ADDRESSING OYSTER MORTALITIES IN FLORIDA'S OFF-BOTTOM OYSTER AQUACULTURE INDUSTRY

FINAL REPORT

Covering June 2020 – December 2021

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Additional project objective reports:

Oyster Health Assessment
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Phytoplankton Biomass: Quality and Quality
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INTRODUCTION

Intensive aquaculture of the eastern oyster *Crassostrea virginica* has increased dramatically over the past decade in Florida. This increase is associated with decreased supplies from commercial fisheries and higher prices for single oysters. In 2013, the Florida Governor and Cabinet approved water column leases removing regulatory barriers to off-bottom oyster aquaculture in support of this new industry. Off-bottom culture has proven highly successful for mid-and north Atlantic growers over the past 25 years but is relatively new to growers in states along the Gulf of Mexico coast. Since 2014, more than 125 Florida growers are operating on over 400 acres of water column aquaculture leases located primarily in the Panhandle. In 2019, these leases produced 4.2 million oysters with a sales value (farmgate) of $0.45-0.55 per single oyster (FDACS Division of Aquaculture internal data).

Unexplained oyster mortality events in the spring and summer of 2018 and 2019 accounted for 50-80% loss of adult oysters reaching market size (~2.5-3 inches) at farms located in Franklin and Wakulla Counties. At an oyster growers' meeting held in Crawfordville (January 24, 2020), possible causes for mortalities were discussed. Although consensus was not reached, growers agreed that a systematic approach was needed to better understand factors associated with these events. Further, successes and knowledge gained from these relatively small-scale farms are critical to the future of oyster culture enterprises in Florida.

A variety of factors, including water quality, disease, toxins, or poor management practices, may account for oyster mortalities. However, diversity in location, gear, seed stock, and operational scale make it challenging to extricate potential causes. In response to industry concerns, "sentinel" farms were established at two lease locations to begin to explore production challenges. A monitoring and assessment plan was initiated at these farm sites to examine basic but important relationships between production and health of cultured oysters, and key environmental factors. Research and Extension faculty were engaged that have the combined expertise in bivalve aquaculture (Sturmer), aquatic animal health (Kane), marine phycology (Phlips), and county Extension (Lovestrand).

These preliminary efforts sought to better understand the interactions of environmental and health stressors potentially impacting production of aquacultured oysters. Specific project objectives were to:

1) Monitor oyster production at commercial farms located at aquaculture lease areas in the Panhandle,
2) Monitor basic water quality parameters and phytoplankton abundance at these lease areas,
3) Assess prevalence and severity of shell parasitism and Dermo disease, and
4) Analyze water quality, phytoplankton, oyster health and production data to discern risk factors for mortality events and relate the importance of monitoring to minimize losses and optimize production.

METHODS

Study Sites

Four farm sites were identified to participate in this study. Two sites were located at the Alligator Harbor Aquaculture Use Zone (AUZ) in Franklin County, where oyster mortalities have occurred in previous years. The Alligator Harbor AUZ is characterized by high salinity with values typically exceeding 30 ppt. The other two sites were located at the Oyster Bay AUZ in the adjacent county, Wakulla County, where salinities are considered to be medium, with values ranging from 15-25 ppt. About 50 % of the state's production of cultured oysters comes from these two areas (FDACS Division of Aquaculture internal data, 2019). Growers were selected based not only on location of farms but willingness to participate in the project.

Oyster Stocks

Two lines (stocks) of single-set oyster seed – triploids produced by crossing diploid oysters with sperm from traditional Louisiana-based (LA) tetraploid broodstock and from the new Florida-west coast (FL) tetraploid broodstock – were spawned at the Auburn University (AU) Shellfish Lab and shipped to the UF/IFAS Shellfish Aquaculture facility in Cedar Key on June 12, 2020. This allowed for evaluating the performance of triploid oysters using two different genetic stocks. Documentation provided by AU included pathology reports from the Virginia Institute of Marine Sciences, veterinary certification letters, and verification of ploidy for both stocks.

Oyster seed (R2, retained on a 2 mm screen) were stocked into upwellers at a commercial nursery and maintained by UF staff. They were transferred to drum barrel silos after a month and continued to be nursed until they reached a size to be retained on a 12 mm screen (R12), a seed size typically purchased by growers. On September 18, oyster stocks were sieved, and numbers estimated both by volume and weight; it was determined there were adequate juvenile oysters to distribute to participating growers.

On September 29, 1,050 oysters of each stock were delivered to each of the growers. Because of the variability in the estimates of numbers, oyster stocks were hand counted the prior day. A sample of 50 from each stock were measured and weighted. The Florida stock averaged 20.7 $\pm$ 2.9 mm in shell height (SH) whereas the LA stock averaged 18.8 + 2.7 mm SH. Both stocks averaged 1.4 $\pm$ 0.6 grams in whole wet weight (WW).

Oysters of each stock were initially deployed into one 9 mm mesh Vexar bag. The growers at Alligator Harbor (AH-A, AH-B) and one grower at Oyster Bay (OB-A) used cylindrical floats attached to the sides of each bag for support. The second Oyster Bay grower (OB-B) placed bags inside a 4-slot floating cage. Growers provided cultured gear and maintenance for the duration of the culture period. They also determined when to split bags based on average bag fullness. Color-coded tags and zip-ties were provided to identify ploidy stocks throughout the culture period and to distinguish the stocks from other oysters being cultured on the farm.

Sampling and Monitoring

Water quality Water temperature and salinity were measured at the two lease areas. At the Alligator Harbor AUZ, continuous data were obtained from a monitoring station using YSI 6600 multi-parameter sondes maintained by the Department of Environmental Protection, Aquatic

Preserve staff. At the Oyster Bay AUZ, Onset HOBO Pendant water temperature and conductivity data loggers were placed inside a culture bag at the OB-A farm site.

Growth and mortality Sampling by UF personnel was initiated in January 2021 and conducted bimonthly from then until the majority of oysters reached market size (~70-75 mm SH). At any sampling, oysters in replicate bags from each farm and stock were hand counted for live and dead to determine percent mortality. Dead oysters were not returned to the bag. Additionally, 15 live oysters were randomly selected to measure shell height and whole wet weight, then returned to the bag. Between site visits, participating growers observed oysters regularly and flipped gear on a routine basis allowing the bags and oysters to aerial dry to control biofouling organisms.

At harvest, the final sample period of this study, live and dead counts were conducted on all replicate bags to estimate mortality. Growth was determined by measuring 15-40 live oysters from each replicate bag for shell height, length, width, and whole wet weight. Shell measurements were used to calculate fan ratio (shell length/shell height) and cup ratio (shell width/shell height) to determine if external shell appearance was appealing for the half shell market. Additionally, 7-20 of these oysters were used to determine wet and dry meat weights, and biofouling. Condition index, an estimate of meat yield, was calculated using wet meat weight, dry meat weight, and shell weight values (Abbe and Albright 2003). Pictures were taken to document appearance of external and internal shells as well as meats.

Data Analyses The statistical package R Studio was used to analyze growth data of the two stocks per farm location to determine the effects of triploid stocks. Normality was tested using the Shapiro-Wilk goodness of fit test. Equal variance was tested using the Bartlett Test of Homogeneity of Variances. For response variables that did not satisfy the Shapiro-Wilk Test of Normality ($p \geq 0.05$), the data was analyzed using the Wilcoxon Rank Sum Test. For response variables that did satisfy normality, the data was analyzed using a T-test. Percentage data were transformed using the arcsine square root transformation before analysis. Probability values were not calculated for response variables when a sample period had less than three replicate culture bags per stock.

Environmental differences between the two farm locations in salinity (ppt) and temperature (°C), were compared by with linear models of the data (i.e., two-way analyses of variance) to compare mean differences between *sample period* and *sites*, as well as the interaction between the two. Specific comparisons between sites and sampling period were examined post-hoc with a Tukey test.

$$Temperature, Salinity \sim \text{Normal}\ (\mu)$$

$$\mu = sample\ period \times site \qquad \text{Eq. 1a, 1b}$$

A generalized linear modeling (GLM) framework was used to examine how environmental and site-selection factors affected responses in 1) growth rate in shell height (mm/day), 2) growth rate in weight (g/day), and 3) survival rate. The regression models for growth rates (Eq. 1) were fit with normal error distributions (i.e., using an identity link) based on their quantile-quantile distributions. These were thus and thus linear models that compared differences in means with analysis of variance/covariance. The regression model for survival rate used a logistic model with a logit link given that the response was binary (i.e., number of oysters alive compared to number dead).

$$Growth\ rate_{Shell\ height}, Growth\ rate_{Weight} \sim \text{Normal}\ (\mu)$$

$$\mu = sample\ period \times (stock \times site + dermo\ index + polydora\ index) \quad \text{Eq. 2a, 2b}$$

$$Survival\ rate \sim Alive : dead \sim \text{Binomial}\ (\mu)$$

$$\text{Logit}\ (\mu) = sample\ period \times (stock \times site + dermo\ index + polydora\ index) \quad \text{Eq. 3}$$

The growth and survival rate models (Eq. 2 and 3) assessed effects of the four *sampling periods* (categorical with four levels), *stock type* (categorical: FL or LA), *site* (categorical: OB or AH), the interaction of *stock* × *site*, and two disease indexes. The disease indexes were calculated by multiplying mean severity × mean prevalence.

The potential interaction effect of sample period and all factors were examined, including the potential three-way interaction of *sample period* × *stock* × *site*, given that (1) oysters were aging and growing in each successive sampling period and (2) the different sampling periods include seasonal effects. For example, sample period 1 was between October 2020 and January 2021, and mean air temperature was approximately 19°C (66ºF); meanwhile sample period 4 was in the July of 2021 where the mean temperature was approximately 28°C (82ºF). Although GLMM analyses do not required a balanced sampling design, the uneven replication should also be noted. Replicates for site-sampling period ranged from 2 to 7, and no measurements were taken in sampling period 4 from the Alligator Harbor site. Also, sample periods were not of equal duration, and they were not replicated across multiple years.

Significant factors in the models were identified by simplifying from the full models shown in Eq. 1 and Eq. 2 using backwards stepwise removal of the least significant term to produce the minimum adequate model (Crawley 2015). Laplace approximation was used to estimate likelihood and test statistics based on GLM fitting and inference protocols (Bolker et al. 2009). The effect sizes for the logistic regression for survival were calculated from exponentiating the log-linked model estimates. Confidence intervals were estimated with the more conservative unconditional standard error to account for model uncertainty (Burnham 2002, Grueber et al. 2011). Analyses were conducted with R (version 4.1.1) using the base stats, LME4 (Bates et al. 2015) and MASS (Vinet and Zhedanov 2011) packages. The data and R code for the plots, tables, and models are included in the supplementary material and allow for full reproduction.

Phytoplankton quality and quantity Phytoplankton sampling kits and data sheets were provided to one grower at Alligator Harbor (AH-A) and another at Oyster Bay (OB-A). Samples were collected and preserved weekly from late March through June (spanning the period when previous mortalities occurred). One to two sets of samples per lease area were analyzed each month at the UF Algal Ecology Laboratory for abundance and species composition, noting presence and density of harmful algal bloom species. A separate report for this project objective was submitted by Dr. Edward Phlips.

Oyster health During each sample period, 12 oysters per stock per grower were collected and transported to the UF Aquatic Pathobiology Laboratory for processing. At harvest, 20 oysters per treatment were collected. Oysters were assessed for prevalence and severity of shell parasitism associated with boring sponge, mud worms (*Polydora* spp.), biofouling, empirical meat condition, and prevalence and severity of Dermo (*Perkinsus marinus*) disease as previously described by Kane et al. (2018) and Kim et al. (2006). A separate report for this project objective was submitted by Dr. Andrew Kane.

RESULTS

Water Quality

Environmental factors, such as temperature and salinity, are known to strongly influence oyster performance (Shumway 1996). Monthly summaries of water temperatures and salinities, including averages, standard deviations, minimum and maximum values, are presented in Tables 1 and 2 for the Alligator Harbor AUZ and in Tables 3 and 4 for the Oyster Bay AUZ.

Seasonal differences followed expected patterns with monthly average water temperatures at the Alligator Harbor AUZ high during September (76.9°F), October (78.1°F), and May (78.3°F), and maximum values of 79.5-87.6°F obtained in these months. During the same period, temperatures at the Oyster Bay AUZ were slightly lower with averages of 75.5°F in September, 77.4°F in October, and 78.2°F in May, and maximum values of 77.9-85.2°F obtained in these months. However, the growout period at the Oyster Bay farms extended into June and July with water temperatures averaging 83.6°F and 83.0°F, respectfully, and maximum values of 86.5-88.5°F reached in those months. Temperatures never reached or exceeded 90°F at either site, which are considered stressful for oysters.

At the Alligator Harbor AUZ, monthly salinity averages did not vary among seasons, ranging from 27.8 ppt in April to 32.2 ppt in December, with a maximum value of 33.2 ppt in the latter month. These values are less than those in previous years and may be related to higher rainfall that occurred during this study period, which ameliorated salinity extremes (>35 ppt) that characterize this water body. At the Oyster Bay farms, monthly salinity averages varied from 16.5 ppt in July to 26.1 ppt in December. This is a lower salinity water body influenced by adjacent rivers and seasonal variations due to rainfall, runoff, and prevailing winds.

Continuous water temperature and salinity data for both sites are displayed in Figures 1 and 2, respectively. Averages for each sampling period are also provided. Although average temperatures of 66.5-65.7°F for both AUZs in sample period 1 were higher than those in sample period 2 (64.3-6.4°F), water temperatures declined over the first four months in the study and increased throughout the remaining culture period. Higher salinities for both sites were found in the first sample period (averages: 30.7 ppt, AH; 23.2 ppt, OB). Salinities were similar in the next two sample periods at Alligator Harbor (averages: 28.3 ppt, sample period 2; 29.0 ppt, sample period 3), while average salinities at Oyster Bay declined in sample periods 2 through 4.

Oyster Growth and Mortality

Alligator Harbor

Sample Period 1 – January 26, 2021;118 days from plant

Since planting, grower at the AH-A site had split oysters into three 14 mm bags for the LA stock and two 14 mm bags for FL oysters. Bags were flipped and shaken every 7-10 days. Due to the limited number of replicates, statistical analysis was not conducted on data in this sample period. Triploid stocks had similar shell heights and whole weights; averages and standard deviations for these measurements are found in Table 5. Growth over this period was 0.39 mm SH/day for FL oysters and 0.36 mm SH/day for LA oysters. Survival was high (99.1-99.6%) with interval mortalities of 0.9±0.1% for FL oysters and 0.4±0.3% for LA oysters. After sampling, oysters from each stock were blended and equally divided into three 14 mm bags for each stock (334/bag, FL; 365/bag, LA).

Although it was not determined until the next sampling period that the grower at the AH-B site had mistakenly combined oysters with those received in July for another stock comparison study, it was decided not to report any data from this grower.

Sample Period 2 – March 30, 2021;182 days from plant;64 days from sample period 1

Two months later, oysters reached market size as averages were 75.6±3.2 mm for FL stock and 71.1±4.8 mm for LA stock with no significant differences (p=0.477) observed (Table 5). Growth had slowed during this period with rates of 0.14-0.15 mm/day obtained for both FL and LA oysters. However, overall growth (from planting) was exceptional, most likely the highest reported from any Florida Gulf coast site (0.30 mm SH/day, FL; 0.29 mm SH/day, LA) with market-sized oysters reached in six months. Whole weights for each stock were similar (39.1-39.6 grams) and did not differ (p=0.754). Both interval and cumulative mortalities were low for both stocks (Table 5).

To continue to observe performance of these oysters under stressful conditions (high water temperatures, high salinities) typically observed in spring at this lease area, 450 oysters from each stock were hand counted and placed back into three bags each at 150/bag. The remaining oysters were divided into three more bags (about 160/bag FL and 180/bag for LA). The grower was allowed to harvest oysters from the latter bags, but not from the bags stocked at 150.

Sample Period 3 (Harvest) – May 25, 2021; 238 days from plant; 56 days from sample period 2

At 7.8 months from planting seed, oysters from three replicate bags of each genetic stock were harvested; overall growth, survival, and mortality were determined (Table 6). Growth in terms of shell height, shell length, whole wet weight, wet meat weight, dry meat weight, and condition index did not differ between stocks (p>0.05). However, Florida triploids had higher values compared to Louisiana triploids for shell width (p=0.0001). Growth rates (0.15-0.16 mm SH/day) during this last sample interval of 56 days were the same for both stocks. Growth rate of the LA oysters over the entire culture period of 238 days was 0.26 mm SH/day, as compared to 0.22 mm SH/day for the FL oysters.

Fan ratios of 0.66 and above and cup ratios of 0.33 and above are considered favorable by industry experts for half shell oysters designated for raw bars. Ratios for both stocks were within or exceeded the favorable ranges, but fan ratios differed significantly, while cup ratios did not differ between stocks (Table 6).

Over these additional 56 days from the prior sampling period, interval mortality increased to 28.0±10.9% for the FL stocks and 30.7±20.1% for the LA stocks but did not differ between stocks. Cumulative mortality for the growout period of 238 days was similar between stocks (30.2%, FL; 32.0%, LA) and lower than mortalities observed of adult oysters at this lease location in previous years.

Oyster Bay

Sample Period 1 – January 25, 2021; 117 days from plant

Since planting, the grower at the OB-A farm had split oysters into four 14 mm mesh bags for each stock. Bags were stocked light (about 5.5 lbs each) and desiccated every 7-10 days. The Florida stock had similar average shell height and whole weight as compared to the Louisiana stock; averages and standard deviations for these measurements are found in Table 7. Growth rates over the 117-day period were 0.19 mm SH/day for the FL stock and 0.17 mm SH/day for the LA stock. Genetic stock had no effect on mortality, which was low, during this period.

At the OB-B farm, oysters were initially stocked into an unknown number of baskets on an adjustable long line. At some point, stocks were moved from one lease to another within the Oyster Bay AUZ and each stock placed into one 14 mm bag inside a floating cage. Due to the limited number of replicates, statistical analysis was not conducted on data in this sample period. Conversely at site B, LA oysters had higher average shell height (41.3 mm) and whole weight (9.0 g) compared to FL oysters (36.4 mm, SH; 7.9 g, FL) (Table 7). Average growth rates obtained for FL oysters were 0.13 mm SH/day and for LA oysters were 0.19 mm SH/day, 33-50% lower than rates obtained for oysters at Alligator Harbor over the same period. Unfortunately, it was difficult to assess if the transfer of oysters affected mortality, which was high for the FL stock (18.8%), while mortality was minimal for the LA stock (0.9%). After sampling, the stocks were split into two bags and placed back into a floating cage.

Sample Period 2 – March 29, 2021; 181 days from plant; 64 days from sample period 1

At farm site OB-A, triploid stocks had similar shell heights and whole wet weights (Table 7). Growth rates over this 64-day period were 0.17 mm SH/day for FL and LA oysters, similar to rates obtained in the prior sampling period. Genetic stock had no effect on interval mortality, which again was low (<1%), during this sample period. Cumulative mortality was also similar between stocks (1.5%, FL; 0.9%, LA). After sampling, oysters of each stock were divided based on weight into seven 14 mm bags.

At farm site OB-B, a similar increase in shell height was observed in FL and LA oysters (Table 7). In addition, average whole weights were similar. Statistical analysis was not conducted on data in this sample period due to the limited number of replicates. Growth rates over this 64-day period were higher at this site (0.31 mm SH/day, FL; 0.26 mm SH/ day, LA) compared to the OB-A site. Interval mortality was low for LA oysters (0.8$\pm$0.3%) and slightly higher for FL stocks (4.6$\pm$0.1%), whereas cumulative mortalities were 23.4% (FL) and 1.8% (LA). After sampling, FL oysters were restocked into two 14 mm bags (264/bag) and LA oysters into three 14 mm bags (272/bag); bags were placed inside two floating cages.

Sample Period 3 – June 1, 2021; 245 days from plant, 64 days from sample period 2

Shell height of oysters at farm site OB-A continued to be similar between stocks (Table 7). However, during this sample period FL oysters had significantly higher (p=0.033) whole weight (54.4$\pm$3.9 g) compared to LA oysters (49.4$\pm$3.9 g,). Growth rates were higher than in the previous sampling period with a slight difference between stocks (0.21 mm SH/day, FL; 0.24 mm SH/day, LA). There was a significant effect (p=0.032) of genetic stock on mortality with lower mortalities for FL oysters (8.1$\pm$2.3%) compared to LA oysters (12.9$\pm$4.3%) during this sample period. Cumulative mortalities were 9.6% for FL oysters and 13.8% for LA oysters. After sampling, 100 oysters of each stock were placed into seven replicate bags (10.8-11.9 lbs/bag, FL; 9.3-11.0 lbs/bag, LA). The remaining oysters were split according to the grower's protocols. The seven bags were followed for an additional six weeks until harvestable-sized oysters were obtained.

At farm site OB-B, average values for shell height, whole weight, interval survival, and growth rates were similar between stocks (Table 7). Again, statistical analysis was not conducted on data in this sample period due to the limited number of replicates. Cumulative mortality for LA oysters was comparable to site A (8.5%), whereas FL mortality was higher at 29.5%. After sampling, FL oysters were restocked into three 14 mm bags (155/bag) and LA oysters into five 14 mm (160/bag) and placed inside two floating cages.

Sample Period 4 (Harvest) – July 13, 2021; 287 days from stock, 42 days from sample period 3

At 9.4 months from planting seed, oysters from seven replicate bags of each genetic stock were harvested at Site OB-A. Overall growth, survival, and mortality were determined (Table 8). Growth in terms of shell height, shell length, wet meat weight, dry meat weight, and condition index did not differ between stocks. However, FL oysters had significantly higher shell width and whole wet weight. Growth rates (0.22-0.24 mm SH/day) during this interval of 42 days were the same for both stocks as were growth rates over the entire culture period of 287 days (0.19 mm SH/day, FL; 0.20 mm SH/day, LA). Fan and cup ratios of both stocks exceeded industry's standards; however, cup ratios were higher for LA oysters compared to FL oysters, while fan ratios were similar between stocks. There was a significant effect of genetic stock on interval mortality as LA oysters had higher mortalities (26.4±5.1%) than FL oysters (15.9±1.8%) during this sample period. Cumulative mortality increased to 40.1% for LA oysters compared to 25.4% for FL oysters.

At site OB-B, overall growth, survival, and mortality were determined from three replicate bags of FL oysters and five replicate bags of LA oysters (Table 8). Genetic stock had no effect on shell height, shell length, whole wet weight, wet meat weight, dry meat weight, and condition index. Louisiana oysters had significantly higher average shell width (27.5 mm) compared to Florida oysters (26.1 mm). Growth rates during this interval of 42 days were higher than those at OB-A site for LA oysters (0.33 mm SH/day) and FL oysters (0.25 mm SH/day). Growth rates over the 287-day culture period were within the range of those at OB-A (0.20 mm SH/day, FL; 0.21 mm SH/day, LA). Both stocks fell within industry's standards for shell shape, however, cup ratios were higher for LA oysters compared to FL oysters, while fan ratios were similar between stocks. There was not a significant effect of genetic stock on interval mortality (32.0±3.2%, FL; 31.0±7.2%, LA) as values were similar over this sampling period but higher than those observed at Site OB-A during the same period. Cumulative mortality of 39.5% for LA oysters fell within the range found for oysters at site OB-A; the highest cumulative mortality at the two Oyster Bay farms was for FL oysters (61.5%), which reflected the early loss of oysters during lease transfers prior to the first sampling period.

Relationships

Comparisons of temperature and salinity indicated that most pairwise comparisons were statistically significant (ANOVA and post-hoc p-values were almost all less than 0.05) due to the high statistical power from the large number of repeated measurements (n=1,487–4,411 samples per period; Table 9). Some of these appeared meaningful while others negligible. Mean differences in temperature between site and period were indicated to be significant in the ANOVA and post-hoc test with AH consistently higher than OB. However, the magnitude of the mean difference was less than 1% and differences between sites were less than <0.5°C for all periods (Figure 3A; Table 9). The effect of salinity was more pronounced. On average, AH had approximately 8.5 ppt higher salinity than OB, a difference of approximately 40% (Figure 3B, Table 9). Meanwhile differences in salinity in periods were all less than 3 ppt.

Growth rates for both shell height and weight were affected by the factors of sampling period and site, but not affected by the factors of stock type, Dermo index, nor *Polydora* index (Tables 10&11). The health indexes were calculated by multiplying mean severity × mean prevalence (also referred to as weighted prevalence). Strong correlations were observed between severity and prevalence for Dermo (Figure 4A, slope=1.25, R^2=0.75) and *Polydora* (Figure 4B,

slope=0.77, R^2=0.70). Although the sampling period and site were significant for both of the growth rate ANOVAs, the direction and magnitude of these effects differed between the two analyses. For, shell height growth by far the fastest growing period observed was sample period 1 at AH, which was dramatically higher than the corresponding growth rate at OB as well as 1.6–2.7 times higher than the shell height growth rate observed in any of the other sampling periods (Figure 5A). In comparison, shell height growth at the OB site was lower in the initial periods but significantly higher in sample periods 2 and 3 (Table 11). For the response variable of weight growth, sample period was the largest driver of weight growth with a clear trend of faster weight growth in the later periods; sample periods 3 and 4 had weight growth rates approximately 10-times higher than in sample periods 1 and 2 (Table 11, Figure 5B). Weight growth was also affected by site, with overall weight growth rate being higher at AH, although this effect was relatively small compared to sampling period (Table 11, Figure 5C).

Survival rates were also affected by sampling period and site, and, to a lesser extent, stock (Tables 10&11). The first two periods had almost 100% survival, and survival rates dropped by >25% in sample periods 3 and 4. Overall survival was higher for FL oysters compared to LA oysters. Moreover, the site × sampling period interaction was determined in the GLM to be significant with oysters at OB having higher survival rates in sample periods 2 and 3 (note that no comparisons were made for period 4). Interestingly, Dermo index was determined to have a slight be significantly significant positive effect on survival. A post-hoc assessment of survival rate plotted against Dermo index indicates that, given the small sample sizes per period ($n < 10$), several points exert a large degree of leverage on the regressions (Figure 6). Although a regression developed with all the data shows this relationship to be negative, regressing by individual sampling periods are horizontal or slightly positive, which would explain the GLM result. Ultimately, the primary driver appears to be sampling period, as also indicated by the GLM results. This is shown in Figure 6 by the downward shift of the mostly horizontal regression lines.

DISCUSSION

Water Quality

Temperature and salinity are dominate factors influencing the biology and physiology of eastern oysters in estuaries along the Atlantic and Gulf of Mexico coasts, yet eastern oysters are well known for their broad tolerance of temperature and salinity (Shumway 1996). It is critical to understand the potential impacts of temperature and salinity changes, particularly the higher salinities experienced in Alligator Harbor, to better understand production of cultured oysters in Florida. Previous studies have shown that optimal salinity and temperature combinations for oyster health and productivity are population dependent, and most of these studies, until recently, have been conducted on the Atlantic coast (Buford et al. 2014). In this study, we had the opportunity to evaluate triploid oysters, which were created using tetraploids developed from natural populations collected in Apalachicola Bay. In LaPayre et al. (2016), salinity and temperature (season) were found to critically control oyster growth and mortality in Louisiana public reefs, suggesting that seasonal changes affecting water quality have profound impacts on oyster populations. In this study, potential stressful conditions occurred at the Alligator Harbor AUZ as temperatures reached 87ºF and salinities exceeded 30 ppt in the last sample period.

Slightly higher water temperatures were experienced at the Oyster Bay AUZ during the months of June (maximum 88.5°F) and July (maximum 86.5°F) but salinities during this period were moderate averaging 18.4 ppt in June and 16.5 ppt in July.

Growth

Measuring oyster growth through morphology and biomass is highly influenced by environmental conditions. According to Wadsworth et al. (2019), shell morphometry (i.e., shell height, length, and width) is also influenced in aquaculture by how they are handled, whereas biomass (i.e., whole, tissue, and shell weight) is an indicator of food quality, food availability, oyster filtration rate, and fecundity. When determining growth in this study, both shell height and whole wet weight were measured to account for effects of environmental conditions.

Average shell height of Florida and Louisiana triploid oysters from plant to harvest at one farm (AH-A) located at the Alligator Harbor AUZ and one farm (OB-A) located at the Oyster Bay AUZ are compared in Figure 7. The largest differences in oyster growth occurred between lease locations, although growth also displayed temporal variation. Oyster growth rates are reported to usually increase with increasing temperature, as well as with increasing salinity, and are dependent on initial size (Harding 2007, Kraeuter et al. 2007). In this study, growth rates at Alligator Harbor were highest over the sample period with the lowest average water temperatures and declined as water temperatures increased. Nonetheless, growth was continuous over the entire culture period, as monthly temperature averages were above 65°F for more than 60% of the study period and rarely dropped below 50°F, in contrast to more temperate regions, where a period of no growth occurs in winter (Kraeuter et al. 2007).

Overall growth rates of 0.27 mm/day SH at the AH-A farm site and 0.20 mm/day SH at the OB-A site compared with those measured in other locations across the U.S. Atlantic (reviewed by Kraeuter et al. 2007) are among the highest reported (0.06 mm/day for the East coast). Growth rates at both lease sites were within the range of those reported by Sturmer et al. (2018) in documenting triploid oyster production at multiple farms in Florida during 2016-18. In comparison, triploids at three farm sites along the Gulf of Mexico coast had an average growth rate of 0.23 mm/day (Bodenstien et al. 2021). In another Gulf study, monthly growth rates from 40 years of monitoring data from Louisiana's public oyster reefs ranged from 0.003 to 0.29 mm/day and differed between basins and among seasons (Lowe et al. 2017); overall, growth was maximized at a lower temperature and salinity.

Differences in growth rates between lease locations in this study were likely due to differences in salinity and phytoplankton abundance, as variations in water temperature over sampling periods at the two locations were minor. Growth rates (SH) were initially higher at the Alligator Harbor farm site, which had high salinity (29.4 ppt overall average) and high phytoplankton biomass, as evidenced in the SEEDIT report by Dr. Phlips in which mean total phytoplankton biomass was 590±338 µg carbon/liter compared to 93±32 µg carbon/liter at the Oyster Bay site. Callam et al. (2016) reported that high salinity (an environmental stressor) can cause triploid oysters to grow more quickly but such a stressor could also contribute to higher mortality rates. Growth did vary seasonally at the Alligator Harbor farm site as rates were statistically higher during the first sampling period (0.36-0.39 mm/day) compared to the next two sampling periods (0.14-0.16 mm/day), but rates (in terms of shell metrics) typically decrease as oyster size and age increase. In contrast, growth rates at Oyster Bay over the last two sampling

periods (0.20-0.25 mm/day) were significantly higher than the first two sample periods (0.17-0.19 mm/day).

Average whole wet weight of Florida and Louisiana triploid oysters from plant to harvest at one farm (AH-A) located at the Alligator Harbor AUZ and one farm (OB-A) located at the Oyster Bay AUZ are compared in Figure 8. Unlike rates determined for shell height, mean growth rates measured in whole wet weight increased as oysters increased in shell height and age with the highest rates observed over the last sampling periods at both farm locations and for both triploid stocks (0.53-0.66 g/day). Average oyster weight increased two-fold at Alligator Harbor over the last 56 days of culture and more than tripled over the last 106 days of growout at Oyster Bay. Overall rates for the entire culture period ranged from 0.28-0.31 g/day at Alligator Harbor to 0.26-0.28 g/day at Oyster Bay. In comparison, mean growth rate for triploid oysters as cited in 29 published studies (148 experiments) was 0.16 g/day (Wadsworth et al. 2019). Further, differences in whole weight between ploidy stocks were notable at the Oyster Bay site during the last two sample periods, unlike Alligator Harbor where whole weights were similar, suggesting that population responses under more favorable environmental conditions (such as lower salinities) may be population dependent.

Mortality

Interval and cumulative mortalities of Florida and Louisiana triploid oysters from plant to harvest at one farm (AH-A) located at the Alligator Harbor AUZ and one farm (OB-A) located at the Oyster Bay AUZ can be seen in Figure 9. Environmental factors may have also played a role in driving oyster mortality, although oysters at both lease locations were affected similarly. As water temperatures increased, so did mortality of oysters at both sites and for both triploid stocks. In this study, mortalities at Alligator Harbor increased from 0.9-1.3% to 28.0-30.7% over the last sample period with temperatures and salinities averaging 74.6^{o}F and 29.0 ppt, respectively; whereas at Oyster Bay, mortalities of 9.6-13.8% increased to 15.9-26.6% over the last sample period with average temperatures of 83.5^{o}F and salinities of 17.8 ppt. Additionally, it is important to note that commercial farmers at Alligator Harbor reported higher mortalities over this same time period.

Higher water temperatures have been correlated with increased oyster mortality, although higher water temperature alone may not be lethal (Cheney et al. 2000). Bodenstein et al. (2021) found that, as water temperatures increased, oyster mortality (of both ploidies) increased; however, no significant relationship was found between salinity and mortality. In other studies, the combination of high water temperatures and high salinities resulted in higher mortality rates than any other temperature/salinity combinations in Louisiana (La Peyre et al. 2016, Rybovich et al. 2016, Lowe et al. 2017). Of those studies, only La Peyre et al. (2016) quantified mortality rates at the high salinity and high temperature combination, indicating that salinity was the most significant predictor of oyster mortality, and water temperature having a significant positive relationship with mortality. Further, areas of fast growth in Louisiana were associated with increased mortality; thus, commercial production was suggested to be dependent on locations that provide both adequate growth and limited mortality (Sehlinger et al. 2019). The protistan parasite *Perkinus marinus* that causes dermo disease is also responsible for oyster mortality at high salinities (Rybovich et al. 2016). Although in this study, parasite weighted prevalence (prevalence x severity) was ranked low on the Mackin scale (see Dr. Kane's SEEDIT health

report), which may have resulted in increased stressful conditions but would not be considered lethal.

Differences in mortality rates may also be due to differences in other environmental conditions, such as turbidity, food quality, and oxygen (Shumway 1996), or population genetic differences. A positive response to survival was found at Oyster Bay for the genetic lines, with significant differences in mortalities between Florida and Louisiana triploids at the third and final sampling periods. These differences demonstrate the potential for selective breeding; selecting for resistance in tetraploid, as well as diploid, parents could create a triploid line with higher resistance to summer mortality events (Callam 2013).

SUMMARY

Ultimately, the interaction of multiple stressors must be observed to understand their effects on the growth and mortality of triploid oysters in Florida' subtropical conditions. Water quality (temperature, salinity, parasitism prevalence and severity) explanatory (independent) variables were identified that most influenced oyster production; those relationships are described. Oyster response (dependent) variables included measures of growth and mortality observations. Determining the effects of differences in environmental conditions and population responses remains critical in developing more informed management decisions.

In this pilot study, "sentinel" farms were established at two aquaculture lease areas in the Panhandle to begin examining basic but key relationships between environmental and production variables. Salinity influenced oyster growth in terms of shell height, whereas temperature and salinity affected weight gain. Higher salinities at the Alligator Harbor AUZ were associated with faster growth but also higher mortality, and Dermo and *Polydora* indexes. However, neither Dermo nor *Polydora* parasitism demonstrated any patterns related to mortality. Results of phytoplankton monitoring during months when oyster mortalities typically occur (see Dr. Phlips' report) showed that dinoflagellate species associated with harmful algal blooms were not prevalent. Time from plant to harvest was six months at the Alligator Harbor site and 9.4 months at the Oyster Bay site, resulting in some of the highest growth rates reported for the eastern oyster (Figure 10). Lower mortality was observed in triploids using local (Florida) tetraploid stocks than Louisiana stocks. Mortality differences between genetic stocks indicate potential to develop a triploid line with higher resistance to environmental stressors and mortality events.

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Table 1. Monthly average water temperatures, including standard deviation, maximum and minimum values, from October 1, 2020, to May 25, 2021, at the Alligator Harbor (AH) Aquaculture Use Zone.

Month	Water Temperatures (°F)		
	Average ± SD	Maximum	Minimum
October	78.1 ± 3.1	84.1	70.1
November	70.7 ± 3.8	78.1	60.9
December	58.2 ± 3.0	65.7	48.3
January	57.8 ± 3.9	65.7	50.5
February	60.0 ± 4.6	73.4	49.6
March	69.2 ± 4.3	81.8	59.5
April	71.8 ± 3.6	80.7	60.7
May	78.3 ± 3.1	87.6	61.4

Table 2. Monthly average water salinities, including standard deviation, maximum and minimum values, from October 1, 2020, to May 25, 2021, at the Alligator Harbor (AH) Aquaculture Use Zone.

Month	Water Salinities (ppt)		
	Average ± SD	Maximum	Minimum
October	29.1 ± 1.3	31.1	24.9
November	30.6 ± 0.6	31.9	28.6
December	32.2 ± 0.7	33.2	29.7
January	30.5 ± 1.4	32.3	0.2
February	28.3 ± 1.3	30.3	26.0
March	28.0 ± 0.6	29.4	25.9
April	28.9 ± 0.8	30.8	27.8
May	28.7 ± 1.2	30.5	25.3

Table 3. Monthly average water temperatures, including standard deviation, maximum and minimum values, from October 1, 2020, to July 13, 2021, at the Oyster Bay (OB) Aquaculture Use Zone.

Month	Water Temperatures (°F)		
	Average ± SD	Maximum	Minimum
October	77.4 ± 3.6	84.3	69.1
November	69.8 ± 4.2	78.3	58.8
December	57.0 ± 3.1	64.8	48.3
January	57.5 ± 4.0	68.8	48.5
February	59.5 ± 4.8	72.4	48.3
March	68.2 ± 5.0	88.5	48.5
April	70.9 ± 4.0	80.0	58.0
May	78.2 ± 3.2	85.2	70.3
June	83.6 ± 2.2	88.5	72.7
July	83.0 ± 1.5	86.5	76.7

Table 4. Monthly average water salinities, including standard deviation, maximum and minimum values, from October 1, 2020, to July 13, 2021, at the Oyster Bay (OB) Aquaculture Use Zone.

Month	Water Salinities (ppt)		
	Average ± SD	Maximum	Minimum
October	18.5 ± 0.6	20.1	16.3
November	23.3 ± 3.3	27.3	13.7
December	26.1 ± 0.5	27.5	24.2
January	22.2 ± 1.2	25.3	19.1
February	23.4 ± 0.6	25.0	21.4
March	17.8 ± 1.8	21.1	13.8
April	17.1 ± 3.9	25.7	12.2
May	21.3 ± 2.1	30.6	15.2
June	18.4 ± 0.5	19.4	16.7
July	16.5 ± 0.7	17.9	14.0

Table 5. Averages and standard deviations of growth (shell height, whole wet weight), survival, and mortality over two sampling periods for Florida and Louisiana triploid oysters cultured at one farm (A) within the Alligator Harbor (AH) Aquaculture Use Zone. Parameter values with different letters indicate significant differences (p<0.05) among genetic stocks.

Alligator Harbor Aquaculture Use Zone						
Sample Period	**Triploid stocks** (replicates)	**Shell Height (mm)**	**Whole Wet Weight (g)**	**Interval Survival (%)**	**Interval Mortality (%)**	**Cumulative Mortality (%)**
1/26/2021	**Florida** (n=2)	66.9 ± 1.5	25.9 ± 2.8	99.1 ± 0.1	0.9 ± 0.1	0.9
	Louisiana (n=3)	61.8 ± 3.7	25.2 ± 2.9	99.6 ± 0.3	0.4 ± 0.3	0.4
3/30/2021	**Florida** (n=3)	75.6 ± 3.2[a]	39.6 ± 4.0[a]	98.7 ± 0.9[a]	1.3 ± 0.9[a]	2.2
	Louisiana (n=3)	71.1 ± 4.8[a]	39.1 ± 3.0[a]	99.1 ± 0.5[a]	0.9 ± 0.5[a]	1.3

Table 6. Averages and standard deviations of growth (shell height, whole wet weight), survival, and mortality at harvest for Florida and Louisiana triploid oysters cultured at one farm (A) within the Alligator Harbor (AH) Aquaculture Use Zone. Parameter values with different letters indicate significant differences among genetic stocks if probability values (p) are less than 0.05.

Alligator Harbor Aquaculture Use Zone, 5/25/2021			
Grower	**A** (n=3 per stock)		
Triploid Stocks	**Florida**	**Louisiana**	**Probability Values (p)**
Shell Height (mm)	84.3 ± 3.7[a]	79.5 ± 2.2[a]	0.311
Shell Length (mm)	53.4 ± 2.2[a]	54.4 ± 1.8[a]	0.586
Shell Width (mm)	25.7 ± 0.4[a]	23.0 ± 0.1[b]	0.0004
Whole Wet Weight (g)	75.3 ± 5.4[a]	69.0 ± 2.8[a]	0.149
Wet Meat Weight (g)	7.7 ± 0.2[a]	6.4 ± 3.5[a]	0.979
Dry Meat Weight (g)	1.9 ± 0.03[a]	1.5 ± 0.3[a]	0.226
Condition Index	9.3 ± 0.5[a]	8.6 ± 0.9[a]	0.272
Fan Ratio	0.64 ± 0.01[a]	0.69 ± 0.01[b]	0.002
Cup Ratio	0.31 ± 0.01[a]	0.29 ± 0.01[a]	0.142
Interval Survival (%)	72.0 ± 10.9[a]	69.3 ± 20.1[a]	0.887
Interval Mortality (%)	28.0 ± 10.9[a]	30.7 ± 20.1[a]	0.887
Cumulative Mortality (%)	30.2	32.0	

Table 7. Averages and standard deviations of growth (shell height, whole wet weight), survival, and mortality over three sample periods for Florida and Louisiana triploid oysters cultured at two farms (A, B) within the Oyster Bay (OB) Aquaculture Use Zone. Parameter values with different letters indicate significant differences ($p<0.05$) among genetic stocks.

Oyster Bay Aquaculture Use Zone							
Sample Period	**Grower** (replicates)	**Triploid Stock**	**Shell Height (mm)**	**Whole Wet Weight (g)**	**Interval Survival (%)**	**Interval Mortality (%)**	**Cumulative Mortality (%)**
1/25/2021	**A** (n=4)	**Florida**	42.9 ± 2.4[a]	14.5 ± 5.1[a]	99.1 ± 1.0[a]	0.9 ± 1.0[a]	0.9
		Louisiana	36.4 ± 2.8[a]	10.5 ± 1.3[a]	99.8 ± 0.2[a]	0.2 ± 0.2[a]	0.2
	B (n=1)	**Florida**	36.4	7.9	81.2	18.8	18.8
		Louisiana	41.3	9.0	99.1	0.9	0.9
3/29/2021	**A** (n=4)	**Florida**	53.7 ± 1.4[a]	24.3 ± 1.3[a]	99.4 ± 0.4[a]	0.6 ± 0.4[a]	1.5
		Louisiana	49.2 ± 3.2[a]	20.9 ± 3.7[a]	99.3 ± 0.2[a]	0.7 ± 0.2[a]	0.9
	B (n=2)	**Florida**	56.1 ± 0.5	20.0 ± 3.3	95.4 ± 0.1	4.6 ± 0.1	23.4
		Louisiana	55.1 ± 3.5	21.4 ± 3.5	99.2 ± 0.3	0.8 ± 0.3	1.8
6/1/2021	**A** (n=7)	**Florida**	67.1 ± 2.2[a]	54.4 ± 3.9[a]	91.9 ± 2.3[a]	8.1 ± 2.3[a]	9.6
		Louisiana	64.9 ± 2.3[a]	49.4 ± 3.9[b]	87.1 ± 4.3[b]	12.9 ± 4.3[b]	13.8
	B (FL, n=2; LA, n=3)	**Florida**	67.0 ± 1.8	46.0 ± 2.8	93.9 ± 1.0	6.1 ± 1.0	29.5
		Louisiana	66.1 ± 2.5	45.1 ± 5.1	93.2 ± 3.0	6.8 ± 3.0	8.5

Table 8. Averages and standard deviations of growth (shell height, whole wet weight), survival, and mortality at harvest for Florida and Louisiana triploid oysters cultured at two farms (A, B) within the Oyster Bay (OB) Aquaculture Use Zone. Parameter values with different letters indicate significant differences among genetic stocks if probability values (*p*) are less than 0.05.

Oyster Bay Aquaculture Use Zone, 7/13/2021						
Grower	**A**			**B**		
Triploid Stocks (replicates)	**Florida** (n=7)	**Louisiana** (n=7)	**Probability Values (p)**	**Florida** (n=3)	**Louisiana** (n=5)	**Probability Values (p)**
Shell Height (mm)	76.2 ± 2.5[a]	75.1 ± 1.2[a]	0.466	77.6 ± 2.4[a]	80.1 ± 3.3[a]	0.097
Shell Length (mm)	56.7 ± 2.0[a]	56.2 ± 1.0[a]	0.556	54.0 ± 0.6[a]	55.2 ± 1.1[a]	0.144
Shell Width (mm)	28.8 ± 1.1[a]	27.2 ± 0.7[b]	0.007	27.5 ± 0.2[a]	26.1 ± 0.7[b]	0.019
Whole Wet Weight (g)	82.3 ± 6.9[a]	75.7 ± 2.5[b]	0.047	75.9 ± 3.8[a]	81.9 ± 7.2[a]	0.245
Wet Meat Weight (g)	9.6 ± 1.0[a]	8.8 ± 0.9[a]	0.113	7.4 ± 0.7[a]	7.0 ± 0.8[a]	0.446
Dry Meat Weight (g)	2.0 ± 0.2[a]	1.8 ± 0.2[a]	0.192	1.3 ± 0.1[a]	1.3 ± 0.2[a]	0.580
Condition Index	8.7 ± 0.4[a]	8.4 ± 1.3[a]	0.710	7.0 ± 0.5[a]	6.7 ± 0.7[a]	0.555
Fan Ratio	0.75 ± 0.01[a]	0.75 ± 0.01[a]	0.667	0.70 ± 0.02[a]	0.69 ± 0.02[a]	0.553
Cup Ratio	0.38 ± 0.01[a]	0.36 ± 0.01[b]	0.003	0.36 ± 0.01[a]	0.33 ± 0.01[b]	0.002
Interval Survival (%)	84.1 ± 1.8[a]	73.6 ± 5.1[b]	0.0001	68.0 ± 3.2[a]	69.0 ± 7.2[a]	0.799
Interval Mortality (%)	15.9 ± 1.8[a]	26.4 ± 5.1[b]	0.0001	32.0 ± 3.2[a]	31.0 ± 7.2[a]	0.799
Cumulative Mortality (%)	25.4	40.1		61.5	39.5	

Table 9. Environmental comparisons for salinity and temperature at Alligator Harbor (AH) and Oyster Bay, with estimated confidence intervals and number of measurements (n).

Measurement	Period	Mean AH	Mean OB	Abs. diff.	LowCI	UpCI	n
Temperature (°C)	1	19.13	18.70	0.42	0.15	0.70	2808
	2	17.75	17.37	0.39	0.15	0.62	1487
	3	23.67	23.18	0.49	0.30	0.68	1305
Salinity (ppt)	1	31.11	23.09	8.02	7.92	8.12	4411
	2	28.93	20.42	8.51	8.37	8.64	1925
	3	28.99	20.10	8.89	8.73	9.04	1798

Table 10. Data summary showing means of survival rate and growth rates by sample period, stock, and site, with number of replicate measures indicated.

Sample period	Stock	Site	Replicates	Survival	Shell height (µm / day)	Weight (mg / day)
1	FL	AH	2	0.992	392	208
1	FL	OB	4	0.992	188	111
1	LA	AH	3	0.995	365	202
1	LA	OB	4	0.998	166	77
2	FL	AH	3	0.987	136	221
2	FL	OB	4	0.994	169	153
2	LA	AH	3	0.991	145	217
2	LA	OB	4	0.993	169	162
3	FL	AH	3	0.720	155	638
3	FL	OB	7	0.919	209	470
3	LA	AH	3	0.693	151	534
3	LA	OB	7	0.871	246	445
4	FL	OB	7	0.841	217	665
4	LA	OB	7	0.736	242	626

Table 11. Model outputs growth rate in shell height (µm / day), growth rate in weight (mg / day), and survival rate (alive:dead). Differences between means were tested with generalized linear models for growth rates and logistic generalized mixed model for survival (binomial with logit-link). Fixed effects in the full models included the effects of sample period (categorical 1–4), site (Alligator Harbor [AH] or Oyster Bay [OB]), stock type (Florida [FL] or Louisiana [LA]), and disease indexes for Dermo and *Polydora*. Potential interactions between stock and site were also tested, and the interaction between sample period and all the other effects. The adequate models are shown for each response variable assessed as determined by backwards removal of insignificant effects. Effect estimates for the regression models are shown in relation to the intercept levels of sample period 1, the AH site, the FL stock. Effect estimates and confidence intervals for the survival rate GLM are show are given in odds ratios as calculated by exponentiating the logit-linked model.

Model	Effects	Estimate	LowCI	UpCI	z-score	P
Shell height growth	(Intercept)	376	339	412	20.31	<0.001
	Period 2	-235	-284	-186	-9.39	<0.001
	Period 3	-223	-272	-174	-8.89	<0.001
	Period 4	53	17	89	2.87	0.006
	Site: OB	-199	-245	-153	-8.43	<0.001
	Period 2 × Site: OB	227	164	291	7	<0.001
	Period 3 × Site: OB	274	213	334	8.81	<0.001
Weight growth	(Intercept)	199	149	248	7.86	<0.001
	Period 2	43	-14	100	1.49	0.142
	Period 3	369	316	421	13.67	<0.001
	Period 4	549	489	608	18.05	<0.001
	Site: OB	-102	-147	-57	-4.41	<0.001
Survival rate	(Intercept)	0.99	4.69	5.76	19.03	<0.001
	Period 2	0.56	0.3	1.1	-1.67	0.095
	Period 3	0.01	0	0	-15.33	<0.001
	Period 4	0.01	0	0	-12.03	<0.001
	Site: OB	1.19	0.5	2.8	0.41	0.679
	Stock: LA	0.68	0.6	0.8	-4.09	<0.001
	Dermo index	1.63	1.2	2.2	3.44	0.001
	Period 2 × Site: OB	1.27	0.4	3.7	0.44	0.662
	Period 3 × Site: OB	3.91	1.6	9.4	3.03	0.002

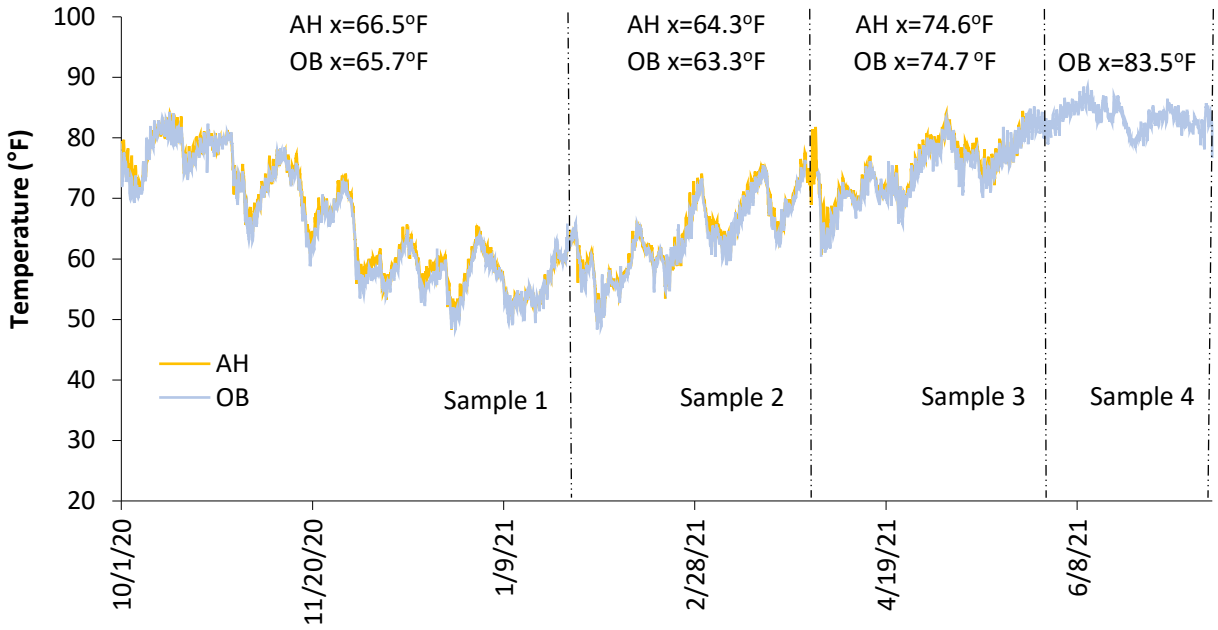

Figure 1. Continuous water temperature (°F) data from October 1, 2020, to May 25, 2021, at the Alligator Harbor (AH) Aquaculture Use Zone and from October 1, 2020, to July 13, 2021, at the Oyster Bay (OB) Aquaculture Use Zone. Means for each sample period are displayed.

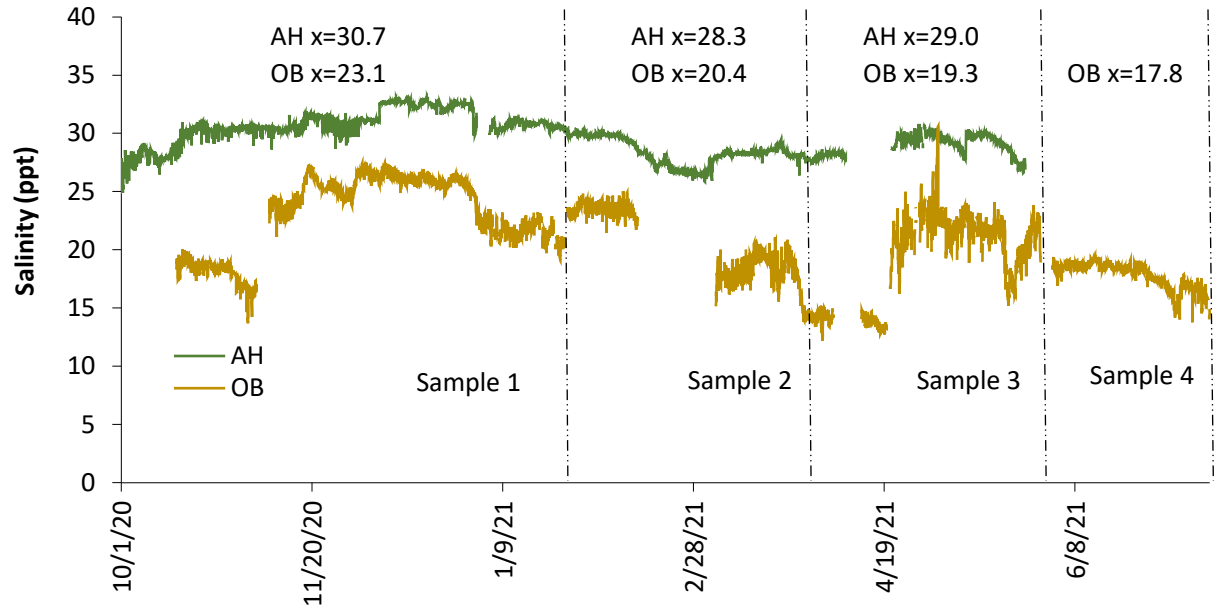

Figure 2. Continuous water salinity (ppt) data from October 1, 2020, to May 25, 2021, at the Alligator Harbor (AH) Aquaculture Use Zone and from October 1, 2020, to July 13, 2021, at the Oyster Bay (OB) Aquaculture Use Zone. Means for each sample period are displayed.

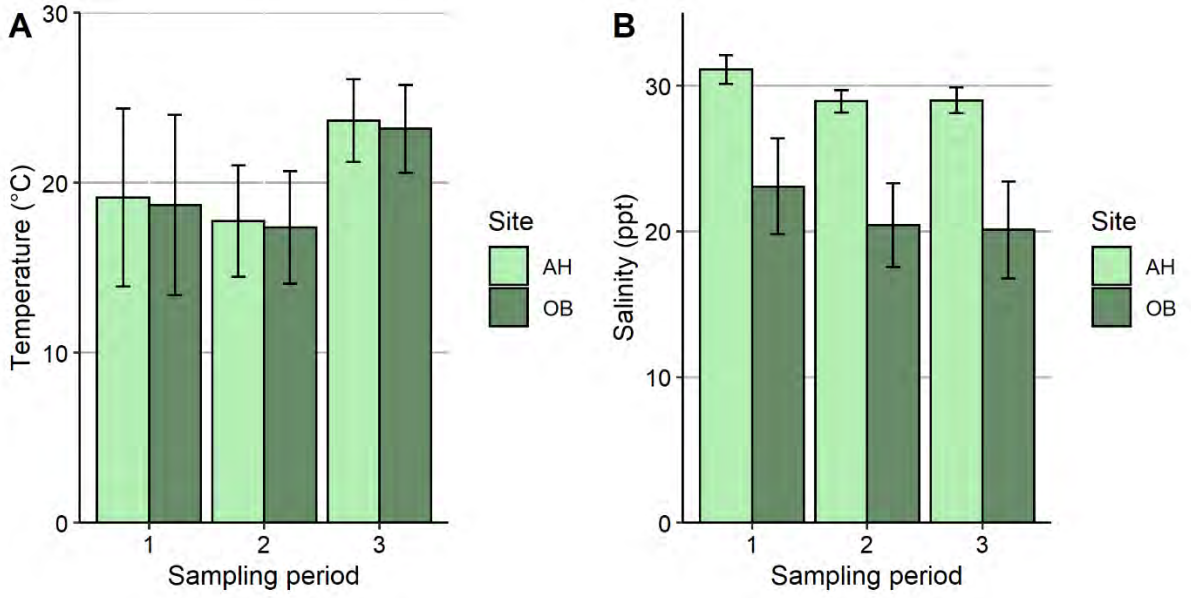

Figure 3. Environmental comparisons for **A**) mean (±SD) temperature and **B**) mean (±SD) salinity by site location (Alligator Harbor [AH] and Oyster Bay [OB]) and sampling period.

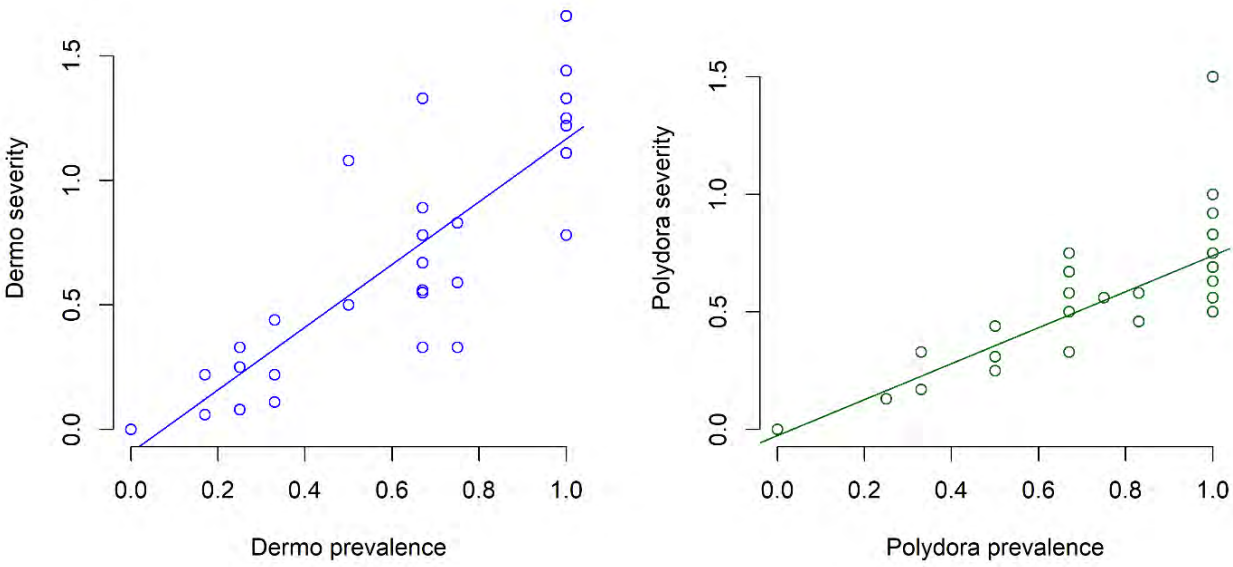

Figure 4. Linear relationship between disease severity and prevalence for **A**) Dermo and **B**) *Polydora*.

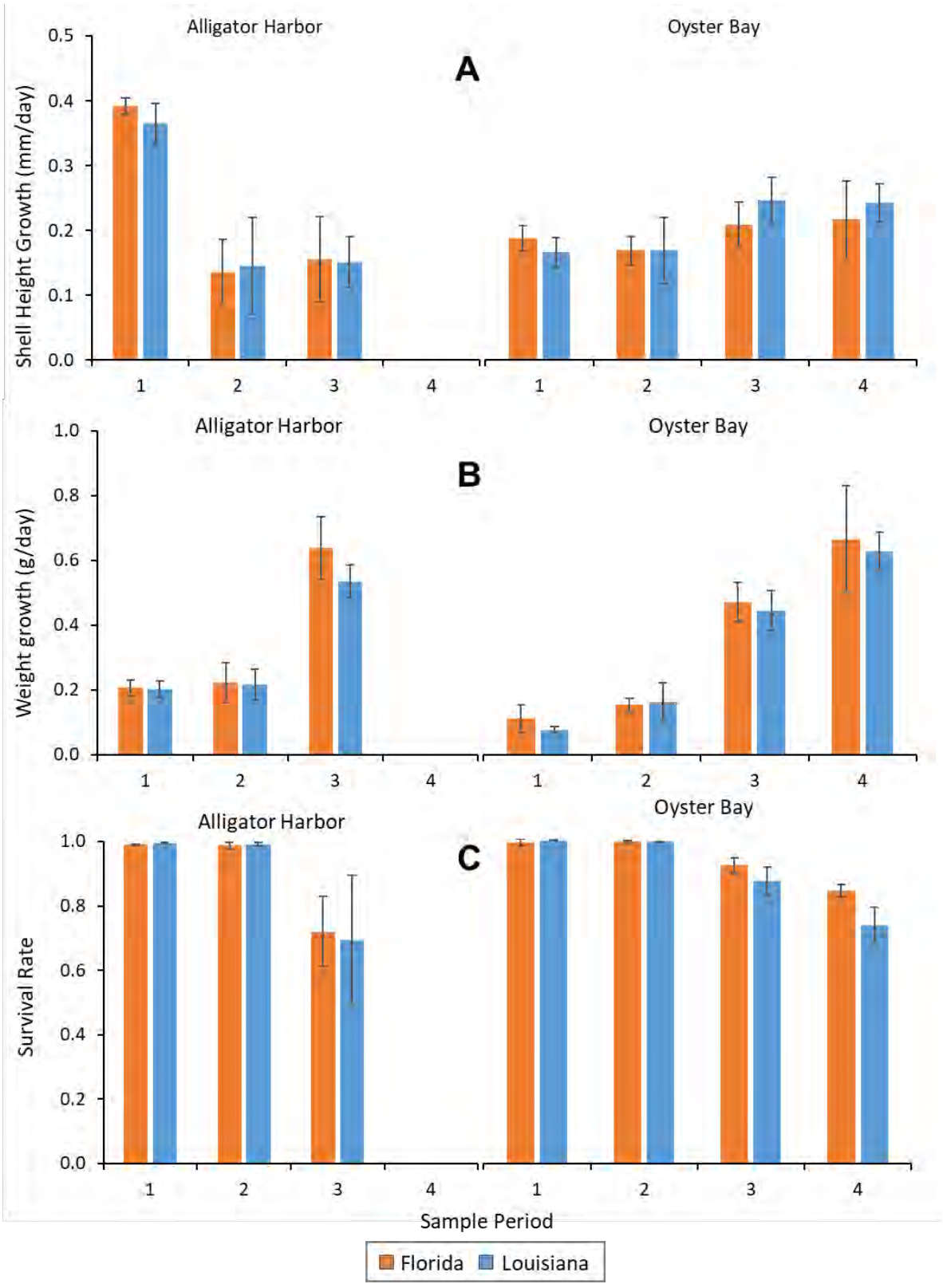

Figure 5. A) Mean (±SD) daily growth rate in shell height, **B**) daily growth rate in weight, and **C**) survival rate by sampling period, site, and stock type.

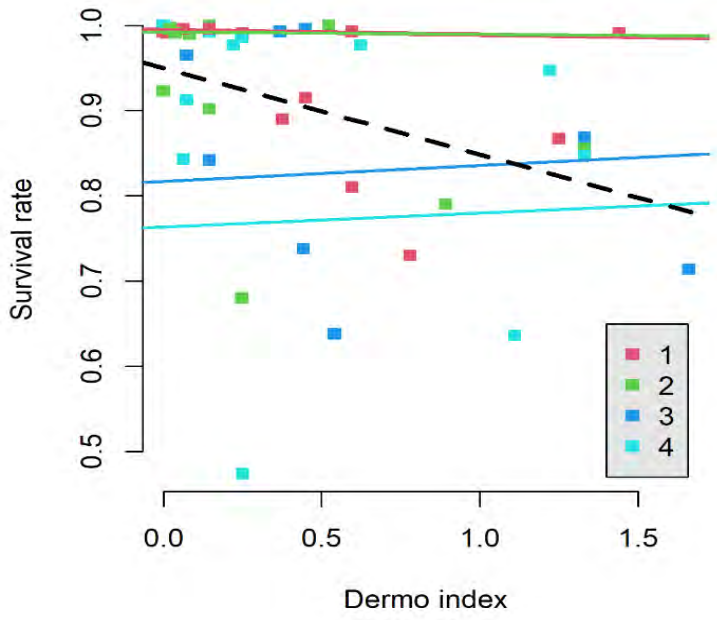

Figure 6. Proportional survival per oyster sample bag plotted against Dermo index and colored by sampling period. Regression lines are shown for all periods (black dashed line) and for individual periods 1 through 4.

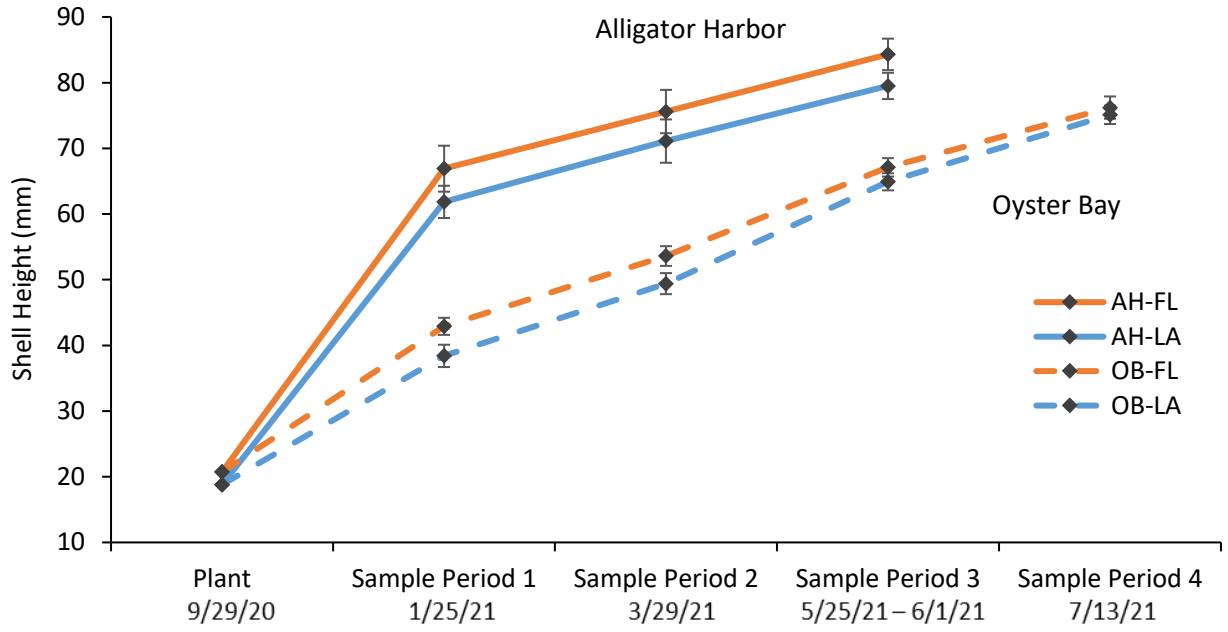

Figure 7. Average shell height (mm) of Florida (FL) and Louisiana (LA) triploid oysters from plant to harvest at farms (AH-A, OB-A) located at the Alligator Harbor (AH) Aquaculture Use Zone (AUZ) and Oyster Bay (OB) AUZ. Error bars denote 95% confidence intervals.

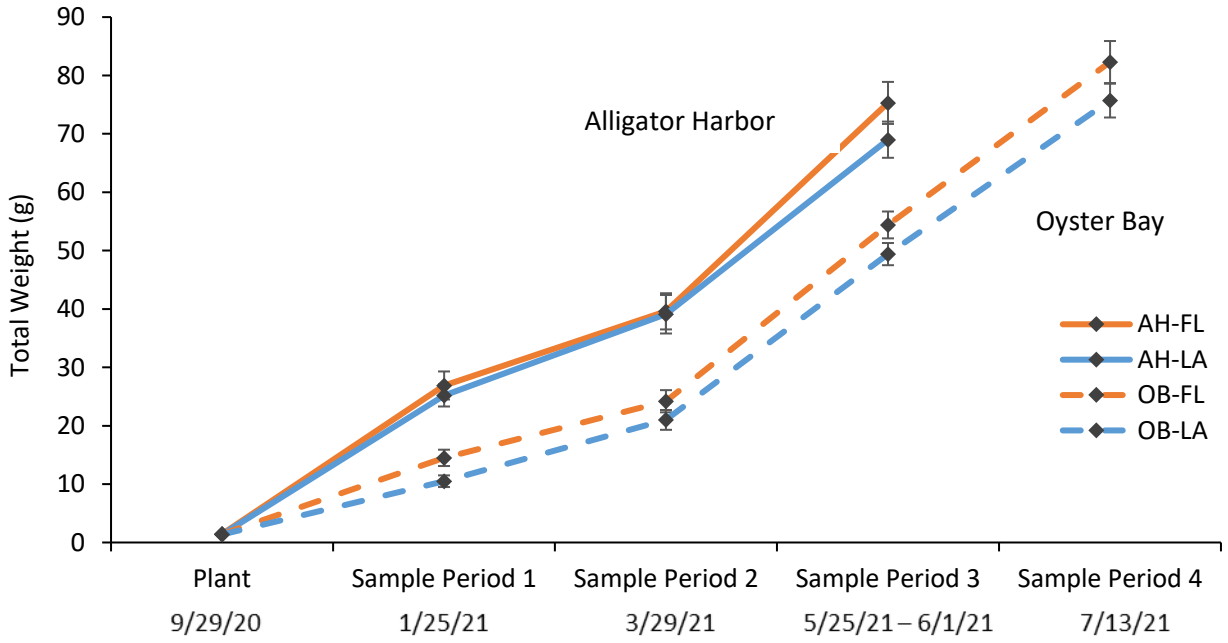

Figure 8. Average whole wet weight (grams) of Florida (FL) and Louisiana (LA) triploid oysters from plant to harvest at farms (AH-A, OB-A) located at the Alligator Harbor (AH) Aquaculture Use Zone (AUZ) and Oyster Bay AUZ. Error bars denote 95% confidence intervals.

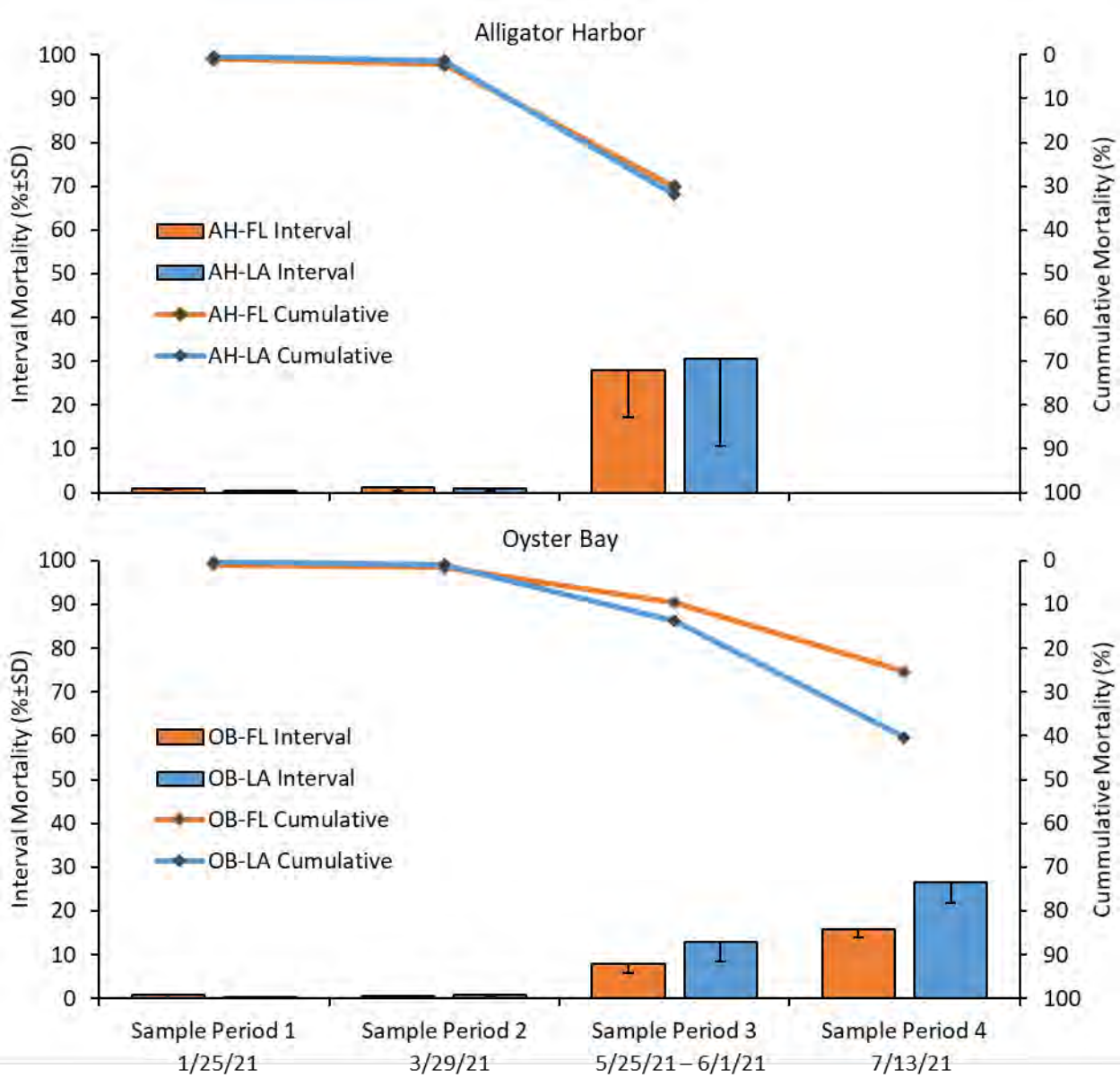

Figure 9. Interval and cumulative mortalities of Florida (FL) and Louisiana (LA) triploid oysters from plant to harvest at farms (AH-A, OB-A) located at the Alligator Harbor (AH) Aquaculture Use Zone and Oyster Bay (OB) Aquaculture Use Zone. Error bars denote 95% confidence intervals.

A

B

Figure 10. Triploid oysters (Florida stock on the left and Louisiana stock on the right) cultured at **A**) Alligator Harbor Aquaculture Use Area, Farm A, harvested March 25, 2021, and **B**) Oyster Bay Aquaculture Use Area, Farm A, harvested July 13, 2021.

Oyster FARMS Project

- SEEDIT -

Support for Emerging Enterprise Development Integration Teams

UF/IFAS

Oyster Health Assessments: Final Report

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February 28th, 2022

Appreciation to Ross Brooks for assistance with data management and visualizations, and Rebecca Rash and Lauren Hintenlang for sample processing and data collection.

Project Background

Shellfish aquaculture represents an important industry in Florida that supports sustainable harvests of high-quality, high-dollar protein. Oyster growers in Panacea, Florida voiced concerns over production issues and mortalities at a meeting held in Crawfordville this past January (2020). In response, this project will initiate a monitoring and assessment plan to generate preliminary information relating water quality to growth, survival, and health of cultured oysters. This Fall, “sentinel” farms will be established by working with industry partners in Franklin and Wakulla Counties. The following methods will be employed at commercial farms located at the Alligator Harbor and Oyster Bay aquaculture lease areas during 2020-21.

Water quality: Environmental factors are known to strongly influence oyster performance. During the Summer 2020, temperature data loggers (HOBO) will be deployed inside oyster bags/baskets at up to 20 farms to observe differences related to location, gear, and management practices. During field trials to be conducted from the Fall 2020 through Summer 2021 at four farms (two at each lease area), continuous data will be obtained from YSI multi-parameter sondes at Alligator Harbor and HOBO conductivity loggers at Oyster Bay.

Phytoplankton quality and quantity: Phytoplankton sampling kits will be provided to one participating grower at each growing location. Samples will be collected and preserved weekly from March through June (spanning the time period when recent mortalities were observed). Two sets of samples per farm will be analyzed monthly at the UF Algal Ecology Laboratory for abundance and species composition, noting presence and density of harmful algal bloom species. Additional preserved samples that coincide with an oyster mortality or harmful algal bloom event will be analyzed.

Oyster production: Juvenile (20mm SL) triploid oysters (n=500) will be provided in the Fall (2020) to participating farms. At planting, oysters will be measured for shell metrics. Growers will provide culture gear and maintenance. Oysters from three replicate bags at each farm will be sampled at bimonthly intervals by UF staff beginning in January through June/July 2021. Between site visits, growers will observe oysters and report if high mortality occurs. At each sampling period, dead oysters will be removed, counted, and measured.

Oyster health: Oysters will be collected pre-deployment and each sampling period from triplicate bags at participating farms at both growing locations. Oysters will be transported to the UF Aquatic Pathobiology Laboratory to assess the prevalence and severity of shell parasitism associated with boring sponge, worms and clams, biofouling, empirical meat condition, and Dermo prevalence and severity. The oyster health component is directed by Dr. Andy Kane, UF Aquatic Pathobiology Laboratory.

Project findings will be shared with industry members at workshops and may lead to the development of management practices resulting in higher or more reliable production efficiency.

UF Project Partners:

Leslie Sturmer, UF/IFAS Extension and Florida Sea Grant
Andy Kane, UF Department of Environmental and Global Health
Ed Phlips, UF/IFAS Fisheries and Aquatic Sciences
Ruth Francis-Floyd, UF/IFAS College of Veterinary Medicine
Erik Lovestrand, UF/IFAS Franklin County Extension

Oyster Health Assessment

Aims: To evaluate oyster health from two different oyster lineages at the start of the project and throughout grow-out at two aquaculture farms in Wakulla County. Oyster health outcomes will be evaluated with the project team relative to growth and survivorship *in situ*, water quality, phytoplankton dynamics, management variables, among others.

Deliverables: Oyster samples from both seed stocks, and from grow-out at two sentinel aquaculture farms, will be provided to Andy Kane at UF Aquatic Pathobiology Laboratory. Initial seed stocks were evaluated for health parameters "pre-planting," prior to deployment at the sentinel farms (this report). Up to four bimonthly health evaluations from replicate grow-out samples from both farms will conducted January through July.

Oyster health endpoints reported include height, condition index, Dermo prevalence and severity, and shell boring parasite prevalence and severity. Approaches to discern oyster condition and health metrics are described below:

Oyster Height:

Oyster "size" is measured as height, observed to the nearest millimeter using a caliper. Height (mm) is measured as the maximum distance between the umbo and the ventral valve margin (Figure 1).

Figure 1. External view of oyster dimensions to measure height.

Condition Index:

Oyster meat and liquor from each animal is shucked from the cup valve into pre-weighed aluminum dishes. Dishes are dried for 72 hours or until dry weights are stable, and weights are recorded to the nearest milligram. Shell cavity volume (cm^3) is determined based on whole oyster weight minus shucked shell weight, assuming that meat and liquor density is approximately 1.0 ($1g/cm^3$). Condition Index (CI) is based on: CI = (Dry weight of tissue x 100) ÷ Cavity volume.

Prevalence and Severity of *Perkinsus marinus* (Dermo):

Processing. Oysters are kept cool (42-48°C) and dry post-harvest, and are processed within 48-hrs of sampling. A 0.5 mm biopsy of mantle tissue is taken aseptically from just anterior to the labial palps (Figure 2) and is placed in sterile culture tubes with sterile Ray's fluid thioglycollate media (RFTM) with Streptomycin and Penicillin. Tissue samples are incubated at room temperature in darkness for 6 days. Tissues can be read at 6 days, or placed at 4˚C for up to 3 months prior to reading.

Examination. After incubation to swell hypnospores (to aid microscopic observations), mantle tissue is removed from the culture tube and stained on a microscope slide with Lugol's iodine. Stained tissue is then examined for the presence and density of stained hypnospores (Figure 3).

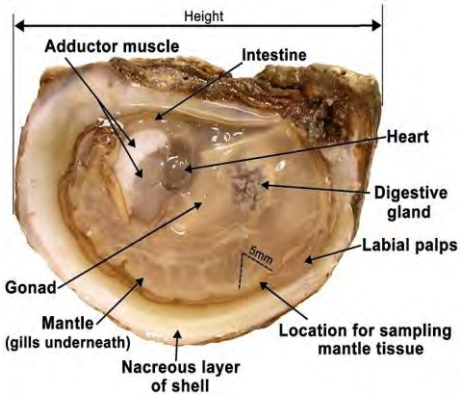

Figure 2. Oyster internal anatomy, showing location for sampling mantle tissue for Dermo analysis.

Calculation definitions:

Dermo severity: Based on the number or density of hypnospores, i.e., infection intensity within a section of mantle tissue discerned using light microscopy. *Dermo Severity Ranking Scheme* is shown in Table 1.

Dermo prevalence: This is a percentage, where the total number of positive cases ÷ total number of cases.

Dermo intensity: Mean severity of positive cases.

Dermo weighted prevalence: Dermo prevalence x Dermo intensity.

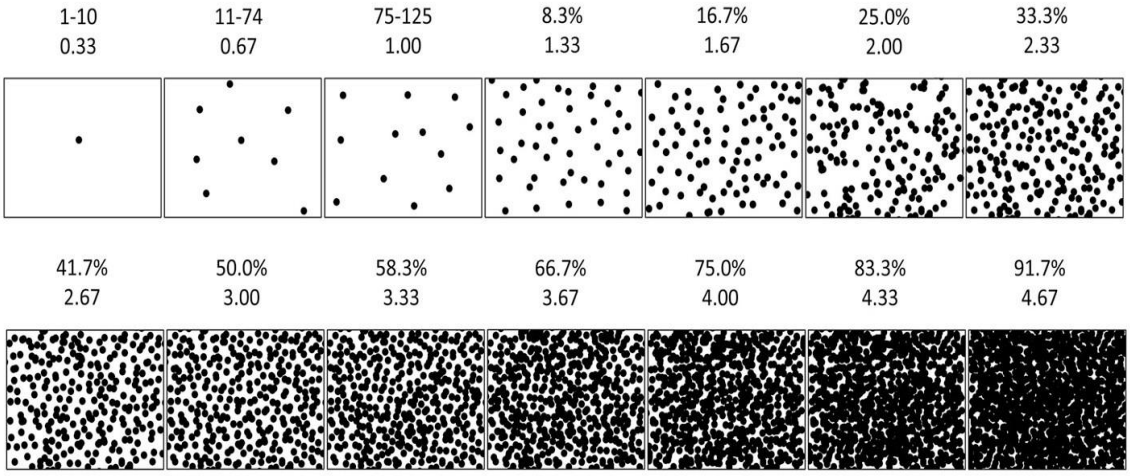

Figure 3. "Dermo Dots" cartoon to systematically rank Dermo severity in stained oyster mantle tissue sections under low magnification (4-10x) using light microscopy. Scores of 0.00 to 1.00 are based on numeric counts of individual hypnospores. Scores higher than 1.00 are based on density of hypnospores. Numbers above each panel show numbers of hypnospores or percent total area represented by hypnospores under the microscope, and corresponding severity score. Refer to the Dermo Severity Ranking Scheme (Table 1) for textual descriptions of ranked severity scores.

Table 1. Dermo infection intensity scale for hypnospore observations used to discern severity rank (Mackin scores, 0-5):

RANK	OBSERVATION
0.00	No hypnospores present
0.33	1-10 hypnospores
0.67	11-74 hypnospores
1.00	75-125 hypnospores
1.33	>125 hypnospores but much less than 25% of tissue is hypnospores
1.67	<25% of tissue is hypnospores
2.00	25% of tissue is hypnospores
2.33	>25% but much less than 50% of tissue is hypnospores
2.67	>25% but <50% of tissue is hypnospores
3.00	50% of tissue is hypnospores
3.33	>50% but much less than 75% of tissue is hypnospores
3.67	>50% but <75% of tissue is hypnospores
4.00	75% of tissue is hypnospores
4.33	>75% but much less than 100% of tissue is hypnospores
4.67	>75% of tissue is hypnospores but some oyster tissue is still visible
5.00	Nearly 100% of tissue is hypnospores

Shell Parasites

Live, and sometimes dead, oyster shell serves as substrate for a variety of boring organisms including *Polydora websteri* (boring annelid), *Diplothyra* sp. (boring clam), and *Cliona celata* (boring sponge; Figure 4). The degree of boring by these shell parasites can affect shell integrity that is vital to the live oyster against predation, and growth dynamics associated with enhanced production output and product quality.

This project discerns the prevalence and severity of *Polydora, Diplothyra* and *Cliona* from seed and grow-out oysters evaluated in the study. The approach to ranking the different shell parasites is presented in the following pages. Comprehensive monitoring of oyster shell parasitism and shell integrity provides is also important to inform monitoring and restoration efforts.

Figure 4. Left valve of wild-harvested *Crassostrea virginica* from Apalachicola Bay showing examples of shell parasitism with worms, clam and sponge. White chalky deposits and yellow areas are also observed (not observed in the current study). Green arrows point to *Diplothyra* clam holes; red arrowheads point to *Polydora* worm tubes. Just beneath the "eye" of the shell (site of adductor muscle attachment), "**M**" reveals a mud blister associated with a *Polydora* worm tube encased by the oyster's deposition of new nacre; asterisks (*) indicate areas of white chalky deposits. Boring *Cliona* sponge holes can barely be seen centrally, between the three asterisks.

- ***Diplothyra* clam observations**

Visual assessment of the degree to which the boring clam, *Diplothyra*, has colonized the shell and can be observed on the internal surface of either or both valves and ranked. Examples of *Diplothyra* clam ranks are provided in Figure 5.

Rank 1: ≤2 count of *Diplothyra* spots are seen in the shell.

Rank 2: 3-10 count of *Diplothyra* spots are seen in the shell.

Rank 3: 11-19 count of *Diplothyra* spots are seen in the shell.

Rank 4: 20-29 count of *Diplothyra* spots are seen in the shell.

Rank 5: ≥30 count of *Diplothyra* spots are seen in the shell.

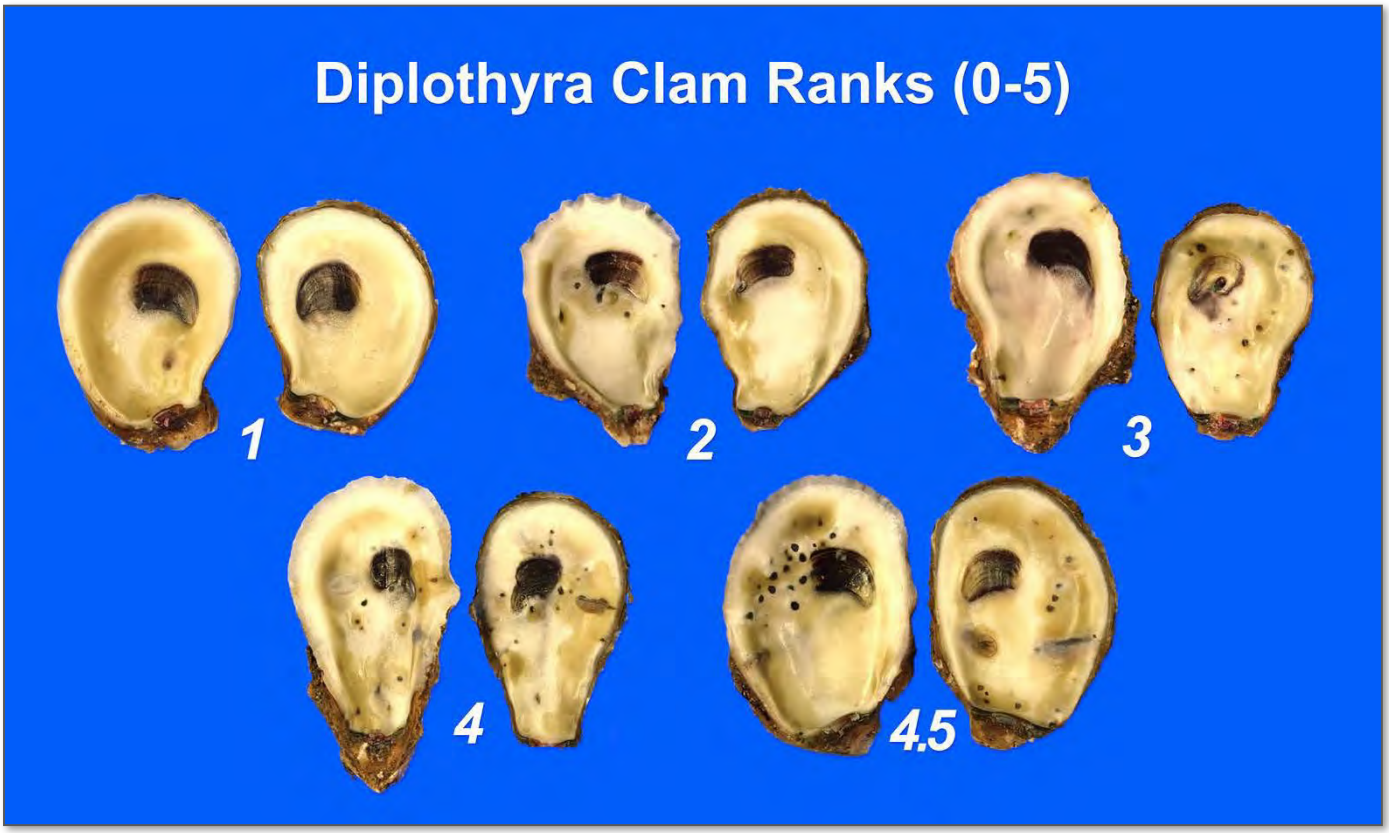

Figure 5. Examples of *Diplothyra* clam ranks, scaled from 1-5, based on visual assessment of individual, freshly shucked oysters. Ranks of 0 (no *Diplothyra* observed with the naked eye) and 5 (severe, maximal amount of *Diplothyra* that can be observed) are not shown. Half ranks were assigned for specimens where observations fall between whole rank observations.

◆ *Polydora* mudworm tubes/mud blister observations

Visual assessment of the amount of *Polydora* tubes and mud blisters observed/colonized on the shell interior based on percent area affected. Examples of *Polydora* worm ranks are provided in Figure 6.

Rank 1: <5% of the shell has *Polydora*-associated tubes or mud blisters.

Rank 2: ~15% of the shell has *Polydora*-associated tubes or mud blisters.

Rank 3: ~25% of the shell has *Polydora*-associated tubes or mud blisters.

Rank 4: ~35% of the shell has *Polydora*-associated tubes or mud blisters.

Rank 5: ≥50% of the shell has *Polydora*-associated tubes or mud blisters.

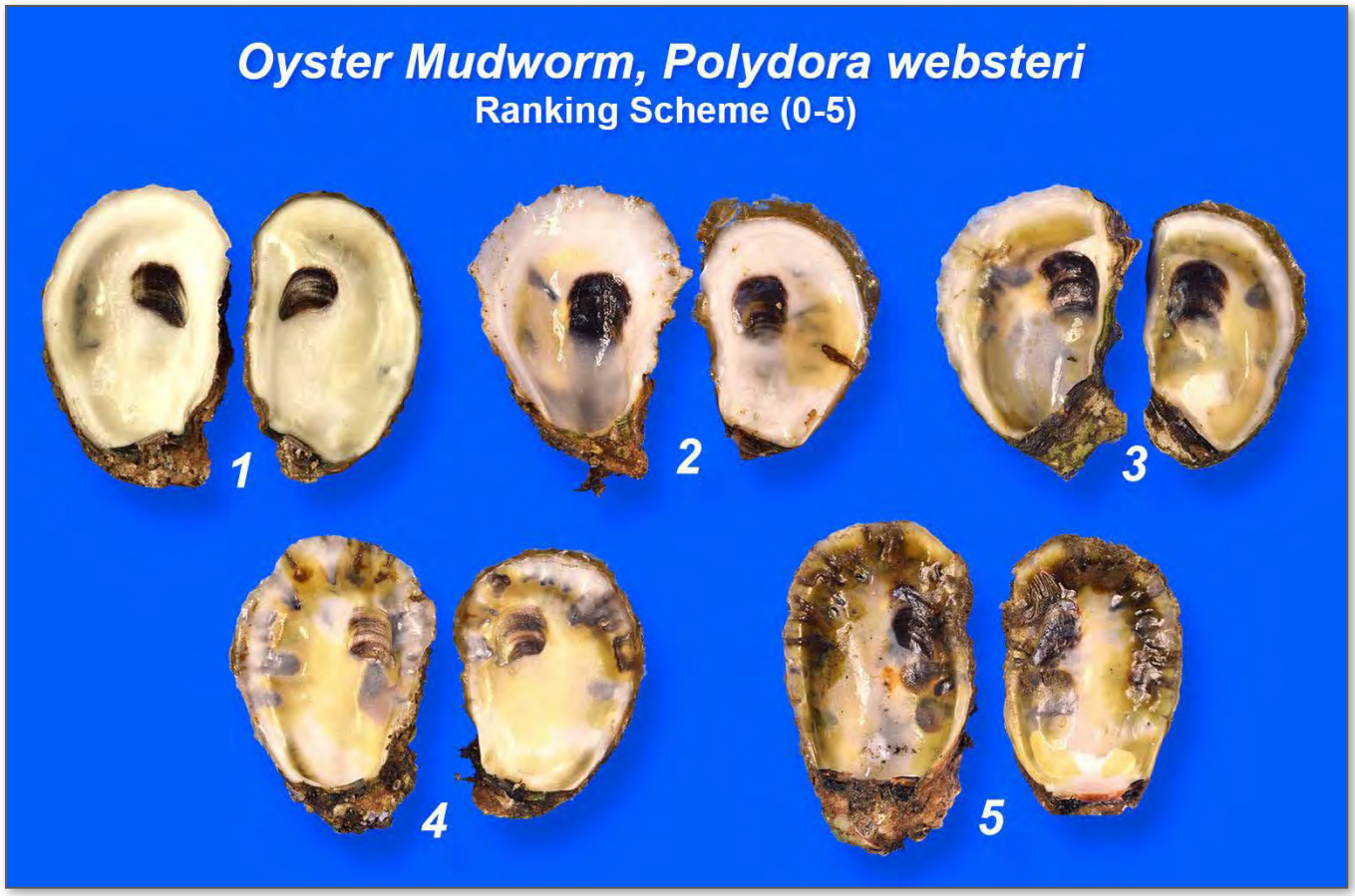

Figure 6. Examples of *Polydora* observation ranks, scaled from 0-5, based on visual assessment of the interior of both valves of individual, freshly shucked oysters. Rank of 0, where no *Polydora* are observed, is not shown. Specimens with worm observations between whole rank scores are given half-rank scores. Cases revealing darker, i.e., fresher evidence of shell parasitism, can add half-rank weight to influence final rank for each animal.

Cliona Sponge Breakthrough Observations:

Visual assessment of sponge breakthrough as observed with the naked eye, on the interior shell surface. Most sponge damage that can be visually observed on the internal surface of the shell occurs in the left (cup) valve. *Cliona* rank scores are primarily based on severity observations (surface area) of sponge breakthrough on the left shell valve; observations from the right valve are considered in the final score for the individual case. Examples of *Cliona* ranks are provided in Figure 7.

Rank 1: <10% of the shell has *Cliona* sponge spots.

Rank 2: ~25% of the shell has *Cliona* sponge spots.

Rank 3: ~50% of the shell has *Cliona* sponge spots.

Rank 4: ~75% of the shell has *Cliona* sponge spots.

Rank 5: >90% of the shell has *Cliona* sponge spots.

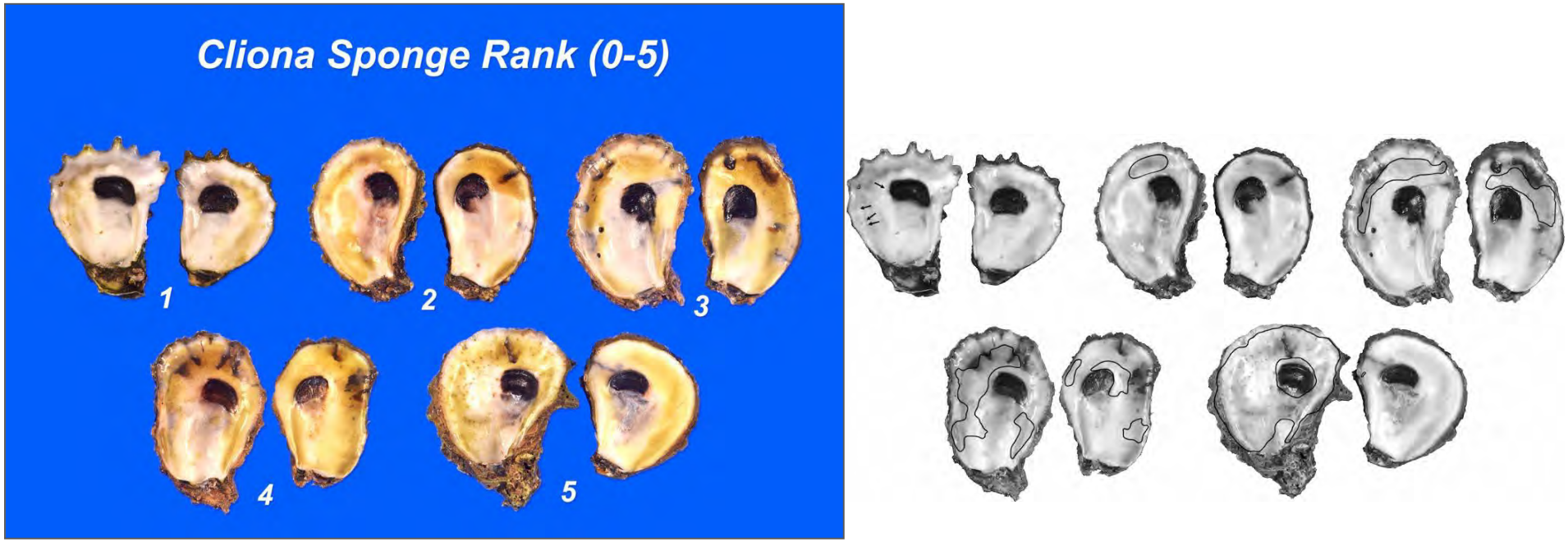

Figure 7. Left: Examples of *Cliona* sponge breakthrough on the internal surface of the shell, scaled from 0-5, based on visual assessment of individual, freshly shucked oysters. Half ranks are assigned for specimens where observations fall between whole rank observations. Relatively high density of sponge spots in areas of a specimen may increase the final score for that case by a half rank. Rank of 0, where no *Cliona* is observed, is not shown in this figure. **Right**: Arrows and outlined areas show sponge breakthrough on the inside of the shell relating to corresponding ranked examples to the left.

Oyster Health Assessment Results:

Oyster health was assessed from seedstock and from sub-sampled aquacultured populations over time from two locations: Alligator Harbor and Oyster Bay. Oyster health data were generated from **340 oysters over 5 collection timepoints** in this study: seedstock were evaluated from collection on 9/28/20 (Collection 1); grow-out samples were collected on 1/26/21 (Collection 2), 3/30/21 (Collection 3), 5/25/21 and 6/1/21 (Collection 4) and 7/13/21 (Collection 5). Samples (n=24 each) were processes from each of two farms (Alligator Harbor and Oyster Bay) for Collections 2 and 4; whereas only one out of the two Alligator Harbor farms contributed samples for Collection 3, and for Collection 4, one of the two Alligator Harbor farm contributed n=12 (instead of n=24) (Table 2).

Table 2. Oyster Count by Farm/Location and Collection

Farm	Collection 1	Collection 2	Collection 3	Collection 4	Collection 5	Grand total
Seed (no farm)	40					**40**
AH-A		24	24	24		**72**
AH-B		24		12		**36**
OB-A		24	24	24	24	**96**
OB-B		24	24	24	24	**96**
Grand total	**40**	**96**	**72**	**84**	**48**	**340**

Oyster size, based on height from the umbo to the ventral margin (Figure 1), was determined for each oyster examined throughout the study. Height data by location and collection are summarized in Table 3. Note that oyster height data reflect only subsamples analyzed for health, and may be representative of larger population subsamples. Individual oyster size (height) data with means, from both locations over time, are shown in Figure 8.

Table 3. Mean oyster height (mm, ±SE)

Location	Collection 1	Collection 2	Collection 3	Collection 4	Collection 5	Grand mean
Seed (no location)	22.5 (±0.5)					**22.5 (±0.5)**
AH-A		66.1 (±1.9)	74.5 (±1.9)	81.8 (±2.2)		**74.1 (±1.4)**
AH-B		57.3 (±2.0)		71.3 (±2.4)		**62.0 (±1.9)**
OB-A		42.5 (±1.0)	53.1 (±1.2)	67.6 (±1.5)	74.7 (±2.0)	**59.5 (±1.5)**
OB-B		41.3 (±1.8)	57.0 (±2.0)	68.4 (±1.8)	85.3 (±2.0)	**63.0 (±1.9)**
Grand mean	**22.5 (±0.5)**	**51.8 (±1.4)**	**61.5 (±1.5)**	**72.4 (±1.2)**	**80.0 (±1.6)**	**59.5 (±1.1)**

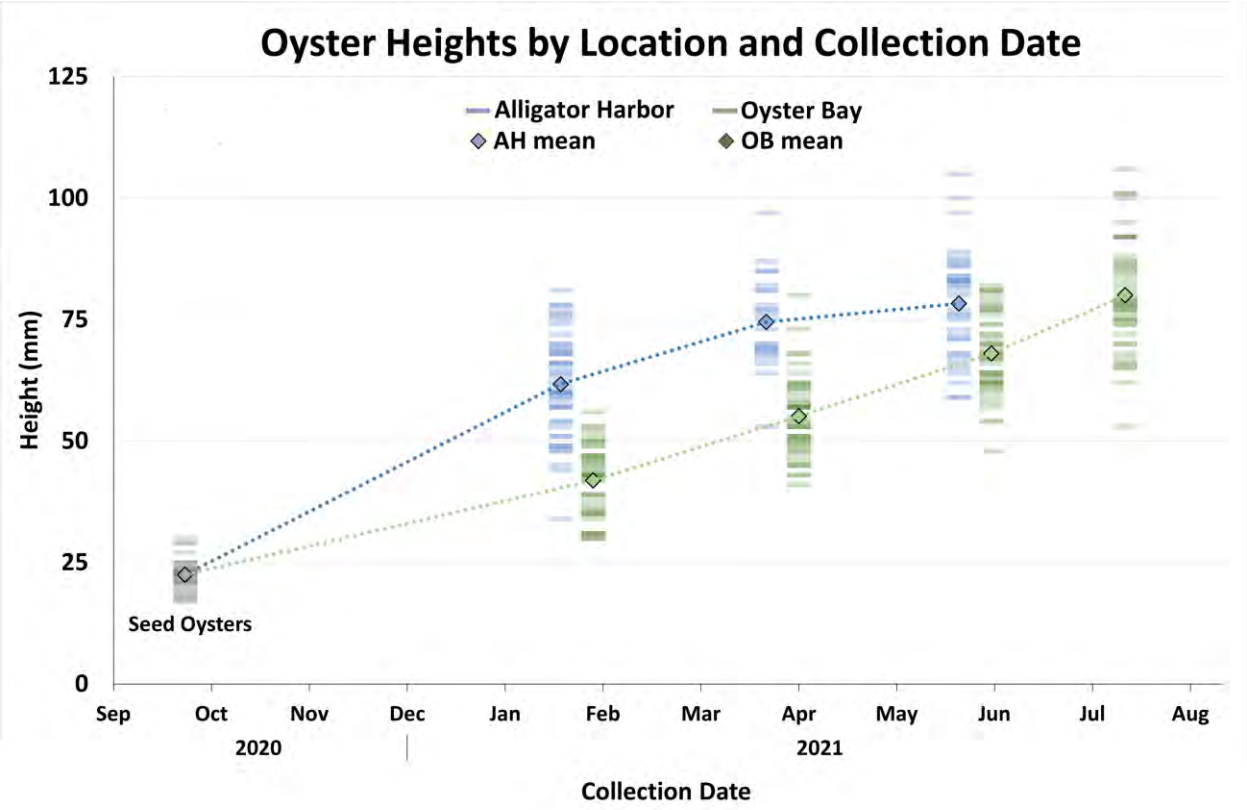

Figure 8. Oyster height data for **Seedstock,** and for subsamples collected from **Alligator Harbor** and from **Oyster Bay,** throughout the study period. Light shaded bars represent individual oyster data; darker bars indicate more specimen representation; diamonds indicate means for each location data set.

Oyster condition was determined using a Standard Condition Index (*CI) as well as our visual "Empirical" Visual Condition Index, as described above. Standard Condition Index data from this study are summarized in Table 4 and Figure 9, and are shown as frequency responses over time in Figure 10. For comparison, Visual Condition scores were also determined for each specimen and are shown in Table 5. The relationship between Standard CIs and APL Visual Condition scores is shown in Figure 11.

Table 4. Mean Standard Condition Index by location and collection

Location	Collection 1	Collection 2	Collection 3	Collection 4	Collection 5	Grand mean
Seed (no location)	7.2 (±0.3)					**7.2 (±0.3)**
AH-A		7.9 (±0.2)	9.3 (±0.3)	11.5 (±0.5)		**9.6 (±0.3)**
AH-B		7.5 (±0.3)		7.7 (±0.7)		**7.5 (±0.3)**
OB-A		7.4 (±0.2)	12.2 (±0.3)	11.0 (±0.3)	9.7 (±0.4)	**10.1 (±0.2)**
OB-B		6.8 (±0.2)	10.0 (±0.2)	10.2 (±0.3)	9.6 (±0.5)	**9.2 (±0.2)**
Grand mean	**7.2 (±0.3)**	**7.4 (±0.1)**	**10.5 (±0.2)**	**10.4 (±0.3)**	**9.7 (±0.3)**	**9.1 (±0.1)**

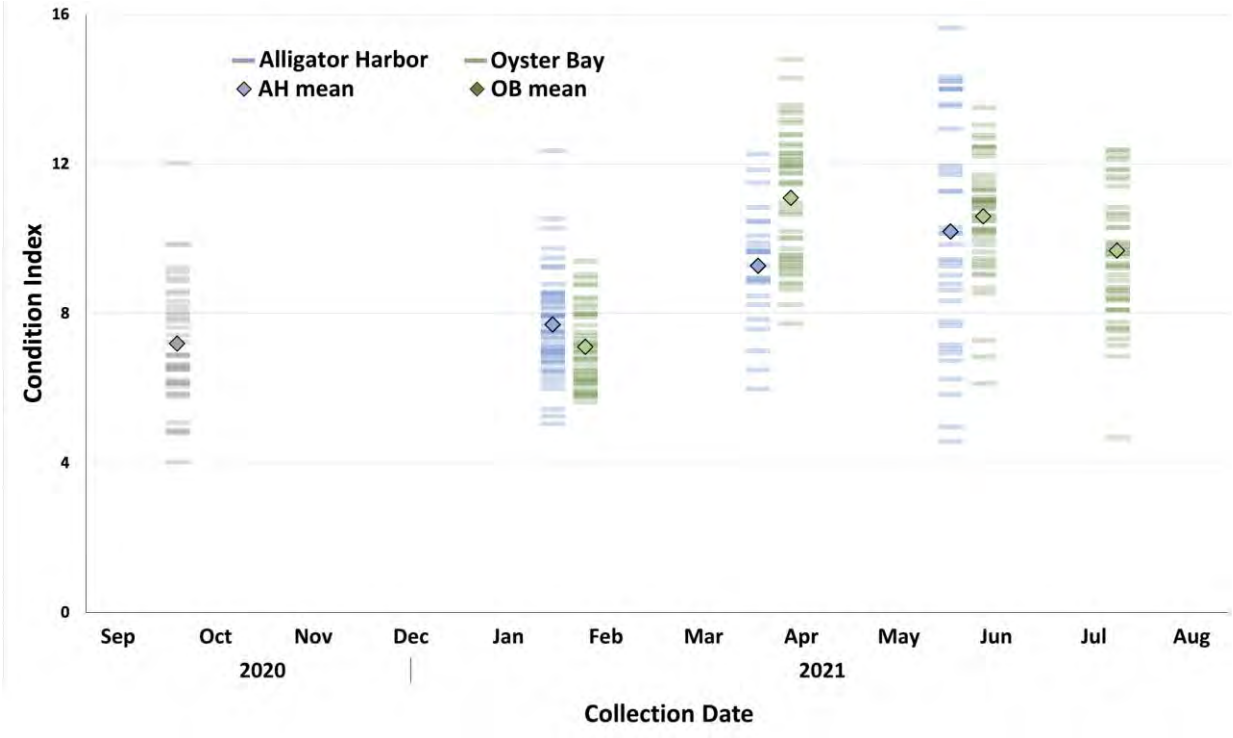

Figure 9. Standard condition data for **Seedstock,** and for subsamples collected from **Alligator Harbor** and from **Oyster Bay,** throughout the study period. Light shaded bars represent individual oyster data; darker bars indicate more specimen representation; diamonds indicate means for each location data set.

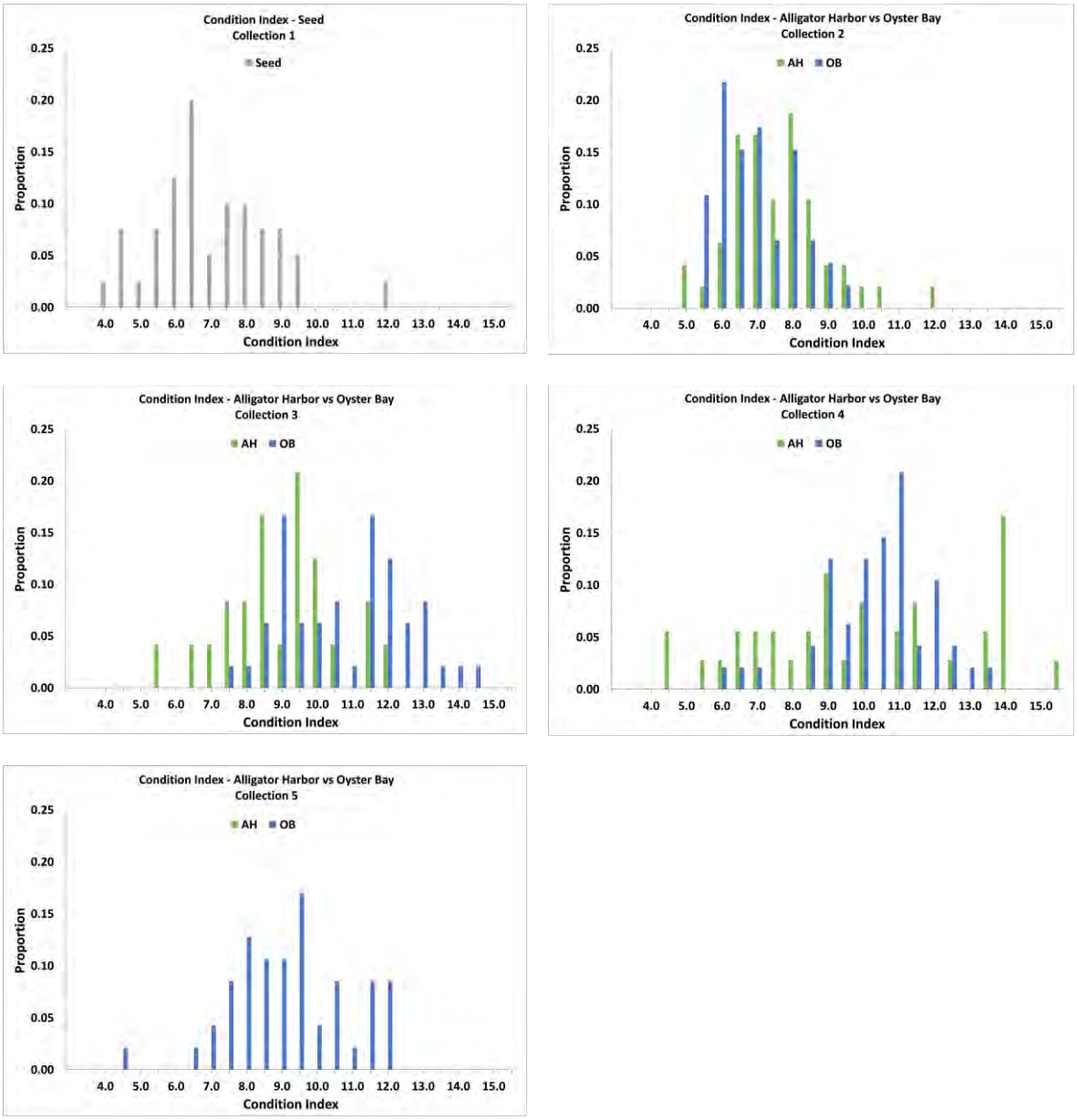

Figure 10. Frequency of Standard Condition Index data for oysters by location (**Alligator Harbor** and **Oyster Bay**) for Collections 1-5 over the study period. Data are proportional responses (relative frequencies) over the range of condition indices observed (x-axes). Collection 5 represents only **Oyster Bay** specimens; none were provided from **Alligator Harbor** for that collection.

Table 5. Mean oyster visual meat ranks by location and collection						
Location	**Collection 1**	**Collection 2**	**Collection 3**	**Collection 4**	**Collection 5**	**Grand mean**
Seed (no location)	2.7 (±0.1)					**2.7 (±0.1)**
AH-A		2.4 (±0.1)	2.6 (±0.1)	3.0 (±0.1)		**2.7 (±0.1)**
AH-B		2.2 (±0.0)		2.8 (±0.1)		**2.4 (±0.1)**
OB-A		3.0 (±0.0)	3.3 (±0.1)	3.3 (±0.1)	3.5 (±0.1)	**3.3 (±0.0)**
OB-B		2.6 (±0.1)	2.7 (±0.1)	3.3 (±0.1)	3.2 (±0.1)	**3.0 (±0.0)**
Grand mean	**2.7 (±0.1)**	**2.5 (±0.0)**	**2.9 (±0.1)**	**3.1 (±0.0)**	**3.3 (±0.0)**	**2.9 (±0.0)**

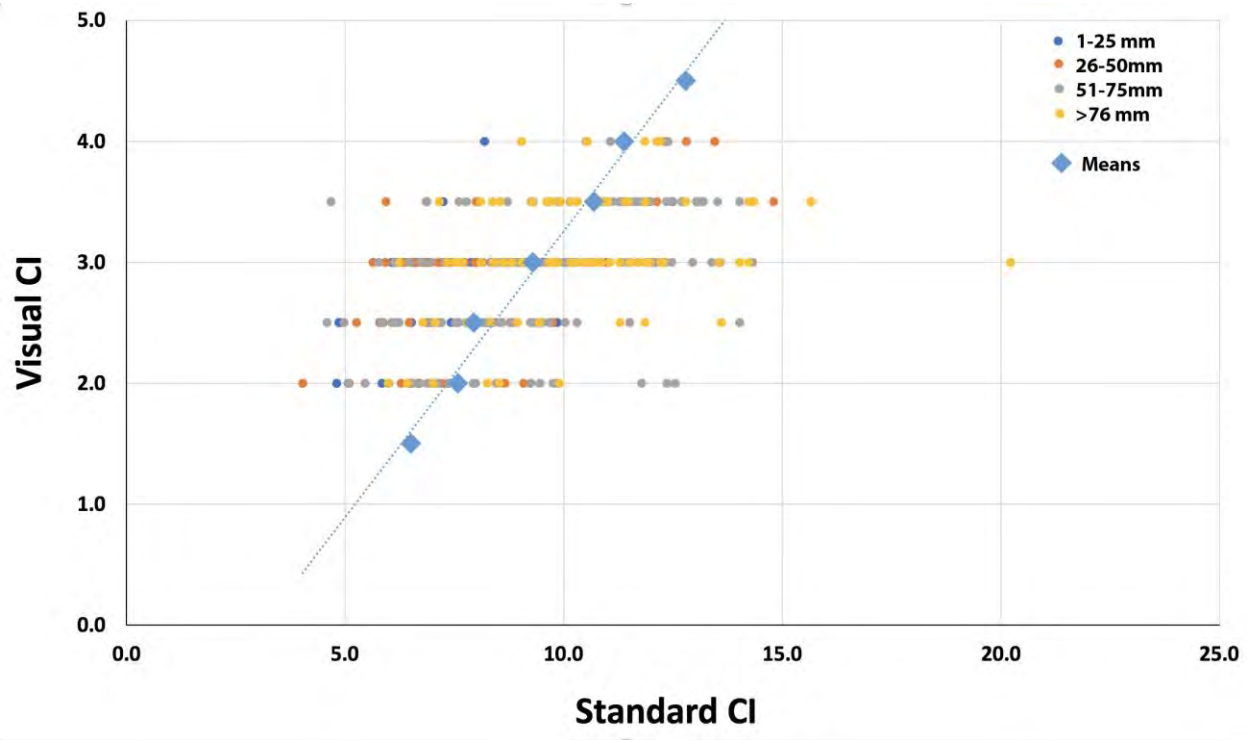

Figure 11. Relationship between Standard Condition Index (CI) and Visual Condition scores for all 340 oyster specimens examined for health. Visual Condition scores range from 1.0-5.0 with 0.5 intervals and are shown by oyster size bin (see Legend). Mean CIs aligned well with Visual Scores (R^2=0.996) based on n=340 from all collections.

Shell parasitism associated with boring worms (*Polydora*), clams (*Diplothyra*) and sponge (*Cliona*) was assessed from the internal surface of both shell valves from each specimen as described above. Only *Polydora* worms or worm damage were recorded from internal shell observations from all collections, all timepoints. Parasites or associated shell damage from *Diplothyra* clams and *Cliona* sponge were not observed on internal shell surfaces from any of the specimens throughout the study. Prevalence of *Polydora* in subsampled oysters, by location over time, is summarized in Table 6 and Figure 12. Severity of *Polydora* observations is shown in Table 7.

Table 6. Polydora Prevalence by location and collection

Location	Collection 1	Collection 2	Collection 3	Collection 4	Collection 5	Grand mean
Seed (no location)	0.55					**0.55**
AH-A		0.58	0.71	0.83		**0.71**
AH-B		0.92		0.75		**0.86**
OB-A		0.46	0.71	0.33	0.67	**0.54**
OB-B		0.33	0.63	0.71	0.96	**0.66**
Grand mean	**0.55**	**0.57**	**0.68**	**0.64**	**0.81**	**0.64**

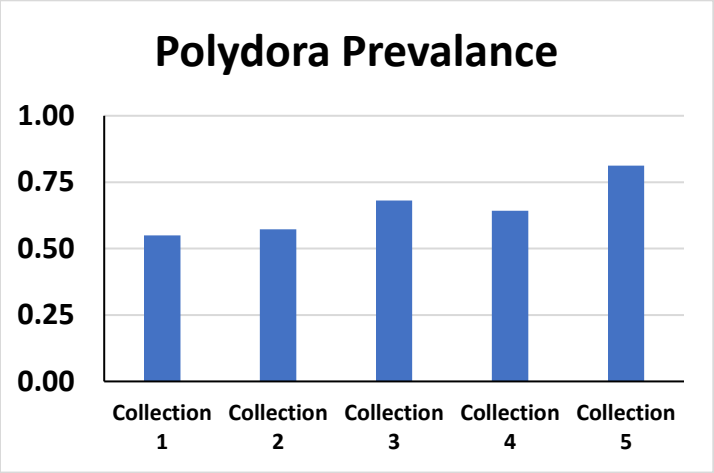

Figure 12. Prevalence of *Polydora* worms observed from internal shell surfaces, Alligator Harbor and Oyster Bay combined, by collection over time. At the time of Collection #5, 81% of oysters examined for health assessment in the study had *Polydora* excavations, worm tubes and/or mud blisters.

Table 7. Mean *Polydora* severity ranks by location and collection.

While *Polydora* prevalence ranged from 55-81% throughout the study (Table 6, Figure 12), infection severity was, on average, relatively mild (severity scores ranged from 0.43 to 0.9 out of 5.0).

Location	Collection 1	Collection 2	Collection 3	Collection 4	Collection 5	Grand mean
Seed (no location)	0.69 (±0.1)					**0.69 (±0.1)**
AH-A		0.34 (±0.1)	0.52 (±0.1)	0.51 (±0.1)		**0.46 (±0.0)**
AH-B		0.83 (±0.1)		0.56 (±0.1)		**0.74 (±0.1)**
OB-A		0.31 (±0.1)	0.48 (±0.1)	0.22 (±0.1)	0.67 (±0.1)	**0.42 (±0.0)**
OB-B		0.27 (±0.1)	0.34 (±0.1)	0.5 (±0.1)	1.14 (±0.1)	**0.56 (±0.1)**
Grand mean	**0.69 (±0.1)**	**0.44 (±0.0)**	**0.45 (±0.0)**	**0.43 (±0.0)**	**0.9 (±0.1)**	**0.53 (±0.0)**

Dermo disease prevalence and severity was determined for all specimens submitted in this study for health assessment. Dermo prevalence (Figure 13) over time generally increases as oysters increase in size and continue to filter water and grow, regardless of environmental conditions. Weighted prevalence (sample prevalence * infection intensity) for Dermo by collection is shown in Figure 14. Intensity of Dermo infections, when present, appears to affect overall oyster condition, based on Standard CIs (Figure 15).

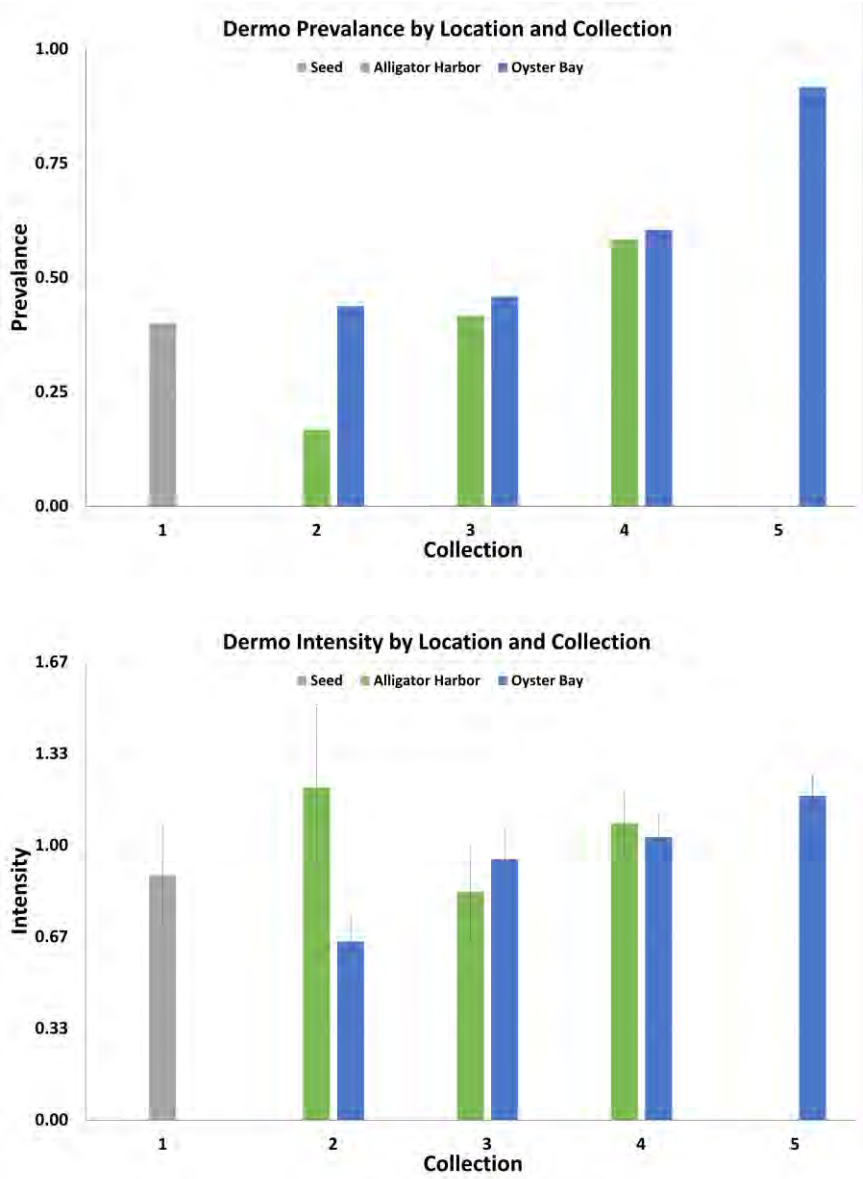

Figure 13. Prevalence (top) and intensity (bottom) of Dermo observed in oyster **Seedstock** (Collection 1) and for samples collected from **Alligator Harbor** and from **Oyster Bay** (Collections 2-5). Intensity data are means ±SE. [I note that prevalence, compared with *weighted prevalence*, tends to provide a more sensitive indicator of Dermo population responsiveness to changes in environmental and growth conditions.]

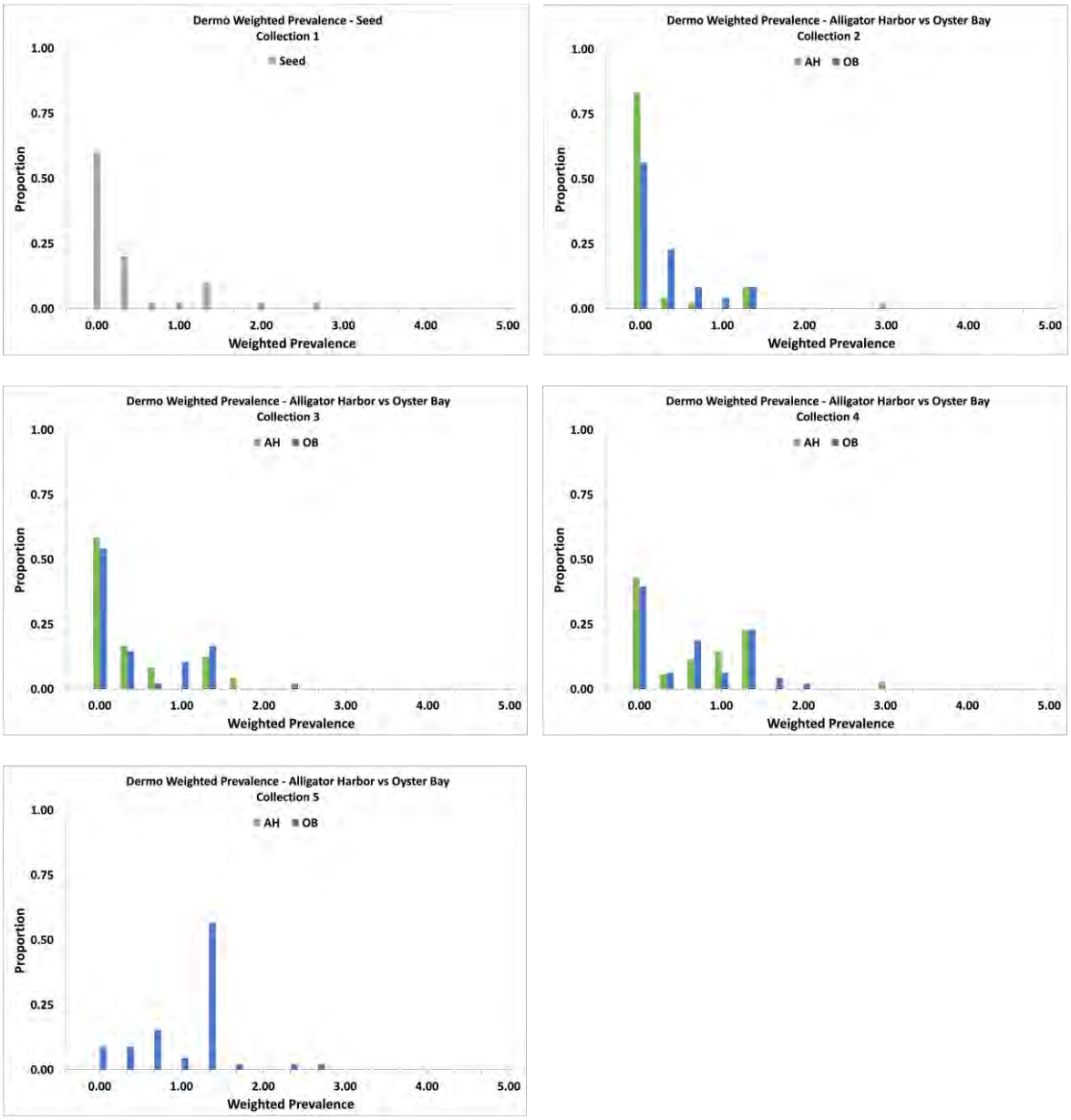

Figure 14. Dermo weighted prevalence for oysters subsampled by location from **Alligator Harbor** and **Oyster Bay** for Collections 1-5. Data are proportional responses (relative frequencies) over the range of weighted prevalences observed (x-axes). Collection 5 represents only **Oyster Bay** specimens (none were sampled from **Alligator Harbor** for that collection).

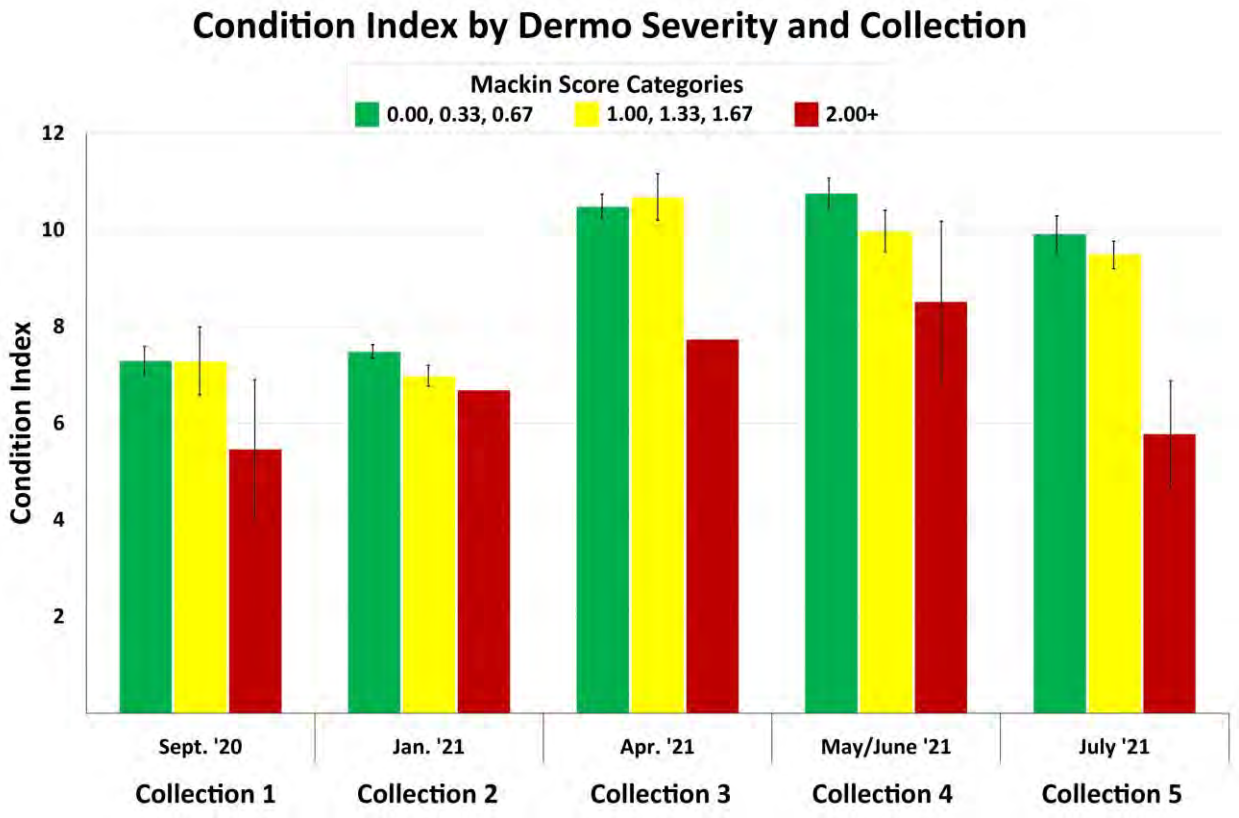

Figure 15. Standard Condition Index (CI ±SE) for oysters subsampled from Alligator Harbor and Oyster Bay (combined) by "Dermo Categorical Rank," by collection. The **GREEN YELLOW RED** presentation of Dermo severity scores for each collection reveals the distribution of "minimal," "moderate" and "relatively severe" Dermo cases with corresponding average CIs. Data indicates that overall physiologic oyster health is lower when Dermo severity is higher. "Stoplight" visualization of Dermo severity scores adapted from *Oyster Sentinel*.

Collections 1-5 Composite Images:

Figure 16. Seedstock from ***Collection 1 (9/28/20)***, representing two triploid lineages (FL3N and LA3N). Twenty from each lineage were randomly subsampled and submitted for health assessment. [*nb: Composite images are shown with labels as they arrived at the APL. The Location/Farm Source labeling system for certain oyster subsamples transported to the APL for health assessment was reversed/inaccurate.*]

Differences in height ($p<0.02$) and Dermo prevalence and Dermo weighted Prevalence ($p<0.02$) were noted between strains in Collection 1. LA-strain seedstock (left, labeled FL 3N) averaged 23.4 mm height, 60% Dermo prevalence and 0.60 Dermo Weighted Prevalence, compared with FL-strain seedstock (right, labeled LA 3N) that averaged 21.5 mm height, 20% Dermo prevalence and 0.12 Dermo Weighted Prevalence.

Figure 17. Oyster subsamples from ***Collection 2 (1/26/21),*** *Oyster Bay and Alligator Harbor*, submitted for health assessment. [nb: *Composite images are shown with labels as they arrived at the APL. The Location/Farm Source labeling system for certain oyster subsamples transported to the APL for health assessment was reversed/inaccurate.*]

Figure 18. Oyster subsamples from ***Collection 3 (3/20/21)***, Alligator Harbor and Oyster Bay, submitted for health assessment. [nb: *Composite images are shown with labels as they arrived at the APL. The Location/Farm Source labeling system for certain oyster subsamples transported to the APL for health assessment was reversed/inaccurate.*]

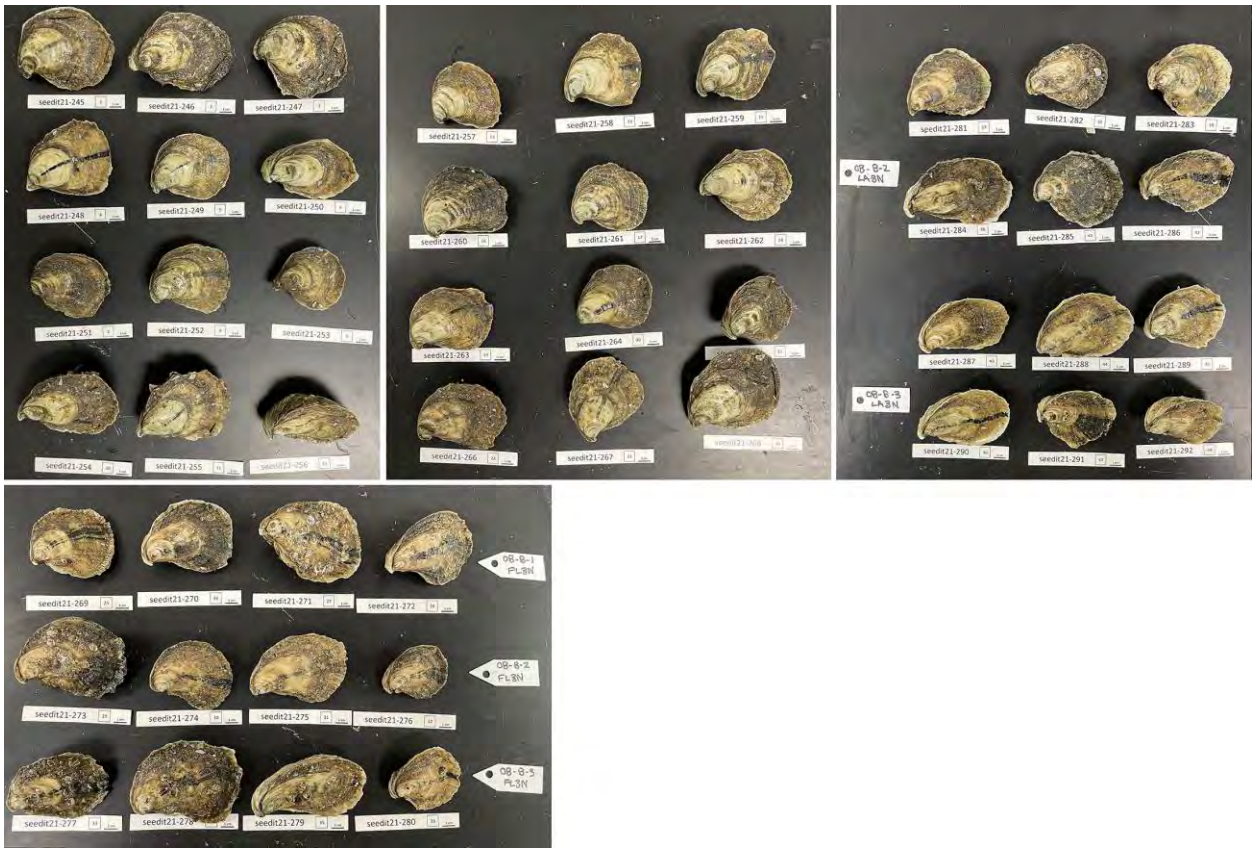

Figure 19. Oyster subsamples from ***Collection 4 (6/1/21)****, from Oyster Bay (Alligator Harbor not shown)*, submitted for health assessment. [nb: *Composite images are shown with labels as they arrived at the APL. The Location/Farm Source labeling system for certain oyster subsamples transported to the APL for health assessment was reversed/inaccurate.*]

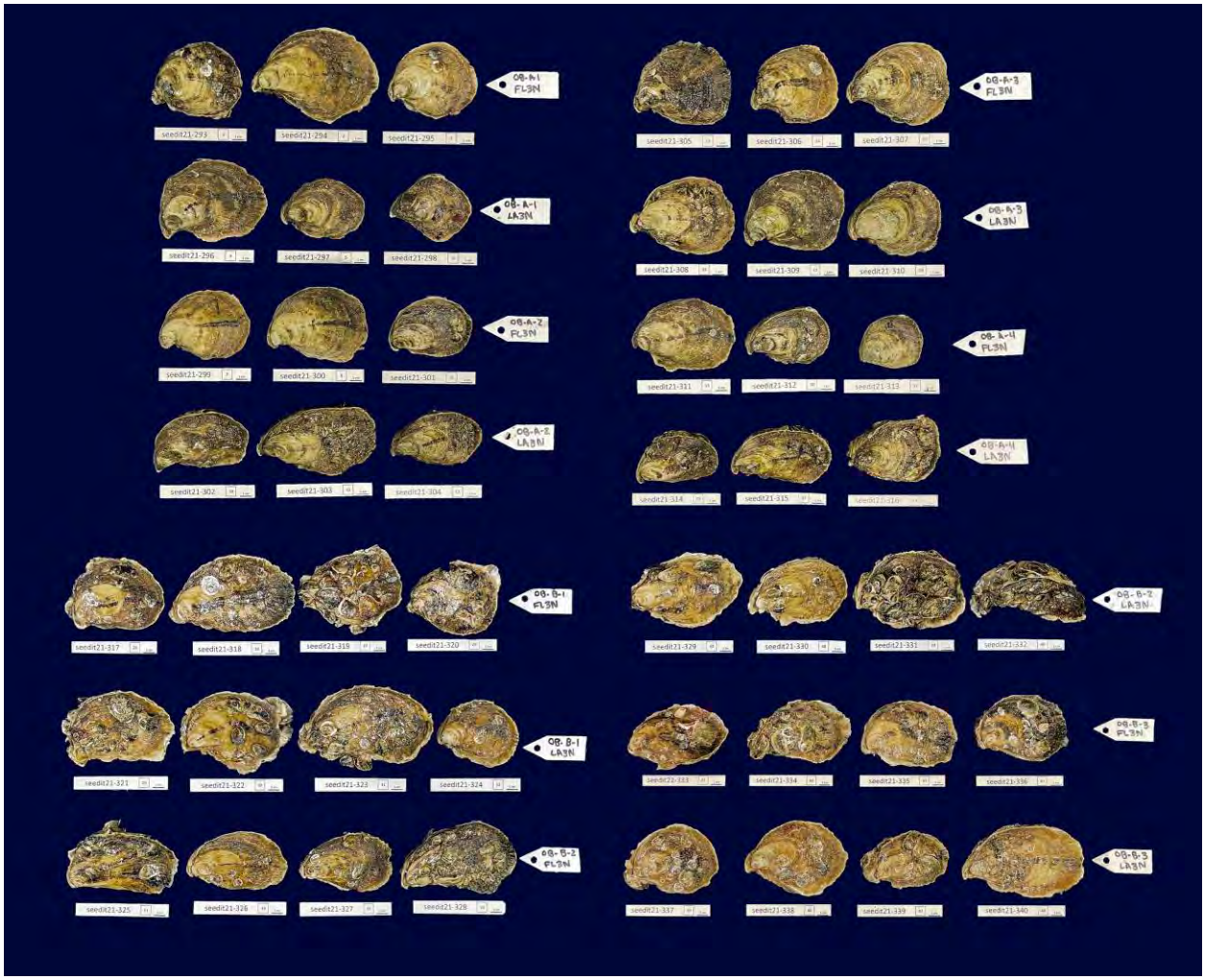

Figure 19. Oyster subsamples from ***Collection 5 (7/13/21)*** from Oyster Bay submitted for health assessment. This collection, has specimens that show biofouling on the exterior surfaces of shells. Biofouling consisted primarily of oyster spat, barnacles and *Cliona* sponge. Spat biofouling was more severe on subsamples labeled OB-B, compared with subsamples labeled OB-A. Barnacle set was relatively minimal compared with spat set, and was recent based on size of barnacles. Cliona growth (minimal to mild) was present on the external surface of several specimens. *Cliona* observations from the external surface of the shells was not included in the health assessment since protocol focused solely on internal shell surface observations. [nb: *Composite images are shown with labels as they arrived at the APL. The Location/Farm Source labeling system for certain oyster subsamples transported to the APL for health assessment was reversed/inaccurate.*]

Figure 20. Observations of biofouling on the exterior of oyster shells from Collection 5. Shells show notable spat settlement. The specimen. on the right shows yellow *Cliona* sponge growth on top off the spat.

Addressing Production and Market Challenges in Florida's Oyster Aquaculture Industry

Objective 2 – Phytoplankton Quality and Quantity

Final Report 2021

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Fisheries and Aquatic Sciences Program, SFFGS, IFAS, University of Florida

Introduction

The objective of this component of the study is to determine the composition and biomass of phytoplankton communities in five coastal regions of Florida associated with bivalve mariculture activities. The focus of the effort is on the trophic state of the regions and potential threats for the health of bivalves represented by the presence of harmful algal species.

Methods

General phytoplankton composition was determined using the Utermöhl method (Utermöhl, 1958). Samples preserved in Lugol's were settled in 19-mm diameter cylindrical chambers. Phytoplankton cells were identified and counted at 400× and 100× with a Leica phase contrast inverted microscope. At 400×, a minimum of 100 cells of a single taxon and 5 grids were counted. At 100×, a total bottom count was completed for taxa >20-30 µm in size. Fluorescence microscopy was used to enumerate picoplanktonic cyanobacteria (e.g., *Synechococcus* spp. and spherical picocyanobacteria spp.) at 1000x magnification (Phlips et al., 1999). Subsamples of seawater were filtered onto 0.2-µm Nucleopore filters and mounted between a microscope slide and cover slip with immersion oil.

Cell biovolumes (μm^{-3} $cell^{-1}$) were estimated by assigning combinations of geometric shapes to fit the characteristics of individual taxa (Smayda, 1978; Sun and Liu, 2003). Specific phytoplankton dimensions were measured for at least 30 randomly selected cells. Species which vary substantially in size, such as many diatom species, were placed into size categories. Phytoplankton biomass as carbon values (µg carbon ml^{-1}) was estimated by using conversion factors for different taxonomic groups applied to biovolume estimates, i.e., 0.065 x biovolume (μm^{-3} ml^{-1} x 10^{-6}) of diatoms, 0.16 x biovolume of dinoflagellates and 0.22 x biovolume of

cyanobacteria and other phytoplankton taxa (Strathmann, 1967; Ahlgren, 1983; Sicko-Goad et al., 1984; Verity et al., 1992; Work et al., 2005).

Results and Discussion

Mean total phytoplankton biomass over the study period at the two sampling sites were 590 µg carbon L^{-1} at Site AH and 108 µg carbon L^{-1} at Site OB (Table 1). These mean values fall within the range of mean values observed in the lower Caloosahatchee estuary (Phlips et al 2021) and lower Tampa Bay on the west coast of Florida (Badylak et al. 2007), but are higher than values observed in the open water region of the Cape Canaveral shelf environment of the off the east coast of Florida (i.e. 122 µg carbon L^{-1}) (Tate et al. 2020). The mean values are considerably lower than in the northern Indian River Lagoon, where mean values were over 3000 µg carbon L^{-1} for the period from 2011 to 2020 (Phlips et al. 2021). The peak biomass observed during the study was 1000 µg carbon L^{-1} at Site AH (Fig. 1). By comparison, peak biomass levels in the northern Indian River Lagoon reached levels over 20,000 µg carbon L^{-1}.

The range of mean biomass values observed in this study are roughly equivalent to 3-8 µg chlorophyll *a* L^{-1}, based on relationships observed in a previous study of the Caloosahatchee estuary (Mathews et al. 2015, Phlips personal communications). From the perspective of trophic state indices for coastal marine systems, the range would be indicative of oligotrophic to lower mesotrophic conditions (ICWA 2021), which would generally be considered good water quality conditions from the perspective of general phytoplankton biomass levels, in terms of overall ecosystem function (TCWA 2021).

In order to examine differences in the structure of phytoplankton communities at the two sampling sites, biomass time-series were sub-divided into four major groups, i.e. dinoflagellates, diatoms, cyanobacteria and all "other" taxa (Fig. 1). These groups provide the basis for evaluating potential threats to ecosystem health represented by key functional groups of phytoplankton.

Dinoflagellates

Site AH had the highest levels of dinoflagellates biomass (Fig. 1, Table 1). The dinoflagellate peaks observed at both AH and OB were dominated by harmful algal bloom

(HAB) species *Karlodinium veneficum* (Fig. 1), as reflected by its prominence in the Top-20 list of biomass observations for individual taxa (Table 2).

Karlodinium veneficum has been observed to produce the toxin karlotoxin in a number of coastal ecosystems around the world (Lassus et al. 2016, Pace et al. 2012). Karlotoxin is ichthytoxic (i.e. harmful to fish) that produces strong hemolytic activity (Bachvaroff, et al. 2009, Goshorn et al. 2004, Műller et al. 2019, Neilsen 1993, Nielsen and Stromgren 1991). The toxin has been linked to incidents of fish mortalities (Abbott and Ballentine 1957, Deeds et al. 2002, Deeds et al. 2004, Landsberg 2002, Place et al. 2012), and has been shown to have lethal and adverse or sublethal effects on a wide range of marine invertebrates, including mussels and scallops (Daugbjerg et al. 2000, Landsberg 2002, Lassus et al. 2016). *K. veneficum* was a prominent feature at Sites AH and OB (Fig. 1), with peak cell densities of 363,000 and 181,000 cells L^{-1}, respectively (Table 1). However, these values are well below peak values associated with serious harmful bloom events in other ecosystems, e.g. 10^7-10^8 cells L^{-1} (Place et al. 2012).

Among the HAB dinoflagellate species not on the Top-20 list, a number of *Prorocentrum* species and *Dinophysis caudata* were observed in the sampling regions (Table 3). Many of these species have been linked to the production of the toxin okadaic acid (DSP, diuretic shellfish poison) and have been linked to issues with bivalve production (Landsberg 2002, Lassus et al. 2016). Another HAB species observed at very low levels is *Cochlodinium polykrikoides*. It has been reported to negatively affect larval bivalves (Griffith et al. 2019). None of the observations of these three taxa were observed at high levels of biomass during the study period.

Another HAB species observed in the study region was *Akashiwo sanguinea*, although the cell densities were well below levels of concern (Table 3). *A. sanguinea* is cosmopolitan in distribution and has been observed to form blooms in coastal ecosystems around the world (Badylak et a. 2014a, Hallegraeff 2003, Horner et al. 1997, Lassus et al. 2016), including the Gulf of Mexico and the Atlantic coast of Florida (Badylak et al. 2014, Hart et al. 2015, Mathews et al. 2016, Phlips et al. 2010, Phlips et al. 2012, 2021a, 2021b, Quinlan and Phlips 2007). *A. sanguinea* plays a major role in the ecology of many marine environments, including coastal ecosystems with variable salinities, where its euryhaline character makes it competitive (Badylak et a. 2014, Matsubara et al. 2007). While *A. sanguinea* has not been reported to be toxic, blooms of the species have been associated with mass mortalities of invertebrates and fish in various regions of the world (Bricelj et al. 1992, Cardwell et al. 1979, Harper and Gullen 1989, Kahru et

al. 2004, Landsberg 2002, Lassus et al. 2016, Schumway 1990). One of the harmful impacts of intense *A. sanguinea* blooms is the potential for the development of hypoxic conditions (Hallegraeff 2003). *A. sanguinea* is also known to produce large quantities extracellular carbohydrate polymer (Badylak et al. 2014b), that can be ecosystem disruptive, including impacts on benthic and pelagic grazer populations (Galimany et al. 2020, Gobler et al. 2013, Smayda 2008, Sunda et al. 2006).

Besides the HAB species discussed above, the Top-20 lists contain several other dinoflagellate taxa at Site OB, at mostly lower biomass levels and none are currently known to produce toxins (Table 2). These include small-celled gymnoid-type spp. and *Scrippsiella.*

Diatoms

Diatoms were major elements (I.e. % of total biomass) of the phytoplankton communities at both Sites AH and OB (Table 1). At Site AH, diatoms dominated the phytoplankton community in terms of biomass (Fig. 1, Table 1). Among the eight diatom species on the Top-20 list of biomass observations for individual taxa, three were most prominent, i.e. *Tropidoneis lepidoptere*, *Leptocylindrus danicus* and *Thalassionema nitzschioides* (Table 2). Nine diatom taxa were on the Top-20 list at Site OB, most prominently two species of the cospmopoliton genus *Skeletonema*.

A high percent contribution of diatoms to biomass is generally considered a positive feature of coastal food webs (Wasmund et al. 2017), with the possible exception of certain HAB species. These include *Pseudo-nitzschia* species known to produce the neurotoxin domoic acid (ASP-Amnesiac Shellfish Poison) in Florida waters (Badylak et al. 2006, Bates et al. 2018) (Table 3), which threaten bivalve production systems in terms of human and aquatic animal health (Bates et al. 2018, Landsberg 2002). *Pseudo-nitzschia* was observed twice at Site AH, but not at high abundances (Table 3), such as blooms observed in southwest Florida, e.g. 4×10^7 cells L^{-1} (Bates et al. 2018), and the northern Indian River Lagoon, 5.3×10^8 cells L^{-1} (Phlips et al. 2021). Some species of *Chaetoceros* are also considered potentially harmful to fish and invertebrates because of the presence of stiff spines that can be physically damaging, leading to lethal or sub-lethal negative impacts (Lassus et al. 2016). Biomass levels of the latter group were generally low both study sites (Tables 3).

Other Taxa

The "other" taxa observed at the sampling sites were largely dominated by nanoplanktonic (i.e. > 2 – 20 μm) species, including cryptophytes, as reflected in their prominence on the Top-20 lists (Table 1). These taxa were particularly important at Site OB (Table 1 and 2). None of the "other" taxa are currently identifiable as HAB species.

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Fig. 1. Time series of phytoplankton biomass at the five sampling sites. Biomass levels are divided into four major groups: dinoflagellates (red), diatoms (yellow), cyanobacteria (blue), and all "other" taxa (green). Letters associated with peaks in biomass refer to the dominant taxa, i.e. Am, *Amphora*; CR, cryptophytes; Cp, *Cerataulina pelagica*; G – *Guinardia;* kv, *Karlodinium veneficum*; L, *Leptocylindrus*; N, Nanoplankton; Sc, *Skeletonema costatum;* Tr, *Tropidoneis*.

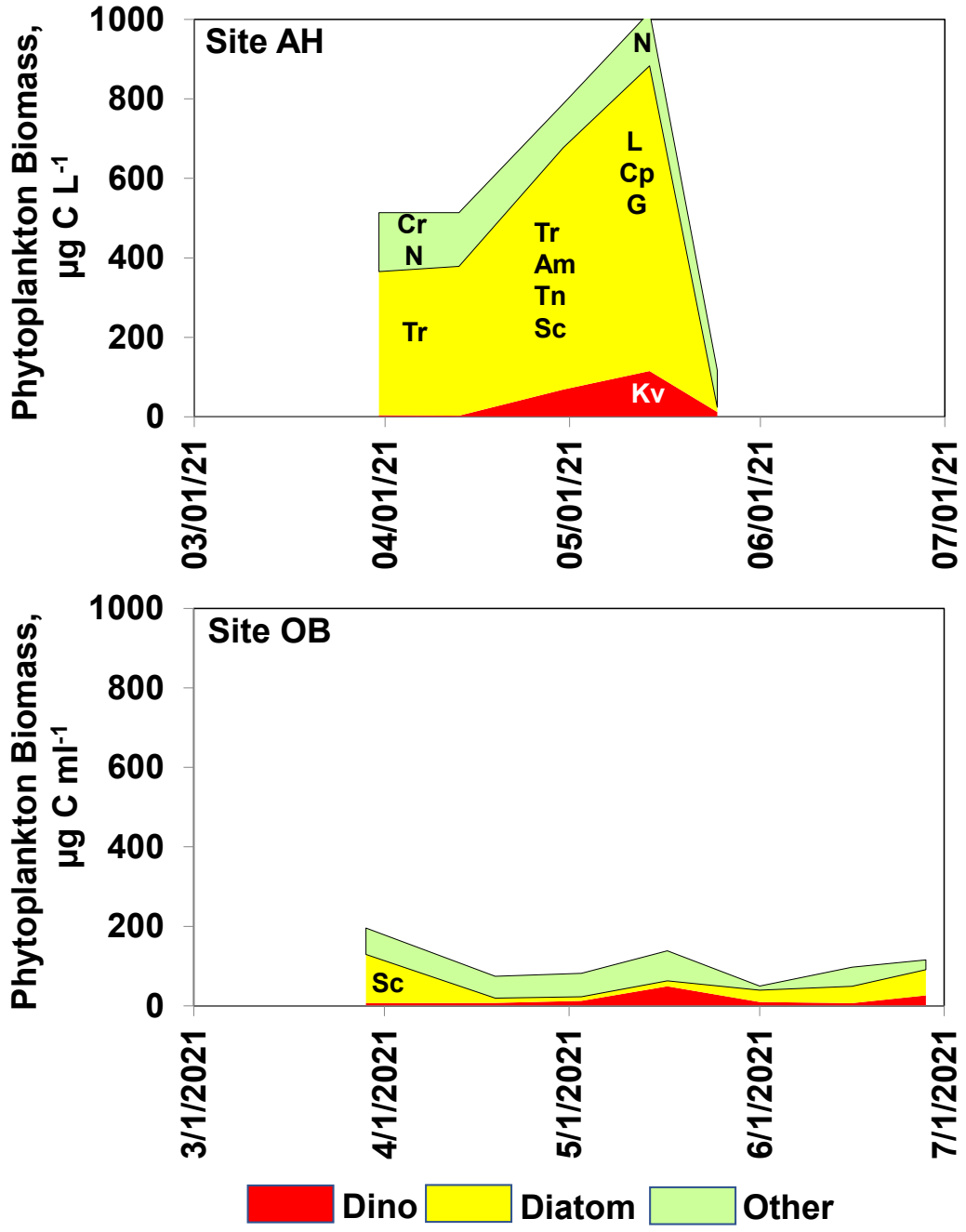

Table 1. Mean biomass (µg carbon L^{-1}) by phytoplankton group and total. Standard deviations are in parentheses. Percent contribution of each group for each site is shown below the mean values.

Site	Dinofl	Diatom	Other	Total
AH	41 (21) 6.3%	425 (286) 61.3%	125 (22) 32.4%	590 (338)
OB	17 (15) 15.7%	42 (38) 38.9%	48 (22) 44.4%	108 (45)

Table 2. Top-40 biomass observations of individual taxa for each of the five sites. Columns show frequency of occurrence in the Top-40, range of biomass values for the entries in the Top-40 and the highest cell density observed. Taxa in red are listed as harmful algal bloom (HAB) species by the IOC (Lassus et al. 2016).

Site AH				
Species	Group	Frequency in Top-20	Biomass µg C L^{-1}	Peak Density 10^3 Cells L^{-1}
Nannoplankton (2µ - 5µ)	Nanoplankton	4	70-105	33740
Tropidoneis lepidoptere	Diatom	3	111-312	2540
Leptocylindrus danicus	Diatom	2	40-238	1088
Thalassionema nitzschioides	Diatom	2	78-216	3628
Karlodinium veneficum	Dinoflagellate	2	47-94	363
Cryptophyte spp.	Cryptophyte	2	50-65	7075
Cerataulina pelagica	Diatom	1	227	1270
Guinardia striata	Diatom	1	183	181
Amphora/Entomoneis spp. cf.	Diatom	1	107	907
Skeletonema cf. *costatum*	Diatom	1	78	2721
Leptocylindrus minimus	Diatom	1	45	3084

Site OB				
Species	Group	Frequency in Top-20	Biomass µg C L^{-1}	Peak Density 10^3 Cells L^{-1}
Nannoplankton (2µ - 5µ)	Nanoplankton	3	35-46	14875
Cryptophyte spp.	Cryptophyte	3	17-28	3084
Skeletonema cf. *costatum*	Diatom	1	78	5442
Karlodinium veneficum	Dinoflagellate	1	47	181
Skeletonema menzeilli	Diatom	1	43	5432
Pyramimonas sp.	Chlorophyte	1	23	553
Pleurosigma/Gyrosigma sp.	Diatom	1	16	36
Gymnoid spp.	Dinoflagellate	1	12	363
Guinardia flaccida	Diatom	1	12	1
Cerataulina pelagica	Diatom	1	11	63
Cyclotella sp.	Diatom	1	10	181
Scrippsiella sp.	Dinoflagellate	1	9	9
Leptocylindrus minimus	Diatom	1	8	535
Rhabdonema sp.	Diatom	1	7	2
Prorocentrum micans	Dinoflagellate	1	7	2
Thalassionema nitzschioides	Diatom	1	6	272

Table 3. Complete list of HAB species observed in the study at the two sampling sites. List includes number of times observed, biomass range for each taxa, highest cell density, the toxin or HAB factor associated with each taxa and the effects of the HAB factor. Taxa in red are confirmed HAB species. The taxa in black are possible HAB taxa, pending additional taxonomic details. Taxa in blue have physical features that can be associated with HAB effects.

SITE AH					
Species	**Freq. of Obs.**	**Biomass Range µg C L^{-1}**	**Highest Density Cells L^{-1}**	**Toxin or HAB factor**	**Effects**
Prorocentrum texanum	4	1-2	600	Okadaic acid	DSP
Karlodinium veneficum	2	47-94	362,800	Karlotoxin	Neurotoxin, Ichthyotoxic
Akashiwo sanguinea	1	2	200	High biomass	Physical Disruption/Low O_2
Cochlodinium polykrikoides	1	<1	200	Oxidant	Cell damage
Gymnoid spp.	2	6-12	361,000	e.g. saxitoxin	Neurotoxins
Pseudo-nitzschia spp.	2	2-5	362,000	Domoic acid	Neurotoxin - ASP
Chaetoceros spp.	2	1-3	1,269,800	Spines	Physical Disruption
Chaetoceros simplex	1	6	181,400	Spines	Physical Disruption
Chaetoceros subtillis	1	4	544,200	Spines	Physical Disruption
Chaetoceros tenuissimus	1	3	541,200	Spines	Physical Disruption

SITE OB					
Species	**Freq. of Obs.**	**Biomass Range µg C L^{-1}**	**Highest Density Cells L^{-1}**	**Toxin or HAB factor**	**Effects**
Prorocentrum texanum	5	<1-1	400	Okadaic acid	DSP
Dinophysis caudata	2	1-2	200	Okadaic acid	DSP
Karenia mikiemoto	1	<1	200	Cytotoxins	Haemolysis
Karlodinium veneficum	1	47	181,400	Karlotoxin	Neurotoxin, Ichthyotoxic
Prorocentrum micans	1	7	1,600	Okadaic acid	DSP
Akashiwo sanguinea	1	5	600	High biomass	Physical Disruption/Low O_2
Gymnoid spp.	5	4-11	362,800	e.g. saxitoxin	Neurotoxins
Chaetoceros spp.	2	2-7	562,000	Spines	Physical Disruption