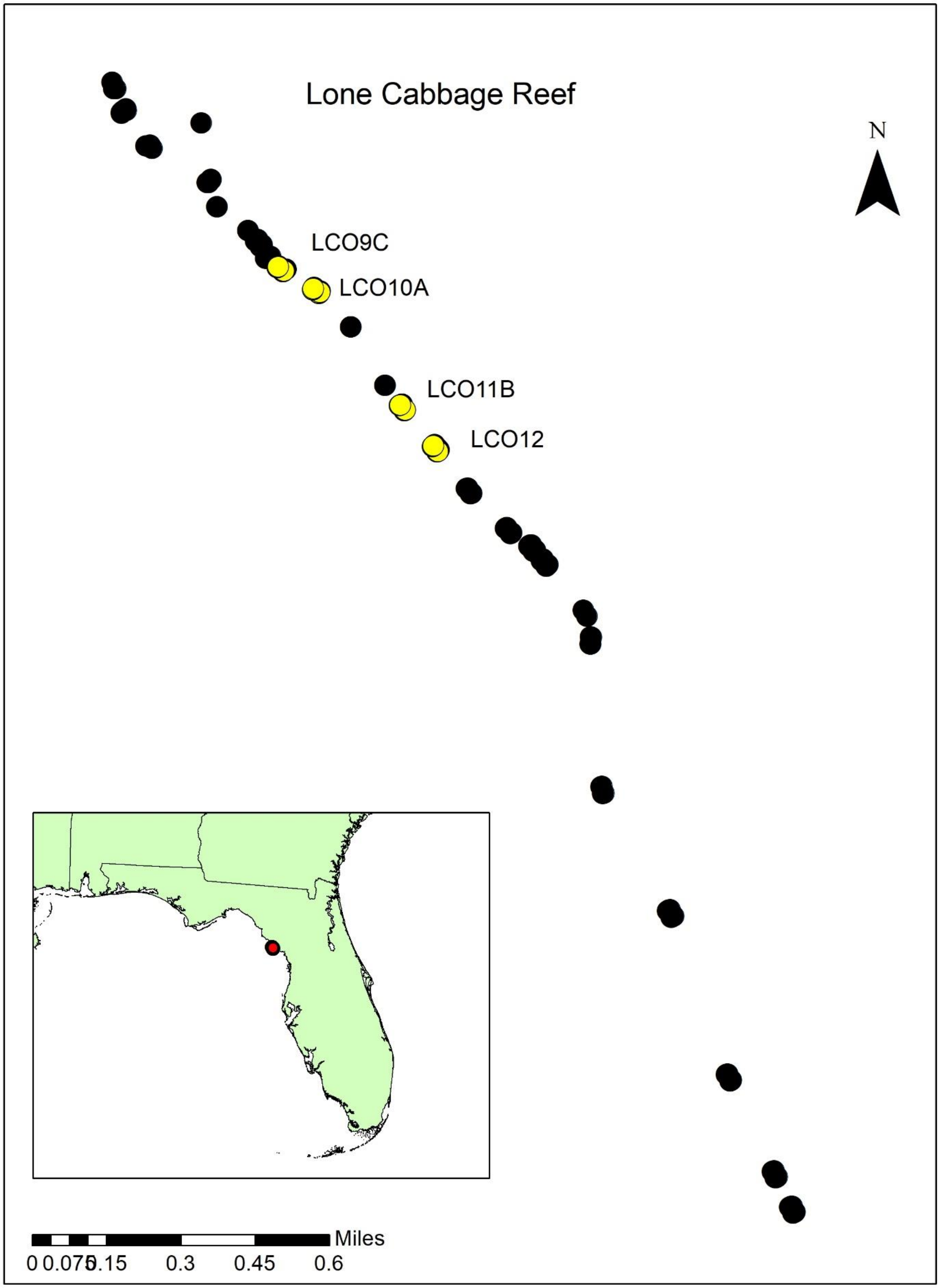

Figure 1: Map of Lone Cabbage Reef in relation to the southeast US (inset map) and Suwannee Sound (larger map). The reef in Suwannee Sound is indicated by black dots and the four stations used in the repeated measures analyses are in yellow.

Analysis – Three repeated transects per station

Station-level

We analyzed the data at the station (individual reef element) level using generalized linear models with a negative binomial distribution (Moore et al. 2020). For this repeated measures analyses we only use data from the three transects per station that have been sampled in all periods. These transects are fixed in space and the same transects have been repeated each year at the closest possible location based on available GPS instrument. Because transect lengths differ between station (because the transects are the length of the station, and stations vary in size) we include transect length as an offset to account for difference in transect lengths between stations. Because our main question of interest is whether or not live oyster counts change over time, we first fit a simple model describing live oyster count as a function of time (Period). Previous analyses have shown no significant differences in live oyster counts between stations, and this pattern continued for Period 24, so no random effects were included.

We found a positive trend in live oyster counts over time, and on average live oyster counts increased from period 22 to period 24. We found live oyster counts during period 24 are the highest observed in the 9 years of monitoring on Lone Cabbage Reef. Observed live oyster counts were lowest in period 7 (Summer 2013) followed by period 16 (Winter 2017-2018), both before restoration. Period 17, the first sampling period post restoration, had an increase in live oyster counts compared to pre-restoration sampling, and this positive trend in oyster counts has continued each sampling period thereafter (period 18-24). Log-transformed beta coefficients from this model are presented in Table 1. In order to interpret these values as estimated numbers of oysters, the beta values must be backtransformed (i.e., for period 24 exp(3.4310) = 30.9). This value suggests that the live oyster count in period 24 per meter of transect is about 31 live oysters. Because the transect is 15-cm wide the average number of oysters is about 160 per m^2. As an example of model fit Figure 2 shows the predicted live oyster count using this simple negative binomial GLM per meter of transect for each period (red dot) with 95% confidence intervals shown in red. The observed oyster counts for each station are shown in black. This plot shows the increase in predicted live oyster count over time. While the 95% confidence intervals on predicted oyster count per meter do overlap for all post-restoration periods, the trajectory of the oyster counts is still positive across all periods post-restoration.

Table 1: Beta coefficients and standard errors for the top model (count_live ~ period + offset(tran_length)). Estimates are log-transformed.

Period	Dates	Beta coefficient	Standard Error
7	Summer 2013	-0.3502	0.3433
10	Winter 2014-2015	2.6219	0.3358
11	Summer 2015	2.5320	0.3359
16	Winter 2017-2018	0.9549	0.3376
18	Winter 2018-2019	2.2379	0.3360
20	Winter 2019-2020	2.8344	0.3358
22	Winter 2020-2021	3.0891	0.3357
24	Winter 2021-2022	3.4310	0.3356

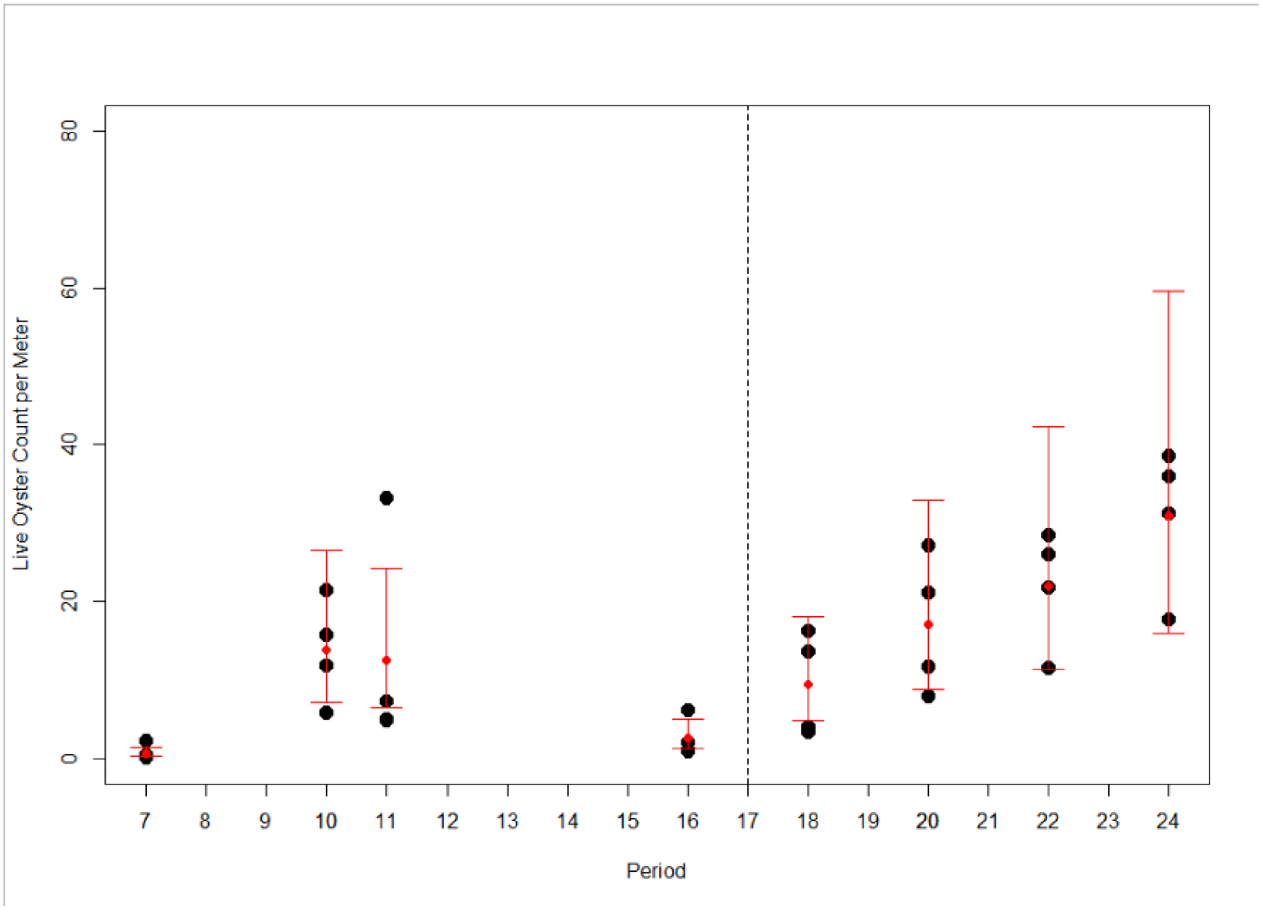

Figure 2: Predicted live oyster counts with 95% confidence intervals from the top model (count_live ~ period + offset(tran_length)) using data aggregated across transects by station. The predictions are per meter of transect. The black dots are the observed oyster counts for each of the four stations during each period. The vertical block dotted line is the period when the large restoration took place on Lone Cabbage Reef.

Transect-level

The same models were also fit to data at the individual transect level. At the transect level, the available data are counts for each of the three transects for each of the four stations (reef elements) during each period. Predicted live oyster counts from the simple negative binomial GLM fit to these transect level data are like the station-level analyses; however, the standard errors are smaller, which results in narrower 95% confidence intervals. Figure 3 provides the predicted live oyster counts with 95% confidence interval by period using the transect level data.

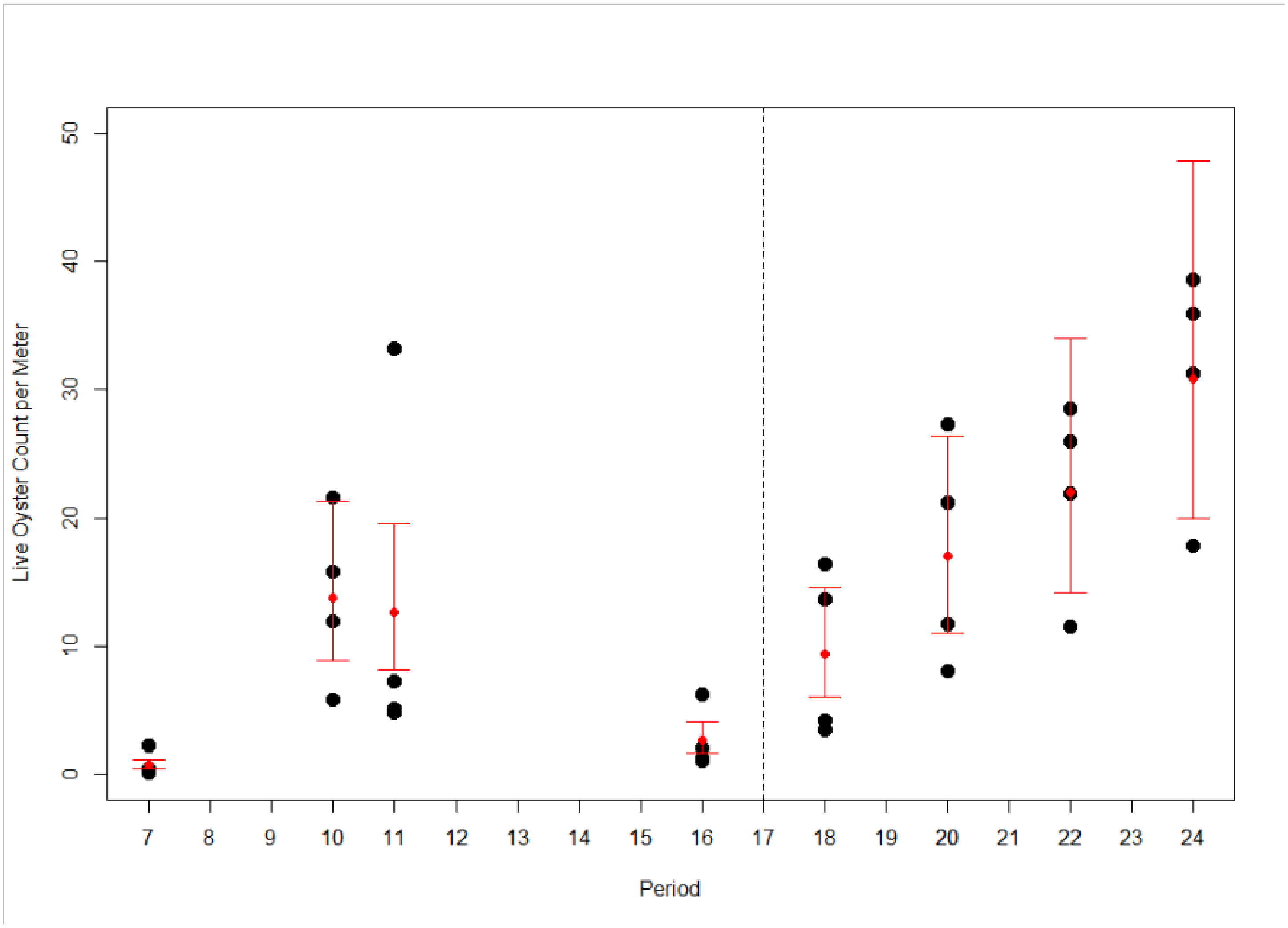

Figure 3: Predicted live oyster counts with 95% confidence intervals from the top model (count_live ~ period + offset(tran_length)) using data from each transect within each station. The predictions are per meter of transect. The black dots are the observed counts for each of the four stations during each period. The vertical block dotted line is the period when the large restoration took place on Lone Cabbage Reef.

Analysis – All transects per station

Station-level

The repeated measures analyses above is based on oyster counts from the exact same three transects that have been conducted on each reef element (station). However, at these same reef elements additional transects were also sampled each year at varying locations along the reef. For these additional transects, the location along the reefs varied each year as well as the number of additional transects. This results in a variable number of transect sampled at these stations over time. These additional transects provide information about live oyster counts across the stations as a whole. We fit the same basic negative binomial GLM to all transects from these stations across periods. Overall, the results were similar showing an increase in predicted live oyster counts post-restoration. However, when all transects are included there is little difference between period 20 and 22, but there is an observed increase in live oyster counts through period 24 (Figure 4), but confidence intervals do overlap for periods 20, 22, and 24. Beta coefficients from this model are included in Table 2 and predicted estimates in Figure 4. These coefficients and results can be interpreted the same as above.

Table 2: Beta coefficients and standard errors for the top model (count_live ~ period + offset(tran_length)). Estimates are log-transformed.

Period	Dates	Beta coefficient	Standard Error
7	Summer 2013	-0.3502	0.3339
10	Winter 2014-2015	2.6219	0.3262
11	Summer 2015	2.5320	0.3262
16	Winter 2017-2018	0.9549	0.3280
18	Winter 2018-2019	2.0813	0.3262
20	Winter 2019-2020	3.1169	0.3259
22	Winter 2020-2021	3.1203	0.3258
24	Winter 2021-2022	3.5020	0.3259

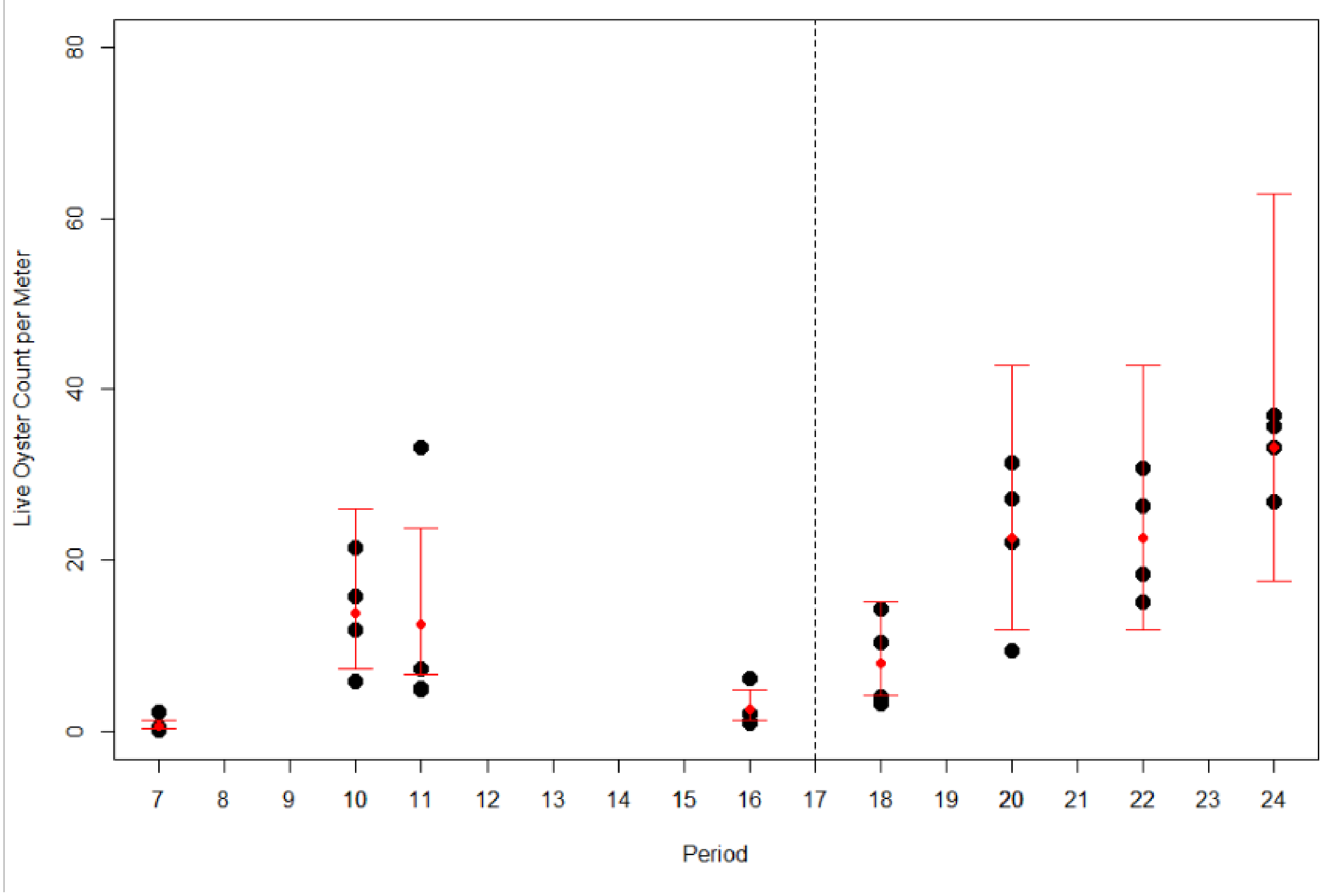

Figure 4: Predicted live oyster counts with 95% confidence intervals from the top model (count_live ~ period + offset(tran_length)) using data aggregated across transects by station. The predictions are per meter of transect. The black dots are the observed counts for each of the four stations during each period. The vertical block dotted line is the period when the large restoration took place on Lone Cabbage Reef.

Transect-level

Again using all of the transects sampled across the four repeated measure station, the same model was fit to the data at the transect level. At the transect level the predicted live oyster counts remain at the highest level for period 24, but a decline in live oyster counts is predicted between periods 20 and 22. Precision of predicted counts is highest for these results because of the larger number of observations included in the prediction. Overall data fit to this model again suggests an overall positive trend in live oyster counts since reef construction for these sites.

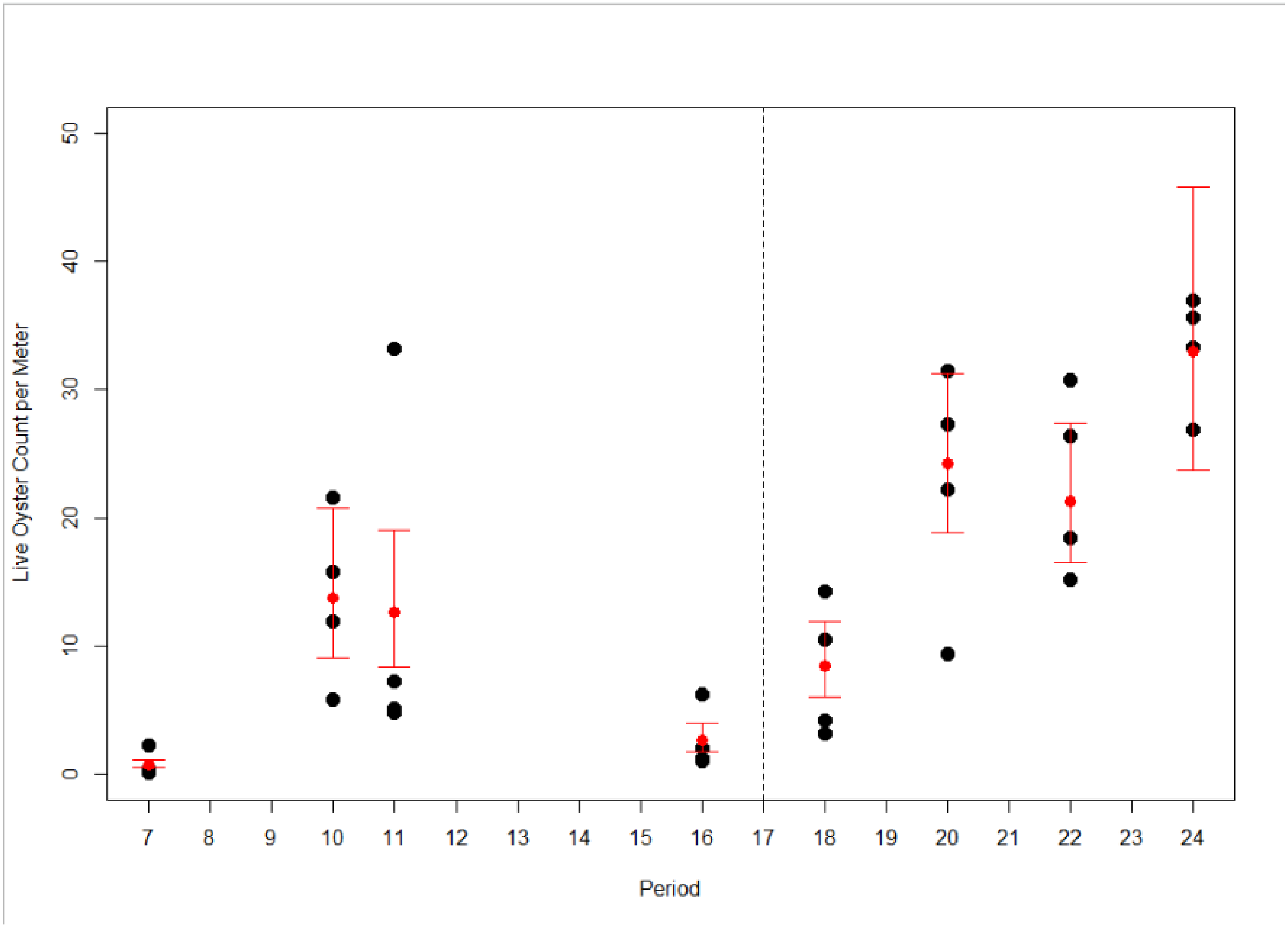

Figure 5: Predicted live oyster counts with 95% confidence intervals from the top model (count_live ~ period + offset(tran_length)) using data from each transect within each station. The predictions are per meter of transect. The black dots are the observed counts for each of the four stations during each period. The vertical block dotted line is the period when the large restoration took place on Lone Cabbage Reef.

Part 2

Assessing trends in oyster counts on restored and unrestored reefs in Suwannee Sound

We analysed available oyster count data from all Lone Cabbage stations (restored and unrestored reefs) by fitting generalized linear models with a negative binomial distribution to these data (see details in Moore et al. 2020). Models described specific hypotheses through combinations of parameters including Period (a measure of time), Strata (open or closed to fishing + restored or unrestored site), and latent effects of commercial fishery trips and landings or river discharge influenced counts. Because transects varied in length across the study area, we used the natural log of transect length as an offset in all models to account for differences in sampling area.

We first predicted live oyster counts, excluding covariates, to understand whether live oyster counts varied over Period (as a measure of time), restored/non-restored (whether sites where counts were completed had been restored or not), fishing/non-fishing (whether sites were open to commercial fishing or not) or strata (combination of restored and fishing). Based on AIC there is no strongly weighted model (Table 1) suggesting that all candidate models fit the observed count data equally.

Table 2: Model selection table.

Model	K	AIC	Delta_AIC	AICWt	Cum.Wt	LL
period * fish	5	3880.62	0	0.24	0.24	-1935.31
period + fish	4	3880.92	0.3	0.21	0.45	-1936.46
period + rock	4	3881.15	0.53	0.19	0.64	-1936.58
period	3	3881.2	0.58	0.18	0.82	-1937.6
period + strata	6	3882.48	1.86	0.1	0.92	-1935.24
period * rock	5	3883.13	2.51	0.07	0.99	-1936.57
period * strata	9	3886.27	5.65	0.01	1	-1934.14

Results (beta parameters) from models testing three broad hypotheses (1) whether live oyster counts were different across strata (restored-closed to fishing, restored-open to fishing, unrestored-open to fishing, restored-closed to fishing), (2) if counts differed across areas open or closed to fishing, (3) if oyster count differed for oyster reefs that had been restored or not restored were compared. None of these models were statistically significant (all beta $p > 0.05$). This suggests that across all combinations of different factors we hypothesized could influence oyster counts, we are not able to statistically detect differences. This does not mean that differences do not exist. We used the models with the lowest AIC value (Table 1) to predict trends in oyster count over time (Period). We found that over time live oyster counts in areas closed to fishing have been increasing, and live oyster counts in areas open to fishing have been decreasing (Figure 1). We also predicted a slight increase in the live oyster counts on wild oyster bars and stable counts on restored oyster bars (Figure 2).

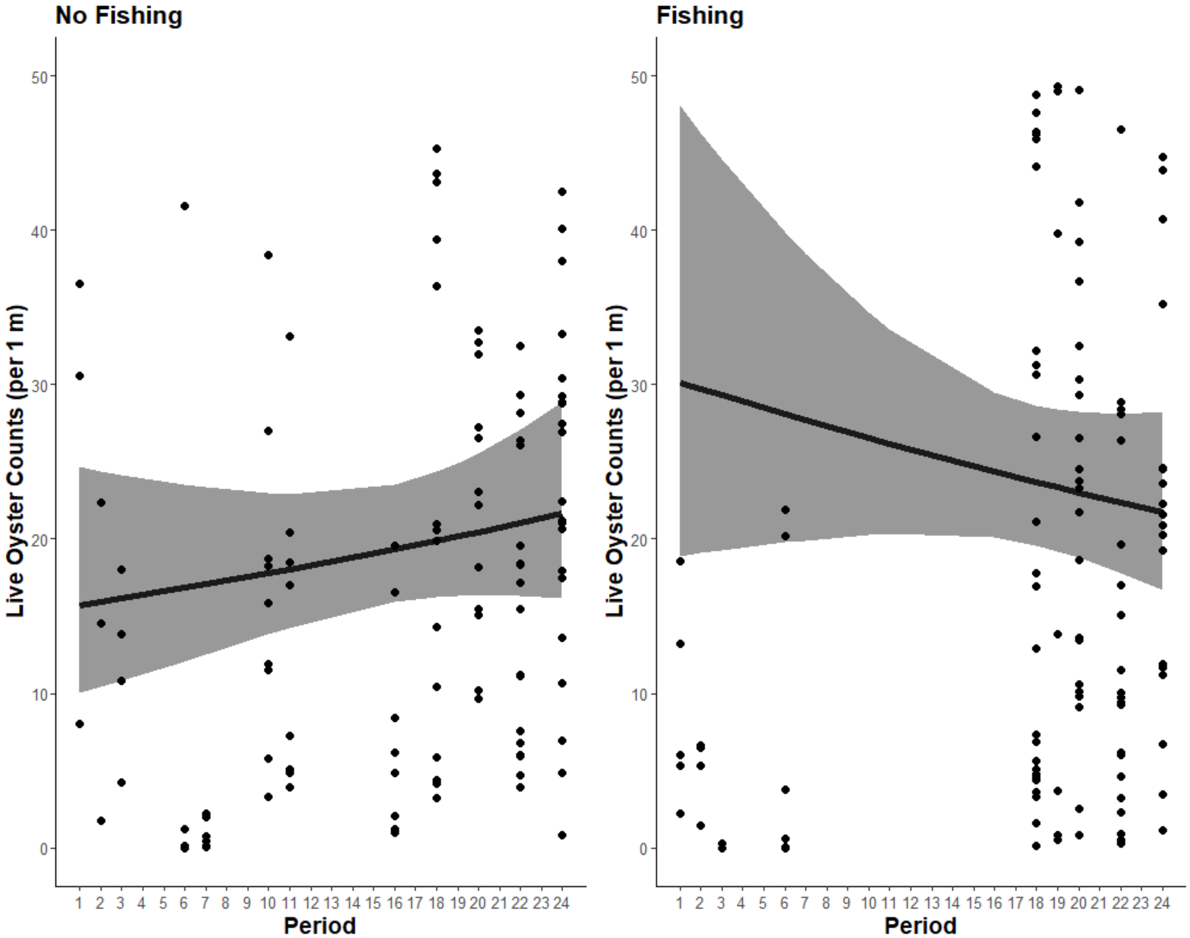

Figure 6: Observed (black dot) and predicted (solid black line) counts of live oysters per 1 meter of transect (y axis) over period of time (x axis) for sites closed to fishing (left panel) and open to fishing (right panel). The grey ribbon is the predicted 95% confidence interval.

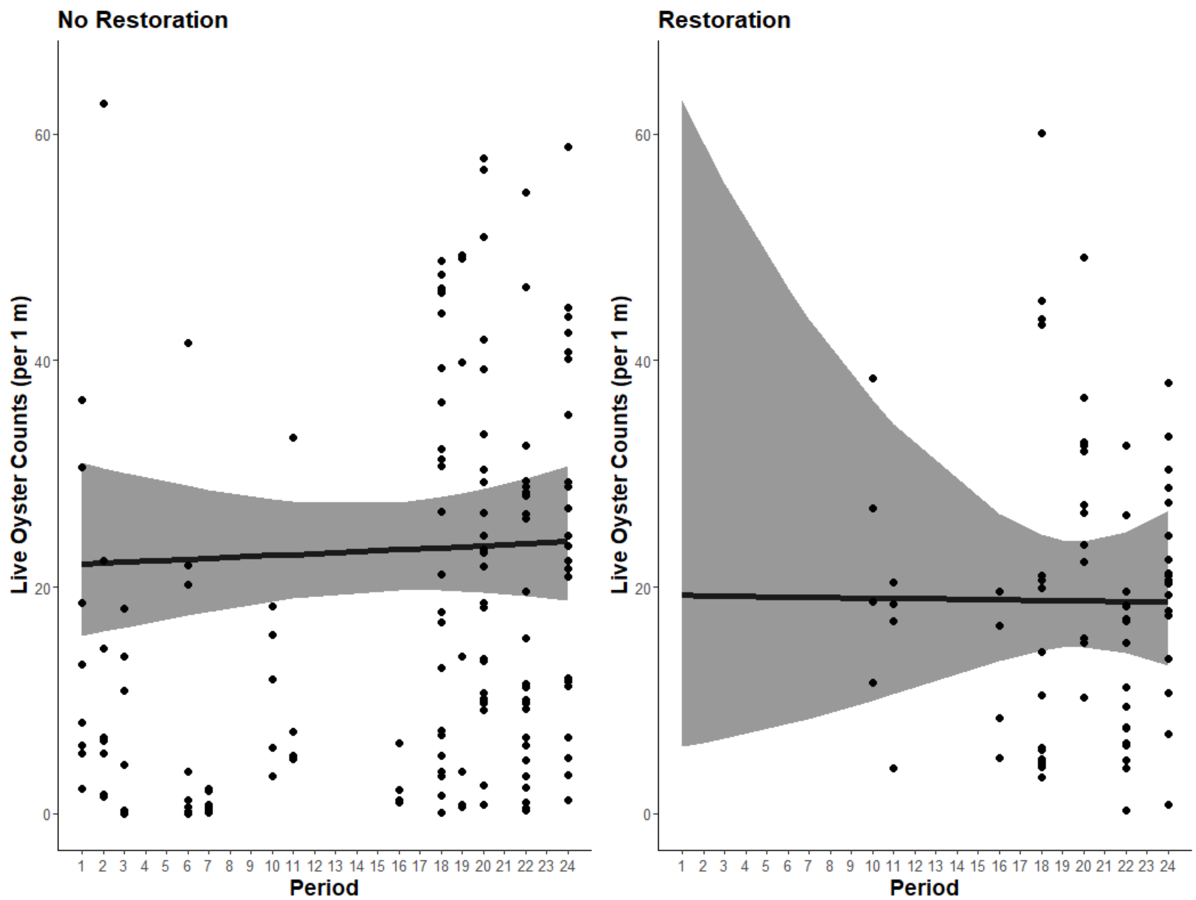

Figure 2: Observed (black dot) and predicted (solid black line) counts of live oysters per 1 meter of transect (y axis) over period of time (x axis) for sites that received rock restoration (left panel) and sites that were not restored (right panel). The grey ribbon is the predicted 95% confidence interval.

Adding covariates

We explored basic covariates of management interest (like Moore et al. 2020) related to river discharge (as a proxy for nutrients, salinity, and other water quality factors). To account for antecedent flow effects, we assessed how discharge in the current year, one-year lag of discharge (one year prior), and two-year lag of discharge (discharge two years prior) influenced live oyster counts. We also assessed commercial fishing activity metrics (i.e., fishery effort and landings at same lag-time intervals). The relationships between commercial fishing and intertidal oyster reefs is complicated because oysters on intertidal reefs rarely reach legal size (76.2-mm) likely because of feeding time limitations when exposed to air. Over the past 12 years of oyster research in Lone Cabbage, < 100 legal size oysters have been measured out of several thousand oysters measured. However, as discussed in Moore et al. (2020) there could be aspects of source-sink dynamics that are occurring that could create linkages between intertidal oyster population trends and subtidal oysters harvested.

We added these covariates to a simple model (but one with AIC support; live counts ~ period + offset(log(transect length)) and tested how individual covariates improved model fit (Table 2).

Table 3: Model selection table. All models are count_live ~ period + covariate + offset(log(transect length)) model as the base model with different covariates added to this model to compare fit.

Covariate	K	AIC	Delta_AIC	AICWt	Cum.Wt	LL
Trips 2-year lag	4	3867.64	0.00	0.91	0.91	-1929.82
Landings 2-year lag	4	3872.55	4.92	0.08	0.99	-1932.28
Trips 1-year lag	4	3880.05	12.42	0.00	0.99	-1936.03
None	3	3881.20	13.56	0.00	0.99	-1937.60
Total Discharge 2- year lag	4	3881.37	13.73	0.00	1.00	-1936.68
Annual Discharge 2-year lag	4	3881.37	13.73	0.00	1.00	-1936.68
Annual Discharge 1-year lag	4	3881.78	14.15	0.00	1.00	-1936.89
Total Discharge 1-year lag	4	3881.80	14.17	0.00	1.00	-1936.90
Landings 1-year lag	4	3882.27	14.63	0.00	1.00	-1937.13
Trips	4	3882.47	14.83	0.00	1.00	-1937.23
Landings	4	3883.14	15.51	0.00	1.00	-1937.57
Annual Discharge	4	3883.16	15.52	0.00	1.00	-1937.58
Total Discharge	4	3883.16	15.53	0.00	1.00	-1937.58

We found model fit could be improved when we included information on oyster fishing trips with a 2-year lag (Table 2).

We then used this model to predict live oyster counts based on commercial oyster trips two-years prior (Figure 3) or commercial landings two-years prior (Figure 4) and both show increasing trend – more commercial fishing trips or landings resulted in higher counts. We are still working to develop hypotheses about this relationship. It could be that in years with more oysters, there are more landings, as the fishery is simply responding to fluctuations in oyster population. This would assume that intertidal and subtidal oyster populations are following similar patterns over time. We are continuing to develop ideas.

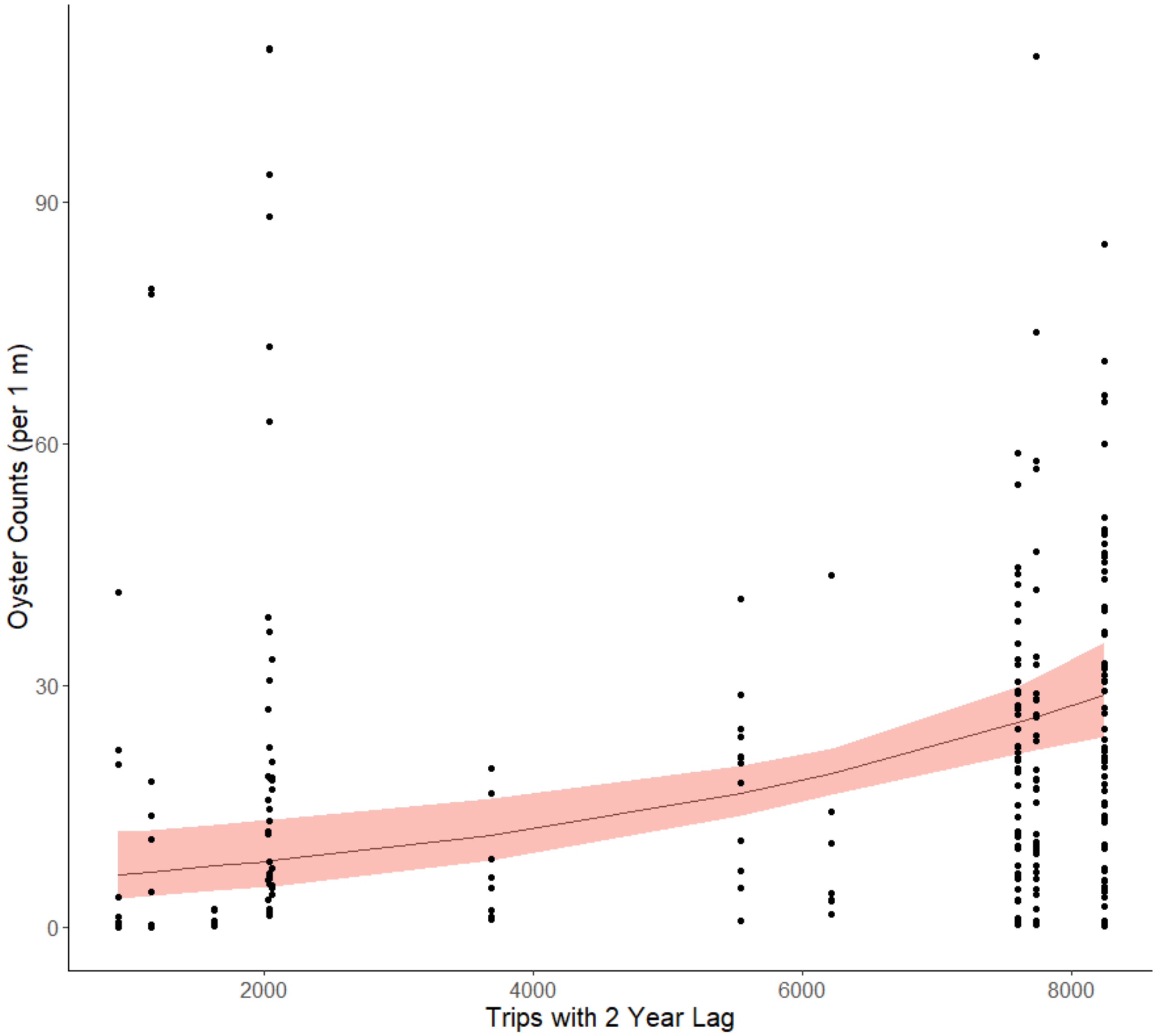

Figure 3: Observed (black dot) and predicted (solid black line) counts of live oysters per 1 meter of transect (y axis) over commercial fisheries trips two years prior (x axis). The pink ribbon is the predicted 95% confidence interval.

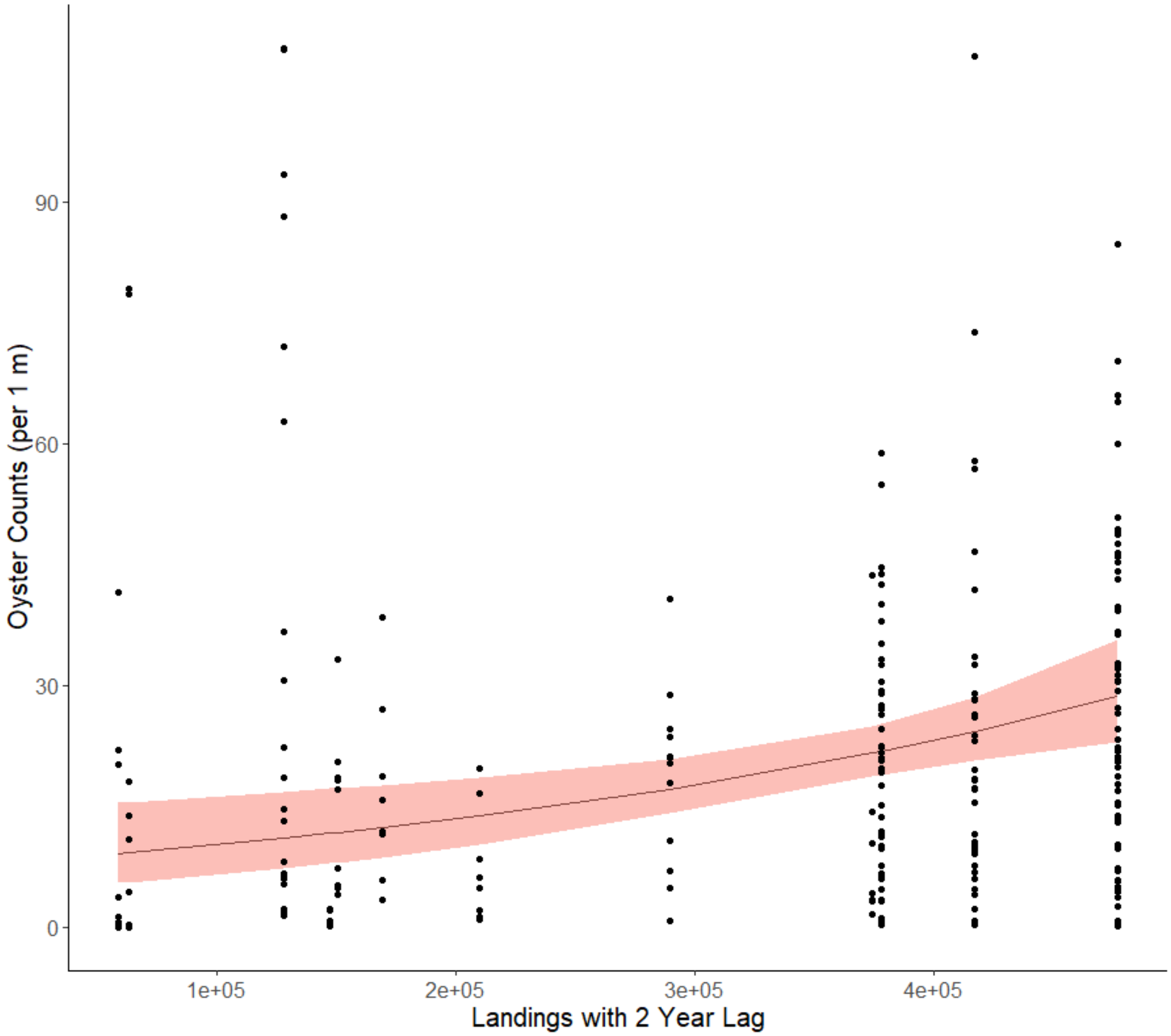

Figure 4: Observed (black dot) and predicted (solid black line) counts of live oysters per 1 meter of transect (y axis) over commercial fisheries landings two years prior (x axis). The pink ribbon is the predicted 95% confidence interval.

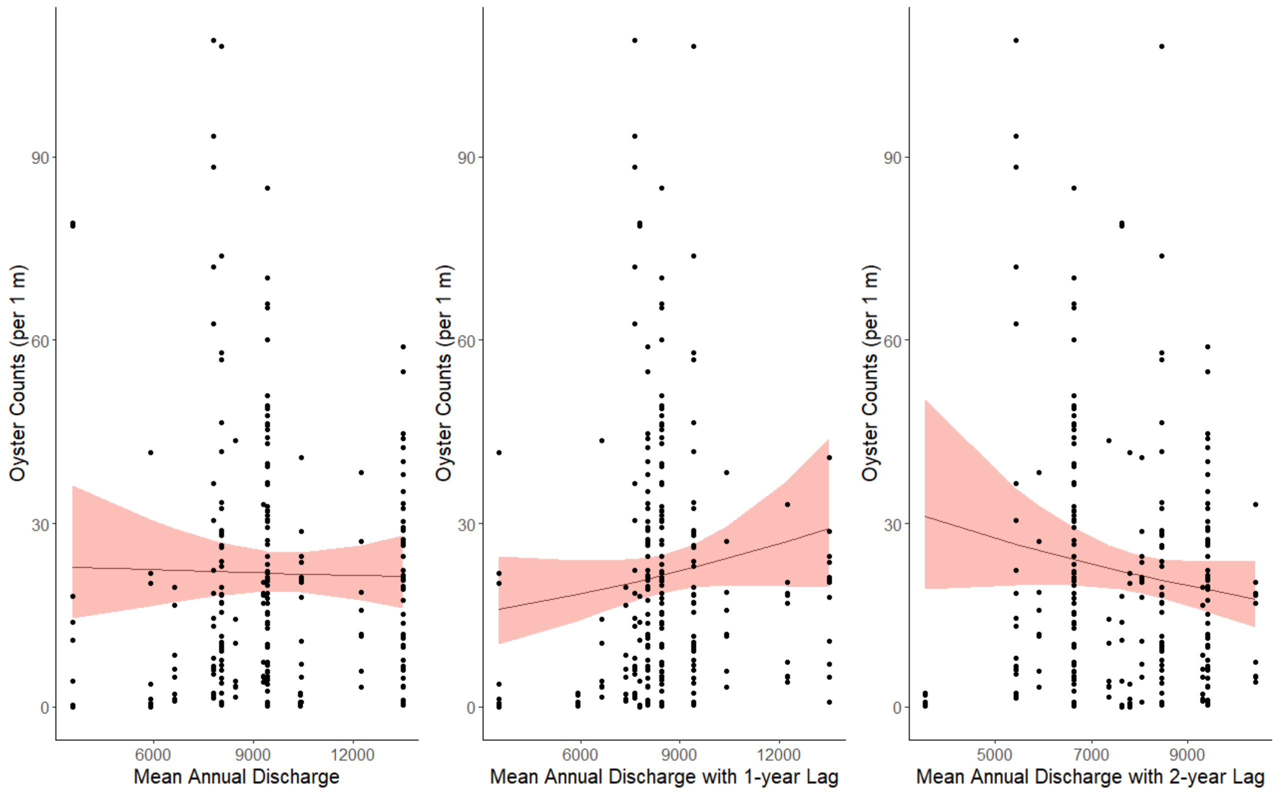

Figure 5: Observed (black dot) and predicted (solid black line) counts of live oysters per 1 meter of transect (y axis) over three different measures of Suwannee River discharge from USGS Wilcox gage. The x-axis of the left panel is mean annual discharge, center panel is mean annual discharge with a 1-year lag, and right panel is mean annual discharge with a two-year lag. The pink ribbon is the predicted 95% confidence interval.

River discharge

We assessed how river discharge, and antecedent river discharge influenced live oyster counts. We found a positive relationship between antecedent flow conditions with a one, but not two-year time lag and no relationship within river discharge for the same year counts are collected.

Overall trends

We fit a separate GLM to data from all sites prior to period 17 (pre-restoration), and the post restoration periods (period 18-24) for restored and un-restored sites. The beta coefficient for Period for the pre-construction sites (all before period 17) was -0.95 (SE = 0.03) a significant decline in oyster counts over time ($p=0.007$). Fitting separate GLM models to the post-construction, unrestored reefs, the beta coefficient for period is -0.05 (SE = 0.04), which is not significant ($p = 0.21$). Post-construction, for restored reefs, the beta coefficient for period is -0.01 (SE = 0.04), which is also not significant ($p = 0.75$). However, the key result is that the restored and unrestored reefs now have overlapping beta coefficients suggesting the live oyster counts per meter of transect are similar. Critically, both restored and unrestored reefs have negative slopes indicating declines, however the unrestored reefs are declining at a faster rate than the restored reefs, though these declines are not statistically significant. This is an area for further assessment.

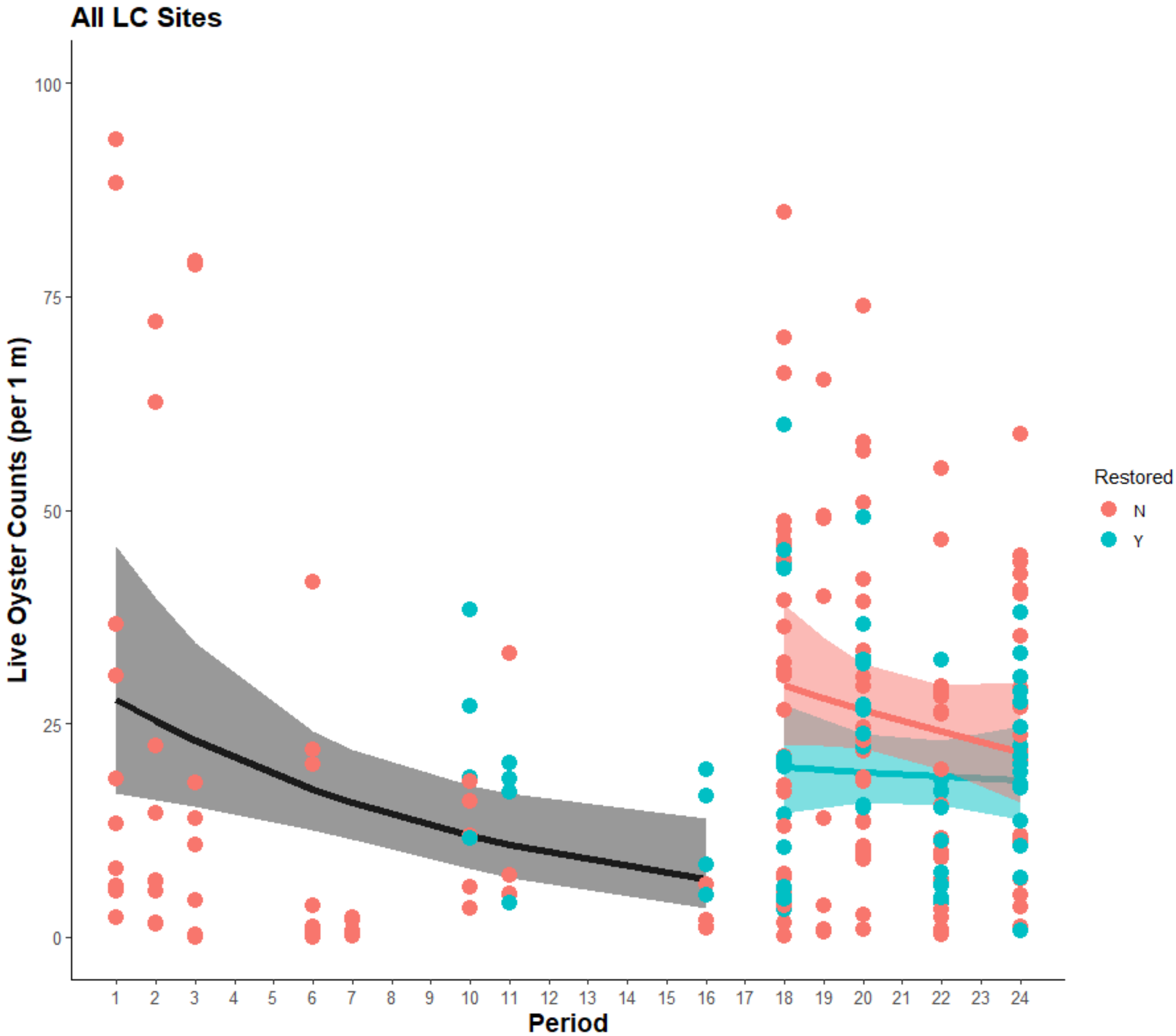

Figure 6: Observed (red dot = not restored, blue dot = restored) and predicted (solid black line for years prior to restoration, red or blue line for years after restoration) counts of live oysters per 1 meter of transect (y axis) over period of time (x axis). The blue dots prior to period 17 are the pilot project reefs and are not included in the GLM represented by the black line and grey error bar. The ribbon is the predicted 95% confidence interval.