Dermo infection (*Perkinsus marinus*) in Sun-Cured Oyster Shells; Informing Oyster Restoration in Texas

Final Report

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Prepared by the Environmental Institute of Houston at the University of Houston – Clear Lake and the University of Houston, Honors College, in cooperation with the Galveston Bay Foundation.

University of Houston Clear Lake

Environmental Institute of Houston

TEXAS COASTAL MANAGEMENT PROGRAM

NOAA NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION U.S. DEPARTMENT OF COMMERCE

GALVESTON BAY FOUNDATION

HONORS

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Contents

List of Figures

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List of Abbreviations

EIH	Environmental Institute of Houston
FAIN	Federal Award Identification Number
GBF	Galveston Bay Foundation
GLM	Generalized Linear Model
GLO	Texas General Land Office
NOAA	National Oceanic and Atmospheric Administration
OSP	Office of Sponsored Programs
OSRP	Oyster Shell Recycling Program
RFTM	Ray's Fluid Thioglycollate Method
TPWD	Texas Parks and Wildlife Department
UH	University of Houston
UHCL	University of Houston-Clear Lake

EXECUTIVE SUMMARY

Oyster reefs provide critical functions for a healthy coastal ecosystem in Galveston Bay, Texas. Oyster reefs have declined to a fraction of their historical coverage resulting in reef restoration becoming a focus for resource managers, commercial oyster industry, researchers, and NGOs. Oyster reef restoration is accomplished by introducing hard substrate, typically in the form of reclaimed shucked shells (or cultch), back into the local environment to be colonized by native spat. The Galveston Bay Foundation's Oyster Shell Recycling Program began in 2011 and they currently collect discarded oyster shells from 35 recycling partners. Recycled oyster shells should be sun-cured (or quarantined) prior to use in restoration projects because they can harbor invasive species and disease. Dermo infection, caused by the spore-forming protozoan parasite *Perkinsus marinus* is a density-dependent limiting factor to oyster population growth. Currently sun-curing recommendations are based on a single study conducted in South Carolina in 2002. With the increasing interest and number of oyster restoration projects this project was developed to investigate current best practices to assess the risk of infecting native oyster reefs with Dermo infection through restoration projects.

This study was purposefully designed to test a "worst-case scenario" for Dermo infection in sun-cured oysters in Texas. Oysters with elevated Dermo infection were obtained and deployed whole, either in the interior or top of four experimental shell piles, to demonstrate the sun-curing process of un-shucked oysters that may enter the recycling pathway. Two of the piles were fenced to limit access by wildlife and two were left unfenced. Oysters were individually numbered and tracked throughout the study. Half of the oysters were tracked for Dermo infection using the Ray's Fluid Thioglycollate Method, while the other half were evaluated for tissue decomposition using percent coverage of tissue and tissue condition categories. Oysters were deployed a total of 35 weeks from October 2022 to June 2023. Temperature and relative humidity monitors were co-located with deployed oysters. The piles were also monitored using game cameras for the first 6 weeks of deployment to assess potential disturbance due to foraging wildlife.

Oysters deployed on the tops of the unfenced piles were depredated by feral hogs within the first 31 hours of deployment. There was a significant difference in the tissue condition and decomposition between the oysters deployed on the tops of the fenced and unfenced piles. Temperatures were higher and more variable on top of the piles, while relative humidity was generally higher in the interior of the piles. Oysters in the interior of the piles were slower to desiccate compared to those at the top of the piles, but once desiccated the oysters in the interior of the piles continued to degrade. More decomposing insects, such as maggots, were observed in association with interior oysters, and they had a lower percent cover of tissue compared to those at the top of the piles. There was a significant decrease in the Dermo infection intensity after the first week of deployment and throughout the study. Oysters in the interior of the piles had significantly less Dermo infection intensity than the top of the piles. No dermo infection was detected in the interior of the piles after the 6th week of deployment, while it was detected on the top of the piles until the 31st week of deployment.

This study used oysters with a historically high initial level of Dermo infection collected from Confederate Reef, which is currently closed to harvest. It is likely that commercially sourced oysters that typically end up in the recycling pathway would have lower background Dermo infection levels, but this hypothesis should be tested further. It is unknown how frequently un-

shucked oysters are found in the recycled shell materials, but oyster recycling staff have observed them regularly while collecting shells. Future audits of oysters entering the recycling pathway (from both commercial and retail sources) should be conducted to quantify the amount of tissue entering the curing piles. For the curing site used in this study, the presence of a robust feral hog population seems to help to remove oyster tissue resulting in expedited curing treatment, but not all sun-curing locations have feral hog populations. Most of the tissue of oysters deployed in the interior of the piles was gone by the 16th week, which corroborates results from the previous study on which current recommendations are based. Alternatively, most oysters deployed at the top of the fenced piles had tissue remaining through the 35th week of our study. The previous study did not evaluate oysters on top of the piles. It was thought that UV light and lower relative humidity levels helped to speed up tissue decomposition and *P. marinus* mortality rates, but our results bring this into question. It is understood that the decomposition rate is positively correlated with higher temperatures. Similar to the 2002 study, we found that the interior temperature was generally lower than the external temperature of the piles. However, we found the oysters deployed in the interior of the piles decomposed more quickly. Therefore, perhaps other factors may have a higher influence on decomposition such as humidity and insect interaction than temperature.

While Dermo infection intensity ratings were typically low after the first week of deployment, *P. marinus* is known to be able to infect an oyster with as few as ten cells. *P. marinus* is and has historically been found in all bays and estuaries in the northern Gulf of Mexico, so there is no concern for introducing *P. marinus* through restoration efforts into an area in Texas where it does not already exist. Background Dermo infection levels in Texas are high relative to much of the northern Gulf of Mexico and Dermo infection reduces growth and reproduction of infected oysters. Oyster spawning season extends from late Spring through early Fall and the success of an oyster restoration project is typically measured by the recruitment of spat, and the growth of the reef post-restoration. Therefore, to aid in the success of a restoration project the reef substrate material should not contribute to local sources for *P. marinus* exposure to newly recruited oysters. To this end, timing the deployment of the recycled shell to the beginning of the non-spawning season could ensure that should residual tissue remain, there is ample time for it to break down, and any released *P. marinus* dies before new spat settles at the restoration site. The viability of the *P. marinus* spores observed throughout this study is unknown. Future laboratory-based studies to expose uninfected oysters to the desiccated but infected tissues from oysters gathered at the sun-curing site are needed to determine the viability and risk level of *P. marinus* associated with the recycled shell material.

Perkinsus marinus is not the only risk associated with the use of recycled oyster shells for restoration projects, however it was the only risk evaluated in this study. While there are a variety of treatments that can be used to sterilize the recycled shells such as heat treatment, and freshwater, bleach, or acid soaks these are not logistically reasonable for large-scale shell recycling programs. Dermo infection is monitored across the northern Gulf of Mexico by a variety of organizations, but consistent monitoring in Galveston Bay has not occurred since 2010. The results of this study suggest that resource managers and practitioners that have active depredation of oyster tissue at the top of their piles, as seen in this study, may consider curing their shell material for a minimum of 3 months provided that is deployed for curing during "warm-weather" months (April – September). Shell deployed for curing during "cold-weather" months, should continue to follow existing recommendations of curing for 6 months due to the reduced rate of tissue degradation during cold-weather months.

INTRODUCTION AND BACKGROUND

The Eastern Oyster (*Crassostrea virginica*) is a species of oyster native to Texas. Oyster reefs are in decline with an estimated 85% loss world-wide (Beck et al. 2011) and 60-80% loss locally, in Galveston Bay (GBF 2023). Healthy oyster reefs are an important component of Texas Bays providing numerous ecosystem services such as shoreline stabilization, water filtration, habitat creation, and it is one of Texas' most economically important fisheries (Beck et al. 2011, Bidegain et al. 2017, Coen et al. 2007, DePiper et al. 2017, Grabowski et al. 2012) (Figure 1). However, reefs face a myriad of natural and anthropogenic stressors. Natural pressures on oyster populations include predation (Grabowski et al. 2012, Hill and Weissburg 2013, Hanke et al. 2017), sedimentation (Du et al. 2019, Hanke et al. 2021, Saoud and Rouse 2000) extreme weather events (Du and Park 2019, Hanke et al. 2022), and disease (Craig et al. 1989). Whereas anthropogenic stressors on oyster populations are mainly derived from overfishing, habitat loss, and pollution (Beck et al. 2011, Jackson et al. 2001, Worm et al. 2006). Resource managers, academics, and non-governmental organizations work together to address these threats through regulation and restoration.

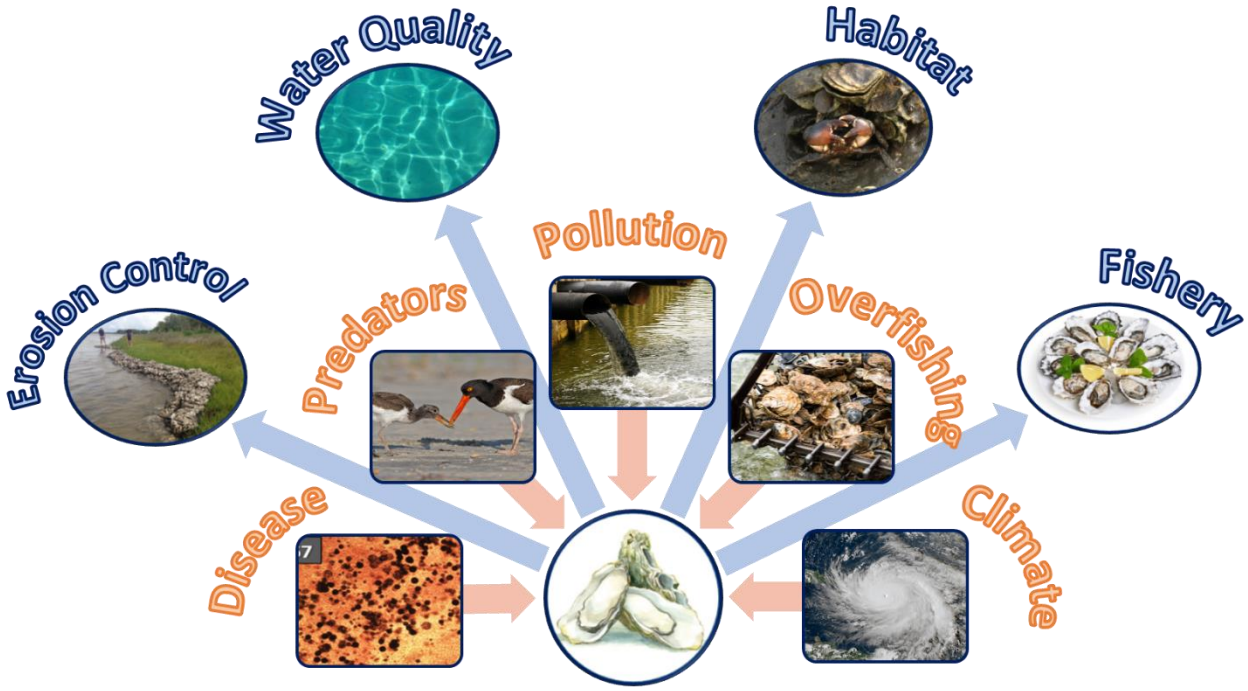

Figure 1. Eastern Oysters (*Crassostrea virginica*) provide many ecosystem services (blue arrows), but they also face threats (orange arrows).

Oyster reef restoration can be accomplished by introducing hard substrate, typically in the form of reclaimed oyster shells (or cultch), back into the local environment to be colonized by native spat (Coen and Luckenbach 2000). The Galveston Bay Foundation (GBF) gathers shells from local seafood restaurants through the Oyster Shell Recycling Program (OSRP) for reuse in reef restoration in Galveston Bay, Texas (GBF 2022) (Figure 2). The Galveston Bay Foundation piloted the OSRP in 2011 with a single restaurant. Over the last decade, GBF has expanded its operations and now collects an average of 150 tons (300,000 pounds) of shells per year from over 36 restaurants ranging from the Inner Loop of Houston to Galveston Island. To date, GBF

has collected over 1,650 tons (3,300,000 pounds) of oyster shell and returned approximately 840 tons of these recycled shells to Galveston Bay to help replenish hard substrate and sustain the local oyster population. The Galveston Bay Foundation's shell-based reef restoration and shoreline protection efforts have resulted in 0.80 acres of oyster habitat creation (Laroche et al. 2022) and 2,600 linear feet of shoreline protection (Hanke et al 2022). With the goal of acquiring larger volumes of shell to support larger reef restoration efforts, it is imperative to test and validate these sun-curing procedures. The information derived from this study will help ensure that clean and safe shell is returned to Galveston Bay and other state waters. With any conservation effort, it is important to make sure practitioners are not inadvertently introducing or increasing disease in native reefs.

Figure 2. Schematic illustrating the oyster shell recycling pathway through the Galveston Bay Foundation's Oyster Shell Recycling Program (OSRP) and some example images of each step. a. photo of oyster recycling bins from participating restaurants that are picked up by the OSRP. b. photo of the recycling bins being emptied at the sun-curing site c. photo of a dump truck load of recycled shell being emptied at the sun-curing site. d. photo of a large-scale oyster restoration using sun-cured oyster shells, and e. photo of a volunteer oyster restoration event where bags of the sun-cured oysters are placed back into the bay.

Dermo disease is caused by the spore-forming protozoan parasite *Perkinsus marinus*. Oysters can become infected when they ingest any life stage of *P. marinus* (Volety and Chu 1994) (Figure 3). Once ingested, *P. marinus* proliferates within the tissues of the oyster host. It can be transmitted from an infected oyster to surrounding oysters either through excretion or when decomposing tissue from dead oysters release spores into the water column (Bidegain et al. 2017). Dermo infection rates are highest when the water is warm and salinity is high, so late summer tends to be the peak of *P. marinus* loading in Texas bays (Calvo et al. 2003, Craig et al. 1989, Silvy et al. 2020). Dermo infection does not harm people that ingest the oysters, but the infection can impair oyster growth and reproduction, eventually causing mortality. Because Dermo infection can be transferred from decomposing oyster tissue, many restoration programs

are mandated to quarantine or sun-cure before re-introducing recycled oyster shell back into the bay.

Recycled oyster shells may harbor invasive species and disease-causing organisms (including *P. marinus*), therefore the OSRP currently follows best practices recommended by the Texas Parks and Wildlife Department (TPWD) which includes a minimum of six months of land-based sun-curing. The current best practices are based off of a study conducted by Bushek et al (2004) in South Carolina, which used oysters from a reef in Galveston Bay (Confederate Reef) with historically high levels of Dermo infection. This study demonstrated Dermo infection prevalence declined significantly after one month and was virtually eliminated after three months (Bushek et al. 2004). To expand on the limited previous work evaluating Dermo infection persistence in sun-cured oysters, this project was developed to track Dermo infection presence, prevalence, and intensity in sun-cured oysters with considerations for location within the pile and the influence of foraging wildlife.

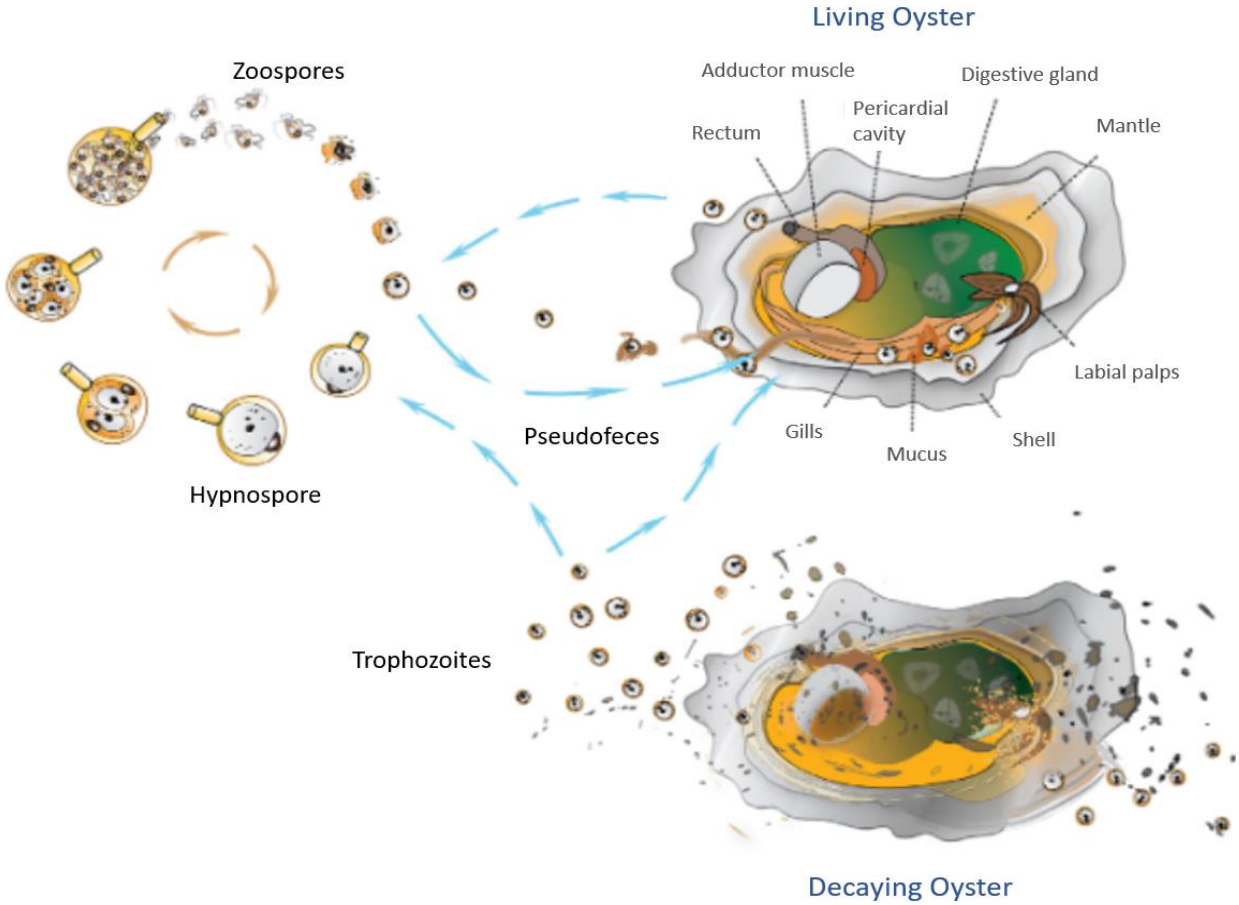

Figure 3. Lifecycle and infection mechanisms of Dermo infection (*Perkinsus marinus)* in Eastern Oyster (*Crassostrea virginica*) adapted from: Fernández et al. 2018

Objectives

The objectives of the study were to:
1) track the prevalence and severity of Dermo infection in sun-cured oysters,

2) evaluate the influence of location of oysters within curing piles on Dermo infection prevalence and severity, and
3) evaluate the impact from wildlife foraging during the sun-curing process.

METHODS

This study was purposefully designed to test a "worst-case scenario" for Dermo infection in sun-cured oysters in Texas. Oysters with elevated Dermo infection were used and deployed whole to demonstrate the sun-curing process of un-shucked oysters that may enter the recycling pathway.

Study Site

Oysters were collected from Confederate Reef in Galveston Bay on October 6, 2022. This reef has historically high Dermo infection rates (Silvy et al. 2020) and was sampled at the end of the summer. Oysters were processed the same day as collection. Once processed (see Field Methods section below for detailed processing steps), oysters were kept on ice overnight and deployed at the GBF's Red Bluff Curing Site (Figure 4) on the following day, October 7, 2022. The GBF created four replicate piles approximately 6 feet wide by 3 feet tall of recycled oyster shells collected through their OSRP. Two of the piles were fenced (piles A & C in Figure 4) and two were left unfenced (piles B & D in Figure 4) to evaluate potential influence by wildlife access. Fenced piles were surrounded by four-foot high, 4-gauge wire fence panels with four-inch square mesh and then reinforced by a layer of chicken wire to exclude smaller animals.

Field Methods

Initial processing consisted of cleaning the exterior of the oysters using a stiff hand-held brush and fresh water and knocking off other shell fragments or spat. When clean, oysters were measured (length and width). Then the oysters were shucked (i.e., the abductor muscle was detached from the lid only) and the oyster was tilted to allow water to drain from the open shell. The shucked and drained oysters were weighed, and initial tissue condition was recorded for each oyster. Initial tissue condition was categorized as either shrunken (e.g., small, dehydrated appearance) or plump (e.g., round, lush, creamy color) based on Ray (1966). Additional tissue condition categories were added after the first week of deployment and included "liquified", "desiccated", and "no tissue" (Figure 5). A 5-mm biopsy punch was used to take a sample of the mantle tissue which was placed in a prepared vial with 10mL of NaCl Thioglycollate medium inoculated with Chloromycetin/Nystatin solution and incubated in the dark at room temperature for 7 days (per Ray 1966).

Oysters were individually numbered, and the shells were closed around the tissue with bailing wire and deployed in either the interior or top of one of four replicate piles of recycled oyster shell at the GBF Red Bluff Curing Site. This was done to mimic a situation where a whole un-shucked oyster was included in the shell recycling material. After initial deployment, half of the oysters (n = 40) from each deployment location were sampled for Dermo infection ("Dermo" oysters) and the other half were sampled for tissue condition and decomposition ("Tissue" oysters) weekly for the first six weeks, then every other week for six months, and once a month for two more months (covering a total of 8 months deployment). To monitor pile status, game cameras (HyperFire 2, Reconyx, Holmen, Wisconsin, USA) were set to take three photos, one

second apart when motion was detected. Game cameras were deployed for the first 6-weeks of the study and downloaded during each weekly check (Figure 4).

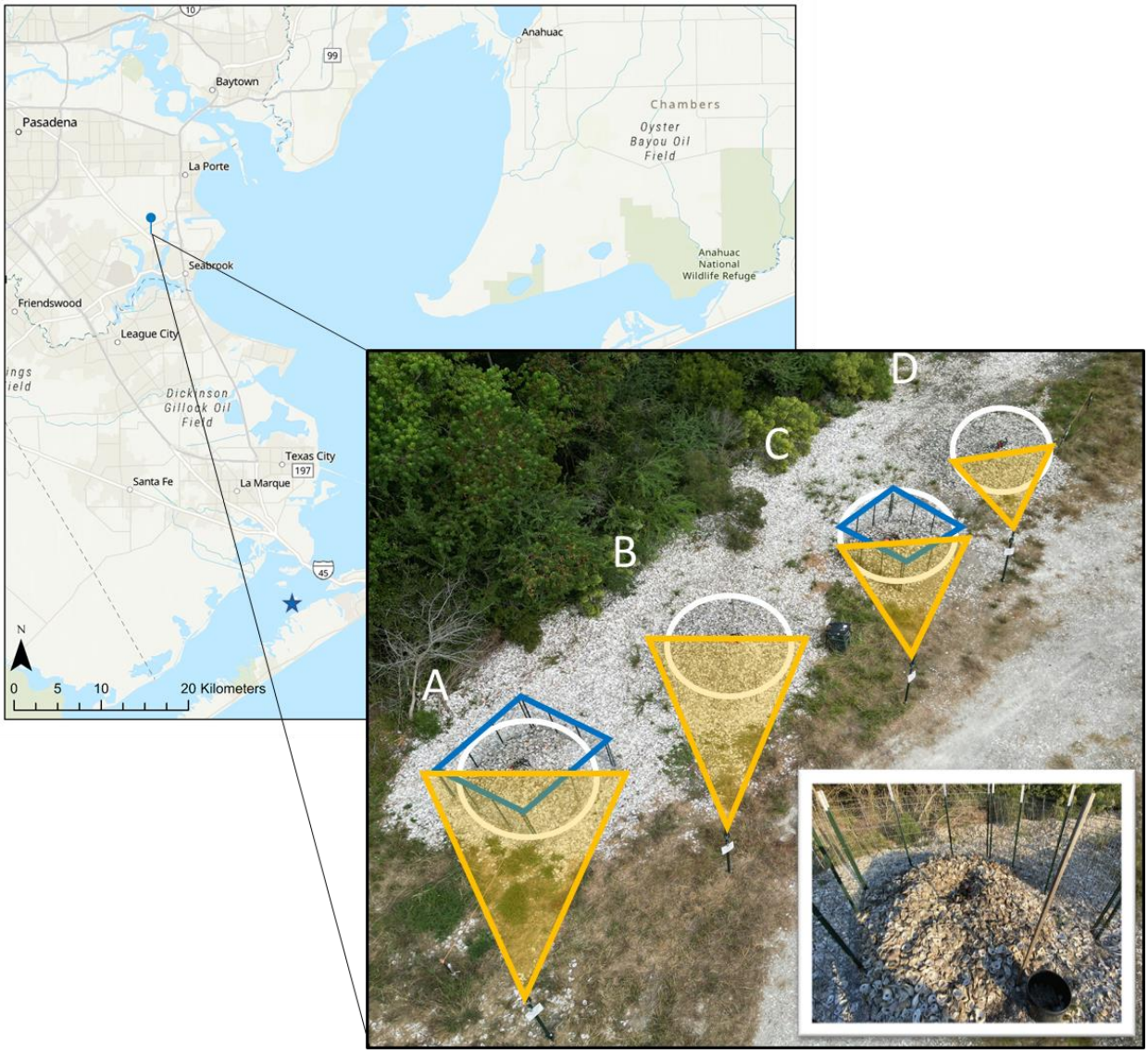

Figure 4. Map of Red Bluff curing site utilized by the Galveston Bay Foundation, and site of the sun-curing experimental piles. Aerial image showing the four experimental piles, A and C were fenced (blue squares), and B and D were unfenced. Game cameras were deployed for the first 6-weeks of the study to document wildlife interactions (yellow triangles = approximate field of view of game cameras). Inlayed photo of a fenced pile with the center dug out to deploy the interior oyster treatment. Blue star on map indicates location of Confederate Reef where oysters were procured for the study.

At each check, all "Dermo" oysters were evaluated for tissue condition (Figure 5). If tissue was present, a 5-mm biopsy punch was used to take a sample for Dermo infection analysis. Additionally, at each check all "tissue" oysters were weighed and percent cover of tissue on the shell and tissue condition was recorded. Temperature and relative humidity sensors (U23-001 HOBO Pro v2, Onset, Bourne, Massachusetts, USA) were co-located with each group of oysters in the interior of the piles and deployed on the top of pile C to capture the ambient conditions.

Figure 5. Examples of the five tissue condition categories used to describe decaying oyster tissue deployed at GBF's sun-curing site from the on-going Texas General Land Office study by GBF and UHCL.

Laboratory Methods

Oyster tissue samples were evaluated following Ray's Fluid Thioglycollate Method (RFTM) after being incubated for 7 days (Ray 1966). The tissue was removed from the incubation vial, macerated on a glass slide, then stained with Lugol's solution and covered with a cover slip. Samples were viewed under a dissecting microscope (5x power), *P. marinus* spores were counted, and a Dermo infection intensity rating was assigned using the Mackin (1961) scale, as modified by Craig et al. (1989) which ranges from 0 (e.g., no *P. marinus* spores detected) to 5 (e.g., nearly 100% of the tissue is comprised of hypnospores) (Figure 6).

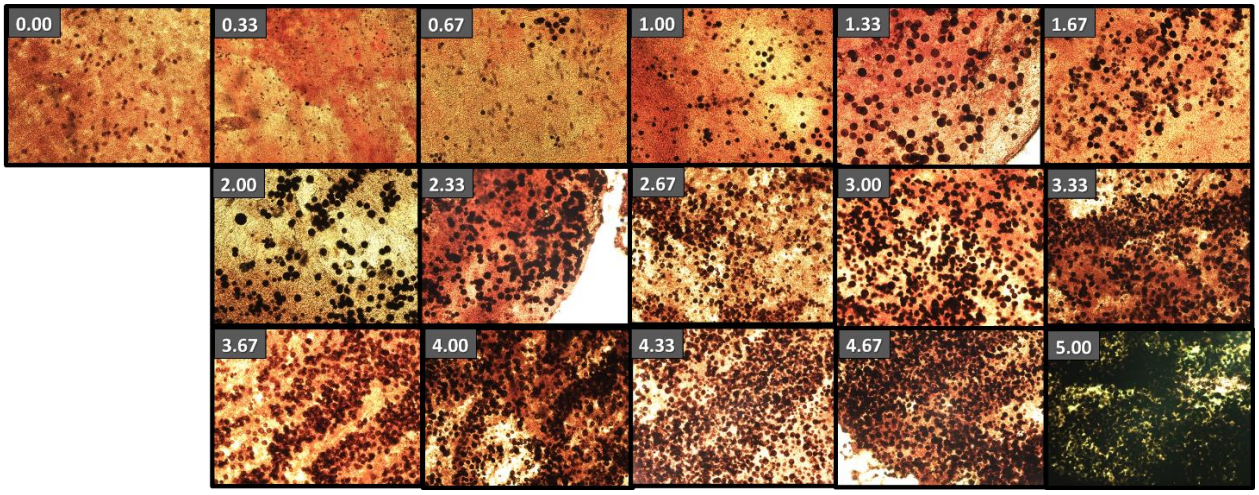

Figure 6. Examples of tissue pathology slides showing the range of Dermo intensity rating using the Ray's Fluid Thioglycollate Method (RFTM).

Data Analyses

All data were tested for normality prior to statistical analysis (Shapiro and Wilk 1965). If data were determined to be non-normal, nonparametric statistical methods described below were used. Statistical analyses were conducted using R Studio (2022.07.2 build 576). The relationship between the presence or absence and intensity of Dermo infection and categorical variables were evaluated using either the Kruskal-Wallis Rank Sum Test (Myles and Wolfe 1973) with subsequent post-hoc Pairwise Wilcoxon Rank Sum Test (when applicable) or a binomial Generalized Linear Model (GLM) for detection prediction analysis (R package *pscl*). For all statistical tests, we used $\alpha = 0.05$ to determine statistical significance. All means are reported ±1 SE, unless otherwise noted. We used a Friedman Rank Sum Test (Myles and Wolfe 1973) to evaluate repeated measures of Dermo infection intensity by study week.

RESULTS

A total of 96 oysters were collected from Confederate Reef in West Bay, Galveston Bay (29.26349˚ N, -94.91654˚ W - WGS84) and processed on October 6, 2022. The water temperature at the time of collection (9:35 am) was 25.4 deg C and salinity was 26.37 psu. A sub-set of 80 of the collected oysters were utilized in the sun-curing study. Average length was 93.7 mm ± 1.26 and the average total (shell and tissue) weight after being shucked and drained was 161.5 ± 4.88 g (Figure 7).

Deployed oysters used were live at the time of shucking and initial tissue condition was recorded with 51% ($n = 41$) as plump, and 49% ($n = 39$) as shrunken with a minimum of 50% coverage by the tissue. Additionally, 35% ($n = 28$) were observed to be “milky” in color indicating development for spawning, while 65% ($n = 52$) were “watery”. Forty of the oysters were used to track the prevalence and intensity of Dermo infection, two of which were below legally harvestable size (76.03 and 75.98 mm). We included these smaller oysters in the study because of their elevated Dermo infection intensity rating of 1.0. The average initial Dermo infection intensity was 0.9665 ± 0.08 (Figure 8). There was no correlation between length of oyster and Dermo infection intensity ($F = 1.193$, $p = 0.2775$, one-way ANOVA).

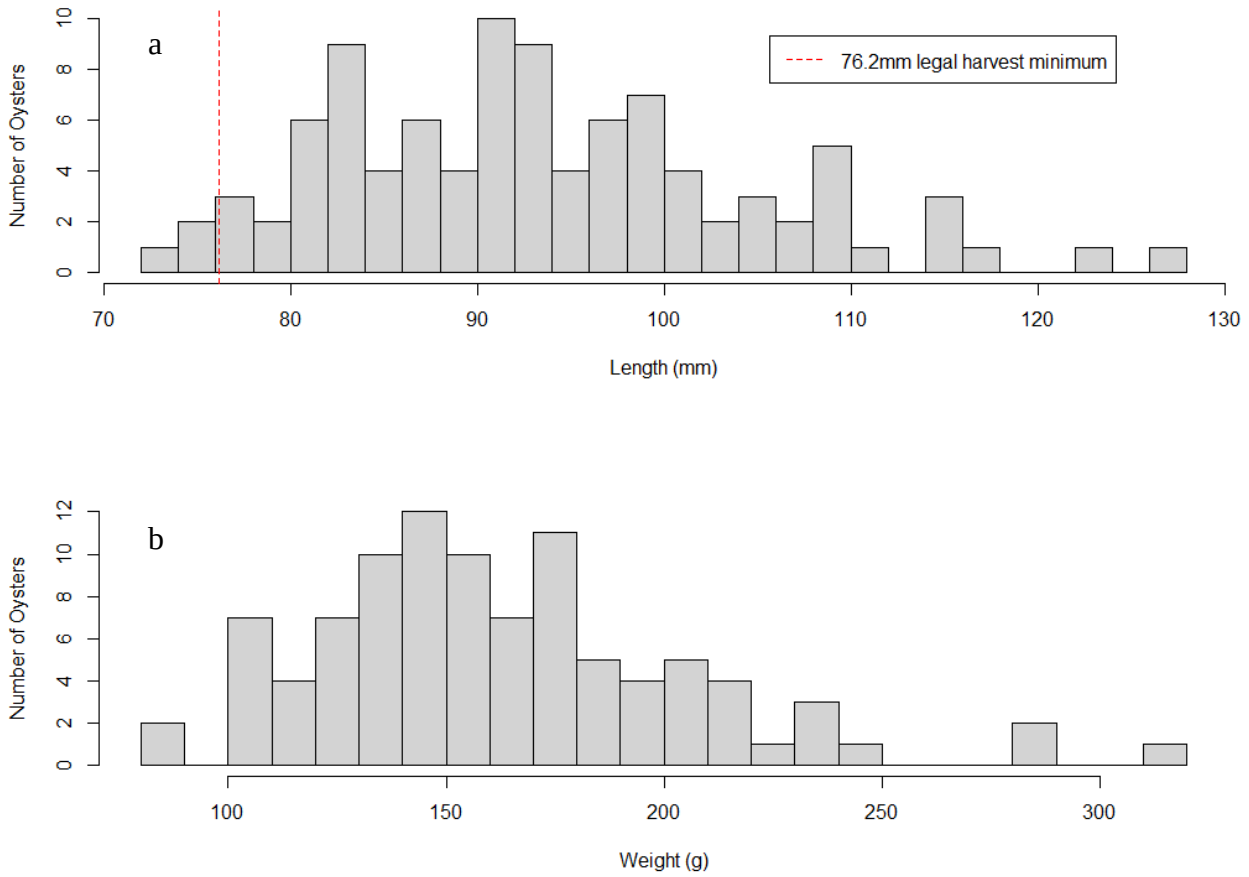

Figure 7. Histograms illustrating the frequency of oysters by a) length (mm) and b) post-shuck weight of the shell and tissue (g) for the 80 oysters collected from Confederate Reef in West Bay, Galveston Bay and used in the Sun-Curing project.

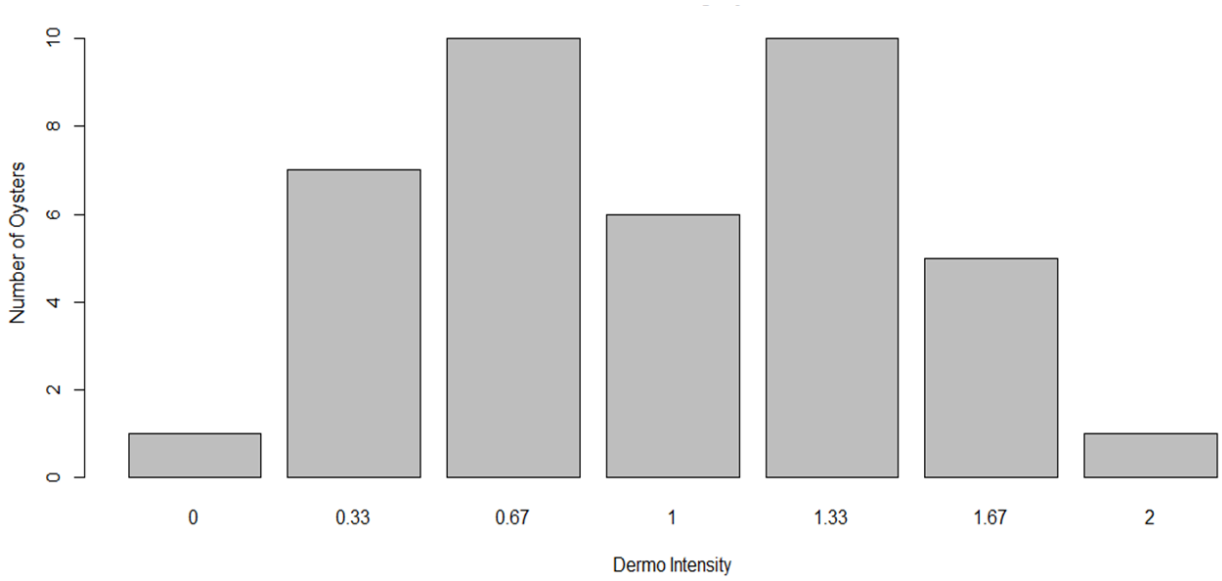

Figure 8. Distribution of initial dermo infection intensity scores for the 40 oysters used to track dermo infection prevalence and intensity.

Oysters were deployed in the experimental curing piles on October 7, 2022, and the experiment ran for 35 weeks, through June 8, 2023. Some of the oysters deployed at the top of the piles were removed from the experiment due to depredation by feral hogs. These oysters are denoted as "N/A" tissue condition in Figure 10. As a result, there were only four oysters sampled for tissue in the tops of the unfenced piles after the first week of deployment and only one after the second week. Further analysis of tissue condition only included oysters deployed in fenced piles as a result. Camera traps deployed at each pile captured initial interaction between the deployed oysters and feral hogs which occurred just 3 hours after deployment. Depredation of study oyster tissue by feral hogs, occurred just 31 hours after initial deployment (Figure 9). During some oyster sampling visits the research team could hear the feral hogs in the nearby tree line and they would occasionally appear to seemingly check to see if the coast was clear for them to scavenge any new oyster shell.

While there were wildlife interactions observed for fenced piles (primarily from vultures) deployed oysters were not compromised/depredated. Through camera trap footage review it was clear that the impact from wildlife to the unfenced piles only affected the top of the pile and there was no physical disturbance to the oysters deployed in the interior of the piles. Oysters that were depredated and the shells were not recovered were assumed to have zero percent tissue cover. While some "Dermo" oysters were depredated we failed to detect any statistically significant difference in Dermo infection intensity between oysters deployed at the top of fenced piles versus not fenced piles (chi-squared = 0.1878, p = 0.6647, Kruskal-Wallis rank sum test), therefore all piles were pooled for further Dermo infection analyses.

The temperature (˚F) and relative humidity (%) recorded throughout the deployment time varied on a diurnal cycle as well as a seasonal cycle, as the experiment ran from early Fall through early Summer (Figure 11 & Figure 12). Temperatures were higher and more variable on top of the piles, while relative humidity was generally higher in the interior of the piles. In fact, following rain events it was not uncommon for the relative humidity in the interior of the piles to stay at or near 100% for days or even weeks. The 19 sampling events spanned a wide range of temperature and relative humidity conditions with the highest recorded being 49.2 ˚C (120.6 ˚F) and 100% and the lowest recorded being -8.8 ˚C (16.2 ˚F) and 14.9% respectively.

Figure 9. Unfenced piles B and D when wildlife interaction compromised deployed study oysters 31 hours post-deployment**.**

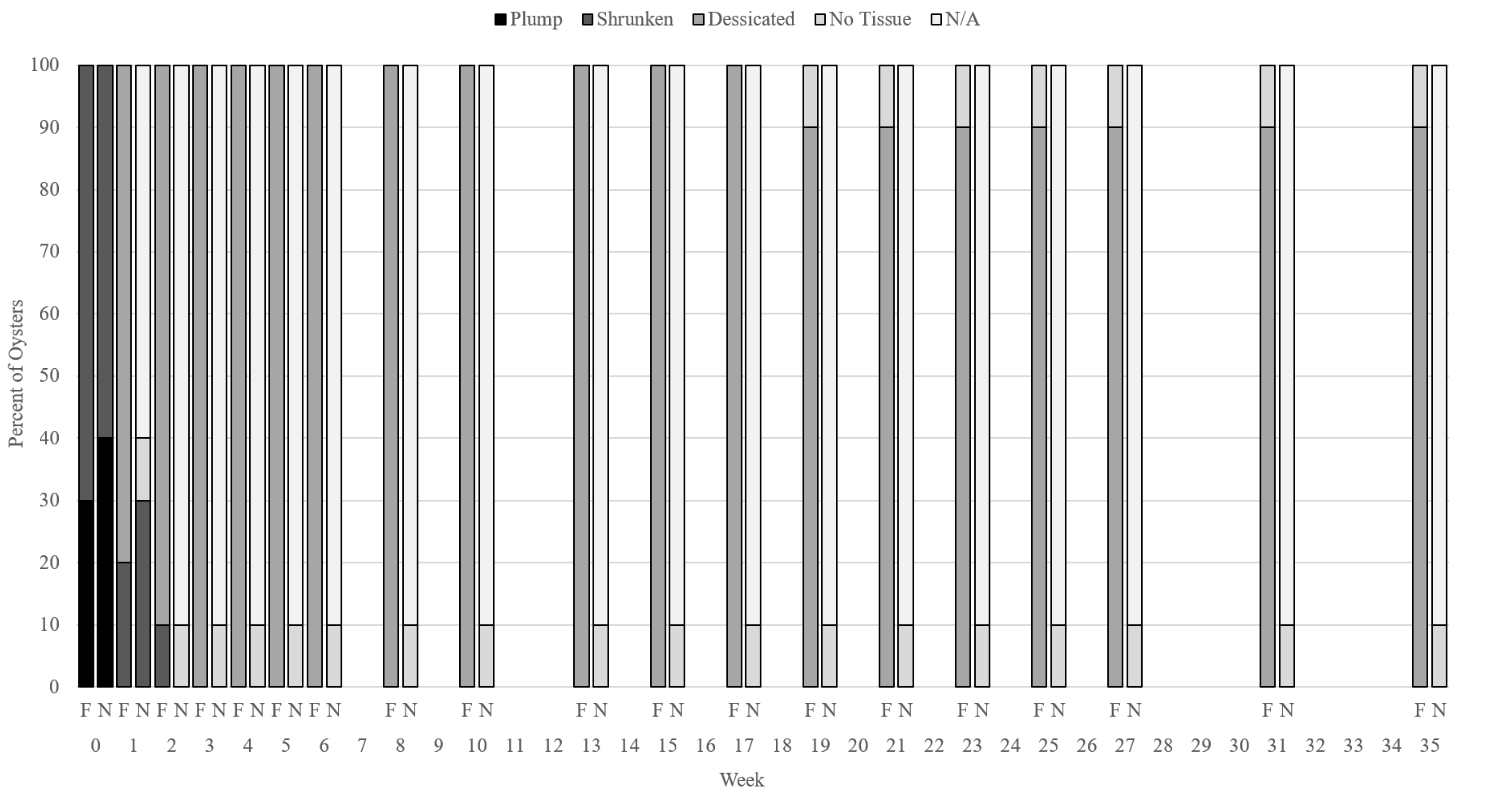

Figure 10. The percentage of "tissue" oysters deployed on the tops of the piles by tissue condition category by sampling week and whether the pile was fenced = "F", or not = "N". N/A represents oysters that were depredated by feral hogs and therefore no longer trackable for tissue condition.

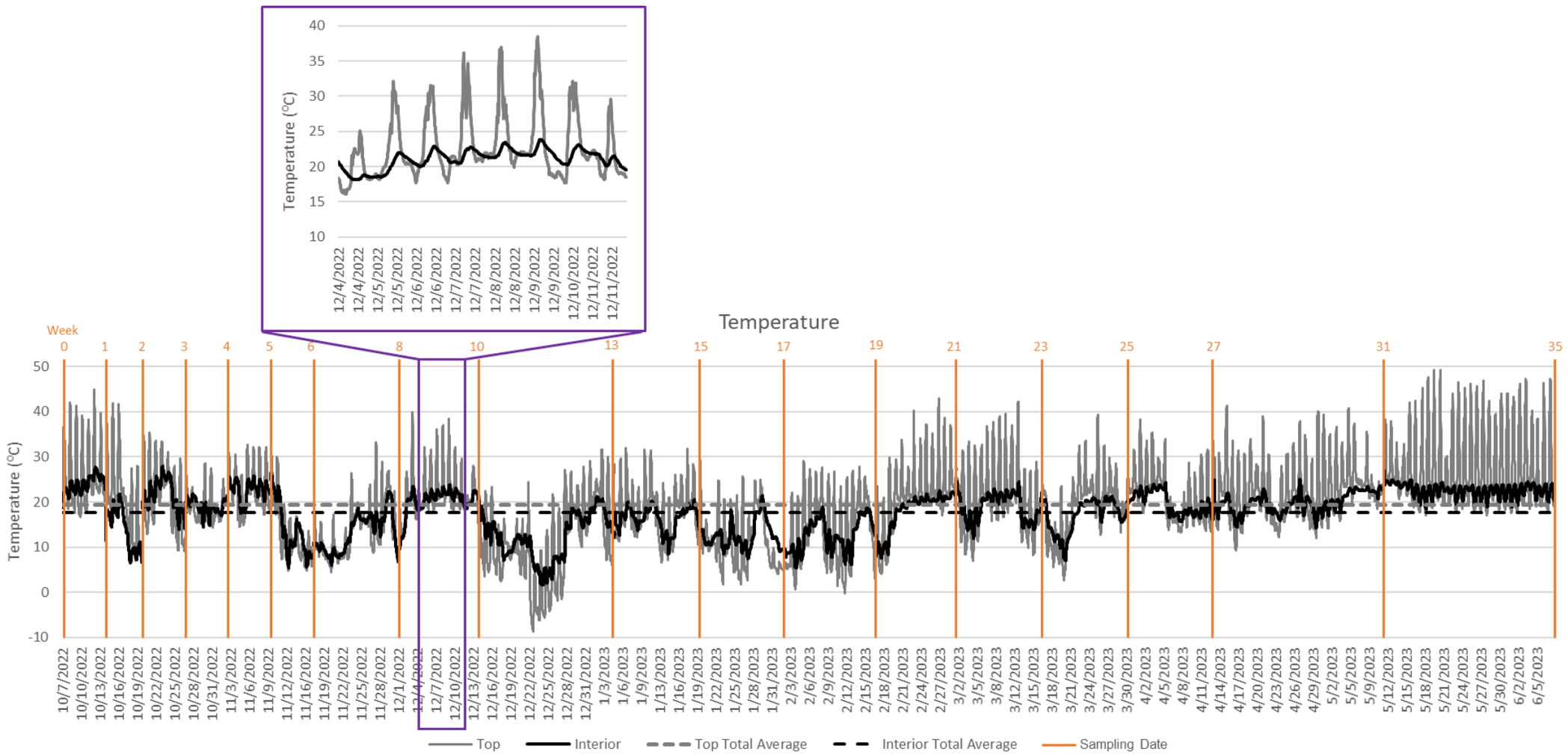

Figure 11. Time series of temperature data as the average of the sensors co-located in the interior of each of the four experimental piles (black line) with the sensor located at the top of pile C (grey line - ambient conditions). Dashed black line is the total overall mean temperature inside of the piles and the dashed grey line is the total overall average temperature on top of the piles for the duration of the study. Orange bars indicate sampling dates. Purple border inlayed graph shows an expanded view of temperature data to demonstrate the difference in diurnal variability inside versus on top of the piles.

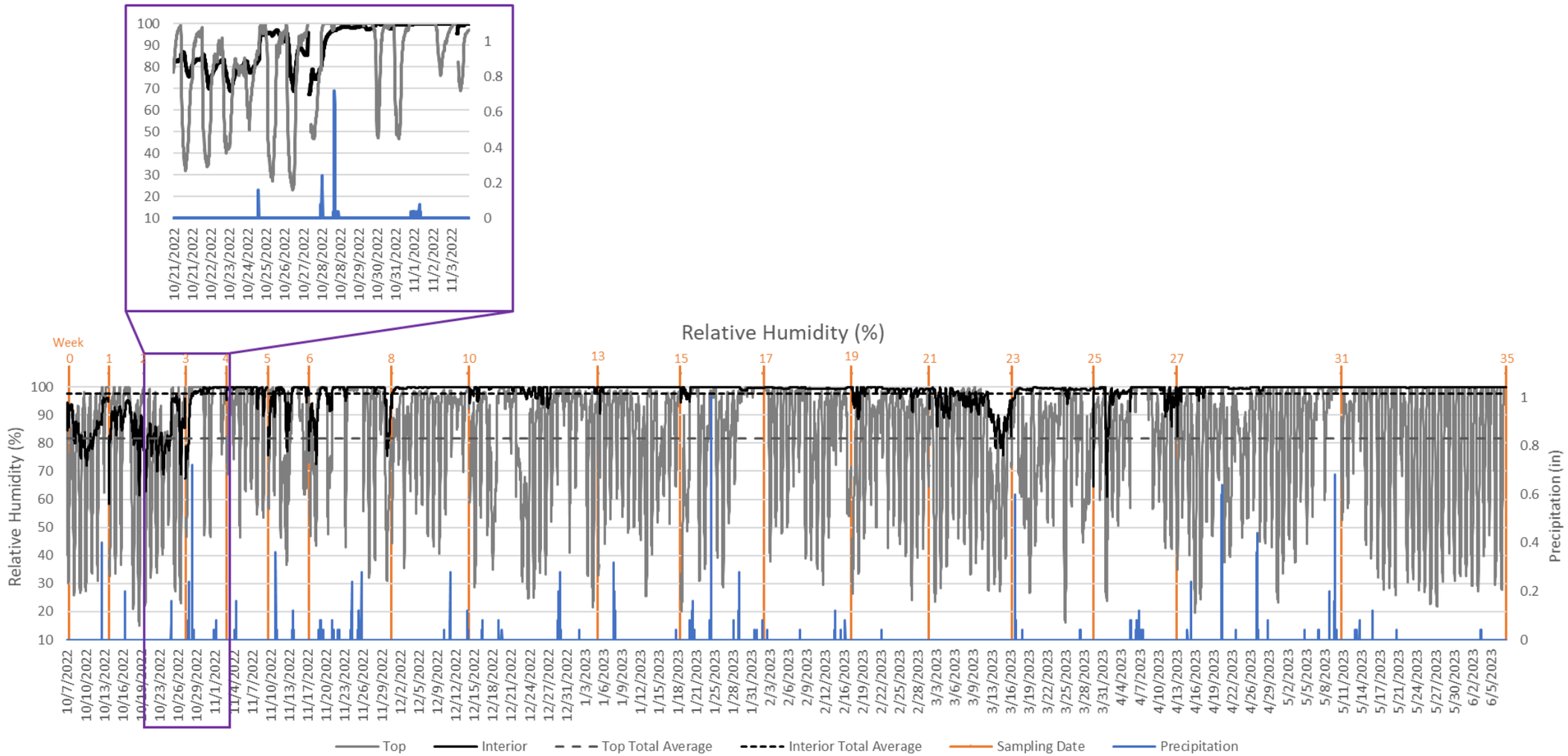

Figure 12. Time series of relative humidity data as the average of the sensors co-located in the interior of each of the four experimental piles (black line) with the sensor located at the top of pile C (grey line - ambient conditions). Dashed black line is the total overall mean relative humidity inside of the piles and the dashed grey line is the total overall average relative humidity on top of the piles during the duration of the study. Blue bars illustrate precipitation in inches. Orange bars indicate sampling dates. Purple-border inlayed graph shows an expanded view of relative humidity data to demonstrate the difference in diurnal variability inside versus on top of the piles.

Oysters in the interior of the piles were slower to desiccate compared to those at the top of the piles, but once desiccated the oysters in the interior of the piles continued to degrade and had a lower percent cover of tissue throughout the study compared to those at the top of the piles (Figure 13). A sub-set of the oysters in the interior of the piles became liquified (e.g. Figure 5) during weeks 1 through week 3. This liquified tissue condition state of decomposition was not observed in the oysters deployed at the top of the piles; those oysters transitioned directly to a desiccated state. At week 4, all remining tissue was in the desiccated condition and at week 6 the percent of oysters with no tissue as well as the percent cover of tissue for the oysters that had desiccated tissue present stabilized until week 19 (Figure 13). At week 19 we see an increase in oysters with no tissue (decrease in oysters with tissue condition desiccated) regardless of location, and this sampling week corresponds with the first period with consistent warming (Figure 11). The next observed reduction in percent of oysters with no tissue for the interior oysters occurred at week 31 which corresponded with elevated relative humidity levels (Figure 12).

Anecdotally, the research team noted the presence of maggots (as either pupae or larvae) as well as other insects, all of which were more prevalent in oysters deployed in the interior of the piles. There were 97 occurrences of oysters with maggots observed in the interior of the piles, while the top of the piles only had 47 occurrences of oysters with maggots observed. Maggots were most prevalent in the first week of deployment and their presence dropped off through week 13 of deployment (Figure 14). There also appeared to be a relationship with the presence of maggots and the Dermo infection intensity. Dermo infection intensity was significantly lower for oysters that had no maggots observed (chi-squared = 43.082, $p < 0.0001$, Kruskal-Wallis rank sum test) (Figure 15) with the probability of maggots present being highest 68% with a Dermo infection intensity of 1.0, and lowest (10%) with a Dermo infection intensity of 0 ($z = 5.12$, $p < 0.0001$, Generalized linear model).The mechanism(s) driving this correlation is(are) unknown.

Dermo infection intensity significantly decreased after the first week of deployment regardless of location (top versus interior) within the pile (x^2 (18) = 301.68, $p < 0.0001$, Friedman test with pairwise Wilcoxon signed-rank test; p-adjusted using the Bonferroni multiple testing correction method). Throughout the study (week 1 – week 35) there was a significant decrease in Dermo infection intensity measured for oysters deployed in the interior of the piles compared to the top of the piles (chi-squared = 10.086, $p = 0.0015$, Kruskal-Wallis test) with no Dermo infection detected in the interior of the piles after week 6 (Figure 16). Dermo infection continued to be detected in tissue sampled from the oysters deployed at the top of the piles through the 31st week of deployment. Individual tissue condition and Dermo infection intensity for each oyster by week are summarized in Appendix A.

All oysters, including the "tissue" oysters were sampled for Dermo infection at the initial (week 0) and at the end (week 35) and the only oyster that was positive for Dermo infection (lowest intensity rating = 0.33) at week 35 was a "tissue" oyster deployed in the top of pile C. Interestingly, during the initial Dermo test, no Dermo infection was detected for this oyster.

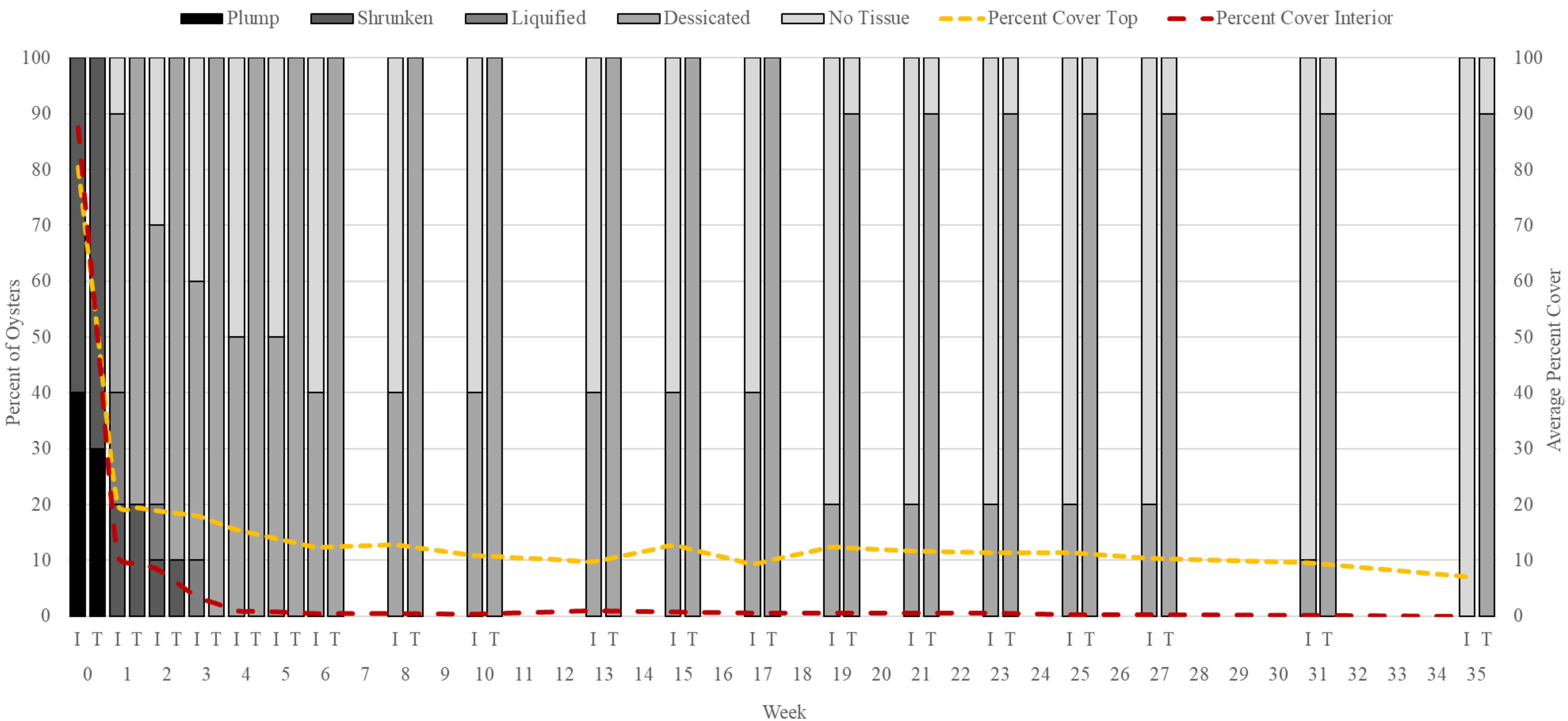

Figure 13. Percent of oysters deployed in fenced piles by tissue condition category of oysters monitored for tissue condition by sampling week and oyster deployment location. The average percent of the oyster shell that was covered by tissue by week and location are plotted (yellow short-dash line = oyster deployed at the top of the fenced piles, and red long-dash line = oysters deployed at the interior of the fenced piles).

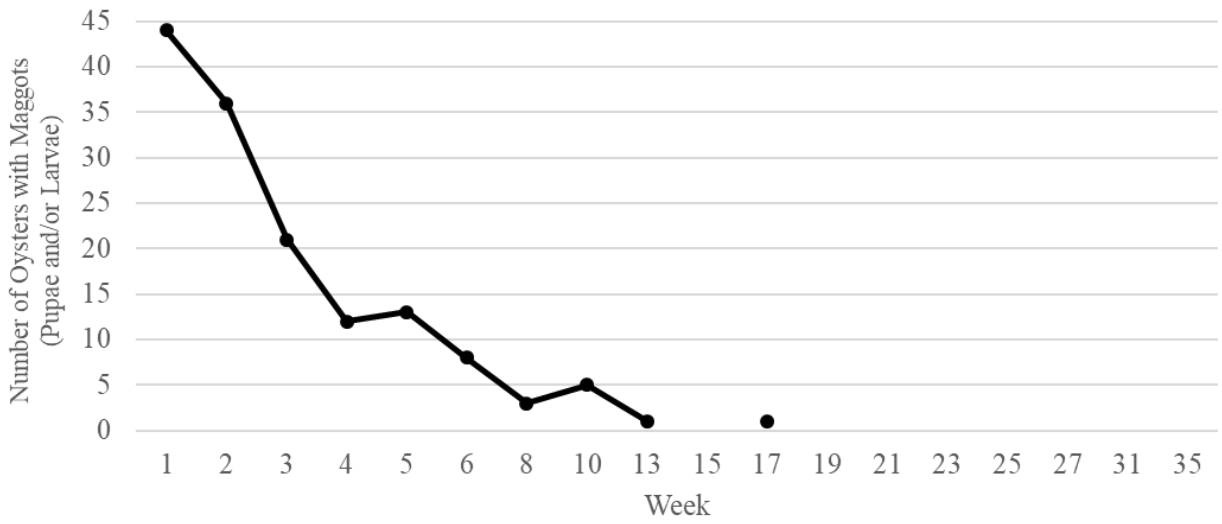

Figure 14. Number of oysters with maggots (pupae and or larvae) observed by week in all oyster piles combined.

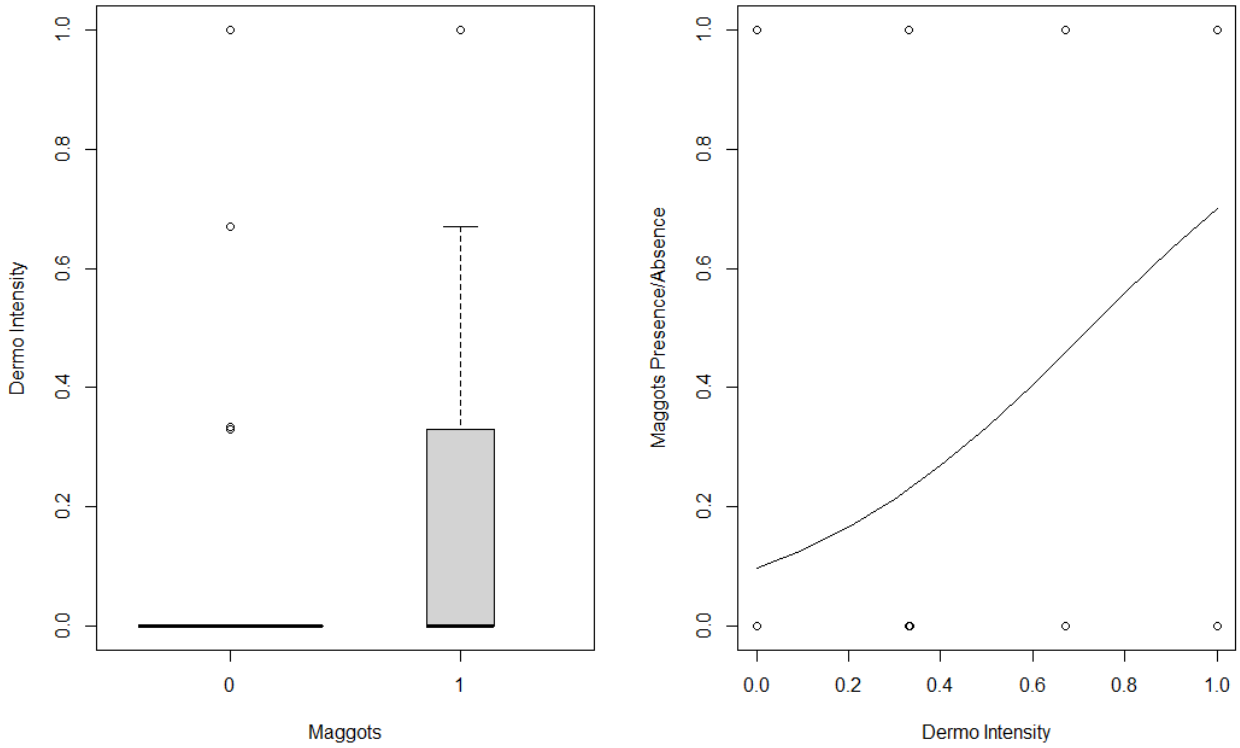

Figure 15. a) Boxplot of Dermo infection intensity for oysters with maggots observed (1) versus not observed (0). b) Fitted binomial Generalized Linear Model (GLM) applied to the probability of maggots being present by the Dermo infection intensity with detection probability curve.

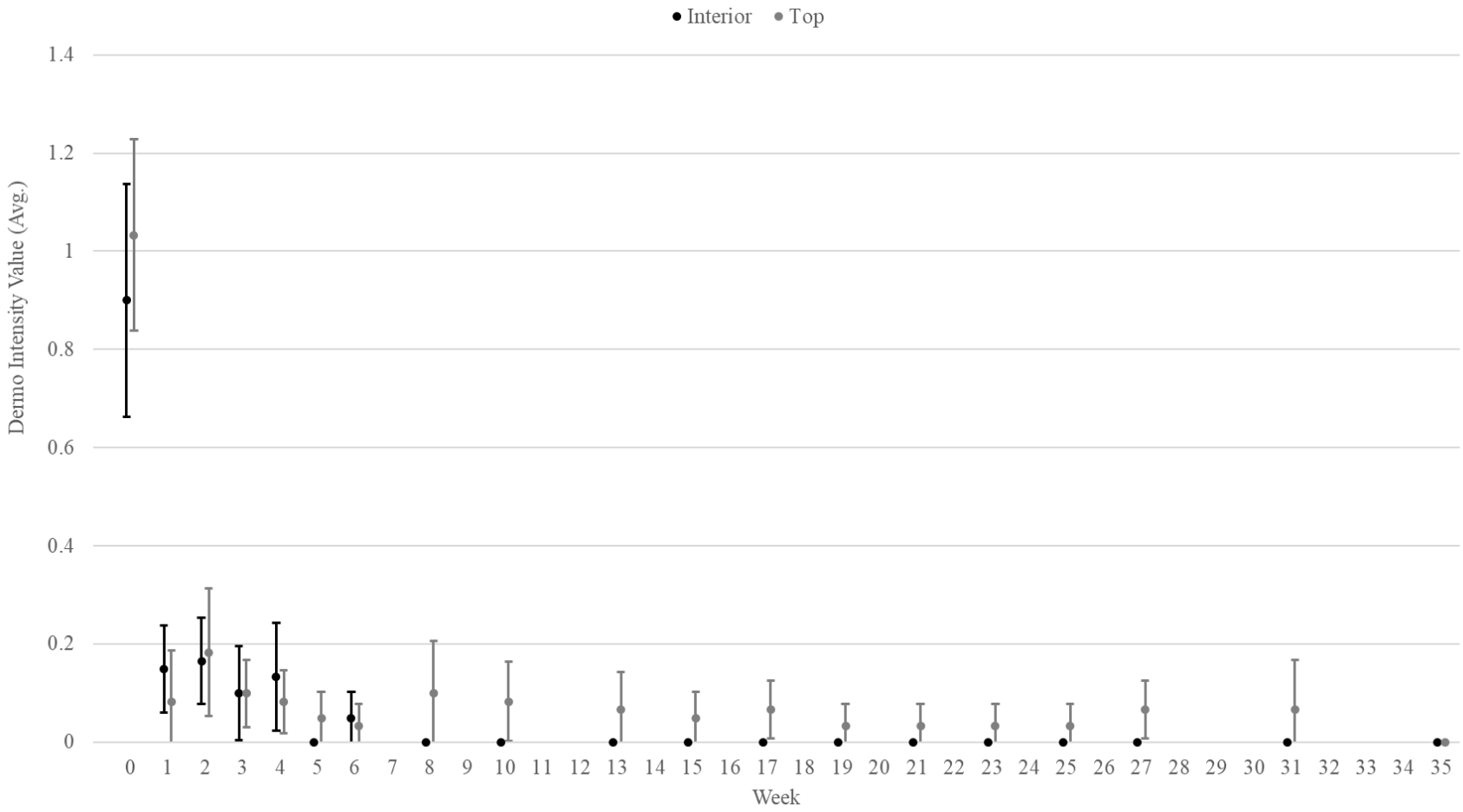

Figure 16. Average dermo infection intensity values by week and location for all piles. Error bars represent 95% confidence interval.

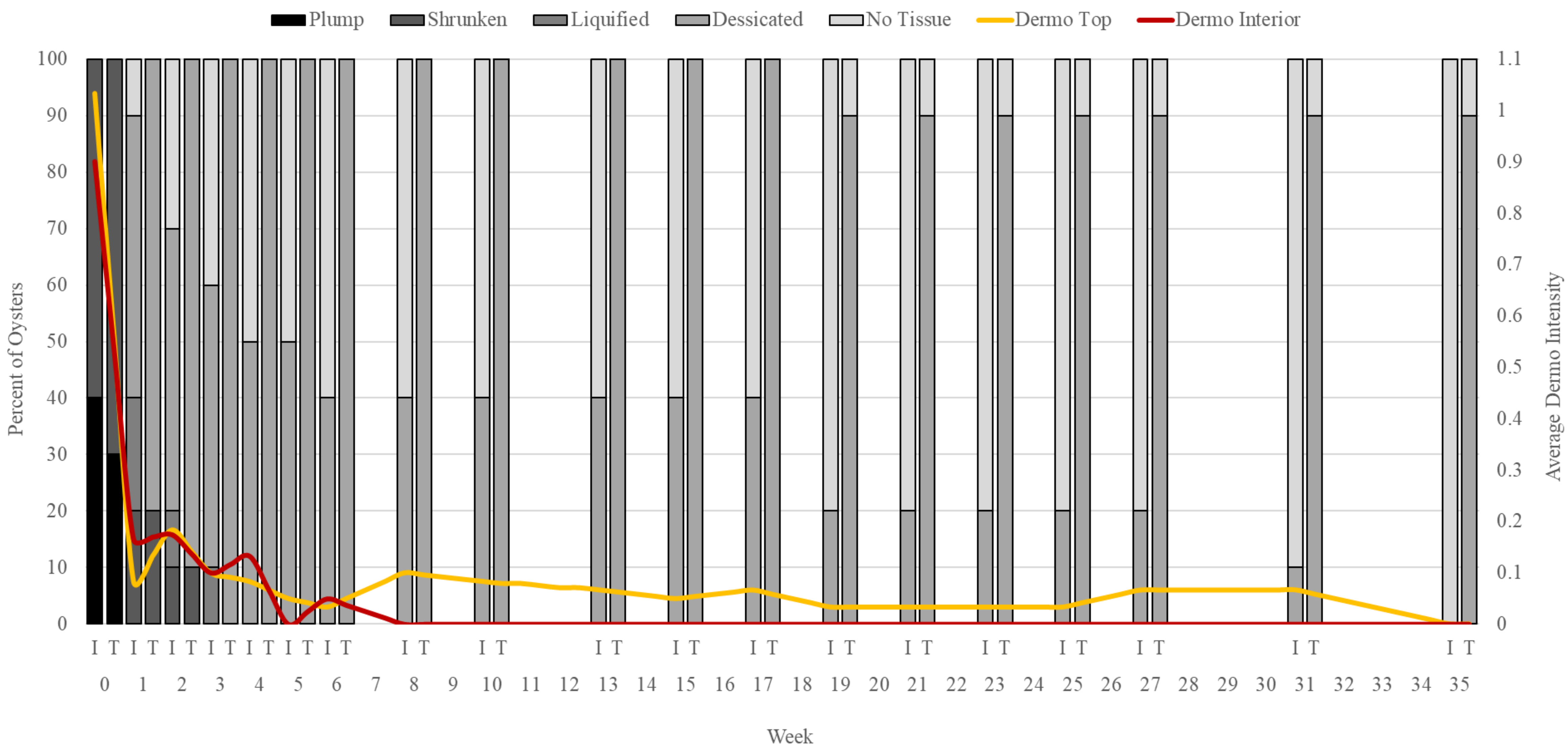

Figure 17. Percent of oysters monitored for tissue condition in fenced piles by tissue condition category by sampling week and oyster deployment location. The average Dermo infection intensity for oysters from all piles by week and location are plotted (yellow line = oyster deployed at the top, and red line = oysters deployed at the interior).

DISCUSSION

This study was designed to test the worst-case-scenario by including oysters with a high initial level of Dermo infection, and deploying them wired-closed, simulating whole un-shucked oysters deployed in the sun-curing piles. Dermo infection in commercially harvested oysters that could end up in the oyster recycling pathway are not regularly monitored because Dermo infection poses no threat for human consumption. A preliminary study by Schubert and Hanke (2023) that evaluated Dermo infection in commercially sourced oysters found that 21-22% of oysters tested were positive for Dermo infection, while the oysters used to monitor Dermo infection in the study had an initial positivity rate of 97.5%. Furthermore, the oysters deployed in this study were meant to mimic whole un-shucked oysters that were included in the recycling process. It is unknown how frequently un-shucked oysters are found in the recycled shell materials, but it is reasonable to assume that it can happen, particularly if a restaurant is unable to sell oysters before their holding time. There are few references to un-shucked oysters that have been observed in shell piles in the literature (Bushek 1997, Bushek 1998, Bushek et al. 2004), and GBF OSRP staff have made note of un-shucked oysters on many occasions while collecting shell (personal communication: S. Batte, GBF).

The impact of wild animals on the sun-curing process was tested in this study using fenced and unfenced piles. Initial analysis of game camera photos conducted by Smith et al. (2023) suggests that a number of types of wildlife interact with the oyster piles including feral hogs, vultures, opossums, deer, coyotes, and songbirds. The number of interactions at unfenced piles was much higher than at fenced piles and the highest number of interactions for feral hogs occurred in the first week post-deployment, while the highest number of interactions by vultures occurred in the third week post-deployment (Smith et al. 2023). Within the first week of deployment oysters deployed at the top of the unfenced piles were depredated by feral hogs which resulted in the removal of all oyster tissue for those affected oysters and therefore the assumed removal of *P. marinus*. Feral hogs have quickly spread globally and have well documented negative impacts on the environment including but not limited to, competition with and predation of native species, habitat damage, disease transmission, and fecal bacteria in local waterways (Massei et al. 2011). We may have found the one positive impact that feral hogs can have on the environment, consumption of decaying oyster tissue at sun-curing sites; however, we recognize that this is not a sufficiently redeeming quality to allow their continued habitation. For the Red Bluff curing site, the presence of a robust feral hog population helps to remove oyster tissue and subsequent *P. marinus* resulting in potentially expedited treatment, but not all sun-curing locations have feral hog populations, and their detrimental impacts to the surrounding environment probably outweigh their help consuming rotting oyster tissue. There were also instances of feeding observed by vultures and opossums (although it is possible the opossums were feeding on the associated insects). Their impact was restricted to just the oysters deployed on the top of the piles; game camera footage suggests that all these wild animals only access the top few inches of the oyster piles.

The temperature and relative humidity sensors that were co-located with the deployed oysters helped to characterize the differences in the ambient conditions that the oysters were exposed to in the interior of the piles compared to the top of the piles. These data may help explain the differences in tissue decomposition and Dermo infection presence and prevalence between these

two deployment locations. Historically sun-curing recommendations for quarantining recycled oysters to be used in restoration projects were aimed at making sure the shell was exposed to the sun for UV irradiation to damage/degrade *P marinus*, as has been shown in laboratory-based studies (Ford et al. 2001, Buschek and Howell 2000). Our results indicate that the interior of the piles was more humid and supported a more consistent (yet lower) temperature environment compared to the top of the piles which mirror results presented in Buschek et al. (2004).

Tissue decomposition was more rapid for oysters deployed in the interior of the piles. Also, some oysters deployed in the interior of the piles experienced a liquification decomposition stage, which seemed to correspond with high maggot presence and resulted in loss of tissue when the liquified material leaked out of the oyster shells or were consumed by insects making it unsampleable at the next check. Anecdotally the research team noted the presence of maggots as well as other insects; all of which were more prevalent in oysters deployed in the interior of the piles. These insects may play an important role in the degradation of oyster tissue in curing piles. The potential relationship between the Dermo infection and the presence of maggots may be a result of timing, as both the maggot presence and the Dermo infection intensity was higher in the earlier weeks of the study and then declined.

There appeared to be continuous tissue decomposition through the first three weeks of deployment, then there was little to no change from week 4 through week 17 which corresponds to November through February when temperatures were the lowest during the deployment. The rate of decomposition may have been higher if the oysters were deployed in warmer months. Because most recycled oyster shell for the GBF OSRP comes from restaurants, the volume of recycled shell is variable throughout the year, but there tends to be a peak in March each year with elevated levels through August (personal communication: S. Batte, GBF). Similar to Bushek's 2004 study, most (greater than 50%) of the tissue in the deployed oysters in the interior of the piles was gone by the 16th week, which was the end of their study, but alternatively the majority of oysters deployed at the top of the piles had tissue remaining throughout the 35th week of our study (top deployment location was not evaluated in Bushek et al. 2004).

Much of the oyster shell collected through the GBF's OSRP comes from participating restaurants, and it is not uncommon for un-shucked, uneaten oysters, and/or shell with varying amount of tissue remaining to be included in with the recycled shells (personal communication: S. Batte, GBF). Other studies have found that desiccated oyster tissue found in shell piles exhibited Dermo infection (Bushek et al. 1994, Bushek et al. 2004). Additional studies to better evaluate the background levels of Dermo infection present in oysters that enter recycling pathways are needed. Understanding the presence and intensity of Dermo infection in oysters that can be recycled will help to evaluate the underlying source level of Dermo infection in sun-cured oysters. An audit of the amount of tissue present in a typical load of recycled shell, with quantification of the number of un-shucked oysters would be helpful in extrapolating the amount of tissue and therefore Dermo infection present in recycled shell piles.

We observed a significant decrease in the presence and prevalence of Dermo infection after the first week of deployment. While the intensity ratings for these tissues were typically low (average Dermo infection intensity of 0.33), studies have shown that infection can be initiated with as few as ten cells (Valiulis 1973, Bushek et al. 2004) and an overall infective dose

estimated at 50 cells (Bidegain et al. 2016). *Perkinsus marinus* can survive for 3 to 14 days in seawater (Chu et al. 2002, Chu and Lund 2006). Transmission of *P. marinus* has been shown to be highest from dead/decaying oysters during periods of high temperatures when oyster die-offs are occurring from Dermo infection (Calvo et al. 2003). As temperatures rise and extend for longer periods throughout the year due to global warming, Dermo infection and transmission rates are expected to increase (Craig et al. 1989).

There was a significant difference in the Dermo infection presence and prevalence depending on where they were deployed with the oysters in the interior of the piles having no Dermo infection detected after week six. While we refer to some analyses in terms of the presence and absence of Dermo infection, we cannot confirm absence, rather in these cases, we did not detect Dermo infection in the tissue sample that was used during that sampling event. The GBF OSRP currently sun-cures their recycled oyster shells for 6 months and mechanically mixes the piles after 3 months of curing. The experimental piles included in this study were not mechanically turned. Additionally, the experimental piles used in this study were relatively small (~6 ft wide by ~3 ft tall) and previous work suggests that the size and shape of the shell pile during sun-curing may alter the decomposition of tissue and subsequent Dermo infection (Bushek et al. 2004). Typical sun-curing piles at the GBF OSRP Red Bluff site are spread out flat, up to two feet tall to increase the proportion of oysters exposed to the top/sun. It was thought that UV light and lower relative humidity levels helped to speed up the decomposition and *P. marinus* mortality rates (Bushek et al. 2004, Diggles 2020, Diggles et al. 2021) but our results do not support this hypothesis. Bushek et al. 2004 suggested that tissue decomposition rates are likely to decrease with an increase in shell pile size, but they did not test oysters at the top of the piles. It is understood that the decomposition rate is positively correlated with higher temperatures and similar to our study they found that the interior temperature was generally lower than the external temperature of the piles. However, we found that the oysters deployed in the interior of the piles actually decomposed more quickly than those deployed on the tops of the piles; therefore, perhaps other factors have a higher influence on decomposition such as humidity and insect interaction than temperature. Anecdotally researchers noticed that once the tissue became desiccated the insect interactions decreased and decomposition slowed. It would be interesting to involve an entomologist in future studies to investigate the interactions between the insects and decomposition of the oyster tissue at the sun-curing site.

Perkinsus. marinus is and has historically been found in all bays and estuaries in the northern Gulf of Mexico (Craig et al. 1989). Consequently, there is no concern for introducing *P. marinus* through restoration efforts into an area in Texas where it does not already exist. Background Dermo infection levels in Texas are high relative to much of the northern Gulf of Mexico (Craig et al. 1989). Dermo infection reduces growth and reproduction of oysters (Dittman et al. 2001). Oyster spawning season extends from late Spring through early Fall when water temperatures are elevated. The success of an oyster restoration project is typically measured in the recruitment of spat, and the growth/size of the reef/oysters post-restoration. Therefore, to aid in the success of a restoration project, the reef substrate material should not contribute to the local source for *P. marinus* exposure to newly recruited oysters. To reduce this risk, timing the deployment of the recycled shell to the beginning of the non-spawning season (cooler temperatures) should ensure that if any residual desiccated tissue remains, there is ample time for it to break down and any released *P. marinus* die before new spat settles at the restoration site.

The viability of the spores observed throughout this study is unknown. Bushek et al. (2004) attempted to monitor the viability of the *P. marinus* in oysters deployed in sun-curing piles and they suggest that the parasites likely did not enlarge during the RFTM incubation period, bringing their viability into question. Future laboratory-based studies to expose uninfected oysters to the desiccated but infected tissues from oysters gathered at the sun-curing site is needed to determine the viability and risk level of the recycled shell material.

Typically, the RFTM requires that a tissue sample is collected from the mantle of the oyster for analysis, however depending on the decomposition pathway and rate, identifying or discerning the tissue types or even if something is in fact oyster tissue became difficult. Therefore, samples were taken from any available tissue using best professional judgment. It is unknown how the types of tissue sampled may have impacted our ability to observe the Dermo infection present in the remaining tissue as a whole.

Perkinsus marinus is not the only risk of using recycled oyster shells for restoration projects. With the global seafood market and the popularity of boutique oyster bars and restaurants, oyster shells that enter the recycling pathway can come from nearly anywhere in the world. There can be non-native polychaetes, algae, sponges, tunicates, gastropods, viruses, bacteria, and protozoans associated with raw and discarded oyster shells (Diggles 2021). While there are a variety of treatments that can be used to sterilize the recycled shells such as heat treatment, and freshwater, bleach, or acid soaks these are not logistically reasonable for large-scale shell recycling programs (Diggles 2021, Bushek 2000). Sun-curing or desiccation for 4 to 6 months remains the preferred method to treat large volumes of recycled shell. Our results support previous studies recommendations that the prevalence of Dermo infection is correlated to the decomposition rates of tissue (Bushek et al. 2004). Therefore, we expect that deployment of recycled oyster shell in hotter and wetter months will help decomposition happen more quickly after initial deployment, and result in more rapid declines in the potential for additional Dermo infection in wild oysters.

Recommendations

Dermo infection is monitored across the northern Gulf of Mexico by a variety of organizations and reported to the Oyster Sentinel database (https://data.oystersentinel.cs.uno.edu/). There has not been any Dermo infection monitoring in Galveston Bay since 2015, and no consistent monitoring since 2010. There is a need for year-round monitoring of Dermo infection in oysters of Galveston Bay as seasonal cycles of infection and associated environmental variables can aid in existing oyster reef management, and restoration strategies as well as help researchers and managers understand the potential impacts of declining freshwater inflow and increasing salinity and water temperatures on Dermo infections.

The fact that there are feral hogs that are habituated to shell dumping and are utilizing the tissue as a food source at the Red Bluff Curing Site provides a benefit by removing decaying tissue, effectively removing the Dermo infection. However, it is unknown if *P. marinus* can survive the digestive tract of a feral hog, and if so, if it can remain viable in the hog feces. Because the

oyster tissue from the tops of the piles was effectively gone due to depredation by feral hogs within 2 weeks of deployment, we assumed that oysters at the tops of the piles were free of *P. marinus* after 2 weeks. The oysters in the interior of the piles had no Dermo infection detected after the 6th week of deployment. Depending on the demand for oyster shells, the results of this study suggest that resource managers and practitioners that have active depredation of oyster tissue at the top of their piles, to the extent that tissue is quickly removed, cure their recycled oyster shell material for a minimum of 3 months as long as the shell is deployed during "warm-weather" months (April – September). For oysters deployed during "cold-weather" months, the results suggest continuing the current practice of 6 months deployment with a mechanical rotation at 3 months be continued until additional studies can be completed to better understand the seasonal component and determine how temperature may impact the decomposition of the oyster tissue and subsequent Dermo infection prevalence. Should the feral hog population cease to exist on the Red Bluff Curing Site property, the tissue decomposition and Dermo infection of oysters on the top of the piles is expected to increase, and we recommend returning to the cold-weather curing protocol.

Lessons Learned

Our study showed depredation by feral hogs and vultures impacts oysters at the tops of sun-curing piles. We deployed oysters in the same plastic mesh bags that GBF uses for its oyster gardening (GBF 2023) and attached the bags to wire cable in an attempt to avoid losing the study oysters, but the feral hogs were able to rip through the bags and remove the bailing wire to access the oyster tissue. This was important to our study design as one of our goals was to determine the impact that wildlife has on the sun-curing process. Future studies should consider using a sturdier container that will allow the oysters to be exposed to the ambient environment at the top of the piles but protect them from depredation as not all sun-curing sites have feral hogs, or the same wildlife present.

Additionally, the development of the tissue condition categories was a "work-in-progress" as we observed the tissues throughout the initial weeks of deployment. We did not have previously defined condition categories beyond the initial "plump" and "shrunken" as defined by Ray (1966). As a result, the field team had to spend significant time in the field together standardizing the evaluation of these categories, and re-evaluation using photos in the initial weeks was required after the categories were finalized. Future studies may consider using these categories to standardize the process of documenting tissue degradation in oysters deployed in sun-curing piles.

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Appendix A – Data Summary Table

Summary table of Dermo intensity values and tissue condition category by oyster by week of the study. Data are organized by the wildlife access type (fenced or not fenced), the pile (A, B, C, or D), location of deployment within the pile (interior or top), the data type being monitored (Dermo or Tissue), and the oyster number. The top row of data for each oyster number displays the tissue condition category by week (P = plump, S = shrunken, L = liquified, D = desiccated, NT = no tissue, and N/A = not sampled because it was removed from the study due to depredation). The second row of data for each oyster number is the Dermo intensity by week, note: Dermo intensity was measured for all oysters at their initial deployment (week 0), and for all oysters with tissue remaining at week 35, otherwise only "Dermo" oysters were monitored for Dermo intensity each week of the study.

Wildlife Access	Pile	Location	Oyster Type	Oyster No	Week 0	1	2	3	4	5	6	8	10	13	15	17	19	21	23	25	27	31	35
Fenced	A	Interior	Dermo	2365	P	D	D	D	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	0.33	0.33	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				2373	P	L	D	D	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	0	0	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				3953	P	S	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT
					1.67	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				4032	P	D	D	D	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	0.33	0.33	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				4046	S	D	D	D	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					1.33	0.67	0.33	0.67	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			Tissue	2326	S	L	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				2358	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	NT
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				2370	P	D	D	D	D	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				3955	S	D	D	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				3971	S	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Top	Dermo	2359	P	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT
					1.33	0.33	0	0.33	0	0.33	0	0	0.33	0	0	0	0	0	0	0	0	0	0	0
				2360	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					1	0	0.67	0	0.33	0	0.33	1	0.67	0.67	0.33	0.33	0	0	0	0	0.33	1	0
				3952	P	L	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT
					1	0	0.33	0	0	0	0	0	0.33	0	0	0	0	0	0	0	0	0	0	0
				3954	P	D	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT
					1.33	0	0	0	0	0	0	0	0	0	0.33	0	0	0	0.33	0	0	0	0
				4041	S	L	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT
					1.67	1	1	0.33	0.33	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0
			Tissue	2343	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
				2357	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
				3742	S	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT	NT
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				3750	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
				3960	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0

Appendix A Cont.

Wildlife Access	Pile	Location	Oyster Type	Oyster No	Week																	
					0	1	2	3	4	5	6	8	10	13	15	17	19	21	23	25	27	31	35
Not Fenced	B	Interior	Dermo	2335	P	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT
					0.33	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				2342	P	L	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					1	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				3745	S	L	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.33	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				4036	P	S	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				4050	P	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			Tissue	2368	P	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				3962	S	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				3966	P	S	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				3972	P	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					1.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				4048	S	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Top	Dermo	2331	S	L	S	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT
					1	0	0	0	0	0	0	0	0	0.33	0	0.33	0	0.33	0	0	0	0	0
				2345	P	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					1.33	0.33	0	0	0	0	0	0.33	0	0	0	0	0	0	0	0	0	0	0
				2361	S	L	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT
					0.33	0	0	0.33	0	0	0.33	0	0	0.33	0	0.33	0	0.33	0	0	0.33	0	0
				3958	S	L	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					1.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				4035	P	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					0.33	0	0	0.33	0	0	0	0.33	0.33	0	0.33	0	0	0	0.33	0.33	0.33	0	0
			Tissue	2347	P	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				2348	S	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				2363	S	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				4040	S	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				4042	P	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix A Cont.

Wildlife Access	Pile	Location	Oyster Type	Oyster No	Week																		
					0	1	2	3	4	5	6	8	10	13	15	17	19	21	23	25	27	31	35
Fenced	C	Interior	Dermo	2329	P	L	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT
					0.33	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				2374	S	D	D	D	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				4027	P	D	D	D	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					1	0	0.33	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				4028	P	S	D	D	L	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				4038	S	D	D	D	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					1.33	0.33	0.33	0.67	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			Tissue	2333	S	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				2340	P	S	L	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				3957	S	L	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				3975	P	S	S	L	D	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT	NT
					0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				4033	P	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT
					0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Top	Dermo	2341	P	L	D	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT
					0.67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				2346	P	S	S	S	S	S	S	L	L	D	D	D	D	D	D	D	D	NT	NT
					0.67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				3961	S	L	L	L	L	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.33	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				3970	P	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					1.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.33	0
				3973	P	L	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT	NT
					0.67	0	0	0.33	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			Tissue	2334	P	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					N/A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.33
				2354	S	S	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
				3951	P	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
				3959	S	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
				4039	P	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0

Appendix A Cont.

Wildlife Access	Pile	Location	Oyster Type	Oyster No	Week																	
					0	1	2	3	4	5	6	8	10	13	15	17	19	21	23	25	27	31	35
Not Fenced	D	Interior	Dermo	2351	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT
					2	0.33	0.67	0	0.33	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0
				2371	S	S	L	L	L	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT
					1.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				2372	P	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT
					0.67	0	0.33	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				3965	P	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT
					1.33	0	0	0	0	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0
				4049	S	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT
					1.67	0	0	0	0	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0
			Tissue	2337	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
				2339	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
				3728	P	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
				4034	P	S	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				4037	S	S	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT
					0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Top	Dermo	2330	P	S	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT
					1.33	0	0.33	0	0	0	0	0	0	0	0	0.33	0.33	0	0	0	0	0	0
				2338	P	D	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					1.67	0	0.67	0.33	0.33	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0
				2375	P	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT
					0.67	0	0	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0.33	0	0
				3968	S	S	D	L	L	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT	NT
					1	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				4029	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT
					1.67	0	0	0	0	0	0	0.33	0	0	0	0	0.33	0	0	0.33	0	0	0
			Tissue	2349	S	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				2350	S	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					1.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				2353	P	S	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				3967	S	S	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				4047	P	S	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-