A COMPARISON OF THE EFFECTS OF PETROLEUM SUBSTANCES ON THE SETTLEMENT OF THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*

A Thesis

by

KAREN SUE ALSEPT

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF MARINE RESOURCES MANAGEMENT

August 2012

Major Subject: Marine Resources Management

A Comparison of the Effects of Petroleum Substances on the Settlement of the Eastern Oyster, *Crassostrea virginica*

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Approved by:

Chair of Committee,	Thomas L. Linton
Committee Members,	Sammy Ray
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ABSTRACT

A Comparison of the Effects of Petroleum Substances on the Settlement of the Eastern Oyster, *Crassostrea virginica*. (August 2012)

Karen Sue Alsept, B.S., Old Dominion University

Chair of Advisory Committee: Dr. Thomas Linton

In Galveston Bay, Texas, the eastern oyster, *Crassostrea virginica*, is found throughout the bay both intertidal along mudflats and subtidal where their self-built reefs extend vertically deeper. The eastern oyster is an important ecological and economical resource and as such has led to studies regarding their community structure to permit effective creation of artificially built reefs and restoration of existing ones. The presence of the oil and gas industry coupled with increased oyster mortality led to investigations to determine the effects of petroleum substances on the setting, growth, and mortality of the eastern oyster. Many of those studies indicated increased settlement and increased growth of oysters on substrate coated with oil. A field conducted experiment was used to assess the settlement of oyster larvae on cleaned oyster shells coated with two different types of petroleum substances (mineral oil and motor oil), comparing viscosities, in a shallow bayou in Galveston, Texas, where the eastern oyster dominates the intertidal zone. Oyster shells were used as cultch material and divided into three groups; a non-treated control group, mineral oil treated group, and a motor oil treated group. Nekton assemblages, distributions of the ivory barnacle, *Balanus eberneus*, and

Dermo disease infection were assessed. Settlement of oyster larvae occurred in all three groups with no significant difference of preference; algae and sediment present on the shells coupled with the presence of predators most likely caused reduced numbers of spat settlement. Species richness was equal among the groups but varied in evenness of individual species.

ACKNOWLEDGEMENTS

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I would also like to thank my friends and colleagues of our Graduate Researcher to Researcher Group who sacrificed their time to ensure that I succeed and the Marine Sciences and Marine Biology departments' faculty and staff for answering my many questions.

Finally, I would like to thank my friends, Speedy and Loraine, for the use of their house and dock to conduct my experiment.

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CHAPTER I

INTRODUCTION

Estuaries and ecological services

Estuaries support a diverse marine community by encompassing many different habitats, each contributing to the overall productivity. Common habitat types include seagrass beds, intertidal marshes and mud flats, depending on the geographical location. Oftentimes, intermixed in these communities are oyster reef communities that play an important role in estuarine systems because of the number of ecosystem services they alone provide (Stunz et al., 2010; Yeager and Layman 2011).

The eastern oyster, *Crassostrea virginica* (Gmelin 1791), is abundant along the coast of the Gulf of Mexico, from Florida to Texas, and is an ecologically important species providing food and refuge for many marine invertebrates and fish by building dynamic reef habitats (Mann et al., 2009; Soniat et al., 2004; Stunz et al., 2010). Oysters provide filtering services that cleans the water by reducing organic matter in the water column when feeding (Tamburri et al., 2008). They can pump an estimated 2 gallons of water an hour, improving water quality in estuarine environments. For these reasons, oysters are considered a keystone species of the communities in which they reside (Barnes et al., 2010; Knights and Walters, 2010; Smith and Hackney, 1989).

Eastern oysters thrive in a proper mixture of fresh water and salt water and respond to seasonal and weather events that change water characteristics,

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This thesis follows the style of Marine Environmental Research.

specifically, variation in salinity and temperature. Although other abiotic factors affect oysters, these two parameters often lead to predictions regarding *C. virginica* life cycle activities. For example, a change in temperature (rise or fall) can initiate a spawning event and various ranges in salinity have been linked to the oyster's growth and survival during different stages of their life cycle (Kennedy et. al, 1996).

Estuaries receive fresh water through rivers, bayous, and land drainage; salt water is introduced from the ocean. Galveston Bay is a large estuary in southeast Texas that receives salt water from the Gulf of Mexico and fresh water from the Trinity River, San Jacinto River, and Buffalo Bayou. Oyster reefs are scattered throughout Galveston Bay with dense aggregations located near the center of the bay. Their harvest has been a major economical resource for Texas; for example, oyster harvest for Texas in 2010 resulted in over 19 million dollars. Annual harvests vary each year for many reasons including previous years of drought that result in a reduced freshwater inflow and increased salinity, or a flood event that results in an increased freshwater inflow and decreased salinity (Powell et al., 2003). Disease infection and predation which have also been linked to temperature and salinity are also major contributors of increased mortality rates of the eastern oyster.

Stressors

Disease caused by a protozoan parasite, *Perkinsus marinus*, commonly referred to as "Dermo" is common in oyster communities along the Gulf of Mexico and is one major cause of high mortalities (Ray, 1954; Kennedy et al., 1996). The southern oyster drill, *Thias haemastoma*, an aggressive predator, can also cause high mortality among

oyster communities. Both thrive during warm months with increased salinity (Soniat et al., 2009). Anthropogenic stressors caused by fishing effort, coastal development and pollution from industry have reduced oyster reef habitats changing sediment characteristics, topology, and ultimately reducing species diversity and abundance. Extensive research to develop artificial oyster reefs and restoration of natural oyster beds continue to be priority for ecosystem managers due to an increase awareness of the ecological value to overall ecosystem health provided by oyster habitats as well as their economic value to coastal fishery landings (Tamburri et al., 2008; Geraldi et al., 2009).

Life cycle of Crassostrea virginia

The eastern oyster reproduces by broadcast spawning, dispersing eggs and sperm into the water where fertilization occurs. After fertilization, oyster larvae spend 2 to 3 weeks in the pelagic environment until they are ready to settle on a hard surface, possibly by sensing cues from conspecifics in the local population and cues of other sessile invertebrates like the barnacle that inhabits the same environment (Kennedy et al., 1996). During this pelagic period, they are vulnerable to predation and the surrounding physical environment (Geradi et a., 2009; Tamburri et al., 2008).

Purpose of study

The petroleum industry with strong presence in the Gulf of Mexico has been of particular concern because of potential damage to oysters due to oil spills and drilling processes that reach the oyster reefs along the coast. These concerns have engaged scientists to study the effects of petroleum substances on the recruitment, survival and growth of *C. virginica*.

One of the early studies conducted regarding suspected mortality of oysters due to oil fields suggested that petroleum did not cause mortality of oysters (Mackin and Hopkins, 1950). As a result of continued studies regarding pre-settlement activities, the mechanisms by which oysters respond, congregate, and attach to a common hard substrate (hereinafter referred to as "setting") are shown to be important factors when trying to understand the oyster habitat.

Several studies show the relationships between the impacts of petroleum substances on oyster larval recruitment but many vagaries exist but appear dependent on whether the study was done in a laboratory environment or field. For example, one field study showed an increased in settlement of the eastern oyster in the field but not in the laboratory suggesting that the laboratory environment is missing key environmental factors. Differences in settlement patterns have also been shown to vary whether the oily substrate was located intertidally or subtidally possibly due to varied immersion times of the substrate (Smith and Hackney, 1989; Banks and Brown, 2002). Some results have shown increased oyster set and higher growth rates on substrates coated with petroleum treatments. Hulathduwa and Brown (2006) suggest the degradation of hydrocarbons leaves a bacterial film that is an added food source comparable to the biofilm that develops in the natural environment and has been suggested to be a "cue" for oyster larvae to settle (McCoy and Brown, 1998). Others suggest that an initial "sticky" coating might allow for more oysters to set (Banks and Brown, 2002).

Research has also suggested that colonized adult oysters emit chemical cues to which the larvae respond when searching for a hard substrate (Tamburri et al., 2008;

Zimmer-Faust and Tamburri, 1994). Barnes et al. (2010) also suggests that water soluble cues are responsible for larval settlement. It should be pointed out that these studies focus on the biological movement of oyster larvae, but the physical transport of the larvae by currents and tidal activity also influence oyster larvae setting (Kim et al., 2010; Lenihan, 1999) but those points were not considered during this study.

The experimental design for this study was based on one method of farming oysters used by various groups (volunteers, commercial fishers, state agencies, etc.) with the intention of restoring oyster reefs or building artificial oyster reefs. Cultch (hard substrate for larvae to attach such as oyster and clam shells) are placed in bags and then hung from a dock during spawning season to collect spat (newly settled oyster larvae ~0.3mm – 25mm). Once they have grown beyond 25 mm they are considered juvenile oysters until they reach harvestable or adult size > 75 mm. The juvenile oysters are termed "seed" oysters because they can be deposited on oyster beds, potentially increasing the oyster population.

This was a field conducted experiment, in an intertidal bayou in Galveston Bay, where salinity is greater than 25 ppt on average (Fig. 1). Growth rates and reproductive potential are usually high in this salinity, but predators and disease are often greater compared to a lower salinity environment (Kennedy et. al., 1996). Although the higher salinities typically contribute to less dense aggregations of oyster beds, this site had several clusters of live adult oysters in this intertidal zone, available for colonization.

Fig. 1 Galveston Bay. Site selection-Sydnor Bayou (HARC, 2010).

Study objective, questions, and hypothesis

The objective of this study was to examine differences in settlement patterns and growth of the eastern oyster, *Crassostrea virginica* with the goal of understanding how different petroleum substances with different viscosities impact the recruitment potential of oyster larvae through increased or decreased settlement. Crude oil was used in previous studies but refined motor oil was used in this study due to its availability (oil companies contacted would not provide crude oil for proposed study since crude is a hazardous substance). Also used in this study was mineral oil (white oil) which appears to be non-toxic to the environment and has viscous characteristics similar to motor oil.

The following questions were posed: 1) Does motor oil and mineral oil similarly increase or decrease settlement of *C. virginica* larvae and are the results different from a

non-treated group? 2) Are there taxonomic differences associated with each substance as attractants for such organisms as barnacles, crabs, and worms that are common in oyster reef habitats?

The research hypothesis was: Since mineral oil and motor oil are viscous petroleum substances, both treatments would achieve the same oyster settlement result compared to a non-treated control group and both treatments would have similar taxonomic associations.

Site characteristics were first assessed and adult populations were monitored for spawning activity in March, 2011, to implement oyster larvae setting experiment from May – September, 2011. Two petroleum oil treatments (motor oil and mineral oil) were applied and one non-treated group was used as the control.

CHAPTER II

MATERIALS AND METHODS

Site selection

The location of the study is in Sydnor Bayou (Fig. 2), a bayou connected to Galveston Bay in Galveston, Texas (29˚15'57.05 N, 94˚52'04.81 W). The site is shallow, < 1m, with a *Spartina alterniflora* marsh edge that contains pockets of adult oysters scattered along the edge on the mudflats in the intertidal zone.

Fig. 2. Sydnor Bayou, Galveston, Texas. Dock location (HARC, 2010).

A dock was selected that would allow for monitoring without the concern for possible damage from recreational boating activities (Fig. 3). Since the bayou is shallow, no motorized water crafts can access the area.

Fig. 3. Dock at Sydnor Bayou. Experiment site.

Determining spawning time

Crassostrea virginica reproduce by broadcast spawning; they are known to respond primarily to temperature changes (rise or fall) that initiates a spawning event usually in late spring/early summer and late summer/early fall. However, oysters can spawn throughout the summer (Banks and Brown, 2002; Kennedy et al., 1996). To estimate oyster spawning time, oysters were inspected from the established population for gonad development and activity between March and early May, 2011. A test strip consisting of a polypropylene rope with 6 oyster shells attached was placed at the dock in early April and inspected throughout the month for spat settlement. Because barnacles (a sessile invertebrate like the oyster) usually spawn in early spring (compared to oysters that spawn in late spring/early summer), the test strip was also used to assess the amount of barnacles; it should be noted, however, that barnacles can spawn year round (Banks

and Brown, 2002). The final site inspection occurred on May 4th, 2011. The test strip shells were densely covered with barnacles and two spat; gonadal development of the colonized eastern oysters showed ~25% spawned. The eastern oyster reproduces by broadcast spawning and fertilization occurs in the water. After fertilization, the larval stage lasts approximately two weeks and then requires a hard substrate to attach (Fig. 4).

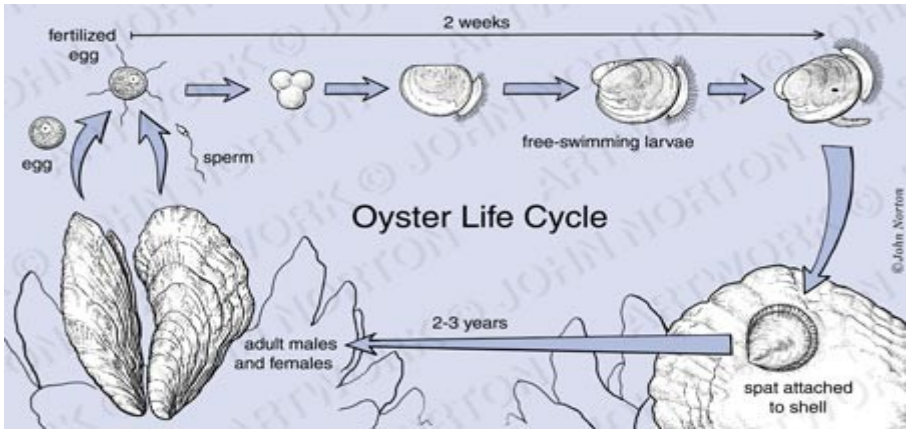

Fig. 4. Oyster life cycle. Illustration © John Norton (Norton, 2001).

Materials

Oyster shells, collected at Texas A&M University at Galveston, were cleaned, dried, and used as cultch material (defined in chapter I). These shells were from previously harvested oysters collected during research in Galveston Bay that were allowed to dry in the sun. The oyster shells varied in size from small (<70 mm), medium (70-90 mm), and large (>90 mm).

Thirty oyster shells were placed into 1, ~10 mm polyethylene net bag. Each group (non-treated control – mineral oil – motor oil) consisted of five bags and each bag

received the same number of shells from the different size groups. The bags will be referred to as spat bags from hereinafter because of their primary purpose which is to collect spat. Harness assemblies consisting of polypropylene rope and stainless steel hooks were used to hang the spat bags from underneath the dock; the bags were held approximately 10 inches off the bottom (Fig. 5). They were covered during high tide and uncovered during low tide, representing an intertidal zone where the adult oysters in this geographical area predominantly reside.

Fig. 5. Spat bag position. Hung from underneath the dock.

Petroleum oil treatments

SAE 50 motor oil was used for treatment on one group and high viscous food grade mineral oil (white, 350 FG) was used for the second group. The third "control" group had no oil treatment. The motor oil and mineral oil groups' cultch material were soaked in their respective treatments for 12 hours and then drained for two hours prior to

transfer to the site. The spat bags were hung from underneath the dock in an "X" pattern (Fig. 6 and Fig. 7) and each position was randomly given a number between 1 and 5 using an excel random number generator tool. The groups were spaced 10 feet apart on the 30 x 10 ft dock. Sydnor Bayou is a shallow, mixed environment and each group was expected to have equal opportunity for larvae attachment although, larval densities were not measured.

Fig. 6. Experimental design showing "X" pattern. Each group is indicated by a color, green (non-treated control), red (mineral oil), black (motor oil).

Fig. 7. Experimental design. Harness assemblies with spat bags attached.

Settlement patterns and growth

One spat bag from each group was removed at the end of each month from May through September, 2011, with the last bag having a total of 5 months immersion. Settlement patterns were determined by counting the number of spat which attached to each shell; a measurement was also taken for growth rates. Although the size of the settled spat was expected to grow beyond 25 mm after several months of immersion, the term "spat" was used to describe all settlement counts from spat bags during the experiment. A random number generator (excel tool) was used again to determine which bag from each group was removed each month.

Three new spat bags, one of each oil treatment and the non-treated control group, were deployed for only one month intervals in June, August, and September to compare spawning activities and differences that might occur due to length of immersion. The June bags were to take advantage of spawning if it were still occurring. Since another spawning event can occur in late summer, new bags were placed in August and September using the same treatment procedure stated in the petroleum oil treatments section of methods.

Predators-species abundance and diversity

Predators are a constant threat to oysters and although many predators are seasonal, the oyster larvae and spat stages are the most vulnerable due to their size and weak shell. Blue crabs, mud crabs, and fish such as the goby quickly find where oysters are located and crabs are known to kill eastern oysters particularly spat and juvenile oysters (White and Wilson, 1996). A spat bag from each group was removed at the end

of May and placed in a plastic bag to transport to the lab for inspection; any organisms that accompanied the spat bags were identified but not retained and not used for analyses since many fell off during removal of the bags from the dock. In the following months, a 5 gallon bucket was placed in the water under the bag to catch organisms as they fell off during removal. This was to allow for a more accurate account of organisms. The samples were bagged, labeled, and placed in a freezer until identification.

Fouling organisms, predators, pests and algae

Barnacles are considered a fouling organism when attached to an oyster shell because competition for space occurs that can limit oyster spat settlement due to reduced available substrate surface. Although, studies suggest that both the eastern oyster and barnacle settle in response to cues emitted through waterborne activities that might facilitate the settlement of each other (Barnes et al., 2010; Tamburri, 2008). A common barnacle of Galveston Bay is the ivory barnacle, *Balanus eburneus,* and has been known to inhabit areas similar to the eastern oyster but further from shore compared to the eastern oyster which occupies mudflat areas along the marsh edge (Bushek, 1988). Barnacle coverage was considered heavy if barnacles covered over 75% of the inside of the shell. Since heavy algae that can coat the oyster shell used as cultch can be an inhibitor of oyster larvae settlement, a range of light, moderate, and heavy coverage was given (further described in Chapter III). Sediment can also inhibit spat settlement and was noted as present or not.

Common worms that inhabit oysters and leave worm tubes, such as from the *Serpulidae* family were assessed as light, moderate, or heavy and given percentages of

coverage similar to algae. Other polychaetes such as the mud worm, polydora, were assessed as present or not. Serpulid worms leave a calcareous tube on the shell of the oyster and the mud worm enters through the shell opening and forms mud tube blisters in the shell of the oyster. The later can lead to death of an oyster due to energy routed in repairing the irritated area which in turns leads to weakening of the oyster and can weaken its shell. Other worm tubes were also assessed.

Disease-Perkinsus marinus *(Dermo)*

Since this site had high salinity and an expected increase in temperature as the summer progressed, two controlling parameters that increase the presence of disease (Soniat et al., 2009), tissue samples for analyses were taken to examine whether *P. marinus* infected the new generation of oysters and samples of the colonized oysters were tested for Dermo. Samples were taken twice in March, and once in August and once in September, for the established populations of oysters. The spat and juvenile oysters that were collected from the spat bags in August and September were also tested for the disease. Samples were also taken from established intertidal populations from similar areas in the west side of Galveston Bay (Sportsman Road and Confederate Reef) for disease prevalence and intensity comparison as an added value to this study (discussed in the Appendix).

Physical parameters

Temperature and salinity play an important role in the biological activities of oysters and affect their reproduction, growth and survival. Measurements were taken during removal of bags to monitor throughout the experiment. While adult oysters

survive better in lower salinities ranging from 15 ppt to 22 ppt, larval development has been shown to be positively impacted in higher salinity environments (Shumway 1996). It was expected to have continued temperature and salinity increase throughout the summer.

Statistical analyses

The sample sizes of spat collected were small, so to determine significance, a non-parametric Kruskal-Wallis *H* test, $x^2 > 5.99$ and $p \geq 0.05$ or the Mann-Whitney *U* test was used to determine significance between groups. Percentages were assigned to portray bulk information regarding algae and barnacle coverage.

CHAPTER III

ANALYSES/ RESULTS

Oyster larval setting

In order to determine the total numbers of spat that settled, each of the 30 shells in each cultch bag were examined from May – September, 2011. The shells were rinsed and examined and all oyster spat were counted and measured. As stated earlier, all oyster counts from bags will be referred to as "spat" regardless of size. It was reasonable to determine when spawning occurred by comparing month to month samples using size as an indicator.

The results indicated that settlement patterns did not differ significantly among treatments after comparing monthly spat counts (Fig. 8, Table 1). The overall total percentage of spat counts for the five months resulted in no significant difference among groups, determined using statistical methods (non-parametric Kruskal-Wallis, $x^2 > 5.99$, $P > .05$). The mineral oil had 40% of the total spat count, followed by the non-treated control group with 34% and then the motor oil group with 26% of the total spat count.

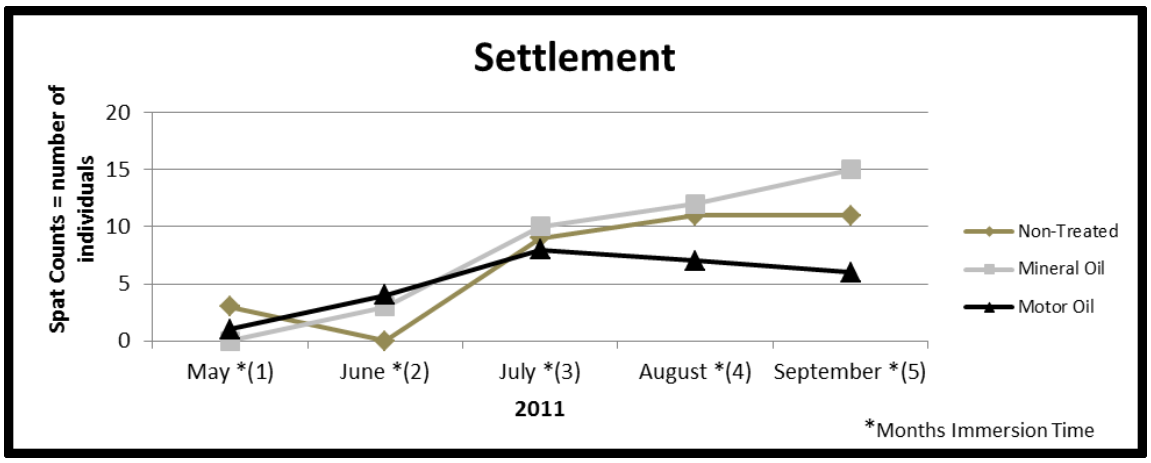

Fig. 8. Settlement of *Crassostrea virginica* (spat count). Fifteen spat bags were deployed in early May, 2011, and divided into three groups of five bags each. One bag was removed each month from each group.

Table 1

Settlement of *Crassostrea virginica* (spat count). Fifteen spat bags were deployed in early May, 2011, and divided into three groups of five bags each. One bag was removed each month for each group.

Months	Immersion Time	Non-Treated	Mineral Oil	Motor Oil
May	1 Month	3	0	1
June	2 Months	0	3	4
July	3 Months	9	10	8
August	4 Months	11	12	7
September	5 Months	11	15	6

Oyster growth as an indicator for spawning time

Using vernier calipers, shell measurements were taken of each spat by measuring the oyster shell height from umbo to bill over the curve of the right valve to the nearest millimeter (Fig. 9 and Fig. 10). An average monthly growth rate was determined by measuring spat that were collected from the bags that were retrieved after deployment of one month (Table 2).

Fig. 9. Measuring an oyster.

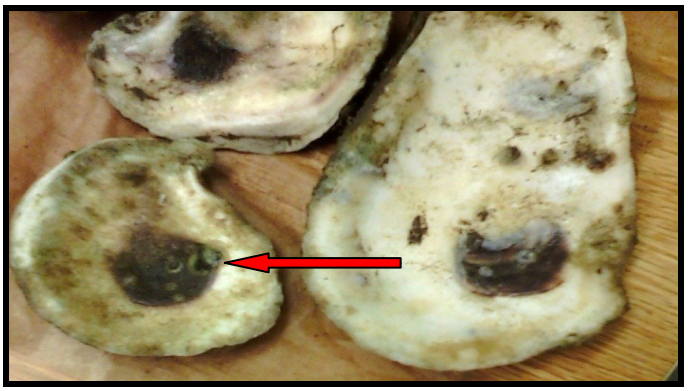

Fig. 10. Spat on an oyster shell. The shell was removed from a spat bag.

Table 2

Spat size (mm) (one month). Three new spat bags were placed in the water for each group in June, August and September. Spat bags were deployed for one month intervals to monitor spawning and activities of species associated with the spat bags. May is included for the monthly analyses.

Month	Immersion Time	Non Treated	Mineral Oil	Motor Oil
May	1 Month	6, 9, 6	0	6
June	1 Month	0	4, 12, 14, 16	15
August	1 Month	0	0	0
September	1 Month	8	0	0

Table 3

Spat size (mm) (five month). Fifteen spat bags divided into three groups of five bags each were deployed in early May. One bag was removed each month for each group from May - September. The "red" numbers indicates a dead oyster as determined by either a fresh box, spat scar or left valve remaining on shell.

Month	No Oil	Mineral Oil	Motor Oil
May	6, 6, 9	0	6
June	0	9, 15, 23	10, 10, 17, 18
July	16, 29, 35, 35, 37, 38, 43, 46, 49	5, 9, 11, 21, 24, 24, 28, 29, 30, 31	5, 7, 12, 18, 21, 24, 28, 44, 52
August	15, 24, 26, 30, 30, 30, 32, 39, 40, 45, 53	15, 21, 25, 26, 31, 32, 33, 38, 40, 43, 48, 52	19, 32, 32, 47, 52, 53, 60
September	25, 30, 33, 33, 33, 35, 45, 48, 52, 58, 62	7, 15, 20, 24, 25, 25, 28, 28, 32, 36, 38, 41, 50, 54, 58	10, 18, 30, 31, 32, 44

The smallest measurement was 4 mm (June), the largest was 16 mm (June), and the average growth rate determined was 9.6 mm per month (Table 2). Any boxes (both shells of the oyster remaining intact but no oyster meat), spat scar (marking left on the attachment surface after the spat was removed), or the left valve remaining (oysters normally attach to the substrate on their left valve side), were measured and included in the settlement data analyses but not the monthly growth rate analyses or overall growth analyses.

Monthly recruitment was estimated using a growth rate of 16 mm or less per month since the highest monthly growth rate was 16 mm in the June bag deployed for one month (Table 3).

September had no live oysters under 16 mm. Although there were boxes under 16 mm for the mineral and motor oil groups, it could not be determined what month those oysters had settled since the bags had been in the water for five months. The total number of spat collected for June was almost twice as much as May and, although a new bag was not positioned in July for monthly analyses, the spat collected from the July bag that was immersed for two months had sizes than 15 mm for 22 of 28 collected spat suggesting that the spawning event occurred in the middle of May (Table 2 and Table 3).

Oyster growth among treatments

The growth was averaged to be 9.6 mm per month although there were some less and even as high as 16 mm (Table 3). It could not be determined exactly at what time the oyster larvae had settled during the four weeks of immersion. The motor oil treatment had the greatest growth of 52 mm at the end of the first three months, July, and at the

end of the fourth month (60 mm), August. The control group had the highest growth at the end of the five month experiment of 62 mm at the end of September. The motor oil group's largest spat was >10mm compared to the largest control group oyster after three months immersion time and >25mm compared to the mineral oil group's largest oyster for that month (Table 2) .

Barnacle setting

The inside of each shell in each bag was assessed for coverage of the ivory barnacle, *Balanus eburneus* since oyster larvae prefer the inside of oyster shells (communication with Ray 2011) although they will set on the outside of the shell as well (Fig. 11).

Fig. 11. Example of heavy barnacle cover on an oyster shell. The shell was removed from a spat bag retrieved during study.

Barnacle setting varied among months and among treatments but with no significant difference among the treatments. One month immersion time during May, 2011, resulted in minimal heavy barnacle coverage for all three treatments with 10% or

less barnacle coverage in all three groups. The greatest difference was during the month of June when the motor oil group only had 7% coverage compared to 33% and 27% in the non-treated and mineral oil groups respectively (Fig. 12). As the months progressed, barnacle size increased with very few new barnacles indicating that barnacle spawning had occurred early in the experiment.

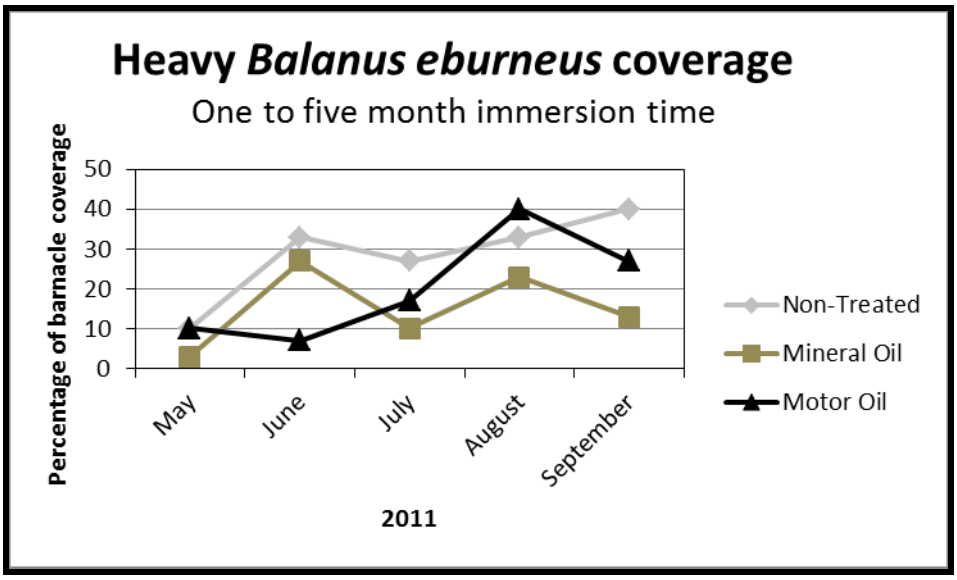

Fig. 12. Heavy barnacle coverage for the ivory barnacle, *Balanus eburneus*. Shown for cultch bags deployed for five consecutive months for all three treatments. Heavy barnacle coverage = barnacles coverage of over 75% of each oyster shell = n/30 shells.

The spat bags that were deployed for only one month intervals showed little *B. eburneus* coverage for the months of June, August, and September.

Algae and sediment

The algae growth on the inside of each shell was assessed as light, moderate or

heavy. If the algae covered up to 25% of the shell, the range was determined to be light. If the algae covered 50 % of the shell, the range was determined to be moderate. Any algae coverage greater than 50% was determined to be heavy. The sediment coverage was noted when initially examining shells prior to rinsing. A heavy algae cover was present on the shells from the spat bags that increased throughout the summer with the greatest amount in July and August. The September spat bags for all three groups had little heavy algae (Fig. 13).

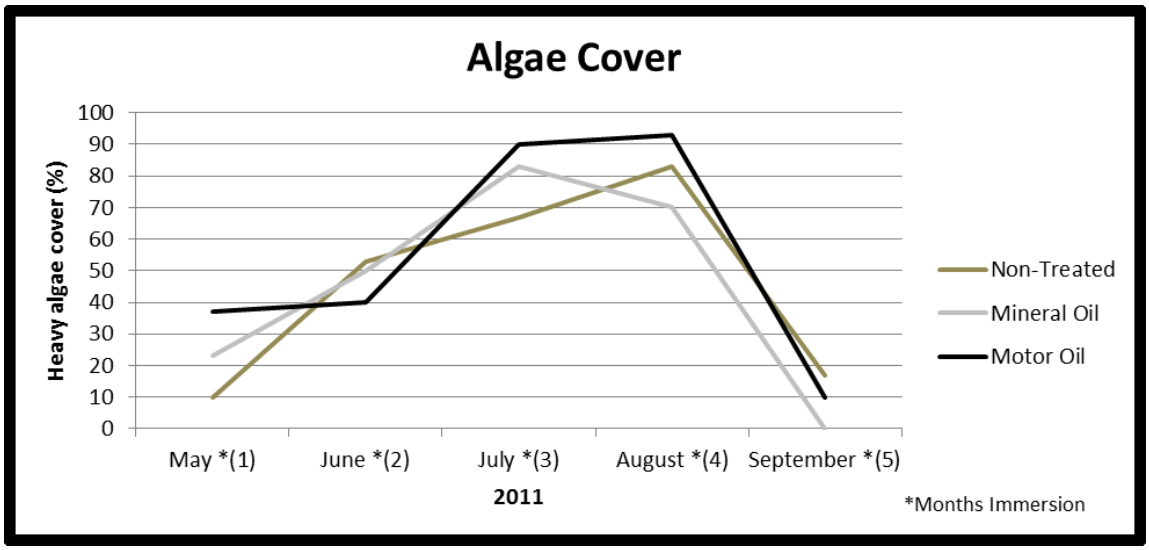

Fig. 13. Algal cover (five month). Fifteen spat bags were deployed in the water in early May, 2011, and divided into three groups of five bags each. One bag was removed monthly until the last bag was removed at the end of September.

New spat bags were deployed for one month intervals for the months of June, August and September. The new June bag showed similar heavy algal cover to the June

bag that was immersed for two months. The new September bag that was deployed for only one month was also similar to its respective September bag that was immersed for five months. The August bags that were deployed for one month had less algal cover (30 % – 40%) compared to the August bags that were deployed for four months (70 % - 90 %) (Fig. 14).

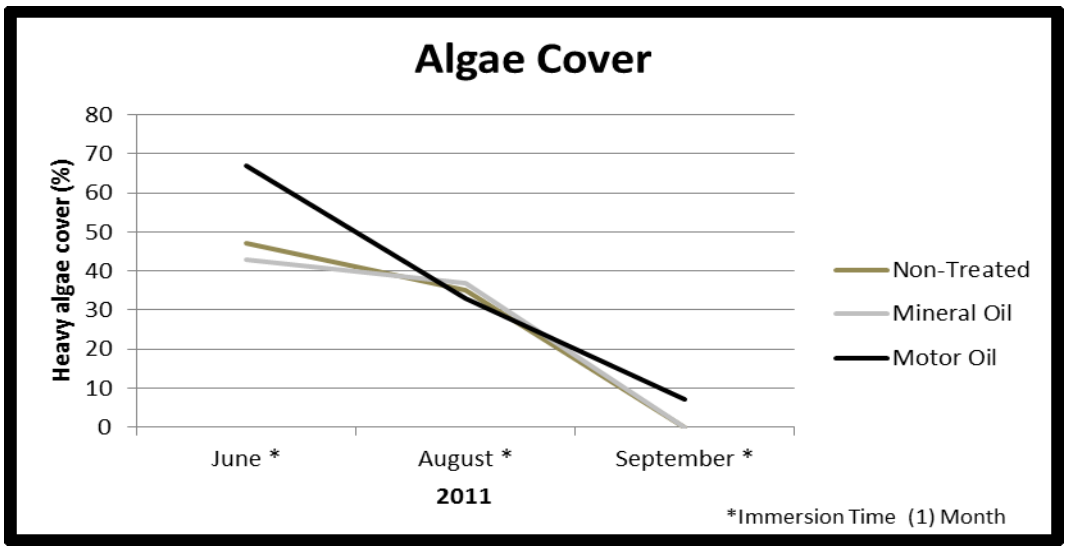

Fig. 14. Algae cover (one month). Three new spat bags were deployed in June, three in August and three in September, one for each group. The bags were removed after deployed for one month.

Sediment was present in a loose layer in all spat bags. There did not appear to be any difference of sediment coverage among the groups. The September bags showed an accumulation of sediment in the bottom of the bags.

Predators-species abundance and diversity

The community of invertebrates and fish were collected for each sample spat bag and were identified through visual inspection and taxonomic key references. A dissecting microscope was used if necessary depending on the size of the individual.

Serpulid worms were present on most shells for all three groups, although very light for the first month of the experiment (Fig. 15). The motor oil treated group appeared to have less serpulid worms compared to the non-treated and mineral oil groups.

Fig. 15. Oyster shell showing Serpulid worm casings. The white, calcareous tubes were left from Serpulid worms and the dark softer tubes were left by other worms.

Tunicates were irregular shaped and suspected to be *Mogula* sp. and appeared early in the experiment in May and June, 2011, in all groups. May had the greatest amount of tunicates of which the non-treated control group had the greatest amount of ~35 tunicates both May and June combined. The mineral oil group had ~23 tunicates,

both months combined and the motor oil treated shells had the least number of tunicates, ~15.

Mussels appeared to be Atlantic paper mussels, *Amygdalum papyrium*, and were present in all three groups. The greatest amount was during the last two months, August and September with the greatest number of mussels belonging to the motor oil treated group. Very few hook mussels, *Ischadium recurvum,* were found in the spat bags during the experiment.

Nekton assemblages were analyzed to study relationships between petroleum substances and their oyster habitat as well as assessing populations of possible predators and commensual animals. Crustaceans and fish were collected during spat bag removal (Table 4). The May sample collection had some animals in each bag and were noted but not used for abundance data since many organisms fell into the water during retrieval and none were kept for identification. A five gallon bucket was used during continued sampling from June through September, 2011. The bucket was placed in the water under the bag prior to disconnecting the cultch bag from the harness assembly. This was to minimize loss of species upon retrieval in order to get an account of species abundance and diversity that are commonly found in an oyster reef habitat (Humphries et al., 2011; Shervette and Gelwick, 2008).

Crabs, shrimp, and fish were seen on all three bags removed in May but, not included in data analyses since the numbers and species were not recorded. Species identifications and numbers of the nekton assemblages were taken for the samples collected for the months of June, July, August, and September, 2011 for the consecutive

month analyses and for June, August, and September for the one month immersion time spat bags. The total crustaceans consisted of 138 blue crabs, *Callincetes sapidus,* 46 various mud crabs (the stone crab, *Menippe adina,* oyster shell mud crab, *Panopeus*sp. and the flatback mud crab, *Eurypanopeusdepressus*) (Fig. 16) and 71 shrimp of the genus, *Palaemonetes* sp., suspected to be daggerblade grass shrimp and marsh grass shrimp. Sixty fish of the family Gobiidae were identified to be the naked goby, *Gobiosoma bosc.* Species richness was equal among groups but evenness of the species varied.

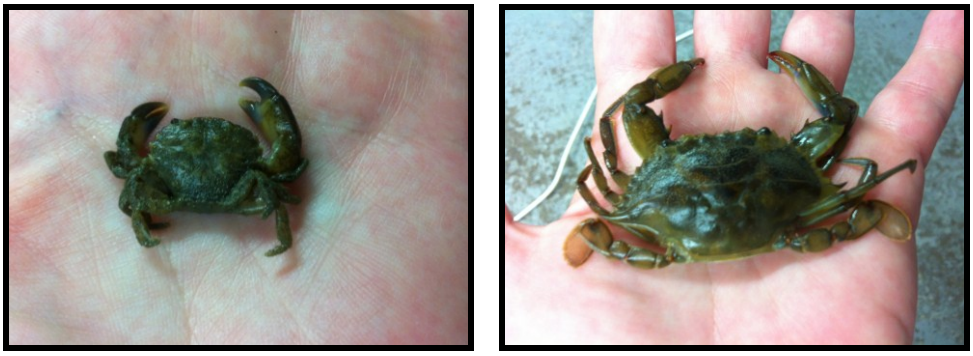

Fig. 16. Predators. Animals removed during spat bags during study. Left – mud crab. Right – blue crab.

Table 4

Crustaceans and fish collected for all treatments.

Common name	Scientific name	Total number of individuals	Non-treated control	Mineral oil	Motor oil
Crustaceans					
Blue crab	*Callinectes sapidus*	138	40	54	44
Mud crabs	Xanthidae sp.	46	27	8	11
Porcelain crab	*Petrolisthes armatus*	1	0	1	0
Shrimp	*Palaemonetes* sp.	71	27	28	16
Fishes					
Naked goby	*Gobiosoma bosc*	60	31	15	14

After comparing the different immersion times, varied distribution of shrimps, crabs (blue and mud), and fish were noted. Shrimp abundance was greatest in June for both the two month and one month immersion times compared to the other months. The greatest number of shrimp was present in bags that were only deployed for one month for all groups. There was no significant difference among treatments and abundance of shrimps. Blue crabs were present in both the consecutive month bags and one month bags, again with no significant difference among groups and blue crab abundance. Mud crabs were most abundant in bags that were in the water for three, four, and five months which totaled 41 crabs which indicates a preference to inhabit where oysters are present and is consistent with studies conducted by Shervette and Gelwick (2008), investigating seasonal and spatial variations in fish in oyster habitats. The one month immersion bags only totaled 5 mud crabs, all groups combined. The naked gobies were similarly

distributed like the mud crabs with the greatest abundance in months 3-5 of the consecutive month bags, totaling 52 fish. The one month bags only totaled 7, all groups combined. Twice as many fish were associated with the non-treated control group compared to the mineral and motor oil groups. The behavior of the naked goby in response to oyster habitats is consistent with the studies by Breilburg (1991). Overall, all three groups had the same species richness but varied in evenness (Table 5).

Table 5

Species breakdown by month and by treatment. The left side of the table represents bags removed after 2, 3, 4, and 5 month immersion times. The right side of the table represents new bags that were deployed June, August and September with only one month immersion time, * = months immersion time.

Month	Mud Crabs	Blue Crabs	Fish	Month	Shrimp	Mud Crabs	Blue Crabs	Fish
JUNE *(2)				**JUNE *(1)**				
Non-Treated	1	8	0	Non-Treated	15	1	6	1
Mineral Oil	0	15	0	Mineral Oil	13	0	6	0
Motor Oil	0	8	1	Motor Oil	1	0	7	0
JULY *(3)								
Non-Treated	6	2	10					
Mineral Oil	5	5	6					
Motor Oil	1	6	1					
AUGUST *(4)				**AUGUST *(1)**				
Non-Treated	9	3	9	Non-Treated	2	0	1	0
Mineral Oil	2	3	5	Mineral Oil	7	0	3	0
Motor Oil	2	8	5	Motor Oil	3	0	0	0
SEPTEMBER *(5)				**SEPTEMBER *(1)**				
Non-Treated	9	8	9	Non-Treated	1	0	12	2
Mineral Oil	1	9	3	Mineral Oil	3	1	13	1
Motor Oil	5	7	4	Motor Oil	2	3	8	3

Disease-Perkinsus marinus *intensity and prevalence*

Tissue samples for disease analyses were taken to examine whether the protozoan parasite *Perkinsus marinus,* commonly referred to as "Dermo," prevalence and intensity varied between the colonized intertidal oysters and the new generation of spat and juvenile oysters collected to include when discussing mortality. Samples were taken twice in March for adult oysters and, once in August and September for established adult oysters. Samples were also taken from spat and juvenile oysters collected from spat bags collected in August and September.

Ray's (1966a) method of oyster tissue culture was used for analyses. A piece of mantle tissue was taken for analyses, incubated in fluid thioglycolate medium for about 7 days, then stained with Lugol's iodine and observed at 40X and 100X magnification. An intensity value is given a range from no cells to heavy, each with a corresponding numerical value of 0.00 – 5.00.

Colonized adult oysters: Two samples of the adult eastern oyster population were taken in March, 2011 to assess overall characteristics of the colonized community and Dermo analyses. The oysters were in banana stock-like clusters along the upper intertidal region of the mud flat. They were somewhat elongated, thick-shelled, exhibited sharp recent growth with light spat, light juvenile oysters and consisted of several large boxes (defined earlier in chapter III, p. 19) that were filled with mud. Organisms included hook mussels, serpulid worms, mud worms, barnacles, and yellow boring sponge (a common pest of oysters in high salinity environments). Two types of green algae were covering many of the oysters, filamentous algae and sea lettuce. The

first sample taken on March 1, 2011, from the site area of colonized oysters showed a difference among cultures which alluded to a possible difference between the mediums; this sample was not used for analyses. The second sample taken on March 11, 2011, consisted of 10 market size oysters > 75 mm. Seven out ten market size oysters were found to be infected with dermo resulting in a 70% prevalence of infection. Although there was 70% prevalence, the cells were smaller than what is usually seen in Dermo infected oysters. The intensity value varied between 0.00 and 2.00, no cells (N) and light – moderate (L-M$^-$). Continued sampling at this site and at similar sites with similar characteristics, specifically salinity and temperature, were conducted for comparison. This information is discussed in the appendix as an added value to this study.

Spat and juvenile samples from study (spat bags): Samples of spat and juvenile oysters were taken in August from the control, mineral oil, and motor oil groups as follows: control group, 8 juvenile oysters cultured and none were infected; mineral oil group, 10 spat and juvenile oysters cultured and no Dermo cells present except for two possible pre-Dermo cells in five of the 10 cultured tissue samples; motor oil group, 6 spat and juvenile oysters cultured and no Dermo cells were noted except for one possible pre-Dermo cell found in one oyster. A spat and juvenile sample were also taken in September from the experimental groups as follows: control group, 11 oysters were sampled with zero infected; mineral oil group, 11 oysters were sampled with zero infected except for two possible pre-Dermo cells in two samples; motor oil group, 4 oyster cultures were taken and no Dermo cells were noted except for three possible pre-Dermo cells in one oyster sample.

Physical parameters

Water salinity was taken at the time of deployment of cultch bags (early May) and throughout the experiment during sampling events with a refractometer. A local temperature was also taken if possible. If a temperature was not taken, a temperature from a nearby location (Sammy's Reef) that is monitored was used.

The initial salinity was 29 ppt and steadily increased throughout the summer with a final salinity of 40 ppt at the end of September. The temperature steadily increased throughout the summer with a significant peak from May to June (Fig. 17).

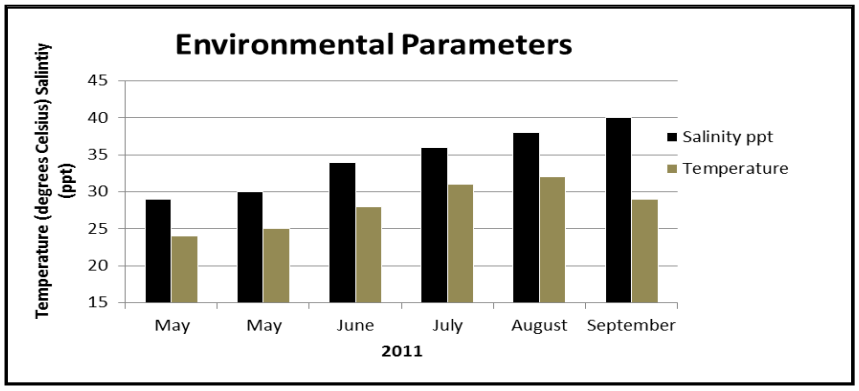

Fig. 17. Environmental parameters. Salinity and temperature were taken at the initial deployment of bags and throughout the experiment during retrieval of cultch bags.

CHAPTER IV

DISCUSSION

Settlement of spat

Settlement patterns did not vary significantly among the three groups nor between the mineral oil and motor oil groups. The surfaces created by petroleum products have been suggested as creating a coating similar to a biofilm coating, to which larvae of both oysters and barnacles are attracted (Barnes et al., 2010; Campbell et al., 2011) but the experiment did not show a significant differential settlement preference among treated shells. The algae growth coupled with sediment build up on the oyster shells was probably the primary reason for spat not to settle, both have been found to inhibit larvae settlement (Tamburri et al. 2008). A limited larval supply could also result in reduced numbers. No oyster larvae sampling was done to determine densities. Furthermore, the abundance of predators could have reduced the numbers of larvae during their pre-settlement stage through larval predation activities.

Growth of oysters

Growth of oysters during their early life cycle can be rapid during the summer months depending on food availability. If spawning occurred around mid-May then settlement would follow at approximately 16 days later at the beginning of June. Using a monthly growth average of 9.8 mm, the average juvenile oyster would then be ~39.2 mm. All three groups exhibited comparable growth rates with no apparent difference between the two treatments or to the non-treated group indicating that there was no

change in food source or availability caused by the exposure to petroleum substances.

Barnacles

Barnacle coverage peaked in June and decreased throughout the rest of the experiment in the non-treated control and mineral oil groups but the opposite occurred for the motor oil group. Since the one month only immersion time for June showed significantly less coverage, the results suggest that settlement of the barnacle differentiated between a bag with conspecifics (probably had some barnacles since May) and a bag without any barnacle settlement. Decrease in barnacle abundance could be due to predators such as blue and mud crabs. The decrease in abundance of barnacles with increased immersion times for two of the three groups is consistent with the results of Brown and Swearingen (1998). It is not clear why the motor oil group had less barnacle coverage in June; this is inconsistent with research that showed facilitated recruitment of barnacles on oily substrate (Smith and Hackney, 1989). Shells without heavy barnacle cover were still available in the spat bags for oyster larvae to settle and since barnacles and oysters have been found to settle together, the barnacles did not seem to act as an inhibitor of spat set due to competition for space.

Algae and sediment

Algae growth was prevalent in all groups in the five month bags and one month bags during optimal predicted settling periods. Although bacterial films have been shown to facilitate recruitment of oyster larvae, an excess growth of algae reduces the clean surface area for larvae to attach. Due to the shallow bayou and soft sediment, silt was always present and could have been a determinant factor that limited spat

settlement.

Predators

All treatments were colonized by the same species although the abundance of each varied among treatments. Although the predators like the blue crabs and mud crabs that were present were small, they can reduce the numbers of oyster larvae settlement and post-settlement spat because of the larval and spat size.

CHAPTER V

CONCLUSION

Summary

This study did not show dissimilar settlement patterns between the mineral oil and motor oil group nor against the non-treated control group. Further studies would need to be performed to support the hypothesis if the "viscosity" of petroleum substances was the primary driver that leads oyster larvae to settle. The eastern oyster, *Crassostrea virginica*, did not appear to have a preference since spat was discovered in all three groups. No inhibition of animal colonization was determined as all three groups were equal in species richness. This study adds to the recent importance given to the complexity of oyster reefs and their diversity of nekton assemblages (Humphries, et al., 2011). The viscosity of the substance alone cannot be used as a determining factor of increased or decreased settlement of oysters from this study. Sediment and algae cover most likely caused reduced oyster settlement as well as mortality of larvae and spat through predation.

Other analyses

A laboratory study combined with field studies that compare similar sites might offer insight to the settlement response of the eastern oyster regarding viscous substances. Since many studies also include the differences of oyster activities associated with intertidal and subtidal oyster habitats, including that aspect would be another beneficial study.

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APPENDIX

Continued Dermo comparison of nearby sites as an added value to this study.

Since the study area did not show the expected levels of Dermo infection, continued sampling of the area followed and was compared to similar "high salinity, high temperature" areas. Two similar sites were assessed, Sportsman Road and Confederate Reef (both in west bay, Galveston).

August

A sample was taken in August of colonized oysters from the intertidal area in Sydnor Bayou. They were heavily encrusted with algae and silt with light barnacle coverage, hook mussels, heavy polydora and boring sponge. Ten market size oysters and three sub-market size oysters (<75 mm) were analyzed for Dermo infection. The market size oysters showed an 80% prevalence with an intensity range from 0.00 – 4.00 (N – M-H), no cells to moderate heavy. The cells were still very small when compared to samples taken from similar nearby locations in the west bay area of Galveston Bay. The sub-market sample showed 67% prevalence of Dermo infection with an intensity range between 0.00 and 1.67 (N – L-M), no cells to light moderate infection.

September

A sample was collected from the adult population at the end of September of 4 market and 7 sub-market size oysters. 10 market size oysters were not collected due to the oyster clusters consisting of half mud-filled boxes. The oysters were heavily encrusted with silt and algae with very heavy boring sponge, moderate large barnacles

and heavy polydora. Two of the four market size oysters were infected with Dermo indicating a 50% prevalence and only one of seven of the sub-market size were infected, 15% prevalence. The cells intensity range was from very light to light moderate with some possible pre-Dermo cells. Overall, Dermo appeared low for this sample.

October

To compare similar environments, a sample was taken at the end of October, 2011 at the experimental site, Sydnor Bayou and at Sportsman Road, west end of Galveston, Tx. Both areas salinity was 37 ppt and 38 ppt, and temperature was 20 and 21 degrees Celsius, respectively. Samples were taken from both areas, intertidal where the eastern oyster dominates.

Sydnor Bayou's sample characteristics were similar to previous samples; heavy algae, heavy boring sponge, serpulid worms, mussels, barnacles and heavy *Polydora* mud worms were present. The market sample showed 83.3 % prevalence and the Dermo cells were mostly very small to small and distorted and the intensity was very light to light to moderate infection. The sub-market sample showed 30.8 % prevalence showed very light infection of Dermo and the cells were very small to small.

Sportsman Rd's sample characteristics had a moderate coat of silt; light serpulid worms, moderate algae and very few market samples were collected. Only 3 market size oysters were cultured with a prevalence of 100 %. The intensity of infection was light-moderate to moderate with fairly well enlarged Dermo cells as well as variable sizes including very small to small cells. The sub-market sample showed 71.43 % prevalence with intensity range of light-moderate to moderate with variable cell sizes.

January

Samples were taken at Sydnor Bayou and Confederate Reef (west end), salinity and temperature was 30 ppt and 17.8 degrees Celsius for Sydnor Bayou and 22 ppt and 15 degrees Celsius for Confederate Reef.

The oysters were heavily coated with silt from Sydnor Bayou with heavy boring sponge, light serpulid worms, heavy polydora and very light barnacles. The market size oysters showed 54.55 % prevalence and the intensity of Dermo was very-light to moderate infection in those cultures infected. The Dermo cells were mostly very small to small, some variable and some fairly well enlarged. The sub-market oysters showed 31.58 % prevalence with only 6 of 19 oysters infected. The intensity was very – light to moderate infection with small Dermo cells.

Confederate Reef's sample had a light coat of algae and silt, heavy boring sponge and serpulid worms. The market size oysters showed 100 % prevalence with Dermo intensity of light-moderate to moderate-heavy infection. The Dermo cells were very small to small and fairly well enlarged. The sub-market sample showed 90.5 % prevalence with Dermo intensity of very - light to light – moderate infection, very small to small Dermo cells.

Summary

All sites showed Dermo prevalence in the market and sub-market oysters but the prevalence and intensity of infection varied between areas and Sydnor Bayou showed the least. The Dermo cells found in the tissue samples of oysters from Sydnor Bayou were very small and many were distorted compared to the other two sites. In high

salinity, high temperature environments, infection of Dermo is expected but this particular small bayou is surrounded by a golf course on both sides and houses on one side so the water nutrient load could be different which could also affect algae growth and so forth. Gray et al (2009) did not find a correlation between land use development and intensity levels of Dermo disease but small, almost isolated bayous that support intertidal oyster communities such as this one would benefit from continued studies of oyster disease infection.

VITA

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