Oyster recruitment in Barataria Bay following the Deepwater Horizon oil leak and opening of the Davis Pond freshwater diversion structure.

W. B. Stickle, C. Brunner, Xinmei Guo and Barry Aronhime, Department of Biological Science, Louisiana State University, baton Rouge, LA 70803

Abstract

This experiment investigates the recruitment of oyster spat (*Crassostrea virginica*) in hydrocarbon-contaminated and salinity stressed Barataria Bay. These results will help us to determine if and how exposure to hydrocarbons and mortality due to freshwater discharge from the Davis Pond Freshwater diversion structure affects oyster recruitment at Barataria Bay through the use of plankton samples and tiles that have been treated with seawater passing through oysters. Plankton samples are designed to determine the availability of oyster larvae for settlement to oyster spat. The pretreatment of the tiles simulates oyster shells as a substrate for oyster larval recruitment and settlement as spat in two sites that were previously exposed to the Deepwater Horizon oil leak (Mangrove Point and Bay Batiste), one that was subjected to salinity stress that killed adult oysters (Hackberry Bay), and one site with little oyster mortality in 2010 (Creole Bay). Six deployments of tiles for three weeks at all locations will allow us to begin to assess the seasonal impact of this recruitment process. The results of this experiment will be helpful in assessing the current and future state of oyster populations at oil impacted and salinity stressed locations on the Louisiana coastline.

Introduction

The Deepwater Horizon (DWH) MC252 oil spill released 4.4×10^8 +/- 20% barrels of light south Louisiana crude oil (Crone and Tolstoy, 2010) into the Gulf of Mexico. By the time the well was successfully capped on July 15, 2011, it was leaking 53,000 barrels per day. Approximately 665 miles of coastline were contaminated (http://www.popularmechanics.com/science/energy/coal-oil-gas/bp-oil-spill-statistics). The impacts and implications of this spill are currently being investigated and may not be fully understood for many years to come.

The Eastern Oyster (*Crassostrea virginica*) is a mollusk vital to the Gulf-Coast region Economy. Oyster reproduction is affected by a number of factors, including the presence of biofilms, pheromones, seasons, and salinity. This experiment investigates the effects the sharp and rapid decrease in salinity levels as a result of the opening of the Davis Pond freshwater Diversion structure had on the eastern oyster (*Crassostrea virginica*) population of Barataria Bay, Louisiana. Natural oyster beds of *C. virginica* can tolerate a salinity range of 10-30 psu in the estuaries of the Gulf of Mexico (Berrigan et al. 1991). Areas where the salinity is predominately less than 10 psu hinder oyster spawning and result in a decreased numbers of oyster larvae in the water column, which results in less oysters settling as spat (Supan, 2002). Although areas where the salinity is predominately greater than 15 psu facilitates increased spat numbers, the survival rates are less due to significant predation (Supan, 2002). Garton and Stickle also observed increased predation as a result of increased salinity in laboratory studies by Garton and Stickle (1980). On 30 April, 2010 an immense diversion of freshwater from the Mississippi River was re-routed into Barataria Bay, a significantly productive estuary in the Mississippi River Delta complex to prevent oil from entering the inland marshes of Barataria Bay in order to prevent what would be a nearly impossible oil cleanup of the maze of Louisiana marshes. A study was conducted in order to assess the initial effects of the Davis Pond freshwater diversion (FWD) on Barataria Bay, Louisiana. A number of physical and chemical properties were measured in Barataria Bay in July 2010 and September 2010, subsequent to the Davis Pond FWD. Samples were taken for chemical, spectroscopic, and bacterial analyses. In addition, dissolved oxygen, temperature, and salinity were measured. The results showed that the Davis Pond FWD might have ameliorated some of the harmful effects of the oil, as the levels of dissolved organic matter were decreased. However, the measured salinity showed substantial spatial and temporal variations. Specifically, salinity was much lower in the interior of Barataria Bay (Bianchi 2011). We sought to investigate the effects of salinity stress caused by the Davis Pond FWD and resulting decreased salinity implications for the eastern oyster populations in this area.

The purpose of this experiment is to evaluate the recovery of oyster reefs from oil pollution and low salinity by monitoring the number of pediveliger larvae in the water column and spat settlement on artificial tiles at each of the four sites to determine how Deepwater Horizon oil pollution and changes in salinity since the opening of the Davis Pond Fresh Water Diversion have affected oyster spat recruitment. In addition, the number of ectoprocts, barnacles, and mussels recruited was also noted at each site. The results of this experiment will provide useful data for the future of the oyster fisheries in Barataria Bay, LA.

MATERIALS AND METHODS

Site Description

We chose four experimental sites in Barataria Bay. Each site was characterized by different ranges of salinity as well as different exposures to leaked oil as a result of the Deep Water Horizon Spill (DWH). Mangrove Point (Site 1) exhibited high salinity and was heavily impacted by oil. Creole Bay (Site 2) exhibited mesohaline salinities and was deemed healthy, meaning it was not oiled as a result of the DWH. East Hackberry Bay (Site 3) was salinity stressed as a result of the opening if the Davis Pond diversion structure but was not impacted by Deepwater Horizon oil. Bay Batiste (Site 4) exhibited mesohalane salinities and was oiled as a result of DWH.

Veliger larval abundance

A 0.5m diameter 80µ mesh plankton net was used to determine the number of veliger larvae present in the water column. Plankton tows were conducted during the deployment and retrieval of oyster spat collecters durinr the Fall Deployment season. This mesh size has been routinely used to collect oyster veliger larvae in previous oyster larval studies (Galtsoff 1964) A flowmeter was positioned in the center of the net opening in order to quantitate the volume of seawater filtered during a 10 minute plankton tow on each deployment and sampling date. The initial and final readings of the flow meter were recorded to calculate the amount of water filtered through the net, and the samples collected in the net were poured into two collecting jars with 10% formalin to preserve the plankton samples.

Calculations of seawater filtered

$$\text{Distance Travelled (m)} = \frac{(\text{flow meter}_{\text{final}} - \text{flow meter}_{\text{initial}})}{999{,}999} \times 26{,}873$$

$$\text{Vol. of water filtered } (m^3) = \underline{3.14 \times (\text{diameter of net in m})^2} \times \text{Distance Travelled (m)}$$

D stage Veliger Counts

Two containers of sample water are poured into a 1400 mL graduated cylinder to determine the volume of the plankton sample. The sample and a stitrring bean was placed in a 2l beaker on a stirring plate. After 5 minutes of stirring, a 5 mL Hensen Stempel Pipette is used to collect a sample of water from the cylinder, and the water is transferred to a 1810 Ward Counting Wheel. The counting wheel was placed under a dissecting microscope, and turned towards one of the ends. Once the number of veliger larvae were counted in the viewing area, the counting wheel was turned to a new viewing area, and the veliger larvae were counted.This continued until the end of the counting wheel chamber was reached. The counting process was repeated four more times for a total of five replicates.

The number of D stage veligers per m^3 of seawater was calculated with the equations given below:

$$\text{Total veligers in sample} = \frac{\text{vol.}_{\text{total}} \text{ sample in mL}}{5 \text{ mL}} \times \text{\# veliger in 5 mL sample}$$

$$\text{\# Veligers}/m^3 \text{ of water} = \frac{\text{Total veliger in sample}}{\text{Vol. of water filtered } (m^3)}$$

Materials for Spat Settlement

15cm x 15cm unglazed quarry tiles were used in this experiment. The ridged sides of the tiles were placed in the water facing outwards to allow access to a surface more conducive to oyster settlement than a smooth surface.

We built 12 structures to hold the collection tiles. The structures were made using 2 “diameter and10’ long PVC poles, with 2 30” crosspieces cut in half lengthwise to form channels and attached to each pole so that the tiles would fit tightly in between the crosspieces. Four tiles were placed on each side of the crosspiece apparatus for each pole. Ziplock ties that ran through holes drilled in the crosspieces were used to secure the tiles in place. In addition, buoys were attached to the tops of the poles to make the poles easier to find upon collection and to distinguish our collection structures from other poles used to designate the oyster reef areas of the oystermen.

Collection Methods

Oyster spat settlement on tiles

This project is modeled on two earlier studies of oyster spat settlement in Barataria Bay, LA (Brown and Swearingham 1998; and Banks and Brown 2002). The oyster spat collection tiles were conditioned in a seawater table with 0.5 of a sack of oysters obtained from Barataria Bay 1 week prior to deployment. The tiles were placed ridged side up in the tank. The Instant Ocean synthetic seawater was maintained in the salinity range of 14-19 psu.

Tiles were deployed and collected three weeks later once every 3 weeks on June 9 (Spring deployment), August 24, September 14, October 5, October 26, and November 21, 2011(Fall deployment). The last collection was on December 12, 2011 for a total of 6 replicates over time. The tiles were placed ~ 1 m below the seawater surface so settlement occurred on subtidal substrates. We used 4 ice chests to transport the tiles, one for each site. Tile racks were constructed to keep the tiles apart. Tiles were transported in the ice chests while covered with seawater to keep them wet. A total of 12 structures were used, placing 3 at each site and labeling each structure A, B, and C at each of 4 sites: Mangrove Point (site 1), Creole Bay (site 2), Hackberry East (site 3), and Bay Batiste (site 4). 8 tiles were secured in each structure, with the ridged sides facing outwards. 4 tiles on each structure remained in the water for the duration of the spring deployment and again during the fall deployment, while the other 4 were collected every 3 weeks and replaced with newly conditioned tiles. Once the tiles were in place between the crosspieces of each structure, zip ties were used to pull the ends of the crosspieces together on either side to prevent the tiles from slipping out of the sides of the structure. Zip ties were also secured around the middle of the crosspieces for additional support. The tiles retrieved from the water were carefully placed in the tile racks to be brought back to the lab for analysis.

We attached a Hobo temperature/salinity probe to each of the B structures at all 4 sites. The probe was programmed to record temperature, salinity, and conductivity data every 15 minutes. We captured the data from each probe every trip. In addition to the data recorded by the probes, we recorded longitude and latitude for each site, and we used a Model 85 YSI conductivity meter to measure the temperature, salinity, conductivity, and dissolved oxygen at each site at that point in time.

Data Analysis

Tiles

Upon return to the lab, the tiles were carefully placed in a seawater table rough side up until analysis within 24 h. Each tile was carefully analyzed for the presence of oyster spat, ectoprocts, barnacles, and mussels using both the naked eye and a dissecting microscope (10x). Barnacles were counted by percent coverage, while oyster spat, ectoprocts, spat, and mussels were individually enumerated.

Statistical Analysis

Upon completion of this project data will be subject to ANOVA and *a posteriori* tests using SAS to perform the analyses. We will consult with Dr. Barry Arronhime in our Department and a Ph.D. student of Dr. Ken Brown who was coauthor on the two settlement projects which served as the basis for our experimental design.

Results

Veliger larvae abundance

Veliger data were not normally distributed (Shapiro-Wilk W = 0.70, p<0.0001). We used a gamma distribution in Proc Genmod (generalized linear models) to analyze the data. There were significant date (chi-square = 441.38, p <0.0001, df = 5), site (chi-square = 158.93, p<0.0001, df= 3), date*site interaction (chi-square = 317.80, p<0.0001, df=14) effects. We used a post hoc test using z scores. Significant differences by date at each site occurred regularly. D stage veliger larval abundance exhibited peaks on October 5, 2011 at Mangrove Point, Creole Bay, and Hackberry Bay and a higher peak abundance occurred on October 26, 2011 at Creole Bay, Hackberry Bay and Bay Batiste.

D stage veliger larval abundance peaked on October 26, 2011 at Creole Bay and Hackberry Bay Louisiana but was very low in August and September 2011(F

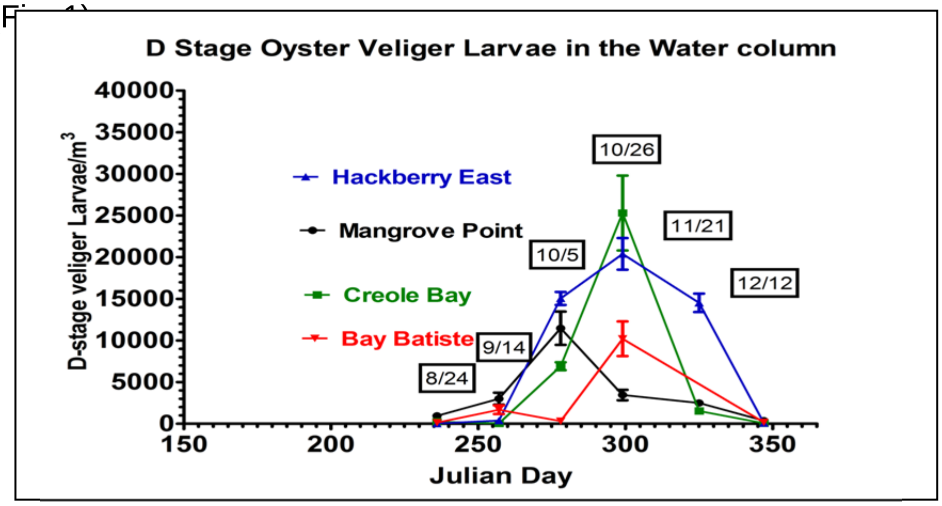

Figure 1. D stage oyster veliger abundance in Barataria Bay Louisiana in the fall of 2011.

Oyster Spat Settlement on Tiles

In comparison to an earlier study in lower Barataria Bay by Banks and Brown (2002) (Fig. 2) oyster spat settlement was low in our study throughout Barataria Bay (Fig. 3).

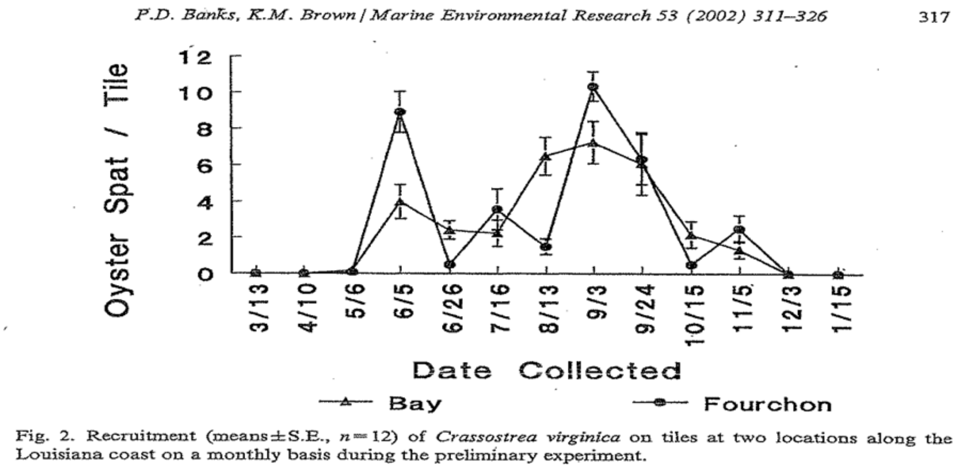

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Fig. 2. Recruitment (means±S.E., $n=12$) of *Crassostrea virginica* on tiles at two locations along the Louisiana coast on a monthly basis during the preliminary experiment.

Figure 2. Banks and Brown (2002). Sampling sites were Bay Champagne (subtidal) and Port Fourchon (Intertidal).

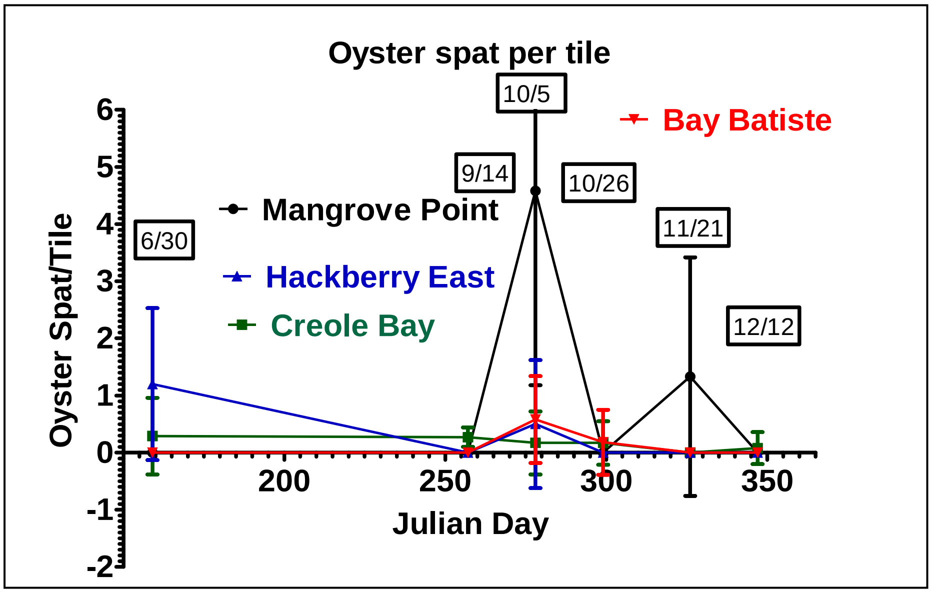


Figure 3. Spat settlement on subtidal tiles during three week deployment periods at four sites in Barataria bay in 2011. The November 21, 2011 collection was made after 28 days deployment due the bad weather on Barataria Bay the previous week.

The sum of oyster spat settlement at the four sites in Barataria Bay was higher than the spat settlement rate over the entire fall season of 2011. Spat settlement at Mangrove point was much higher that at the three sites within the productive oyster reef zone. The cumulative spat settlement as a percent of the sum of the three week deployments was also higher at Mangrove Point (56.4%survival) than at Creole Bay (27.9% survival), Hackberry Bay (25% survival) or Bay Batiste (18.4% survival). Several southern oyster drills (*Stramonita haemastoma*) were observed on the oyster spat settlement structures at Mangrove Point on October 26 but not at any of the other sites.

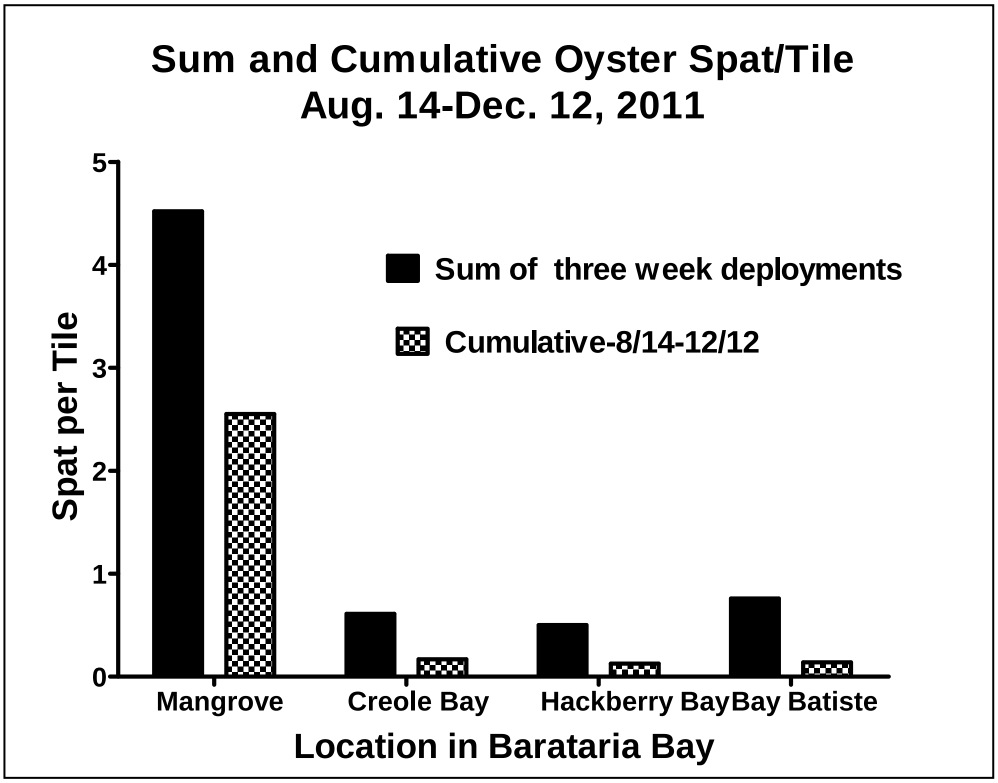


Figure 4. A comparison of the sum of three week deployments at four sites in Barataria Bay and the net spat settlement over the fall season.

A significant number of oyster spat settled at Mangrove Point (26 spat). The size range of the settled spat was correlated with two periods of spat settlement as shown in Figure 3 on October 5, 2011 and November 21, 2011.

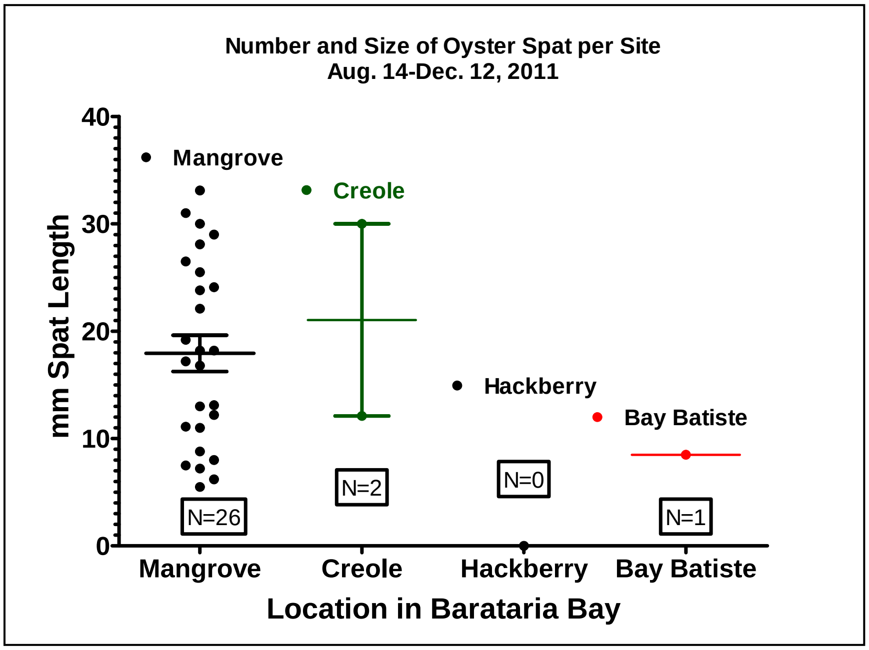


Figure 5. Number and size range of oyster spat that settled on tiles at the four sites in Barataria Bay in the Fall of 2011.

Summary of D-stage Veliger Abundance and Spat settlement in Barataria Bay, LA.

- D stage oyster veliger abundance was highest on October 26th 2011.
- Oyster spat recruitment but was low throughout the fall spat recruitment season in our study compared to earlier recruitment studies in Barataria Bay.
- Recruitment peaked on October 5, 2011 at 4.58±3.40 (12 tiles) spat per tile at Mangrove Point which is a salinity location in Barataria Bay only used for bedding oysters.
- In comparison to Mangrove Point, oyster spat recruitment was low throughout the fall at Creole Bay, Hackberry Bay, and Bay Batiste within the public and private oyster lease areas of Barataria Bay.
- There is an uncoupling of D stage oyster veliger larval abundance in the water column and spat settlement due to some inhibition of larval settlement.
- Oyster spat recruitment was low throughout the fall in our study compared to earlier recruitment studies in Barataria Bay.

Barnacle Settlement on Tiles

The barnacle *Balanus eburneus* (Gould) settled on the tiles throughout the fall of 2011. **Barnacle** settlement data were also not normal (W=0.92, p<0.0001). We used a negative binomial distribution and found significant date (p<0.0001), site (p<0.0001) and date * site (p<0.0001) effects. We used a post hoc test using z scores. Significant differences by date at each site occurred regularly. 10/25 differs from other sampling periods at many sites. Highest barnacle settlement occurred at Mangrove Point with the highest settlement time occurring on October 5, 2011. The highest rates of barnacle settlement occurred on July 2 and October 5, 2011 at our four sampling sites in Barataria Bay.

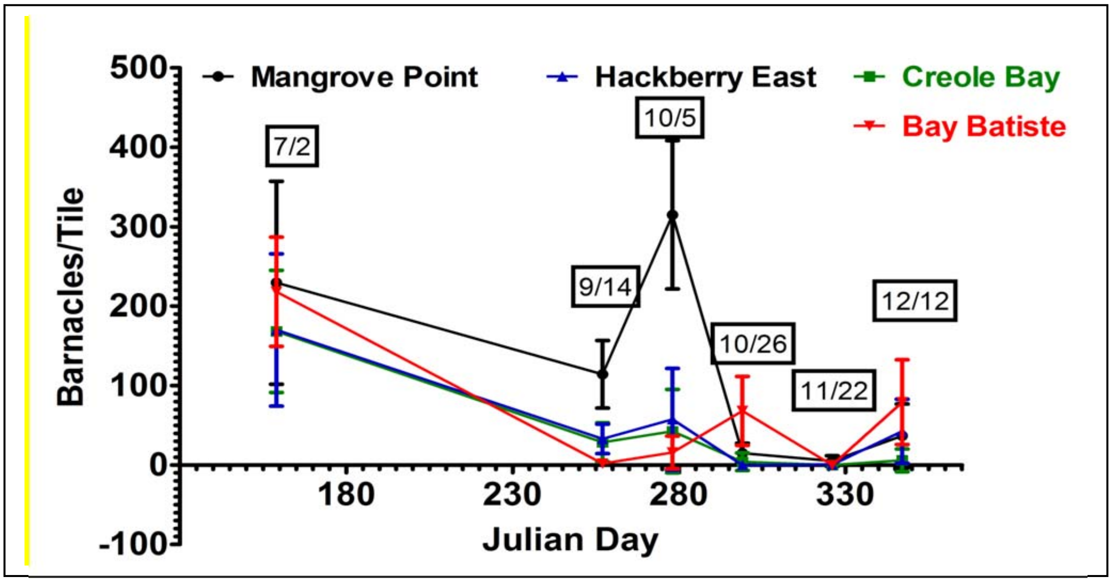


Figure 6. *Balanus eburneus* abundance in Barataria Bay Louisiana in 2011.

Ectoproct Settlement on Tiles

The primary recruitment of the Bryzoan *Membranipora savartii* was on October 26, 2011 at Creole Bay, Hackberry Bay and Bay Batiste. Minimal recruitment occurred at the high salinity site at Mangrove Point.

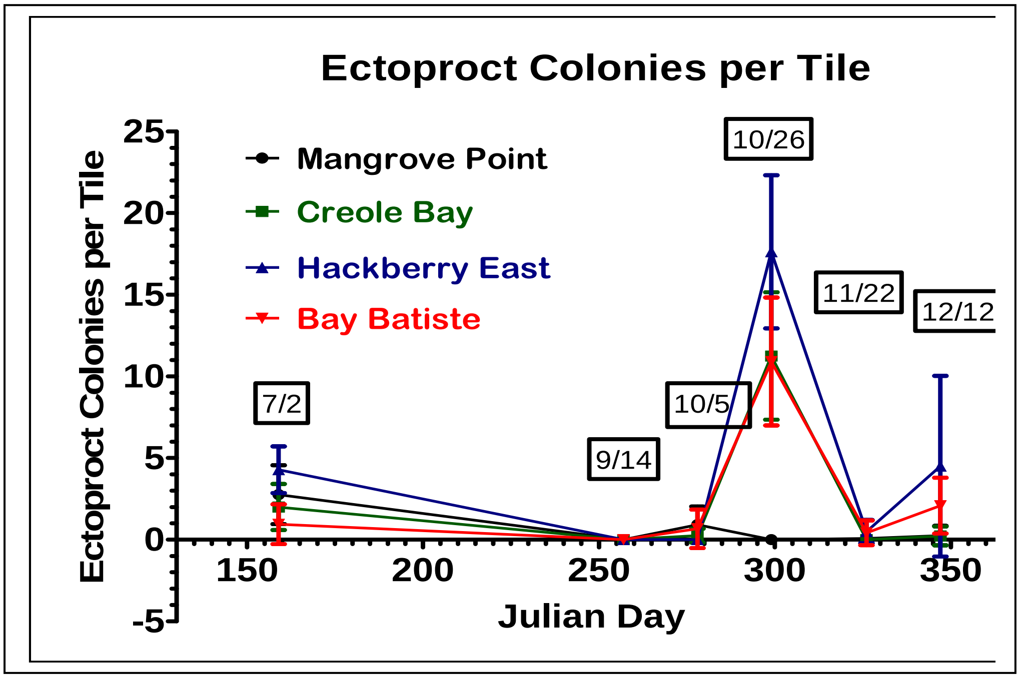


Figure 7. Colonies of the bryozoan *Membranipora savartii* on settlement tiles.

Hooked mussel recruitment

Ischadium recurvum recruited to tiles at Creole Bay, Hackberry Bay, and Bay Batiste during the fall of 2011 but not at the high salinity site at Mangrove Point. The results are based on the tiles which were deployed all fall. Mussels recruited to the tiles on10/5, 10/26, 11/22 and 12/12.

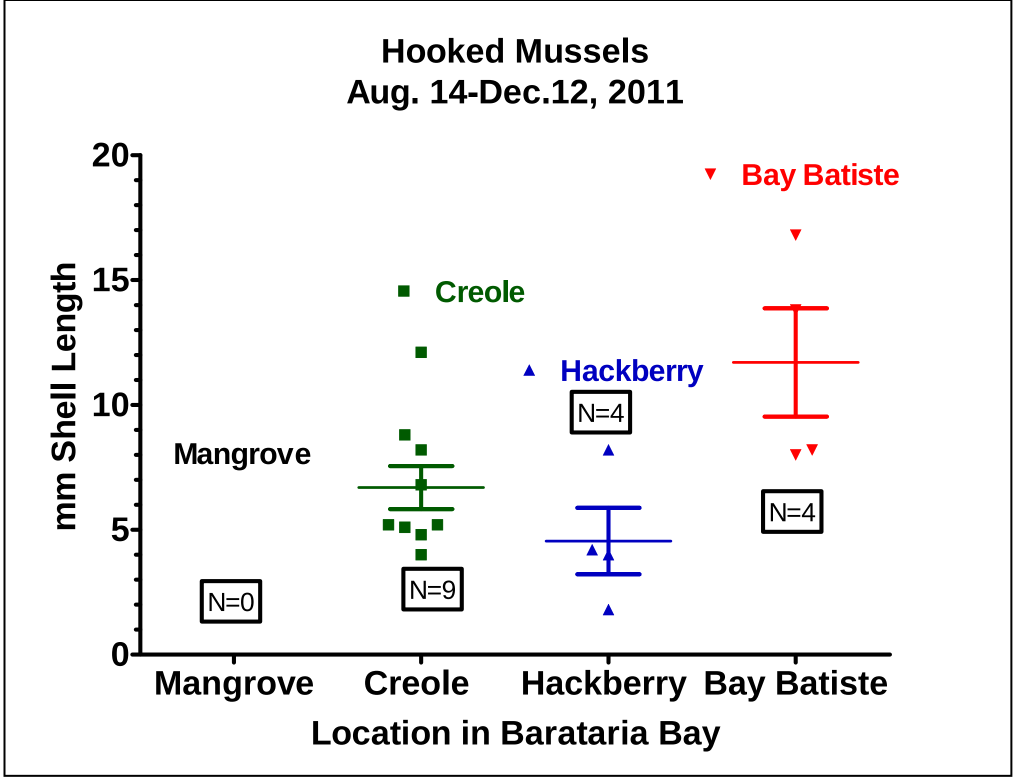


Figure 8. The number and length of hooked mussels, *Ischadium recurvum*

Abiotic Factors at the spat collection sites in Barrrataria Bay during the fall of 2011.

Point in Time Measurements

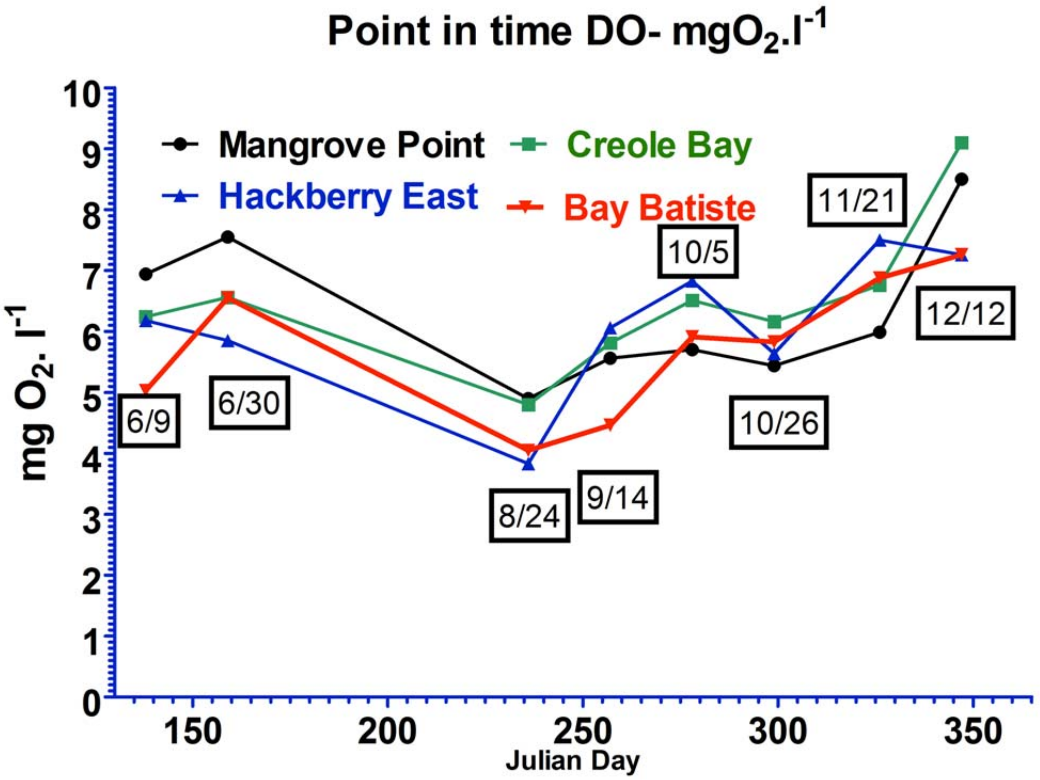


Figure 9. Point in time dissolved oxygen levels at the four spat deployment sites in Barataria Bay in the fall of 2011.

Water Temperature

Seawater temperature followed the same trend at all four collection sites in Barataria Bay and was still above 20°C (20.7-21.7°C on November 21, 2011. Water temperature dropped to 11.4-12.1°C by December 12, 2011.

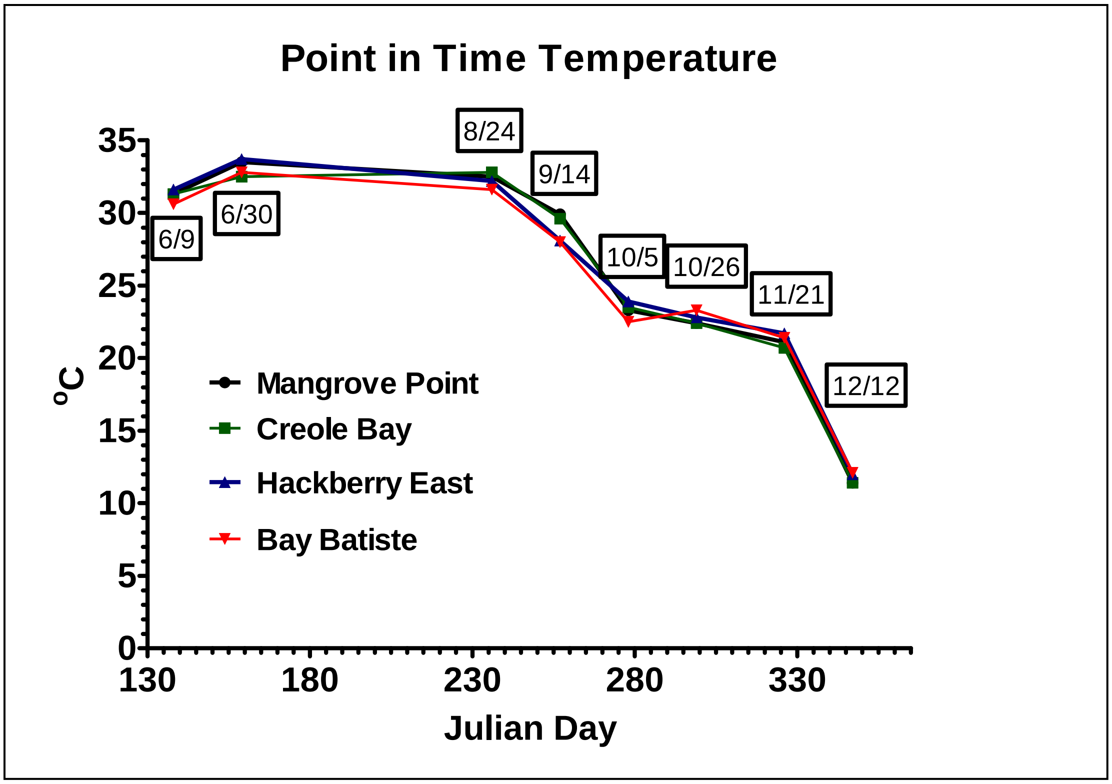


Figure 10. Point in time seawater temperatures at the four spat deployment sites in Barataria Bay in the fall of 2011.

Salinity –Point in time values

In contrast to seawater temperature, ambient salinity varied significantly by site in Barataria Bay in the fall of 2011. The major flood in the Mississippi River drainage system in the spring and summer of 2011 depressed ambient salinities at Mangrove Point in June 0f 2011 because freshwater from the Mississippi River entered the mouth of Barataria Bay.

Salinity at Creole Bay, Hackberry Bay, and Bay Batiste was significantly reduced due to substantial rain and wind Tropical Storm Lee prior to the September 14th collection of spat collectors**.**

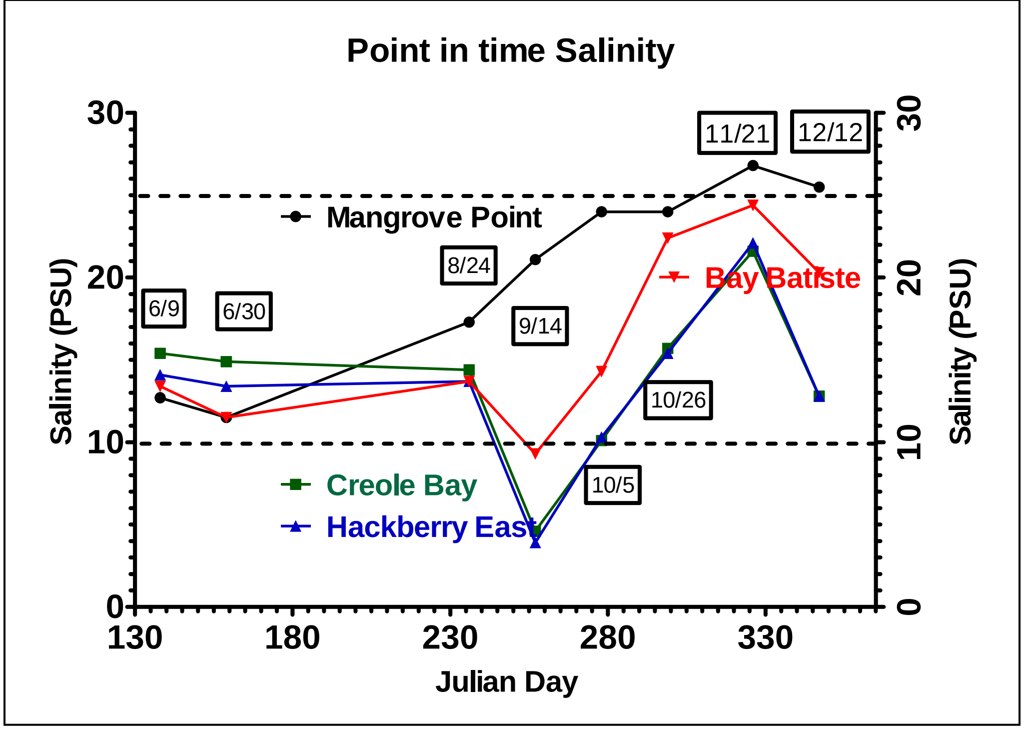
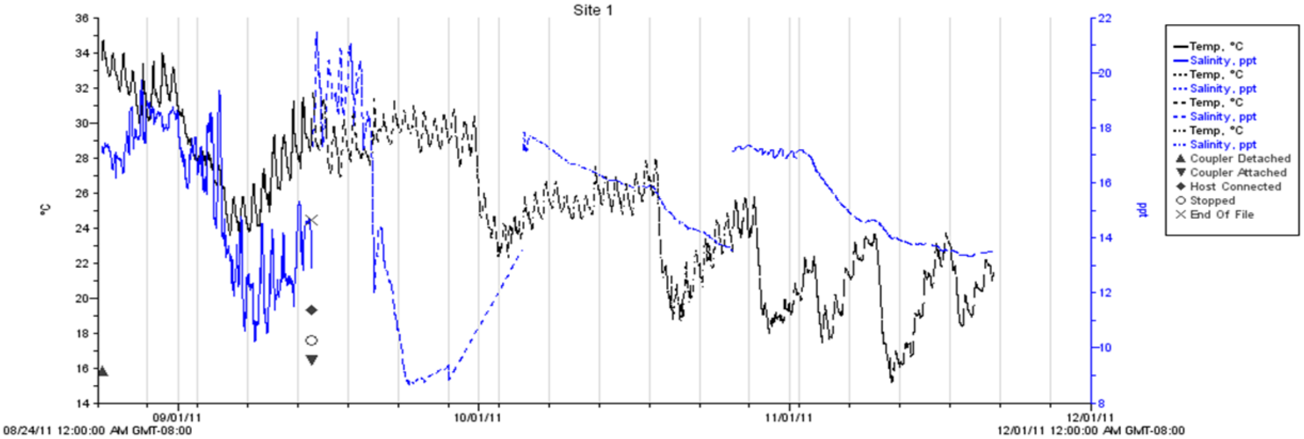


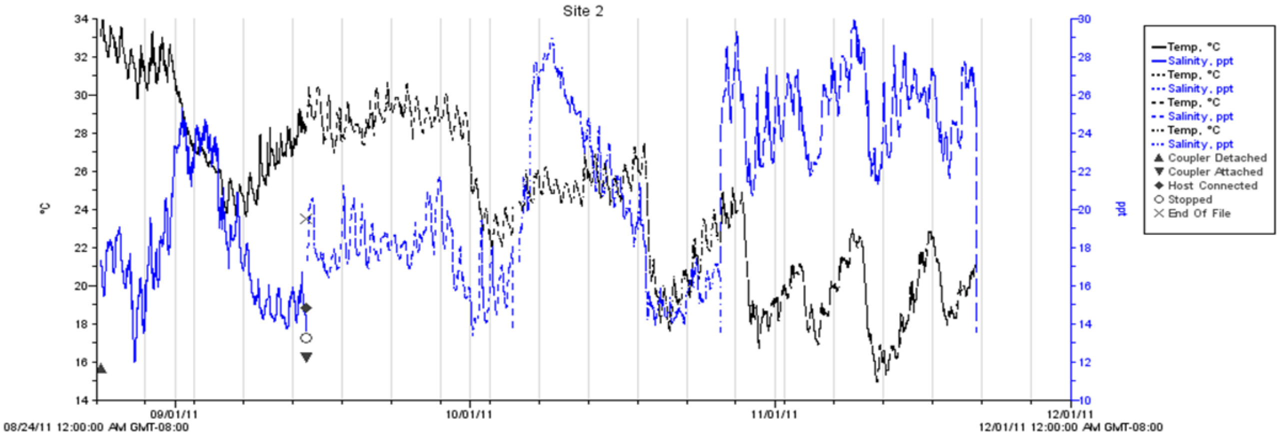
Figure 11. Point in time salinity levels at the four spat deployment sites in Barataria Bay in the fall of 2011.

Hobo data for temperature and salinity

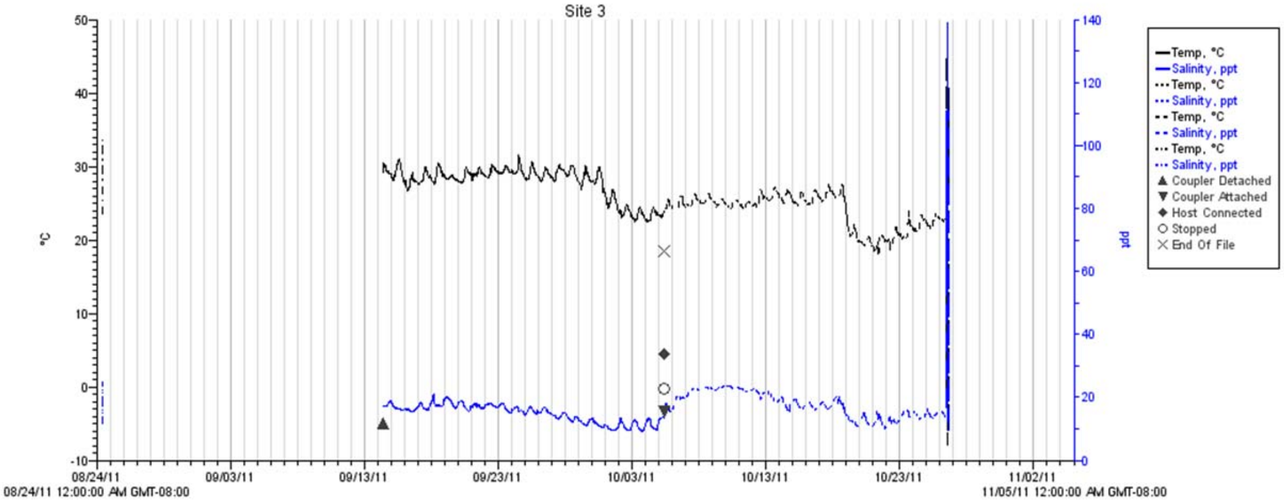
Mangrove Point



Creole Bay

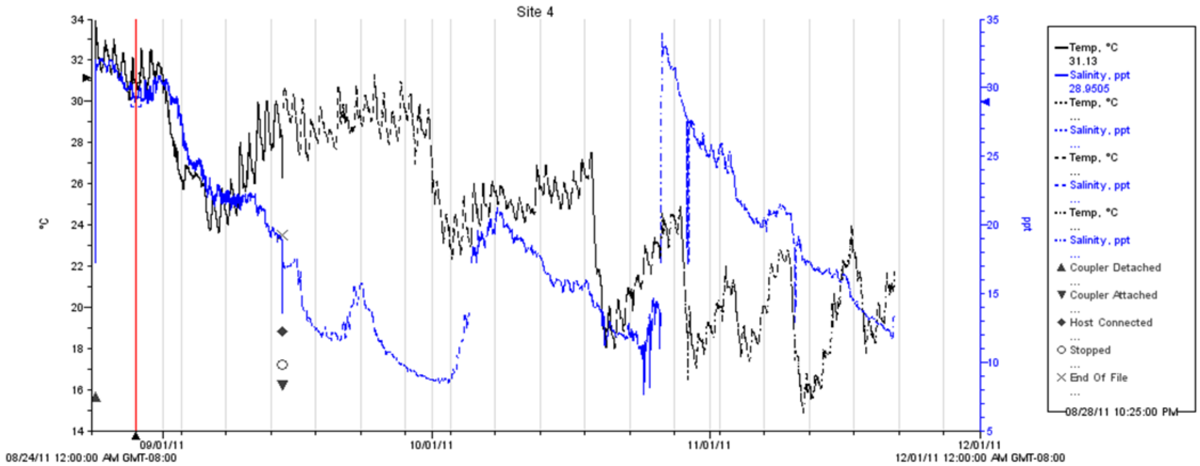


Hackberry East



**The probe set at Hackberry East malfunctioned on more than one occasion. This is the data that was recorded by the probe.

Bay Batiste



Discussion

Tropical storm Lee affected the Barataria Bay area in early September 2011, just prior to our collection on September 14 2011. We attribute the low spat count and anomaly in our results to the large amount of sediment displaced during this storm. Large amounts of sediment collected onto the tiles, preventing colonization of spat and most other organisms.

When looking at all of the results, we found higher larval numbers in the water column which did not correlate with the much lower numbers of oyster spat found to have actually settled on the collection tiles. Possible reasons for lack of settlement despite higher numbers of veliger larvae in the water column include predation by pelagic predators, oyster drills, mud crabs, etc. both pre-settlement and post-settlement. Another possible reason may be the oil byproducts lingering in the water column. Hackney and Smith studied the effects of hydrocarbons on oyster settlement by exposing clam shells to different petroleum treatments and then observing spat settlement (1989).

Further research is necessary to investigate the plausibility of the above suggestions as well as other possible reasons for the incongruence in the veliger larvae in Barataria and the number of spat being allowed to settle and begin the path to maturity.

Additional experiments that could provide useful information about future oyster harvests involve the substrates used by man to encourage spat settlement. It is common practice to use limestone and cultch (oyster shells) to act as substrate for settling spat. During our field studies, the limestone seemed to be effective, as many of the pieces dredged up showed signs of spat settlement. Limestone can be costly and is only effective when it is not buried in the mud bottom, creating an anoxic environment. Testing the efficacy of limestone, cultch, and other possible substrates could provide a more cost effective way for fisherman to encourage spat recruitment.

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