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Oyster Spat Survival in Response to Hydrocarbon Contamination and Predation in Barataria Bay, Louisiana

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OYSTER SPAT SURVIVAL IN RESPONSE TO HYDROCARBON CONTAMINATION AND PREDATION IN BARATARIA BAY, LOUISIANA

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ABSTRACT

Barataria Bay, in southeast Louisiana is home to productive oyster reefs that are both ecologically and economically important, but was threatened by the *Deepwater Horizon* oil spill in April 2010. This study was designed to determine how the oil spill affected long- and short-term oyster recruitment, and spat (juvenile oysters) cellular health. I also investigated how predators in the bay affect spat survival. Four study sites were selected in Barataria Bay: two control and two oiled, each with a low and high salinity location. To determine whether there were long-term effects of the oil spill on oyster recruitment, tiles were placed at each site in 2012 and 2013 and spat recruitment quantified monthly. Results indicate that in 2012, recruitment varied more with salinity, but in 2013 when early summer salinity was lower, recruitment only occurred at the control sites. Tiles with 5 mL of light crude oil absorbed were used to study the short-term effects of oil on oyster and barnacle recruitment in 2013. Spat recruitment was lower on oiled tiles but there was no difference in spat size between treatments; alternately, there was a slight tendency for oil to increase barnacle recruitment. The effect of No (0 ppm), Low (500 ppm) or High (25,000 ppm) oil concentrations in 10 and 20 PSU seawater on spat cellular function was determined. Lysosomal stability was lower in low and high oil treatments suggesting oiling can affect spat health after just 10 days of exposure. The effect of predators on spat survival at each site was determined by quantifying predation on spat with no cage or in predator exclusion cages with 0.5, 1.0, or 3.0 cm mesh openings. The presence of a cage reduced predation of oyster spat. Predation rates were greatest on spat without cages suggesting larger predators such as blue crabs and oyster drills, with access to exposed spat, may play greater roles in post-settlement spat mortality than other predators such as mud crabs. Oyster recruitment and spat survival seems to depend more on salinity and predation than long-term hydrocarbon

contamination. However, the short-term effect of hydrocarbon contamination can detrimentally impact spat recruitment and health; thus, clean-up efforts immediately following an oil spill and continuous monitoring efforts are necessary to maintain healthy oyster populations.

INTRODUCTION

The eastern oyster, *Crassostrea virginica*, comprises a large portion of the fishing industry in northern Gulf of Mexico, supplying approximately 67% of the nation's oysters and supporting a nearly $40 million industry in Louisiana (National Oceanic and Atmospheric Administration (NOAA) 2010a). Not only are they economically important, but oysters also provide numerous ecological services including shoreline stabilization (Meyer et al. 1997, Piazza et al. 2005), carbon sequestration (Grabowski et al. 2012) and water filtration thereby reducing turbidity (Newell and Koch, 2004) and removing nitrogen (Piehler and Smyth 2011). Additionally, these ecosystem engineers aid in the production of additional oysters (Grabowski and Peterson 2007, Geraldi et al. 2013), and provide refuge for a variety of fish and invertebrate species (Lenihan et al. 2001, Peterson et al. 2003a, M. La Peyre et al. 2014).

The value of ecosystem services provided by oyster reefs has been estimated up to $100,000 per hectare per year, in addition to the economic value of harvesting oysters (Grabowski et al. 2012). Although oyster reefs are thus a critical component of the ecology and economics of the northern Gulf of Mexico, oyster reefs in this region are not exempt from the global degradation of oyster communities. Approximately 85% of reefs have been lost worldwide, and though oyster populations in the Gulf of Mexico are considered to be in better condition (fair) than those along the Atlantic coast of North America (poor or functionally extinct), they face many of the stressors that have led to rampant degradation including overharvesting, predation, and other impacts from human activities (Beck et al. 2011). Impacts from human activity can range from sedimentation and anoxic conditions due to development and nutrient input from storm water runoff (Jackson et al. 2001) to more severe, large-scale anthropogenic impacts such as oil spills and freshwater diversions.

The *Deepwater Horizon* (DWH) oil spill occurred on April 20, 2010 and threatened a large portion of oyster communities in the northern Gulf of Mexico. The blowout left the Macondo wellhead open for approximately 90 days before it was capped in mid-July 2010 (National Resource Damage Assessment (NRDA) 2012). During this time it released approximately 4.9 million barrels of oil into offshore oil plumes which reached Louisiana's coastline in May 2010 (NRDA 2012, Emergency.Louisiana.Gov 2010). While cleanup efforts occurred immediately after the blowout, extensive areas of Louisiana's coastline received some level of oiling (NOAA 2010b). Among those areas was Barataria Bay in the southeast portion of the state. Barataria Bay is home to many productive oyster reefs which were impacted by oiling from the *DWH* spill (Department of Health and Hospitals (DHH) 2010). Oysters are sedentary, benthic-dwelling filter feeders that provide extensive habitat but are susceptible to contaminants in the water and sediment. In a typical year, oyster spawning begins in late spring and continues through late summer with oyster recruitment generally occurring during May through October of each year in the northern Gulf of Mexico (Supan 1983, Banks and Brown 2002). Concern arose about the effect of this spill on the oyster industry due to the widespread oiling of productive estuarine habitats and concurrence of the spill and the natural spawning and recruitment period in 2010.

Previous studies have investigated how coastal systems including marshes (Mendelssohn et al. 2012, Pezeshki et al. 2000) and oyster communities (Hulathduwa and Brown 2006, Banks and Brown 2002) respond to hydrocarbon exposure following an oil spill. One study on the fouling community of the Louisiana coast found that recruitment and growth responses varied by species, with greater oyster recruitment and growth on crude-oil exposed surfaces, perhaps due to degradation of oil into biofilms (Banks and Brown 2002). Although there did not appear to be

a long-term effect of hydrocarbon contamination on recruitment of the common barnacle, *Balanus eburneus* (Banks and Brown 2002), recruitment was initially depressed on hydrocarbon contaminated surfaces (McCoy and Brown 1998). While these studies suggest that oysters and common fouling species in areas impacted by the *DWH* oil spill may be resilient to immediate hydrocarbon contamination, natural recruitment patterns were altered by hydrocarbon contamination (McCoy and Brown 1998) and the long-term and cellular impacts hydrocarbon exposure has on these individuals must also be considered.

One concern of hydrocarbon exposure in marine systems is the effect poly-aromatic hydrocarbons (PAHs) will have on communities and species both at ecosystem and cellular levels. PAHs are toxic compounds found in crude oil that can have large deleterious effects on invertebrates as they have both carcinogenic and mutagenic qualities (Peterson et al. 2003b, Ramdine et al. 2012). PAHs readily bind to sediment as well as particulate organic matter found in the water column (Baumard et al. 1999), and have been found to persist in sediment at contaminated sites for many years, up to decades after initial exposure (Peterson et al. 2003b, Short et al. 2007). Bivalves such as oysters can uptake these contaminants through the sediment in which they live or through ingestion during water filtration (Jackson et al. 1994) where they are then accumulated within their tissues (Stegeman and Teal 1973). Unlike some arthropods and fish species (Neff et al. 1976), oysters are unable to quickly metabolize PAHs and because of their inability to move, may be increasingly susceptible to long-term contamination. Oysters are ubiquitous across the muddy, benthic habitats in the northern Gulf of Mexico and persistent PAHs in sediment could exacerbate oyster assemblages' risk to detrimental effects on the individual and population level from prolonged exposure to contaminants from the *Deepwater Horizon* oil spill.

Although mortality in marine species can result from exposure to PAHs (Roberts et al. 1989), bivalves such as oysters usually exhibit non-lethal reactions such as reduced reproductive ability and health (Bender et al. 1988). Oyster responses to stressors such as pollution or PAHs, can include whole-body responses such as decreased condition index (Mahoney and Noyes 1982), cellular and subcellular reactions such as lowered ability to combat disease and infection (Chu and Hale 1994), and decreased lysosomal stability (Ringwood et al. 1998). Many studies have looked at the effect of stressors such as PAHs on both adult and larval oysters. Larval stages of bivalves have been studied as they are more susceptible than adults to decreased health and growth, or even death when exposed to aquatic contaminants and stressors such as metals (Connor 1972) or acidified water (Kurihara 2008). However, the effect of PAHs on oyster spat, or juvenile oysters ≤ 25 mm shell height remains unclear. In previous toxicity studies juvenile bivalves were more sensitive than adults (Ringwood 1990) warranting further investigation into the specific effects of PAHs on these juveniles as the continued production of oyster reefs relies on survival and growth of spat into adult oysters.

Reefs not only provide habitat to settling oyster larvae but to many other invertebrates and fish (Soniat et al. 2004, Grabowski et al. 2005) including fouling species such as barnacles (*Balanus eburneus*) and bryozoans (*Membranipora savartii*) (Brown and Swearingen 1998), commercially important invertebrates like blue crabs (*Callinectes sapidus*) and penaeid shrimp (*Peneaus* spp.) (Hulathduwa and Brown 2006) and resident nekton such as gobies (*Gobiosoma bosc*) and blennies (*Chasmodes bosquianus*) (Harding 1999). Hydrocarbon contamination of oyster reefs has been shown to have an effect on the composition of these communities (Hulathduwa and Brown 2006). Although these resident nekton and invertebrates are impacted by hydrocarbon contamination as are oysters, they also can play a significant role in shaping the

oyster reef community by decreasing survival of oyster spat through predation (Newell et al. 2000, Grabowski 2004, Newell et al. 2007, O'Connor et al. 2008). Common predators include mud crabs (*Panopeus* spp. and *Eurypanopeus depressus*) (Bisker and Castagna 1987, Grabowski 2004, Kulp et al. 2011), stone crabs (*Menippe adina*) (Brown and Haight 1992), blue crabs (*C. sapidus*) (Eggleston 1990), oyster drills (*Stramonita haemastoma*) (Garton and Stickle 1980, Brown and Richardson 1987), and predatory fish such as the black drum (*Pogonias cromis*) (Hulathduwa et al. 2007, Brown et al. 2008), many of which negatively affect oyster recruitment and survival (O'Connor et al. 2008). Thus after the *DWH* oil spill, how the types and sizes of predator populations might be impacted and subsequently affect oyster spat populations remained unclear.

The *Deepwater Horizon* oil spill therefore has potential to impact oyster reef communities by affecting the successful recruitment, growth, and cellular responses of oyster spat either by acute or chronic hydrocarbon and PAH contamination, and by altering the predator populations on the reefs. The purpose of my study is to determine how both hydrocarbon contamination and salinity affect oyster spat in Barataria Bay, Louisiana through effects on life-history and cellular processes, and top-down pressures. I will investigate the long-term effects (2-3 years post-spill) of hydrocarbon contamination from the *DWH* oil spill on oyster recruitment. However, since these long-term studies do not assess acute effects immediately after the spill, I am also interested in understanding how oyster recruitment is affected by immediate hydrocarbon contamination; do settling larvae preferentially wait to settle on uncontaminated surfaces and is their recruitment and growth impacted by the presence of hydrocarbons? I therefore also monitor recruitment to oiled substrates in the field, and my null hypothesis is that there will be no difference in oyster recruitment at each site, neither due to long-term nor short-

term hydrocarbon contamination. I am also interested in studying the effect of hydrocarbon contamination and salinity on oyster spat physiology at cellular and subcellular levels. My null hypothesis is that there will be no difference in oyster spat health among different oil and salinity treatment levels and combinations. My last project is designed to determine the relative impacts predators have on oyster spat survival at each of my four study sites by excluding access of certain sized predators to oyster spat. My null hypothesis is that there will be no difference in oyster spat survival due to excluded predators.

In summary, to determine the effect of salinity, hydrocarbon contamination, and predation on oyster spat densities and survival, a field study was conducted in Barataria Bay. A laboratory study was conducted to determine the impact salinity and hydrocarbon contamination have on oyster spat health and cellular function.

METHODS

Site Description

In Barataria Bay, Louisiana, four sites, two control and two oiled, were chosen based on Shoreline Cleanup Assessment Technique (SCAT) Current Shoreline Oiling data from September 2010 (Figure 1A). Control sites were defined as those that had "no" oil observed and oiled sites were those that received "heavy" oiling from the oil spill. Sites were also selected so that there was a high and low salinity location for each treatment (Table 1). Hackberry Bay is located in the northwestern section of Barataria Bay, Bay Jimmy in the northeastern section, and Grand Isle and Grand Terre are barrier islands at the mouth of the bay (Figure 1B). Sediment at all sites was silt mixed with sand.

During each sampling event, temperature (°C), salinity (PSU), and dissolved oxygen (DO) (mg/L) were measured with a YSI 85 meter. In October of 2011, 2012, and 2013, an Ekman sediment grab was used to collect two sediment samples from each site for total PAH concentration analysis. The concentration of PAHs in sediment was determined using standard tissue preparation, chromatographic cleanup, and gas chromatograph/mass spectrometry (GC/MS) techniques as described in the Association of Analytical Communities International (AOAC International) Official 2007.01 Method (2007). Significant differences in dependent variables over the two years (temperature, salinity, DO, and total PAHs) were analyzed separately at each site using a two-way ANOVA in SAS 9.3.

In both years, Grand Isle and Grand Terre had significantly higher salinity than Hackberry Bay and Bay Jimmy (Tukey *a posteriori test*, $p<0.0001$). However, temporal trends in salinity varied by year (Table 2). In 2013, salinities were lower than 2012 ($p=0.024$), especially in the late spring, due to higher spring rainfall and a resulting freshet, which caused a drop in

salinity at the low salinity sites during May 2013 and at the high salinity sites during June 2013 (Figure 2). Dissolved oxygen varied by site and year as the DO (mg/L) at Grand Isle and Grand Terre, was higher in 2013 than in 2012, but lower in 2013 than in 2012 at Bay Jimmy ($p<0.05$) with no differences between years at Hackberry Bay (Table 2). There were no differences in temperature across sites or years (Table 2).

The sites did vary in total PAHs in 2011, with Bay Jimmy having higher PAH concentrations than Grand Isle and Grand Terre (Tukey *a posteriori tests*, $p<0.001$) and Grand Terre having higher PAHs than Grand Isle ($p<0.0001$). Sediment samples were not taken at Hackberry Bay in 2011. In 2012, PAH concentrations dropped significantly at the two oiled sites, Bay Jimmy and Grand Terre ($p<0.0001$) but there was no difference between the two years at Grand Isle. Differences among sites were less pronounced in 2012, but Bay Jimmy had double, and significantly greater PAH concentrations than all other sites ($p=0.005$). PAH levels at Grand Terre, the other oiled site, were not different than Grand Isle or Hackberry Bay (Table 2). Sediment samples from 2013 were taken but results not received in time for publication of this thesis.

Study sites will henceforth be referred to by their physical descriptions rather than names in the results and discussion section: Grand Isle = control high salinity site; Hackberry Bay = control low salinity site; Bay Jimmy = oiled low salinity site; Grand Terre = oiled high salinity site (Table 1).

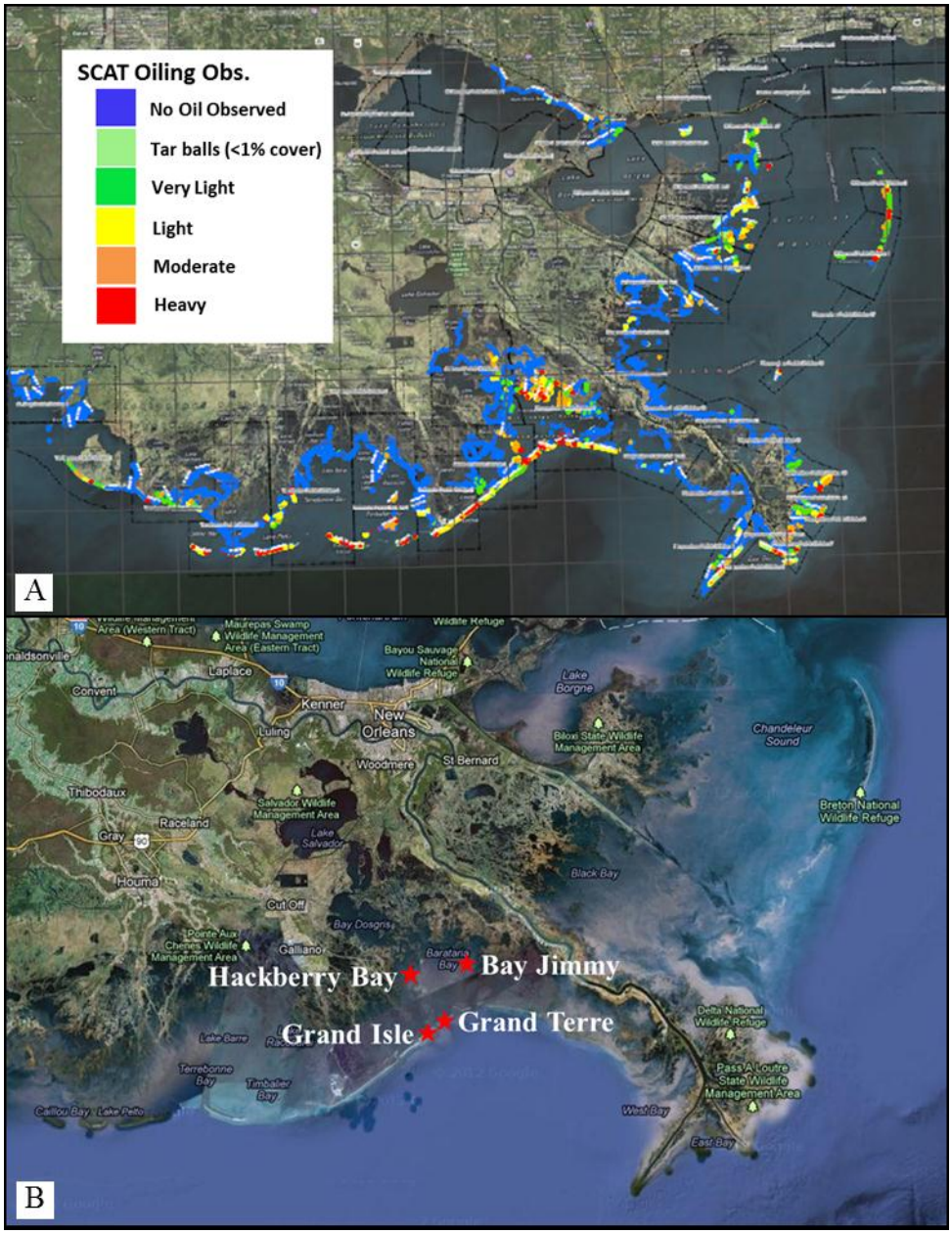

Figure 1. Sites in Barataria Bay selected based on SCAT surface data from 2010 used to assess oiling level (NOAA 2010b) (A). The four sites are: Grand Isle – control high salinity, Hackberry Bay – control low salinity, Bay Jimmy – oiled low salinity, and Grand Terre – oiled high salinity (B).

Table 1. Summary of treatment (oil or control) and salinity (low or high) classifications for each of the four study sites in Barataria Bay, Louisiana

Site	SCAT Data - Oil level	Site Classification	
		Oil	Salinity
Grand Isle	No oil observed	Control	High
Hackberry Bay	No oil observed	Control	Low
Bay Jimmy	Heavy	Oiled	Low
Grand Terre	Heavy	Oiled	High

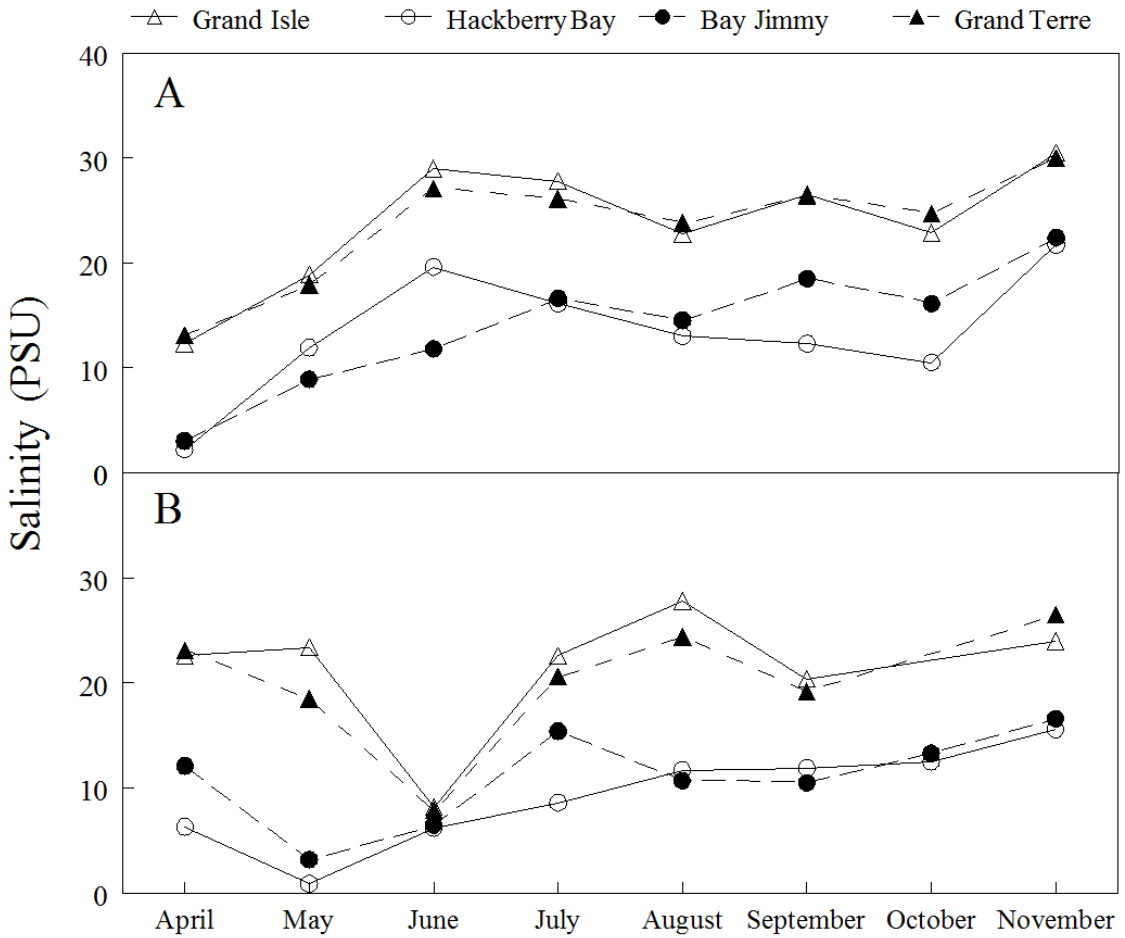

Figure 2. Salinity data collected for each site from April-November in 2012 (A) and 2013 (B). Grand Isle = control high salinity, Hackberry Bay = control low salinity, Bay Jimmy = oiled low salinity, and Grand Terre = oiled high salinity.

Table 2. Yearly water quality data (April – November) and total PAH concentrations in October of each year for each of the four study sites (averages ± standard errors). Statistical results from two-way ANOVAs for each measurement over site and year are also included.

	Temperature (°C)		**Salinity (PSU)**		**Dissolved Oxygen (mg/L)**		**Total PAHs (ng/g)**	
Site	*2012*	*2013*	*2012*	*2013*	*2012*	*2013*	*2011*	*2012*
Grand Isle	29.4 ± 1.04	29.78 ± 1.14	23.84 ± 2.13	20.2 ± 1.73	7.02 ± 0.30	8.05 ± 0.37	137 ± 8.0	115 ± 6.43
Hackberry Bay	28.08 ± 1.12	27.63 ± 0.83	13.41 ± 2.12	9.38 ± 1.09	6.31 ± 0.35	5.97 ± 0.31	--	171 ± 13.31
Bay Jimmy	27.8 ± 1.36	27.92 ± 0.92	13.98 ± 2.13	10.72 ± 1.09	5.88 ± 0.50	6.89 ± 0.44	519 ± 8.81	241 ± 14.58
Grand Terre	29.41± 1.27	28.71 ± 1.10	23.64 ± 1.94	18.45 ± 1.58	6.3 ± 0.20	8.07 ± 0.27	394 ± 22.0	139 ± 9.15
Source	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Site	0.71	0.574	17.35	<0.0001	5.08	0.004	135.8	<0.0001
Year	1.06	0.308	5.39	0.024	14.34	0.0004	323.5	<0.0001
Site*Year	0.08	0.971	0.07	0.978	2.08	0.114	63.16	<0.0001

Long-Term Oyster Recruitment Monitoring

Oyster spat recruitment was monitored as part of the Gulf of Mexico Research Initiative (GoMRI) and Coastal Waters Consortium (CWC) by placing ten 10 x 10 cm cement board tiles at each of the four sites monthly from April to October during the 2012 and 2013 field seasons. Tiles were placed in pairs, by attaching them with cable ties to one of five polyvinylchloride (PVC) poles. Tiles were positioned so that one surface (refuge surface) was placed adjacently to the PVC pole and facing the other tile. The other surface of the tile (exposed surface) was open to the water column. After one month in the field, tiles were collected and replaced with new cement board tiles. Tiles were placed in labeled plastic bags, immediately put on ice, and stored in a cold room until further analysis. The tiles were counted under a dissecting scope, and the number of every live oyster spat and barnacle on each surface (exposed or refuge) of each tile was determined.

Short-Term Oiling Study

To evaluate short-term differences in oyster recruitment on clean versus hydrocarbon-contaminated substrates, five days prior to deployment in the field, forty 10 x 10 cm cement board tiles were dipped into oil and placed in a covered glass container sealed with Parafilm®. We used approximately 600mL of light crude Louisiana oil with a hydrocarbon profile similar to Macondo-252 oil (determined by GC analysis, M. Scott Miles, Pers. Obs.). As each tile absorbed the oil, tiles were once again dipped into the remaining oil one day prior to deployment in the field; oil-soaked tiles absorbed approximately 5mL of oil each.

During sampling trips in June, July and September 2013 ten oil-soaked tiles were attached to five PVC poles and placed at all four sites, approximately 100 meters away from the control tiles. Oil-soaked tiles were collected one month later and the oyster spat and barnacle recruitment quantified as in the (control) recruitment tiles from the long-term sampling.

Cellular and Sub-cellular Responses of Oyster Spat to Oil and Salinity

To investigate how oyster spat respond at cellular and subcellular levels to hydrocarbon contamination in high and low salinity water, a laboratory experiment was conducted in August 2013. Oyster spat (18.61 ± 0.28 mm, average shell height ± SE) were obtained from the Auburn Shellfish Lab at Dauphin Island, Alabama. Approximately 60 spat were then added to each tank and checked every other day for mortality throughout the 10 day duration of the experiment. After 10 days, spat were sampled or preserved for different analyses. The six treatments used in this experiment were high (25,000 µg/L), low (500 µg/L), and no (0 µg/L), concentrations of oil in 200g of sediment in 200 liter tanks of either high (20 PSU) or low (10 PSU) salinity seawater (n=2 per treatment combination).

Condition Index - The average condition index (CI) was determined on 20 spat from each treatment at the end of the experiment. Tissue and shell samples were dried for 48 hours at 60°C and the CI calculated by dividing the dry tissue weight (g) by the volume of the internal shell cavity (whole shell weight (g) – wet shell weight (g)) multiplied by 100 (Lawrence and Scott 1982).

Histology analyses - Histological slides of oyster spat (n=8 per treatment) were prepared and examined using light microscopy for parasites and pathologies such as gregarines and *Perkinsus marinus*, and digestive gland atrophy as described in Kim et al. (2006). Digestive gland atrophy was observed at 100x and ranked from 0-4 with 4 indicating the greatest atrophy (Kim et al. 2006). Ten random microscope fields at 400x were viewed to determine the incidence of brown cells in the digestive gland and to evaluate the epithelial cells. Epithelial cells were evaluated visually for infection (Kim et al. 2006) and structure but then scored from 1-4 with 4 indicating the worst epithelial condition. Scores of 1 or 2 were considered "good" and scores of 3 or 4, "bad".

Lysosome Destabilization Measurements - The digestive gland lysosome destabilization rates and times were measured in a pooled sample of 5 spat (n=6 per treatment). Digestive glands of the five spat were removed and placed immediately on ice with 200 µL Pronase working solution (1g Pronase in 100 mL Tissue Culture Grade Water (TCGW) diluted 1:10 in oyster saline) until approximately 2.0 mm^3 digestive gland was obtained for each treatment. Tissue was dissociated using a pellet pestle motor and another 800 µL of Pronase solution added. After mixing, 100 µL of 5% FBS was added and the samples centrifuged at 20g for 2 minutes at 15°C. The supernatant was collected and centrifuged at 15°C for another 5 minutes at 200g. After discarding the

supernatant, the samples were rinsed 3-5 times by re-suspending the pellet and adding 1 mL of Cell Rinsing Solution (168 mg NaHCO3, 250 mg Glucose, 50 mg Galactose, 50 mg Trehalose with 4.250 g NaCL, and 225 mg KCl or 8.5 g NaCl, 450 mg KCl for 10 and 20 PSU solution, respectively, in 500 mL TCGW), and centrifuging for 5 minutes at 200g. Cells densities were counted and diluted to 1 x 10^5 cells per 1 mL. Neutral Red working solution (10 µL:1 mL) was added to each sample and 100 µL of each sample added to duplicate wells in a 96 well plate in the dark. Plates were centrifuged at 100g for 2 minutes and dye retention within lysosomes was estimated in approximately 100 cells after 15, 30, 45 and 60 minutes. Cells were counted using an inverted microscope at 400x in a dark room, and the time when approximately 50% of the cells were destabilized, Neutral Red Retention (NRR) time was noted. NRR time was determined as the time interval immediately prior to 50% of the cells becoming destabilized.

Predator Exclusion Study

In the 2012 field data, spat recruitment per tile surface was counted and analyzed, and appeared to vary on the exposed and refuge tile surfaces with refuge surfaces having greater recruitment overall (see Results section, Table 8, Figure 11). Results from this study shaped the methods for the 2013 predation experiment designed to determine if observed differences in recruitment on tile surfaces were due to predators. Predation can impact post-settlement mortality, and preliminary data suggested the composition of oyster reef commensal communities in 2012 were different across the four sites (Kay et al. 2013).

To test the relative impacts of different-sized predators at each of the four sites, predator exclusion cages containing pre-set spat tiles were deployed at each field site in August 2013 and collected 2 weeks later. Cement board tiles were placed in buckets at the Louisiana Sea Grant

Oyster Hatchery in Grand Isle, LA in mid-July 2013 with approximately 100 oyster pedi-veligers per tile. Oyster larvae were fed and allowed to settle on tiles for 2 days before being transferred to flow-through tanks for two weeks where they grew into larger spat. After two weeks (4.42 ± 0.12 mm, average shell height ± SE), the number of oyster spat on each surface of the tile was counted and recorded and the tile randomly placed into a labeled predator exclusion cage. Predator exclusion cages were labeled and grouped such that each group contained one of each cage type; the groups were then numbered (1-10) with color-coded cable ties.

Cages were constructed from 30 x 30 cm pieces of Vexar® mesh with 0.5, 1.0, and 3.0 cm mesh openings to exclude common oyster spat predators (Table 3, Figure 3A). Pre-set spat tiles were placed in the cage and 5-6 cm pieces of PVC placed on either side of the tile to ensure the tile was not touching the mesh cage (to prevent abrasion of spat off of tiles and to thwart predators from reaching spat from outside of the cage). Predator exclusion cages (n=10 per size, per site) were cable tied shut after the addition of the preset tile and PVC pieces. Control cages (n=4 per site) were constructed similarly to the exclusion cages, except one of the four sides was left open, with a 12-15 cm wide opening (Figure 3B). This was done to ensure that the cage itself had no effect on oyster spat survival and that predators could still access tiles. Tiles with the no cage treatment (n=10 per site) were attached to PVC poles along with the cages and placed at each field site (Figure 3A). Tiles were collected after two weeks to ensure that predators did not have time to consume all of the oyster spat. Tiles were collected, and placed in separate bags with label IDs corresponding to the site, cage type, and group number. Tiles were placed immediately on ice for laboratory analysis, and the number of oyster spat on each surface of the tile was counted to determine the percent survival of spat per cage type and site. Average spat shell height ± standard errors at the end of the two week period was 12.02 ± 0.04 mm.

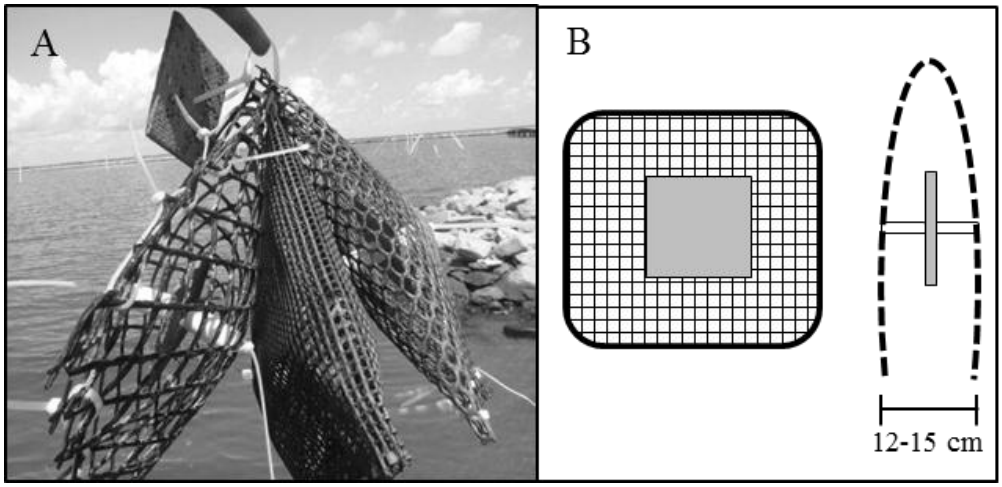

Figure 3. Predator exclusion cages prior to deployment in field. From left: No cage treatment, 3.0 cm, 0.5 cm, and 1.0 cm cages. Size (cm) of cages indicates width of mesh opening. (A). A diagram of control cages: tile in the middle of the cage (left) and a profile image showing the control cage opening on the unsealed portion of the cage created by pieces of PVC (right) (B).

Table 3. Predator exclusion cages with predators intended to be excluded and those with access to spat in each cage. Predators are not listed multiple times; if a predator is listed with access to a cage type, it is assumed that the same predator will have access in larger cages. Size (cm) of cage indicates size of opening in Vexar® mesh.

Cage Type	**Predators Excluded**	**Predators with Access to Spat in Cage**
0.5 cm	Most	Small mud crabs (approximately ≤7.5mm carapace width); flatworms
1.0 cm	Oyster drills, blue crabs	Mud crabs
3.0 cm	Blue crabs	Oyster drills
No cage	None	Blue crabs, fish
Control (1 side unsealed)	None	All

Statistical Analyses

Spat density per tile in 2012 and 2013 was analyzed by site and month using a two-way ANOVA separately for each year. Because of the late recruitment peak (see Results), April-July 2013 data were omitted to avoid excessive zeroes in the data. The average oyster density per tile surface during 2012 was analyzed using a two-way ANOVA comparing site and surface (exposed or refuge).

In the short term oil exposure experiment, spat and barnacle recruitment and growth on oil-soaked tiles were compared to the corresponding measurements on control tiles from the GoMRI sampling. Dependent variables (average number of spat or barnacles per tile surface) were analyzed separately for each month over site (four sites) and treatment (oil-soaked or control) using a two-way ANOVA. Oyster spat size was analyzed using a one-way ANOVA comparing the different spat sizes per treatment (oiled or control).

For all recruitment data (oyster or barnacle), log-transformed dependent variables were used to achieve normality. If a significant departure from normality still existed (Shapiro-Wilk test for normality), the model that produced the lowest AIC value was used.

In the laboratory study, the dependent variables of condition index, number of brown cells, digestive gland atrophy, and lysosomal stability were analyzed separately using two-way ANOVAs comparing oil dose (No, Low or High) and salinity (10 PSU or 20 PSU). The epithelial condition was first analyzed using a two-way ANOVA comparing oil dose and salinity but because normality could not be achieved, it was then analyzed using a "proc logistic" code for a multinomial logistic regression where the probability of spat in a treatment (oil dose and salinity) receiving a "good" score (1 or 2) was determined.

The log-transformed dependent variable of percent oyster spat consumed in no cage, control cages, or any type of cage (0.5cm, 1cm or 3cm mesh) was compared using a one-way ANOVA to determine if there was a cage artifact effect on oyster mortality in the pre-set predation study. Oyster predation among all cage types (0.5cm, 1cm, 3cm or no cage) and sites (four) was compared using a two-way ANOVA.

All statistical analyses were completed in SAS 9.3. If a two way interaction was significant, I compared each level of treatment within each level of the other treatment, using Tukey *a posteriori* tests to further understand differences, following Underwood (1981). The Tukey *a posteriori* test is indicated the first time in each paragraph, but only the p value is given for contrasts later in the paragraph. Where applicable, significant differences in comparisons from Tukey tests are indicated by different letters above histogram bars and the level of significance specified in the figure caption. Otherwise, significant Tukey differences are given within the text of the Results.

RESULTS

Long-Term Oyster Recruitment Monitoring

In 2012, there were differences in recruitment due to site (control or oiled) and month in the field, and a significant month by site interaction (Table 4). There were two recruitment peaks: one in June and the second in September for the high salinity sites (23.4 ± 1.26 PSU, average seasonal salinity ± SE), but for the low salinity sites (12.9 ± 1.41 PSU), there was only one recruitment peak during June. In June, the control low salinity site had significantly greater recruitment than the oiled low salinity site (Tukey *a posteriori* test, $p = 0.005$) and the control high salinity site ($p < 0.0001$). In September, there were no differences in recruitment between the two high salinity sites or the two low salinity sites, but the high salinity sites (26.5 ± 0.05 PSU, average ± SE) had significantly greater oyster spat recruitment than the low salinity sites (15.4 ± 3.1 PSU) ($p < 0.0001$) (Figure 4A).

In 2013, there were no differences in recruitment due to month, but the site and the month by site interaction were significant (Table 4). During 2013, there was very little recruitment at both the low and high salinity oiled sites. The control low salinity site (11.7 PSU) had significantly greater recruitment than any other site during August 2013 (Tukey *a posteriori* test, $p<0.0001$) and peak recruitment occurred one month earlier than the control high salinity site. In both September and October 2013, the control high salinity site (24.07 ± 2.14 PSU, average ± SE) had significantly greater recruitment than all other sites ($p<0.0001$) indicating recruitment lasted twice as long at this site (Figure 4B).

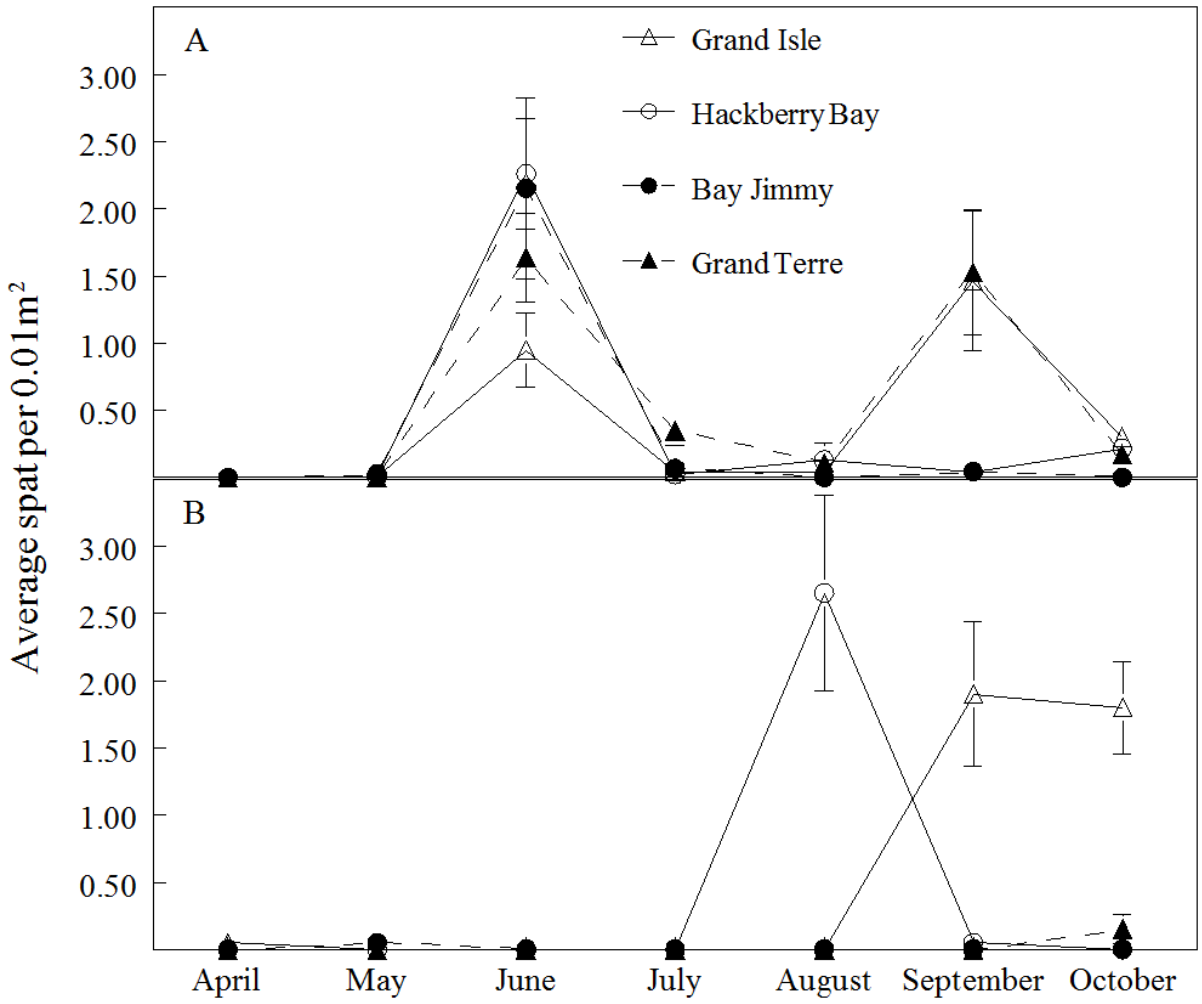

Figure 4. Number of spat per 0.01m^2 at each of the four sites during each month in 2012 (A) and 2013 (B) (average ± standard errors). Grand Isle = control high salinity, Hackberry Bay = control low salinity, Bay Jimmy = oiled low salinity, and Grand Terre = oiled high salinity.

Table 4. Statistics from the two-way ANOVA for oyster recruitment by month and site in 2012 and 2013.

	2012		***2013***	
Source	**F**	**p**	**F**	**p**
Month	49.76	<0.0001	0.24	0.7879
Site	4.72	0.0030	26.88	<0.0001
Month*Site	4.37	<0.0001	21.03	<0.0001

Short-Term Oiling Study

During the short-term oiling study, oyster recruitment occurred in only one month, September 2013. For these data, both the treatment and site effects were significant, but there was also a site by treatment interaction (Table 5). The greatest recruitment occurred at the control high salinity site, and recruitment was significantly lower on oiled than control tiles. However, there was no difference in recruitment among the control and oiled tiles at the control low salinity site and the oiled tiles at the control high salinity site (Figure 5). There was no significant difference in the size of recruited oyster spat on control tiles (1.97 ± 0.21 mm) and oiled tiles (2.66 ± 0.53 mm) (mean shell height over all sites ± standard errors) (one-way ANOVA, $F_{TREATMENT}=2.13$, $p=0.15$).

Barnacle recruitment occurred during all three months with one month (July) having a significant treatment effect, but significant site and treatment by site interactions during all three months (Table 5). In June, barnacle recruitment was greatest at the oiled low salinity site (Tukey *a posteriori* test $p<0.0001$) with high barnacle recruitment at the oiled high salinity site as well. At the oiled low salinity site, barnacle recruitment was significantly lower on the oiled tiles than control tiles, but was significantly lower on the control than the oiled tiles at the oiled high salinity site (Figure 6A). In July 2013, barnacle recruitment was higher on the oiled tiles at three sites: control high salinity and control and oiled low salinity sites, with no difference between treatments at the oiled high salinity site (Figure 6B). In September the greatest barnacle recruitment occurred at the two high salinity sites. At the control high salinity site, there was no difference in barnacle recruitment between control and oiled tiles, but at the oil high salinity site barnacle recruitment was significantly greater on the oiled tiles than the control tiles (Figure 6C).

Table 5. Statistics from two-way ANOVAs for treatment and site for oyster and barnacle recruitment in the short term oil exposure experiment.

	Oysters		**Barnacles**					
	September		*June*		*July*		*September*	
Source	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Treatment	13.08	0.0005	0.00	0.947	81.44	<0.0001	0.44	0.511
Site	21.88	<0.0001	224.15	<0.0001	34.03	<0.0001	159.24	<0.0001
Treatment*Site	10.26	0.002	20.27	<0.0001	23.78	<0.0001	4.08	0.008

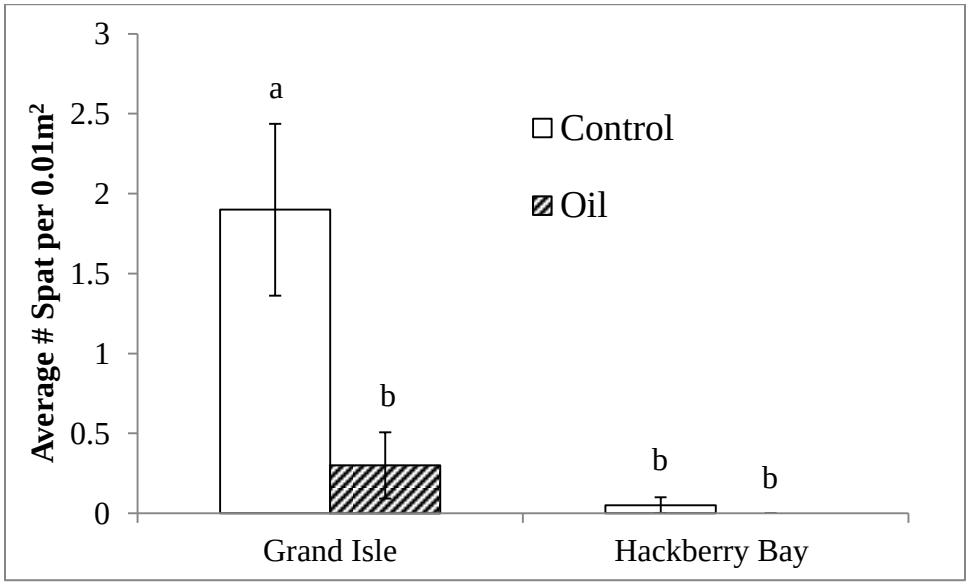

Figure 5. Spat density during the September 2013 short-term oiling study (average ± standard errors). There was no recruitment at Bay Jimmy or Grand Terre. Bars with different letters indicate significant differences in spat abundances (p <0.0001). Grand Isle = control high salinity, Hackberry Bay = control low salinity.

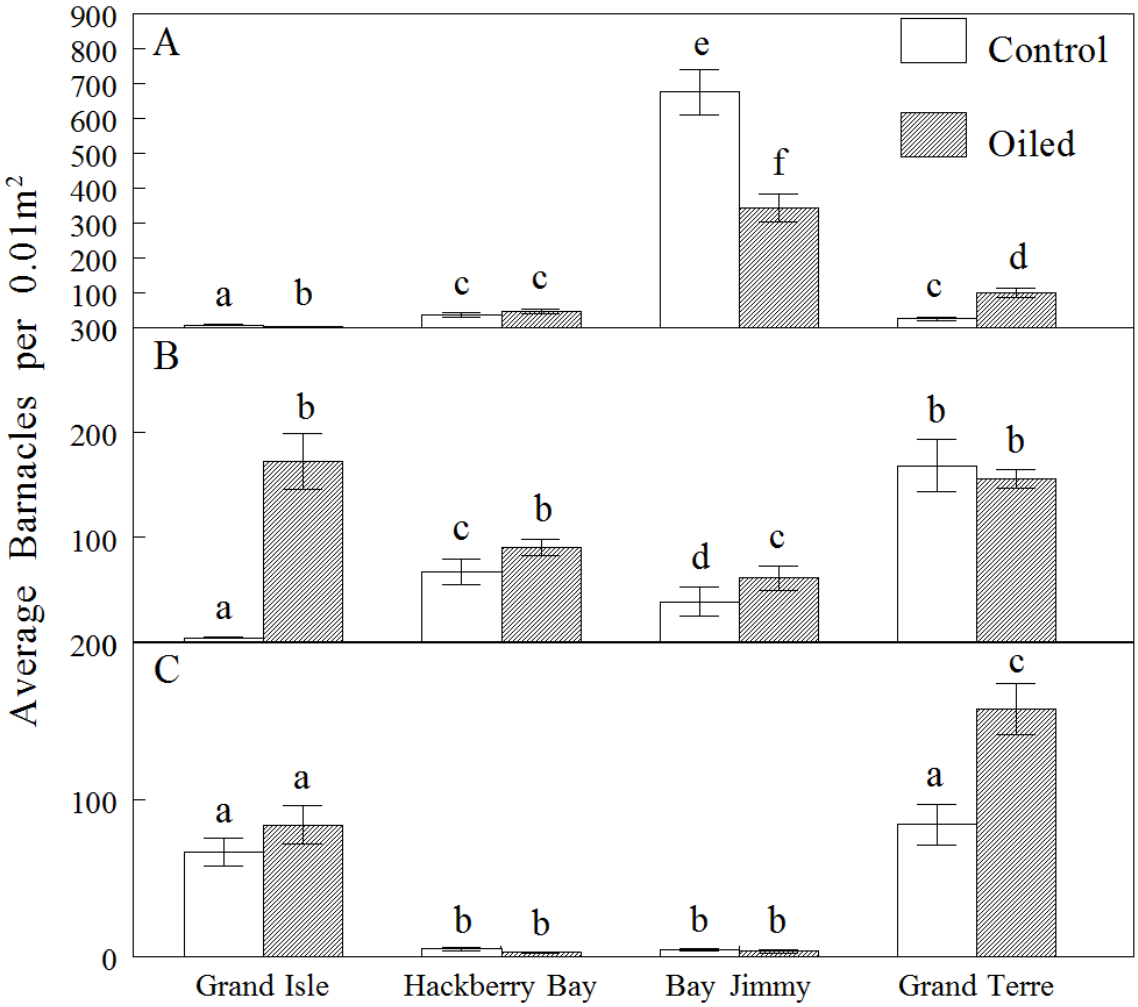

Figure 6. Barnacle density per tile at each site during June (A) July (B) and September (C) 2013 (average ± standard errors). Bars with different letters indicate significant differences in barnacle abundance during each month ($p < 0.05$). Grand Isle = control high salinity, Hackberry Bay = control low salinity, Bay Jimmy = oiled low salinity, and Grand Terre = oiled high salinity.

Cellular and Sub-cellular Responses of Oyster Spat to Oil and Salinity

Very little mortality of oyster spat occurred during the laboratory study. At the end of the experiment, there was no significant difference in oyster spat condition index among the six treatments (Table 6, Figure 7).

Histological analysis showed that in both treatments, oil and salinity had significant effects on the number of brown cells and digestive gland atrophy, but there were significant oil by salinity interactions as well (Table 6). Oyster spat in the low oil, 20 PSU salinity treatment had greater brown cell counts than all other treatments (Figure 8A). In the 10 PSU treatment tanks, oysters exposed to high doses of oil had greater digestive gland atrophy than oysters exposed to low and no oil. Compared to other interactions, oysters exposed to high doses of oil in the 20 PSU treatment tanks had slightly greater digestive gland atrophy scores than oysters in low doses but there was no difference in score between oysters in high and no oil doses and low and no oil doses. All oyster spat in the 20 PSU seawater treatment tanks had greater digestive gland atrophy than oysters in the low and no doses of oil in 10 PSU seawater, but there was no difference in oyster spat in the 20 PSU seawater treatment tanks and oysters in the high oil dose in 10 PSU seawater (Figure 8B). The probability of "good" gastrointestinal epithelial health appeared to increase in treatments with no oil (Figure 9), but there were no significant treatment effects (oil or salinity) on gastrointestinal epithelial condition (Table 7).

There was a significant oil effect on the time at which 50% neutral red retention (NRR) occurred in lysosomes (Table 6). On average, cells from oyster spat in treatments without oil had significantly later NRR times than those in low oil treatments (Tukey *a posteriori* test, $p=0.019$) and those in high oil treatments ($p<0.0001$). There was no difference in NRR time between oyster spat in low oil, 20 PSU seawater and spat in no oil, 10 PSU seawater. Between the two treatments with oil, spat in the high oil treatment had an earlier 50% NRR time than spat in low oil treatments ($p=0.028$) on average. Spat in the 10 PSU seawater with no oil had a greater NRR time than spat in low and high doses of oil. The same was true for spat in 20 PSU seawater; spat in no doses of oil had later NRR times than spat in low and high doses of oil (Figure 10).

Table 6. Statistics from two-way ANOVAs comparing oil and salinity effects on oyster spat health in the laboratory study.

	Condition Index		*Histology*				*Lysosomal Stability*	
			Brown Cells		Digestive Gland Atrophy			
Source	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Oil	0.18	0.834	3.70	0.0254	5.02	0.0069	18.59	<0.0001
Salinity	0.00	0.965	8.32	0.0041	9.05	0.0028	3.86	0.053
Oil*Salinity	0.05	0.620	4.68	0.0097	6.23	0.0021	0.06	0.939

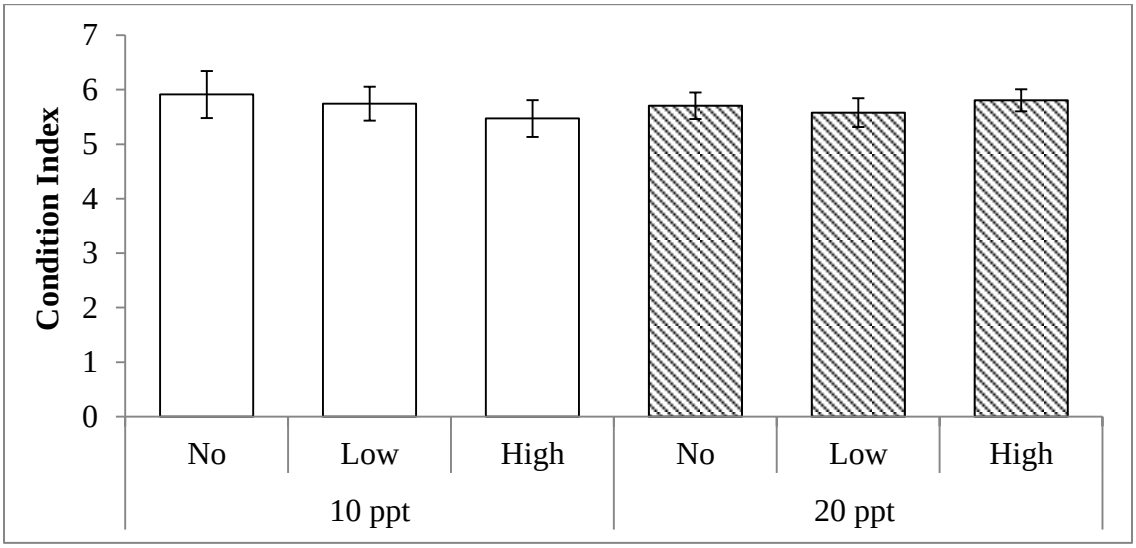

Figure 7. Condition index of oyster spat from each treatment at the end of the salinity and oil laboratory experiment (average ± standard errors).

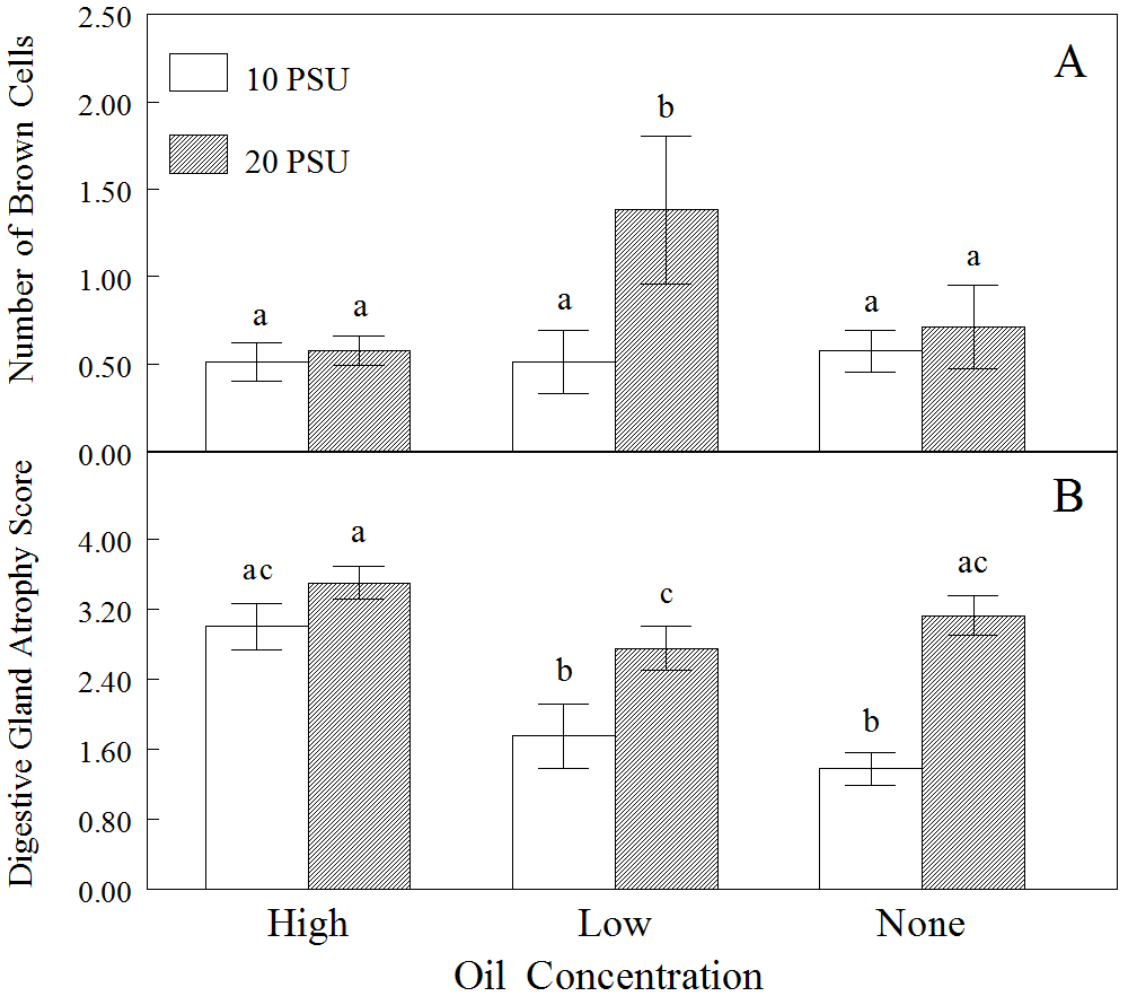

Figure 8. Results from histological analysis of spat in salinity and oil laboratory study. Average number of brown cells in ten microscope fields at 400x for each treatment ± standard errors. Different letters above histogram bars indicate significant differences among treatments (p=0.001) (A). Average digestive gland atrophy score (0-4) per treatment ± standard errors. A higher score indicates greater atrophy. Different letters above histogram bars indicate significant differences among treatments
(p<0.05) (B).

Table 7. Statistics from the multinomial logistic regression of oil and salinity effects on oyster spat gastrointestinal epithelial condition in the laboratory study.

Effect	**Wald Chi-Square**	**p**
Salinity	0.007	0.935
Oil	1.384	0.501
Salinity*Oil	0.339	0.844

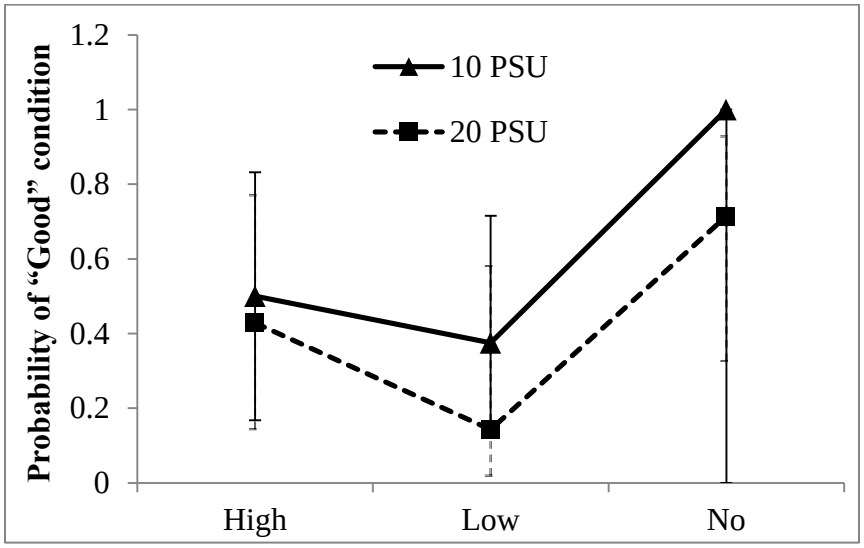

Figure 9. Probability of spat having "Good" gastrointestinal epithelial condition in the salinity and oil laboratory study. Upper and lower confidence limits are shown for each combination of two salinities and three oil treatments. There was no difference in condition probability among treatments.

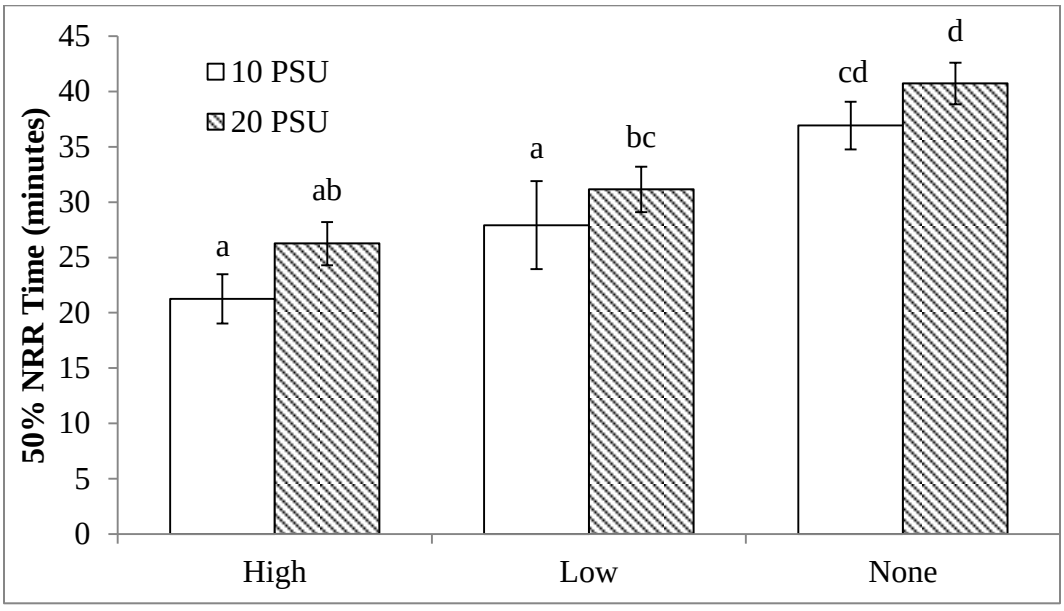

Figure 10. Time (minutes) when 50% of the cells became destabilized in each treatment in the salinity and oil laboratory study (average ± standard errors). Different letters above histogram bars indicate significant differences in NRR time between treatments ($p \leq 0.01$).

Predator Exclusion Study

During 2012, both surface of the tile and site had significant effects on oyster recruitment (Table 8). Oyster recruitment varied by site, but overall was greater on refuge surfaces than on exposed surfaces. At the two control sites, there were no differences in spat recruitment on the exposed and refuge surfaces. However, at the oiled sites, there were differences in oyster recruitment on exposed and refuge surfaces with greater recruitment on refuge surfaces than exposed surfaces at the oiled low salinity site (Tukey *a posteriori* test, $p=0.003$) and oiled high salinity site ($p=0.0001$) (Figure 11). Since this indicates spat survival increases in refuges, I decided to do a study on predation, and place spat in different mesh size cages, to determine whether I could identify specific predators responsible for these differences.

Predation rates among no cage, control cages, and fully enclosed cages (all sizes) were first compared to determine if there was a cage artifact affecting predation. There was a significant cage treatment effect on predation (one way ANOVA, $F_{CAGE}=15.39$, $p<0.0001$) with predation on tiles without cages significantly greater than predation on spat in any mesh size cage or control (3 sides sealed, 1 side open for predators) cage. There were no significant differences in predation on tiles in any type of cage (0.5, 1.0 or 3.0 cm mesh openings) or control cage (Figure 12).

Spat survival in 0.5 cm, 1.0 cm, 3.0 cm mesh cages and no cage treatments were then compared. The site and cage type were both significant effects, but the site by cage interaction was also significant (Table 9). No cage treatments had greater spat predation than any type of cage at three of the four sites: control low salinity, control high-salinity, and oiled high salinity (Tukey *a posteriori* tests, $p<0.005$) but not at the most heavily oiled, low salinity site (Figure 13). Oyster spat predation versus mesh size varied by site. At the control high salinity site there

was no difference in predation rates between 0.5 and 1.0 cm mesh opening cages but predation rates among all other cages were significantly different with mortality increasing as mesh openings increased or were absent (Figure 13C). Predation on spat on tiles with no cage at the control low salinity site was greater than all other cage sizes. In 0.5 cm mesh cages at the control low salinity site, predation was lower than predation on spat in 3.0 cm mesh but not from 1.0 cm mesh cages. On tiles in 1.0 cm mesh cages at this site, predation was not significantly lower than predation in 3.0 cm mesh cages (Figure 13A). There was no difference in predation between the 0.5 and 1.0 cm mesh cages at the oiled low salinity site. There was also no difference in predation in the 3.0 cm mesh and open cages at this site. However, predation in 0.5 cm and 1.0 cm mesh cages was lower than predation on spat in 3.0 cm mesh cages and tiles in no cage (Figure 13B). At the oiled high salinity site there was greater predation on tiles with no cage than any other cage type but there were no differences among the other cages (Figure 13D).

Predation on spat in the same mesh cage size varied by site with spat in 0.5 cm mesh opening cages at the control low salinity site experiencing lower predation than spat in the same size cages at all other sites (Tukey *a posteriori* tests, p=0.0001). However, predation on spat in 0.5 cm mesh opening cages was not different among the oiled low salinity site, oiled high salinity site or control high salinity site. Similarly, predation rates were lower in 1.0 cm mesh cages at the control low salinity site than in 1.0 cm cages at all other sites (p=0.001). Predation on spat in 1.0 cm cages at the control high salinity site was significantly lower than at the oiled high salinity site (p=0.008) but marginally lower than the oiled low salinity site (p=0.056). There was no difference in predation on spat in 1.0 cm mesh opening cages between the two oiled sites. At the oiled low salinity site, predation of spat in 3.0 cm cages was greater than all other sites (p=0.01). Spat predation in 3.0 cm cages was greater at the control high salinity site than at the

control low salinity site ($p<0.0001$) and oiled high salinity site ($p=0.048$), and greater at the oiled high salinity site than the control low salinity site ($p=0.027$). There were no differences in predation rates on tiles without cages at all four sites (Figure 13).

Table 8. Statistics from the two-way ANOVA for oyster recruitment by field site and tile surface (exposed versus refuge) in 2012.

Source	**F**	**p**
Site	3.08	0.0274
Surface	23.36	<0.0001
Site*Surface	1.65	0.1767

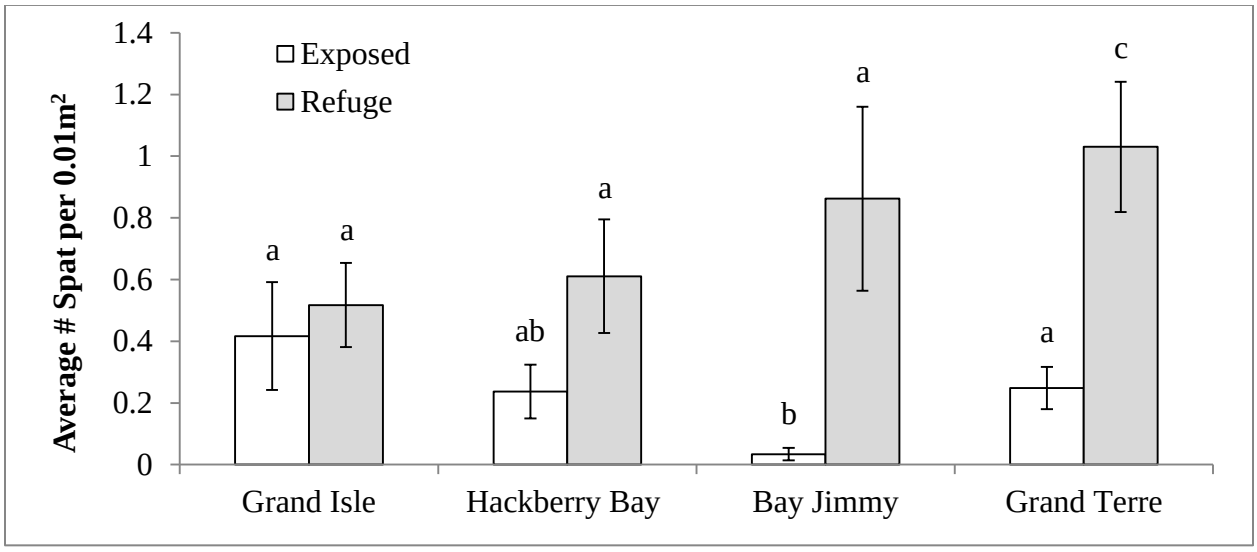

Figure 11. Number of spat per $0.01m^2$ recruitment tile surface (exposed or refuge) at each of the four sites during 2012 (average ± standard errors). Different letters above histogram bars indicate significant differences in recruitment on tile surfaces ($p<0.05$). Grand Isle = control high salinity, Hackberry Bay = control low salinity, Bay Jimmy = oiled low salinity, and Grand Terre = oiled high salinity.

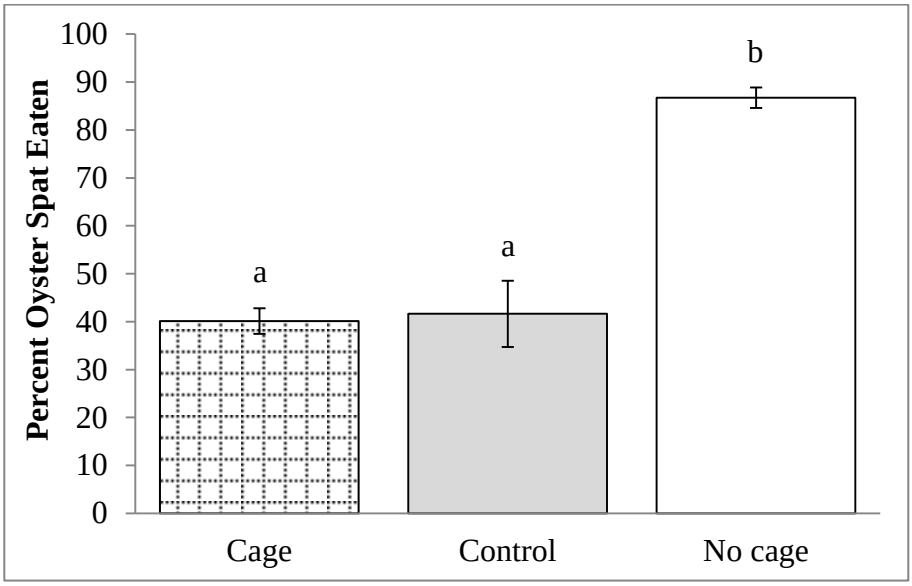

Figure 12. Percent oyster spat eaten in cage, control, and no cage treatments. Histogram bars indicate percent oyster spat consumed, averaged over sites ± standard errors. The cage histogram bar is the average percent spat eaten in 0.5 cm, 1.0 cm, and 3.0 cm (fully enclosed) cages. Letters above histogram bars indicate significant differences in percent oyster consumption per cage type ($p \leq 0.002$).

Table 9. Statistics from the two-way ANOVA for oyster predation (percent eaten) by site and cage type in August 2013.

Source	F	p
Site	31.54	<0.0001
Cage	65.14	<0.0001
Site*Cage	4.42	<0.0001

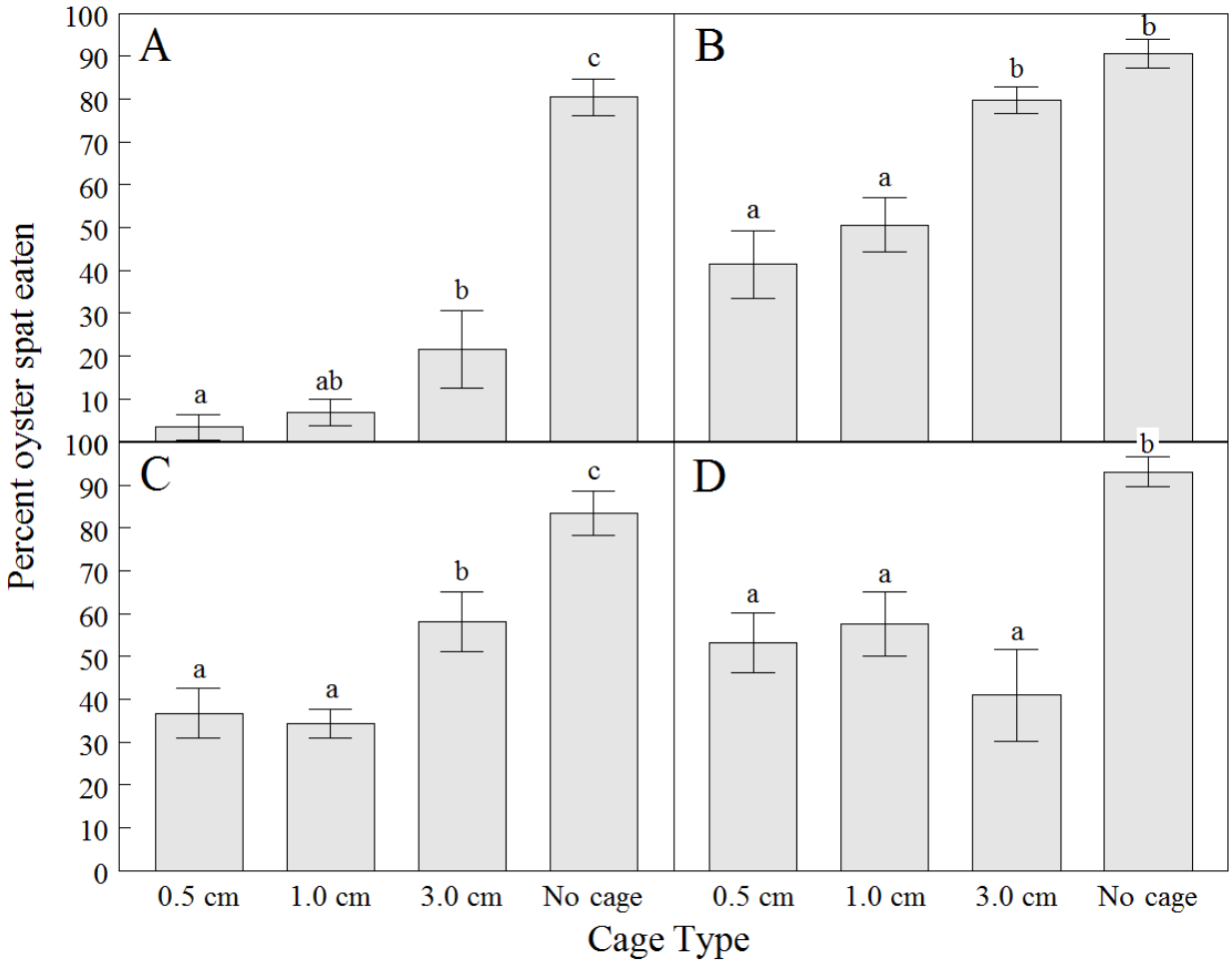

Figure 13. Percent oyster spat consumed per cage type at each site (average percent ± standard errors): control low salinity, Hackberry Bay (A); oiled low salinity, Bay Jimmy (B); control high salinity, Grand Isle (C); oiled high salinity, Grand Terre (D). Different letters above histogram bars indicate significant differences in predation among cage types at each site ($p \leq 0.01$). Differences in predation rates on spat in the same size mesh cage over all four sites, are given in the text.

DISCUSSION

In this study, there were no consistent long-term effects of hydrocarbon contamination on oyster spat recruitment between 2012 and 2013 which could be explained by three possible scenarios leading to differences in recruitment during these two years. One could be due to the temporal gap in sampling post-spill; sampling began two years after the spill, possibly missing any immediate effects of widespread hydrocarbon contamination on spat recruitment. Differences in recruitment in 2012 appeared to be more dependent on salinity, with similar recruitment occurring in the early summer at all four sites, but recruitment occurring only at the high salinity sites in late summer suggesting that long-term effects of hydrocarbon contamination are not typically prevalent and oyster recruitment varies more with physical parameters. Higher salinities in the gulf have been found to increase oyster recruitment and growth (Soniat et al. 2012). However, in 2013, recruitment only occurred in the late summer at the two (low and high salinity) unoiled sites, prompting further investigation into what other factors might influence long-term effects.

Eastern oysters typically spawn during the summer months when salinity is between 10-30 PSU and water temperatures reach approximately 25°C (Hayes and Menzel 1981, Supan and Wilson 2001). However, drops in salinity to less than 10 PSU, as observed during May and June 2013, have the ability to suppress spawning and oyster spat survival (Livingston et al. 2000, Pollack et al. 2011). La Peyre et al. (2013) found that the interaction of high temperatures (>25°C) and low salinity (<10 PSU) led to suppressed recruitment in southeast Louisiana in 2010 and 2011. Average temperatures from April-November exceeded 25°C at all sites in both 2012 and 2013, but in 2013, salinity was less than 10 PSU during the month of June, potentially explaining why delayed recruitment was observed at the control high salinity site, until spawning

and successful recruitment resumed when salinities exceeded 20 PSU in July and August 2013. While 10 PSU is not lower than salinities observed in 2012 at the two low salinity sites, salinity was less than 10 PSU at the control low salinity site in April 2013, dropping to around 1 PSU in May 2013 and remaining below 10 PSU until July 2013. Recruitment was observed at this site in August 2013 when the salinity finally climbed to around 12 PSU, suggesting that extended low salinity can affect oyster recruitment even for populations typically tolerant of lower salinity.

However, the initial stress of low salinity in combination with hydrocarbon contamination could also have resulted in decreased recruitment at the two oiled sites in 2013. In atypical years such as 2013, with increased rainfall and low salinity during early summer when oysters typically spawn, the lingering presence of hydrocarbons combined with lower than normal salinity could impact oyster spawning and successful spat recruitment.

Finally, the lack of consistency in long-term effects of hydrocarbon contamination on oyster spat recruitment suggests the need for continued long-term sampling beginning immediately following an oiling event and continuing for many years after. Oyster recruitment naturally varies from year to year (Brown and Swearingen 1998, Southworth and Mann 2004), so continued studies are necessary to capture how variation in abiotic factors such as salinity and temperature (La Peyre et al. 2013), or hypoxia (Osman 1994) can coincide with the presence of hydrocarbons, which can be re-suspended in the sediment through natural processes like wave action during storms, thereby reducing oyster recruitment (Santschi et al. 2001).

In the immediate, or short-term study of hydrocarbon contamination, results indicate that the presence of hydrocarbons can affect the successful recruitment of fouling species differently. At the site with greatest oyster recruitment in September 2013, lower recruitment on oiled tiles suggests that either oyster spat may preferentially settle on uncontaminated surfaces, or there is

greater post-settlement mortality on these surfaces. Smith and Hackney (1989) and Banks and Brown (2002) found suppressed oyster recruitment on oil coated surfaces in the intertidal zone due to sediment coating and in a laboratory setting, respectively, and results from my study also indicate there can be suppressed recruitment on oiled surfaces in the subtidal zone. Although recruitment was lower on oiled tiles, the effect of oil on oyster spat growth remains debatable: we observed no differences in spat size between the two treatments, yet other studies have found that oiled surfaces can lead to smaller spat sizes (Smith and Hackney 1989) or greater spat growth (Banks and Brown 2002). Spat recruitment appeared to be affected by short-term hydrocarbon contamination in 2013, but growth may have been more dependent on abiotic factors or food supply in southeast Louisiana (Deksheneiks et al. 1993). The pattern of barnacle recruitment on oiled tiles differed from that of oyster spat, suggesting that different fouling species respond differently to hydrocarbon contamination. Of the twelve barnacle recruitment measurements (four sites, during three months) taken in 2013, in two instances oil lowered barnacle recruitment but increased it in five, with no difference in the other five. These data suggest oil has a weak tendency to increase barnacle recruitment which could be explained by the presence of certain organic compounds found in crude oil (Hill and Holland 1985) or by the biological breakdown of oil into a biofilm that may even enhance recruitment (McCoy and Brown 1998, Banks and Brown 2002).

Overall, oil and salinity had a variety of effects on the cellular responses of oyster spat. A lack of differences in condition index of oyster spat in different treatments in the laboratory study is not surprising, as the oysters were fed regularly and the study was designed to look at the short-term cellular responses rather than long-term changes in spat health due to persistently stressful conditions. A previous study also found little difference in condition index of adult

oysters exposed to a variety of PAH and salinity levels, and that cellular responses tended to vary more with low salinity (J. La Peyre et al. 2014). Histological analysis from my study showed that oyster spat health varied with salinity, as spat in higher salinity water (20 PSU) tended to have decreased health than spat in lower salinity seawater (10 PSU), however, there were no consistent or significant trends among salinity and oil dose. A study conducted six months after the *Deepwater Horizon* oil spill, found there were no differences in wild oyster population infection rates due to the presence of PAHs in their habitat, but rather parasite prevalence tended to be more dependent on salinity (Soniat et al. 2011).

Bivalve growth potential has been shown to be negatively correlated with increasing digestive gland atrophy (Lowe et al. 1981) but based on our results we cannot draw conclusions on how oil and salinity affected spat growth potential. Histopathology of the oysters were examined as previous studies have found rates of infection from parasites and pathologies such as *Perkinsus marinus* tend to be greater in adult oysters in higher salinity seawater (Chu et al. 1993) or when exposed to PAHs (Chu et al. 2002), but no significant trends were observed in our study on juvenile oysters. However, lysosomal stability of oyster spat cells was dependent on the oil dose as the time for which cells could maintain stability decreased as oil concentration increased. Previous studies have found that lysosomes respond readily to contamination and stressors in their environment and less to salinity (Ringwood et al. 1998); stressors such as elevated PAH levels can lead to decreased cellular stability (Grundy et al. 1996) and because of these reactions, lysosomes have been shown to be good indicators of bivalve health in response to PAHs (Moore et al. 2006). Decreased lysosomal stability generally indicates a decline in cellular defenses and nutrition, and increased cellular damage (Moore 1982, Ringwood et al. 1998, Ringwood et al. 2003); results from this study suggest that oil has a significant effect on

cellular stability of oyster spat, potentially affecting their ability to grow and survive following an oil spill.

Results from the 2012 sampling revealed that oyster recruitment was lower on exposed surfaces than the refuge surfaces of tiles at the two oiled sites. It was unclear whether these differences were due to the natural preference of oyster larvae to settle on shaded surfaces (Michener and Kenny 1991) or due to post-settlement mortality by predators at each of the study sites. Preliminary data on the commensal community of oyster reefs revealed differences in community composition, perhaps due to the oil spill (Kay et al. 2013), which may result in location-specific predator effects on oyster spat survival requiring further investigation in 2013.

The amount of oyster predation in the control cages (3 sides sealed with one side unsealed creating a 12-15 cm opening for predators) was similar to predation in fully enclosed cages and much lower than predation on the tiles without cages, deviating from what was expected. The control cage was designed to allow predators' access to the oyster spat and to ensure there was no cage artifact affecting oyster spat survival. Cage controls have been used before when monitoring oyster and barnacle recruitment and have found reduced colonization densities on partially caged (control) and exposed tiles compared to fully caged tiles (Brown and Swearingen 1998). A key difference compared to previous predator exclusion studies is that this study deployed tiles pre-set with oyster spat to monitor predator effects whereas other studies have relied on natural spat set, where cage controls were necessary to ensure that reduced spat set on exposed surfaces was due to predation and not preference of oyster spat to settle on shaded surfaces (O'Beirn et al. 1996, Brown and Swearingen 1998). My results show that oyster survival was lower in the no cage treatment than the control cages (partially enclosed spat on tiles), suggesting that observed differences could potentially be attributed to physical parameters

such as altered water conditions like turbidity or light affecting spat survival (Kennedy 1986, Ritchie and Menzel 1969, and Kenny et al. 1990). Shaded surfaces can initially increase oyster recruitment, but as time in the field increases, overall recruitment decreases because of predators (Michener and Kenny 1991). Additionally, this experiment was conducted at subtidal sites, so spat did not face any desiccation threats whereby shading from a cage might increase survival by retaining moisture and reducing thermal stress (O'Beirn et al. 1996). Thus, I argue that the similarity of oyster spat survival in a fully enclosed cage and a control cage suggests that any type of barrier, even an incomplete one, is a sufficient deterrent of predators, thereby increasing oyster spat survival.

Cages have been found to significantly reduce predation (Johnson and Smee 2014) and my results agree as oyster spat predation was greatest on tiles without cages. While smaller predators such as flatworms and xanthid mud crabs can reduce spat survival significantly (Newell et al. 2000, Grabowski 2004, Newell et al. 2007, and Kulp et al. 2011), predators such as blue crabs and oyster drills could easily access spat on tiles in no cage treatments, and may play greater roles affecting oyster spat survival than the afore-mentioned smaller predators. Blue crabs, *Callinectes sapidus*, have been found to exert significant predation pressure on juvenile oysters (Eggleston 1990) and can tolerate lower salinities from 5-15 PSU (Soniat et al. 2012). Another prominent predator is the oyster drill, *Stramonita haemostoma*; although these snails can feed at low salinities, they are more common in high salinity estuarine environments where they have higher feeding rates (Garton and Stickle 1980). At the two low salinity sites, there was a clearer trend towards increasing predation as mesh size opening increased, unlike the high salinity sites, where there was less difference in oyster predation rates among different cage types. These differences in predation rates at different salinity locations indicate that at the low

salinity locations, blue crabs may be the more predominant predator than xanthid mud crabs or oyster drills, on exposed spat. Garton (1986) found that smaller oyster drills tend to feed on spat rather than adult oysters, and at our high salinity sites where these snails are more common, there may be a greater range in the size class of present oyster drills. A variety in the sizes of oyster drills able to feed on oyster spat protected in 1.0 cm and 3.0 cm cages may have led to the comparable predation rates observed in 0.5 cm, 1.0 cm, and 3.0 cm mesh cages at the high salinity sites indicating different predators can have distinctive effects on oyster survival.

Results from this study can help shape oyster lease selection by providing better insight into the relative impacts of predators in different areas in Barataria Bay and the most effective cages to deter predators. The predation study suggests that the presence of any cage type acting as a barrier will help reduce oyster spat mortality from predation. If predation is very high at a specific location in the bay, my results indicate that either blue crabs or predatory snails are likely having the greatest affect depending on salinity, and appropriately designed cages can be deployed to enhance spat survival.

Oysters in the northern Gulf of Mexico were and remain susceptible to effects from the wide-spread oiling that occurred following the *Deepwater Horizon* oil spill. Our long-term monitoring, and data on recruitment and spat health response to short-term oiling exposure can help guide oyster reef clean-up and restoration efforts in the event of a future oil spill in this region. Oyster recruitment is not significantly affected by long-term hydrocarbon contamination when the only pressure is oil, but in stressful years, such as in the event of flooding and water with below-normal salinity, hydrocarbon contamination can have a greater effect on oyster recruitment. Additionally, oysters may be more sensitive to short-term oiling than other fouling species such as barnacles, requiring persistent monitoring following an oil spill. Without careful

attention to the spawning of adult oysters and successful recruitment, there could be a shift toward more oil-tolerant, barnacle dominated communities, significantly affecting the oyster fishery and oyster reef ecology.

Also important to clean up efforts is an understanding of the sensitivity of oyster spat to oil contamination, especially if a spill occurs during the summer months when larvae are naturally settling and growing into spat. Results suggest that if exposure occurs for only a brief amount of time, condition index and histology of oyster spat remain unaffected, but that decreased cellular functioning can occur quickly, with significant effects appearing after less than 2 weeks of exposure. Although spat mortality may not result directly from oiling, a decline in oyster spat health can lead to population declines from decreased nutrition and increased infection.

Oyster populations in the northern Gulf of Mexico appear to be in better condition than other populations around the world, but are still susceptible to large-scale pollution events as evident from the *Deepwater Horizon* oil spill in 2010, in addition to the pressures they face from other human activities such as harvesting and altered habitat. Additionally, the energy and fishing industries of Louisiana's economy will continue to overlap in productive coastal areas for years to come suggesting further chances of another oil spill affecting vital estuarine habitats such as oyster reefs. As harvesting, management, and subsequent restoration of these ecologically and economically important species continue, it is important to have a full understanding how hydrocarbon pollution, salinity, and predators can affect oyster recruitment, overall survival and health to better manage this valuable resource.

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VITA

Maria grew up in Raleigh, North Carolina and later attended the University of North Carolina at Chapel Hill (UNC) where she graduated with a Bachelor of Science degree in Biology in 2010. While at UNC, Maria had the chance to study abroad in Brazil and spent a summer working in the Amazon and Atlantic rainforests helping her discover her appreciation for field and conservation ecology. Upon graduating, Maria completed a summer internship with the Albemarle-Pamlico National Estuary Program (APNEP). It was while interning with APNEP that Maria decided to pursue a career focusing on coastal and marine ecosystems. Thereafter, Maria accepted a job at the UNC Institute of Marine Sciences (IMS) in Morehead City, NC in Dr. Charles "Pete" Peterson's lab. While at IMS, Maria worked as a post-graduate research assistant and an AAUS certified diver on an extensive oyster restoration project in Pamlico Sound, NC. After working at IMS for two years, Maria was recruited to Louisiana State University to work on a project studying the long-term effects of the *Deepwater Horizon* oil spill on oyster communities in southeast Louisiana. Her research has been presented at two national conferences, and will aid in the better understanding of oyster reef management and restoration. Maria plans to continue studying marine systems to protect and manage these valuable and diverse communities.