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Gene Expression of Stress-Related Biomarkers and Disease Presence in Gene Expression of Stress-Related Biomarkers and Disease Presence in Crassostrea Virginica

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GENE EXPRESSION OF STRESS-RELATED BIOMARKERS AND DISEASE PRESENCE IN *CRASSOSTREA VIRGINICA*

By: Jaycie Keylon

A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

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ABSTRACT

Crassostrea virginica, the Eastern oyster, is an environmentally and economically important species along the East coast and in the Gulf of Mexico. Environmentally, oysters are filter feeders, cleaning the water of contaminants. They also provide habitat and shelter for other marine species. Oysters are of significant economic value as seafood to nearby communities as well. Eastern oyster populations have been significantly adversely affected by the parasite *Perkinsus marinus*, which causes Dermo disease. Dermo affects oysters during times of warm temperatures and high salinities and has the potential to wipe out entire populations of oysters. Throughout this study, the presence of Dermo was followed by collecting oysters from different sites on the Mississippi Gulf Coast. Gene expression of heat shock protein-70, galectin, anti-apoptosis protein, and sodium hydrogen exchanger protein was evaluated because these genes can indicate environmental and immune stress. In oysters studied here, Dermo was not found, which can be explained by the environmental conditions at the time of harvest. Furthermore, significant alterations in stress and immune related gene expression were not detected, which was also consistent with the absence of Dermo.

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ABBREVIATIONS

ANOVA	Analysis of Variance
BMG	BM Gautier
GCB	GC Biloxi
HSP-70	Heat shock protein 70
JHBSL	JH Bay St. Louis
JSOS	JS Ocean Springs
MMB	Maritime Museum Biloxi
PCR	Polymerase Chain Reaction
RTFM	Ray's Thioglycolate Fluid Medium
SLOS	SL Ocean Springs
SSHS	St. Stanislaus High School
TSPC	TS Pass Christian

1. INTRODUCTION

1.1 Crassostrea virginica

Crassostrea virginica, the Eastern oyster, is found along the East Coast of the United States and in the Gulf of Mexico including the Mississippi Sound. Oysters are a very important species because they provide habitat and shelter for other organisms and filter pollutants from the water. Oysters and their reefs also protect shorelines from erosion. Oyster reefs promote diversity and stability by affecting water circulation and flow patterns. This improves water quality and nutrient cycling in the water. Eastern oysters support the economy when harvested and sold as food thus providing a variety of jobs to fishermen in coastal towns (Galtsoff, 1964).

1.2 Dermo

Dermo is an infection caused by the parasite *Perkinsus marinus.* Dermo is one of the leading causes of oyster mortality in the Mississippi Gulf Coast. *C. virginica* oysters have been heavily affected by Dermo disease. *P. marinus* infects the hemocytes of the oysters and degrades the oyster's tissue (Tasumi & Vasta, 2007). Hemocytes play a major role in the immune system of invertebrates. They can be compared to the phagocytes of vertebrates. Dermo adversely affects the digestive gland and mantle of the oyster. Once ingested, the parasite proliferates. This proliferation is associated with the pathogenesis of the disease as the parasite multiplies and takes nutrients from the host. The parasite infects and continues to replicate until it is released through the feces or the oyster dies. Once released, the parasite continues the same process in more oysters. Dermo infection causes great stress on the oysters including: tissues become pale, gamete production decreases, mantle and digestive tissues shrivel, and blood vessels are blocked. Though

oysters can persist through many years of active Dermo infection, they become very stressed and nonproductive (Busca et al., 2009; Lenihan et al., 1999).

Dermo prevalence is significantly affected by environmental conditions like temperature, salinity, hypoxia, and food availability. At higher temperatures, the lysozymes, or the chemical defense of the oysters, are reduced allowing more infections to occur (Chu & La Peyre, 1993). Because of this temperature sensitivity, more Dermo infections occur in the warmer months of the year (summer and spring). Environmental pollutants, lack of available food, and high salinities are also associated with increased prevalence of Dermo (Pernet et al., 2019). The more stressed the oysters are, the easier it is for Dermo to cause infection. Dermo is a leading cause of death in oyster populations, so much so that some populations are considered ecologically extinct (Schulte, 2017). Throughout this study, we tested for the presence of Dermo as well as followed the expression of stress and immune related genes in oysters collected from the Mississippi Sound. We hypothesized that differential gene expression could be indicative of infection based on a related stress or immune response.

1.3 Stress and Immune Related Genes

Throughout this study, gene expression of heat shock protein-70, galectin, anti-apoptosis protein, and sodium hydrogen exchanger protein was measured because these genes are known stress and immune-related genes that can provide insight into how the oyster is responding to stress or infection. Gene expression can provide information about general markers of disease resistance that may be associated with an unsuccessful immune response (Nikapitiya et al., 2014).

Heat shock protein-70 (HSP-70) is upregulated in response to stress. It prevents protein aggregation, promotes the folding of proteins to the native state, and refolds aggregated proteins (Geraci et al., 2011). It controls the activity of regulatory proteins as well. In response to stress and disease, HSP-70 inhibits apoptosis. By inhibiting apoptosis, the protein becomes more stable in conformation (Mayer & Bukau, 2005).

Galectin is a carbohydrate binding protein that exists intra- and extracellularly. Galectin recognizes endogenous ligands and is involved in inflammation by mediating adaptive immune responses (Tasumi & Vasta, 2007). Galectin is expressed in hemocytes, where Dermo infects, and promotes the phagocytosis of infectious agents in the host cell (Tasumi & Vasta, 2007).

Anti-apoptosis protein is key to survival in variable situations as it mediates the protective effects of apoptosis and keeps the cells alive (Clapp et al., 2012). Apoptosis is programmed cell death. Anti-apoptosis protein promotes the survival of affected cells by maintaining the integrity of the mitochondrial membrane and preventing the activation of the apoptosis cascade (Busca et al., 2009). Anti-apoptosis protein is upregulated in response to stress (Jones et al., 2019).

Sodium hydrogen exchanger protein is another important stress-related gene. It is a membrane protein that transports sodium into the cell and hydrogen out to keep homeostasis within the cell (Vallés et al., 2015). Sodium hydrogen exchanger protein is upregulated in oysters under stress, specifically low-salinity conditions (Vallés et al., 2015). Throughout the study, 18S ribosomal RNA was used as a reference gene because it is uniformly expressed in all tissues of the oyster.

1.4 Goals

The goals of this study were to determine presence or absence of Dermo in Eastern oysters collected at various sites in the Mississippi Sound and measure stress and immune related gene expression in the same oysters. It was hypothesized that stressed oysters would have increased expression of stress and immune-related genes which could indicate environmental stress and predict the presence of infection. It was also hypothesized that the more stress an oyster was under, the more likely it would be to have had Dermo infection. We used condition index, Dermo presence/absence, and gene expression to evaluate our hypotheses.

2. MATERIALS AND METHODS

2.1 Oysters

The oysters from this study were provided by the Mississippi Oyster Gardening Program from eight sites along the Mississippi Gulf Coast. The sites were: St. Stanislaus Bay St. Louis (SSHS), JH Bay St. Louis (JHBSL), TS Pass Christian (TSPC), Maritime Museum Biloxi (MMB), GC Biloxi (GCB), JS Ocean Springs (JSOS), SL Ocean Springs (SLOS), and BM Gautier (BMG). Figure 1 shows the location and latitude and longitude of each collection site. Ten oysters from each site were provided, giving a total of 80 oysters for the study. These oysters were deployed as spat on shell in cages during the summer of 2020 and were harvested in December of 2020.

1. SSHS Lander
2. SSHS Bay St. Louis
3. JH Bay St. Louis
4. TS Pass Christian
5. MM Biloxi
6. GC Biloxi
7. JS Ocean Springs
8. SL Ocean Springs
9. BM Gautier
10. Grand Bay Lander

Site	Description	City	Coordinates
1	St. Stanislaus High School (Lander)	Bay St. Louis	30.304968, -89.325304
2	St. Stanislaus High School (SSHS)	Bay St. Louis	30.305025, -89.325315
3	JHBSL	Bay St. Louis	30.334387, -89.331403
4	TSPC	Pass Christian	30.331054, -89.283668
5	Maritime Museum (MMB)	Biloxi	30.392968, -88.857867
6	GCB	Biloxi	30.416986, -88.908997
7	JSOS	Ocean Springs	30.418516, -88.836062
8	SLOS	Ocean Springs	30.343711, -88.722355
9	BMG	Gautier	30.363593, -88.637757
10	Grand Bay (Lander)	Moss Point	30.369654, -88.420534

Figure 1. Map of the Oyster Gardening sites and Lander sites of oysters used in this study. From 1-10 the sites are St. Stanislaus High School (Lander), St. Stanislaus High School (Bay St. Louis), JH Bay St. Louis, TS Pass Christian, MM Biloxi, GC Biloxi, JS Ocean Springs, SL Ocean Springs, BM Gautier, and Grand Bay (Lander). Latitude and Longitude coordinates of the sites are provided.

2.2 RNA/DNA Extraction

A ZYMO kit was used to extract DNA and RNA from the oyster samples. About 0.2 mL of the digestive gland and mantle tissue from each oyster were dissected and placed in an epitube. DNA/RNA Shield was added to preserve the genetic integrity and expression of the samples. Proteinase K and PK Digestion Buffer were added to each sample to digest contaminating proteins and nucleases. The samples were incubated at room temperature so the tissues could lyse. After incubation, the samples were centrifuged, and the supernatants were collected. DNA/RNA Lysis Buffer was added to the supernatant to prevent degradation of the nucleic acids. After centrifuging in a spin-away filter tube, the tube contained the RNA and the filter contained the DNA. To both the RNA and DNA, 96% ethanol was added. DNAse treatment was added to the RNA flow through to digest the remaining DNA. Flow through is what is left in the spin column after centrifuging after each step. To both the RNA and DNA flow throughs, DNA/RNA Prep Buffer and DNA/RNA Wash Buffer were added, centrifuged, and flow through was discarded between each step. At the end of the extraction process, RNase free water was added to the filters of both the RNA and DNA and centrifuged and the flow through represented the eluted RNA and DNA, respectively.

2.3 Quantification

The extracted RNA and DNA were quantified with a NanoDrop. The NanoDrop determines the concentration and purity of each sample. The 260/280 value was noted, which also determined how pure the samples are. The 260/280 value of DNA should be between 1.8 and 1.9 while the 260/280 value of RNA should be between 1.9 and 2.1. Once concentrations were recorded, each sample was diluted with DI water to reach a final concentration of 100 ng/µL.

2.4 Primers

This study utilized PCR, gel electrophoresis, and quantitative PCR (qPCR). Both PCR and qPCR needed forward and reverse PCR primers. During PCR, the DNA or reverse transcribed RNA is denatured and made into single stranded DNA (ssDNA). During annealing, the ssDNA is cooled, and the forward and reverse primers bind to their complementary sequences on the template DNA. Then, the primers are extended by Taq polymerase synthesizing new strands of DNA. The primers optimized to measure Dermo (DNA) and gene expression of 18S, HSP-70, galectin, anti-apoptosis protein, and sodium hydrogen exchanger protein are shown in Table 1. Primer melting temperatures and amplification efficiency for each gene are also shown.

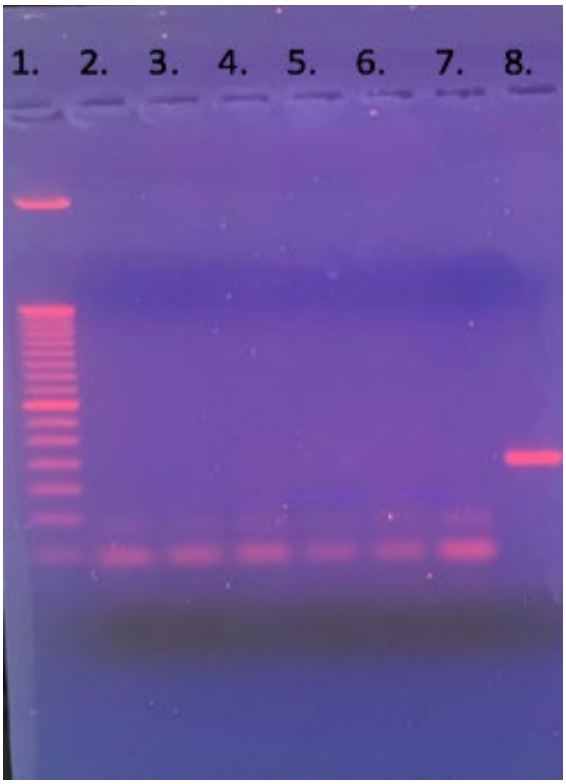
Gene	Accession	Primer Sequence		Product length	Efficiency (%)	Primer Melt Temperature	Reference
18s	NR_145818.1	F	5'-TGGTTCATTCCGATAACGAACGA-3'				
		R	5'-CGCCACTTGTCCCTCTAAGAA-3'				
HSP-70	AJ271444.1	F	5'-CAAGCAGCGTCAGAGGATAGC -3'	65 bp	108.27	61.13 60.54	A
		R	5'-CCTGTTTGACGGTGAAGACGTA-3'				
Galectin	DQ779197.1	F	5'-CACTCTCCGCTGATATGGTGG-3'	155 bp	105.76	64.13 63.87	B
		R	5'-GGCTTGATGTCCAGCGAGT-3'				
Anti-Apoptosis Protein	XM_022459049	F	5'-AAAATGAACCGGCAACTCGC-3' 5'-TTCTGCCATCTGCCGAACTT-3'	129 bp	98.87	60.04 59.96	C
		R					
Sodium Hydrogen Exchanger Protein	XM_022459049.1	F	5'-CTCGCTCTGGATGCTGCTAT-5'	127 bp	84.81	59.68 60.03	D
		R	5'-AAATGGCTATTGCCAGGGCT-3'				
Perkinsus marinus	MK253307.1	F	5'-CGCCTGTGAGTATCTCTCGA-3'	90 bp			
		R	5'-GTTGAAGAGAAGAATCGCGTGAT-3'				

Table 1: Forward and reverse sequences, Genbank accession numbers, product lengths, amplification efficiencies, and melt curve maximum temperatures for the genes 18S, HSP-70, galectin, anti-apoptosis protein, sodium hydrogen exchanger protein, and *Perkinsus marinus* PCR products used in this study. References: A (Rathinam et al. 2000), B (Tasumi & Vasta 2007, Nikapit), C (Jones et al. 2019, Zhao et al. 2012), D (Jones et al. 2019)

2.5 Using PCR to quantify Dermo DNA

After the samples were extracted and DNA quantified, PCR was run on each oyster sample from gill and mantle tissues. A Master-Mix was created to minimize pipetting error throughout the PCR. The Master-Mix contained 25 uL of PCR Master, 5 uL of 10 uL stock forward and reverse primers, and 10 uL of DI water per sample. PCR Master assists in DNA amplification. Only 5 uL of each DNA sample was used in this PCR process. With each PCR experiment, a negative control consisting of water only and a positive control of DNA from Dermo plasmids (Dermo plasmid DNA, kindly provided by Dr. Roxanna Smolowitz, Roger Williams University), and a 18S control was run.

Once PCR was completed on all of the DNA samples, gel electrophoresis was run on each of the samples. The gel contained agarose, 1X TAE Buffer, and ethidium bromide to stain the gel. A ladder was added to the first well of each of the gels to be able to evaluate the presence or absence of Dermo by size. The sample bands were compared to the ladder. The Dermo band size was expected to be 90 base pairs long. Once finished, the gel was visualized on a gel imager or a UV plate. The results of the gels were consistently inconclusive. Positive and negative controls were run on each gel. However, some of the positive controls appeared as negative and the negative as positive, or nothing appeared at all (see Figure 2 for example gel).



1. Ladder
2. Negative Control
3. BMG5
4. SSHS5
5. MMB5
6. JHBSL5
7. Positive Control
8. 18S

Figure 2. The gel above shows an example of a gel result where the negative control appeared as a positive band.

To further evaluate the presence or absence of Dermo, we decided to change course. Instead of using the gels to determine Dermo presence, we made a standard curve from the Dermo plasmid and ran qPCR. Normally, to determine the presence or absence of disease, Ray's fluid thioglycolate medium (RFTM) culture assay is done. In this method, the oyster tissues are stained with iodine to determine Dermo abundance (Audemard et al., 2019). We chose to use the qPCR method over the RFTM method because qPCR is much more specific and can detect infection at much lower levels than RFTM. For qPCR, the Dermo plasmid was diluted to represent different levels of infection on the Mackin scale. The Mackin scale is a way to

determine the intensity of a Dermo infection in an oyster (Mackin et al., 1950). From there, we used the cycle threshold (Ct) values (see 2.8) and melting points of the samples compared to the plasmid standard curve to determine presence or absence of Dermo (Figure 3).

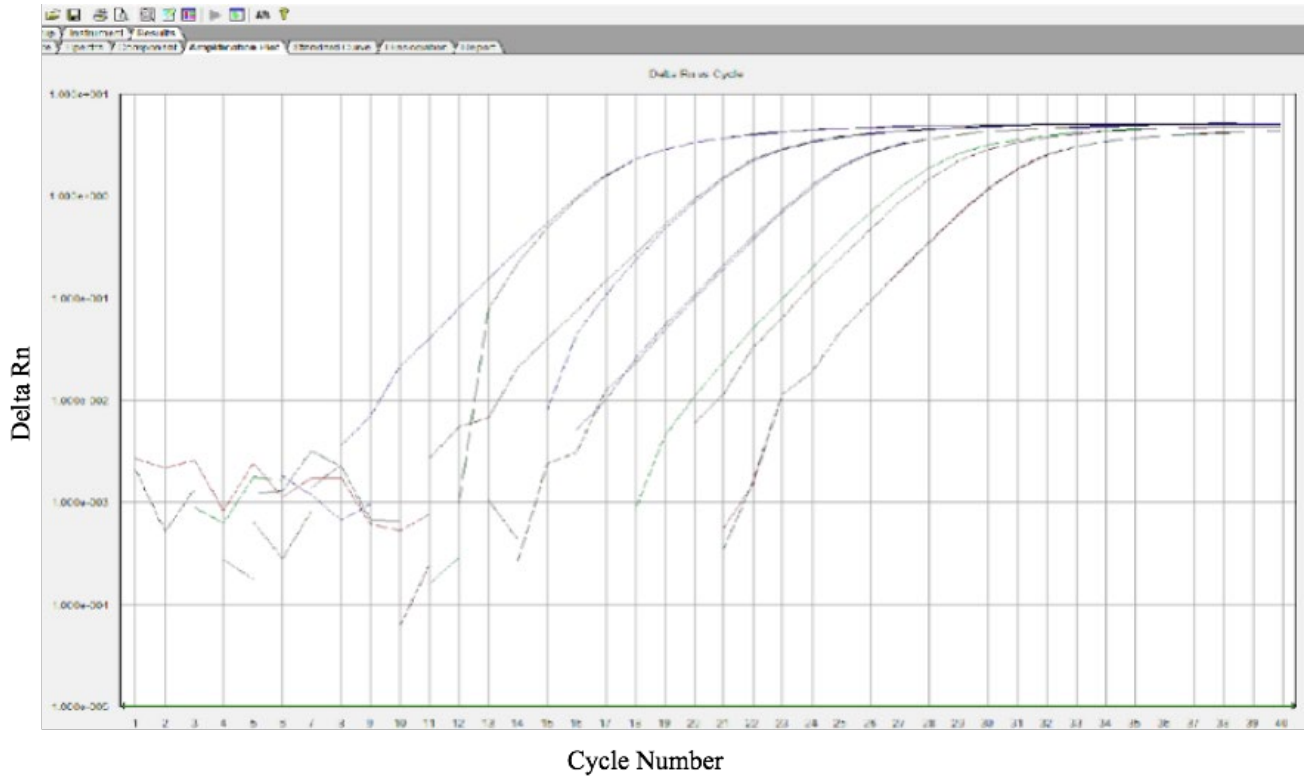


Figure 3. Standard curve of Dermo infection ranging from Mackin scale of infection 1 to 5. 1 is the lowest possible infection (far right line) and a 5 is the most severe infection (far left line).

2.6 Gene expression of stress genes in oysters

RNA isolated from oyster gill and mantle was reverse transcribed to generate cDNA to use in qPCR. A Master-Mix was prepared using RNase free water, 10X RT Buffer, 25 mM $MgCl_2$, dNTP mix, random hexamers, RNase inhibitor, and Multiscribe RT. The Master-Mix was added to 2.5 ul of the RNA samples. The RNA had to be converted to cDNA because

although gene expression measures the amount of RNA, PCR is based on starting with a double stranded template, the cDNA.

2.7 qPCR using SYBR Green

After the RNA was converted to cDNA, qPCR using SYBR Green was run on all of the samples. A Master-Mix was prepared using nuclease free water, SYBR Green, forward primer, and reverse primer. qPCR was used to evaluate gene expression of HSP-70, galectin, anti-apoptosis protein, and sodium hydrogen exchanger protein as well. An 18S control was run with each of the plates to serve as a reference gene. Once all of the samples were run, the data was evaluated according to the Delta Delta Cycle Threshold (ΔΔCT) values and melt temperatures.

2.8 Statistics

Cycle threshold (Ct) values are determined through qPCR. Ct values indicate the cycle number required for fluorescent signals to cross the threshold and exceed background noise. Ct values are inversely proportional to the amount of starting material. The lower the Ct value, the more concentrated the sample was to begin with, and vice versa. Ct values were found for 18S, the housekeeping gene, and the genes of interest. ΔCt was found by subtracting the Ct value of the housekeeping gene (18S) from the Ct value of the gene of interest. ΔCt values show the difference of expression between two genes. ΔΔCt values indicate the difference between the control and the gene of interest. This value can be used to find fold change ($2^{-\Delta\Delta Ct}$). Fold change is useful in determining which genes were differentially expressed. To test significant differences between the sites, one-way ANOVA analysis was run. One-way ANOVA analyzes the means of unrelated groups and provides a p-value. If the p-value of the data is less than 0.05, the data are

significantly different. If the p-value is greater than 0.05, the data are not considered significantly different as compared to each other.

3. RESULTS AND DISCUSSION

Throughout this study, we used a combined approach of condition index, Dermo presence, and gene expression to assess oyster health in oysters collected in December as part of the Mississippi Oyster Gardening Program. Condition index was assessed in a separate study, but at the same sites as the oysters from this study. Dermo presence was initially assessed by gel electrophoresis but confirmed with qPCR. Expression of stress and immune related genes HSP-70, galectin, anti-apoptosis protein, and sodium hydrogen exchanger protein were also measured by qPCR. Differential expression of genes could indicate high environmental stress or infection. These genes were chosen based on previous studies. Anti-apoptosis protein was upregulated in high temperatures while sodium hydrogen exchanger protein was down-regulated in low temperatures (Jones et al., 2019). In oysters, galectin is a participant in inflammation and immunity and is upregulated in response to stress (Tasumi & Vasta, 2007). HSP-70 was upregulated in short term exposure to high stress and downregulated with increased exposure to the stressors (Jones et al., 2019). We used 18S ribosomal RNA as the reference gene because it is uniformly expressed in all conditions and tissues. Based on previous studies, EF1a, another common housekeeping gene, was not effective as it is upregulated in response to low salinities, so we decided to use 18S as our reference gene considering the oysters exposure to different environmental stressors (Jones et al., 2019). Overall, we did not see significant differential expression in the genes of interest because the environmental conditions the oysters were in were

not extreme enough to affect gene expression. Dermo presence was also low due to the environmental conditions, so the genes were not differentially expressed in response to an infection either.

Condition index for the oysters was assessed and is shown in Figure 4. Oyster condition index is determined by the dry meat weight divided by the internal cavity volume (Lawrence, 1982). Low condition index and presence of Dermo can be correlated because Dermo can be more prevalent in stressed oysters (Pace et al., 2020). Based on this condition index, JH Bay St. Louis and TS Pass Christian oysters had significantly lower condition index as compared to oysters collected at three other sites. Oysters from sites with lower condition index were hypothesized to be infected with Dermo.

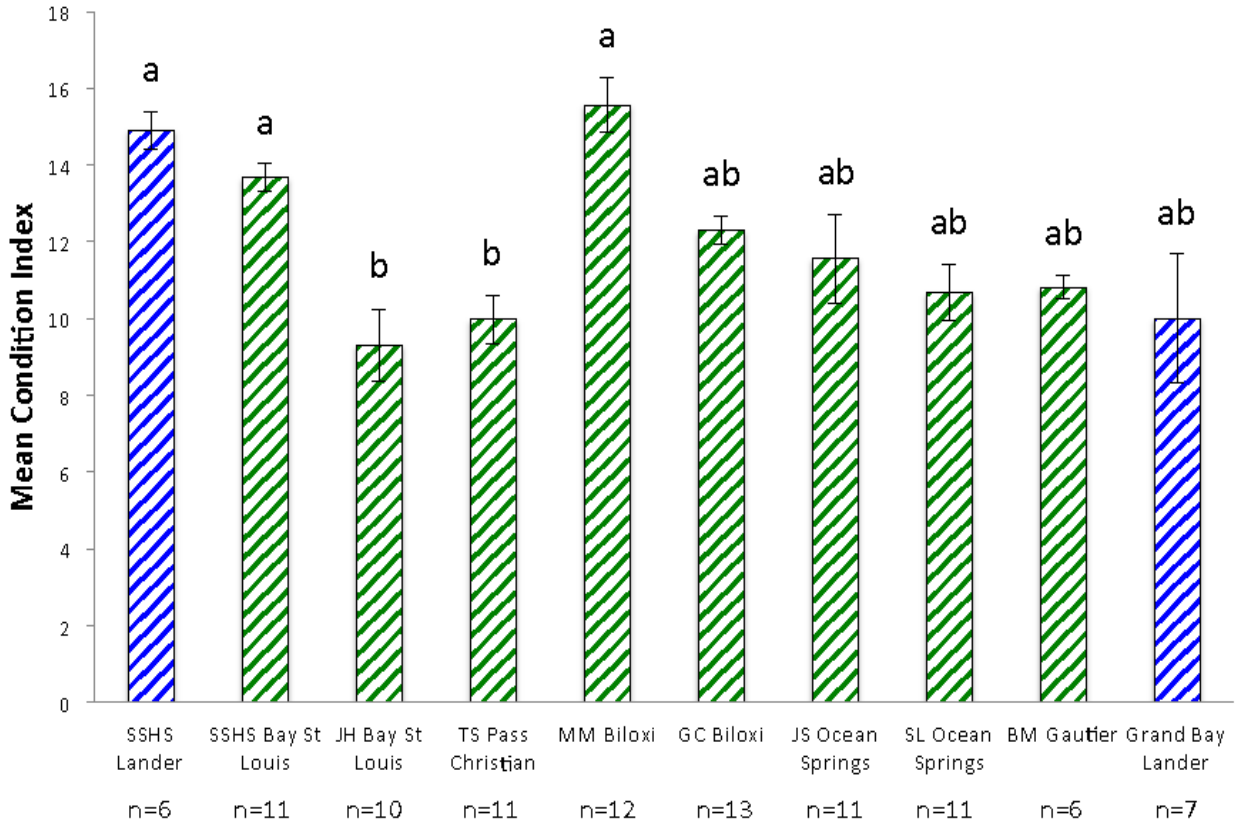


Figure 4. Condition index measured in oysters from each of the oyster gardening sites, including oysters that were deployed as biosensors in landers in Bay St. Louis and Grand Bay. JH Bay St. Louis and TS Pass Christian oysters had the lowest condition index. Different letters above the bars denote means that are significantly different following 1-way ANOVA ($p<0.05$; n = 6 - 13).

Initially, we attempted to measure the presence of Dermo infection in the oysters using gel electrophoresis. However, the gels showed evidence of possible contamination and positive and negative results were inconclusive. Therefore, we decided to use qPCR to quantitate Dermo DNA in the oyster mantle and gill tissues. We decided to run the oyster samples through qPCR because qPCR can detect extremely low levels of Dermo infection, and we had Dermo plasmid DNA available to construct qPCR standard curves (Faveri et al., 2009). We anticipated based on

the environmental conditions unfavorable to Dermo (i.e., low salinity) and the gel electrophoresis results that there would be a very small incidence of infection in the oysters, if any.

Previous studies used positive Dermo samples to construct a Mackin scale of infection to compare future results to (Faveri et al., 2009). The Mackin scale of infection was first created using oysters from coastal Louisiana. Originally it was thought that the mass mortality events of oysters were due to oil and gas pollution. It was then discovered that Dermo was actually causing the mortality events (Soniat 1996). From there, the Mackin scale of infection was created based on percent infection in the oyster population and weighted prevalence of the infection in each oyster. The scale starts at zero indicating no infection and ends at five indicating the most severe and prevalent infection (Mackin et al., 1950; Mackin and Hopkins, 1962). Using provided positive Dermo plasmid controls, we could determine a quantitation standard curve and melting temperature of the 90 base pair PCR product. A melt curve analysis allowed us to distinguish between primer dimers (T_m = range 70.8 -72.9 ^{0}C) and actual presence of Dermo (Figure 5). Samples that contained the Dermo PCR product had a melt temperature of 78^{o}C. Only two of the eighty samples had possible Dermo infection. Both of the samples were from JH Bay St. Louis. Even then, the infection seemed to be on the edge of detection as the infection presence was not supported in both technical replicates. The melting point of the two positive samples (78^{o}C) aligns with the melting point of the positive samples on the standard curve. JH Bay St. Louis oysters did have the lowest condition indices, though, which supports the possible presence of Dermo infection. While a low condition index does not automatically mean the oyster is infected with Dermo, it does indicate that the oyster is under excess stress. Excess stress in the oysters makes it easier for them to be infected with diseases like Dermo.

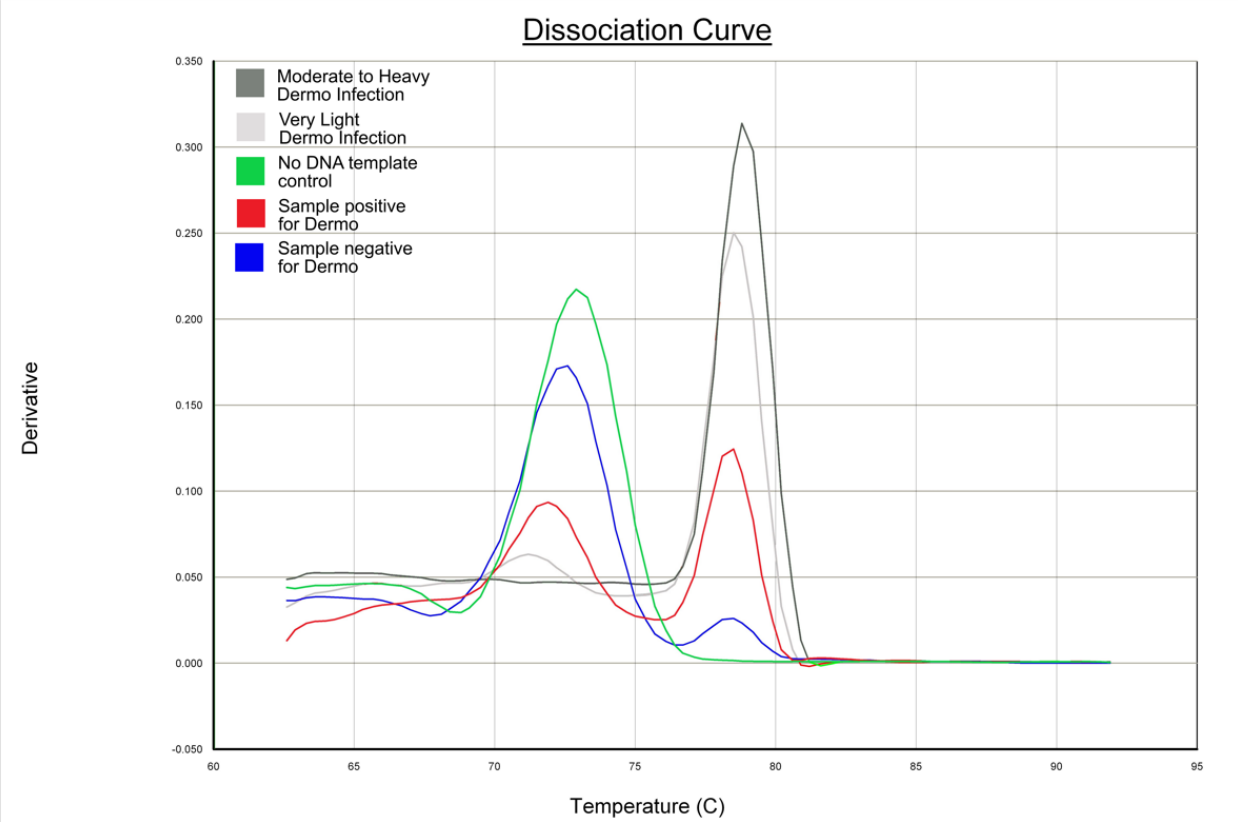


Figure 5. The dissociation curve of Dermo plasmids shows that positive infection appears at 78.1-78.5^{0}C and negative samples appear between 70 and 72^{0}C.

Stress and immune-related gene expression were also measured to assess potential oyster stress. There was significant difference in gene expression in gill and mantle tissue in oysters collected across the sites for HSP-70, galectin, and sodium hydrogen exchanger protein (Figure 6). Ten oysters from each of the eight sites were analyzed totaling 80 oysters. Anti-apoptosis protein showed a significant difference between JS Ocean Springs and SL Ocean Springs, but these results were not consistent with Dermo presence or differences in condition index. The ΔCt values at each site show the expression of the genes compared to a control. JSOS and SLOS

showed significant differences in expression of anti-apoptosis protein. Site JSOS is on a more sheltered bayou than SLOS suggesting possible differences in water quality and stressors, but overall, condition index and expression of the other genes did not indicate extreme stress on the oysters. Condition index was analyzed versus Ct values as well (Figure 7). The graphs indicate there was not significant up or down regulation with the stress and immune related genes compared to condition index. This indicated that the oysters were not under any out of the ordinary stress.

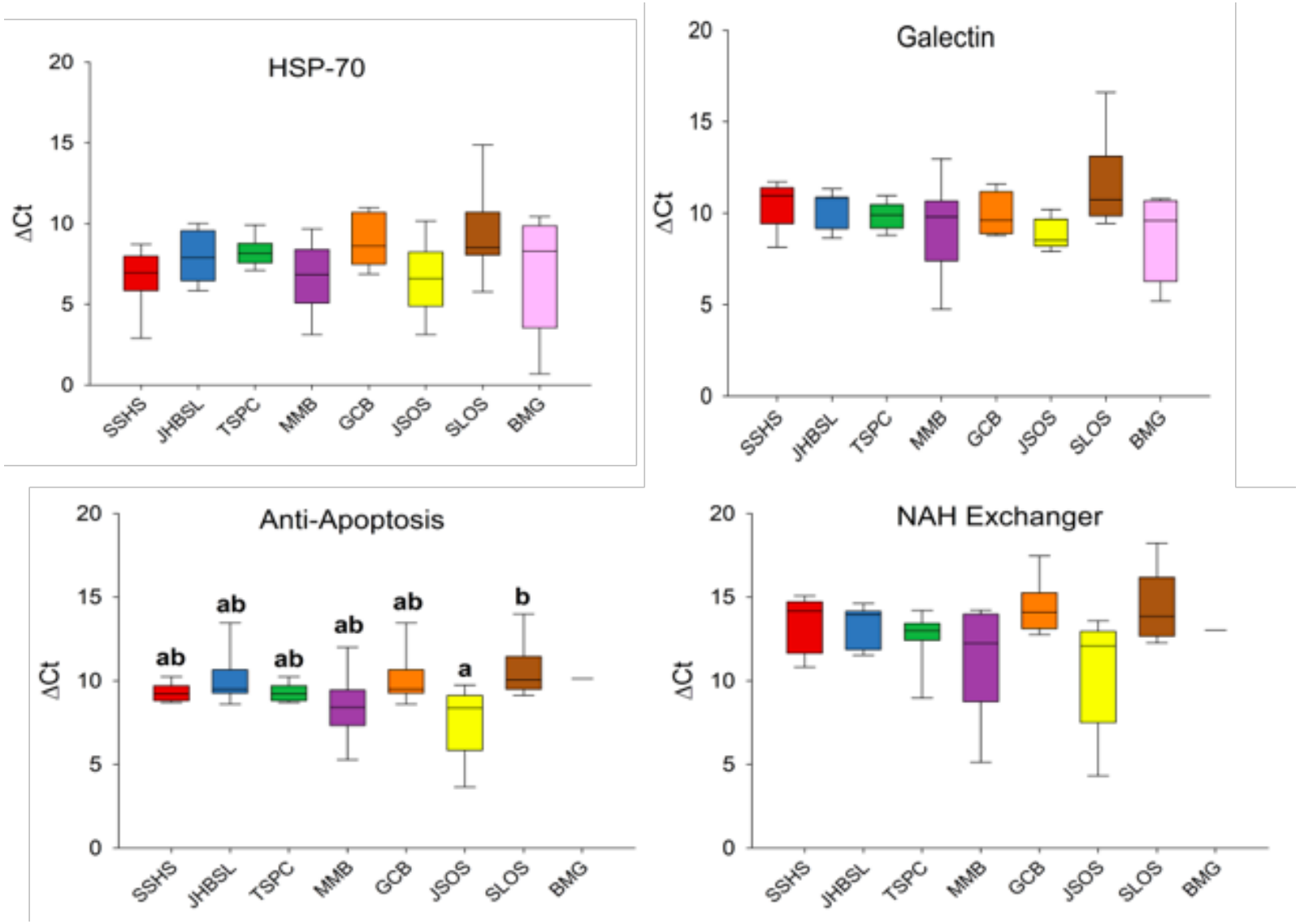


Figure 6. ΔCt values for HSP-70, galectin, anti-apoptosis protein, and sodium hydrogen exchanger protein. Different letters denote means that are significantly different following 1-way ANOVA ($p < 0.05$).

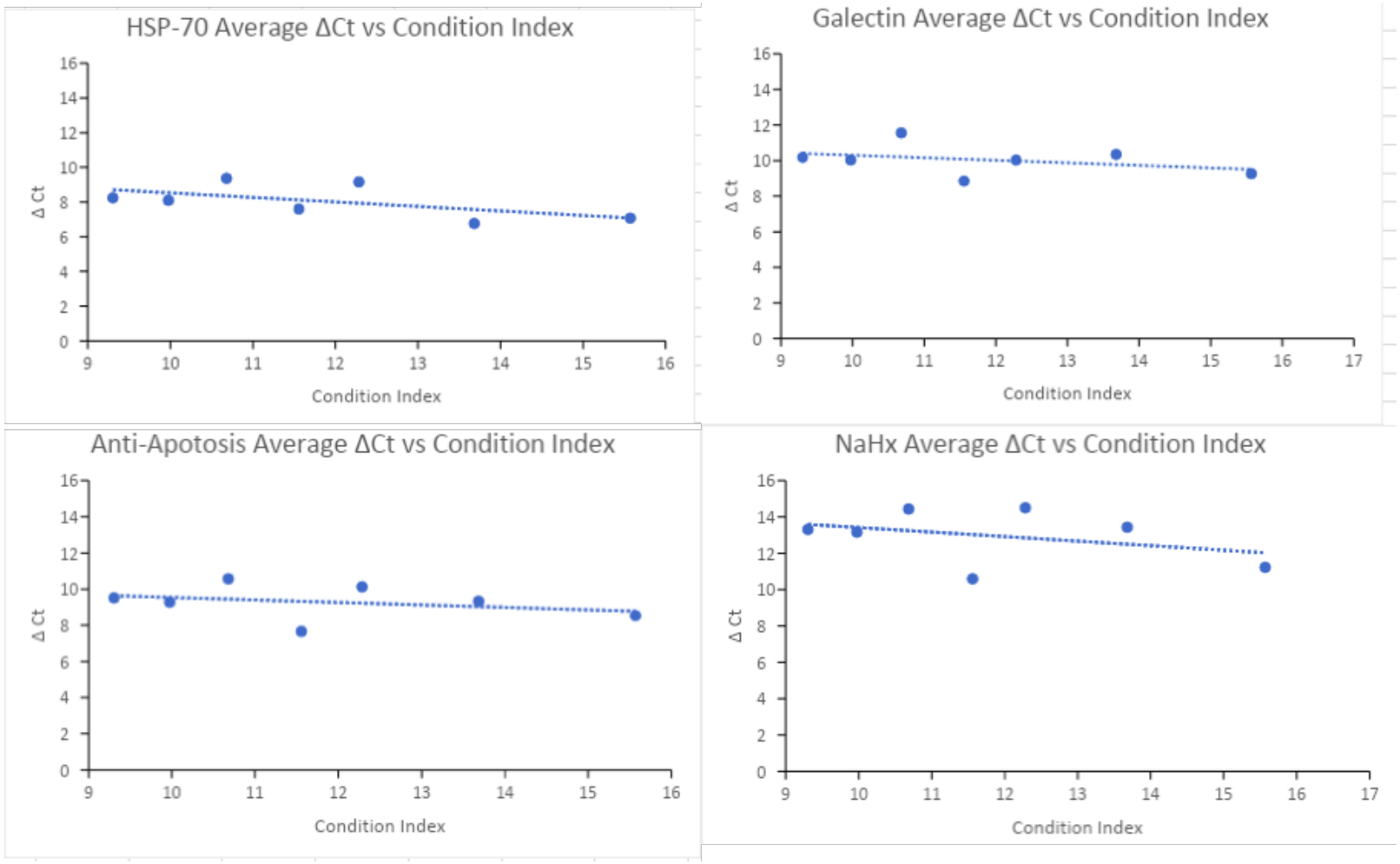


Figure 7. Average Ct values of HSP-70, galectin, anti-apoptosis protein, and sodium hydrogen exchanger protein in relation to condition index.

We found that there was almost no Dermo found in these oysters, which is not surprising. JH Bay St. Louis oysters showed some Dermo infection and had low condition indices. The condition indices and gene expression did not suggest extreme stress, though. Aside from anti-apoptosis protein expression, there were no site-specific differences in oyster stress-related gene expression. The absence of high levels of stress and Dermo infection can be attributed to the fact that these oysters were harvested in the winter. There were mass mortality events in oysters in the Mississippi Sound due to Dermo in the summer months of 2016 and 2019 (Pace et al., 2020). After these mass mortality events, there were obvious changes in the condition index of the oysters. Shell height, shell weight, gonadal thickness, and adductor muscle diameter have been used in the past to determine condition index. After the 2016 mortality event, oyster gonads became thinner and the adductor muscle diameter to shell height ratio suggested a change in the shape of the oyster shell. This condition index analysis after a Dermo mortality event shows how infection can alter the condition index of the oysters. Because the condition indices of the oysters from this study are not concerningly low or out of the ordinary, it can be further determined that these oysters did not suffer Dermo infection (Pace et al., 2020). To better understand how Dermo infection is correlated to warmer temperatures, this study could be done again in the summer months to see if the results would be different. Dermo is also most prevalent in high salinities, but flooding events that occur mostly in the summer months could prevent Dermo from infecting the oysters, but could still cause excess stress on the oyster. Anti-apoptosis protein and sodium hydrogen exchanger protein are upregulated in low salinity conditions. There may be more differential expression in the stress and immune related genes in summer as well as a higher prevalence of Dermo infection. We are now studying the effects of low salinity, pH, and hypoxia on juvenile oysters to see how such stress can impact development.

4. REFERENCES

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