

Project Oyster Pensacola: Assessment of oyster survival, water quality and the fish and invertebrate community associated with oysters

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Technical Report of Project Oyster Pensacola

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## Introduction

The Bream Fishermen Association (BFA) was established over 50 years ago by local fishermen and residents to promote environmental stewardship in northwest Florida and south Alabama. Data collected by this organization represents some of the earliest records of water quality in the region. In recent years, the BFA samples water quality quarterly at 48 fixed stations to support Florida Department of Environmental Protection's Status and Trends Program. These data provide land managers, state, and federal agencies a detailed account of conditions within the Pensacola and Perdido watersheds.

Citizen science, engagement and education are important aspects of the BFA mission. The organization provides quarterly newsletters and evening meetings with guest speakers to keep the community apprised of local aquatic issues. The BFA operates closely with academia, (colleges and universities), K12 educators (homeschool organizations, middle and high schools) and businesses which provides an interesting and diverse pool of volunteers who assist in data collection. In August 2017, Don McMahon, owner of the Pensacola Bay Oyster Company (PBOC), spoke about his newly established aquaculture venture in lower Escambia Bay. He had leased an area at Magnolia Bluffs along Scenic Highway to secure floating cages for juvenile oyster grow-out. He presented his project, projections, and business model to BFA membership, and then conceded he had lost his first crop due to low salinity.

Eastern oysters, *Crassostrea virginica*, are widespread throughout Atlantic and Gulf estuaries. Adult oysters tolerate a wide range in temperature with significant populations from Atlantic Canada to Panama (Carlton and Mann 2009). Oysters have been an important food resource since pre-colonial times. However, areal coverage and biomass have been in decline for decades (zu Ermgassen et al. 2012). The oyster fishery in the Gulf of Mexico (GOM) represents about 64% of the US total (NMFS 2014). In 2012, the oyster fishery was the third highest revenue producer behind the shrimp and menhaden fisheries, representing 10% of total landing revenues in the GOM (NMFS 2014). In the Pensacola Bay system, wild harvest oyster landings have ranged from a peak of 491,000 pounds of oyster meat in 1985 to less than 3,000 since 2013 with no commercial harvest since 2018 (FWC Commercial Fisheries Landings Summaries).

Salinity is a key environmental factor affecting oyster success. While oysters tolerate and can survive for short periods in salinities below 5, oyster populations are often greatest between 10-20 PSU (Shumway 2009). At salinities above 25, disease, predation and competition with other organisms can limit populations even though growth and reproduction rates are often high (Shumway 2009). In addition to being a food source, oysters provide essential ecosystem services. This includes filtration and sequestration of organic matter and nutrients, which ameliorates the negative effects of phytoplankton blooms caused by anthropogenic nutrient pollution (Newell et al 2005). Microbes in both the sediments and the oysters also mediate ecosystem processes related to nutrient cycling, such as denitrification (Kellogg et al. 2013, Caffrey et al. 2016). Oysters are a foundation species, producing reefs or hard substrate that protects coastline (Grabowski et al. 2012, zu Ermgassen et al. 2012) and serve as habitat for over 200 species of fish and invertebrates (Karnauskas et al. 2013).

The freshwater inflow in 2017 which ruined the PBOC's first crop was driven by above average rainfall, particularly June and August which were about 3 times greater than the 30-year average for those months (52 cm compared to 18 cm). As a result, most area creeks and rivers were flooded. In Pensacola Bay and other parts of the estuary, stratification, the separation between freshwater on the surface and saltwater on the bottom, was extreme.

During the August 2017 meeting with Don McMahon, participants discussed whether hanging oysters in cages off docks would improve local water quality. These efforts have been undertaken in Chesapeake and Mobile Bays ([www.cbf.org/how-we-save-the-bay/programs-initiatives/maryland/oyster-restoration/oyster-gardening/](http://www.cbf.org/how-we-save-the-bay/programs-initiatives/maryland/oyster-restoration/oyster-gardening/), [www.restoreourshores.org/living-shoreline/oyster-mats-gardening/](http://www.restoreourshores.org/living-shoreline/oyster-mats-gardening/)). The PBOC agreed to provide oyster spat for this effort if BFA agreed to organize the effort and secure the state permits from various state agencies. Florida Fish and Wildlife Commission (FWC) oversees regulations concerning deployment of caged shellfish, including oysters, in surface waters. FWC permit requirements include information about cage size, types of oysters (ploidy) and the physical address of the oyster cages. The BFA and PBOC sponsored an education through engagement program to encourage improvement of local water quality in Escambia and Santa Rosa counties to recruit citizen volunteers and fulfil permit requirements. Interested citizens, schools and organizations were invited to participate in a half day face-to-face meeting where they learned about oyster biology, proper care and instructions for hanging cages, potential predators and the methods and data the BFA would collect on survival and growth during this project. This workshop afforded the public a better understanding of the important role that oysters serve in the environment, while in turn, BFA collected information needed for state permit requirements and was able to access private property for this project.

This report describes characteristics of water quality at Project Oyster Pensacola (POP) sites and oyster metrics including growth, survival, and recruitment. We also describe the composition of the biofouling fish and invertebrate community on cages 1 year after deployment and examine how water quality affected oysters. In addition, an in-depth study examined high frequency variability in water quality parameters at one of the POP sites in lower Pensacola Bay (Site X) between January and March 2019. Goals of the project were to increase awareness by local citizens about water quality and the importance of oysters in improving water quality and to provide information for planning future oyster restoration projects.

## Methods

### Site Selection and Deployment

BFA biologists visited each candidate location once in either late January or early February 2018 to evaluate its suitability for oysters. Water quality, presence of wild oysters, bottom type (such as presence or absence of seagrasses) and conditions of the shoreline (natural, riprap or bulkhead) were evaluated. If salinity was below 10, the site was deemed unsuitable. Within the Perdido and Pensacola Bay systems, 25 locations were determined to have acceptable conditions and were selected for the pilot study (Fig. 1). The project lasted for 18 months.



Figure 1 – Map of sampling locations for Project Oyster Pensacola

Cages were constructed of commercially available aquaculture cloth with dimensions of 18" x 6" x 4" and a mesh size of ½". Cages were hung off the docks using either 1 to 2 lines to keep them suspended in the water column and away from any pilings as well as from reaching the sediment surface. Cages, ropes, a brush to remove biofouling, and 75 baby oysters were distributed to participants at the distribution site on May 12, 2018. Cages were deployed at 24 sites. All were stocked with 75 triploid oysters provided by PBOC except for Site N which was stocked with 92 triploid oysters. Before deployment in cages, individual oysters were counted, weighed, and measured for height (distance from umbo to distal) and width. Average oyster height and width were 2.5 cm and 1.7 cm, respectively. This provided the baseline morphometrics of the spat deployed in each cage.

## Monitoring

Three surveys of water quality were done after oyster deployment: during the summer of 2018 (June-July), October 2018, and January 2019. At locations where the water depth was greater than 1 m, surface and bottom temperature, salinity, dissolved oxygen and pH was recorded. At the shallow sites, only surface readings were taken. A Secchi disk was used to measure visibility and water depth.

During the water quality surveys following oyster deployment, survival of oysters was assessed at each site. Numbers of living, dead and wild oysters were recorded. Triploid oysters could be





*Photo 1 - Triploid Oyster*

distinguished from wild oysters by the distinctive cupped shape of the triploid oysters (Photo 1). Ten triploid oysters were haphazardly selected for measurement of height, width and weight. Dead oysters were removed from cages. Sites with 100% oyster mortality were not revisited in later surveys. Between May and July 2019, all locations with surviving oysters were surveyed for the invertebrate and fish community composition. Measurements were made of height and width of all living and recently dead triploid and any wild oysters found in or on cages. The biofouling community associated with oysters and cages was surveyed. Fish and invertebrates were identified to the lowest possible taxa. Counts of larger organisms were made when possible, and relative abundance of settled organisms including tube dwelling amphipods, calcareous tube dwelling worms, barnacles, and bryozoans were noted.

## High frequency study

Site X in lower Pensacola Bay was selected for an in-depth study of high frequency variability in water quality. High frequency measurements of conductivity, temperature, dissolved oxygen, light and photosynthetically active radiation (PAR) were made between January 28, 2019 and March 21, 2019. Readings were made at 15-minute intervals using two types of sensors: HOBOT brand conductivity and light sensors and MinDot dissolved oxygen and PAR sensors. All sensors recorded temperature. Sensors were deployed on a float just below the water surface in about 1 m of water.

The site was visited weekly to download data, clean sensors, measure water quality data with YSI multimeter, light attenuation with a LiCor 4 Pi sensor and collect grab water samples. Water samples were filtered for chlorophyll *a* and dissolved nutrients (nitrate plus nitrite, ammonium and dissolved inorganic phosphate). Chlorophyll *a* was run on 90% acetone extracts using the Welshmeyer (1994) method. Nitrate plus nitrite was determined using the vanadium reduction method of Schnetger and Lehnert (2014). The Holmes et al. (1999) fluorometric method was used for ammonium analysis, while the molybdate method of Parsons et al. (1984) was used for dissolved inorganic phosphate. Salinity was calculated from HOBOT conductivity and temperature data using the OCE package in R.

## Analysis

Mean of surface water quality parameters were calculated and used in correlation analyses. Only triploid oyster values were used for oyster growth, size, and mortality. Oyster mortality was calculated using the method described in Ragone Calvo et al. (2003). The average daily mortality and cumulative mortality are reported below. Growth rates were calculated as described in La Peyre et al. (2013). The increase in growth between sampling periods was normalized to a 30-day standard month. These rates were averaged and reported below.

For analysis of oyster growth and mortality, sites were binned into 3 categories based on average salinity at the site. These were defined as oligohaline where mean salinity was less than 10, mesohaline where mean salinity between 10 and 20, and euryhaline where mean salinity was greater than 20. Sites were also grouped based on whether they were in an enclosed area such as a bayou or canal (enclosed) or in the open bay (open). Differences among salinity zones were evaluated using Kruskal-Wallis rank sum test and the multiple comparisons Dunn Test since

cumulative mortality, maximum oyster height and wild oyster numbers had a non-normal distribution. Analyses were done using R (R Core Team 2019) using packages tidyr (Wickham and Henry 2020), FSA (Ogle et al. 2020) and RColorBrewer (Neuwirth 2014).

## Results

### Water Quality

Salinity across all sites ranged from 1.1 to 29.0 with the lowest salinities in Perdido and Blackwater Bays (Fig. 2) and the highest salinity was in Big Lagoon. The range in salinity at the individual sites was highest at Site X, located in lower Pensacola Bay on the Gulf Breeze Peninsula (Fig. 3). This was also the location of the high frequency study described below.

Four locations were defined as oligohaline (average salinity less than 10) and were located in the East, Blackwater and Escambia Bays and upper Perdido Bay (Table 1). Most locations were mesohaline with an average salinity between 10 and 20. Euryhaline locations were predominantly in Santa Rosa Sound (5 locations) along with Big Lagoon and Pensacola Bay (Table 1).

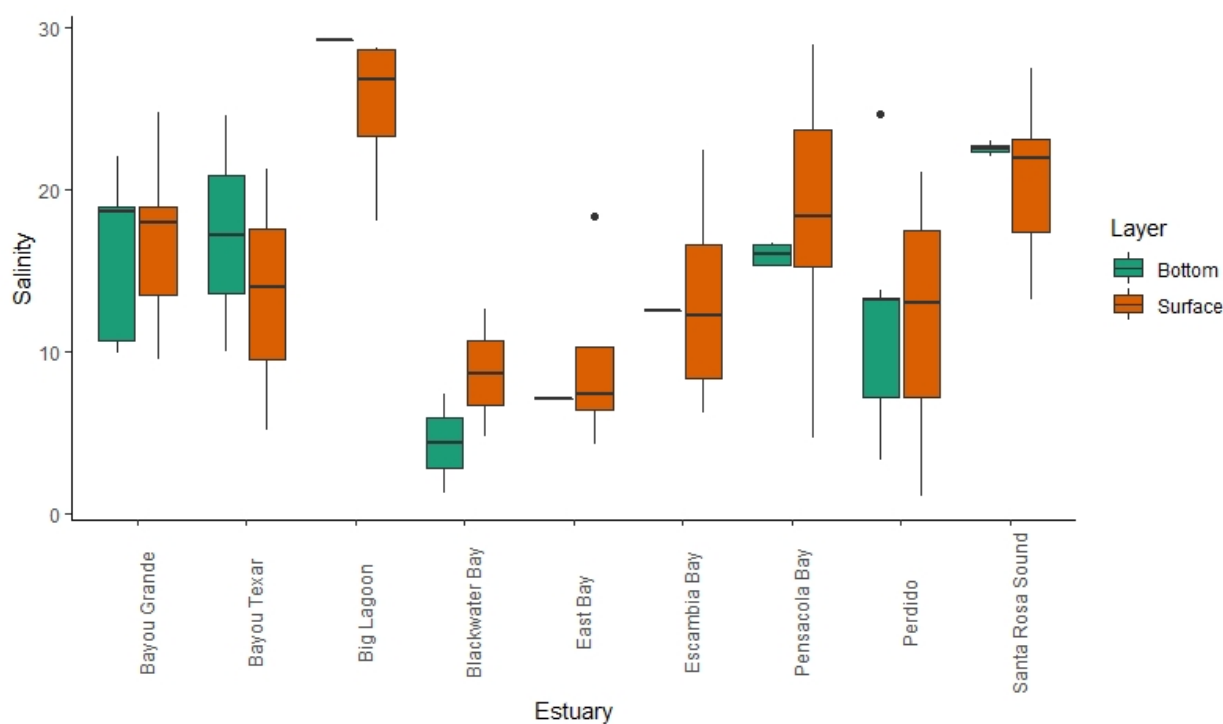


Figure 2 – Box plot of salinity in different subestuaries of the Pensacola and Perdido Bay system. Line represents median, upper and lower bounds of box represent 25<sup>th</sup> and 75<sup>th</sup> percentiles, lines represent 10<sup>th</sup> and 90<sup>th</sup> percentiles. Dots represent outliers. Bottom samples are in green and surface samples are in orange.

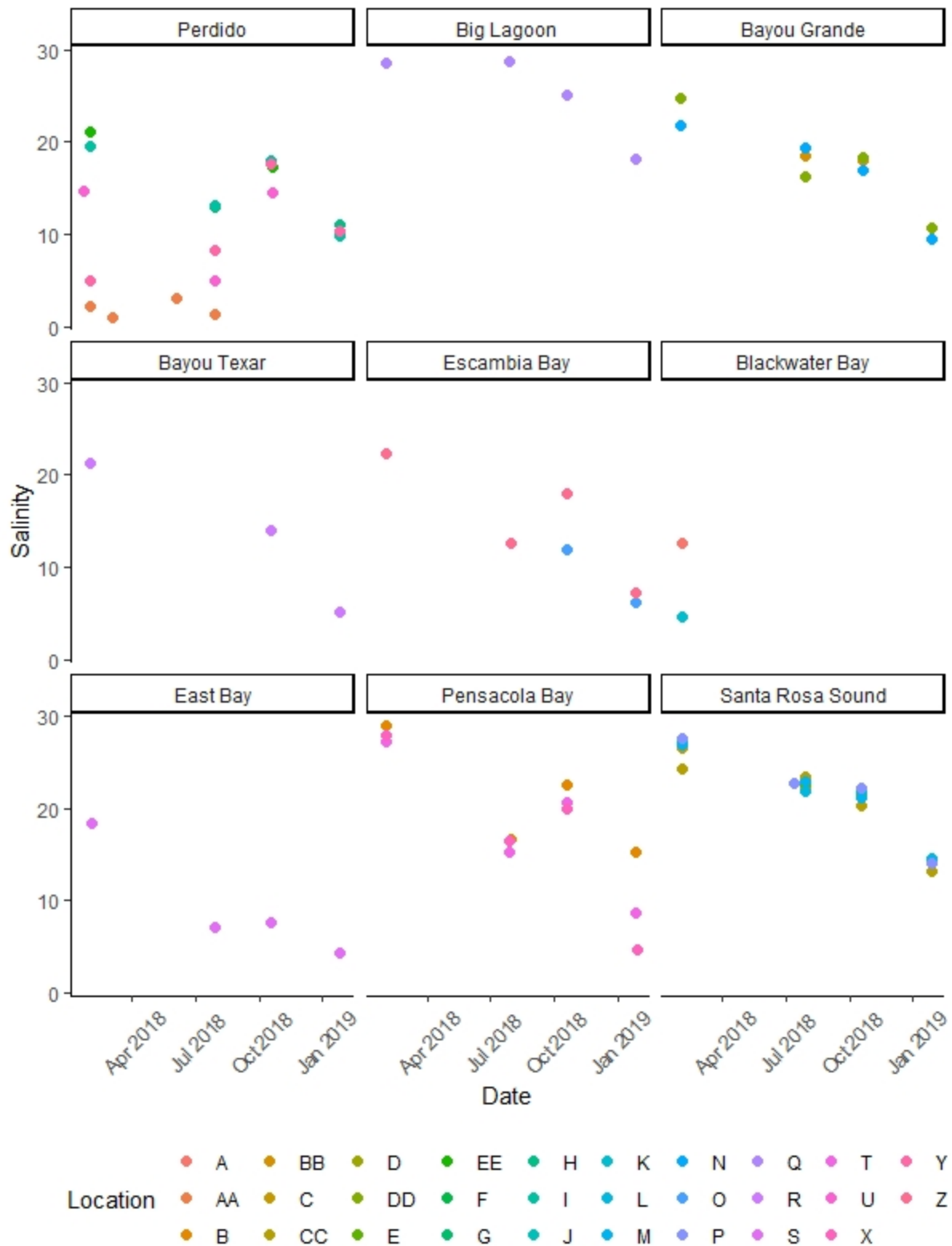


Figure 3 – Surface salinity on individual sampling dates by subestuary at Project Oyster Pensacola sampling locations between January 2018 and February 2019.

Table 1 – Table of Project Oyster Pensacola sampling locations, mean salinity and salinity zone where oligohaline is a mean salinity less than 10, mesohaline is a mean salinity between 10 and 20, and euryhaline is a mean salinity greater than 20. Type is defined by sites that are more enclosed such as bayou and canal locations compared to the open bay locations.

Estuary	Location	Mean depth (m)	Mean Salinity	Salinity Zone	Type
Bayou Grande	N	2.2	16.9	Mesohaline	enclosed
Bayou Grande <sup>1</sup>	DD	1.1	17.5	Mesohaline	enclosed
Bayou Grande	BB	1.0	15.4	Mesohaline	enclosed
Bayou Texar	R	2.1	13.5	Mesohaline	enclosed
Big Lagoon	Q	1.7	25.1	Euryhaline	open
Blackwater Bay	K	n.d. <sup>2</sup>	4.8	Oligohaline	enclosed
Blackwater Bay	A	0.3	12.7	Mesohaline	enclosed
East Bay	S	0.6	9.4	Oligohaline	open
Escambia Bay	O	1.0	9.1	Oligohaline	enclosed
Escambia Bay	Z	1.0	15.1	Mesohaline	open
Pensacola Bay	T	0.8	18.0	Mesohaline	open
Pensacola Bay	X	1.0	17.3	Mesohaline	open
Pensacola Bay	B	0.8	20.8	Euryhaline	open
Perdido	AA	1.4	1.9	Oligohaline	open
Perdido	U	0.9	11.4	Mesohaline	open
Perdido	Y	0.6	10.3	Mesohaline	enclosed
Perdido	EE	0.8	15.4	Mesohaline	open
Perdido	G	1.2	13.6	Mesohaline	enclosed
Perdido	H	1.1	13.9	Mesohaline	enclosed
Perdido	I	1.2	15.1	Mesohaline	enclosed
Perdido	J	n.d.	15.1	Mesohaline	enclosed
Santa Rosa Sound	CC	0.7	20.3	Euryhaline	open
Santa Rosa Sound	C	1.1	21.1	Euryhaline	open
Santa Rosa Sound	D	n.d	21.1	Euryhaline	open
Santa Rosa Sound	E	1.2	19.4	Mesohaline	open
Santa Rosa Sound	F	n.d.	19.4	Mesohaline	open
Santa Rosa Sound	M	1.5	21.3	Euryhaline	open
Santa Rosa Sound	L	0.8	21.4	Euryhaline	enclosed
Santa Rosa Sound	P	0.7	21.6	Euryhaline	open

<sup>1</sup> Actual location in Bayou Davenport, a very small urban bayou next to Bayou Grande

<sup>2</sup> n.d. – no data

Temperature showed a typical seasonal pattern with highest values in the summer and lowest during winter (Fig. 4). Spatial variation among sites could be substantial as in January 2018 when Site AA was 9.8°C and Site B was 15.6°C.



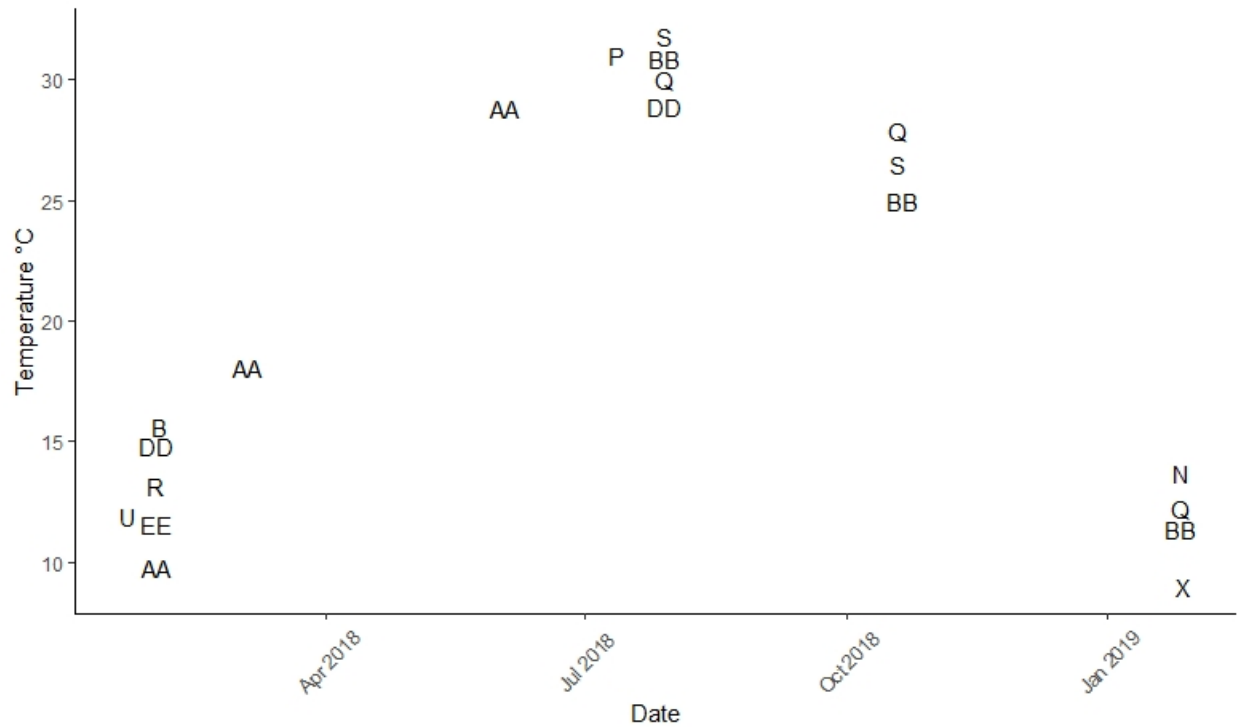


Figure 4 – Temperature on individual sampling dates at Project Oyster Pensacola sampling locations between Jan 2018 and February 2019.

At locations where bottom water quality was measured (depths greater than 1 m), dissolved oxygen at the surface was higher than at the bottom, except in Bayou Grande (Fig. 5). Surface water oxygen concentrations were usually close to saturation, except in Perdido Bay where mean percent saturation was 79% at the surface and 54% in the bottom. This was particularly apparent at Site AA in Perdido Bay where mean oxygen was 56% saturation at the surface and 30% at the bottom. It is unclear how much effect these lower bottom water oxygen had on oyster survival and growth since cages were deployed so that oysters would be near the surface. Spatial variability in dissolved oxygen was high both between sites and within sites (Fig. 6). Oxygen levels were consistently high at sites near seagrass beds such as Big Lagoon, Pensacola Bay Site B and Santa Rosa Sound.

Not surprisingly, oxygen concentrations were generally higher in the winter than summer. The correlation coefficient between oxygen concentration in terms of percent saturation and temperature was  $r = -0.54$  ( $p < 0.001$ ). Since this relationship is based on percent saturation and not absolute concentration in mg/L, it reflects greater oxygen consumption such as higher microbial respiration during the summer months. The relationship between oxygen concentration in mg/L and temperature was much stronger  $r = -0.87$  ( $p < 0.001$ ) which reflects both increased respiration and the fact that water at higher temperatures holds less oxygen.

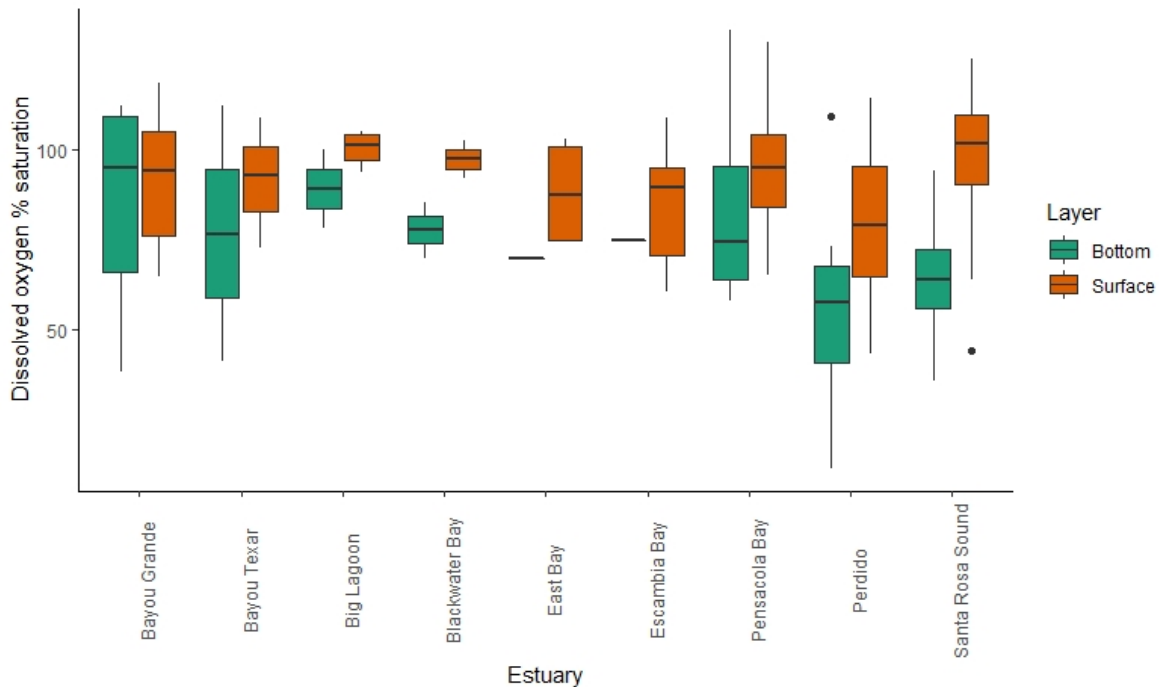


Figure 5 - Box plot of dissolved oxygen (%) in different subestuaries of the Pensacola and Perdido Bay system. Line represents median, upper and lower bounds of box represent 25<sup>th</sup> and 75<sup>th</sup> percentiles, lines represent 10<sup>th</sup> and 90<sup>th</sup> percentiles. Dots represent outliers. Bottom samples are in green and surface samples are in orange.

Salinity and pH were positively correlated ( $r = 0.70$ ,  $p < 0.001$ , Fig. 7). Variability in pH was generally greater when salinity was low. The carbonate buffering capacity of seawater maintains pH in seawater near 8.1. Blackwater streams rich in tannins often have low pH values, so freshwater often has lower pH values than seawater. In addition, pH like oxygen is influenced by primary production (pH decreases) and respiration (pH increases).

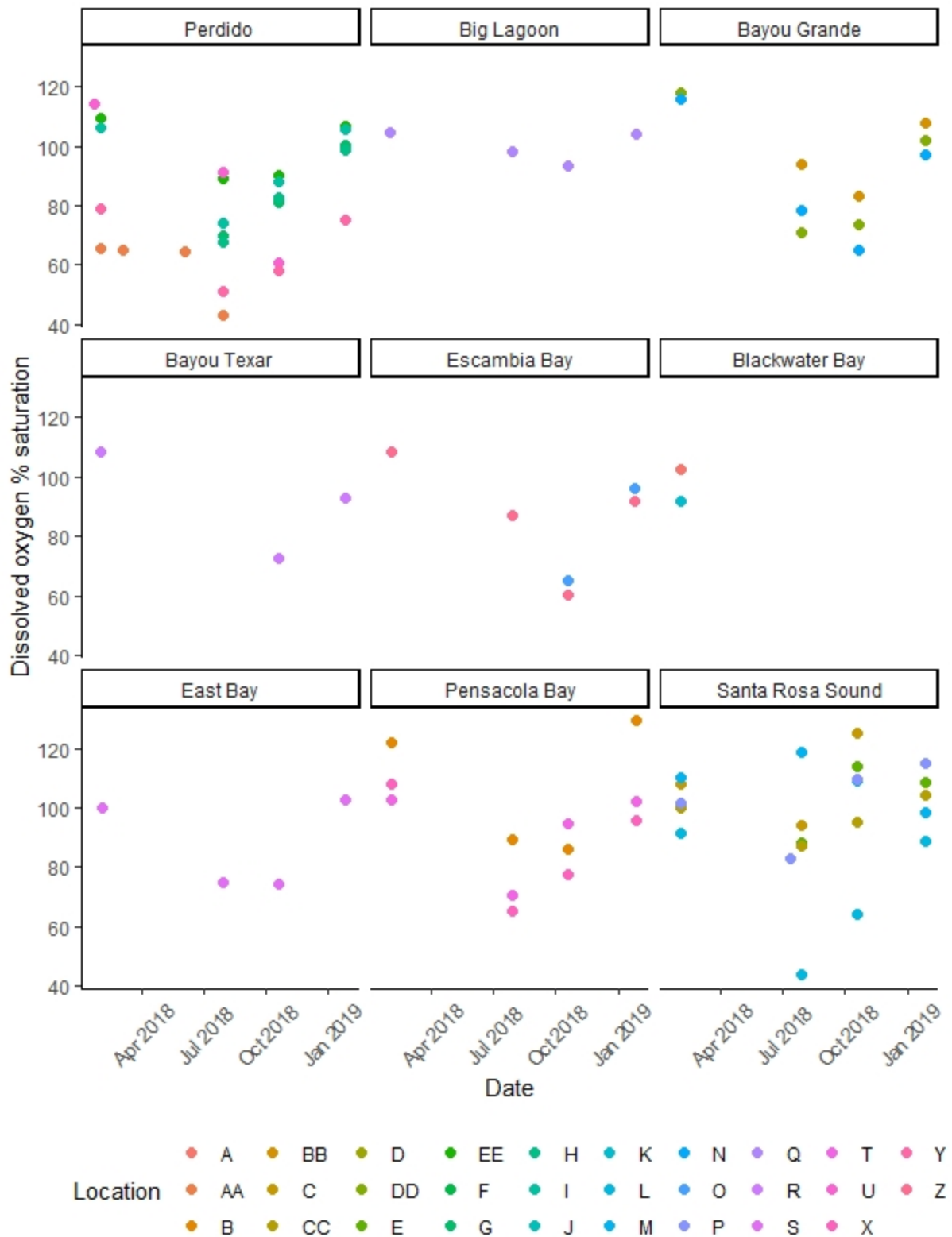


Figure 6 – Surface dissolved oxygen on individual sampling dates by estuary at Project Oyster Pensacola sampling locations between January 2018 and February 2019.

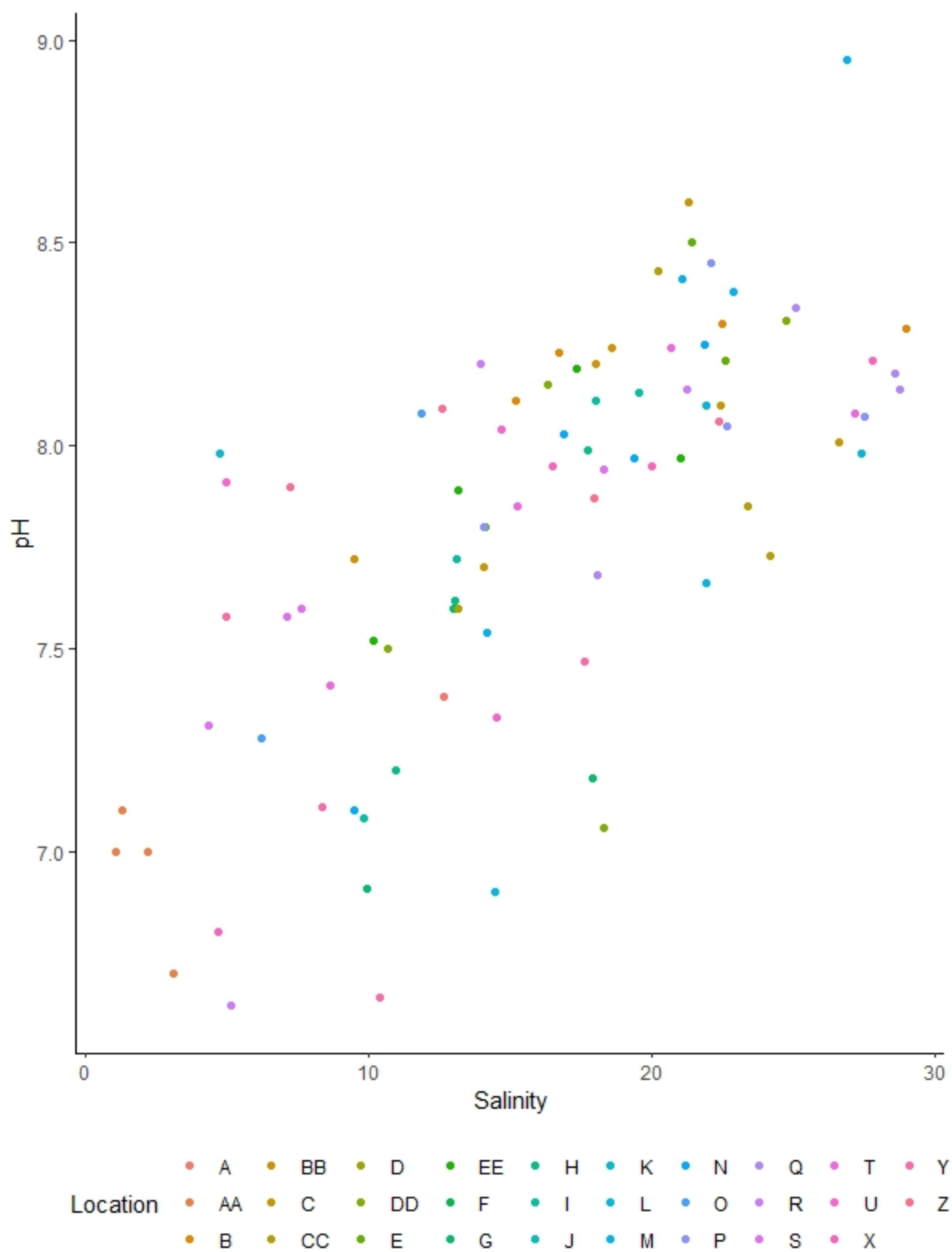


Figure 7 – pH versus salinity on individual sampling dates by estuary at POP sampling locations.

## Oyster Population Dynamics

All sites began with 75 oysters, except for site N which started with 92 oysters (Fig. 8). No oysters survived at sites A and K in Blackwater Bay where there was 100% mortality after the first quarter. Mortality was also high at Site AA in Perdido and Site Z Escambia Bay (Figs. 8 & 9). Cumulative mortality was highest at oligohaline sites, particularly those in canals (Fig. 10). Mortality was lowest in the mesohaline zone with no differences between the open bays and enclosed locations like Bayous and canals (Fig. 10). However, rates were not significantly different among the salinity zones ( $p = 0.57$ ).



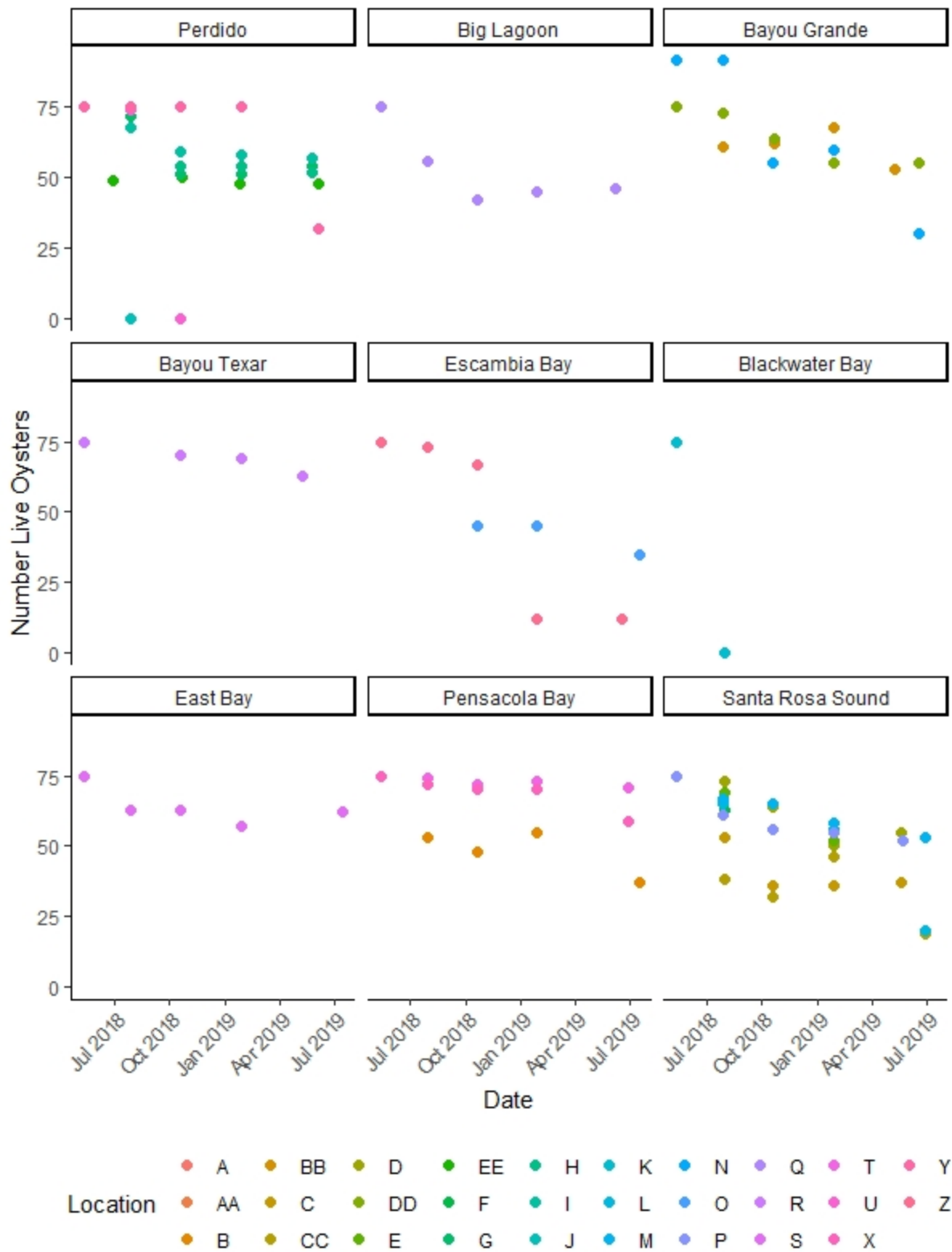


Figure 8 – Number of live oysters at POP sites by subestuary between May 2018 and July 2019.

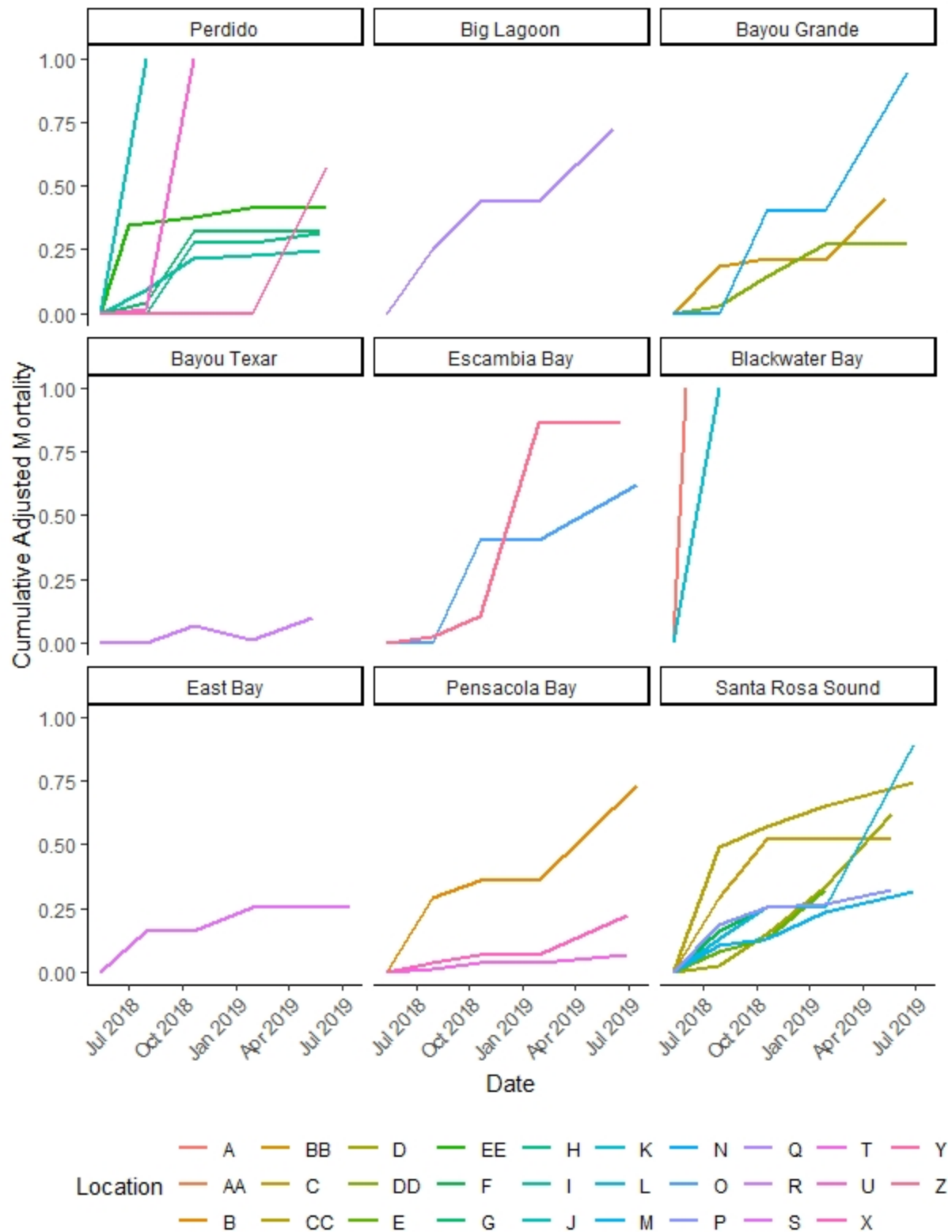


Figure 9 – Cumulative mortality at POP sites by subestuary between May 2018 and July 2019 where 1 is 100% mortality

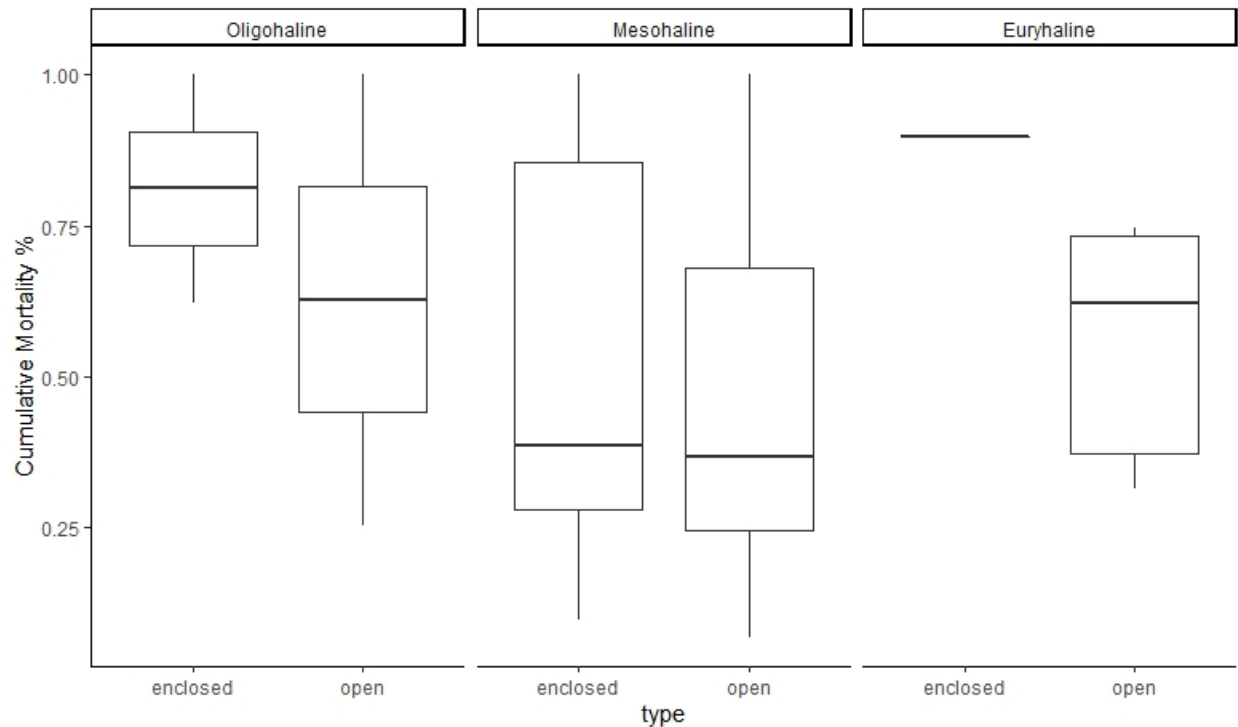


Figure 10 – Cumulative mortality (1 = 100% mortality) of oysters in different salinity zones where Oligohaline is a mean salinity less than 10, Mesohaline is a mean salinity between 10 and 20, and Euryhaline is a mean salinity greater than 20. Type is defined by sites that are more enclosed such as bayou and canal locations compared to the open bay locations.

The biggest increase in growth occurred between deployment in May 2018 and the summer measurement in July 2018 (Fig. 12). During this period, average growth rates ranged from about 12.0 mm/mo at oligohaline locations to 17.8 mm/mo at the euryhaline open bay locations. These rates were significantly higher ( $p < 0.001$ ) than rates during the other seasons. The May-July 2018 growth rates were similar between euryhaline and mesohaline locations ( $p = 0.13$ ) and significantly higher than at oligohaline sites ( $p = 0.012$ ,  $p = 0.06$  for euryhaline and mesohaline, respectively). Rates in Fall 2018 (July to October 2018) and Winter 2019 (October 2018 to January 2019) were between 7 and 32 percent of the initial rates. Growth rates during the last sampling interval generally were between 2 and 3 mm/mo (Fig. 12). During the fall and winter, euryhaline open locations had consistently high growth rates which led to overall larger sized oysters than other locations (Fig. 13). Salinity was not the only factor which may have affected oyster growth. The maximum oyster height was lower at sites with low dissolved oxygen (Fig. 14). If the 4 sites with mortality immediately following deployment were excluded, the correlation between maximum oyster height and dissolved oxygen was 0.57 ( $p = 0.01$ ).

Oyster weight increased over the course of the study, with the greatest weights at Site Q in Big Lagoon and Site B in Pensacola Bay of 129 and 81 g, respectively (Fig. 15). Average final weights of oysters from other locations was between 20 and 40 g (Fig. 15). The relationship between height and weight was similar across all sites (Fig. 16).

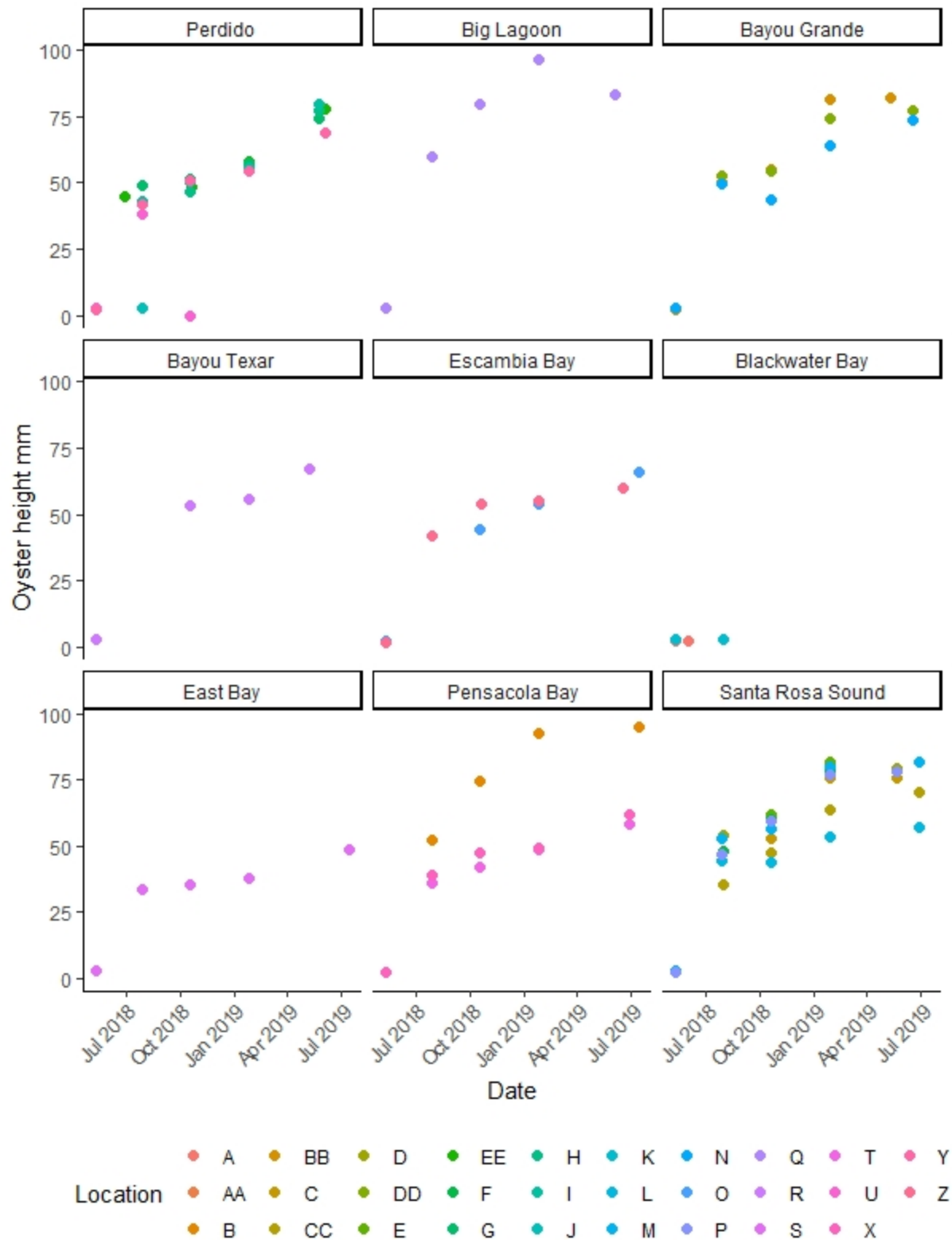


Figure 11 – Average height in mm of oysters at POP sites by subestuary between May 2018 and July 2019.

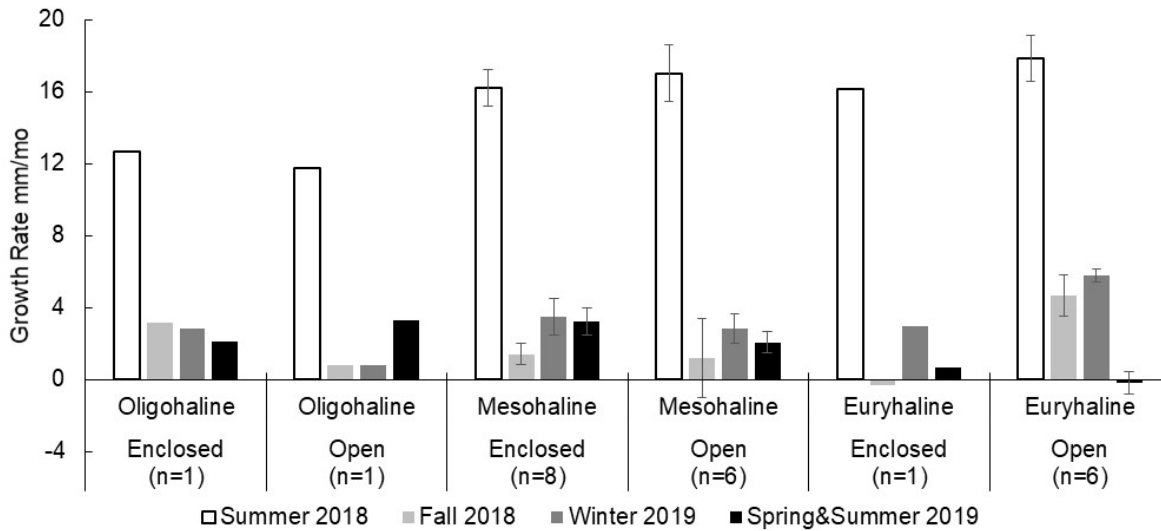


Figure 12 – Average monthly growth rate in mm/mo during the different seasons from oysters in different salinity zones where where Oligohaline is a mean salinity less than 10, Mesohaline is a mean salinity between 10 and 20, and Euryhaline is a mean salinity greater than 20. Sites such as Bayous and canals are enclosed compared to the open bay locations. The number of samples is given as n. Standard error (S.E.) is shown.

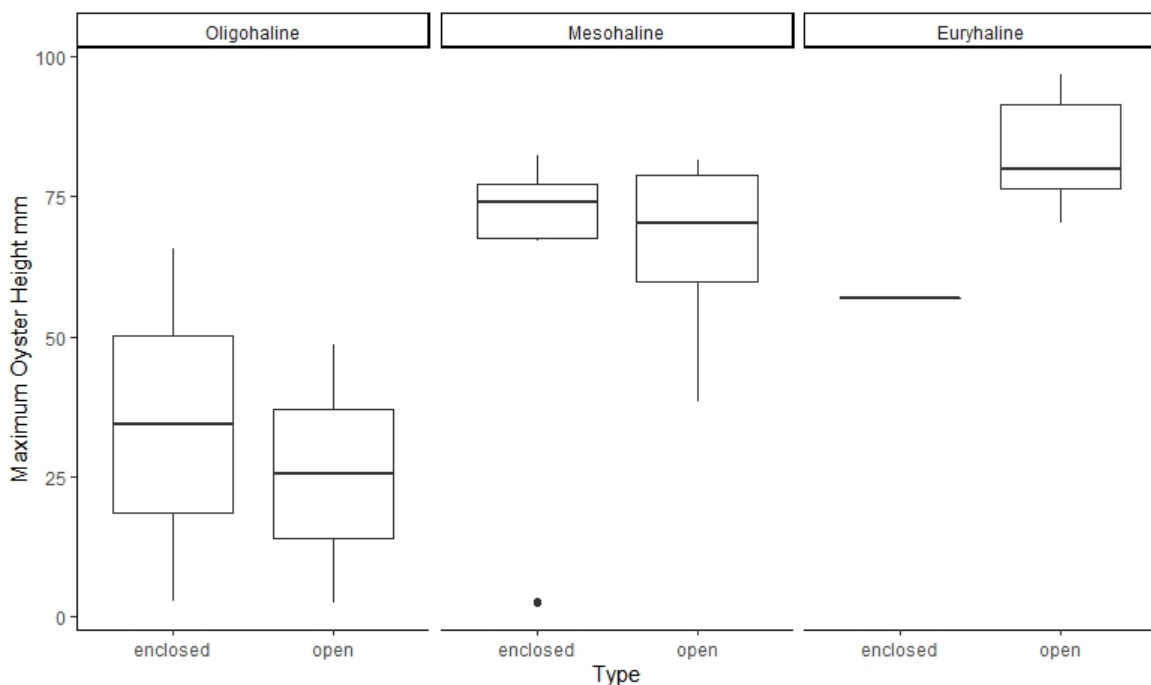


Figure 13 – Maximum oyster height in different salinity zones where Oligohaline is a mean salinity less than 10, Mesohaline is a mean salinity between 10 and 20, and Euryhaline is a mean salinity greater than 20. Type is defined by sites that are more enclosed such as bayou and canal locations compared to the open bay locations.



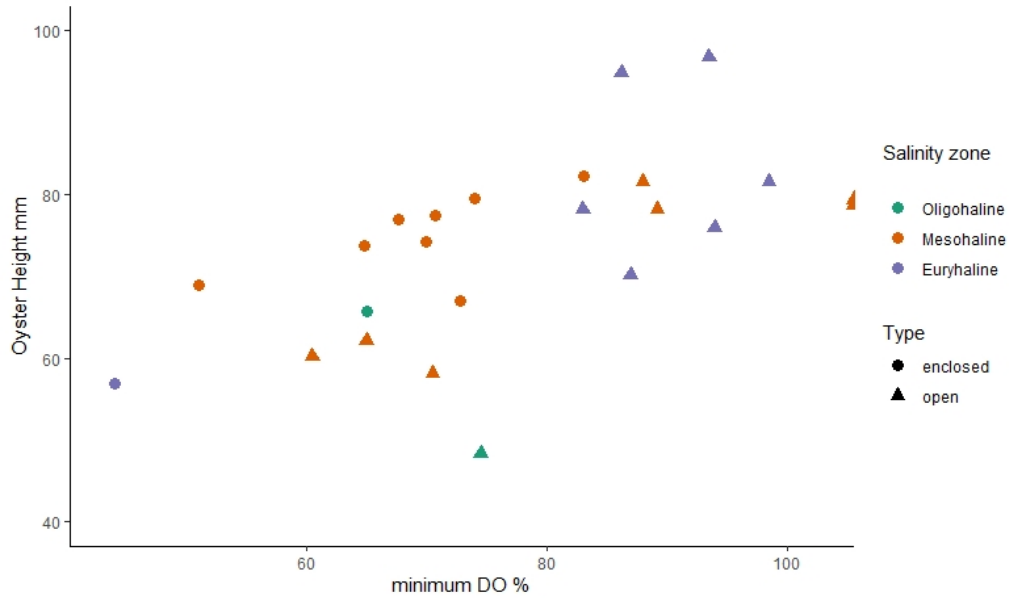


Figure 14 - Maximum oyster height versus minimum dissolved oxygen in different salinity zones where where Oligohaline is a mean salinity less than 10, Mesohaline is a mean salinity between 10 and 20, and Euryhaline is a mean salinity greater than 20. Type is defined by sites that are more enclosed such as urban bayou and canal locations compared to the open bay locations.

Recruitment was highest at Site B in Pensacola Bay where over 300 wild oysters were attached to the cage during the summer 2019 survey (Fig 17). Recruitment at Santa Rosa Sound and Big Lagoon sites was also high between 100 and 200 oysters at some locations. Recruitment at the urban bay sites was variable with high recruitment at Site DD in Davenport Bayou near Bayou Grande. In contrast, Perdido Bay, Escambia and East Bays had very few recruits, 3 or less (Fig 17).

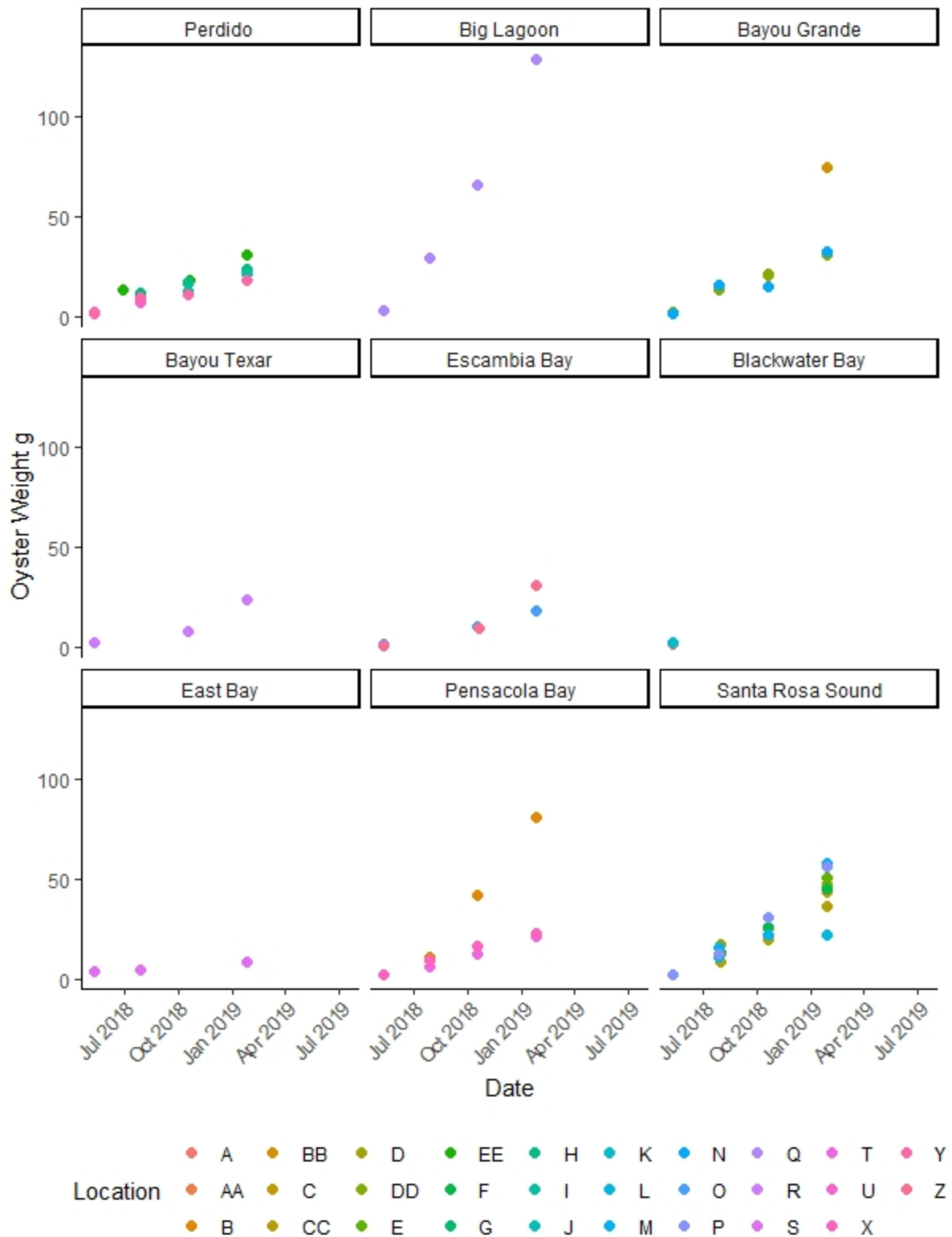
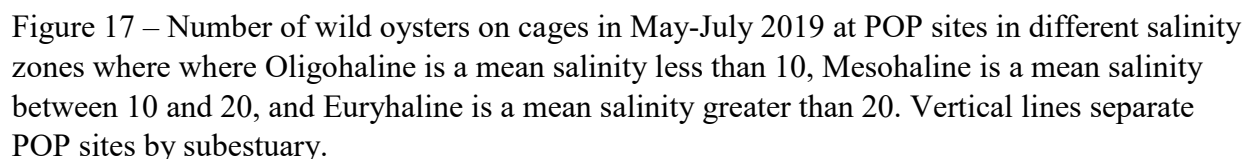
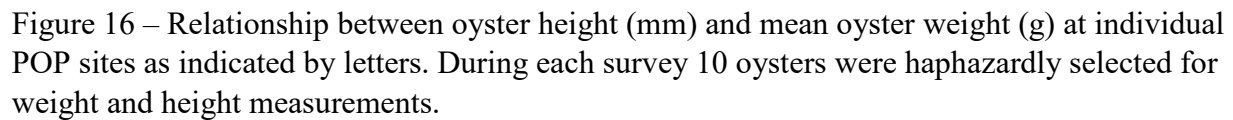


Figure 15 – Average weight of oysters at POP sites by subestuary between May 2018 and July 2019. Ten oysters were haphazardly selected from cages for weight measurements.



## Factors Influencing Oyster Survival and Growth

There was no relationship between mortality and mean salinity (data not shown) with sites having similar mean salinity and relatively low mortality such as Site R, but 100% mortality at Site A (Fig. 9). However, mortality was higher at oligohaline sites than higher salinity sites (Fig 10). This relationship was driven by high mortality at sites A and K in Blackwater Bay and Site AA in Perdido Bay, all sites where salinity was sometimes below 5. A similar pattern has been observed in Breton Sound, Louisiana following openings of the Bonne Carre Spillway (La Peyre et al. 2013).

The factors affecting mortality were variable. Wildlife, specifically otters, at a mesohaline site in Blackwater Bay were reportedly able to extract and ingest oysters; while at another mesohaline site in Perdido Bay, Hurricane Michael (October 2018) generated enough wave action to bounce the oyster cage onto the dock where it stayed long enough to cause 100% mortality. Predation in the euryhaline sites may have been significant based on observations during water quality and growth monitoring. During the water quality and oyster surveys, we observed that any cages which touched the bottom in the higher salinity zones had oyster drills on them. While some sites such as those in Perdido had consistently undersaturated dissolved oxygen concentrations, there was no relationship between dissolved oxygen and oyster mortality. However, oyster growth rates were lower at low dissolved oxygen concentrations (Fig 14).

Growth rates were higher at salinities above 10 compared to oligohaline sites (Fig. 11).

Maximum oyster height and weight were positively correlated with mean salinity with  $r = 0.72$  ( $p < 0.001$ ) and  $r = 0.73$  ( $p < 0.001$ ), respectively (Figs. 18, 19). We also observed the greatest recruitment of wild oysters at POP locations when salinity was above 20 (Figure 20)

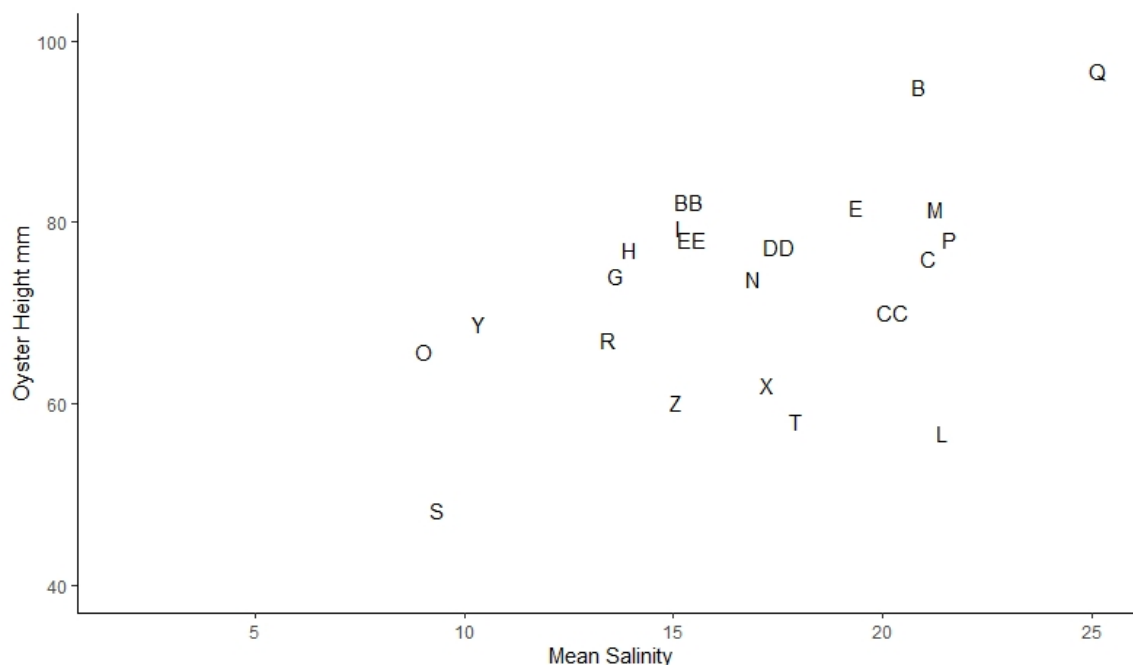


Figure 18 – Maximum oyster height versus mean salinity at POP sites

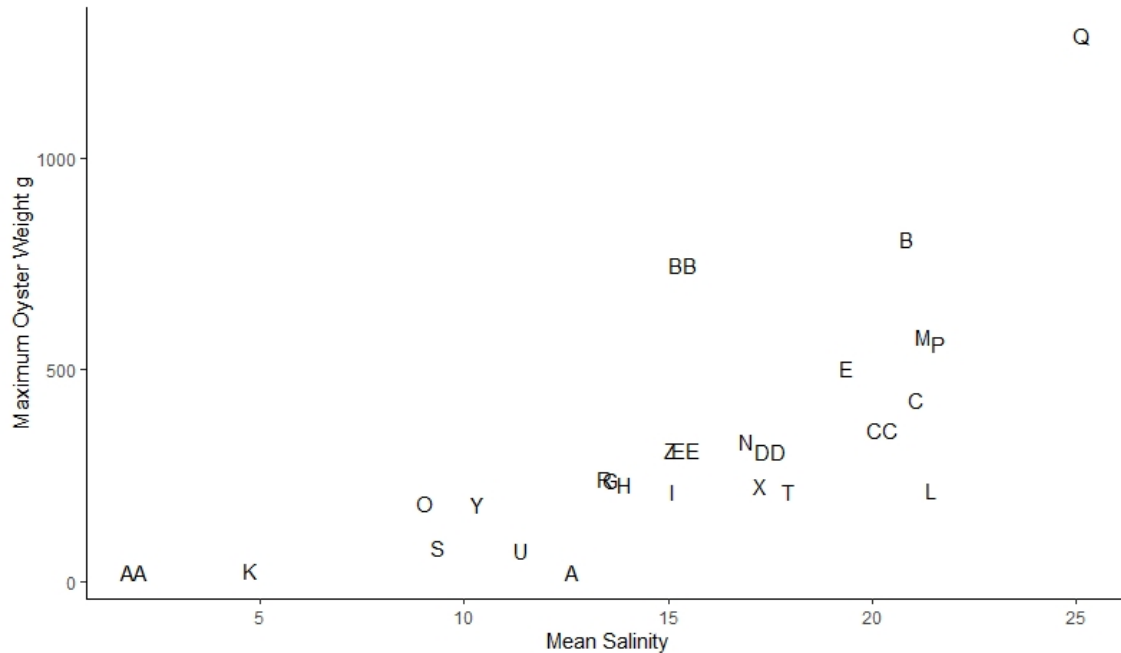


Figure 19 – Maximum oyster weight versus mean salinity at POP sites

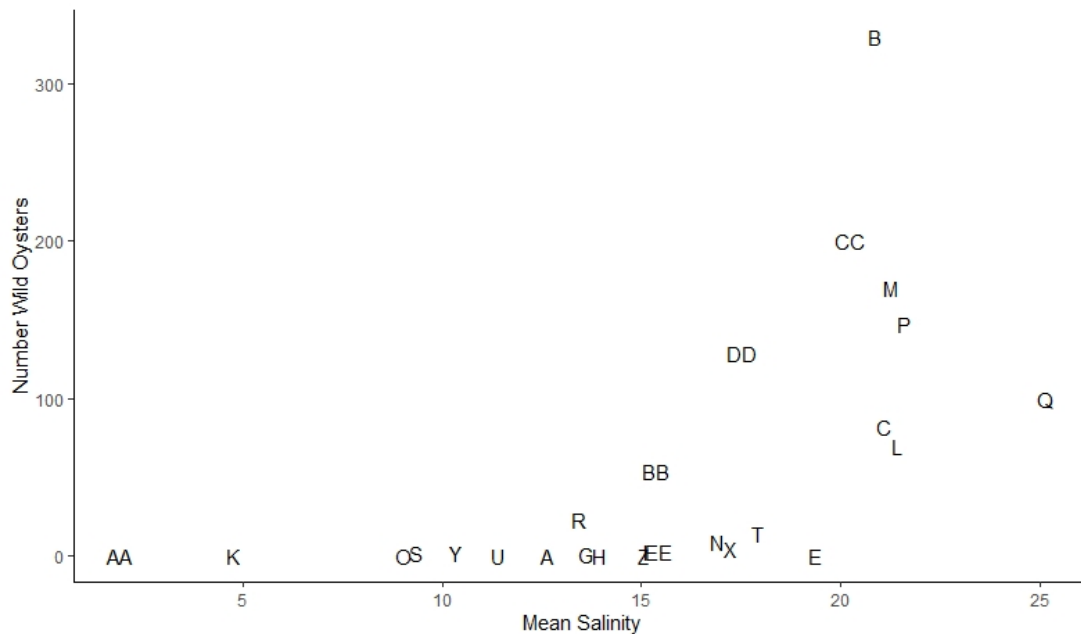


Figure 20 – Number of wild oysters in May/June 2019 versus mean salinity

### Biofouling Community Composition

Fish were associated with oyster cages at 17 of the 22 sites. Blennies were the dominant taxa observed and were found at 14 of the 22 sites. They were particularly abundant at sites O and B, which also had the overall highest fish counts (Fig. 21). Lizardfish were also abundant at site O. Other species associated with the cages included gobies, Pinfish, skillettfish and one unidentified fish.



Crustaceans were also very abundant on and in the cages. Counts were made of all individuals except for amphipods which were often too numerous to count. Where amphipods formed a colony on the cages, counts of the individual colonies were recorded rather than numbers of individuals. Barnacles were the second most common crustacean, occurring at all locations except Site T (Fig. 22). They were most abundant at Site B with 357 individuals on the cage. Mud crabs were also common, occurring at all sites except H. They were most abundant at Site B with 175 individuals.

The most abundant mollusks were mussels (both smooth and ribbed) which were found at 19 of 22 sites (Fig. 23). They were particularly abundant at Site O, B, C, and E. Oyster drills and oyster drill egg cases were found only at euryhaline sites (Q, CC and P). Few mollusks were found at mesohaline sites. Other invertebrate taxa on or inside cages included abundant bryozoans, sabellid “feather duster worms” and other polychaetes (Fig. 24). Marine species such as anemones, corals and sponges were observed at some of the euryhaline sites (Fig. 24).

The highest overall abundance of fish and invertebrates including number of oysters was at Sites B and O. There were consistent and significant differences in the community composition between mesohaline and euryhaline sites based on an analysis of similarity ( $R = 0.405$ ,  $p = 0.04$ ). A non-metric multidimensional scaling plot (Fig. 25) shows the open euryhaline in a small, tight grouping. Site L, in a canal was more similar to the mesohaline sites which showed no consistent differences between open or enclosed sites (Fig. 25).

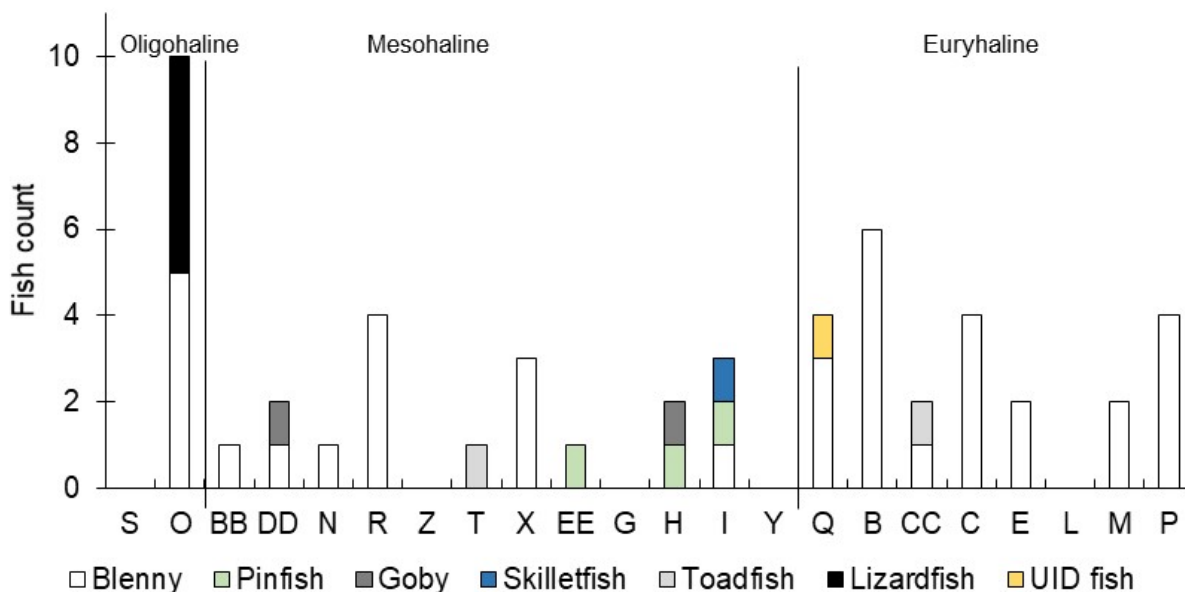


Figure 21 – Count of Fish at POP sites in May-July 2019

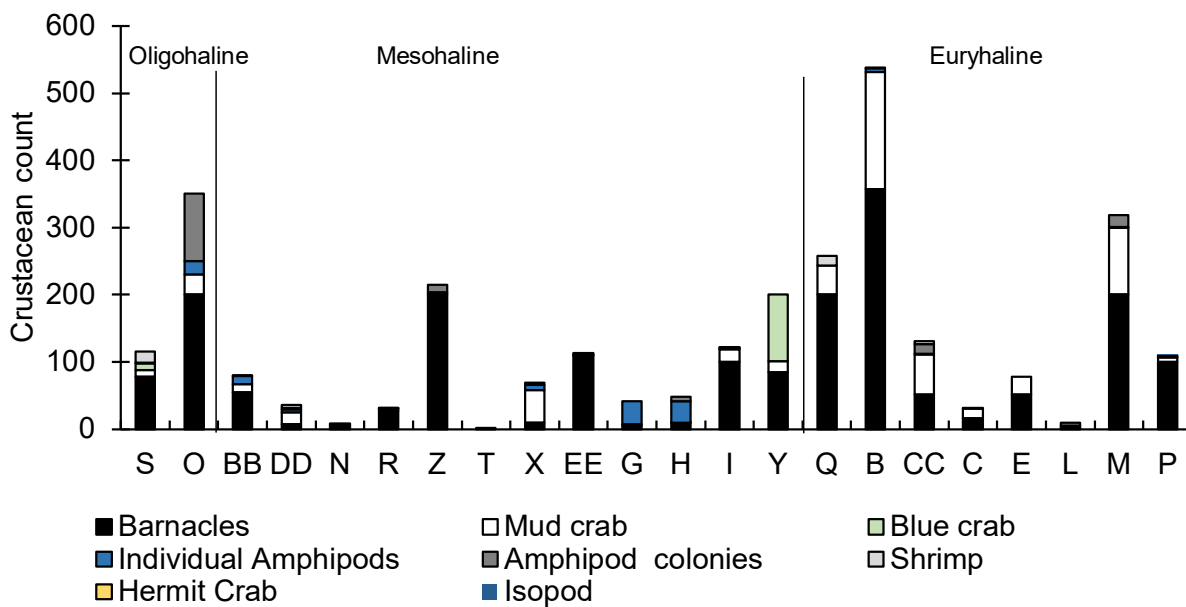


Figure 22 – Count of crustacean taxa at POP sites between May and July 2019

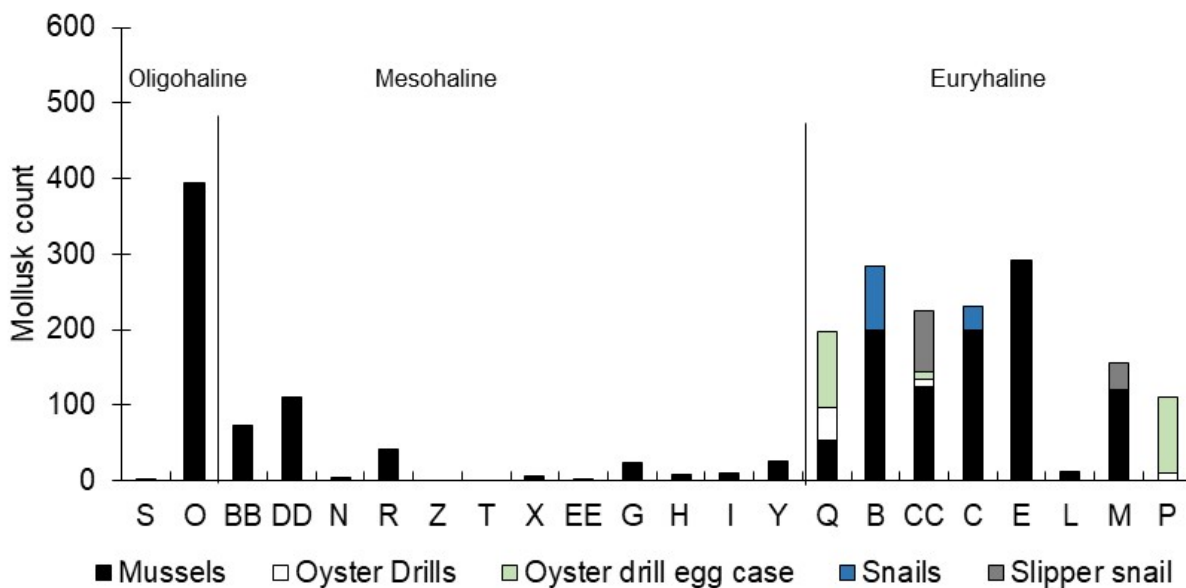


Figure 23 – Count of Mollusks at POP sites between May and July 2019

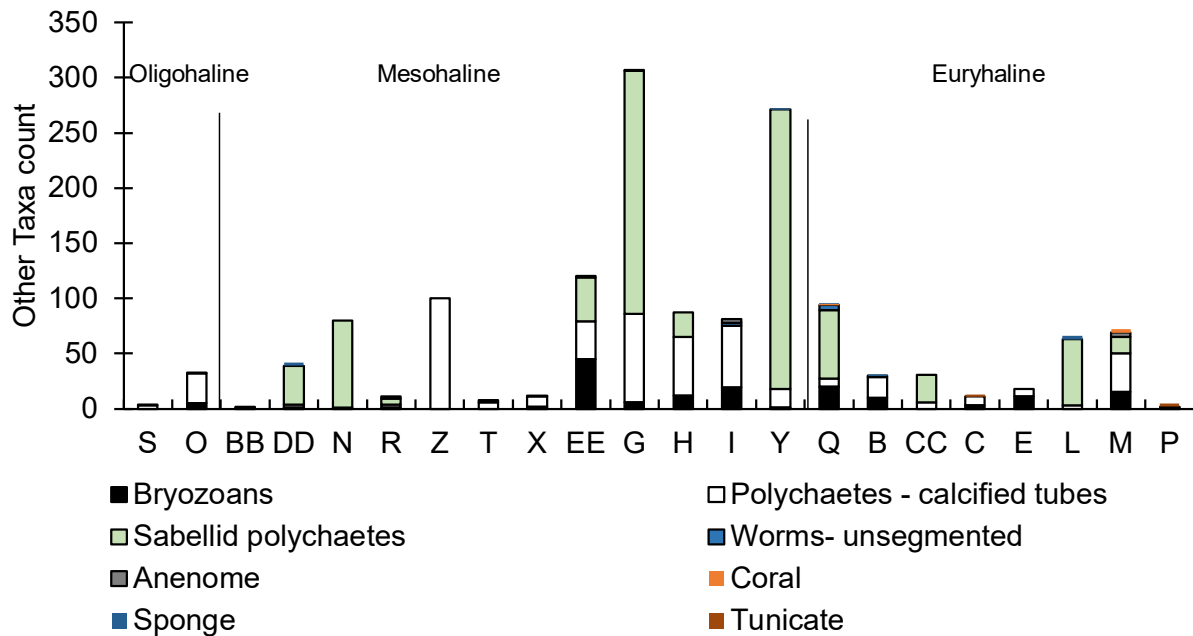


Figure 24 – Count of other invertebrate taxa at POP sites between May and July 2019

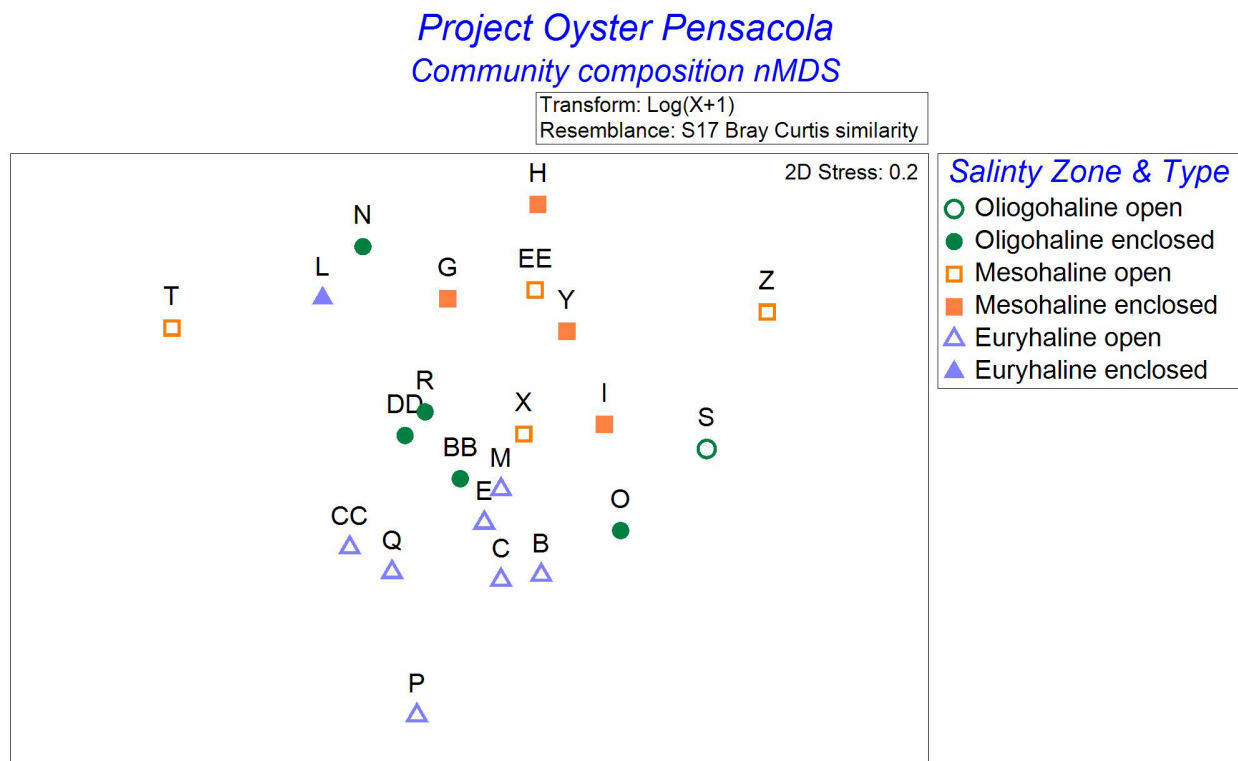


Figure 25 – Nonmetric multidimensional scaling plot of the community composition at POP sites.

## Short Term Variability in Water Quality

POP sites were sampled quarterly for water quality. An intensive study at Site X in lower Pensacola Bay allowed us to examine high frequency variability in salinity, temperature, dissolved oxygen and light. This data along with precipitation from the Pensacola Airport (NOAA <https://www.ncdc.noaa.gov/cdo-web/datatools/lcd>), Escambia River discharge (USGS <https://waterdata.usgs.gov/fl/nwis/uv?02376033>) and NOAA water elevations (NOAA <https://tidesandcurrents.noaa.gov/waterlevels.html?id=8729840>) illustrate how weather events and freshwater inputs affect the physical environment. We evaluated chemical and biological conditions with the weekly grab samples for nutrients and chlorophyll. High frequency measurements of dissolved oxygen data reflect primary production and respiration at the site. The intensive study period occurred between January 28, 2019 and March 21, 2019.

Rain events with amounts greater than 2 cm occurred on Jan 22, Feb 26 and March 3 (Fig 26). Several smaller events also occurred throughout the study. At the beginning of the study, Escambia River discharge was high, over 300 m<sup>3</sup>/s until February 6<sup>th</sup> when it declined to about 150 m<sup>3</sup>/s. Rain events at the end of February and beginning of March had a small effect on discharge which increased slightly on March 10<sup>th</sup> (Fig 26).

Frontal passages affected water levels, particularly the front associated with January 22<sup>nd</sup> event which led to lower water levels in advance of the front and then high-water levels following frontal passage. This pattern was not observed during the end of February rain event. Instead, water levels were depressed during a dry cold front on March 6<sup>th</sup> (Fig. 26).

Over the course of the study, salinity levels gradually increased from a low of 5 to about 15 PSU (Fig. 27). The highest salinity of 19.9 occurred on February 20<sup>th</sup>. High frequency tidal variability is apparent in the record, something which is missed in the weekly or quarterly sampling. One example is February 26 and March 3 rain events when salinity was depressed for a 2-day period. Passage of cold fronts resulted in declines in water temperatures of several degrees. This was particularly pronounced following the March 6<sup>th</sup> dry cold front.

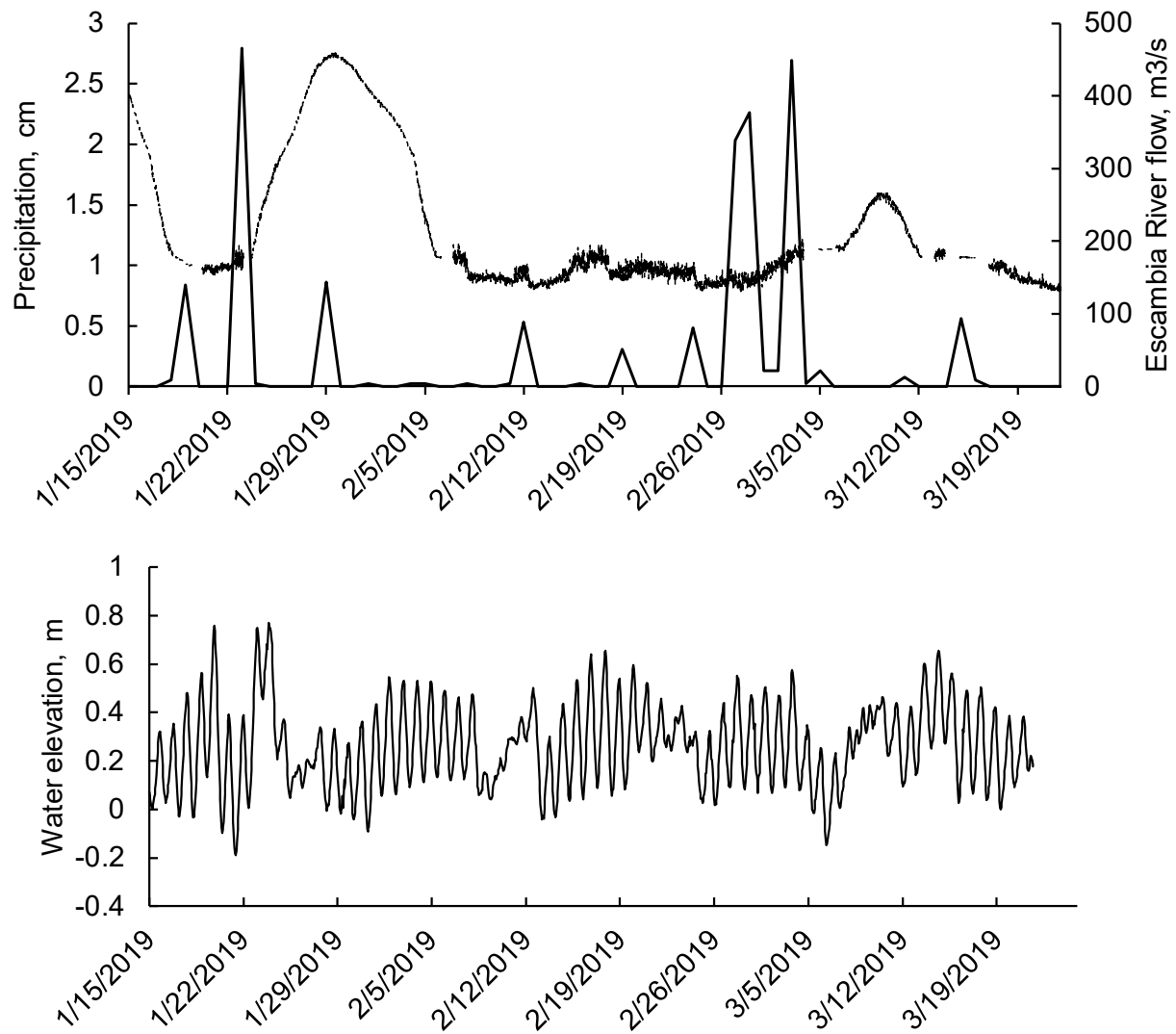


Figure 26 – Rainfall at Pensacola airport in cm during study period (NOAA <https://www.ncdc.noaa.gov/cdo-web/datatools/lcd>). Escambia River discharge from Molino in m<sup>3</sup>/s (USGS <https://waterdata.usgs.gov/fl/nwis/uv?02376033>). Observed water elevation at Pensacola (NOAA <https://tidesandcurrents.noaa.gov/waterlevels.html?id=8729840>)



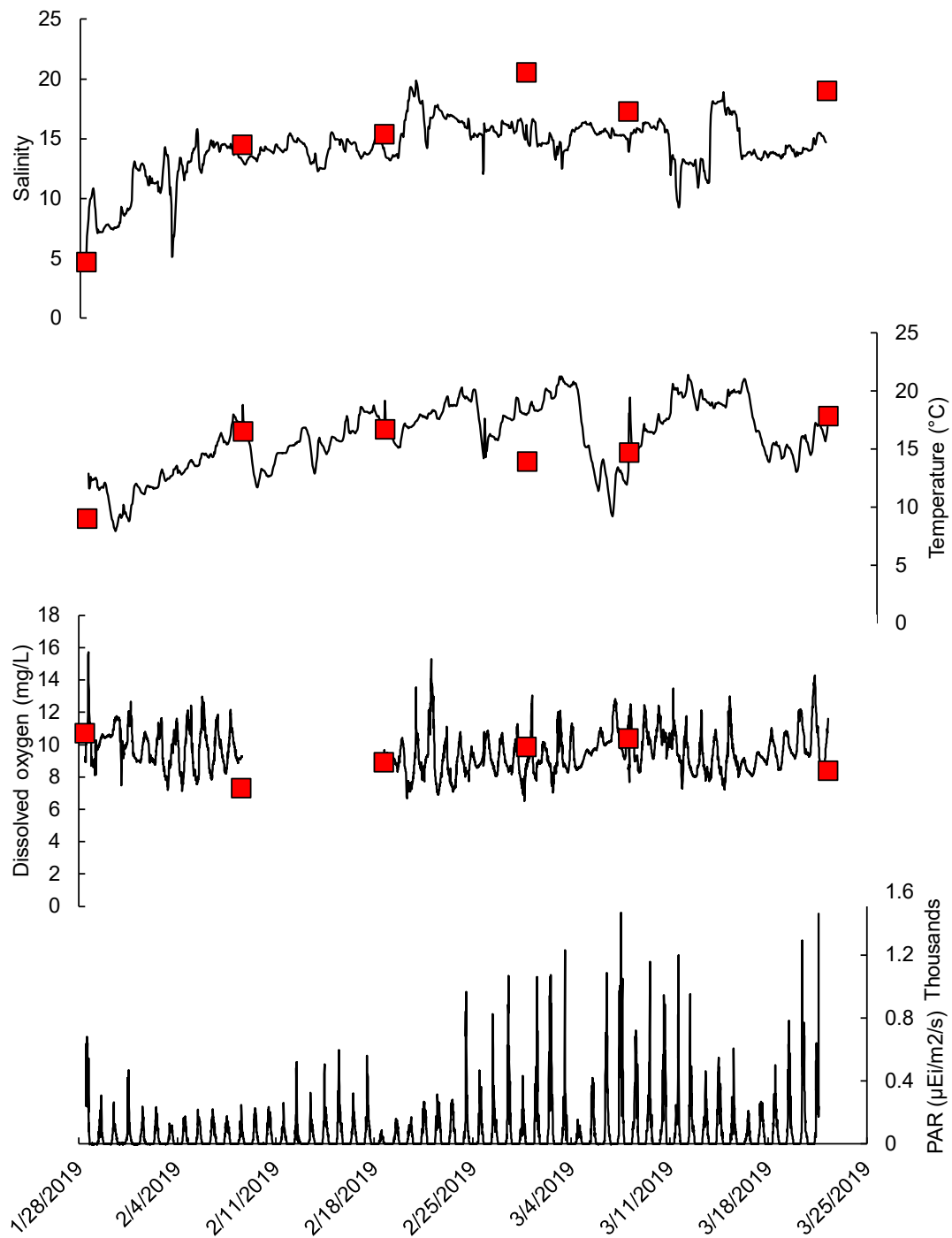


Figure 27 - Short term water quality variability at Site X in Pensacola Bay between January 28, 2019 and March 21, 2019. Sensor data indicated by black line and discrete samples collected with YSI in red boxes.

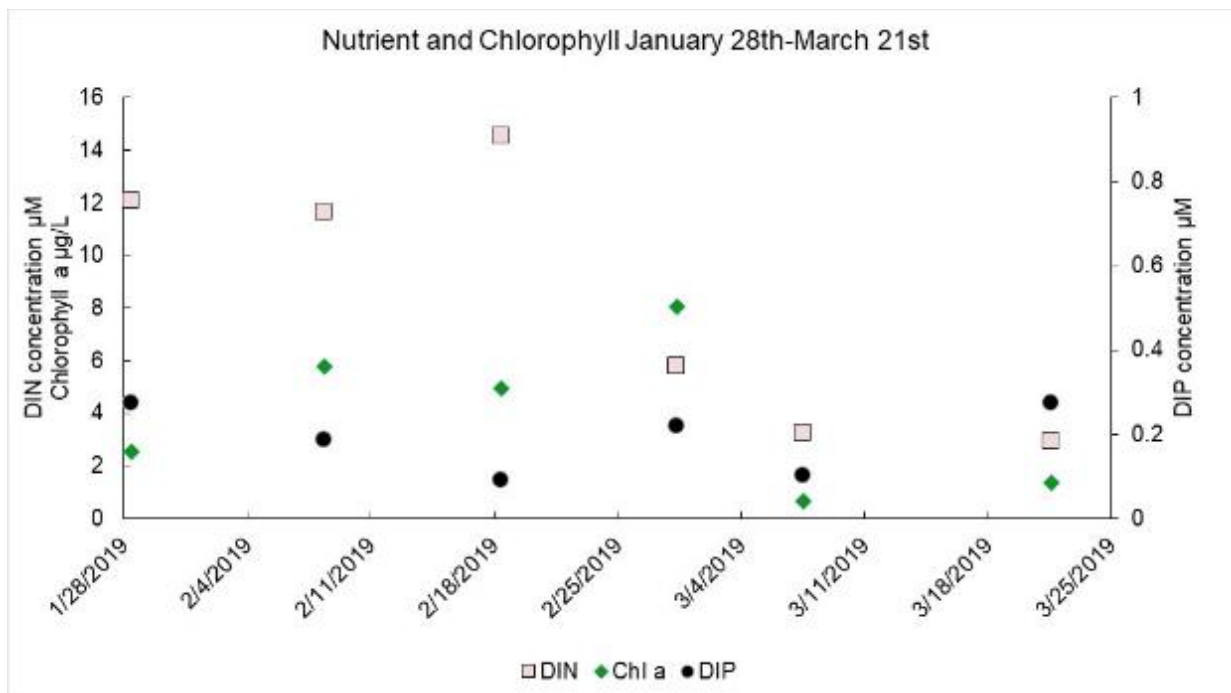


Figure 28 - Discrete dissolved nutrient and Chlorophyll a samples collected at Site X in Pensacola Bay between January 28, 2019 and March 21, 2019. DIN =  $\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$ , DIP is dissolved inorganic phosphate.

Diurnal changes in dissolved oxygen occurred due to photosynthesis during daylight hours. Concentrations increased by 2 or 3 mg/L in late afternoon compared to values at dawn (Fig. 27). The lowest oxygen concentration was 6.8 mg/L. However, no long term trend in dissolved oxygen concentrations occurred over the 8 week time series. Photosynthetically active light increased over the course of the study as daylength increased. Cloudy days with low light such as March 5<sup>th</sup>, March 16<sup>th</sup>, or 17<sup>th</sup> often had lower daytime dissolved oxygen.

Nutrient concentrations were generally low at this site (Fig. 28), similar to previous studies in Pensacola Bay (Murrell et al. 2007, Caffrey and Murrell 2016). Inputs of dissolved nutrients, both nitrogen and phosphorous, are necessary for the growth of phytoplankton. Nitrate was the dominant contributor to dissolved inorganic nitrogen (DIN) (data not shown). Through early March it represented 80-90% of DIN. Later in the study it was between 40 and 67% of DIN. Dissolved inorganic phosphate (DIP) was low throughout the study (Fig. 28), typical of the mesohaline region (Murrell et al. 2007). The DIN:DIP ratio was above 16 on all dates except for the final sample on March 21 when it was 11. High DIN:DIP ratios (>16) can indicate phosphorus limitation of phytoplankton growth, while low ratios (<16) indicate nitrogen limitation of growth. This pattern of potential P limitation in the spring has been observed in other studies of Pensacola Bay (Murrell et al. 2002, Juhl and Murrell 2008).

Chlorophyll a which is found in all primary producers is the most common proxy for phytoplankton biomass. Concentrations gradually increased over the first 4 weeks of the study to a peak of 8 μg/L, then declined to about 1 μg/L for the rest of the study. This coincided with a

decrease in DIN, so nutrient limitation could have affected the growth. Grazing by zooplankton may also contribute to the decline in phytoplankton biomass (Murrell et al. 2002, Juhl and Murrell 2005).

## Conclusions

Project Oyster Pensacola was very successful at engaging the local community with water quality issues (Fig. 29). This project generated much interest from the 25 waterfront homeowners who participated in the study and granted permission to access properties for long-term monitoring. Another unforeseen and welcome outcome were the 32 volunteers which helped build and assemble cages, particularly those who participated in monitoring activities.

BFA members and citizen scientists generated several interesting results. Oysters were successful across diverse geographic locations, from open bays to urban bayous and from Perdido Bay to Santa Rosa Sound. Oyster growth and salinity were positively correlated as were dissolved oxygen and oyster growth. The high frequency sonde data shows the rapid and dynamic response to weather events. We also learned that fish and invertebrate communities were quite different between mesohaline and euryhaline locations.



Figure 29 – Citizen scientists measuring water quality and oysters for Project Oyster Pensacola

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