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Factors Affecting Dermo Disease (*Perkinsus marinus*) in Eastern Oysters (*Crassostrea virginica*) in Galveston Bay, Texas

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Abstract: The oyster disease Dermo (*Perkinsus marinus*) affects the viability of oyster reefs of Galveston Bay, Texas. Documenting the relationships between distribution and prevalence of Dermo disease in the eastern oyster (*Crassostrea virginica*) and environmental conditions is beneficial to management of the eastern oyster in Galveston Bay. We sampled four sites located in Galveston Bay every other month from November 2014 through September 2015. The focus of the study was the relationship of water quality parameters (i.e., fresh-water flow, salinity, water temperature, and water turbidity) to prevalence and parasite concentration of Dermo disease in oysters. Dermo was present in oysters at all reefs sampled, and Dermo prevalence was greatest at April Fool and Confederate reefs, but declined after heavy rainfall. Linear regression analysis indicated water variables such as temperature, salinity, turbidity, and fresh water inflow explained different amounts of the variability in the Mackin Dermo Intensity Scale among sampled reefs. We found combinations of low fresh-water inflow, high salinity, and high temperatures accounted for majority of the variance of Dermo in oysters located in Galveston Bay.

Key words: Dermo disease, eastern oyster, prevalence and parasite, water quality parameters.

1. Introduction

Oyster disease is a reoccurring problem affecting the viability of oyster reefs of Galveston Bay, Texas. Two pathogens, *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo disease) which are spore forming protozoan parasites have caused massive die-offs in populations of the eastern oyster in Galveston Bay [1]. Little information is known about the life cycle of MSX, but the major life stage is a multinucleated plasmodium which infects the oyster tissue [1]. Dermo disease, previously identified as, *Dermocystidium marinum*, caused by *Perkinsus marinus* which has three life stages, can cause infection in oysters [2, 3] and eventually the oyster's death. Oyster death is caused by Dermo spores which grow within oyster tissue and eventually lyses it [1]. Dermo disease is transmitted from an infected oyster to surrounding oysters when decomposing tissue from dead oysters releases spores into the water column [4]. Despite a potentially fatal disease to oyster populations, Dermo is harmless to humans [5].

The earliest known incidence of Dermo in oysters was reported at the 1893 Chicago World's Fair in oysters shipped from Louisiana. Tissues from some of these oysters had been stored and preserved by New Orleans' Cabildo Museum, where examined, and parasitic spores were found [1]. Dermo was later described by Mackin [6] based on examination of infected oysters from Gulf States [1]. Activity of Dermo increases at high salinities (>10 to 12 ppt [1]). This usually occurs due to reduced rainfall or freshwater discharge from coastal rivers that ultimately lead to an increase in salinity, which triggers a rise in Dermo disease prevalence and intensity, producing increased oyster mortality [7].

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Temperature also is a key factor affecting the prevalence of the disease, because pathogen growth is halted below 20 °C [8] and magnified above 20 °C. A decrease in fresh-water inflow during warmer months can lead to combined increased salinity and temperature potentially leading to an increase in Dermo activity [9, 10].

Dr. Sammy Ray of Texas A&M University—Galveston along with his former Ph.D. student, Dr. Thomas Soniat, studied the relationship between water temperature and salinity to the prevalence of Dermo in oysters from the Gulf Coast during the 1990s through the early 2000s. From 1998 until his death in 2011, Dr. Ray continued to monitor the prevalence of Dermo disease in oysters from Galveston Bay using the modified RFTM (Ray's fluid thioglycollate method [6, 11]). This method involves the examination of oyster tissue that has been stained using Lugol's solution. The monitoring results are provided on oystersentinel.org, a web based database which lists the results of pathogen monitoring using the eastern oyster to monitor the health of oyster reeds along the Gulf of Mexico. The web site provides historical temperature and salinity readings, as well as Dermo prevalence in market and under market sized oysters [12].

2. Objectives

To better understand the effects and relationships between environmental variables and Dermo disease on the eastern oyster population in Galveston Bay, we collected oysters from 5 sites within Galveston Bay to determine the prevalence of Dermo disease bimonthly from November 2014 to September 2015. Specific objectives of our study were to determine: (1) prevalence of Dermo disease in oysters, (2) spatial location of Dermo infected oysters, (3) concentrations of the Dermo within infected oysters, and (4) effects of water quality (i.e., fresh-water flow, salinity, water temperature, and water turbidity) on prevalence of Dermo disease in oysters in Galveston Bay.

3. Study Area

All reefs sampled were located in north of Galveston Island and Bolivar Peninsula (Fig. 1). These reefs were chosen because each of them represents a different section of the estuary where oysters are normally produced including the northwest (April Fool Reef), southwest (Confederate Reef), northeast (Fishers Reef), and southeast (Frenchy's Reef, alternate: Hannah's Reef) in Galveston Bay. We collected oysters at four study sites (April Fool Reef [29.476666, -94.914322], Fishers Reef [29.658300, -94.838800], Frenchy's Reef [29.527800, -94.606900], replaced later with Hannah's Reef [29.478459, -94.726181], and Confederate Reef [29.263208, -94.917583]). Researchers and technicians from Dr. George Guillen's lab (Environmental Institute of Houston, University of Houston at Clear Lake) provided boats and crew to access sample sites. All samples were collected under Dr. Guillen's permit (SPR-0504-383) from the TPWD (Texas Parks and Wildlife Department).

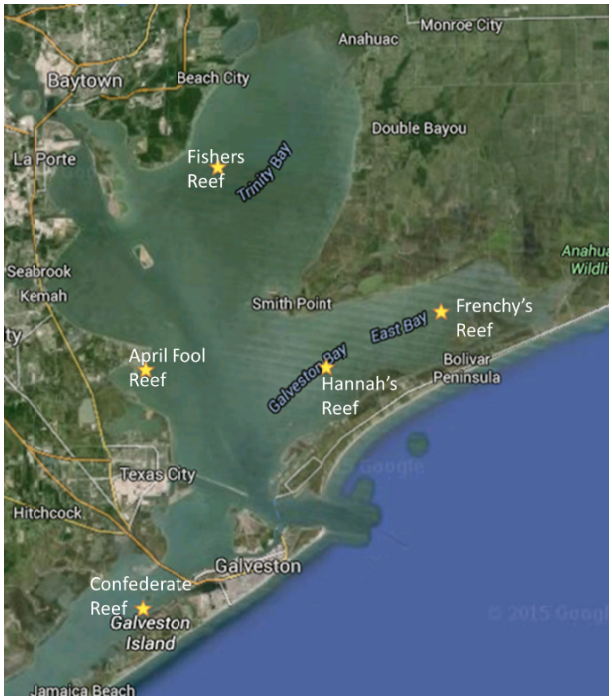

Fig. 1 Locations of April Fool Reef, Fishers Reef, Frenchy's Reef, replaced later with Hannah's Reef, and Confederate Reef where oysters were collected.

3.1 April Fool Reef

Located in the south of the city of San Leon, Texas, April Fool Reef is approximately a five-minute boat ride from the city. It was accessed from the boat ramp at the Topwater Grill in San Leon. It was chosen due to its proximity to the Houston Ship Channel and the possible effects of boat traffic and turbidity to the reef. April Fool Reef was sampled six times bimonthly from November 2014 to September 2015. It is characterized as an "alongshore reef" [13]. It was perhaps historically a part of the chain of reefs known as Redfish Reef, which culminated in Redfish Island and became divided into smaller reefs as a result of dredging [13]. Historical Dermo data, including temperature, salinity, and Dermo prevalence are available for this reef from 1998 to 2011 (http://www.oystersentinel.org). Prior to this study, oysters were collected and processed by Dr. Sammy Ray (Professor, Texas A&M University-Galveston) from this site. Historical salinities have ranged from 2.0 ppt (June 2001) to 32.0 ppt (October 1999). Water temperatures recorded at the reef have ranged from 9.8 °C (January 2003) to 32.8 °C (August 1999). This reef has a history of Dermo infection in market sized oysters which peaked during November 1999 with a prevalence of 2.87 (http://www.oystersentinel.org).

3.2 Fishers Reef

Fishers Reef is closest to the mouth of the Trinity River and the Houston Ship Channel and selected because of its proximity to a source of fresh-water inflow. It was accessed within 15 min by boat from Thompson's Boat Ramp and Marina in Baytown, Texas and sampled six times bimonthly from November 2014 to September 2015. Fishers Reef is characterized as a transverse ridge reef [10]. Dermo data, including temperature, salinity, and Dermo prevalence are available on this reef from 1998 to 2011 (http://www.oystersentinel.org). Historical salinities have ranged from 0.2 ppt (July 2007) to 32.7 ppt (September 2011), and water temperatures have ranged from 7.6 °C (January 2010) to 32.8 °C (August 2003; oystersentinel.org). It has consistently shown Dermo prevalence levels under 1.0 (Mackin Dermo Intensity Scale, hereafter Mackin Scale [10]) since 1998, with the only exception in September 2011 when it was 3.53 (oystersentinel.org).

3.3 Confederate Reef

Confederate Reef is located in west Galveston Bay and was accessed by a public boat ramp at the end of 8-mile road in Galveston, Texas. It was selected because it is a tidal reef, submerged at high tide and exposed at low tide. Confederate Reef was sampled six times bimonthly from November 2014 to September 2015. Dermo data, including temperature, salinity, and Dermo prevalence are available on this reef from 1998 to 2011 (http://www.oystersentinel.org). Historical salinities have ranged from 8.7 ppt (June 2015) to 42.0 ppt (August 2009). Temperatures have ranged from 6.0 °C (January 2010) to 36.0 °C (August 2006). Confederate Reef has shown high levels of Dermo prevalence consistently from 2008 until present with levels of Dermo prevalence above 0.33 (Mackin Scale) until June of this year. It reached its peak Dermo prevalence of 3.03 (Mackin Scale) in August 2010.

3.4 Frenchy's Reef

Frenchy's Reef has been a commercially harvested oyster reef since at least 1966 [14]. It was chosen because it is a public reef, and susceptible to the pressures of commercial fishing, unlike the other reefs sampled. It is located in the north of the Bolivar Peninsula (oystersentinel.org). It was accessed from the Stingaree Restaurant Boat Ramp and sampled only four times bimonthly from November 2014 to May 2015, at which time it was replaced with an alternate reef (Hannah's Reef) after dredging efforts at Frenchy's Reef yielded no live oysters. Frenchy's Reef was approximately a 15-minute boat ride from the boat ramp. It was part of a $3.8 million reef restoration effort in 2011, in which 53,519 m^3 of

cultch (oyster shell and river rock) spread over 72 ha of public reef [15]. Water temperature, salinity and Dermo prevalence data are available on this reef from 1998 to 2011 (http://www.oystersentinel.org). Historical salinities have ranged from 2.1 ppt (October 2002) to 28.0 ppt (March 2000). Water temperatures have ranged from 8.1 °C (January 2003) to 31.3 °C (August 2003). Dermo prevalence levels have never reached above 1.96 (Mackin Scale) except in June 2011, when it was 2.06 (oystersentinel.org).

3.5 Hannah's Reef

Hannah's Reef was selected as the alternative site to Frenchy's Reef (commercially harvestable reef, see above) and because of its close proximity to Frenchy's Reef in Galveston Bay. Hannah's Reef was chosen as an alternate because it is closed to commercial harvest and oysters were presumed to be more readily collected. It was sampled twice, once in each June 2015 and September 2015. Water temperature, salinity, and Dermo prevalence data are available on this reef from 1998 through our collections from November 2014 to September 2015 (oystersentinel.org). Historical salinities have ranged from 4.0 ppt (November 2002) to 30.0 ppt (March 2000). Water temperatures have ranged from 8.1 °C (January 2010) to 31.1 °C (August 2010). Dermo prevalence reached its peak at Hannah's Reef in September 2010 with a prevalence level of 2.87 (Mackin Scale; oystersentinel.org).

4. Field Methods

4.1 Oyster Collection

Boats used included a 6.7 m Twin Vee with a 2012 (130 hp) Evinrude E-tec motor, and a 6.7 m JH Performance with a 2009 (150 hp) Yamaha (4 Stroke) motor. We sampled each site every other month starting in November 2014 and ending in September 2015.

A 30 × 30-mm mesh size oyster dredge (provided by Dr. Thomas Soniat, University of New Orleans; Fig. 2) was pulled behind a boat for three to ten minutes in slow circles and repeated three to eight times as necessary to collect a total of 20 market-sized and smaller oysters. If a reef was accessible by wading, more than 20 oysters were collected by hand. Oysters were placed on ice in a cooler for up to 24 h until the samples were processed.

Location of sample sites was determined using a boat-mounted Hummingbird 1158C model GPS (Global Positioning System). Salinity (0.1 ppt) and water temperature (0.1 °C) were measured using an YSI pro plus meter (Yellow Springs Instruments Yellow Springs, Ohio) one foot below the surface. At

Fig. 2 Oyster dredge used to collect oysters from oyster reefs in Galveston Bay.

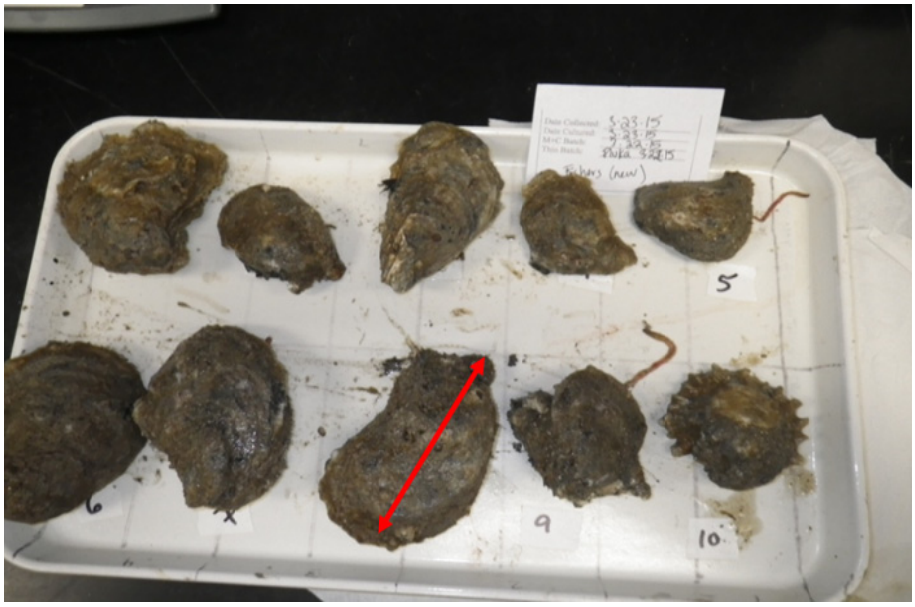

Fig. 3 Red line shows measurement with calipers taken from hinge of oyster shell to beak.

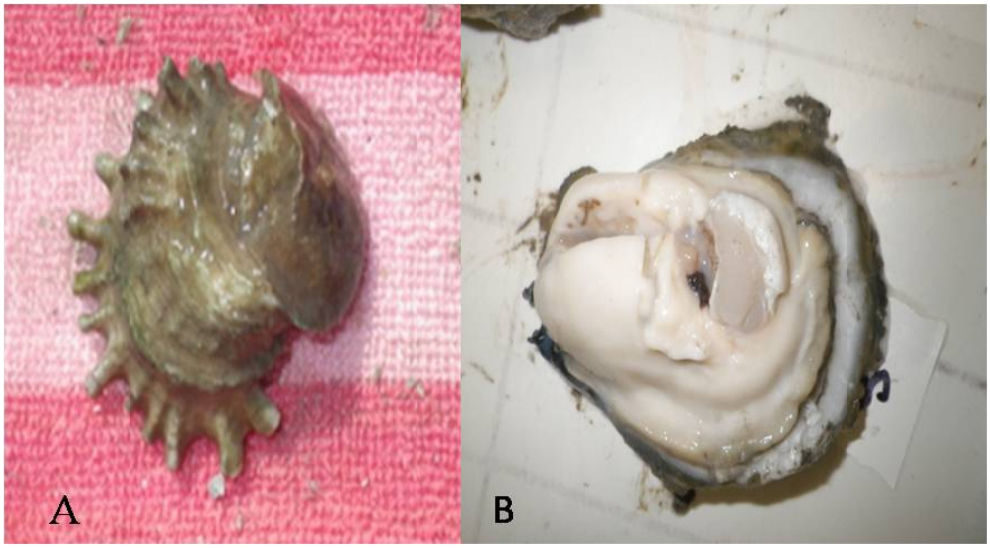

Fig. 4 Oysters with sharp beak (A) and with smooth shell with plump meat (B).

each site, water quality data were collected during each oyster collection. Turbidity was recorded using a Secchi tube (0.1 mm).

4.2 Oyster Sample Processing

Once collected, oysters were taken to Dr. George Guillen's lab in Clear Lake, Texas for processing. Oysters were numbered from 1-20 for each site separately. Each numbered oyster shell was measured (35) from hinge to beak with calipers (0.1 mm). Data recoded for each oyster included the following: date of collection, date of processing, bill condition, length of shell (Fig. 3) of each oyster, and if shell was either sharp to the touch at the edge of the beak an indicator of new growth (Fig. 4A) of the oyster shell was dull and smooth to the touch an indicator no new growth (Fig. 4B). Each oyster was shucked using an oyster knife and gloves. The oyster meat was left in the cupped half shell and meat condition was recorded (as either shrunken (small, shrunken, dehydrated appearance) or plump (round, lush, creamy); Fig. 4B). These meat conditions were based on descriptions from Ray's [16] methods.

5. Lab Methods

To detect Dermo in the oysters, we first had to prepare a thioglycollate culture medium, antibiotics, and a Lugol's working solution. This was done in Dr. George Guillen's lab in Clear Lake, Texas.

5.1 Preparation of Thioglycollate (Thio) Medium

Ray [17] developed the thioglycollate culture method for detecting *Dermocystidium marinum* in oyster tissue. This culture technique enlarges Dermo hypnospores so that they may be easily visible under a microscope. Using this method, we prepared the Thio medium by adding 20 g NaCl to 1 L of DI (deionized) water. We then added 29.0 g of thioglycollate to the NaCl-DI water solution and heated it on a low temperature hot plate, mixing it with a glass stirring rod by hand until all solids were dissolved. We dispensed 10 mL of this mixture with a pipette into 40 (25 mL) screw cap culture tubes. Caps were left loose on the tops of the tubes, which were then placed into test tubes racks (40 tubes each). Tubes were then autoclaved at 15 psi for 15 min. After the tubes cooled, the screw caps were tightened, each tube was labeled with date and time of Thio medium creation, and the tubes were then stored in the dark at room temperature until needed. Excess Thio, approximately 60 mL, was kept in a beaker and refrigerated for up to 30 days, to use if needed for additional tubes.

5.2 Preparation of Antibiotics

Later, 9 mL of DI water was added to a 5-million-unit vial of Stock Nystatin (Sigma N6261) and shook by hand. The reconstituted mixture was allotted equally (2.5 mL) into each of 4 vials. These were labeled with the date and Nystatin Stock 1, 2, 3, or 4 and frozen (up to 365 days) until needed. To prepare the Chloromycetin/Nystatin working solution, we first added 4.5 mL of DI water to a 1 g vial of chloromycetin (Sigma C3738, Chloramphenicol Succinate Sodium Salt) and shook it by hand to re-constitute it. The chloromycetin solution was then added to the Nystatin Stock vial along with 17.5 mL of DI water. This mix was labeled as Chloromycetin/Nystatin working solution with date prepared and then refrigerated up to 365 days until needed. This mixture of antibiotics was necessary to prevent tissue degradation.

5.3 Preparation of Lugol's Working Solution

To prepare Lugols working solution, we added 40 mL of distilled or DI water to 10 mL of 1 N Iodine Stock solution. Iodine and Lugol's working solution were kept at room temperature in a dark cabinet until needed. Lugol's working solution serves to be used as a stain for the tissue samples.

5.4 Oyster Tissue Processing

Just before oyster tissue was added, we removed the working solution of Chloromycetin/Nystatin from the refrigerator and shook it to re-suspend the mixture. We then added 0.05 mL of the Chloromycetin/Nystatin working mixture to each Thio tube and inverted the tube to mix the solutions together. From each oyster (Fig. 5A), we removed a 5-mm^2 piece of anterior mantle using a scalpel and tweezers, added it to the tube of Thio-Chloromycetin/Nystatin mixture, and labeled the tube to identify the reef and number of the oyster from which tissue was taken. Tubes to which tissue was added were stored in the dark at room temperature for a week. Then a 1-mm^2 sub-sample of the tissue in the tube was placed on a slide, masticated using tweezers, and 1-2 drops of Lugols iodine solution were applied to the tissue and blended well using the tweezers (Fig. 5B). Each slide was given an identification number corresponding to its oyster and then placed in a pan (Fig. 6). We then placed a cover slip on each slide and examined the tissue under magnification (4×) using a light microscope. A Dermo prevalence rating based on the Mackin Dermo Intensity Scale [6] as modified by Craig, et al. [18] was recorded for each slide.

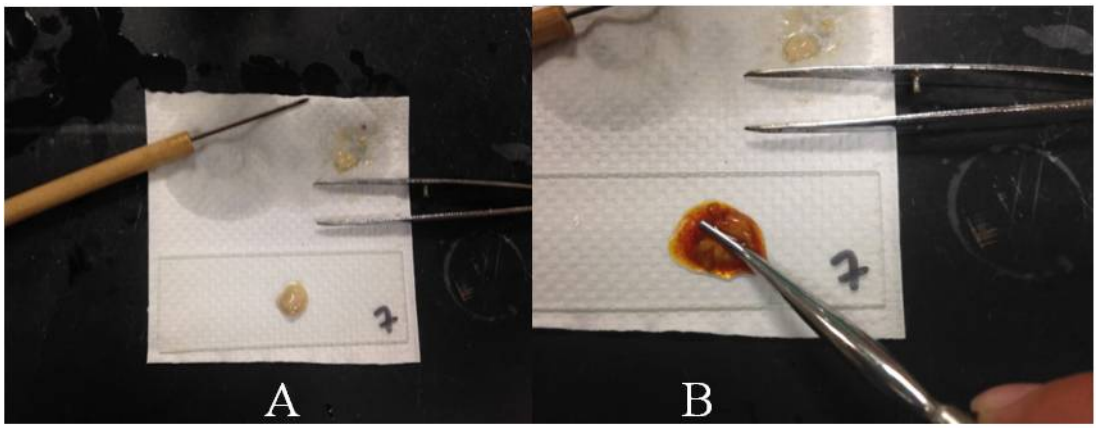

Fig. 5 A 5-mm^2 piece of anterior mantle removed from an oyster placed on a glass slide (A) and tweezers used to blend Lugol's iodine solution into a 1-mm^2 sample of the oyster tissue (B).

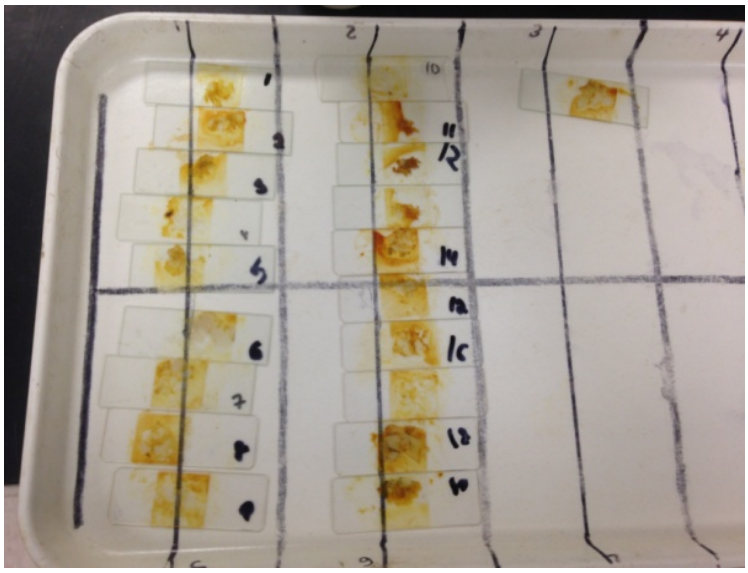

Fig. 6 Oyster tissue slides in pan numbered by oyster from which they were obtained.

5.5 Mackin Dermo Intensity Scale

The Mackin Scale values (Table 1): 0 = no observable hypnospores; 1 = slight infection of tissue with hypnospores; 3 = moderate infection of tissues with hypnospores; 5 = heavily infected tissue [6]. These prevalence ratings, along with temperature and salinity data collected at the field site were uploaded to oystersentinel.org.

6. Statistical Analysis

We used a best subsets regression analysis (MiniTab 17.0; State College, Pennsylvania, USA) to determine which individual or combination of the water-quality variables (fresh-water flow, water temperature, salinity, and turbidity) best accounted for the variation in the Mackin Dermo Intensity Scale values we obtained for our four study reefs. We also assumed fresh-water flow may have had an effect on the other 3 water variables. To illustrate these relationships, we used the scatterplot feature of "Graph" in MiniTab with a regression line.

Because there was a potential delayed effect of fresh-water flow affecting values for the Mackin Dermo Intensity Scale measurements we obtained, data on fresh water flow were used (Trinity River gage readings at Romayor, Texas located 82.5 km north of Galveston Bay) for 2 months prior to our

Table 1 Scale of infection intensity for Dermo (*Perkinsus marinus)* (adapted from Mackin [6] by Craig, et al. [18]).

Letter designation	Infection intensity	Numerical valuea	Description
N	Negative	0.00	No hypnospores present
VL	Very light	0.33	1-10 hypnospores
L-		0.67	11-74 hypnospores
L	Light	1.00	75-125 hypnospores
L+		1.33	>125 hypnospores but much less than 25% of tissue is hypnospores
LM-		1.67	<25% of tissue is hypnospores
LM	Light/moderate	2.00	25% of tissue is hypnospores
LM+		2.33	>25% but much less than 50% of tissue is hypnospores
M-		2.67	>25%, but <50% of tissue is hypnospores
M	Moderate	3.00	50% of tissue is hypnospores
M+		3.33	>50%, but much less than 75% of tissue is hypnospores
MH-		3.67	>50%, but <75% of tissue is hypnospores
MH	Moderately heavy	4.00	75% of tissue is hypnospores
MH+		4.33	>75%, but much less than 100% of tissue is hypnospores
H-	Heavy	4.67	>75% of tissue is hypnospores, but some oyster tissue is still visible
H		5.00	Nearly 100% of tissue is hypnospores

[a]The Mackin Scale values: 0 = no observable hypnospores; 1 = slight infection of tissue with hypnospores; 3 = moderate infection of tissues with hypnospores; 5 = heavily infected tissue.

collections. The Romayor, Texas gage was the closest gage located on the Trinity River to Galveston Bay. We then used these fresh-water flow values as a variable in our best subset regression analyses. For example, fresh-water flow in meters for the month of September 2014 was regressed with the mean Mackin Dermo Intensity Scale measurements that we recorded in November 2014.

7. Results

7.1 Oyster Collection

At April Fool Reef, during each sampling period, 20 or more oysters were dredged from the reef. Therefore, 20 of the largest oysters were kept for analysis. Oysters were generally market-sized (76 mm) or above and clumped together with barnacles found on the outside of their shells.

At Fishers Reef, during each sample period at least 10 oysters were dredged from the reef. During the first two sampling trips, oysters were pulled from a mud and silt bottom, and were large and solitary. During the November 2014 collection, a commercial oyster boat was seen harvesting from the reef. During the last four sampling trips, live oysters were collected easily (only one to three passes with the dredge). The last two sampling trips brought upwards of 30 oysters in the dredge, but all the oysters were dead (Fig. 7). High mortality at this site can possibly be attributed to large fresh-water inflows starting in May 2015 (Fig. 8).

At Confederate Reef, 20 oysters were collected by hand while wading. There were numerous shore birds observed at this reef, as well as sport fish such as trout (*Cynoscion nebulosus*) and red drum (*Sciaenops ocellatus*).

Frenchy's Reef was sampled four times from November 2014 to May 2015. Oyster boats (Fig. 9) were observed dredging oysters at the site during November 2014 on the first sampling trip. During subsequent sampling trips, it became increasingly hard to find oysters. During May 2015, dredging yielded only six oysters, and these were attached to a piece of debris. Several dredge pulls resulted in the bringing up of debris such as shingles, glass, and plastic, and spat sized oysters. Because of the low yield of live dredged

Fig. 7 A dead oyster found at Fishers Reef during July 2015 collection trip.

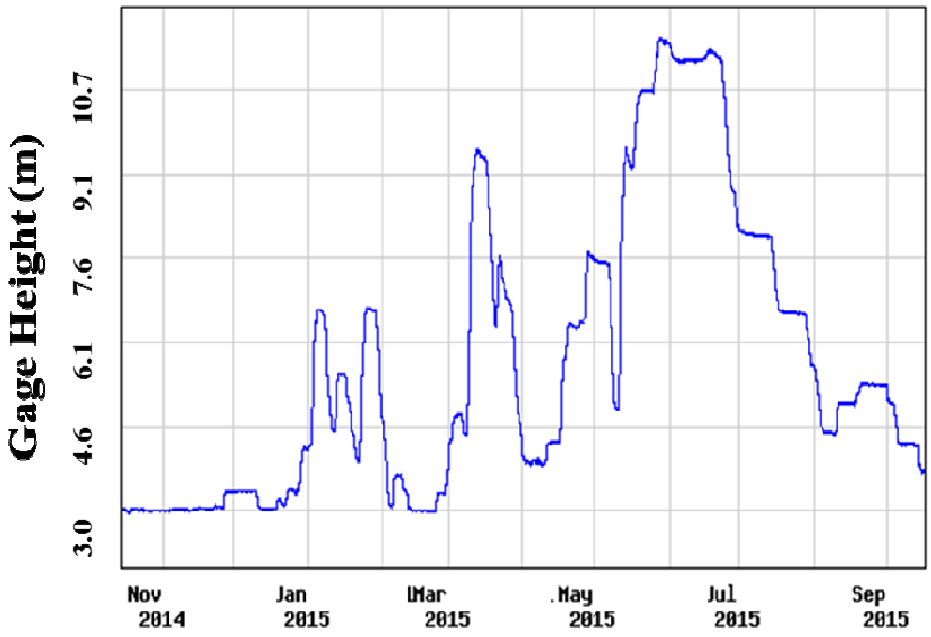

Fig. 8 Height (m) of the Trinity River gage at Romayor, Texas from November 2014 to September 2015.

oysters, oysters from this area of Galveston Bay were substituted with oysters dredged at the alternative site, Hannah's Reef for the remaining sample dates (July 2015 and September 2015).

Hannah's Reef was sampled once each in July 2015 and September 2015. It is situated between two private oyster leases that were identified by white PVC (Polyvinyl Chloride) pipes and black flags. Twenty oysters were relatively easy to harvest (one to two pulls with the dredge). Several recreational fishing boats were observed during each sampling trip.

7.2 Dermo Prevalence

During the first sample trip in November 2014, oysters were collected and analyzed for Dermo from the original four sites in Galveston Bay. April Fool Reef exhibited an average Dermo prevalence of 0.55 on the Mackin Scale (Table 2; Fig. 10). This means there was an average of between 1-74 hypnospores in the cultured tissue sub-sample. Oysters collected at Confederate Reef had an average Dermo prevalence of 0.85 on the Mackin Scale which was an average of 11-125 hypnospores in the tissue samples collected.

Fig. 9 Commercial oyster boats at Frenchy's Reef, in November 2014.

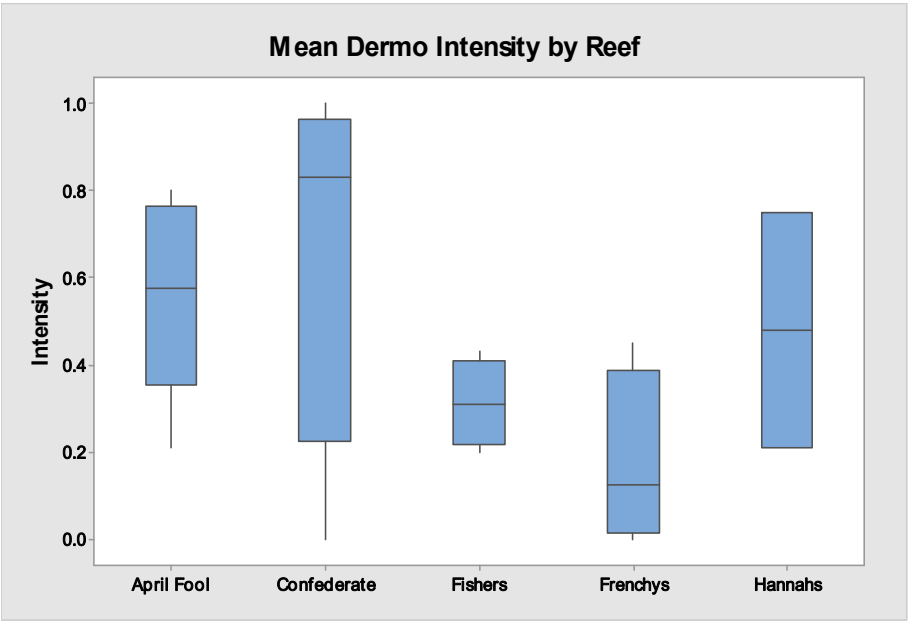

Fig. 10 Mean Dermo intensity by reef.

Table 2 Mean intensity of Dermo in oyster collected from November 2014 to September 2015 at five reefs (April Fool, Confederate, Fishers, Frenchy's, and Hannah's) in Galveston Bay, Texas.

Date	April Fool	Confederate	Fishers	Frenchy's	Hannah's
November 2014	0.55	0.85	0.35	0.05	N/A
January 2015	0.75	0.30	0.27	0.20	N/A
March 2015	0.80	0.95	0.20	0.00	N/A
May 2015	0.60	0.00	0.43	0.45	N/A
July 2015	0.21	0.81	All dead	N/A	0.21
September 2015	0.40	1.00	All dead	N/A	0.75

N/A refers to sample trip where reef substitution was necessary.

Fishers Reef showed an average Dermo prevalence of 0.35 on the Mackin Scale for an average between 1-74 hypnospores in the tissues sampled. Frenchy's Reef averaged 0-10 hypnospores for a Dermo prevalence of 0.05 on the Mackin Scale.

During the second sample trip in January 2015, oysters were collected and analyzed from the same four sites in Galveston Bay (Table 2). April Fool Reef had an average Dermo prevalence of 0.75 on the Mackin Scale with an average of between 11-125

hypnospores in the tissue samples collected. Confederate Reef showed an average Dermo prevalence of 0.30 on the Mackin Scale with an average of 0-10 hypnospores in the collected tissue samples. Fishers Reef had an average Dermo prevalence of 0.27 on the Mackin Scale. The oysters collected had an average of between 0-10 hypnospores present in their tissues. Frenchy's Reef had an average Dermo prevalence of 0.20 on the Mackin Scale with an average of 0-10 hypnospores present in the tissue samples collected.

During March 2015, oysters were again collected and analyzed from the same four sites in Galveston Bay (Table 2). April Fool Reef had an average Dermo prevalence of 0.80 on the Mackin Scale with an average of between 11-125 hypnospores in the tissue samples collected. Confederate Reef had an average Dermo prevalence of 0.95 on the Mackin Scale. Oysters collected at Confederate Reef had an average of 11-125 hypnospores in the tissue samples collected. Fishers Reef had an average Dermo prevalence of 0.20 on the Mackin Scale with an average of between 0-10 hypnospores present in the collected tissue samples. Frenchy's Reef had an average Dermo prevalence of 0.0 on the Mackin Scale. There was an average of 0 hypnospores present in the tissue samples collected.

During May 2015, oysters again were collected and analyzed from four sites in Galveston Bay (Table 2). April Fool Reef showed an average Dermo prevalence of 0.60 on the Mackin Scale with an average of between 1-74 hypnospores found in the tissue samples collected. Confederate Reef had an average Dermo prevalence of 0.0 on the Mackin Scale which means there was an average of 0 hypnospores found in the tissue samples collected. Fishers Reef showed an average Dermo prevalence of 0.43 on the Mackin Scale with an average of between 1-74 hypnospores found in the collected tissue samples. Frenchy's Reef had an average Dermo prevalence of 0.45 on the Mackin Scale, meaning there was an average of 1-74 hypnospores present in the tissue samples collected.

During the fifth sample trip in July 2015, oysters were collected and analyzed from four sites in Galveston Bay (Table 2). April Fool Reef had an average Dermo prevalence of 0.21 on the Mackin Scale meaning there was an average of between 0-10 hypnospores found in the tissue samples collected. Confederate Reef had an average Dermo prevalence of 0.81 on the Mackin Scale with an average of 11-125 hypnospores found in the tissue samples collected. All oysters collected at Fishers Reef were dead and therefore no tissue was available. Frenchy's Reef was not sampled during July 2015 because of the inability to dredge oysters from this area of Galveston Bay; therefore, oysters were dredged at Hannah's Reef for the subsequent sample dates (July 2015 and September 2015). Hannah's Reef had an average Dermo prevalence of 0.21 on the Mackin Scale. There was an average of between 0-10 hypnospores found in the tissue samples collected at Hannah's Reef.

During the sixth and final sampling trip in September 2015, oysters were collected and analyzed from four sites in Galveston Bay (Table 2). April Fool Reef showed an average Dermo prevalence of 0.40 on the Mackin Scale with an average of between 1-74 hypnospores found in the tissue samples collected. Oysters collected at Confederate Reef had an average Dermo prevalence of 1.00 on the Mackin Scale meaning there was an average of 75-125 hypnospores found in the tissue samples collected (Fig. 11). Again at Fishers Reef all oysters collected were dead and therefore no tissue was available. Also, Frenchy's Reef was not used as a sample site during this trip and Hannah's Reef had an average Dermo prevalence of 0.75 on the Mackin Scale indicating there was an average of between 11-125 hypnospores found in the tissue samples collected.

7.3 Water Temperatures

During our sampling period, water temperatures (Table 3) at April Fool Reef ranged from 12.1 °C

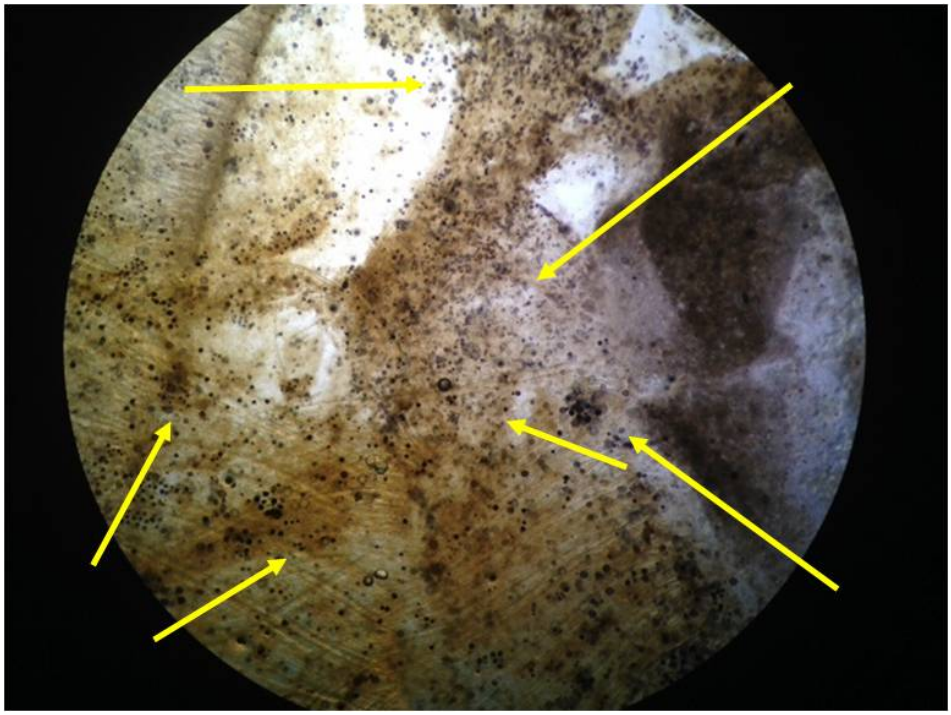

Fig. 11 Dermo spores found in an oyster at Confederate Reef from the September 2015 sample.

Table 3 Water temperature (°C), salinity, and turbidity by date of oyster collection at each site.

Date	Site	Temp.	Salinity	Turbidity
November 2014	Frenchy's	18.87	19.58	0.350
January 2015	Frenchy's	15.30	11.15	0.623
Mach 2015	Frenchy's	20.80	12.00	0.000
May 2015	Frenchy's	27.30	4.64	0.140
July 2015	Hannah's	30.50	3.81	0.137
September 2015	Hannah's	27.70	14.34	0.420
November 2014	Fishers	10.20	19.94	0.732
January 2015	Fishers	13.60	7.80	0.460
March 2015	Fishers	19.50	10.00	0.474
May 2015	Fishers	23.00	4.32	0.100
July 2015	Fishers	31.30	0.45	0.126
September 2015	Fishers	8.54	28.70	0.660
November 2014	Confederate	18.94	27.49	0.450
January 2015	Confederate	18.40	24.50	0.586
March 2015	Confederate	22.10	18.42	0.203
May 2015	Confederate	29.70	8.73	0.160
July 2015	Confederate	32.20	29.89	0.231
September 2015	Confederate	28.00	22.74	0.460
November 2014	April Fool	12.10	20.99	0.866
January 2015	April Fool	13.00	15.99	0.720
March 2015	April Fool	20.10	10.00	0.468
May 2015	April Fool	23.80	8.43	0.150
July 2015	April Fool	32.00	10.78	0.304
September 2015	April Fool	13.15	29.60	0.480

(November 2014) to 23.8 °C (May 2015). The average water temperature at April Fool Reef was 19.0 °C. Water temperatures at Confederate Reef ranged from 18.4 °C (February 2015) to 32.2 °C (July 2015) with an average water temperature of 24.8 °C during the sampling period. At Fishers Reef, water temperatures ranged from 10.2 °C (November 2014) to 23.0 °C (May 2015) during the sampling period. The average water temperature at Fishers Reef was 13.6 °C. At Frenchy's Reef, water temperatures ranged from 15.3 °C (February 2015) to 27.3 °C (June 2015). The average water temperature at Frenchy's Reef was 20.5 °C. Water temperatures ranged from 27.7 °C (September 2015) to 30.5 °C (July 2015) at Hannah's Reef with an average water temperature of 29.1 °C for the 2 months sampled.

The overall average water temperatures were lowest at Fishers Reef (13.6 °C) followed by April Fool Reef at 19.0 °C with Confederate Reef having the highest average water temperature (24.8 °C). Fishers Reef was closest to the Trinity River, whereas Confederate Reef was the furthest from the Trinity River.

7.4 Water Salinities

At April Fool Reef, salinities (Table 3) ranged from 8.4 ppt (May 2015) to 20.9 ppt (November 2014). The average salinity at April Fool Reef was 15.97 ppt. Salinities at Fishers Reef ranged from 4.3 ppt (May 2015) to 19.9 ppt (November 2014). The average salinity at Fishers Reef was 11.87 ppt. Confederate Reef had salinities that ranged from 8.7 ppt (June 2015) to 29.9 ppt (July 2015). The average salinity at Confederate Reef was 21.96 ppt. Salinities at Frenchy's Reef ranged from 4.6 ppt (June 2015) to 19.2 ppt (November 2014). The average salinity at Frenchy's Reef was 11.83 ppt. Salinities ranged from 3.8 ppt (July 2015) to 14.3 ppt (September 2015) at Hannah's Reef during the sampling period. The average salinity at Hannah's Reef was 9.08 ppt.

For those reefs having salinities recorded for all 6 sampling periods, Fishers Reef had the lowest average salinity at 11.87 ppt followed by April Fool Reef at 15.97 ppt. Confederate Reef had an average salinity of 31.96 ppt. As with water flow, Fishers Reef was closest to the Trinity River and Confederate Reef was furthest from the Trinity River.

7.5 Water Turbidity

Water turbidity (Table 3) at April Fool Reef ranged from 0.150 to 0.866 m. The average turbidity of April Fool Reef was 0.498. Turbidity at Confederate Reef ranged from 0.160 to 0.586 m. Confederate Reef had an average turbidity of 0.348. Turbidity at Fishers Reef ranged from 0.100 to 0.732 m. The average turbidity at Fishers Reef was 0.425. Turbidity at Frenchy's and Hannah's reefs ranged from 0.000 to 0.632 m. Average turbidity at Frenchy's Reef was 0.278 m and 0.279 m at Hannah's Reef.

For those reefs having water turbidity readings for all 6 sampling periods, Confederate Reef had the lowest average turbidity (0.348 m) with April Fool Reef and Fishers Reef having the highest turbidity readings (0.498 m and 0.425 m, respectively). Confederate Reef was furthest from the Trinity River where water flow probably did not increase average turbidity readings as it did at April Fool and Fishers reefs as they were closest to the Trinity River.

7.6 Relationships between Variables Collected

Best subsets regressions indicated which water variables explained differing amounts of the variability in the Mackin Dermo Intensity Scale for the reefs sampled (Table 4). For April Fool Reef, the water flow gage at Romyor, Texas explained 61.8% (adjusted R-square) of the variability in the Mackin Scale (Table 4). The three water variables of temperature, turbidity, and the water flow gage explained 92.0% of the variability in the Mackin Scale results for April Fool Reef (Fig. 12). For Confederate Reef (Fig. 13), salinity explained 20.6% of the variability in the Mackin Scale. If all water variables were included, 72.4% of the variability was explained.

Table 4 Best subset regression values (adjusted *R*-square [*R*-sq (adj)]) for Salinity (Sal), temperature (Temp), gage height (Flow) and turbidity (Turb).

Reef	No. Variables	*R*-sq (adj)	Sal.	Temp.	Flow	Turb.
	1	61.8			X[a]	
	1	4.5		X		
	2	83.0	X	X		
April Fool	2	65.4			X	X
	3	92.0		X	X	X
	3	88.3	X		X	X
	4	84.2	X	X	X	X
	1	46.9			X	
	1	13.2		X		
	2	55.7		X	X	
Frenchy's/Hannah's	2	53.3			X	X
	3	36.5	X	X	X	
	3	33.7		X	X	X
	4	0.0	X	X	X	X
	1	20.6	X			
	1	0.0				X
	2	3.3	X			X
Confederate	2	0.0	X		X	
	3	0.0		X	X	X
	3	0.0	X	X		X
	4	74.2	X	X	X	X
	1	44.5			X	
Fishers	1	0.0		X		
	2	95.9		X	X	

[a]X indicates this variable was use in this run of the best subset regression.

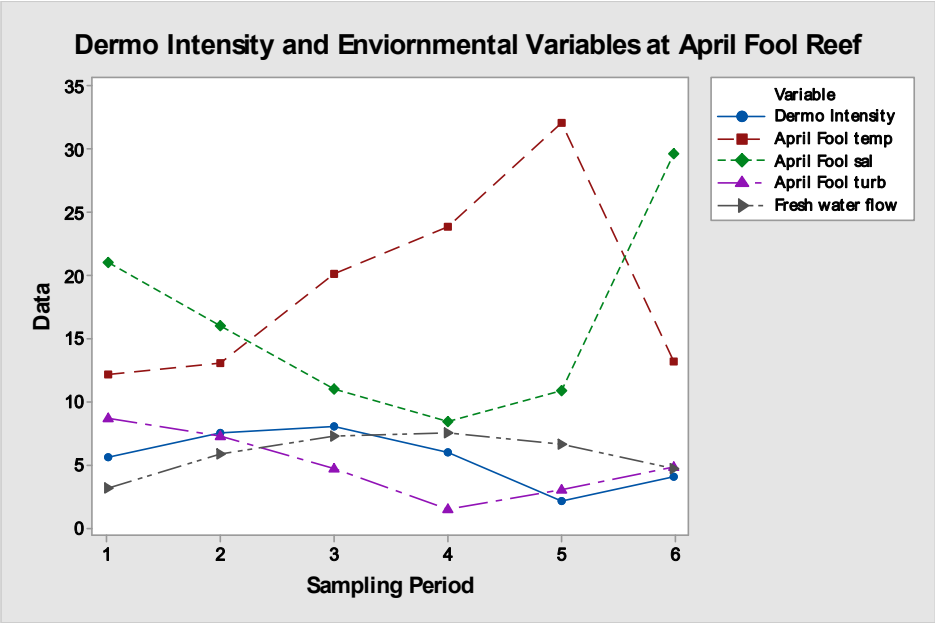

Fig. 12 Graph of Dermo intensity and environmental variables at April Fool Reef.

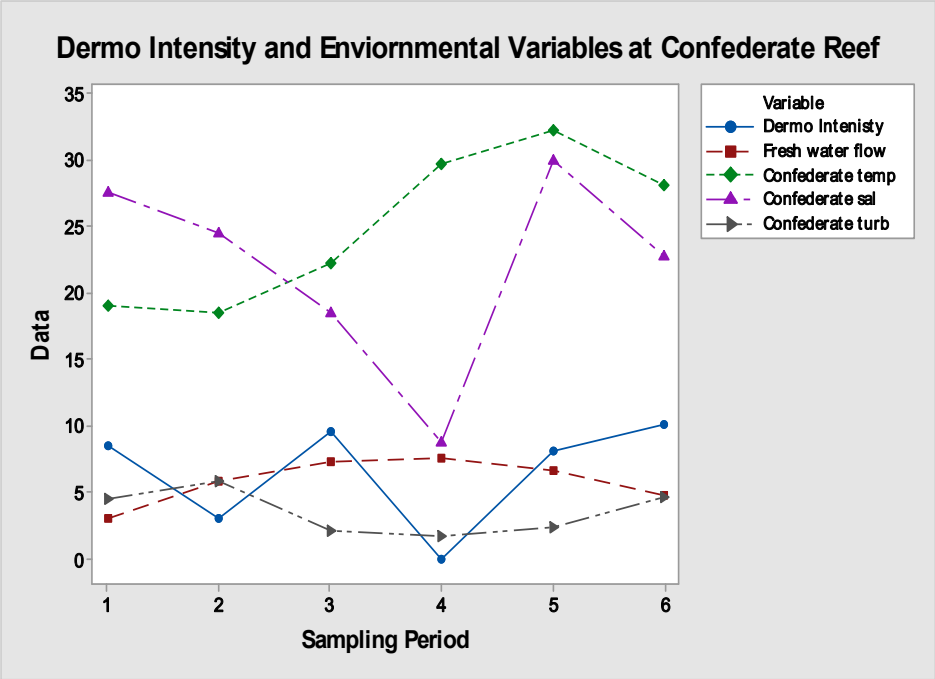

Fig. 13 Graph of Dermo intensity and environmental variables at Confederate Reef.

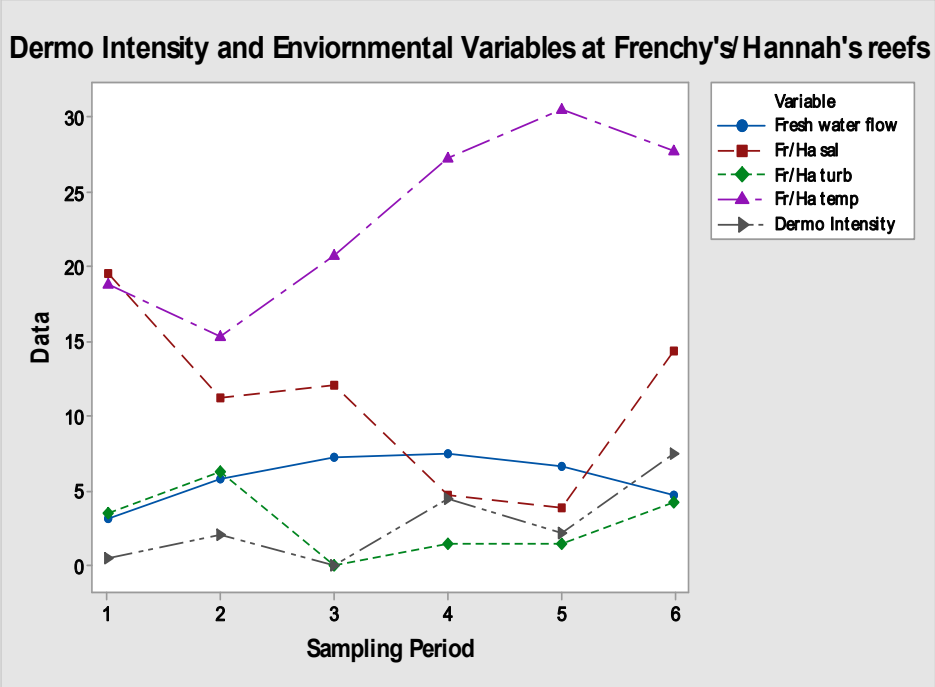

Fig. 14 Graph of Dermo intensity and environmental variables at Frenchy's and Hannah's reefs.

For Frenchy's and Hannah's reefs, water flow explained 46.9% of the variability in the Mackin Scale (Table 4). Adding water temperature to the regression only increased the explained variability to 55.7% (Fig. 14).

Because all oysters were dead for the July and September 2005 samples at Fishers Reef, we used a best subset regression using only the November 2014 and January, March and May 2005 water variables and Mackin Scale data (Table 4). Water flow accounted for 44.5% of the variability in the Mackin Scale data (Fig. 15).

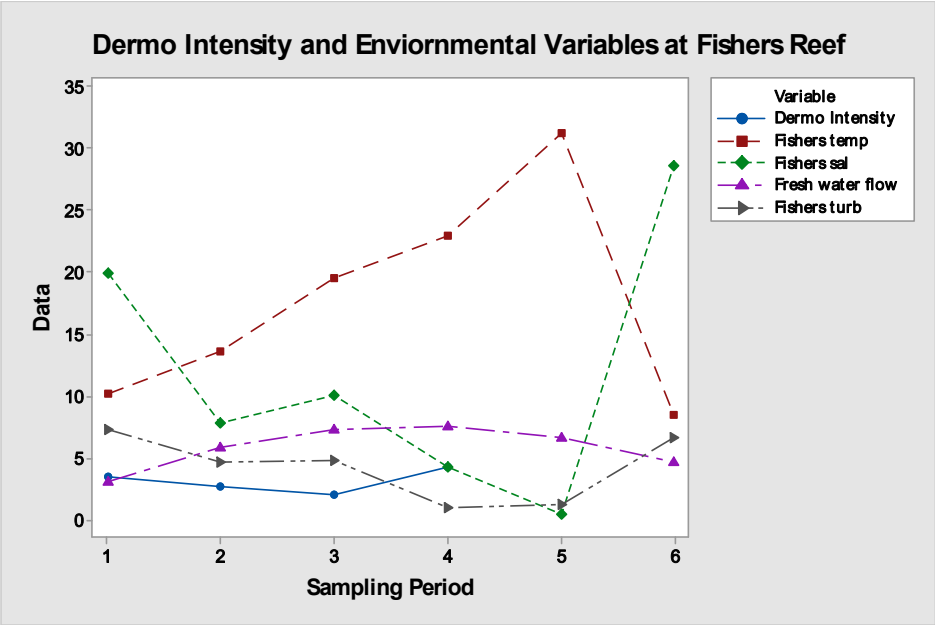

Fig. 15 Graph of Dermo intensity and environmental variables at Fishers Reef.

8. Disscussion

8.1 Fresh-Water Inflows

The results of our study revealed that oyster reefs exposed to high fresh-water inflows had lower occurrences and intensities of Dermo infection. Reefs exposed to normal fresh-water inflows on a regular basis have lower levels of Dermo [5]. However, reefs exposed to fresh-water inflows for extended periods of time (> 48 h), such as Frenchy's Reef, exhibited complete mortality. Therefore, fresh-water inflow exhibited the highest association and influence on disease prevalence for this reef. Water flow at April Fool Reef also attributed a majority of the variability (61.8%) in Dermo prevalence and intensities. Trinity River discharge, water temperature, and turbidity explained 92.0% of the Dermo intensities at April Fool Reef. The high percentages of variability accounted for by water flow contributed to the close proximity of both Frenchy's and April Fool Reef to the inflows from the Trinity River. For Frenchy's and Hannah's reefs, water flow explained 46.9% of the variability in the Mackin Scale. Adding temperature to the regression only increased the explained variability to 55.7%. Since both of these reefs are located in parts of the bay that are blocked from direct inflows from the Trinity River by peninsulas, the influence of water flow at these two reefs was minimized. Culbertson [9] also found that in the two oyster reefs she studied high to moderate amounts of dead oysters. She related this to heavy fresh-water inflows and low salinities for extended periods of time (> 48 h).

8.2 Water Salinity

Our results revealed that at high salinities, when combined with other variables such as extreme temperature, turbidity, and water flow, oysters sampled had a higher prevalence of Dermo. When combined, salinity accounted for higher levels of Dermo at Confederate Reef. At Confederate Reef, only salinity (20.6%) explained any of the variability in the Mackin Scale. However, when salinity, temperature, water flow, and turbidity were combined they accounted for 72.4% of the variability. Dermo is a warm water pathogen that spreads rapidly and can inundate oysters at temperatures above 25 °C [19]. Prevalence and intensity of Dermo have been found to positively correlate with salinity [6, 20, 21]. Lower

Dermo prevalence is often found in conjunction with lower salinities and high Dermo prevalence is often related to increased salinities above 25 ppt [5]. In New England, where the disease is prevalent, activity of Dermo is primarily regulated by temperature [19].

8.3 Water Temperature

Our results found as water temperature increased the prevalence of Dermo increased. For Frenchy's and Hannah's reefs, water flow explained 46.9% of the variability in the Mackin Scale scores. When water temperature was added to the regression model it increased the explained variability to 55.7%. Both of these reefs are located close to shore and protected on at least one side by Bolivar Peninsula, this could potentially decrease water flow and raise temperatures. Dermo is said to vary on a seasonal scale, with higher Dermo intensities being found in warmer months and lower intensities found in cooler months [5]. Quigg, et al. [5] also found that at temperatures lower than 25 °C there were lower Dermo intensities, and at temperatures greater than 25 °C Dermo intensities were higher. In contrast, Cook, et al. [22] found that in a short term study in Delaware Bay the regression plots showed a slight increasing trend, but neither slope was statistically different from zero. Further, Ewart and Ford [9] declared that temperature was never a limited factor for the Gulf of Mexico.

8.4 Water Turbidity

Turbidity as a variable by itself was unimportant at all reefs in explaining the variability in the Mackin Scale intensity scores. High turbidity levels can lower amounts of dissolved oxygen and cause higher water temperatures [23]. This probably explained when water flow, temperature, and salinity were combined, 72.4% of the variability in the Mackin Scale scores was explained at Confederate Reef. There is little information known about the direct effects of turbidity on the Dermo. Oysters are said to grow best when suspended solids are in low concentrations. Sediment increase in the water column can smother larval oysters and disturb their filtration process, which can make then vulnerable to disease [24-26].

9. Summary and Conclusions

The eastern oyster (*Crassostrea virginica*) is an economically and ecologically important shellfish throughout its range, especially to the Gulf Coast of Texas. It faces a myriad of threats from abiotic and biotic sources. When oyster tissue was collected and analyzed for the presence and prevalence of Dermo disease, salinity, temperature, turbidity and fresh-water inflow, or combinations thereof, were found to affect Dermo prevalence and disease intensities.

Based on our study, the following conclusions were drawn:

(1) High salinities are associated with a higher occurrence and intensity of Dermo in oyster tissue.

(2) Higher amounts of fresh-water inflow were associated with Dermo disease intensity in Galveston Bay.

(3) However, extreme fresh-water inflow killed oysters at Fishers Reef.

(4) There is a 2-month lag time in Dermo disease reduction after heavy fresh-water inflow events in Galveston Bay.

(5) The intensities and prevalence of Dermo disease in Galveston Bay increased as water temperature approached high levels (> 28 °C).

Based on the results of our 12-month study, we conclude that low fresh-water inflow, high salinity, and high temperatures can create conditions conducive to an increase in the occurrence and prevalence of Dermo in oysters located in Galveston Bay. We also conclude that high fresh-water inflows for a sustained period of time can cause oyster mortality. Further, it can be concluded that low salinities and low temperatures lead to a decreased occurrence and prevalence of Dermo.

Additional research and/or longer-termed studies of the effects of salinities, temperature, fresh-water

inflow, and turbidity would be beneficial to support the conclusions of our study. With further observation and testing, RFTM can be used to provide fisheries management agencies with a solid knowledge of the effects of temperature, salinity, turbidity, and fresh-water inflow on Dermo prevalence, and could be important to preventing oyster mortality, and sustaining a healthy and economically valuable population of oysters in Galveston Bay.

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