Monitoring the survival of hatchery-produced spat and larvae on Louisiana public oyster reefs

Erin R. Leonhardt
Louisiana State University and Agricultural and Mechanical College

MONITORING THE SURVIVAL OF HATCHERY-PRODUCED SPAT AND LARVAE ON LOUISIANA PUBLIC OYSTER REEFS

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Renewable Natural Resources

by
Erin R. Leonhardt
B.S., Roger Williams University, 2009
May 2013

To my loving family and friends.

For all their endless motivation, guidance and enthusiasm.

Acknowledgements

I thank Dr. John E. Supan for his advisement as my major professor, during this endeavor. I gained valuable experiences, as well as, personnel and academic accomplishments under his guidance. The skills and lessons I learned from this experience will help me to achieve future career goals. I also thank my committee members, including Dr. Jerome F. La Peyre and Dr. Robert P. Romaire. Their support and guidance has shaped my academic experience as well.

There are countless people who have helped me with this project and I extend my most gracious thanks to them. I am very grateful for those that have helped me during this process, including Ryan Olson, Laura Brown, Mary Kissner, Roger Smithhart, Jake Heare and Aaron Honig. I would especially like to thank the Sea Grant Oyster Hatchery staff, including Lisa Bourassa, Megan Gima, Justin Leonhardt and Ryan Moring for their friendships and all the time and effort spent working at the hatchery. I would also like to thank the Louisiana Sea Grant program, for funding and support from everyone, as well as, the Louisiana Department of Wildlife and Fisheries for funding and their outstanding help and support with this project. I also thank personnel in the Louisiana State University Department of Experimental Statistics for their assistance on statistical analyses of my data.

Table of Contents

Abstract

Alternative or supplemental management activities may be necessary to restore and enhance oyster production on Louisiana public oyster reefs. The production of wild oysters is variable due to anthropogenic and environmental factors that affect recruitment, growth and survival. The availability and structure of cultch material for larval recruitment and survival is particularly important to maintain oyster production. Beginning in 2011, the Sea Grant Oyster Hatchery on Grand Isle, LA and the Louisiana Department of Wildlife and Fisheries (LDWF) collaborated to test the survival of hatchery-produced spat and hatchery-produced larvae deployed on public oyster grounds and cultch plant sites. In 2011, a preliminary study was conducted on hatchery-produced spat survival in Hackberry Bay, LA, where 100% mortality of hatchery-produced spat was observed. Survival of hatchery-produced spat was also tested in Mississippi Sound, LA (Round Island site) and California Bay, LA, where sampling took place in September and November 2012 and January 2013. No hatchery-produced spat were collected at either of these sites, suggesting 100% mortality. No significant differences were observed between the numbers of wild spat oysters on treated plots, plots with hatchery-produced spat, to untreated plots ($P>0.05$).

In 2012, LDWF released hatchery-produced larvae at four sites at Calcasieu Lake and the sites were monitored monthly using standard LDWF sampling procedures. For most of the sites, few to no spat existed. Possible causes of hatchery-produced spat and larval mortality are sedimentation, predation, water quality and absence of suitable settling material. To increase survival, future studies should focus on ways to minimize causes of hatchery-produced spat and larval mortality.

Chapter 1
Introduction

Oyster Culturing in Louisiana

From 1999 to 2009, Louisiana was the top producer of *Crassostrea virginica* (Eastern oyster) landings in the United States (LDWF 2011). From 1998 to 2008, Louisiana led all Gulf of Mexico states in oyster landings and oyster production; additionally, Louisiana annually exceeds $35 million in dockside sales (LDWF 2010). Louisiana oyster reefs have suffered from large storms or catastrophic events that decimate or damage oyster beds such as from, Hurricane Katrina (August 2005) and the British Petroleum Deepwater Horizon oil spill (April 2010) (McGuire 2006; Beck et al. 2011). Recently, seed oyster (25 to 74 millimeters) production on both public and private leases has decreased (Figure 1) (LDWF 2011).

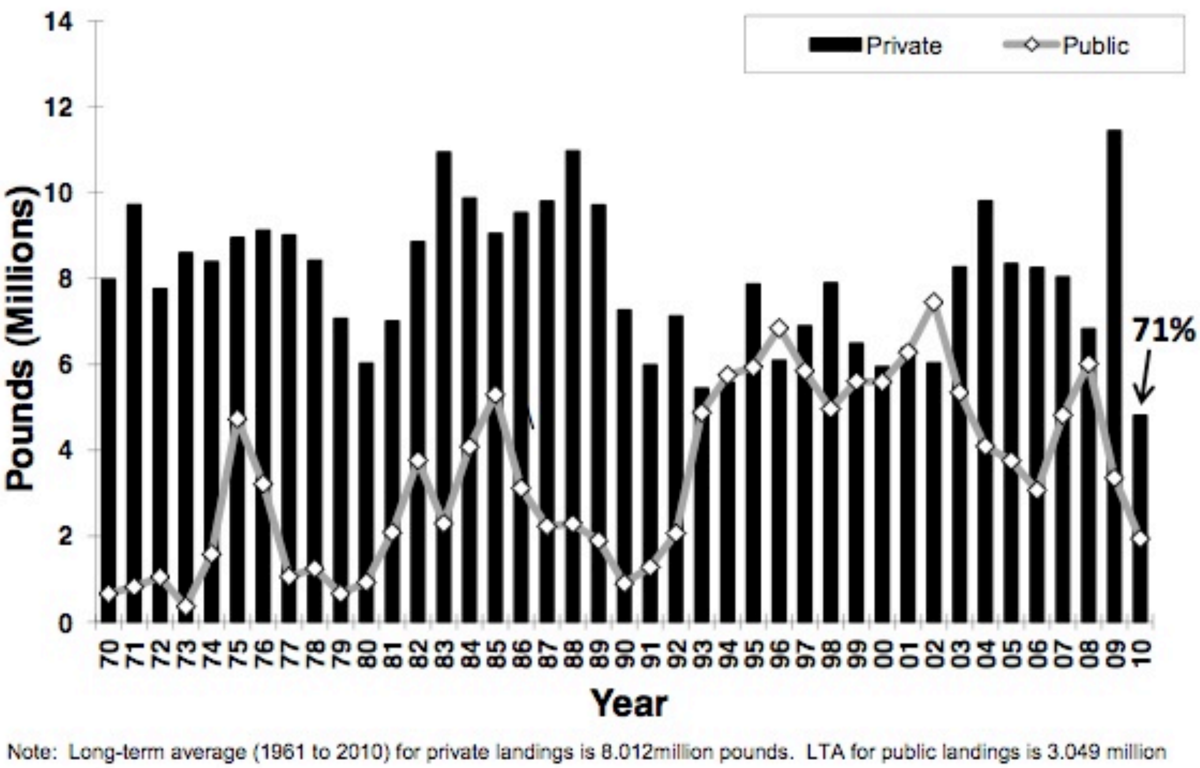


Figure 1. Historical oyster landings from public grounds and private leases in Louisiana from 1970 to 2010. (From: Louisiana Oyster Stock Assessment Report 2011, Oyster Data Report Series No. 17, Louisiana Department of Wildlife and Fisheries, Baton Rouge, Louisiana).

Private lease owners use a traditional oyster culturing technique, seed bedding, to transport seed from public oyster grounds to plant on private leases (Supan 2002). Generally, 80% of oyster production comes from private leases; therefore, the production of seed oysters on public oyster grounds is important to sustain private lease oyster production (LDWF 2011, J. Supan, Associate Professor, Specialist, Molluscan Shellfish, Louisiana State University, personnel communication).

Seed Bedding

There are variables to consider with seed bedding, which include cost and production of wild seed oysters. The costs are associated with the labor, fuel and supplies that are necessary for transporting and planting seed (Melancon & Condrey 1992). Sustainable wild oyster populations depend on a variety of environmental variables including disease, predation, parasites, food quantity and quality, sedimentation and water quality, as well as, population dynamics including recruitment, growth and survival (Soniat et al. 2012). Generally, the combination of two or more environmental variables effects oyster growth and survival significantly more (Heilmayer et al. 2008).

To manage the public oyster seed grounds, the Louisiana Department of Wildlife and Fisheries (LDWF) plants cultch material (VanderKooy 2012). In Louisiana, the production of oyster seed on the majority of public oyster seed grounds is low, even after the use of management strategies such as, planting cultch material (P. Banks, Marine Fisheries Biologist, Louisiana Department of Wildlife and Fisheries, Baton Rouge, Louisiana personnel communication). To compensate for minimal seed production, LDWF is researching new methods to supplement existing management strategies to increase seed production. New methods require the application of oyster hatchery techniques and the production of hatchery-produced spat oysters (less than or equal to 24 mm) and hatchery-produced pediveliger oyster larvae.

Cultch Planting

In Louisiana, the first cultch plant, reported by LDWF, was in 1917 in Sister Lake and up until 1994, clamshell and oyster shell were the primary cultch materials planted (VanderKooy 2012). Oyster shell, however, is used for other applications such as, for industrial and commercial purposes, and is

limited as a cultch material (VanderKooy 2012). Around 1994, there was a restriction to dredge clamshell in Louisiana and the state was forced to consider alternative materials such as, limestone and concrete (VanderKooy 2012).

In the Gulf region, the majority of oyster reef restoration is a response to natural disasters and anthropogenic events that cause devastation to the reefs (VanderKooy 2012). Cultch planting is an oyster reef management strategy and restoration technique to create suitable reef habitat and provide substrate for larval settlement and recruitment (Berrigan 1990; VanderKooy 2012). Cultch planting is the process of deploying suitable material on the seafloor bottom to increase oyster production. States such as North Carolina and Maryland along with states in the Gulf of Mexico, such as Florida, Alabama, Mississippi, Louisiana and Texas, have cultch planting programs for public oyster grounds and some for private lease holders and oyster growers (Chatry 1987; Webster & Merit 1988; Lee 2004; VanderKooy 2012).

Cultch Type

Cultch material should attract larvae for settlement and not negatively affect or alter the marine environment (Soniat & Burton 2005). Many studies have tested cultch material for larval settlement. A study conducted in Terrebonne Parish, Louisiana observed that wild oyster larvae preferred to set on limestone cultch, a calcium-based material, rather than sandstone, a silica-based material (Soniat & Burton 2005). A laboratory and field study, conducted on Grand Terre Island, Louisiana, observed that gypment (a 1:1 mixture of crushed gypsum and Portland cement) performed equal to or better than Mexican limestone or clamshell (*Rangia cuneata*), and Mexican limestone performed just as well as clamshell. Overall, pediveliger oyster larvae prefer to set on materials that are primarily composed of calcium carbonate (Soniat & Burton 2005).

Oyster shell cultch has been observed as an effective cultch material, not only for the shell's attractiveness to larvae, but also the shell's structure (Soniat et al. 2004). Whole oyster shell provides protection from predation and sedimentation; thus, maximizing protection can increase spat survival (Supan 1991; Soniat et al. 2004). Oyster shell also provides structure to keep spat and seed above sediment that can smother spat (Bohn et al. 1995). The orientation of oyster shell was observed to alter

oyster larval recruitment (Soniat et al. 2004). Vertically oriented oyster shell was observed to provide refuge from predation and provide clean surfaces for settlement (Soniat et al. 2004). In addition, oyster shell provides surface area for larval attachment (Bohn et al. 1995).

Hatchery-Produced Oysters

Other culturing methods can be used to supplement or replace production of wild seed (Jones & Jones 1983). One way to raise seed is through the use of oyster hatchery techniques, remote setting techniques and nursery systems (Jones & Jones 1988; Hadley & Whetstone 2007). Oyster hatcheries are commonly used in the United States along the East and West coasts and in other parts of the world to produce larvae, spat and seed oysters. Hatcheries, remote setting facilities and nursery systems alleviate many environmental factors that affect the production and recruitment of oysters (Swartzenberg 1999).

The technique of raising oysters in a hatchery is a developed and accepted method to produce healthy and marketable juvenile and adult oysters (Jones & Jones 1983). In areas where wild spatfall is limiting, it is more advantageous to produce oysters in a hatchery instead of relying on wild seed production (Swartzenberg 1999). Different cultch materials are used to set hatchery-produced larvae (Figure 2) (Bohn et al. 1995). Larvae used for commercial purposes, may be set on micro-cultch to make individual oysters or set on small cultch to make clusters of two or three oysters.

Hatcheries can customize oyster brood production by creating specialized oysters, such as polyploid oysters (Stanley et al. 1981; Swartzenberg 1999). Chromosome manipulation is practiced at hatcheries to create triploid and tetraploid oysters, which can be distinguished from diploid oysters with flow cytometry (Stanley et al. 1981; Allen 1983). Thus, oyster hatcheries can customize oyster stocks, which can be useful for research or commercial purposes.

Remote Setting

A specific hatchery-based production technique called remote setting takes advantage of the delayed metamorphosis of oyster larvae, to store and transport larvae from a hatchery to distant locations (Holiday et al. 1991; Devakie & Ali 2000). Larvae can either be released onto reef substrate at a site or

set onto cultch material in setting tanks. Remote setting hatchery-produced larvae can assist with spat production (Jones & Jones 1988; Chew 1991).

Figure 2. Hatchery-produced spat on whole oyster shell (left) and hatchery-produced seed on poultry shell (right) (Photos courtesy of Louisiana Sea Grant).

Storage duration and temperature affects larval setting success and survival (Devakie & Ali 2000). Optimal storage temperatures and duration periods for the highest percent larval setting success and survival rate differ between oyster species (Devakie & Ali 2000). The tropical oyster (*Crassostrea iredalei*) settlement rate decreases as storage duration increases at varying storage temperatures (Devakie & Ali 2000). This observation differs from the setting success of the Pacific oyster (*Crassostrea gigas*), which after eight days at 5°C the setting rate and post-setting survival decreases (Devakie & Ali 2000).

Optimal temperature and salinity for setting oyster larvae depends on the geographic location and the tolerance of the oyster larvae species. In a remote setting manual written for the United States Gulf Coast, it is recommended that the larval settlement tank water should be between temperatures 25°C and 30°C and salinity should be above 12 ppt (Supan 1991). Larvae from Louisiana, however, were observed to set at 33°C (Supan 1991). Once spat set, they can be transferred to a nursery system or grow-out site.

Nursery Systems

In a nursery system, spat are monitored and grown to a desirable planting size or seed-size. Negative impacts that affect spat growth and survival to seed-size are either eliminated or reduced in a nursery system. Nursery systems can protect spat from predation and siltation (Bohn et al. 1995).

Nursery systems quickly raise spat oysters to seed-size by providing adequate water flow with food-enriched water to the spat (Hadley & Whetstone 2007). The constant flow of water brings phytoplankton and oxygen enriched water to the seed and constantly removes waste (Bohn et al. 1995). One type of nursery is an upweller system, which is a flow-through system that holds spat in silos. Silos have mesh bottoms that allow water to flow from underneath and drain out the top (Figure 3 & Figure 4) (Hadley & Whetstone 2007).

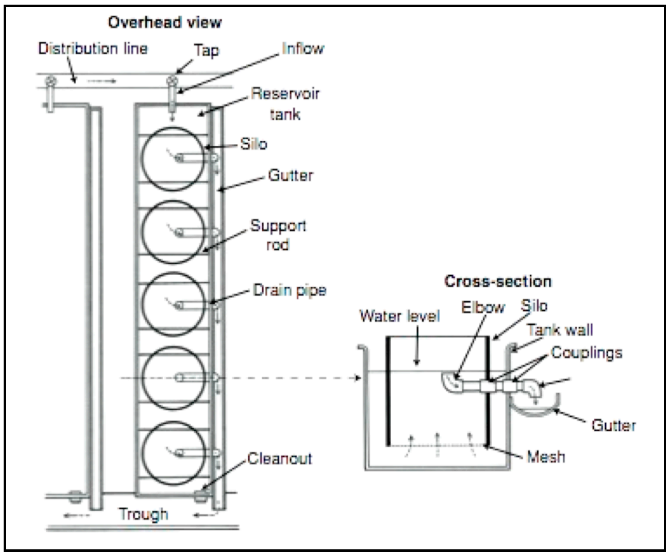


Figure 3. Diagram of an upweller nursery system (From: Hard Clam Hatchery and Nursery Production. Pub. No. 4301. Southern Regional Aquaculture Center).

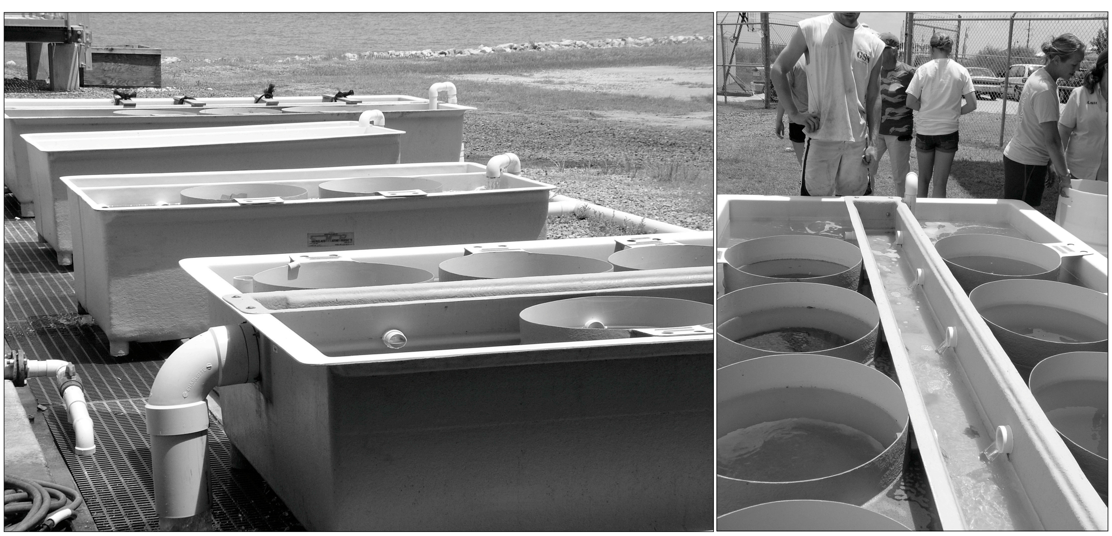


Figure 4. Upweller nursery system at the Sea Grant Oyster Hatchery on Grand Isle, LA. (Photos courtesy of Louisiana Sea Grant).

Site Selection

Site selection is crucial for restoring oyster reefs and involves selecting an area with oyster larval recruits and suitable substrate for larval settlement and survival (Coen & Luckenbach 2000; North et al. 2008). The dispersal of larvae within an estuarine is affected by water flow, circulation patterns and oyster larval swimming abilities (North et al. 2008). The adjacent and surrounding habitat can also affect the survival of larvae after it sets (Grabowski et al. 2005).

Sites should have a firm bottom, rather than a soft bottom, which causes heavier cultch materials to subside underneath mud (VanderKooy 2012). Soft bottom sites that are selected for reef restoration typically receive a suitable base layer of cultch material prior to planting cultch intended for settlement (VanderKooy 2012). A study on the influence of adjacent habitats to restored reefs in North Carolina, observed that mudflat reefs had more shell clusters, increased water flow and isolation from habitats with predators such as crabs (Grabowski et al. 2005). Thus, for these reasons the mudflat reef was suggested to provide greater survival and growth for oysters (Grabowski et al. 2005).

Life Cycle

Spawning, the release of gametes from sexually mature oysters, occurs in nature when water salinities are above 5 ppt to 10 ppt (Kennedy 1996). Previous studies have described that either a temperature change or temperature increase triggers spawning (Medcof 1939; Butler 1956). Natural substances in the water such as, algal ectocrines, were observed to trigger *C. virginica* spawning, under laboratory conditions (Galtsoff 1964). In the Gulf of Mexico, oysters spawn in the spring when temperatures reach 25°C, and in the fall spawning is stimulated by a decrease in water temperature, which may occur in July to August (Hayes & Menzel 1981). In the Gulf of Mexico, it is reported that oysters can reach sexual maturity in one month (Hopkins 1955).

Egg and larval development of *C. virginica* occurs in the water column (Figure 5). After 24 to 48 hours, larvae develop into veligers, with a swimming and feeding organ called a velum (Thompson et al. 1996). The larvae develop an “eye-spot” and a foot and search for suitable substrate for settlement; these larvae are referred to as pediveliger larvae (about 290 to 320 microns in length) (Thomspson et al. 1996).

Once the oyster larvae set, they metamorphose into a spat oyster. According to the LDWF, spat-size is from 0 to 24 mm (P. Banks, personnel communication).

Development depends primarily on temperature and salinity (Thompson et al. 1996). In a laboratory study, Galtsoff (1964) observed the development of trochophore larvae at a salinity of 32.2 ppt and a water temperature of 22.5°C to 24.5°C. Davis (1958) found that for oyster larvae conditioned at 26 ppt to 27 ppt, recently settled spat grow best at 17.5 ppt and growth is hindered at 10 ppt or lower. Adult oysters can survive in salinities from 0 ppt to 42 ppt, however, the optimal salinity range for adult oyster growth is 14 ppt to 28 ppt and growth is limited at salinities under 5 ppt (Shumway 1996). In the northern Gulf of Mexico, oyster larval settlement is optimal at a salinity range of 18 ppt to 22 ppt (Chatry et al. 1983).

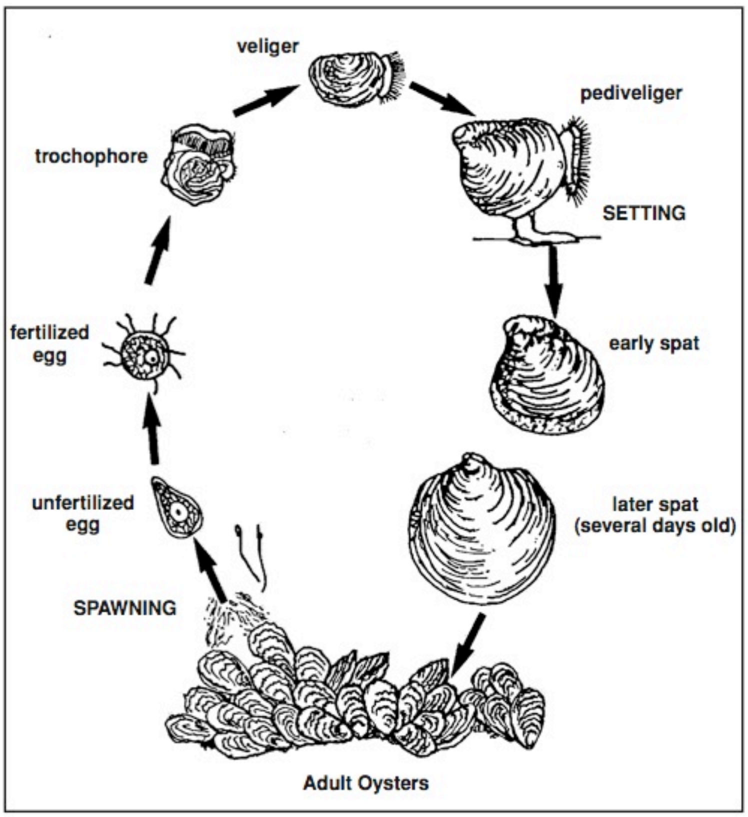


Figure 5. Life cycle of *Crassostrea virginica*. (From: The Oyster Fishery of the Gulf of Mexico, United States: A Regional Management Plan – 2012 Revision. Pub. No. 202, Gulf States Marine Fisheries Commission, Ocean Springs, Mississippi).

Larval Settlement

When cultch material is deployed, it becomes a surface for a variety of microbial organisms and macrofaunal species such as barnacles, bryozoans or boring sponges to settle on (Keough & Raimondi 1995; Barnes et al. 2010). The composition of organisms on substrates may increase or decrease oyster larval settlement (Barnes et al. 2010). Larval settlement can be associated to the chemical structure of biofilms on cultch material (Soniat & Burton 2005). Biofilms can provide positive or negative bioorganic cues for larval settlement and some, such as L-3,4-dihydroxyphenylalanince studied with *Crassostrea gigas* larvae, can induce larval settlement and metamorphosis (Coon et al. 1985; Keough & Raimondi 1995).

Fouling organisms can compete for space and food with larval recruits (Supan 2002). Fouling organisms can have a positive or negative interaction towards oyster larvae recruitment and settlement (Barnes et al. 2010). Cultch that is deployed before an oyster spawning event can attract biofouling organisms, which can negatively interact with oyster larval recruits (Saoud & Rouse 2000).

Oyster settlement is shown to have a relationship with salinity and temperature, which can vary spatially and temporally (Soniat et al. 2012). Numerous studies have observed temperature and salinity ranges for larval settlement, development and growth (Shumway 1996). A temperature of 19°C and 24°C was found to favor larval settlement (Ryder 1885). Davis (1958) found that larvae kept in 17.5 ppt, 15 ppt and 12.5 ppt salinity water had higher spat set than larvae kept at 10 ppt or 7.5 ppt. Spat set was observed in Texas near 35 ppt salinity water (Shumway 1996). In addition, thermal shock can positively or negatively affect larval settlement (Shumway 1996). A thermal shock of 24°C to 29°C did not affect settlement, however, a thermal shock of 15°C and 20°C negatively affected settlement (Diaz 1971, 1973).

Factors Affecting Larval and Spat Survival

Sedimentation

Oysters grown on-bottom are subject to sedimentation (Soniat et al. 2004). Sedimentation is a major issue in Louisiana and causes oyster mortality (Soniat et al. 2004). Increased sediment loads in Louisiana estuaries are commonly caused from storms, freshwater diversions and input from river

systems (Soniat et al. 2004; McGuire 2006). Although, freshwater diversions of the Mississippi River are beneficial for preventing flooding and saltwater intrusion into estuaries, there is a large amount of sediment associated with the diversions (Soniat et al. 2004; McGuire 2006). Large amounts of sediment smother oyster larval recruits and prevent clean, suitable substrate for larval settlement (Soniat et al. 2004; Burke et al. 2008).

Predation

Predation is a main cause of oyster mortalities in the Gulf of Mexico and affects all size classes of oysters (Soniat et al. 2012). Oysters, however, are able to tolerate a wide range of salinity, which may not be suitable for their predators (Brown & Stickle 2002). Common predators for oysters in the Gulf of Mexico include oyster drills (*Stromonita haemastoma*), mud crabs (*Eurypanopeus depressus, Panopeus herbstii*), blue crabs (*Callinectes sapidus*), stone crabs (*Menippe adina*) and black drum (*Pogonias cromis*) (Soniat et al. 2012). Some of these predators are euryhaline and can adapt to salinities of 5 to 15 ppt, such as blue crabs and mud crabs (Soniat et al. 2012). When crab species are juveniles, they occupy small spaces within oyster reefs and prey on spat oysters that occupy the same space (Soniat et al. 2004). Mud crabs and blue crabs commonly prey on spat oysters (Brown et al. 2008). A study conducted in Maryland, found that the flat mud crab (*Eurypanopeus depressus*) and white-fingered mud crab (*Rhithropanopeus harrisii*) preferred to prey on spat oysters less than 8 mm in length and significantly reduced the survival of spat oysters (Kulp et al. 2011). The temperature threshold for southern oyster drills, collected from Caminada Pass near Grand Isle, LA and tested in an aquaria study, was 10°C and 12.5°C (Garton & Stickle 1980). In addition, predation rates of the southern oyster drill did not significantly differ between 10 ppt and 30 ppt (Garton & Stickle 1980). In Louisiana, a salinity range of 5 ppt to 15 ppt limits overall predator abundance (Soniat et al. 2012).

Other predators to oysters are fish species, specifically black drum and sheepshead (*Archosargus probatocephalus*) (Brown et al. 2008). Black drum is described by Louisiana oyster lease owners as one of the main causes of oyster mortality (George et al. 2008). Black drum predation on Louisiana oyster reefs depends on fish size and season (George et al. 2008). Thus, black drum sizes 70 cm to 75 cm were

observed as more effective predators on oysters and drum were observed to consume more oysters in the spring (2005) than in the fall (2004), in Lake Grand Ecaille and Creole Bay (George et al. 2008).

Disease

Dermo (*Perkinsus marinus*), a protistan parasite, is one of the largest causes of oyster mortalities in the Gulf of Mexico and along the Atlantic coast (Brown et al. 2005; La Peyre et al. 2010). The optimal temperatures for Dermo to grow and proliferate is 28°C to 32°C and temperatures of 2°C and 10°C decrease the parasite load (La Peyre et al. 2010). Dermo favors higher salinity water (Brown et al. 2005). A sub-optimal salinity of 3.5 ppt effectively inhibits Dermo, which occurs in Louisiana for short periods (La Peyre et al. 2010). In Louisiana, a decrease in Dermo infection in oysters occurs when there was a decrease in salinity accompanied by low temperatures (La Peyre et al. 2010). During years when the water temperatures in Louisiana are below average, lowering salinity with freshwater diversions could decrease Dermo infection (La Peyre et al. 2010).

Temperature

Temperature affects both reproductive growth and somatic growth (Powell et al. 1995). Growth is determined by how the oyster directs its energy, whether energy is put towards somatic growth or reproductive growth (Powell et al. 1995). When water temperature increases, oysters direct their energy from somatic growth to reproductive growth, however, when water temperature decreases, somatic growth occurs (Powell et al. 1995). High temperatures coupled with low salinities is detrimental to oyster growth and survival (Heilmayer et al. 2008). Temperature also affects filtration rates in oysters, which affects ingestion rates (Powell et al. 1995; Pernet et al. 2008).

Temperature influences the prevalence of predators, susceptibility and presences of disease and ability to cope with stressors (Lannig et al. 2006; Brown & Stickle 2002; La Peyre et al. 2010). Oysters are able to adapt to a wide range of temperatures, which can minimize mortality from predation (Brown & Stickle 2002). Temperature also influences the presence and susceptibility of Dermo (La Peyre et al. 2010). Elevated temperatures such as, 28°C, can weaken defense mechanisms of *C. virginica* towards

pollutants, such as Cadmium, which may limit reproductive abilities (Lannig et al. 2006). Thus, various environmental factors that affect oyster survival can be driven by temperature.

Salinity

In Louisiana, river systems and freshwater diversions provide considerable amounts of freshwater to coastal marshes and bays, which changes salinity levels (McGuire 2006). Salinity can affect the species and number of predators present in an area as well as the presence of Dermo (Brown et al. 2005). High salinity water attracts more predators, which decreases oyster survival (Supan 1991; Brown et al. 2008). Because oysters can survive in lower salinities than most of their predators, selecting a reef site near a freshwater input can positively affect oyster survival. Salinity is also a vector for certain diseases, such as Dermo (Brown et al. 2005). Prolonged period of freshwater, however, lowers salinity and can cause high oyster mortality (Heilmayer et al. 2008). The effect that salinity has on development, recruitment, growth and survival plays a substantial role in oyster production.

To survive periods of low salinity, oysters keep their valves closed (Heilmayer et al. 2008). During valve closure, the oyster is suppressed from feeding and relies on anaerobic metabolism for energy (Heilmayer et al. 2008). After an extended period of closure, carbon dioxide accumulates in the tissues in concentrations high enough to cause mortality (Heilmayer et al. 2008). Accumulation of carbon dioxide, during valve closure, is a potential cause of summer mortality (Heilmayer et al. 2008).

Food

Oysters filter feed on a variety of food sources such as particulate matter, algae, phytoplankton, microorganisms and detritus (Gosling 2003; Tolley et al. 2005). Baldwin and Newell (1991) classified *C. virginica* larvae as omnivores, feeding on numerous suspended particulates. Possible nutrition sources for adult and larval oysters are dissolved organic matter (DOM) and bacteria (Crosby et al. 1990; Langdon & Newell 1996). Phytoplankton, however, has been considered as a main food source and nutrient for oysters and is used for biological processes (Tolley et al. 2005). The presence and species of phytoplankton are important for oyster growth and development (Tolley et al. 2005). Many laboratory studies have tested and shown optimal algal diets for oyster larval growth and development (Langdon &

Newell 1996). Algal diets can affect settlement and juvenile growth; furthermore, protein content is positively correlated with larval settlement and high carbohydrate contents in algae may enhance juvenile growth (Wikfors et al. 1984). It is suggested that there is a minimum food level that supports market-size (greater than 75 mm) oyster populations; therefore, when food is limiting mortality occurs (Powell et al. 1995).

Chapter 2
Preliminary Evaluation of Hatchery-Produced Spat on Louisiana Public Oyster Grounds

Seed bedding is a common method of producing oysters in Louisiana. Wild oyster production, however, can vary due to environmental and anthropogenic influences (Soniat & Burton 2005). Common factors in Louisiana that affect survival and growth of spat and seed oysters include predation, water quality and sedimentation (Powell et al. 1995; Burke et al. 2008; Wang et al. 2008; Soniat et al. 2012).

There are other methods for raising seed that limit environmental impacts. Usually, these methods involve a hatchery facility, remote setting facility or nursery system. These facilities and systems provide reliable methods for raising larvae and seed oysters (Jones & Jones 1983; Swartzenberg 1999; Hadley & Whetstone 2007). These facilities and systems, however, do not exist near all oyster-producing areas.

An alternative method to increase wild seed production is through the use of cultch plants (VanderKooy 2012). Cultch plants require suitable cultch material to attract larval settlement and suitable habitat to promote oyster growth and survival (Grabowski et al. 2005; Soniat & Burton 2005). Often, cultch plants are conducted because suitable cultch material is lacking (Chatry 1987). Oyster shell, the common and most preferred cultch material for productive oyster reefs, is limiting on some reefs (Soniat & Burton 2005; VanderKooy 2012). There are reasons that oyster shell is limiting. In places, such as Louisiana, the return of oyster shell to reefs is limited because shell is sold for industrial or commercial purposes (Piazza et al. 2005). Also, the production of new shell is limited by environmental factors, such as salinity (Chatry 1987). In Louisiana, cultch plants began in 1926 on public seed grounds and oyster reservation areas to recreate reefs and increase oyster production (Chatry 1987). Saltwater intrusion into productive oyster reefs east of the Mississippi River caused a lack of oyster shell substrate (Chatry 1987). Thus, to maintain and increase oyster production it is necessary to plant cultch (Chatry 1987).

Cultch planting by itself may not be as an effective method for increasing oyster production as planting spatted cultch. In early 2000, a study conducted in North Carolina observed planting spatted cultch was more beneficial at producing oysters than traditional cultch planting (Lee 2004). This study

remote set pediveliger larvae, produced by the Sea Grant Oyster Hatchery, onto shell cultch (Lee 2004). After setting, spat were held in a rack nursery system and planted when spat reached fingernail size (Lee 2004). Lee's (2004) results show about a 75% loss of seeded cultch material due to recession in the muddy sediment. Lee (2004) recommends to eliminate the nursery and to plant spatted cultch directly on-bottom, reducing the labor associated with the nursery (Lee 2004). Lee (2004) speculates that direct planting of spatted cultch will provide greater growth and survival. Thus, a more reliable method to produce seed may entail using hatchery-produced spat and planting spatted cultch.

In the summer of 2011, the Louisiana Department of Wildlife and Fisheries (LDWF) and the Sea Grant Oyster Hatchery on Grand Isle, LA collaborated efforts to answer the question "Is it more effective to plant hatchery-produced spat set on cultch than it is to plant traditional cultch material?" To answer this question, a preliminary study tested the survival of hatchery-produced spat planted on Louisiana oyster reefs during the summer of 2011. The Sea Grant Oyster Hatchery raised competent pediveliger larvae for setting on cultch material. Poultry shell cultch and marble aggregate were used because it was commercially available. In addition, the poultry shell was used because crushed shell was used as a method by Louisiana oyster growers to produce single oysters (J. Supan, personnel communication). The LDWF chose study sites within Hackberry Bay, which are part of 1,781.4 hectares (4,402 acres) of public oyster reed reservation ground designated by state legislature that LDWF manages using cultch plants (LDWF 2011; P. Banks, personnel communication).

The percent survival of hatchery-produced spat and seed at deployment sites was evaluated, using the estimated initial number of spat and seed deployed. The null hypothesis was there would be 100% survival of hatchery-produced spat. The alternative hypothesis was there would be 100% mortality of hatchery-produced spat. Recommendations from this preliminary study were used in hatchery-produced spat research conducted in 2012.

Materials and Methods

Site Selection

Hackberry Bay was selected to plant hatchery-produced spat (≤ 24 mm). There were two sites within Hackberry Bay that were planted with hatchery-produced spat, the Hackberry Bay 2004 South Cultch Plant (HB 2004; 29°23.439'N, 90°03.093'W) and the Hackberry Bay 2008 Cultch Plant (HB 2008; 29°25.495'N, 90°01.263'W). There were two deployment locations at HB 2004, one in the Northeast corner and one in the Southeast corner.

The northern site was planted with 1,775 cubic meters of #57 limestone on 4.05 hectares of bottom (LDWF 2011). The southern site was planted with about 3,062 cubic meters of #57 limestone on 10.12 hectares (LDWF 2011). The 2008 cultch plant was planted with approximately 75% #57 limestone, 15% crushed concrete and 10% oyster shell on about 20.23 hectares (LDWF 2011). The survival of hatchery-produced spat deployed in Hackberry Bay was tested in an effort to rehabilitate and restore these public oyster grounds.

Hatchery-Produced Larval Setting

The Sea Grant Oyster Hatchery on Grand Isle, LA produced competent oyster pediveliger larvae using standard techniques (Supan & Wilson 1993) for setting on commercially available marble aggregate and poultry shell. Marble aggregate averaged 18.4 mm by 5.9 mm (n=10) in size, while poultry shell was retained after grading over a 3.175 mm mesh screen. Marble aggregate was rinsed with filtered ambient seawater prior to placement on the bottom of three, fiberglass-reinforced setting tanks (internal tank dimensions 3.0 m x 0.6 m x 0.3 m) at approximately 25 mm deep. Poultry shell was rinsed with filtered ambient seawater prior to placement on the bottom of wooden setting trays (internal tray dimensions 76.2 cm x 35.6 cm x 13.3 cm), lined with 150-micron nylon screen, at approximately 25 mm in depth. All setting tanks were filled with one-micron absolute filtered seawater, to a depth of 25 mm. Approximately 4 million larvae (setting tanks) and one million larvae (setting trays) were suspended in ambient filtered seawater in setting tanks and poured evenly onto cultch in the setting tanks using an 8 L plastic sprinkling can. Larvae were given at least 24 hours to set before moving to the field sites or nursery system. A

culling rake and filtered seawater were used to rinse the spatted cultch down the tank drains into 18 L transportation buckets modified with 1.6 mm drainage holes at their base to allow water drainage and fitted with a cover prior to transport.

A series of calculations were conducted to estimate the number of spat and percent set of spat. The purpose of gathering these values was to assess the validity of marble at attracting spat, and also to determine the number of live spat pre and post spat deployment.

To estimate the initial number of spat set on marble cultch, three 100 mL replicate subsamples were collected during each deployment for later microscopic analyses, using a stereo dissecting microscope at 0.7-30x (Leica EZ4). The mean number of live and crushed spat (spat with damaged shells) from the three replicate samples was calculated. The number of live spat and crushed spat that were deployed at each site was calculated by the equation:

(mean number of live and crushed spat ÷ 100 mL) * total volume (mL).

The percent setting success was calculated by the equation:

(total number of live and crushed spat deployed ÷ initial # of larvae added to setting tank) * 100.

The percent set was divided into two categories, live spat and crushed spat. The mean number of live and crushed spat was estimated from the replicate subsamples and the mean number of live spat was used to estimate the number of live spat deployed. Estimates of percent live and crushed spat were calculated as follows:

(mean number of live spat or crushed spat ÷ total number of live and crushed spat) * 100.

Spat set on poultry shell were removed from the setting trays 24 hours post-setting and raised in the upweller nursery system. Spat raised in the nursery system were subsampled by collecting three 100 mL, dry volume, replicate subsamples from the total volume. The mean number of spat deployed was determined from the replicate subsamples, as well as, the percent survival of spat from the nursery system. These values determined the number and percent of live spat deployed at HB 2008. In addition, twenty-five spat from each subsample were randomly selected and measured to determine mean shell height.

Different spat deployment sizes and cultch types were deployed between different sites. Larger spat (mean sizes 8.1 mm, 11.9 mm and 8.9 mm for three separate deployments on two dates) were deployed on poultry shell at HB 2008 after being raised in the upweller nursery system. Freshly struck spat were set to marble cultch and deployed at HB 2004. Each site was sampled separately for spat survival, because of the differences in deployment size and cultch type.

Hatchery-Produced Spat Deployment and Site Monitoring

Deployment sites were marked with PVC poles (50 mm diameter) and monitored fortnightly for water quality and wild spatfall and monthly for bottom samples. These sites did not have replication, since this was a preliminary study. Surface and bottom salinity and temperature were measured using a hand-held YSI-6050000 YSI Pro Plus Multi-Parameter Water Quality Meter System during initial deployment, fortnightly and during sampling periods, while bottom samples at HB 2008 and HB 2004 sites were only collected monthly (YSI Incorporated, Yellow Springs, OH 45387). Temperature and salinity were supplemented with surface water quality data from the United States Geological Survey (USGS) at the station "Hackberry Bay Northwest of Grand Isle, LA" for 2011 (29°23.900'N, 90°2.467'W). Spat plates were deployed and retrieved fortnightly to monitor wild spatfall; the plates were brought to the lab and assessed using a dissecting microscope (Supan 1983).

Initial spat deployment dates varied, but the final sampling date was the same for both sites, October 5, 2011. The deployment date for larger spat at HB 2008 was July 6 and 13, 2011, and for freshly struck spat at HB 2004 were August 10, 16 and 18, 2011. The July 13 deployment at HB 2008 was not sampled during the study because the marker pole was lost, therefore, the July 6 deployment site will be referred to as the HB 2008 site.

Bottom samples were collected monthly at each site. Bottom samples were collected initially using oyster tongs lined with 3.175 mm plastic mesh, and later by LDWF SCUBA divers. Divers were instructed to collect bottom samples by hand and brought on board. At HB 2004, dive sampling collected 6,500 L (dry volume) of cultch material, while at HB 2008 divers collected all the material around the

marker pole. At both sites, the material was divided into 100 mL volumes and three replicate subsamples from the twelve volumes were randomly selected to examine for live hatchery-produced spat.

Bottom samples were also collected prior to deploying spat to observe the presence or absence of existing wild oysters. One 2 L (dry volume) sample was collected at HB 2008 using oyster tongs, and one three-minute dredge tow was used to collect a sample at HB 2004, as per LDWF standard sampling methods. A maximum of twenty-five oysters were randomly chosen from each bottom sample and measured for shell height (mm).

Results

Pre-deployment bottom samples contained various sizes of wild oysters. The size range was spat-size (≤ 25 mm) to market-size (≥ 75 mm). At HB 2008 (August 4), the mean oyster size was 26.8 mm and the size range was 12 mm to 48 mm (n=25 oysters). In the NE corner of HB 2004 (August 10), the mean oyster size was 45.2 mm and the size range was 11 mm to 118 mm (n=25 oysters). In the SE corner of HB 2004 (August 16), the mean oyster size was 25.3 mm and size range was 19 mm to 59 mm (n=8 oysters). Most of the spat and seed oysters were attached to oyster shell or live market-size oysters.

The mean number and mean size of spat deployed at HB 2008 varied (Table 1). The percent survival of spat raised in the nursery was similar for both mean sizes deployed on July 13.

Table 1. Date, cultch type, mean number and mean size (mm) of spat deployed at HB 2008 (2011). Percent survival of spat was estimated post-nursery.

Date	Cultch Type	# Spat Deployed	Mean size (mm)	Percent Survival (%)
7/6	Poultry shell	142,973	8.1	n/a
7/13	Poultry shell	222,937	11.9	97
7/13	Poultry shell	534,352	8.9	98

For the HB 2004 deployments, the number of larvae added to each setting tank and the percent set varied (Table 2). The greatest number of larvae used for setting was on August 16, with 3,750,000 larvae, however, the percent setting success was less than August 10 and similar to August 18. The greatest

percent setting success was on August 10 (60%), with an initial number of 3,150,000 larvae added to the setting tank. The percent setting success was nearly the same for August 16 and 18.

Table 2. Location, date, mean number of larvae added to setting tank, mean number of live and crushed spat and percent setting success for spat deployed at HB 2004 (2011). Larvae were set to marble aggregate.

Location	Date	# Larvae Added	# Live and Crushed Spat	% set
NE corner	8/10	3,150,000	1,896,468	60
SE corner	8/16	3,750,000	1,393,673	37
SE corner	8/18	2,250,000	680,040	30

The effect of handling on spat survival was observed in spatted marble subsamples. All subsamples had spatted cultch, but some of the spat were observed to be crushed (shattered shell) (Table 3). The percent of live spat was greater than the percent of crushed spat for each sampling period. The percent of live spat remained around 65-70% of the total live and crushed spat, while the percent of crushed spat remained around 30%, for all sampling periods. The mean number of live spat deployed at HB 2004 was approximately one million on August 10 and August 16. The least number of live spat deployed at HB 2004 was just under 500,000 spat on August 18.

Table 3. Location, date, estimated number and percent of live spat, estimated number and percent of crushed spat and total estimated number of spat deployed at HB 2004 (2011). Spat were deployed on marble cultch.

		Live		Crushed		Total
Location	Date	#	%	#	%	#
NE corner	8/10	1,233,546	65	662,922	35	1,896,468
SE corner	8/16	976,301	70	417,372	30	1,393,673
SE corner	8/18	447,102	66	232,938	34	680,040

Surface and bottom temperatures and salinities were identical at each sampling, but varied between sampling periods and sites. During the time of deployment, the temperature and salinity at HB 2008 was 31.5°C and 13.47 ppt (July 6), and was the maximum recorded temperature and salinity for this

site. The minimum temperature at HB 2008 was 22.7°C (October 5), during this preliminary study, and the minimum salinity was 1.57 ppt (August 4). During the times of deployment, the temperatures at HB 2004 were 29.8°C, 30.9°C and 31.8°C and the salinities were 7.71 ppt, 8.42 ppt and 10.63 ppt (August 10, 16 and 18 respectively). HB 2004 had a maximum temperature of 31.8°C (August 18) and a minimum of 23.2°C (October 5), and had a maximum salinity of 10.72 (August 31) and minimum of 7.71 ppt (August 10). The USGS station shows that salinity at Hackberry Bay was less than 5 ppt from mid-July to late July and also in the beginning of August (Figure 6). The salinity increased mid-August, and remained below 15 ppt until September (USGS). The temperature in July fluctuated above and below 30°C and in August was greater than or equal to 30°C (USGS).

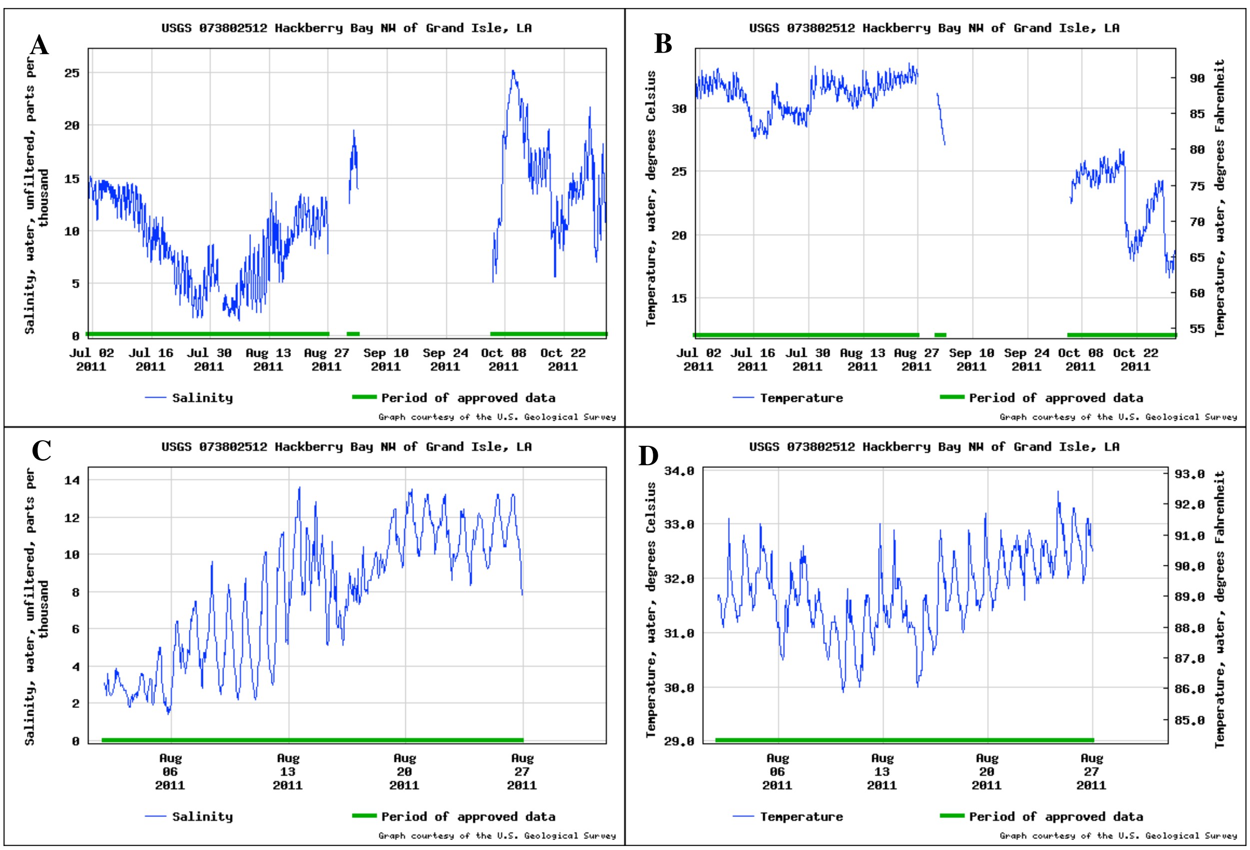


Figure 6. Salinity (A) and temperature (B) from July 1 to October 30 and salinity (C) and temperature (D) in August, at the United States Geological Survey station "Hackberry Bay Northwest of Grand Isle, LA" (2011).

The bottom samples did not contain live hatchery-produced spat and there were no wild spat on spat plates for HB 2008 and HB 2004. There were no spat on crushed poultry shell in the samples at HB

2008, however, dive sampling collected wild spat set to whole oyster shell. The dive sample at HB 2004 contained marble cultch, oyster shell and gravel cultch. The marble cultch, the cultch of interest, had scars from the freshly struck spat. The spat scars appeared to be the original size of the spat deployed. Thus, there was minimal to no growth of spat set to marble aggregate at HB 2004. Overall, there was 100% mortality of spat on marble cultch deployed at HB 2004 and 100% mortality of larger spat set on crushed poultry shell at HB 2008.

Discussion

The outcome of 100% mortality of hatchery-produced spat at Hackberry Bay (2011) can be used as a learning tool to modify future applications of hatchery-produced spat and suggest possible causes of spat mortality. This study can improve knowledge on sampling techniques, handling techniques, site selection and cultch type selection. The result of crushed spat demonstrates the need for better handling techniques for moving and deploying spatted cultch. Improving sampling methods at deployment sites will provide more informative and reliable data.

During this preliminary study, a culling rake moved spatted cultch from the raceway tank to the transportation containers. Subsamples contained crushed spat. The spat was likely crushed from pushing the cultch together with the rake. The marble cultch was moved with a culling rake because water was inadequate to move the cultch. For future applications of setting cultch, practices should follow recommended methods for remote setting larvae (Jones & Jones 1983, 1988; Supan 1991). For applications that use setting tanks such as the ones used for this study, lighter weight cultch, such as the poultry shell cultch, should be used to move the cultch with water and prevent crushing.

Improved sampling techniques provide more accurate and reliable data. Tongs were effective at collecting whole oyster shell and large cultch material. Tongs, however, were ineffective at collecting small-sized cultch material. The space between the tong teeth was larger than the cultch material deployed. The mesh used to line the tong basket was small, but perhaps not small enough. The other method used to collect samples, SCUBA diving, was effective at gathering small cultch material. A benefit to SCUBA diving is that it is a common sampling technique used by the LDWF to assess oyster

stocks on public grounds (P. Banks, personnel communication). There are restrictions to diving, however. Some of these restrictions include certified personal and weather conditions. Water visibility at Hackberry Bay was low. For guidance, divers followed the pole markers to the deployment spot. The best sampling method at a site should be logical and practical.

Easier spat sampling could be achieved if spat samples were deployed in containers or trays at deployment sites. Brown et al. (2008) observed no tray bias in a survival study when seed oysters were placed in trays. Different methods, such as using trays, for studying spat survival would provide easier sampling and reliable data. The LDWF conducts a research project with Nestier trays (LDWF 2011). This study attaches live oysters to the trays and tests survival and mortality of the oysters at varying sites. This is a useful technique for easily assessing survival and mortality.

There are concerns with planting spat on-bottom. Predation is a common cause of oyster mortality in the Gulf region and affects all size classes (Soniat et al. 2012). Common predators include mud crabs, blue crabs, stone crabs, oyster drills and black drum (Soniat et al. 2012). Blue crabs, stone crabs and flat mud crabs dominate high sediment areas (Soniat et al. 2004). Crabs that dominate low sediment areas in Louisiana estuaries are porcelain and stone crabs (Soniat et al. 2004). Hackberry Bay had a soft bottom and high sediment load. Predators that dominate high sediment areas potentially threatened hatchery-produced spat. Mud crabs are small in size and prey on spat oysters (Kulp et al. 2011). It is possible that mud crabs preyed on the larger spat oysters deployed at HB 2008. A predation study at HB 2008 should be conducted to test the effects of mud crabs. The marble cultch at HB 2004, had scars of freshly struck spat. Because there was no growth of hatchery-produced spat at HB 2004, there may have been different causes of mortality. Future studies should observe the effects of predation. To test for predation, spat samples could be placed in a predator exclusion enclosure. Differences in survival between enclosed spat and unenclosed spat would suggest if predation caused mortality. If predation was not a cause of mortality, then other factors should be considered. These factors could be related to water quality parameters, such as temperature and salinity.

In the 2011 Oyster Stock Assessment by the LDWF, hooked mussels (*Ischadium recurvum*) were found at all six Hackberry Bay sampling locations. Hooked mussels compete for food and space for settlement (LDWF 2011). It is possible that competition for food existed in this preliminary study between hooked mussels and hatchery-produced spat. Limited food availability reduces oyster growth and survival (Tolley et al. 2005).

Temperature and salinity affect oyster growth and survival (Heilmayer et al. 2008). At Hackberry Bay, this study recorded salinity a minimum salinity 1.57 ppt and a maximum salinity 13.47 ppt. The salinity and temperature between recorded data was supplemented with data supplied by the USGS station Hackberry Bay NW of Grand Isle, LA. Adult oyster growth is optimal from salinities of 14 ppt to 28 ppt, and growth is limited under 5 ppt (Shumway 1996). In the Gulf region, it is reported that an optimal salinity range for oyster growth and development is from 10 to 22 ppt (Lorio & Malone 1994; Supan 2002). Thus, a salinity of 1.57 ppt is not optimal for growth and development of spat. Low salinity may have strongly influenced survival of freshly struck spat at HB 2004. Prolonged periods of low salinity results in mortality. The salinity at Hackberry Bay, from the time of the first deployment on July 6, remained below 15 ppt until the beginning of September (the only approved data for September is September 3) (USGS). The salinity during mid-July to late July and in the beginning of August was below 5 ppt (USGS). These periods of low salinity coupled with temperatures near 30°C may have caused stress on hatchery-produced spat. Low salinity has been observed to increase stress in oysters, which results in less protection from other environmental variables (Lannig et al. 2006). Furthermore, low salinity combined with high temperature impacts oyster survival.

High temperature coupled with low salinity may have greater impact on oyster survival. In St. Lucie Estuary, Florida, temperatures above 25°C and salinities below 5 ppt had the greatest negative impact on oyster fitness (Heilmayer et al. 2008). At Hackberry Bay in August, the water temperatures during deployment and sampling were greater than 25°C and over 30°C on some dates. These temperatures paired with low salinities likely affected survival of hatchery-produced spat. Spat showed no growth at HB 2004, thus, the combination of temperature and salinity are suggested as a main cause of

mortality at Hackberry Bay. The severity that temperature and salinity had on hatchery-produced spat survival at HB 2008 is difficult to tell. Maybe the larger spat could survive a period of low salinity and high temperature better than the freshly struck spat. In future studies, the effects of temperature and salinity on spat survival and growth should be distinguished from other causes of mortality.

Another cause of potential mortality is from sedimentation. Excessive sedimentation can smother oysters and cause an anoxic condition (Saoud & Rouse 2000; Soniat et al. 2004). At Hackberry Bay, visibility was low and soft sediment was collected in samples. LDWF reported in 2011 that the bottom of Hackberry Bay was mostly “soft silt and clay” (LDWF 2011). High sediment loads are not favorable to spat survival because the spat can be covered by silt and smothered. Larger spat oysters may have refuge from increased sediment loads, whereas, recently settled oysters might not have the ability to overcome sediment. In bottom samples collected pre-deployment, there was a range of oyster sizes. Oysters ranged from spat-size to market-size with mean sizes of 26.8 mm and 45.2 mm, HB 2008 and HB 2004 respectively. For market-size oysters to survive on soft bottom, the oysters continue to grow past the sediment layer (Wheaton 2007). For spat oysters to survive on soft bottom, they need substrate that will protect them from smothering. Soniat et al. (2004) suggests that vertical orientation of shell on reefs can significantly reduce mortality from sedimentation. It is possible that the wild oysters collected in the bottom samples were in vertical orientations, which promoted survival. Hatchery-produced spat had limited vertical orientation from the crushed poultry shell and marble aggregate. Thus, hatchery-produced spat mortality could be a result of limited vertical structure and sedimentation. Survival may be similar or the same as wild oysters, if hatchery-produced spat were deployed as market-size oysters or set to cultch with vertical relief, such as whole oyster shell. Thus, survival and growth on soft bottom, of all oyster sizes, requires cultch with vertical orientation.

Future applications of hatchery-produced spat in Louisiana should test survival on different cultch materials. Limestone significantly attracts oyster larvae (Soniat & Burton 2005). The marble aggregate, deployed at HB 2004, had larval settlement. Survival and growth after deployment, however, did not exist. Soniat and Burton (2005) mention the possibility of texture affecting larval settlement. Although

larvae set to the marble, survival and growth of spat on marble could be limited by texture. Based on surface observations, the marble had a smooth surface, rather than a porous or rough surface. The size of the marble may not be suitable for spat survival. More surface area of cultch material allows the cultch to stay on the seafloor surface (Bohn et al. 1995). Whole oyster shell has optimal surface area (Bohn et al. 1995). Whole shell secures seed to the bottom and prevents seed from sinking (Bohn et al. 1995). Cultch with less surface area has the tendency to sink between crevasses and into sediment (Bohn et al. 1995). The marble cultch may not have optimal surface area for staying on the seafloor surface. Thus, physical characteristics of cultch effects spat survival.

Structural complexity of a reef dictates the success of oyster survival, recruitment and growth (Nestlerode et al. 2007). Larger sized cultch materials, such as whole oyster shell and clamshell, provide more complexity for refuge from predation, sedimentation and hypoxia (Nestlerode et al. 2007). Smaller sized cultch material, such as gravel and shell fragments, are less attractive at providing suitable habitat complexity (Nestlerode et al. 2007). It is possible that the crushed poultry shell and marble cultch lacked the necessary structural complexity for spat survival. The use of larger cultch material at Hackberry Bay would provide structural integrity, possibly increasing survival. The increased surface area of larger cultch material is also beneficial for spat survival (Bohn et al. 1995). The soft bottom at Hackberry Bay is not suitable for planting small cultch material that is easily covered by sediment. Restoration projects at Hackberry Bay will require different cultch materials to set hatchery-produced spat and additional substrate material to create a structurally complex habitat.

Site selection is important for choosing a restoration site. Hackberry Bay was selected to plant spat because this site was near the oyster hatchery and is public seed ground. Sites selected within Hackberry Bay were the firmest areas detected by poling, a traditional means of evaluating oyster water bottom (Supan 2002). The majority of the bay had soft bottom, with minimal hard substrates. Oysters can survive and grow on soft bottoms, however, by growing in height to keep their shell opening above the sediment and silt (Wheaton 2007). Oysters can also survive on soft bottoms by vertical orientation (Soniat et al. 2004). Oysters on muddy reefs have characteristic shell shapes that are narrow, long and

thin (Wheaton 2007). Oysters that grow on more complex and firm bottoms tend to have thicker shells and are more uniform in shape, if they are not crowded (Wheaton 2007). Thus, sites with long and narrow oysters indicate a soft bottom and sites with more uniform and thicker shelled oysters indicate a harder bottom. In most circumstances, muddy bottoms do not favor oyster growth and survival (Soniat et al. 2004). Oysters survive best on complex reefs with hard substrates (Supan 2002). It was valuable to understand the integrity of hatchery-produced spat on soft bottom, since wild oysters can survive on soft bottoms by adapting shell shape. At Hackberry Bay, however, no hatchery-produced spat survived during this preliminary study.

The main contributor to hatchery-produced spat mortality cannot be determined, but previous research may suggest possible causes of mortality. Predation, temperature, salinity, sedimentation, bottom type, shell orientation and cultch type are variables that can affect spat growth and survival. The improvement of handling techniques and sampling techniques will assist with future research on hatchery-produced spat survival. Overall, it is valuable to assess hatchery-produced spat survival under different conditions to determine boundaries and limits.

Chapter 3
Survival of Hatchery-Produced Spat on Louisiana Public Oyster Grounds

Cultch plants are used as a management strategy to restore degraded or deteriorated oyster reefs (Berrigan 1990). Fisheries programs and leaseholders expect planted cultch to increase oyster production (Berrigan 1990). States along the Gulf Coast and East Coast, such as North Carolina, Maryland, Florida, Texas, Mississippi and Louisiana, use cultch plants to enhance and manage oyster production (Webster & Meritt 1988; Berrigan 1990; Lee 2004; VanderKooy 2012). Restoration activities, such as cultch planting programs that are conducted properly and managed well can stimulate and sustain oyster production (Coen & Luckenbach 2000).

Cultch plants can provide economic benefits to the oyster industry. Cultch planting, however, can be laborious and costly with a small net income compared to other aquaculture techniques, such as remote setting (Lee 2004). In 1986 and 1987, the Florida Department of Natural Resources in Apalachicola Bay, Florida showed that reef restoration economically benefitted the industry (Berrigan 1990; Lee 2004). The reef was restored with clamshell (*Rangia* spp.) cultch from Lake Pontchartrain, Louisiana and with alternative cultch, such as Calico Scallop shell, when clamshell was not available. In Florida, cultch planting remains an important part of oyster reef management. The Florida Division of Aquaculture plants oyster shell yearly from contributed local processing plants (FDACS 2012). Each year, since 1999, 250,000 bushels of oyster shell are planted in Florida. Another Gulf Coast natural resource agency with a cultch planting program is the Mississippi Department of Marine Resources (Becker 2011). In August 2011, it began a cultch plant project to capture the fall spat set. The types of cultch planted for this project were crushed oyster shell and limestone. On the East Coast, the Maryland Sea Grant Extension Service describes, specifically for the Chesapeake Bay, the benefits and techniques of planting cultch (Webster & Meritt 1988). It is necessary for public leaseholders in the Chesapeake Bay to plant cultch, because state law demands that leases are located on barren or marginal grounds (Webster & Meritt 1988). These grounds require cultch material to create suitable habitat for seed oyster production.

Cultch replenishment increases oyster production by providing suitable substrate for larval settlement and spat production (Berrigan 1990). The most preferred cultch material is oyster shell (Bohn et al. 1995). Oyster shell is physically and chemically suitable for oyster larvae (Soniat et al. 2004). Additionally, oyster larvae are attracted to calcium carbonate-based materials (Bohn et al. 1995; Soniat et al. 2004; Soniat & Burton 2005). Oyster shell has a wide surface area, which keeps the shell on bottom and from sinking (Bohn et al. 1995). Oyster shell also provides vertical orientation and interstitial spaces for protection from sedimentation and predation (Soniat et al. 2004). In some circumstances, oyster shell is limited as reef and cultch material, thus, alternative cultch are required to provide substrate for larval settlement and recruitment (Piazza et al. 2005). Planting alternative cultch is a method to create oyster reefs and manage seed production.

Oyster shell resources are limited for purchase in Louisiana and alternative cultch are used to compensate for minimal shell resources (VanderKooy 2012). The Louisiana Department of Wildlife and Fisheries conducts cultch plantings on public oyster grounds with materials, such as crushed concrete and limestone (VanderKooy 2012). It is expected that the planted cultch will provide substrate for oyster larvae, which will metamorphose into competent spat.

Louisiana is one of the leading producers for oysters in the United States. The current method of oyster production in Louisiana, seed bedding, relies on the production of naturally-produced oyster seed (Supan 2002). Spat survival, however, depends on numerous environmental and anthropological factors, such as predation, sedimentation, freshwater diversions, disease, cultch type, storms, salinity and temperature (Brown et al. 2003; Soniat et al. 2004; Soniat & Burton 2005; McGuire 2006; La Peyre et al. 2010). Instead of relying on natural seed production, raising seed at a hatchery is a more reliable method for oyster production (Swartzenberg 1999). Furthermore, producing spat at a hatchery minimizes outside influences that affect larval survival and recruitment (Jones & Jones 1983). To better restore Louisiana oyster grounds, an alterative or supplement to cultch plantings may entail planting hatchery-produced spat.

This project involved collaborative efforts between the Sea Grant Oyster Hatchery on Grand Isle, LA and the Louisiana Department of Wildlife and Fisheries. The hatchery produced, competent, pediveliger larvae and set the larvae to poultry shell cultch. The poultry shell was commercially available as pullet shell for chicken ranching and was purchased from a local vendor. Additionally, the poultry shell could be easily and gently moved out of setting tanks with water. Poultry shell was also used to mimic historical accounts of Louisiana oyster growers planting crushed shell to produce single oysters (J. Supan, personnel communication). LDWF deployed the spatted cultch onto 0.405-hectare (one-acre) plots on established cultch plants in southeast Louisiana. The preliminary study from the summer of 2011 provided insight on spat handling techniques and on spat survival on a soft bottom site. In the summer of 2012, hatchery-produced spat were gently transported and deployed on harder bottom at California Bay and Round Island.

California Bay and Round Island sites are both 2011 emergency restoration cultch plants and are observed to have low numbers of spat (P. Banks, personnel communication). These sites were greater than 152.4 m from the existing National Resource Damage Assessment, as a response to the British Petroleum Deepwater Horizon oil spill (P. Banks, personnel communication). Thus, California Bay and Round Island were not located in NRDA study areas (P. Banks, personnel communication). California Bay is part of a cultch planting program that began in 1917 and remains an active site for planting cultch (LDWF 2011). The most recent cultch plants at California Bay were in 2007 and 2009 (LDWF 2011). California Bay is one of a couple sites that are managed by LDWF for biologically sustaining the oyster resources and economically providing opportunities (LDWF 2011). There are multiple sites in Mississippi Sound that are sampled for oyster stock assessment by LDWF (LDWF 2011). Round Island is a location within Mississippi Sound that is assessed for oyster production and planted with cultch material.

Hatchery-produced spat survival was evaluated with quadrat sampling (September 2012) and volumetric sampling (November 2012 and January 2013). The goal of this project was to assess the application of hatchery-produced spat on Louisiana public oyster grounds. The objective was to test the

variability in occurrence (total number of live oysters) between hatchery-produced oyster spat and wild oyster spat at California Bay and Round Island, Louisiana. The null hypothesis was the total number of live hatchery-produced spat would be equal in both treated plots (with hatchery-produced spat) and untreated plots (without hatchery-produced spat).

Materials and Methods

The Sea Grant Oyster Hatchery on Grand Isle, Louisiana produced competent pediveliger oyster larvae using standard hatchery techniques for spat deployment in the summer of 2012 (Supan & Wilson 1993). Spat were set to crushed poultry shell, a calcium carbonate-based material. All crushed shell was graded and retained on a 3.175 mm (1/8 inch) mesh, rinsed with ambient seawater, and transferred into five fiberglass-reinforced setting tanks (internal tank dimensions 3.0 m x 0.6 m x 0.3 m). Crushed shell was added to each tank to sufficiently cover the bottom of the tank 25 mm deep. The cultch was covered with filtered one-micron absolute seawater, with 25 mm deep seawater covering the cultch. Each setting tank received up to approximately four million larvae. Due to hatchery production, it took two to three days to obtain four million larvae per tank; therefore, larvae were fed 4 L of hatchery-produced algae once a day. Algal species included *Chaetoceros calcitrans* and *Isochrysis* sp. (clones T-Iso and C-Iso). Algae were produced by the Sea Grant Oyster Hatchery using standard protocols (Supan & Wilson 1993). The larvae were allowed at least 24 hours to set, and then deployed immediately to the study site(s) or held in the hatchery's nursery system for one to two days until planting.

Three 100 mL dry volume subsamples of spatted cultch were collected to determine the mean initial number of spat deployed at each plot. A stereo dissecting microscope (Leica EZ4) (0.7 to 30x) was used to analyze the spat set. This study focused on hatchery-produced spat survival, thus, only the initial number of live spat deployed at the sites was important. The estimated number of live spat deployed per plot was calculated by the equation;

$$(\text{\# of live spat} \div 100 \text{ mL}) * \text{total volume (mL) of spatted cultch.}$$

Spat were deployed on selected 0.405-hectare (one-acre) plots at California Bay and Round Island in Mississippi Sound, with the assistance of LDWF personnel and vessels (Figure 7).

Approximately 8.7 million hatchery-produced spat were deployed at California Bay and approximately 11.1 million spat were deployed at Round Island (P. Banks, personnel communication). Spat were deployed as freshly struck spat, set on poultry shell.

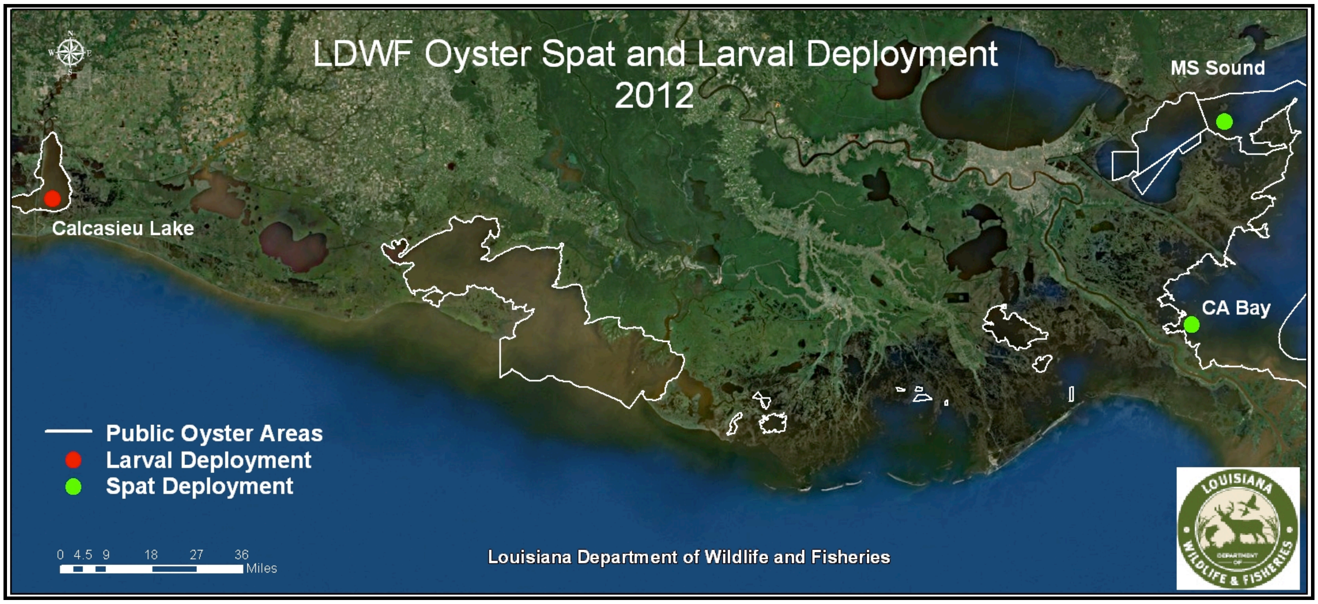


Figure 7. Louisiana Department of Wildlife and Fisheries hatchery-produced oyster spat deployment sites (CA Bay=California Bay, MS Sound=Mississippi Sound, where the Round Island site is located) and larval release site (Calcasieu Lake) (2012).

Spatted cultch was transferred from the hatchery to the sites in 18 L transportation buckets fitted with 1.588 mm drainage holes and covers. The duration of time that spat remained out of water was minimized to avoid desiccation. In one circumstance, a seaplane transferred spat from the hatchery to a vessel waiting at the California Bay deployment site.

California Bay and Round Island are sectioned by LDWF into grids and marked with a central GPS coordinate for each grid. Grids from each site were randomly selected by LDWF for spat deployment. For easier deployment and boat maneuvering, grids were transformed into circular 0.405-hectare (one-acre) plots. To deploy spat, the transportation bucket was arched over the water's surface, while the vessel moved in concentric circles around the central point within the plot (Figure 8). Untreated plots were similar and did not receive hatchery-produced spat.

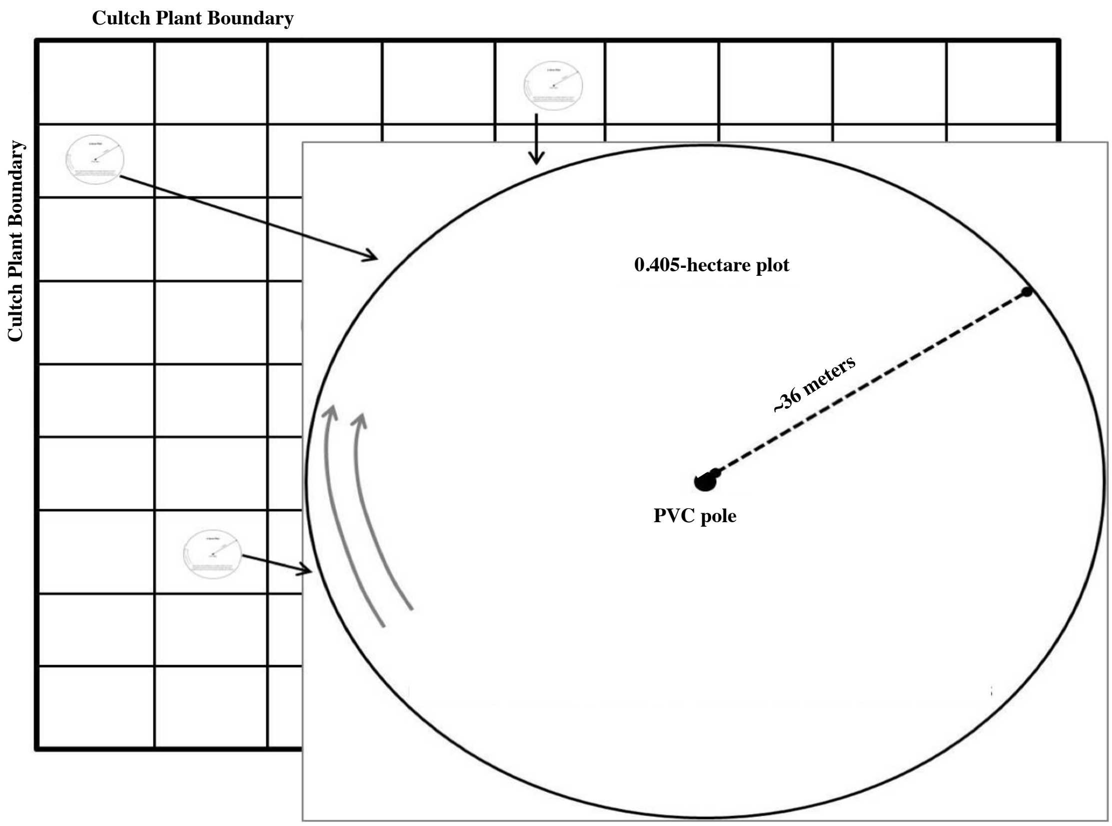


Figure 8. Diagram depicting the deployment of hatchery-produced spat on 0.405-hectare (one-acre) plots.

Three treated and three untreated plots were randomly selected to sample from California Bay and Round Island. The same plots were sampled in September, November (2012) and January (2013), however, one untreated plot at California Bay was reselected due to soft sediment.

September 2012 Spat Sampling

The September 2012 sampling event collected quadrat samples with SCUBA diving. In the preliminary Hackberry Bay study, dive sampling was more effective at collecting smaller cultch material. To improve standardization and sampling methods, experienced LDWF divers were instructed to collect four, quarter-meter square, quadrat samples per sampling location per plot at each site (Figure 9). The vessel crew selected the sampling locations and divers were instructed on where to sample.

The divers collected all solid contents within the quadrat and delivered individual samples to the vessel crew. Each sample was delivered to the vessel in a mesh-sampling bag, a standard collection method used by LDWF. The crew monitored the sampling and either deployed the quadrat for the diver

or allowed the diver to move the quadrat within the sampling location. To make maneuvering and sampling easier for the divers, sampling locations were chosen based on the position of the vessel. Generally, one sampling location was on the port side, one on the starboard side and one off the stern. If it appeared that the port and starboard sampling locations intersected with the stern location, the vessel was moved within the plot to a new location. The crew placed each sample in separate, labeled, burlap sacks and transported the samples to Louisiana State University for analysis. Burlap sacks were labeled as treated (T) or untreated (U), grid/plot number, sampling location (1 through 3 or A through B) and quadrat number (Q1, Q2, Q3 and Q4).

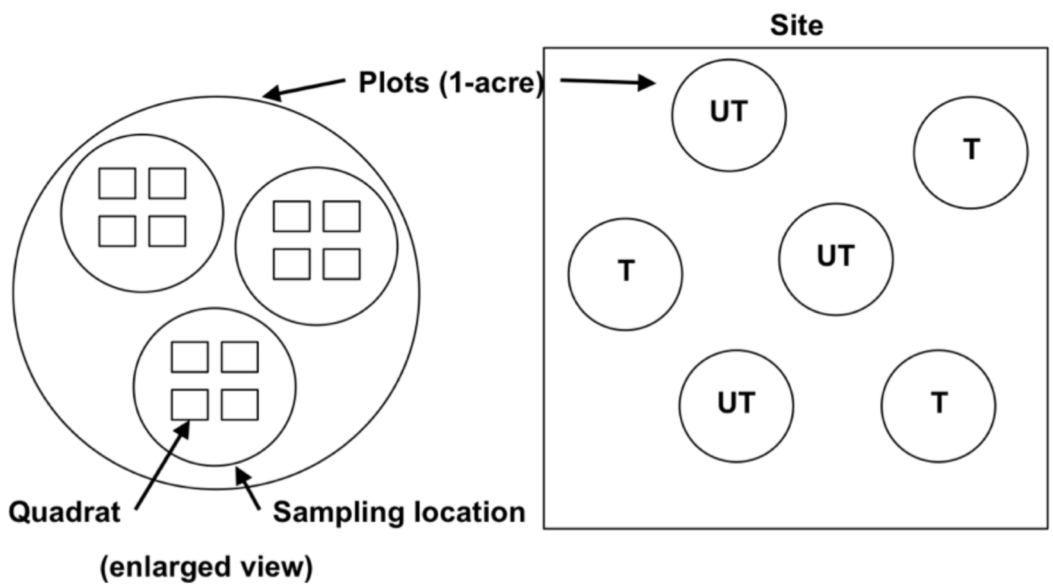


Figure 9. Diagram depicting quadrat sampling in September 2012. Plots were treated (T) with hatchery-produced spat or untreated (UT) without hatchery-produced spat. There were three sampling locations with four, replicate, quarter-meter square, quadrats (total of 12 quadrats) per plot.

The samples were transported for survival and growth analysis of oyster spat. Initially, the samples were brought to LDWF's Lacombe office and stored in a cool, dry room for two days (California Bay samples) or one day (Round Island samples). The samples were then transported to the Louisiana Sea Grant office in Baton Rouge, LA for analysis. Each sample was examined for live hatchery-produced spat, which was identified by the cultch type (poultry shell) or if the oyster was an individual or in a cluster of two or three. Data was recorded on LDWF's oyster sample data sheet (Appendix A). Oysters

were categorized into three size classes (i.e. spat [< 24 mm], seed [25 to 74 mm] and sack [> 75 mm]). Shell height was measured along the oyster's longest vertical axis, from the end of the umbo to the end of the bill. All spat, seed and sack-size oysters were measured.

November 2012 Spat Sampling

The sampling method changed in November, due to weather conditions. Each plot was sampled using a hand dredge to collect three, replicate, volumetric samples per plot. Overall, there were three 18 L replicate samples per plot. The dredge basket was fitted with shrimp trawl webbing (6.53 mm bar mesh size) to collect the cultch material that hatchery-produced spat was set on. The dredge was towed off the vessel's stern and completed as many tows as necessary to collect the samples within the plot.

Samples were transported in individually labeled, burlap sacks. Samples were stored in an outdoor facility before transporting the samples to the Louisiana Sea Grant office for survival and growth analysis. Sacks were labeled as treated or untreated (T or U), site location (C for California Bay or R for Round Island) grid number and replicate number.

Oyster growth and survival data was recorded on LDWF's oyster sample data sheet. The focus was on finding live hatchery-produced spat (< 24 mm). Hatchery-produced spat would be set to poultry shell or appear as an individual oyster or in clusters of two or three oysters, with minimal to no cultch material visible. It was expected that wild spat would be attached to limestone or concrete planted by LDWF.

January 2013 Spat Sampling

The January 2013 spat sampling maintained the same sampling methods from November 2012. Additionally, the same plots from November were sampled in January.

Water Quality

Temperature and salinity data was recorded either by hand held instrumentation or by using a nearby United States Geological Survey (USGS) station(s) during spat deployment at California Bay and Round Island. Data gathered by hand recorded surface and bottom water quality, while, the USGS stations gather surface water quality data. There is no existing USGS station at Round Island, therefore,

two nearby stations were obtained as salinity and temperature references for Round Island. These two stations included "Mississippi Sound near Grand Pass" (Grand Pass) and "Mississippi Sound at USGS St. Joseph Island Light" (St. Joseph Island Light) (Appendix B; Appendix C). The Grand Pass station is located near Oyster Bay in Mississippi Sound (30°7.367'N, 89°15.017'W) and the St. Joseph Island Light is located in Mississippi Sound (30°11.448'N, 89°25.329'W) (USGS). There is partial salinity data for St. Joseph Island Light (Appendix C). There is, however, complete specific conductance data for each station. The closest USGS station to the California Bay site was "Northeast Bay Gardene near Point-A-LA-Hache" (29°35.145'N, 89°36.358'W) (Appendix D). The USGS stations were also used to assess the salinity and temperature over the course of the study, from pre-spat deployment to the end of January.

The recorded on-site surface and bottom water temperature (°C), salinity (ppt), dissolved oxygen (mg/L) and specific conductivity (uS/cm), was measured with a YSI-6050000 YSI Pro Plus Multi-Parameter Water Quality Meter System for hand data collection, while, USGS stations collected supplemental data including, water temperature, salinity, specific conductance and gage height (YSI Incorporated, Yellow Springs, OH 45387). Water quality during the spat deployment at California Bay was recorded by hand, however, water quality during spat deployment at Round Island was estimated with USGS station data.

Ambient seawater temperature (°C) and salinity (ppt) was measured at the Sea Grant Oyster Hatchery on Grand Isle, LA, during spat setting. This data approximates the temperature and salinity in the setting tanks prior to spat deployment or on spat deployment dates. Not all values are available on exact deployment dates; therefore, the closest available data to the deployment date is provided.

Statistical Analysis

For this project, the data of interest was the total number of live hatchery-produced spat oysters. The ANOVA was used to test for significant differences in the number of live spat oysters between treated and untreated plots. All statistical analyses were done on SAS and a significance level of $P<0.05$ was used (procedure PROC MIXED, SAS Institute Inc., 9.3).

Results

The number of spat deployed at California Bay varied for each deployment date (Table 4). Approximately 500,000 spat were deployed in May and August, however, approximately two million spat were deployed in July.

The number of spat deployed at Round Island was similar for both deployments on July 20, approximately one million spat (Table 5). The least number of spat deployed was on July 3, which were approximately 18,000 spat.

Ambient seawater temperature and salinity was measured at the Sea Grant Oyster Hatchery on Grand Isle, LA (Table 6). Not all temperature and salinity data was available from the hatchery during hatchery-produced spat deployments dates; therefore, the temperature and salinity in the setting tanks was approximated with the closest available data. The temperature and salinity in the setting tanks during spat deployments at California Bay was potentially between 27°C to 39°C and 20 ppt to 34 ppt (May), near 28°C and between 26 ppt to 28 ppt (July) and near 32°C and 27 ppt (August). The temperature and salinity in the setting tanks during deployments at Round Island was potentially between 28°C to 31°C and 24 ppt to 26 ppt (July 3) and 30°C and 25 ppt (July 20).

Table 4. Location (treated or untreated plots), deployment date, estimated number of spat deployed, sampling plots and GPS coordinates for the center of each 0.405-hectare (one-acre) plot, as well as total estimated number of spat deployed. For California Bay hatchery-produced spat deployment (2012).

Location	Deployment Date	Spat Deployed	Plot #	GPS Coordinate
CA Bay Treated	5/30/12	408,998	22	29°30.206'N, 89°33.893'W
	7/12/12	2,000,000	8	29°30.024'N, 89°33.685'W
	8/13/12	535,263	50	29°30.433'N, 89°33.414'W
	TOTAL	2,944,261		
CA Bay Untreated			41	29°30.382'N, 89°33.590'W
			54*	29°30.570'N, 89°33.888'W
			46	29°30.482'N, 89°33.889'W
			21**	29°30.209'N, 89°33.998'W

Plot # 54* was replaced for sampling in November by Plot # 21**, which was sampled on November 20, 2012. Plots in September were sampled with SCUBA and collected four quarter-meter square quadrat samples per sampling location per plot (three sampling locations per plot). Plots in November were sampled with hand dredges and collected three 5-gallon buckets dry volume of cultch per plot.

Table 5. Location (treated or untreated plots), deployment date, estimated number of spat deployed, sampling plots and GPS coordinates for the center of each 0.405-hectare (one-acre) plot, as well as total estimated number of spat deployed. For Round Island hatchery-produced spat deployment (2012).

Location	Deployment Date	Spat Deployed	Plot #	GPS Coordinate
RI	7/03/12	18,000	18	30°6.999'N, 89°27.199'W
Treated	7/20/12	1,007,239	23	30°7.099'N, 89°27.610'W
	7/20/12	1,206,271	5	30°6.822'N, 89°27.201'W
	TOTAL	2,231,510		
RI			44	30°7.281'N, 89°27.718'W
Untreated			29	30°7.087'N, 89°26.884'W
			20	30°6.999'N, 89°26.990'W

Plots in September were sampled with SCUBA and collected four quarter-meter square quadrat samples per sampling location per plot (three sampling locations per plot). Plots in November were sampled with hand dredges and collected three 5-gallon buckets dry volume of cultch per plot.

Table 6. Ambient seawater temperature and salinity at the Sea Grant Oyster Hatchery on Grand Isle, LA. Provides closest available data to hatchery-produced spat deployments dates at California Bay and Round Island (2012).

Site	Deployment Date	Hatchery Date	Temperature (°C)	Salinity (ppt)
California Bay	5/30	5/19	27	20
		5/31	39	34
	7/12	7/10	28	28
		7/12	n/a	26
	8/13	8/13	32	27
Round Island	7/03	7/01	31	24
		7/04	28	26
	7/20	7/14	30	25
		7/20	n/a	25

Water quality was measured during deployment at California Bay and was supplemented with USGS station data during deployment at Round Island (Table 7; Table 8). The temperature during all spat deployment dates at California Bay was approximately 30°C (Table 7). The salinity during spat deployment at California Bay was 10 ppt, in May, and 19 ppt and 20 ppt, in July and August, respectively (Table 7). The estimated temperature and salinity at Round Island during all spat deployments was approximately 30°C and 19 ppt (Table 8).

Table 7. Deployment date, surface (S) and bottom (B), temperature, salinity, dissolved oxygen, specific conductivity and gage height (from USGS station data) at California Bay spat deployments (2012).

Deployment Date	Surface/ Bottom	Temperature (°C)	Salinity (ppt)	Dissolved Oxygen (mg/L)	Specific Conductivity (uS/cm)	Gage Height (USGS) (m)
5/30/12	S	29.0	10.3	9.5	n/a	0.12
	B	28.8	10.5	8.3	n/a	
7/12/12	S	30.0	19.5	8.2	34,500	0.09
	B	28.7	20.2	4.0	34,500	
8/13/12	S	30.7	19.2	8.8	34,500	0.08
	B	30.2	20.7	6.0	36,400	

Table 8. Deployment date, United States Geological Station (USGS) stations, temperature, salinity, specific conductance and gage height. The mean temperature, salinity and specific conductance were calculated to determine estimated water quality at Round Island spat deployments (2012).

Deployment Date	USGS Station	Temperature (°C)	Salinity (ppt)	Specific Conductance (uS/cm at 25°C)	Gage Height (m)
7/3/12	Grand Pass	31.2	19.0	30,600	0.42
	St. Joseph Island Light	30.7	n/a	23,300	0.86
	Round Island (estimate)	31.0	19.0	26,950	0.64
7/20/12	Grand Pass	28.7	18.5	29,900	0.38
	St. Joseph Island Light	28.8	n/a	24,500	0.84
	Round Island (estimate)	28.8	18.5	27,200	0.61

Water quality was collected from all treated and untreated sites during sampling periods, September, November and January. All the treated and untreated plots had similar data for surface and bottom water quality; thus, the means of surface and bottom water quality from the plots were used to estimate water quality during each sampling period (Table 9). The salinity at California Bay was approximately the same during the September, November and January sampling periods. At California Bay, the temperature was the highest in September (approximately 28°C) and similar in November and January (approximately 14 to 15°C). The salinity and temperature at Round Island varied during sampling periods. At Round Island, the salinity was the lowest (approximately 3 ppt) in January and

highest in November (approximately 15 ppt). The temperature at Round Island was the highest in September (approximately 27°C) and lowest in January (approximately 12°C).

Table 9. Sampling date, mean surface and bottom water quality between treated and untreated plots, salinity, temperature, specific conductivity and dissolved oxygen at California Bay and Round Island during sampling periods (2012 & 2013).

Sampling Date	Surface/ Bottom	Salinity (ppt)	Temperature (°C)	Specific Conductivity (uS/cm)	Dissolved Oxygen (mg/L)
California Bay					
9/24/12	S	14.4	28.6	25,500	6.6
	B	14.4	28.3	25,400	5.6
11/14/12	S	15.0	14.9	19,900	8.9
	B	15.8	14.9	20,900	8.6
1/22/13	S	13.3	13.7	17,200	10.3
	B	13.4	13.6	17,300	10.4
Round Island					
9/25/12	S	6.1	27.5	11,300	7.0
	B	6.4	27.2	11,700	6.2
11/15/12	S	14.7	15.0	18,900	9.0
	B	15.1	14.8	20,000	8.8
1/23/13	S	3.7	11.9	4,900	10.5
	B	3.8	11.5	5,000	10.5

Sample Analysis

At California Bay and Round Island, quadrat and volume samples did not contain hatchery-produced spat set on poultry shell. Thus, there was 100% mortality of hatchery-produced spat at California Bay and Round Island.

The samples did contain, however, wild oysters that were set on LDWF cultch. Therefore, an ANOVA was used to test a hierarchy of variances between treated and untreated plots, sampling locations (quadrat sampling), plots, quadrats (quadrat sampling) and replicates (volumetric sampling) (Appendix E). The most important test of variance was to determine if planted hatchery-produced spat had an affect on the number of wild spat on treated plots. For quadrat sampling, the variance was tested for the number of live wild spat between plots within treated or untreated (T or U), between sampling locations within

treated or untreated plots and between quadrats (residuals) within sampling locations within treated or untreated plots (Appendix E). For volumetric sampling, the variance was tested for number of live wild spat between plots within treated or untreated (T or U) plots and between replicates (residuals) within treated or untreated plots (Appendix E). The number of live wild spat was significantly different between residuals, for quadrat and volumetric sampling, for all sampling periods at Round Island and California Bay ($P<0.05$) (Appendix E).

The variance, in the number of live wild spat between treated and untreated plots, was tested for each sampling period at each site. There were no significant differences between the number of live wild spat on treated sites to the number of wild spat on untreated sites in September, November and January at Round Island or California Bay ($P<0.05$) (Table 10).

Table 10. Type 3 Tests of Fixed Effects, for number of live wild spat between treated and untreated plots (procedure PROC MIXED, SAS Institute Inc., 9.3). Site, sampling date, effect, numerator and denominator degrees of freedom, F values and P values. Significance level is $P<0.05$.

Site	Sampling Date	Effect	Num DF	Den DF	F Value	Pr > F
Round Island	9/25/12	TreatedUntreated	1	4	0.23	0.6575
	11/15/12	TreatedUntreated	1	4	0.31	0.6078
	1/23/13	TreatedUntreated	1	4	2.87	0.1656
California Bay	9/24/12	TreatedUntreated	1	4	0.30	0.6119
	11/14/12	TreatedUntreated	1	4	1.34	0.3112
	1/22/13	TreatedUntreated	1	4	1.03	0.3679

Quadrat and Volumetric Sample Overview

The quadrat samples from California Bay in September (2012) contained no hatchery-produced oysters and few wild oysters (Figure 10 & Figure 11). From the 36 sampled quadrats on treated plots, six quadrats contained wild oysters set to LDWF planted cultch (Figure 10). The treated plots contained more spat than seed or sack-size oysters (Figure 11). There were no seed oysters found in treated plots. In the 36 untreated quadrats, three quadrats contained wild oysters (Figure 10). There were more spat oysters on treated plots than seed or sack oysters (Figure 11). Overall, the greatest number of wild oysters was spat-size in treated plots (Figure 11).

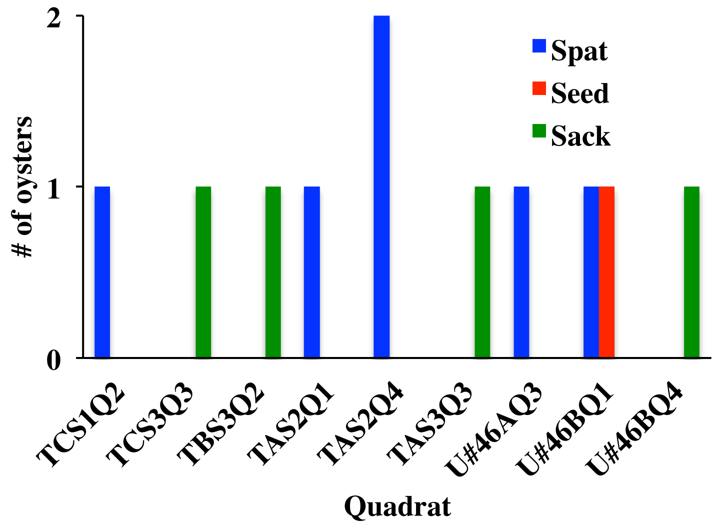


Figure 10. Number of oysters from individual quadrat samples in treated and untreated California Bay plots (September 24, 2012). Oysters are classified by size (spat, seed or sack).

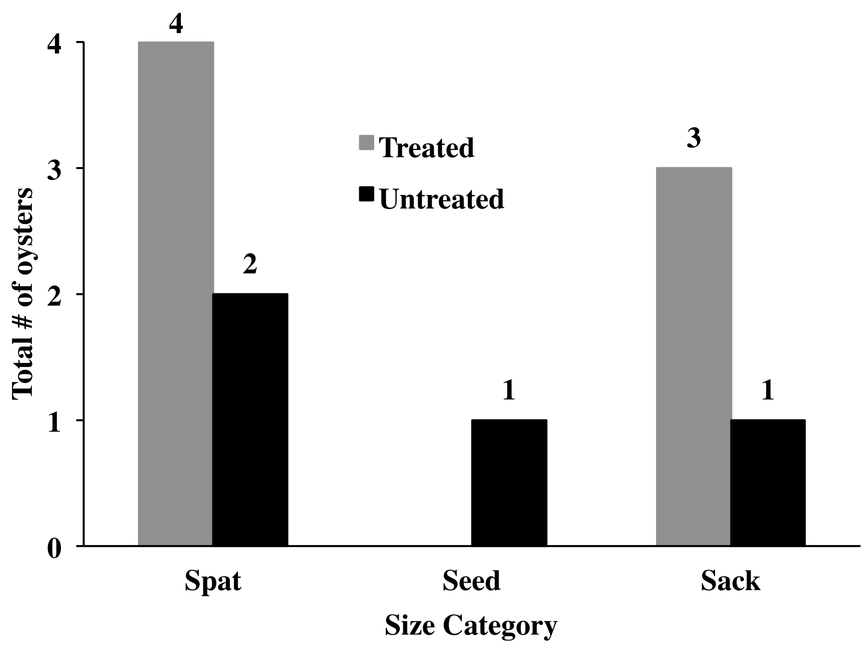


Figure 11. Total number of spat, seed and sack size oysters from quadrat samples in treated and untreated plots at California Bay (September 24, 2012) (n=36 total treated samples; n=36 total untreated samples).

The volumetric samples from California Bay in November (2012) contained wild oysters set to LDWF planted cultch (n=3 replicate, dredge samples per plot) (Figure 12). The number of sack-size oysters was greatest in untreated and treated plots (Figure 13). The number of seed-size oysters was least of oysters collected from treated and untreated plots (Figure 13). There were more oysters in treated plots

than untreated plots for spat, seed and sack sizes (Figure 12). Overall, there were more spat, seed and sack oysters collected from treated plots than untreated plots.

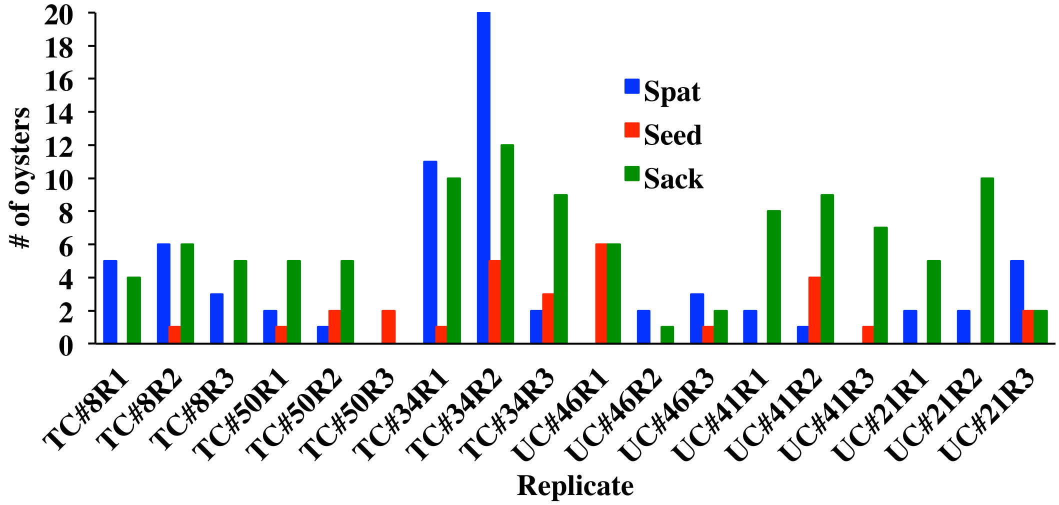


Figure 12. Number of oysters collected from volumetric samples in treated and untreated California Bay plots (November 14, 2012) (grid #21 was sampled on November 20, 2012). Oysters are classified by size (spat, seed or sack).

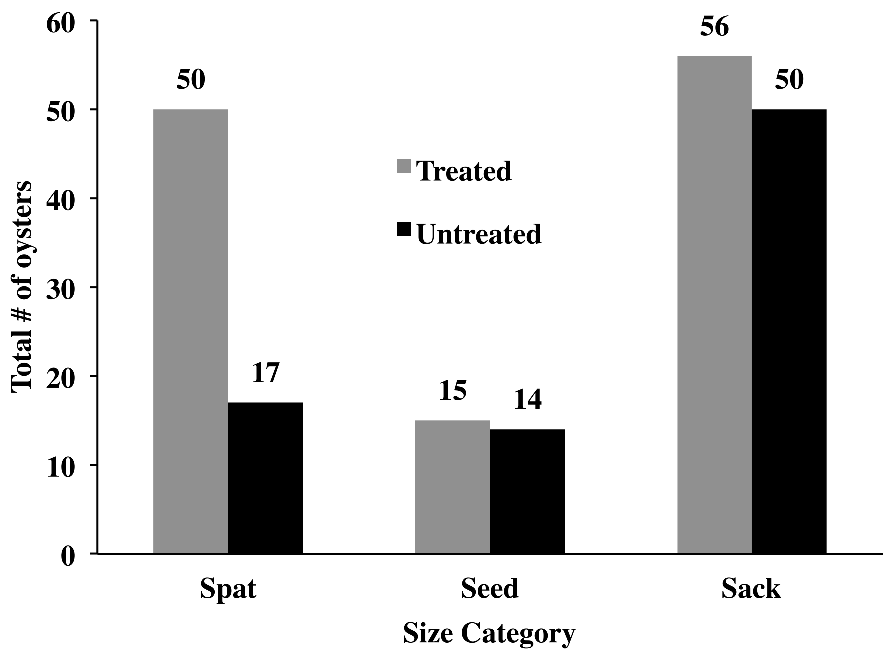


Figure 13. Total number of spat, seed and sack size oysters from volumetric samples in treated and untreated plots at California Bay (November 2012) (n=9 total treated samples; n=9 total untreated samples).

The volumetric samples from California Bay in January (2013) contained wild spat, seed and sack-size oysters set to LDWF planted cultch (Figure 14). The greatest numbers of oysters were sack-size for both treated and untreated plots (Figure 15). The least number of oysters were spat-size for both treated and untreated plots (Figure 15).

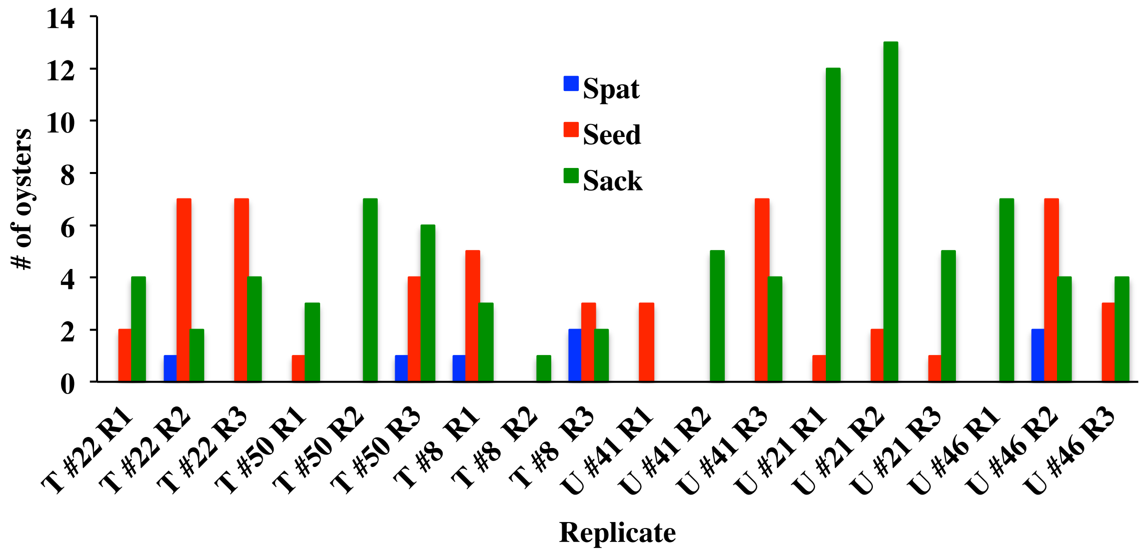


Figure 14. Number of spat, seed and sack size oyster from volumetric samples at California Bay (January 22, 2013).

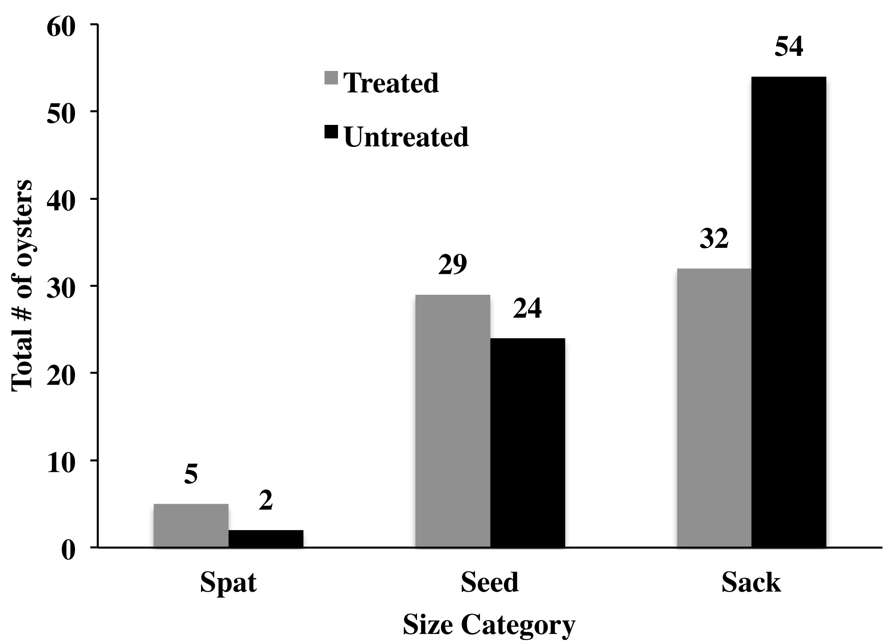


Figure 15. Total number of spat, seed and sack size oysters on treated and untreated plots at California Bay (January 22, 2013).

The quadrat samples from Round Island in September contained wild oysters set to LDWF planted cultch. Most of the quadrat samples from Round Island contained spat and seed-size oysters (Figure 16 & Figure 17). The number of sack-size oysters was the least in both untreated and treated plots (Figure 17). In the untreated and treated plots, there was a greater number of spat and seed-size oysters than sack-size oysters (Figure 17). Overall, the greatest numbers of oysters were spat-size oysters from treated

plots.

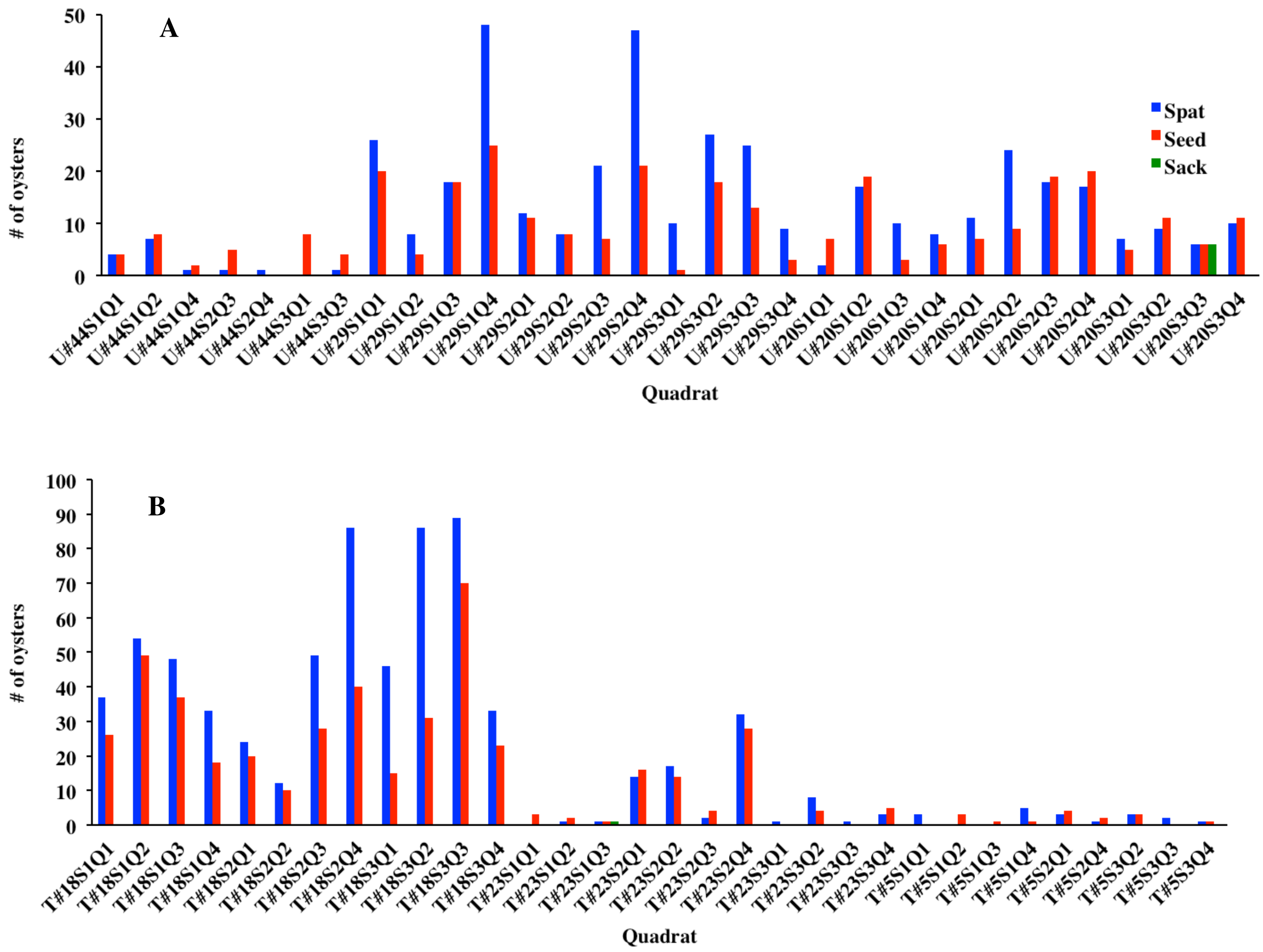


Figure 16. Number of oysters from individual quadrat samples in treated (A) and untreated (B) Round Island plots (September 25, 2012). Oysters are classified by size (spat, seed or sack).

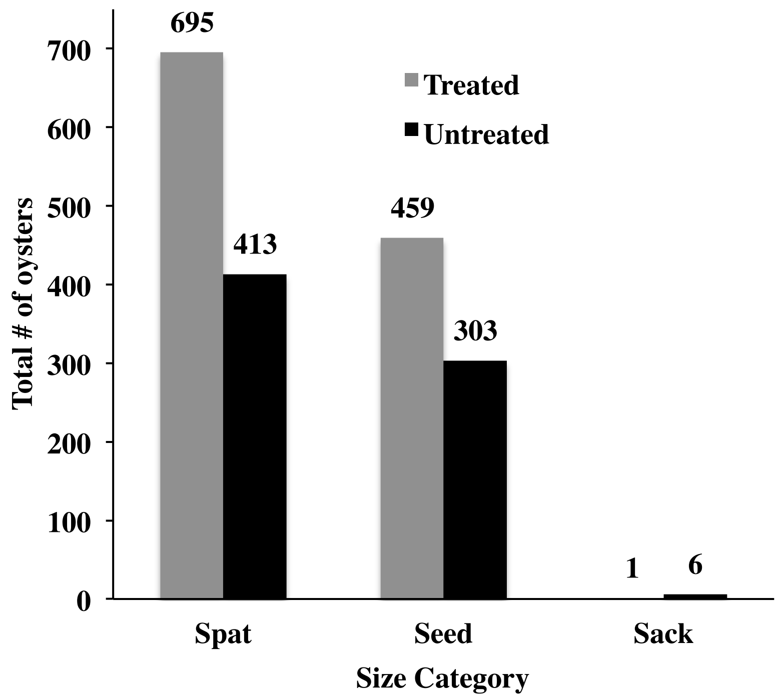


Figure 17. Total number of spat, seed and sack size oysters from quadrat samples in treated and untreated plots at Round Island (September 25, 2012) (n=36 total treated samples; n=36 total untreated samples).

The volumetric samples from Round Island in November contained live wild oysters set to LDWF planted cultch (Figure 18).

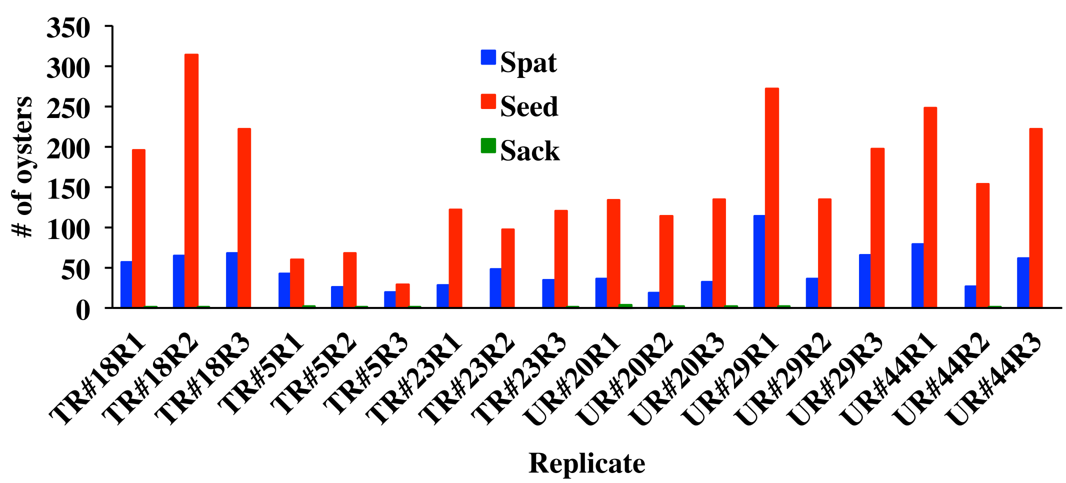


Figure 18. Number of oysters collected from volumetric samples in treated (A) and untreated (B) plots at Round Island (November 15, 2012). Oysters are classified by size (spat, seed or sack).

From both treated and untreated plots, the greatest total number of oysters was seed-size (Figure 19). The least number of oysters from treated and untreated plots were sack-size (Figure 19). Untreated plots

contained more oysters for each size category than treated plots (Figure 19). Overall, the greatest numbers of oysters were seed-size oysters in untreated plots.

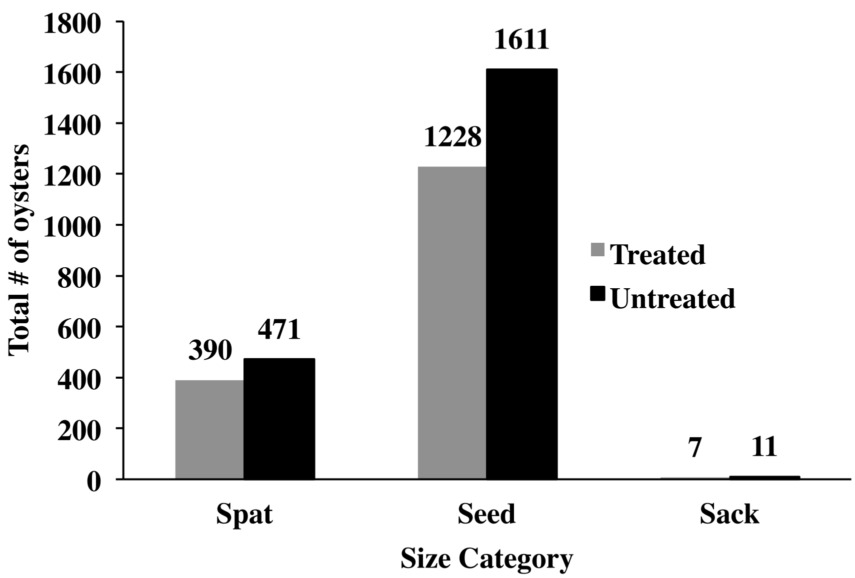


Figure 19. Total number of spat, seed and sack size oysters from volumetric samples in treated and untreated plots at Round Island (November 15, 2012).

The volumetric samples from Round Island in January (2013) contained wild oyster set to LDWF planted cultch (Figure 20; Figure 21).

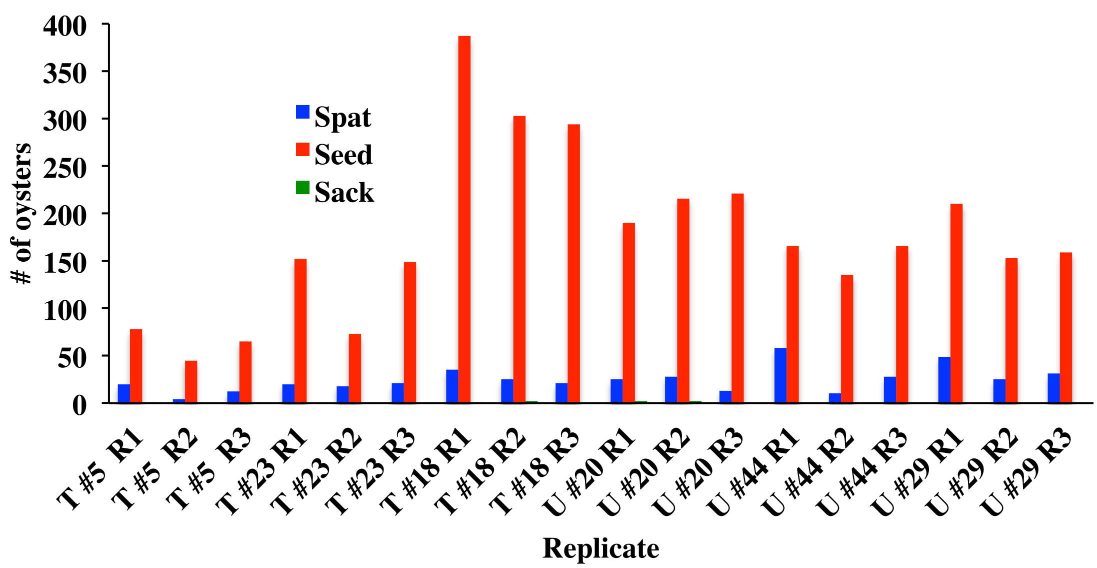


Figure 20. Number of spat, seed and sack size oyster from volumetric samples in treated and untreated plots at Round Island (January 23, 2013).

There were more wild seed oysters than spat oysters in all treated and untreated plots (Figure 20). From both treated and untreated plots, there were more wild seed oysters than spat or sack-size oysters (Figure 21).

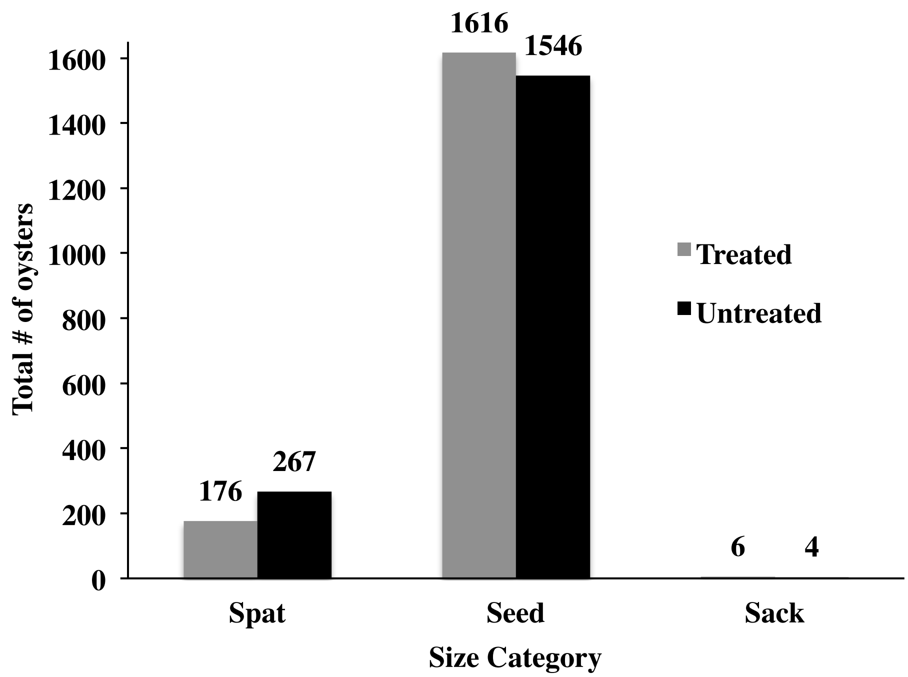


Figure 21. Total number of spat, seed and sack size oysters from volumetric samples in treated and untreated plots at Round Island (January 23, 2013).

Overall, Round Island volumetric samples contained more spat and seed oysters than California Bay and California Bay contained more sack oysters than Round Island.

Observations were made in samples collected at Round Island and California Bay. During sampling, crabs were observed among cultch material from the volumetric and quadrat samples. Crab types included mud crabs, stone crabs and blue crabs. Hooked mussels covered the majority of the oysters from samples at California Bay. Some quadrat and volumetric samples from Round Island contained black-colored cultch material. The dark color indicates that this cultch was buried. There were also shell particulates at some of the Round Island samples. These shell fragments were from deteriorated oyster shell.

Discussion

The high mortality of hatchery-produced spat at Round Island and California Bay is likely related to a combination of events. These events include sedimentation, predation, salinity and temperature and bottom type. Specific observations from the sites, such as buried cultch material, cultch type and fouling organisms, can also account for mortalities.

Hooked mussels were observed on oysters collected from California Bay. Fouling from hooked mussels may be a cause of hatchery-produced spat mortality or an indicator of water quality. Fouling organisms can compete for space and food, threatening oyster survival (LDWF 2011). In the North Pontchartrain Basin oyster stock assessment, LDWF observed high densities of hooked mussels in lower salinity sites (LDWF 2011). LDWF also observed that when salinity increased in eastern Mississippi Sound areas, hooked mussel densities decreased (LDWF 2011). Based on these observations, the presence of hooked mussels found at California Bay may indicate a low salinity area. This corresponds with low salinity recordings by the USGS during the spring and summer of 2012 (Appendix D). The salinity recorded at California Bay corresponds with previous observations and this study's observations of finding hooked mussels in a low salinity area, such as California Bay. Annual statistics from 2002 (USGS) show that California Bay salinity remains low (Appendix D). Hooked mussels are observed to survive low salinities (Melancon et al. 2003). Surviving lower salinities provides an advantage over predators, because the hooked mussels can survive low salinities, but their predators cannot (Melancon et al. 2003). Therefore, the high presence of mussels at California Bay suggests that California Bay is a low salinity site. The mortality of hatchery-produced spat at California Bay may have been stunted, due to competition for food resources and suitable places to survive. The mean salinities for June and July and the salinity levels displayed in the USGS graph generally remain in an optimal range for oyster spat survival and growth in the Gulf of Mexico (Lorio & Malone 1994).

After the first deployment (May 30) of hatchery-produced spat at California Bay, the daily mean salinity remained near 5 ppt, until June 6, and the water temperature remained near 30°C (USGS station data for Northeast Bay Gardene near Point-A-LA-Hache). Low salinities, less than 5 ppt, coupled with

high water temperatures, above 30°C, is not optimal for oyster survival and growth (Heilmayer et al. 2008). Oysters from St. Lucie River Estuary, Florida experienced the most detrimental effects when exposed to salinities of 5 ppt and water temperatures of 30°C (Heilmayer et al. 2008). Under these conditions, oysters had reduced somatic growth and stopped feeding below 5 ppt (Heilmayer et al. 2008). Oysters closed their valves under these conditions, which causes carbon dioxide accumulation and eventual death (Heilmayer et al. 2008). The observations made on St. Lucie River Estuary oysters may be a similar scenario as to California Bay. The immediate exposure of hatchery-produced spat to these conditions is a highly probable cause of May spat mortality. Additionally, prolonged periods of low salinity can cause spat mortality or reduce growth (Lorio & Malone 1994).

In the Gulf region, the optimal salinity for growth and development is at an intermediate range, from 5 ppt or 10 ppt to 15 ppt (Lorio & Malone 1994; Brown et al. 2008). This study observed salinities in this intermediate range and the presence of wild spat on treated and untreated plots during sampling periods. Surface salinity was recorded during the July 12 deployment as 19.5 ppt and the USGS station recorded a mean daily value of 9.4 ppt. On August 13, the last spat deployment at California Bay, surface salinity of 19.2 was recorded and the USGS station recorded a mean daily value of 14.7 ppt. These differences may be attributed to differences in recording locations. After July 12, the salinity remained near 10 ppt, which is optimal for spat growth and survival. After August 13, the salinity increased to over 20 ppt, by the end of the month. High salinity areas are more suitable for predators. Therefore, predation may have affected the survival of spat after the July and August deployments.

Predator abundance and presence was not monitored during this study. Thus, only inferences about predation on spat at California Bay and Round Island can be based on previous studies. A benefit to lower salinity sites is the reduction of predators, such as mud crabs, that prey on spat oysters. Salinities around 15 ppt or higher promotes the presence of predators and fouling organisms (Supan 2002). California Bay may be optimal for spat survival because the salinity remains below those preferred by predators. Crab predation is a common cause of oyster mortality in Louisiana (Soniat et al. 2012). The three-dimensional habitat that oyster reefs and cultch material creates is also a suitable habitat for many

crab species. Mud crabs commonly prey on spat oysters less than 8 mm in length in Louisiana (Kulp et al. 2011; Soniat et al. 2012). Mud crabs can adapt to a wide range of salinities and prefer to prey on spat oysters, which may make them a candidate species for spat mortality during this study. More conclusive predator data should be collected to determine effects on spat survival at California Bay and Round Island.

Round Island is a different type of site than California Bay. Round Island is between the Rigolet's Pass, one of three openings between Lake Pontchartrain, LA, and the Gulf of Mexico. LDWF field crews that sample Round Island describe this area to have a strong water flow because of closeness to the Rigolet's Pass. There is constant exchange of water between Lake Pontchartrain and the Gulf of Mexico. For future spat deployment studies at Round Island, it would be valuable to record surface and bottom water velocities, because the water flow can affect spat survival. Increased water flow may deter predators by removing scents, and water flow carries more food-enriched water (Grabowski et al. 2005). Water flow also has an effect on sedimentation and filter feeding (Soniat et al. 2004). Water flow over horizontal shells was found to form a water boundary layer, but water flow over vertical shells provided areas of reduced flow (Soniat et al. 2004). The limestone and concrete cultch at Round Island may be providing vertical refuge, which may aid in feeding during strong currents. There were more wild spat oysters found at Round Island, indicating that this environment is suitable for supporting spat survival, however, there were no hatchery-produced spat found. The lack of hatchery-produced spat may be a result of limited vertical relief from the poultry shell. Limited vertical refuge coupled with water flow could reduce the ability for hatchery-produced spat set on poultry shell to effectively feed at Round Island.

The salinity at Round Island was estimated from two USGS surface water quality stations, St. Joseph Island Light and Grand Pass. The salinity data for the St. Joseph Island Light does not exist for the deployment dates, thus the Grand Pass site estimated water quality. The salinity on July 3 and July 20 was 19.0 ppt and 18.5 ppt, respectively (USGS). The USGS annual mean monthly salinities, since 2002 (some values are missing) are generally between 15 ppt to greater than 20 ppt. In April and May, the

salinity was less than 15 ppt and greater than 10 ppt (Appendix A). Therefore, Round Island is a higher salinity site than California Bay. This salinity range falls slightly outside of the optimal salinity range for oyster growth and development in the Gulf of Mexico (Lorio & Malone 1994). Oysters can grow and survive in salinities greater than 15 ppt, but negative affects from predation can occur in this range (Supan 2002). The temperature was close to 30°C during both deployments, while the July 3 deployment was slightly greater and the July 20 deployment was slightly less. High temperatures coupled with high salinity can cause stress on the oyster, which may be a cause of spat mortality at Round Island.

The salinity over the course of this study varied at Round Island; whereas, at California Bay, the salinity remained relatively stable. At California Bay, the salinity remained near 13 ppt and 15 ppt in September, November and January sampling periods. Past available USGS monthly mean salinity data, from 2002 to October 2012, shows that there is an overall low salinity level at California Bay, however, at Round Island the salinity level changes throughout the fall and early winter. At Round Island, during the September sampling, the salinity was around 6 ppt, while in November it was around 15 ppt and in January around 4 ppt. Temperature and salinity have a greater effect on oyster survival and growth (Heilmayer et al. 2008). Furthermore, high temperature coupled with low salinity has the most detrimental effects (Heilmayer et al. 2008). The temperature at Round Island in November and January was mostly below 20°C, which has less detrimental effects when coupled with low salinity. Round Island water quality data for this study corresponds with the USGS Grand Pass station and with the St. Joseph Island Light station for November and January (Appendix B and Appendix C). The large changes in salinity at Round Island may be related to freshwater moving out of Lake Pontchartrain through the Rigolet's Pass. Changes in salinity may cause more stress on oysters than other areas, such as California Bay, that have a more stable salinity regime.

The cultch type used for setting hatchery-produced spat may not be an optimal material for either California Bay or Round Island. The 3.175 mm, graded, poultry shell may have the tendency to sink between crevasses of existing cultch material. Muddy areas on the cultch plants could also cause cultch with limited surface area to sink, allowing sediment to smother spat (Bohn et al. 1995; Saoud & Rouse

2000). Buried oyster shell was observed in some of the samples at Round Island. The collection of only buried shell in some samples was an indicator of soft bottom and lack of cultch material on the bottom's surface. Thus, some hatchery-produced spat may have sunk in areas with limited cultch material and were smothered by sediment.

The storage duration and temperature of hatchery-produced spat from the hatchery to the deployment site may have affected spat survival. Live spat were observed in subsamples before leaving the hatchery. A way to test the effect of storage duration and temperature would be to mimic the transportation time and temperature at the lab. Subsamples of the spat could be observed and counted before and after the duration period to test for survival. Subsamples of spat could be deployed in enclosures in the wild, and then after one week, measured for survival and growth each day. In reality, deployment from the hatchery to the planting may take hours. Therefore, additional studies could test the effect of different temperatures during the transport from the hatchery to planting.

Differences between setting tank salinity and site salinity may have affected spat survival at California Bay and Round Island. During California Bay hatchery-produced spat deployments, the salinity in the setting tanks was greater than the salinity at the site, by a difference of 7 ppt to 10 ppt. During Round Island hatchery-produced spat deployments, the salinity in the setting tanks was greater than the salinity at the site, by a difference of 5 ppt to 7 ppt. The water temperature was approximately the same at the hatchery and at the sites (approximately 30°C); therefore, changes in salinity, rather than temperature, may be a more likely cause of mortality. Spat oysters also survive better under certain salinities (Davis 1958). A study conducted by Chanley (1958), found that recently settled spat experienced 100% mortality in a salinity of 2.5 ppt and 50% mortality in a salinity of 5 ppt. Thus, low salinity (< 5 ppt) at the sites could be a cause of hatchery-produced spat mortality. Future studies should test the tolerance of the hatchery-produced spat used in this study, to changes in salinity.

Round Island and California Bay have different site characteristics, but both support wild spat production. Both sites, however, did not support the survival and growth of hatchery-produced spat during this study. Possible causes of mortality, such as predation, sedimentation, salinity and cultch type,

can be suggested as to why the hatchery-produced spat did not survive. There may be other causes of mortality, however, that are unknown or it may be the result of a combination of variables. It is recommended that future site selection for hatchery-produced spat deployment should focus on past and present salinity levels before deploying spat. Future studies can try to separate variables that may cause mortality to better determine the main causes of mortality at specific sites. Hatchery-breeding techniques can be used to provide salinity tolerant larvae to specific sites or use triploid spat to distinguish between wild and hatchery oysters. In addition, whole oyster shell should be used for future efforts using hatchery-produced spat.

Chapter 4
Survival of Hatchery-Produced Larvae on Louisiana Public Oyster Grounds

Larval recruitment depends on numerous environmental and anthropological factors, such as sedimentation, freshwater diversions, cultch type, suitable bottom substrate, storms, salinity and temperature (Brown et al. 2003; Soniat et al. 2004; Soniat & Burton 2005; McGuire 2006; La Peyre et al. 2010). Recruitment, however, is most effected by the abundance of competent oyster larvae in the water column when such conditions are ideal. These factors are site specific and can vary from year to year. One way to augment recruitment, is to utilize hatchery-produced oyster larvae.

The production of oyster larvae at a hatchery can be more reliable than the production of natural larvae. A hatchery can control the majority of factors that affect larval survival to pediveliger stage, such as broodstock selection, egg quality, food availability, larval rigor and water quality (Supan & Wilson 1993; Swartzenberg 1999). Remote setting is a common hatchery technique that takes advantage of the delayed metamorphosis of larvae. Remote setting is the process of storing and transporting oyster larvae from a hatchery to an alternate site (Holiday et al. 1991). Generally, competent pediveliger larvae are wrapped in moist paper toweling and stored in a cooler for transport (Devakie & Ali 2000). Once on site, the larvae can be set to cultch material in a remote setting tank, then transferred to a nursery system or grow-out site. For this study, larvae were transported and deployed immediately onto oyster reefs without the use of remote setting tanks, similar to Maryland (Fredriksson et al. 2013).

Calcasieu Lake, located in the western part of Louisiana and the designated study site for this study, is part of the LDWF oyster management plan and historically is an active management site (LDWF 2011). Oyster harvesting at Calcasieu Lake began before 1967 (LDWF 2011). To manage the area, LDWF and the Louisiana Department of Health and Hospitals developed closure periods and closed areas and developed gear allowances and restrictions (LDWF 2011). Calcasieu Lake was first allowed for oyster harvest by hand or with tongs, then in 2004 with hand oyster dredges (less than or equal to 0.9144 m wide) and then in 2006 with “mechanical retrieval systems for dredges” (LDWF 2011). Calcasieu Lake is part of LDWF’s water bottom assessment and oyster management program, which collects reef

information, such as reef size (LDWF 2011). Calcasieu Lake is a “Public Oyster Area” and is designated as a seed reservation by state legislature (LDWF 2011).

This project involved collaborative efforts between the Sea Grant Oyster Hatchery and the Louisiana Department of Wildlife and Fisheries. Hatchery-produced larvae were provided to the LDWF and released at Calcasieu Lake. Treated sites, sites with hatchery-produced larvae, were selected by LDWF provided that these sites did not effect on-going NRDA (Natural Resource Damage Assessment) studies associated with the British Petroleum Deepwater Horizon oil spill (P. Banks, personnel communication). Samples from the larval release sites were collected using dredge tows by the LDWF. The goal was to determine the application of hatchery techniques on Louisiana public oyster grounds. To satisfy this goal, hatchery-produced larvae were released upon public oyster grounds. The objective was to test the variability in the total number of live spat between treated and untreated sites. The null hypothesis was that the number of live spat on the treated sites would be equal to the number of live spat on untreated sites. The alternative hypothesis was that the number of spat on the treated sites would be greater than the number of spat on the untreated sites. At the larval release sites it was expected that by mid to late Fall, there would be a peak in total number of spat. This peak would suggest that the hatchery larvae released in the summer had survived to spat-size.

Materials and Methods

The Sea Grant Oyster Hatchery on Grand Isle, LA raised competent pediveliger larvae, using standard hatchery techniques (Supan & Wilson 1993). Larvae were wrapped in moist paper toweling and stored in a cooler for transportation to the sites. The larvae were provided to LDWF for release. Larvae were released on-bottom by using a retrofitted garden sprayer with long plastic tubing and a weighted end. Larvae were released on-bottom to provide the pediveligers with immediate settlement substrates and to promote the larvae to settle on site.

There were four treated sites and eight untreated sites at Calcasieu Lake. All the treated sites were located in the south portion of lake, east of the ship channel (P. Banks, personnel communication). The treated sites included the 2009 Cultch Plant, Little Washout, Big Washout and Middle of Lake

(Figure 22). Of these sites, the 2009 Cultch Plant was the only site that was planted with cultch by LDWF in the year 2009. The eight untreated sites included Nine Mile, Commissary Point, Long Point, Northeast Rabbit, Southeast Rabbit, Turner's Bay, West Cove Transplant and West Rabbit sites (Figure 22 & Figure 23). These untreated sites were located in different portions of Calcasieu Lake. Untreated sites that were located in the south portion of Calcasieu Lake, west of the ship channel, were NE Rabbit, SE Rabbit, West Cove Transplant and West Rabbit (Figure 22). Long Point and Nine Mile were located in the south portion of the lake slightly north of the treated sites (Figure 22). The other two sites, Commissary Point and Turner's Bay, were located in the central portion of Calcasieu Lake (Figure 23). LDWF personnel collect monthly stock assessments of these twelve sites. The monthly data used for this study included two sampling periods prior to the initial larval release, JuneA (June 5, 6 and 7) and JuneB (June 20, 21 and 25), and six sampling periods after the initial release, August (August 2, 13, 15), SeptemberA (September 4, 6 and 13), SeptemberB (September 19 and 25), OctoberA (October 3 and 11), OctoberB (October 16, 17 and 25) and November (November 9, 20 and 28) (data provided by LDWF 2012). Sampling periods lasted more than one day for each period, due to time and weather conditions.

The treated sites have similar reef area, except for the 2009 Cultch Plant. The 2009 Cultch Plant consists of 5.67 hectares (14 acres) of reef (P. Banks, personnel communication). The other three treated sites, Big Washout, Little Washout and Middle of Lake, are sites located within one reef. The total reef area for these sites is 390 hectares (965 acres), therefore, each of theses three treated sites are approximately 130.18 hectares (Figure 24). These sites, however, do not appear evenly dispersed among the entire reef, but the 130.18 hectares is the best available estimate (Figure 24). In a separate LDWF project, each treated site was sampled and cultch material was classified as muddy shell, brown shell and black shell (D. Hill, Marine Fisheries, Louisiana Department of Wildlife and Fisheries, St. Charles, Louisiana, personnel communication). LDWF found that Big Washout had primarily muddy shell than brown shell. At Little Washout and Middle of Lake only brown shell was collected in their samples. At the 2009 Cultch Plant, the majority of cultch was black shell, then brown shell and muddy shell.

Figure 22. South portion of Calcasieu Lake, Louisiana. Includes the four treated sites, 2009 Cultch Plant, Big Washout, Little Washout and Middle of Lake (enclosed portion). Includes six of the eight untreated sites, Long Point, Nine Mile, NE Rabbit, SE Rabbit, West Cove Transplant and West Rabbit. (Provided by the Louisiana Department of Wildlife and Fisheries 2013).

Figure 23. Central portion of Calcasieu Lake, Louisiana. Includes two of the eight untreated sites, Commissary point and Turner's Bay. (Provided by the Louisiana Department of Wildlife and Fisheries 2013).

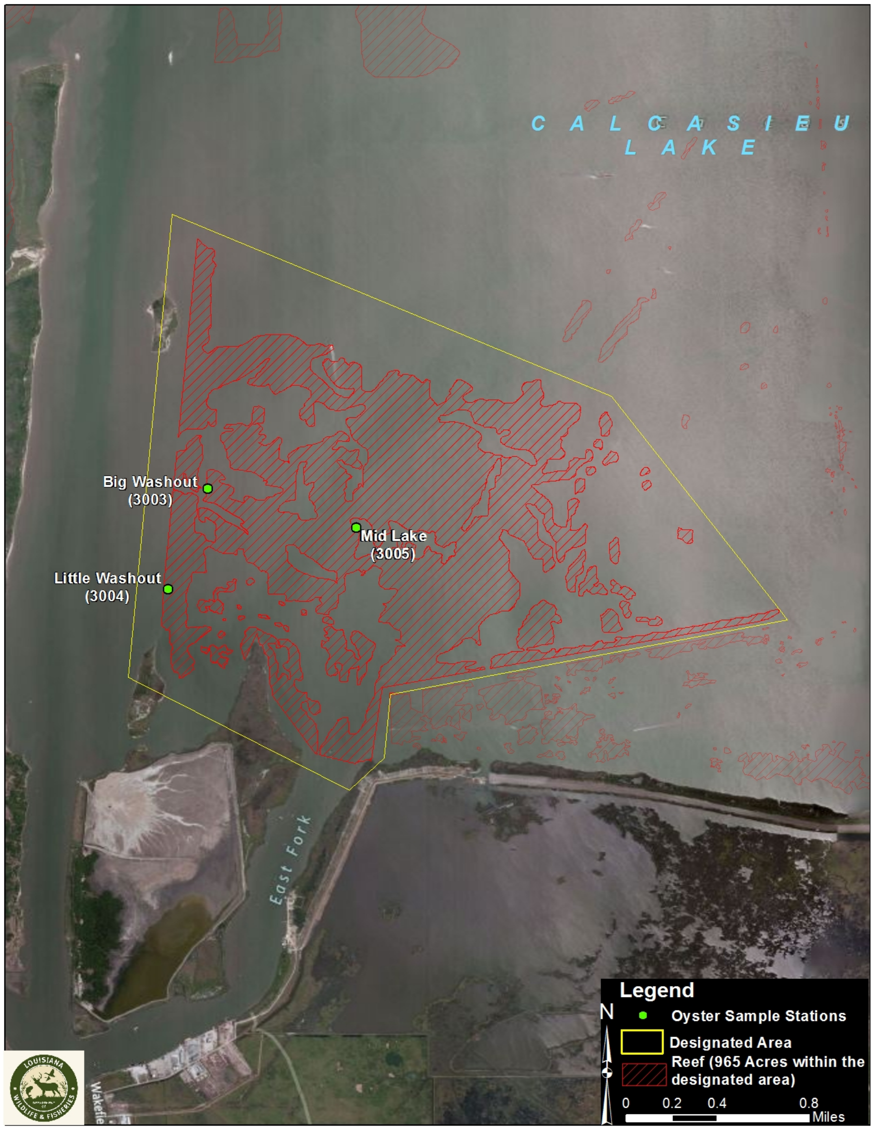


Figure 24. Three of the treated sites at Calcasieu Lake, Big Washout, Little Washout and Middle of Lake (provided by the Louisiana Department of Wildlife and Fisheries 2013). The total reef area, for all three sites, is 390 hectares (965 acres).

All Calcasieu Lake sites were sampled using sampling techniques established by LDWF. The sampling method uses a hand dredge and collects three 3-minute dredge tow samples per site. The dredge dimensions are 60.96 cm long tooth bar, 30.48 cm wide throat, with a 7.62 cm mesh-size bag. The dredge was secured to one side of the boat and towed along the bottom at an optimal angle to collect cultch material. The tows stayed within 244 meters from the central GPS coordinate of each site. The samples

were put into labeled baskets for transport back to the LDWF Lake Charles office. The LDWF oyster sample data sheet was used to record the number of oysters categorized by size class.

Hydrology information was measured at each site using a YSI-6050000 YSI Pro Plus Multi-Parameter Water Quality Meter System data included, surface and bottom water temperature, salinity, dissolved oxygen and specific conductivity (YSI Incorporated, Yellow Springs, OH 45387). Salinity, temperature and specific conductance data was supplemented by the United States Geological Survey (USGS) station "North Calcasieu Lake near Hackberry Bay, LA" (30°1.900'N, 93°17.967'W) (Appendix F).

Ambient seawater temperature (°C) and salinity (ppt) was measured at the Sea Grant Oyster Hatchery on Grand Isle, LA, during larval rearing using a hand-held thermometer and refractometer. The data provided, approximates the temperature and salinity of the larval rearing tanks prior to larval release or on exact release dates. Not all values are available on exact release dates; therefore, the closest available data to the release date is provided.

Statistical Analysis

The number of live spat between treated and untreated sites was analyzed using an ANOVA. If there were significant interactions between month and site, then Tukey's *a posteriori* tests were used to compare all pair-wise combinations for significant differences (Brown et al. 2008). The number of pair-wise combinations is large for this study, therefore, only the significant differences in number of spat from treated sites are reported and only the probability level is reported with the test (P<0.05) (Brown et al. 2008). All statistical analyses were done on SAS (procedure PROC MIXED, SAS Institute Inc., 9.3). Mean number of spat per site per month and standard deviations were also calculated.

Results

Treated and untreated sites were located in different areas of Calcasieu Lake (Table 11; Table 12). The number of larvae released per release date and the total number of larvae released per site varied (Table 11).

Table 11. Untreated sites and GPS coordinates of sites at Calcasieu Lake (2012).

Site	GPS Coordinate
Long Point	29°54.646'N, 93°19.525'W
Nine Mile	29°53.100'N, 93°19.617'W
Commissary Point	29°58.349'N, 93°16.767'W
NE Rabbit	29°51.417'N, 93°22.933'W
SE Rabbit	29°50.583'N, 93°22.533'W
Turner's Bay	30°03.333'N, 93°18.733'W
West Cove Transplant	29°50.850'N, 93°22.183'W
West Rabbit	29°50.817'N, 93°23.700'W

Table 12. Larval release site, release date, estimated number of larvae released, total number of larvae released at each site, GPS coordinate of sites and total number of larvae released for all sites, for Calcasieu Lake hatchery-produced larvae release (2012).

Site	Release Date	Number of larvae released	Total number of larvae released	GPS Coordinate
2009 Cultch Plant	7/20 8/15	8,680,000 14,975,000	23,655,000	29°50.600'N, 93°19.117'W
Little Washout	7/20 8/15	9,900,000 14,975,000	24,875,000	29°51.071'N, 93°20.401'W
Big Washout	7/20 7/30 8/15	8,700,000 130,000,000 14,975,000	153,675,000	29°51.146'N, 93°20.450'W
Middle of Lake	7/20 7/30 8/15	12,500,000 130,000,000 14,975,000	157,475,000	29°51.233'N, 93°19.735'W
		TOTAL	359,680,000	

The mean number of spat collected in the dredge tows varied per site per month (Table 13). The standard deviations for some of the samples were high, which could be a result of sampling methods (Table 13). West Cove Transplant, located west of the ship channel in the south portion of the lake, had the most total mean number of spat oysters out of all the sites combined from JuneA to November (Figure 25). The 2009 Cultch Plant had the highest mean number of spat oysters out of the treated sites from JuneA to November (Figure 25).

Table 13. Treated (*) and untreated sites (n=12) and sampling periods (n=8) with mean number of live spat ± standard deviations (replicates=3), at Calcasieu Lake 2012.

Site	JuneA	JuneB	August	SeptemberA	SeptemberB	OctoberA	OctoberB	November
Turner's Bay	19 ± 7	0	0	9 ± 4	4 ± 1	8 ± 6	21 ± 8	23 ± 15
Commissary Point	21 ± 5	0	0	17 ± 4	7 ± 8	9 ± 4	21 ± 1	42 ± 38
Long Point	0	0	0	2 ± 3	0	0	0.0	1 ± 1
9 Mile	0 ± 2	0	0	0	0	0	0.0	0.0
Middle of Lake*	5 ± 2	0	0	3 ± 4	2 ± 2	2 ± 2	3 ± 2	5 ± 2
2009 Cultch Plant*	56 ± 7	0	0	44 ± 32	15 ± 5	2 ± 3	20 ± 9	1 ± 2
Big Washout*	0 ± 1	0	0	0 ± 1	1 ± 1	0	1 ± 1	0 ± .5
Little Washout*	4 ± 4	0	0	1 ± 1	2 ± 2	1 ± 1	3 ± 2	2 ± .5
SE Rabbit Island	3 ± 1	7 ± 6	22 ± 17	16 ± 4	10 ± 3	11 ± 11	10 ± 10	12 ± 6
West Cove Transplant	8 ± 2	0	74 ± 34	43 ± 23	14 ± 9	70 ± 25	71 ± 12	185 ± 7
NE Rabbit Island	5 ± 4	0	48 ± 14	18 ± 10	14 ± 10	12 ± 4	14 ± 6	5 ± 4
West Rabbit Island	6 ± 3	0	5 ± 4	12 ± 8	15 ± 2	8 ± 5	17 ± 7	11 ± 6

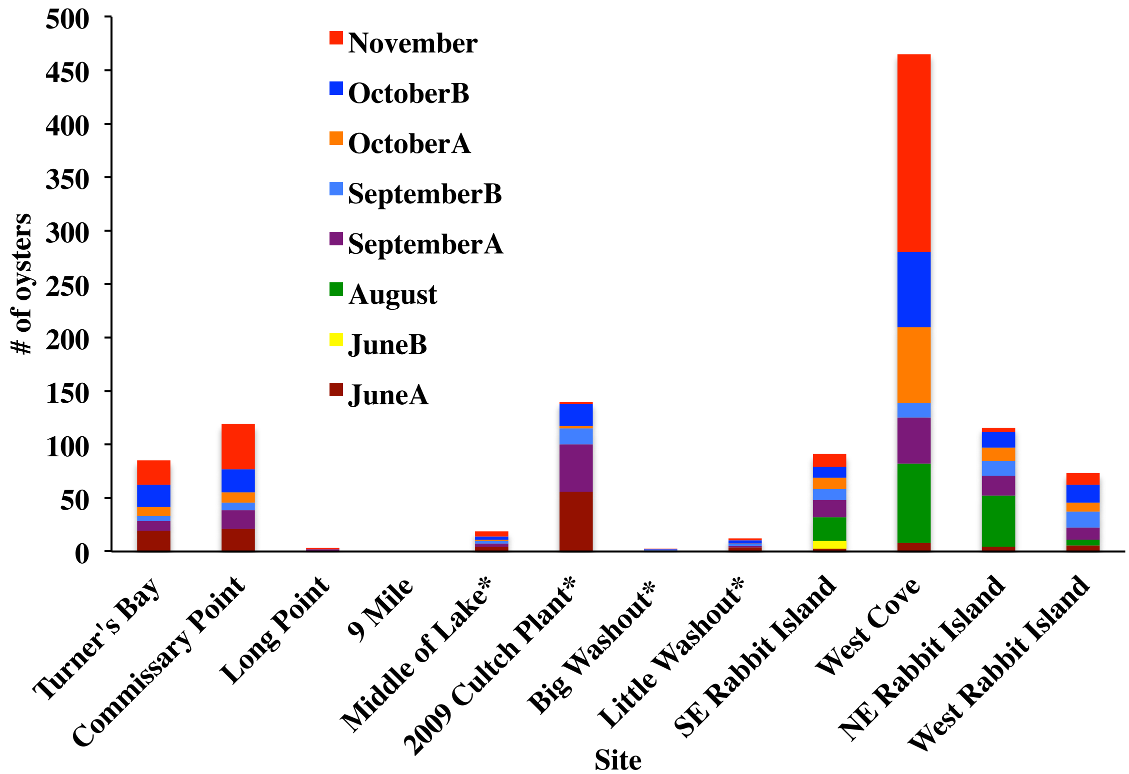


Figure 25. Mean number of live spat on treated (*) and untreated sites (n=12) during sampling periods (n=8), at Calcasieu Lake (2012).

There was a significant month by site interaction (ANOVA, $P<0.05$). For all pair-wise comparisons, the 2009 Cultch Plant was the only treated site that had significantly more live spat for comparisons among sites within months and months within sites (Tukey's *a posteriori*, $P<0.05$). The 2009 Cultch Plant had significantly more live spat than all the other treated plots and untreated plots in JuneA (Tukey's *a posteriori*, $P<0.05$) (Table 14). Additionally, the 2009 Cultch Plant had significantly more live spat than Turner's Bay, Long Point, 9 Mile, Middle of Lake, Big Washout, Little Washout and West Rabbit Island in SeptemberA (Tukey's *a posteriori*, $P< 0.05$) (Table 14).

The 2009 Cultch Plant was the only treated site that had significant differences among months within sites (Tukey's *a posteriori*, $P<0.05$) (Table 15). There were significantly more live spat in JuneA than in November, OctoberA, OctoberB and SeptemberB, significantly more live spat in SeptemberA than JuneB, significantly more live spat in SeptemberA and JuneA than in August and significantly more live spat in SeptemberA than OctoberA (Tukey's *a posteriori*, $P<0.05$). It was expected that there would be an increase in the number of live spat on treated sites in the Fall compared to JuneA, JuneB and August, pre-larval release. The only statistics satisfying this expectation was the 2009 Cultch Plant, which had significantly more live spat in SeptemberA than JuneB and SeptemberA than August (Tukey's *a posteriori*, $P<0.05$).

The surface and bottom water quality data is unavailable for the actual sites at the time of larval release. Therefore, the closest estimates for water quality at Calcasieu Lake were determined with the USGS station "North Calcasieu Lake near Hackberry Bay, LA" (Table 16).

The ambient seawater temperature and salinity data was measured at the Sea Grant Oyster Hatchery on Grand Isle, LA (Table 17). Not all temperature and salinity data was available from the hatchery during larval release dates; therefore, temperature and salinity in the larval rearing tanks is approximated with the closest available data. The temperature and salinity in the rearing tanks during larval release was approximately near 30°C and 25 ppt (June 20), at 29°C and 30 ppt (June 30) and near 32°C and 27 ppt (August).

Table 14. Treated (*) and untreated sites (n=12) and sampling periods (n=8), showing treated sites with significantly more live spat (a and b) (replicates=3), at Calcasieu Lake 2012 (Tukey's *a posteriori*, P<0.05).

Site	JuneA	JuneB	August	SeptemberA	SeptemberB	OctoberA	OctoberB	November
Turner's Bay	19[b]	0	0	9[b]	4	8	21	23
Commissary Point	21[b]	0	0	17	7	9	21	42
Long Point	0[b]	0	0	2[b]	0	0	0	1
9 Mile	0[b]	0	0	0[b]	0	0	0	0
Middle of Lake*	5[b]	0	0	3[b]	2	2	3	5
2009 Cultch Plant*	56[a]	0	0	44[a]	15	2	20	1
Big Washout *	0[b]	0	0	0[b]	1	0	1	0
Little Washout*	4[b]	0	0	1[b]	2	1	3	2
SE Rabbit Island	3[b]	7	22	16	10	11	10	12
West Cove Transplant	8[b]	0	74	43	14	70	71	185
NE Rabbit Island	5[b]	0	48	18	14	12	14	5
West Rabbit Island	6[b]	0	5	12[b]	15	8	17	11

Table 15. All significant differences for the number of live spat among months within sites (Tukey's *a posterior*, P<0.05). The only treated site with significant differences among months was the 2009 Cultch Plant.

	2009 Cultch Plant*	Commissary Point	NE Rabbit	West Cove Transplant
JuneA	JuneB November OctoberA OctoberB SeptemberB	-	-	November OctoberA OctoberB SeptemberA
JuneB	SeptemberA	November	-	November OctoberA OctoberB SeptemberA
August	JuneA SeptemberA	November	JuneA JuneB November OctoberA OctoberB SeptemberB	JuneA JuneB November SeptemberB
SeptemberA	-	-	-	-
SeptemberB	-	-	-	-
OctoberA	SeptemberA	-	-	SeptemberB
OctoberB	-	-	-	SeptemberB
November	-	-	-	-

Table 16. Mean daily temperature, salinity, specific conductance and gage height from the USGS station "North Calcasieu Lake near Hackberry Bay, LA", during larval release dates.

Release Date	Temperature (°C)	Salinity (ppt)	Specific Conductance (uS/cm at 25°C)	Gage height (m)
7/20/12	30.5	7.5	13,000	1.13
7/30/12	31.3	8.5	14,700	1.12
8/15/12	31.1	12.4	20,800	0.94

Table 17. Ambient seawater temperature and salinity at the Sea Grant Oyster Hatchery on Grand Isle, LA. Provides closest available data for larval release dates at Calcasieu Lake (2012).

Larval Release Date	Hatchery Date	Temperature (°C)	Salinity (ppt)
7/20	7/14	30	25
	7/20	n/a	25
7/30	7/30	29	30
8/15	8/13	32	27

Discussion

The 2009 Cultch Plant was the only treated site that was recently planted with cultch, and was the only treated site that had significant differences in the number of live spat between sites and between months. More spat oysters at this site may be the result of a cultch affect, meaning there was suitable cultch material to support larval settlement and growth and survival of spat. There were no other recent cultch plant sites in this study to compare to the 2009 Cultch Plant site. The 2009 Cultch Plant may be a suitable site for spat production because there is optimal habitat including water flow, food, dissolved oxygen, temperature and salinity (Coen & Luckenbach 2000). The additional cultch on the 2009 Cultch Plant could provide structural complexity, which promotes oyster survival and growth (Nestlerode et al. 2007). Structural complexity also provides refuge spaces from predators. Minimized predation from cultch material on the 2009 Cultch Plant could be another reason why there was more spat production. Minimized predation, however, could be a result of the surrounding habitat and not the cultch material (Grabowski et al. 2005).

Dredges are reported as more damaging and destructive than tongs to reefs composed of shell (Harding et al. 2010). The allowance of dredging for oysters in Calcasieu Lake, in 2004, may have

initiated the beginning of oyster reef damage (LDWF 2011). Damaged shell can be less suitable material for larval settlement and spat survival. Broken shell pieces have the tendency to sink, which provides fewer places for settlement and less refuge from sediment. It was expected that the treated sites in Calcasieu Lake would have a greater number of spat oysters post-larval release, however, this was not the result for most of the treated sites among months. Lack of suitable substrate, from dredging, could be the cause of minimal to no spat found on treated sites. The 2009 Cultch Plant was the only treated site that had significantly more live spat after larval release dates; furthermore, sampling period SeptemberA was significantly different from JuneA and August. Thus, supplementing dredged sites with cultch material may be a solution to increase larval settlement and spat production.

The combined effects of low salinity and high temperature during larval release could be a possible cause of mortality. The USGS station used for Calcasieu Lake recorded mean temperatures of 30°C and greater for all three release dates. The salinity for July 20 and July 30 were similar, 7.5 ppt and 8.5 ppt, respectively. The salinity during the August release was higher at 12.4 ppt. Temperatures above 30°C and salinities less than 5 ppt were found to have the most detrimental effects on oyster from St. Lucie River Estuary (Heilmayer et al. 2008). Although the salinity was above 5 ppt in July and for the duration of the study, salinities near 8 and 9 ppt with high temperatures may have affected larval survival and recruitment. In the remote setting manual for the Gulf Coast region, it is recommended that the larval settlement tank water should be between temperatures 25°C and 30°C and salinity should be above 12 ppt (Supan 1991). Larvae from Louisiana, however, were observed to set at 33°C (Supan 1991). Therefore, it may be likely that the larvae released in August did set to cultch material on site because the mean salinity was slightly above 12 ppt and the mean temperature was below 33°C.

Differences between larval rearing tank salinity and site salinity may have affected larval survival at Calcasieu Lake. Transferring hatchery-produced larvae to a different salinity may have caused larval mortality. Oyster larvae are also influenced by the salinity that their parent oysters are acclimated to (Davis 1958). The salinity tolerance of the larvae used in this study may have been conditioned to survive in a specific salinity range. Thus, California Bay or Round Island salinity ranges may not have

favored hatchery-produced larval survival. Future larval release studies should test the salinity tolerance of larvae produced at the Sea Grant Oyster Hatchery.

In a water bottom assessment of Calcasieu Lake, the sites were described to have a variety of bottom substrate and structure (LDWF 2011; D. Hill, personnel communication). Bottom substrates were classified into soft mud, moderately firm mud, firm mud, buried shell, slightly covered shell and exposed reef (D. Hill, personnel communication). During this bottom assessment, Big Washout was characterized with muddy shell and Little Washout and Middle of Lake was characterized with brown shell. Correspondingly, these three sites were used as treated sites during this study. These sites were also depicted by LDWF to occupy the same general area, whereas, the 2009 Cultch Plant occupied a separate area. The three treated sites within the same area had similar bottom substrate, which may validate the similar spat production found at these sites. The muddy shell and brown shell may not provide suitable substrate or habitat for larval settlement. Furthermore, soft mud bottoms can smother oysters with sediment or cause anoxic conditions, which can cause mortality (Saoud & Rouse 2000).

The treated sites are located on the east side of the ship channel in the south portion of Calcasieu Lake, which may have a different bottom type than the west side of the ship channel or the central portion of the lake. The cumulative number of live spat, from JuneA to November, was greatest at the majority of untreated sites that were on the west side of the ship channel and in the central portion of the lake than at the treated sites. Sites west of the ship channel, NE Rabbit, SE Rabbit, West Rabbit and West Cove Transplant, and in the central portion of the lake, Commissary Point and Turner's Bay, should be tested with hatchery-produced larvae to observe for differences in spat production. Thus, the number of live spat oysters collected from treated and untreated sites may be a reflection of different bottom types, as well as, the presence of cultch material.

Suitable cultch material effectively attracts larvae and supports growth and survival. Some sites may have more suitable cultch than other sites. Cultch material that is covered in sediment and fouling organisms can affect larval settlement (Soniat et al. 2004; Barnes et al. 2010). Biofilms on cultch material provide bioorganic cues, which can positively or negatively affect larval settlement (Keough & Raimondi

1995). These factors may have played a role in larval settlement on the treated sites. An analysis of biofilms and composition of organisms covering cultch should be assessed in future studies. Additionally, larvae need available surface area for setting. Horizontal shell provides more surface area for larval settlement, but survival of settled larvae is better on vertical shell (Soniat et al. 2004). It is possible that the cultch material at Big Washout, Little Washout and Middle of Lake did not have suitable or available cultch material for hatchery-produced larvae to set. The only treated site that had significant numbers of live spat was the 2009 Cultch Plant. This site was planted with cultch three years prior to this study. It was not expected that three-year-old cultch would show differences between treated sites, however, there were more spat oysters on the 2009 Cultch Plant. This suggests that the planted cultch material may have some sort of effect on spat survival. The additional cultch could benefit spat production in varying ways. These benefits include providing surfaces for larval settlement, protection from predators and deterrence from sediment overburden. It is difficult to determine the exact role of the 2009 Cultch Plant in promoting spat production. The other information that is unavailable is whether the spat found on the 2009 Cultch Plant is from hatchery-produced larvae. Future studies should apply hatchery-breeding techniques, such as using triploid larvae, to distinguish between wild (diploid) and hatchery-produced spat.

Hatchery-produced larvae were released on-bottom to increase larval settlement on treated sites. Larval swimming behavior combined with water movement, however, may have dispersed hatchery-produced larvae to other sites (North et al. 2008). Oyster larvae vertical swimming behavior plays a significant role in larval transport, which may have occurred during this study (North et al. 2008). In this study, however, competent pediveliger larvae were released on-bottom to minimize the time larvae spent in the water column and immediately provide settlement surfaces. Thus, larval swimming behavior may have affected the number of spat found on treated sites, but efforts were made to provide immediate substrate for settlement on-site.

Supplementing Calcasieu Lake with hatchery-produced larvae in this study did not show great increases in spat production. Whether the larvae died upon release from water quality or did not find

suitable settlement places is undeterminable. Future studies should test hatchery-produced larvae under varying field conditions at a laboratory that mimic release site characteristics. Additionally, improved substrate selection and estimates of wild spatfall and resulting oyster abundance should be obtained prior to releasing larvae on a site. The use of triploid larvae would allow more accurate and reliable assessments of hatchery-produced larval release.

Chapter 5
Summary and Conclusions

There was no survival of hatchery-produced spat in Hackberry Bay (2011), Round Island and California Bay (2012 and 2013). The spat mortality, from the preliminary study at Hackberry Bay, provided valuable insight for site selection and spat handling techniques, which were applied in the 2012 Round Island and California Bay studies. The presence of live, wild spat at Round Island and California Bay suggests that these sites can support spat production, however, the hatchery-produced spat died at these sites. There are likely a combination of environmental variables that caused freshly struck hatchery-produced spat mortality at these sites. Causes of mortality could be from low salinity, high temperature, predation, competition for food and space from fouling organisms, sedimentation, cultch type and bottom habitat.

In Calcasieu Lake, there were spat on treated and untreated sites, however, distinguishing between wild spat and hatchery-produced spat was not possible within the scope of this study. The lack of spat on treated sites may be related to reduced larval survival or settlement. Inefficient larval settlement may be associated with suitable settlement surfaces, water quality or bottom type. After settlement, spat production at Calcasieu Lake is likely related to a variety of environmental variables including, sedimentation, bottom habitat, temperature and salinity, which may be site specific for different portions of the lake. Additionally, hatchery-breeding techniques, such as using genetic markers, should be used to assess the success of larval release.

Future restoration projects in Louisiana that apply hatchery-produced spat and larvae to oyster reefs should continue to improve on site selection, transportation techniques and cultch type. Site selection for spat production should include optimal temperature and salinity regimes during deployment and suitable bottom habitat to support survival and growth. Future studies on spat survival during transportation would provide insight on optimal storage duration and temperature for increasing spat survival. Planting hatchery-produced spat on larger cultch material, such as whole oyster shell, will promote survival and growth of hatchery-produced spat on reefs, while minimizing possible causes of

mortality, such as predation and sedimentation. Selecting sites with suitable, clean cultch material for larval release may promote larval settlement and spat production on treated sites. Applying these recommendations and limiting environmental variables that affect survival, to future hatchery-produced spat deployments and larval releases, could improve survival of spat and larvae on Louisiana public oyster reefs.

Literature Cited

Allen Jr., S. K. 1983. Flow cytometry: Assaying experimental polyploidy fish and shellfish. *Aquaculture*. 33:317-328.

Baldwin, B. S. & R. I. E. Newell. 1991. Omnivorous feeding by planktotrophic larvae of the eastern oyster *Crassostrea virginica*. *Mar. Ecol. Prog. Ser.* 78:285-301.

Barnes, B. B., M. W. Luckenbach & P. R. Kingsley-Smith. 2010. Oyster reef community interactions: The effect of resident fauna on oyster (*Crassostrea* spp.) larval recruitment. *J. Exp. Mar. Biol. Ecol.* 391:169-177.

Beck, M. W., R. D. Brumbaugh, L. Airoldi, A. Carranza, L. D. Coen, C. Crawford, O. Defeo, G. J. Edgar, B. Hancock, M. C. Kay, H. S. Lenihan, M. W. Luckenbach, C. L. Toropova, G. Zhang & X. Guo. 2011. Oyster reefs at risk and recommendations for conservation, restoration, and management. *BioScience*. 61:107-116.

Becker, S. 2011. Department of marine resources rebuilds and restores oyster reefs: Mississippi Department of Marine Resources, 1141 Bayview Avenue, Suite 101, Biloxi, Mississippi 39530. News Release 11-96-SB.

Berrigan, M. E. 1990. Biological and economical assessment of an oyster resource development project in Apalachicola Bay, Florida. *J. Shellfish Res.* 9:149-158.

Bohn, R. E., D. W. Webster & D. W. Meritt. 1995. Producing oyster seed by remote setting. *Northeastern Regional Aquaculture Center Bulletin*. 220:1-10.

Brown, B. L., A. J. Butt, D. Meritt & K. T. Paynter. 2005. Evaluation of resistance to Dermo in eastern oyster strains tested in Chesapeake Bay. *Aquacult. Res.* 36:1544-1554.

Brown, K. M., G. J. George, G. W. Peterson, B. A. Thompson & J. H. Cowan Jr. 2008. Oyster predation by black drum varies spatially and seasonally. *Estuar Coast*. 31:597-604.

Brown, K. M., G. W. Peterson, P. D. Banks, B. Lezina, C. Ramcharan & M. McDonough. 2003. Olfactory deterrents to black drum predation on oyster leases. *J. Shellfish Res.* 22:589-595.

Brown, K. M. & W. B. Stickle. 2002. Physical constraints on the foraging ecology of a predatory snail. *Mar. Fresh. Behav. Physiol.* 35:157-166.

Burke, K., E. Bataller & G. Miron. 2008. Spat collection of a non-native bivalve species (European oyster, *Ostrea edulis*) off the eastern Canadian coast. *J. Shellfish Res.* 27:345-353.

Butler, P. A. 1956. Reproductive cycle in native and transplanted oysters. *Proc. Natl. Shellfish. Assoc.* 46:75.

Chanley, P. E. 1958. Survival of some juvenile bivalves in water of low salinity. *Proc. Natl. Shellf. Assoc.* 48:52-65.

Chatry, M. 1987. Seed oyster production in Louisiana and prospects for enhancement. p. 27-30. *In* Sinderman, C. J. (editor). 1987. Reproduction, maturation, and seed production of cultured species. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 47.

Chatry, M., R. J. Dugas & K. A. Easley. 1983. Optimum salinity regime for oyster production on Louisiana's state seed grounds. *Contrib. Mar. Sci.* 26:81-94.

Chew, K. K. 1991. Hatchery development and remote setting of the Pacific oyster larvae. *Aquacult. Mag.* 17:72-75.

Coen, L. D. & M. W. Luckenbach. 2000. Developing success criteria and goals for evaluating oyster reef restoration: Ecological function or resource exploitation? *Ecol. Eng.* 15:323-343.

Coon, S. L. & D. B. Bonar. 1985. Induction of settlement and metamorphosis of the pacific oyster, *crassostrea gigas* (Thunberg), by L-DOPA and catecholamines. *J. Exp. Mar. Biol. Ecol.* 95:211-221.

Crosby, M. P., R. I. E. Newell & C. J. Langdon. 1990. Bacterial mediation in the utilization of carbon and nitrogen from detrital complexes by *Crassostea virginica*. *Limnol. Oceanogr.* 35:625-639.

Davis, H. C. 1958. Survival and growth of clam and oyster larvae at different salinities. *Biol. Bull.* 114:296-307.

Devakie, M. N. & A. B. Ali. 2000. Effects of storage temperature and duration on the setting and post-set spat survival of the tropical oyster, next term *Crassostrea iredalei* (Faustino). *Aquaculture.* 190:369-376.

Diaz, R. J. 1971. Effects of thermal shock on larvae of the oyster, *Crassostrea virginica* (Gmelin). Master's thesis, University of Virginia, Charlottesville, VA. 36pp.

Diaz, R. J. 1973. Effects of brief temperature increases on larvae of the American oyster (*Crassostrea virginica*). *J. Fish. Res. Bd. Canada.* 30:991-993.

Fredriksson, D. W., C. N. Steppe, L. Wallendorf & R. Mayer. 2013. The design and deployment of a containment barrier system for *In-situ* of *Crassostrea virginica* larvae for possible aquaculture and restoration applications. (abstract). Aquaculture 2013. Nashville, Tennessee.

Galtsoff, P. S. 1964. The American oyster *Crassostrea virginica* Gmelin. *Fish. Bull.* 64:1-480.

Garton, D. & W. B. Stickle. 1980. Effects of salinity and temperature on the predation rate of *Thais haemastoma* on *Crassostrea virginica* spat. *Biol. Bull.* 158:49-57.

George, G. J., K. M. Brown, G. W. Peterson & B. A. Thompson. 2008. Removal of black drum on Louisiana reefs to increase survival of Eastern oysters *Crassostrea virginica*. *N. Am. J. Fish. Manage.* 28:1802-1811.

Gosling, E. M. 2003. Bivalve mollusks: biology, ecology and culture. Oxford; Malden, MA: Fishing New Books. 443 p.

Grabowski, J. H., A. R. Hughes, D. L. Kimbro & M. A. Dolan. 2005. How habitat setting influences restored oyster reef communities. *Ecology.* 86:1926-1935.

Hadley, N. H. & J. M. Whetstone. 2007. Hard clam hatchery and nursery production. Southern Regional Aquaculture Center, Pub. No. 4301. 8p.

Harding, J. M., R. Mann, M. J. Southworth & J. A. Wesson. 2010. Management of the Piankatank River, Virginia, in support of oyster (*Crassostrea virginica*, Gmelin 1791) fishery repletion. *J. Shellfish Res*. 29:867-888.

Hayes, P. F. & R. W. Menzel. 1981. The reproductive cycle of early setting *Crassostrea virginica* (Gmelin) in the northern Gulf of Mexico, and its implications for population recruitment. *Biol. Bull.* 160:80-88.

Holiday J. E., G. L. Allan & J. Frances. 1991. Cold storage effects on setting of larvae of the Sydney rock oyster, *Saccostrea commercialis*, and the Pacific oyster, *Crassostrea gigas*. *Aquaculture*. 92:179-185.

Hopkins, S. H. 1955. Oyster setting on the Gulf Coast. *Proc. Natl. Shellfish. Assoc*. 45:52-55.

Jones, G. & B. Jones. 1983. Methods for setting hatchery produced oyster larvae. British Columbia Ministry of Environment, Marine Resources Branch, Info. Rpt. # ISSN 0229-8120; No. 4. 94 pp.

Jones, G & B. Jones. 1988. Advances in the remote setting of oyster larvae. British Columbia Ministry of Environment, Marine Resources Branch. Info. Rpt. # ISSN 0-7718-8627-6. 88 pp.

Kennedy, V. S. 1996. Biology of larvae and spat. Pages 371-421 *In*: V. S. Kennedy, R. I. E. Newell & A. F. Eble (eds.) The Eastern Oyster *Crassostrea virginica*. Maryland Sea Grant College, University of Maryland, College Park, Maryland.

Keough, M. J. & P. T. Raimondi. 1995. Responses of settling invertebrate larvae to bioorganic films: effects of different types of films. *J. Exp. Mar. Biol. Ecol*. 185:235-253.

Kulp, R. E., V. Politano, H. A. Lane, S. A. Lombardi & K. T. Paynter. 2011. Predation of juvenile *Crassostrea virginica* by two species of mud crabs found in the Chesapeake Bay. *J. Shell. Res*. 30:261-266.

Lannig, G., J. F. Flores & I. M. Sokolova. 2006. Temperature-dependent stress response in oysters, *Crassostrea virginica*: Pollution reduces temperature tolerance in oysters. *Aquat. Toxicol*. 79:278-287.

La Peyre, M. K., S. M. Casas, W. Gayle & J. F. La Peyre. 2010. The combined influence of sub-optimal temperature and salinity on the in vitro viability of *Perkinsus marinus*, a protistan parasite of the eastern oyster *Crassostrea virginica*. *J. Invertebr. Pathol*. 105:176-181.

Lee, L. 2004. Demonstrate on-bottom oyster grow-out method using remotely set seeded cultch. FRG #99-AM-14.

Lorio, W. J. & S. Malone. 1994. The cultivation of American oysters (*Crassostrea virginica*). Southern Regional Aquaculture Center, Pub. No. 432. 8p.

Louisiana Department of Wildlife and Fisheries. 2010. Oyster stock assessment report. Oyster Data Report Series No. 16. Louisiana Department of Wildlife and Fisheries. P.O. Box 98000. Baton Rouge, LA 70898.

Louisiana Department of Wildlife and Fisheries. 2011. Oyster stock assessment report. Oyster Data Report Series No. 17. Louisiana Department of Wildlife and Fisheries. P.O. Box 98000. Baton Rouge, LA 70898.

McGuire, T. 2006. Louisiana's oysters, America's wetlands, and the storms of 2005. *Am. Anthropol.* 108:692-705.

Medcof, J. C. 1939. Larval life of the oyster (*Ostrea virginica*) in Bideford River. *J. Fish. Res. Board Can.* 4:287-301.

Melancon, E. J., B. Asrabadi & D. Diaz. 2003. Response of hooked mussel, *Ischadium recurvum*, to relaying as a remediation technique to reduce befouling on oysters and documentation of its distribution in a Louisiana estuary. (abstract). Workgroup: Public Health and Processing, Oyster Research and Restoration in U.S. Coastal Waters: Strategies for the future.

Melancon, E. J. Jr. & R. Condrey. 1992. Economics of a Louisiana oyster seed bedding fishery and influence of lease yield on expenses to operate. *J. Shell. Res.* 11: 143-147.

Nestlerode, J. A., M. W. Luckenbach & F. X. O'Brien. 2007. Settlement and survival of the oyster *Crassostrea virginica* on created oyster reef habitats in Chesapeake Bay. *Restoration Ecol.* 15:273-283.

North, E. W., Z. Schlag, R. R. Hood, M. Li, L. Zhong, T. Gross & V. S. Kennedy. 2008. Vertical swimming behavior influences the dispersal of simulated oyster larvae in a couple particle-tracking and hydrodynamic model of Chesapeake Bay. *Mar. Ecol. Prog. Ser.* 359:99-115.

Oyster resource development [Internet]. [FDACS] Florida Department of Agriculture and Consumer Science, Division of Aquaculture; c2004 [cited 2012 January 15]. Available from: http://www.floridaaquaculture.com/bad/bad_oysterplant.htm.

Pernet, F., R. Tremblay, I. Redjah, J. M. Sevigny & C. Gionet. 2008. Physiological and biochemical traits correlate with differences in growth rate and temperature adaptation among groups of the eastern oyster *Crassostrea virginica*. *J. Exp. Biol.* 211:969-977.

Piazza, B. P., P. D. Banks & M. K. La Peyre. 2005. The potential for created oyster shell reefs as a sustainable shoreline protection strategy in Louisiana. *Restor. Ecol.* 13:499-506.

Powell, E. N., J. M. Klinck, E. E. Hofmann, E. A. Wilson-Ormond & M. S. Ellis. 1995. Modeling oyster populations. V. Declining phytoplankton stocks and the population dynamics of American oyster (*Crassostrea virginica*) populations. *Fish. Manage.* 24:199-222.

Ryder, J. A. 1885. A new system of oyster culture. *Science.* 6:465-467.

Saoud, I. G. & D. B. Rouse. 2000. Evaluating sediment accretion on a relic oyster reef in Mobile Bay, Alabama. *J. Appl. Aquac.* 10:41-49.

SAS Institute Inc., System Requirements for SAS 9.3 Foundation for Microsoft Windows for x64, Cary, NC: SAS Institute Inc., 2012.

Shumway, S. E. 1996. Natural environmental factors. Pages 467-513 *In*: V. S. Kennedy, R. I. E. Newell & A. F. Eble (eds.) The Eastern Oyster *Crassostrea virginica*. Maryland Sea Grant College, University of Maryland, College Park, Maryland.

Soniat, T. M. & G. M. Burton. 2005. A comparison of the effectiveness of sandstone and limestone as cultch for oysters, *Crassostrea virginica*. *J. Shellfish Res.* 24:483-485.

Soniat, T. M., C. M. Finelli & J. T. Ruiz. 2004. Vertical structure and predator refuge mediate oyster reef development and community dynamics. *J. Exp. Mar. Biol. Ecol.* 310:163-182.

Soniat, T. M., J. M. Klinck, E. N. Powell & E. E. Hofmann. 2012. Understanding the success and failure of oyster populations: Periodicities of *Perkinsus marinus*, and oyster recruitment, mortality, and size. *J. Shellfish Res.* 31:635-646.

Stanley, J. G., S. K. Allen Jr. & H. Hidu. 1981. Polyploidy induced in the American oyster, *Crassostrea virginica*, with cytochalasin B. *Aquaculture*. 23:1-10.

Supan, J. 2002. Extensive culture of *Crassostrea virginica* in the Gulf of Mexico region. Southern Regional Aquaculture Center, Pub. No. 4300. 4p.

Supan, J. 1991. Using remote setting to produce seed oysters in Louisiana and the Gulf coast region. Louisiana Sea Grant College Program, Center for Wetlands Research, Louisiana State University, Baton Rouge, Louisiana 70803-7507.

Supan, J. 1983. Evaluation of a leased oyster bottom in Mississippi Sound. *Gulf Research Reports*. 7:261-266.

Supan, J. E & C. A Wilson. 1993. Louisiana oyster seed alternatives. *World Aquaculture*. 24:79-82.

Swartzenberg, J. 1999. Farm raising of oyster seed: A manual for remote setting oyster larvae on micro cultch and the in-water nursery system. J&B AquaFood. Cooperation with North Carolina Fisheries Resource Grant Program.

Thompson, R. J., R. I. E. Newell, V. S. Kennedy & R. Mann. 1996. Reproductive processes and early development. Pages 335-370 *In*: V. S. Kennedy, R. I. E. Newell & A. F. Eble (eds.) The Eastern Oyster *Crassostrea virginica*. Maryland Sea Grant College, University of Maryland, College Park, Maryland.

Tolley, S. G., A. K. Volety, M. Savarese, L. D. Walls, C. Linardich & E. M. Everham. 2005. Impacts of salinity and freshwater inflow on oyster-reef communities in Southwest Florida. *Aquat. Living Resour.* 19:371-387.

VanderKooy, S. (editor). 2012. The oyster fishery of the Gulf of Mexico, United States: A regional management plan – 2012 revision. Publication No. 202, Gulf States marine Fisheries Commission, Ocean Springs, Mississippi.

Wang, H., W. Huang, M. A. Harwell, L. Edmiston, E. Johnson, P. Hsieh, K. Milla, J. Christensen, J. Stewart & X. Liu. 2008. Modeling oyster growth rate by coupling oyster population and hydrodynamic models for Apalachicola Bay, Florida, USA. *Ecol. Model.* 211:77-89.

Webster, D & D. Meritt. 1988. Stabilizing oyster ground. Oyster Aquaculture Workbook Series. Maryland Sea Grant Extension. Pub. No. UM-SG-MAP-88-04. Last modified October 17, 2012.

Wheaton, F. 2007. Review of the properties of Eastern oysters, *Crassostrea virginica* Part I. Physical properties. *Aquacult. Eng*. 37:3-13.

Wikfors, G. H., J. W. Twarog & R. Ukeles. 1984. Influence of chemical composition of algal food sources on growth of juvenile oysters, *Crassostrea virginica*. *Biol. Bull*. 167:251-263.

Appendix A
Louisiana Department of Wildlife and Fisheries Oyster Sample Data Sheet.

+LDWF Oyster Sample Data Sheet

PROJ _______________ **CSA** ___________ **STATION NAME/NUMBER** ______________________________________

DATE _____________ **TIME** ______________ **GEAR TYPE** _________ **COLLECTORS** _________________________

COMMENTS ____

Air Temp	Wind Direction	Wind Speed	Turbidity			Conductivity	Salinity	Water Temp	DO
					TOP				
					BOTTOM				

Work Group	Size Range (mm)	Live	Dead Valve	Dead Box
		Measure 25 live spat and count the remaining live spat	Measure 25 dead spat & count remaining dead spat	
0	0 – 4			
1	5 – 9			
2	10 – 14			
3	15 – 19			
4	20 – 24			
5	25 – 29			
6	30 – 34			
7	35 – 39			
8	40 – 44			
9	45 – 49			
10	50 – 54			
11	55 – 59			
12	60 – 64			
13	65 – 69			
14	70 – 74			
15	75 – 79			
16	80 – 84			
17	85 – 89			
18	90 – 94			
19	95 – 99			
20	100 – 104			
21	105 – 109			
22	110 – 114			
23	115 – 119			
24	120 – 124			
25	125 – 129			
26	130 – 134			
27	135 – 139			
28	140 – 144			
29	145 – 149			
30	150 – 154			
31	155 – 159			
32	160 – 164			
33	165 – 169			
34	170 – 174			
35	175 – 179			
36	180 – 184			
37	185 – 189			
38	190 – 194			
39	195 – 199			
40	200 – 204			

OYSTER SUMMARY			
	Spat	Seed	Sack
Live			
Dead			
% Mortality			
Total % Mortality			
Seed & Sack % Mortality			

Species	Code	Number
Hooked Mussels	*2135*	
Oyster Drills	*2111*	
Mud Crabs	*2425*	
Blue Crabs	*2003*	
Stone Crabs	*2424*	
Gulf Toadfish	*2109*	

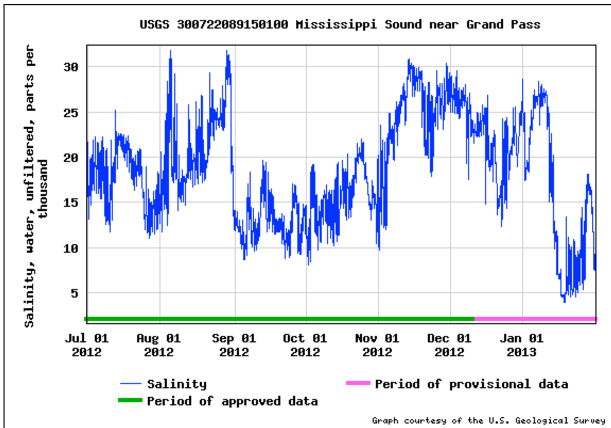
Additional Comments: ____

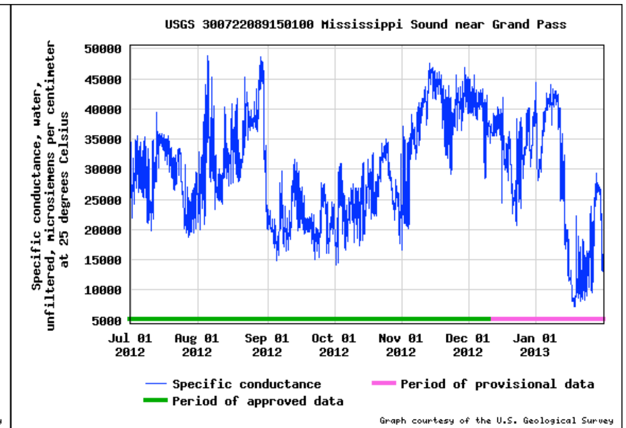
____

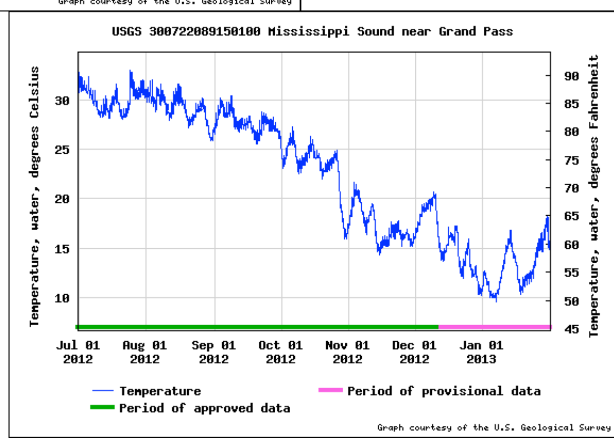
____

Oyster Sample Data Sheet – Rev 3/05

Appendix B
Salinity, Specific Conductance and Temperature at the United States Geological Survey Station Mississippi Sound near Grand Pass, LA (Supplemented for Round Island).







00010, Temperature, water, degrees Celsius,
YEAR Monthly mean in deg C (Calculation Period: 2002-10-01 -> 2012-11-30)

Period-of-record for statistical calculation restricted by user

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2002										25.23	17.53	13.39
2003	10.44	13.44				28.53	28.81	29.82	27.80		20.08	12.47
2004	12.11						29.98	29.27				
2005							29.54					
2006									28.10			
2008										22.97	16.89	
2009		14.51			25.99	29.18	29.47	29.68	28.30	23.79	17.87	12.17
2010	9.75	10.41			26.72		30.21	30.42	29.05		18.94	11.03
2011	10.53	13.08	20.11	23.60	25.34	29.65	29.82		27.03	22.43	17.90	14.02
2012	15.01	15.28	21.01	23.26	26.98	28.30	30.05	29.15	27.73	23.36	17.26	
Mean of monthly Temperature, water	11.6	13.3	20.6	23.4	26.3	28.9	29.7	29.7	28.0	23.6	18.1	12.6

**** No Incomplete data have been used for statistical calculation**

Appendix B Continued.

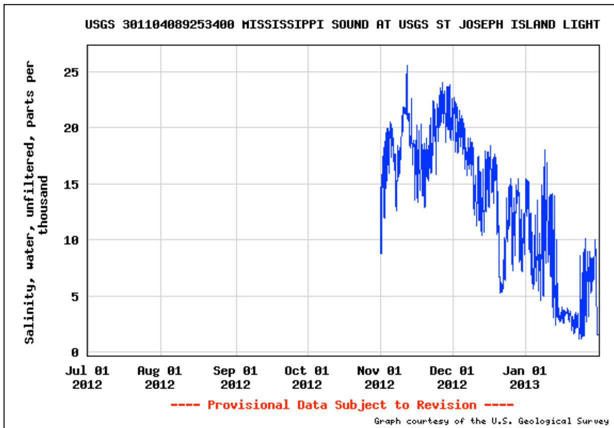
00480, Salinity, water, unfiltered, parts per thousand,
YEAR Monthly mean in ppth (Calculation Period: 2002-10-01 -> 2012-11-30)

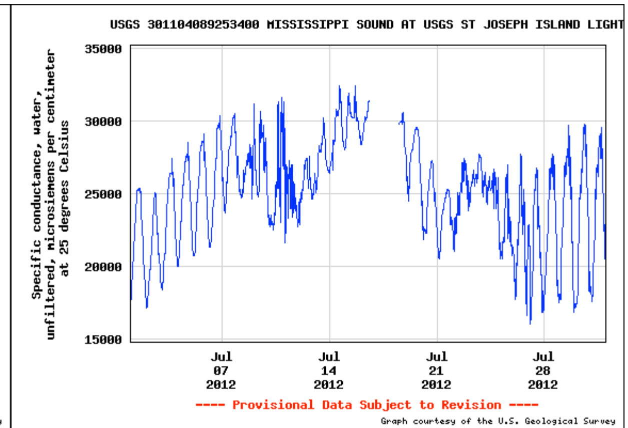
Period-of-record for statistical calculation restricted by user

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2002										14.49	12.68	18.64
2003	17.34	21.30				15.14	10.20	17.51	19.50	20.60	23.88	23.34
2004	20.70						12.45	21.34				
2005							15.59					
2006									26.61			
2009					13.62	17.15	20.72	23.20				
2010							14.12	16.16	17.83		22.82	23.58
2011	24.12	22.85					14.87		17.59	22.92	25.84	25.68
2012			15.07	11.33	14.30	20.34	17.75	21.42	12.93	15.51	24.40	
Mean of monthly Salinity	20.7	22.1	15.1	11.3	14.0	17.5	15.1	19.9	18.9	18.4	21.9	22.8

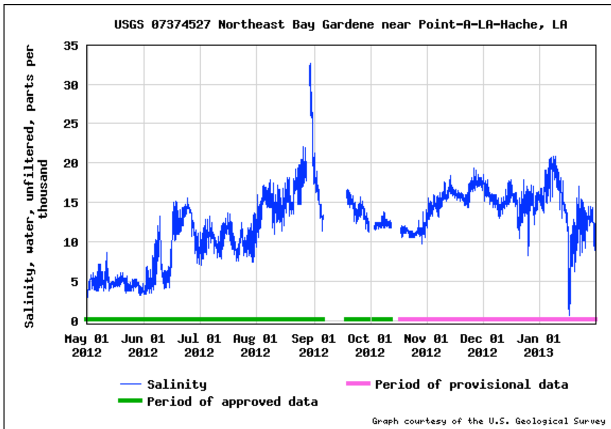
**** No Incomplete data have been used for statistical calculation**

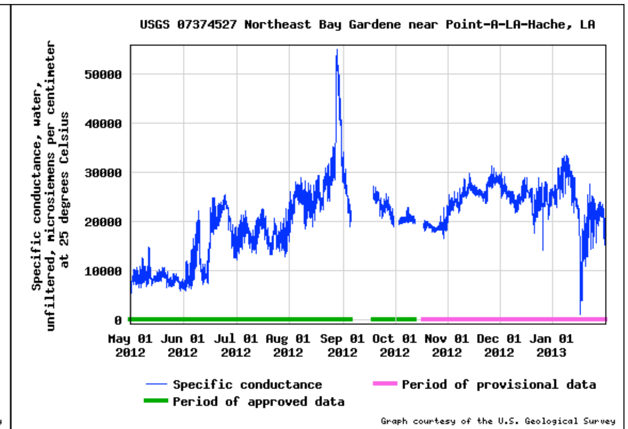
Appendix C
Salinity, Specific Conductance and Temperature at the United States Geological Survey Station St. Joseph Island Light, LA (Supplemented for Round Island).

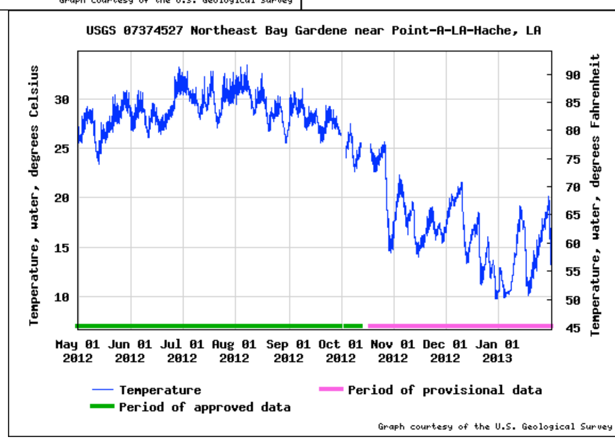




Appendix D
Salinity, Specific Conductance and Temperature at the United States Geological Survey Station Northeast Bay Gardene near Point-A-LA-Hache, LA (Supplemented for California Bay).







00480, Salinity, water, unfiltered, parts per thousand,
YEAR Monthly mean in ppth (Calculation Period: 2002-10-01 -> 2012-07-31)

Period-of-record for statistical calculation restricted by user

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2002										8.18	7.79	7.40
2003	7.12	10.66	7.42	4.90	10.17		4.68	13.32	14.11	12.48		
2004	10.43	8.64	6.61	4.01	3.80	15.09	10.39	10.83	13.37	11.72	12.99	9.47
2005	7.89	7.33		1.94	5.52	7.68	7.03				18.55	19.41
2006	19.19	16.27	14.54	12.71	12.56	15.63	13.79	13.86	15.26	13.02	10.57	
2007	9.76	6.61		7.75						9.37	13.91	16.72
2008	12.88	6.84	4.11	2.25	2.49			9.29	15.73		17.62	
2009										8.28	9.09	
2010			4.99	6.72	4.80							
2011			11.64	7.14					9.73	13.18	18.27	11.96
2012				2.47	4.74	9.16	10.17					
Mean of monthly Salinity	11.2	9.4	8.2	5.5	6.3	11.9	9.2	11.8	13.6	10.9	13.6	13.0

**** No Incomplete data have been used for statistical calculation**

Appendix D Continued.

00010, Temperature, water, degrees Celsius,
YEAR Monthly mean in deg C (Calculation Period: 2002-10-01 -> 2012-09-30)

Period-of-record for statistical calculation restricted by user

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2002										25.58	17.45	13.48
2003	10.68	14.67	19.62	21.83	27.32		29.00	29.83	28.04	23.49		
2004	13.02	13.18	19.92	21.96	26.07	29.32	30.37	29.50	27.97	26.17	20.51	12.79
2005	14.73	15.83	17.52	21.47	25.69	29.35	29.82				18.57	13.17
2006	15.55	14.85	19.43	24.56	26.51	30.13	30.25	30.78	28.37	24.02	17.10	
2007	13.47	12.72	20.17	21.06			29.77			23.91	18.56	16.33
2008	11.70	16.43	17.86	22.72	25.86	29.77	30.11	28.69	27.01	23.02	17.20	14.23
2009		15.38	19.35	21.05	26.08		30.14			23.85	17.91	12.62
2010	9.94	10.65	15.35	22.25	27.50	30.32	30.03	30.34	29.08	23.37	19.03	
2011	11.18	13.89	20.60	23.92		30.23			27.33	22.52	18.43	14.33
2012		15.89	22.01	23.67	27.49	28.62	30.03	29.43	28.02			
Mean of monthly Temperature, Water	12.5	14.3	19.2	22.4	26.6	29.7	29.9	29.8	28.0	24.0	18.3	13.8

Appendix E
Covariance Parameter Estimates for Live Wild Spat at Round Island and California Bay (Procedure PROC MIXED, SAS Institute Inc., 9.3). Significance Level is *P*<0.05.

Round Island September 25, 2012

Cov Parm	Group	Estimate	Standard Error	Z Value	Pr > Z	Alpha	Lower	Upper
Plot (TreatedUntreated)	TreatedUntreated T	678.11	701.82	0.97	0.1670	0.05	178.45	33548
Plot (TreatedUntreated)	TreatedUntreated U	90.8078	103.40	0.88	0.1899	0.05	21.9760	9225.77
Location (Treated*Plot)	TreatedUntreated T	33.2483	41.5259	0.80	0.2117	0.05	7.4075	7930.60
Location (Treated*Plot)	TreatedUntreated U	0	-	-	-	-	-	-
Residual		150.76	27.5244	5.48	<.0001	0.05	108.59	223.45

Round Island November 15, 2012

Cov Parm	Group	Estimate	Standard Error	Z Value	Pr > Z	Alpha	Lower	Upper
Plot (TreatedUntreated)	TreatedUntreated T	168.57	318.98	0.53	0.2986	0.05	25.7787	37262355
Plot (TreatedUntreated)	TreatedUntreated U	327.46	476.02	0.69	0.2458	0.05	63.5667	486357
Residual		434.61	177.43	2.45	0.0072	0.05	223.48	1184.28

Round Island January 23, 2013

Cov Parm	Group	Estimate	Standard Error	Z Value	Pr > Z	Alpha	Lower	Upper
Plot (TreatedUntreated)	TreatedUntreated T	5.5563	59.3950	0.09	0.4627	0.05	1.5033	1E182
Plot (TreatedUntreated)	TreatedUntreated U	0	-	-	-	-	-	-
Residual		152.05	57.4712	2.65	0.0041	0.05	81.5027	378.20

Appendix E Continued.

California Bay September 24, 2012

Cov Parm	Group	Estimate	Standard Error	Z Value	Pr > Z	Alpha	Lower	Upper
Plot (TreatedUntreated)	TreatedUntreated T	0	-	-	-	-	-	-
Plot (TreatedUntreated)	TreatedUntreated U	0.002083	0.009351	0.22	0.4119	0.05	0.000184	3.378E28
Location (Treated*Plot)	TreatedUntreated T	0.04271	0.03236	1.32	0.0934	0.05	0.01454	0.4447
Location (Treated*Plot)	TreatedUntreated U	0	-	-	-	-	-	-
Residual		0.08611	0.01572	5.48	<.0001	0.05	0.06203	0.1276

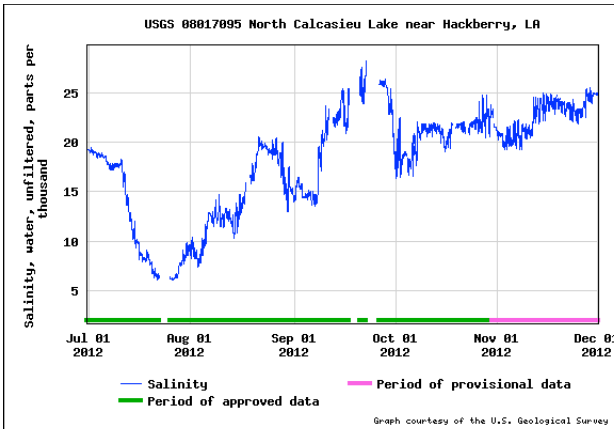
California Bay November 14, 2012

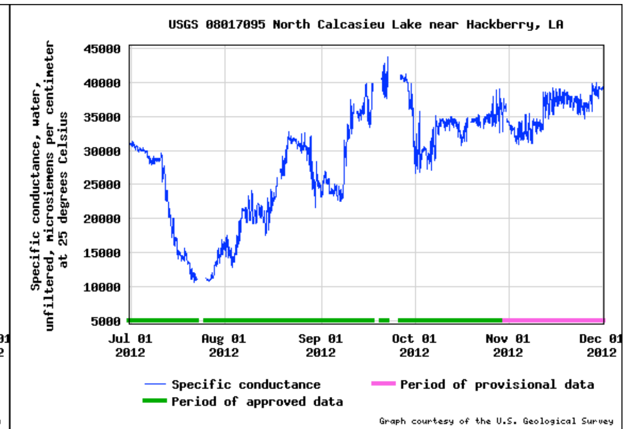
Cov Parm	Group	Estimate	Standard Error	Z Value	Pr > Z	Alpha	Lower	Upper
Plot (TreatedUntreated)	TreatedUntreated T	21.1270	25.6481	0.82	0.2050	0.05	4.8287	3807.22
Plot (TreatedUntreated)	TreatedUntreated U	0	-	-	-	-	-	-
Residual		13.3968	5.0635	2.65	0.0041	0.05	7.1808	33.3211

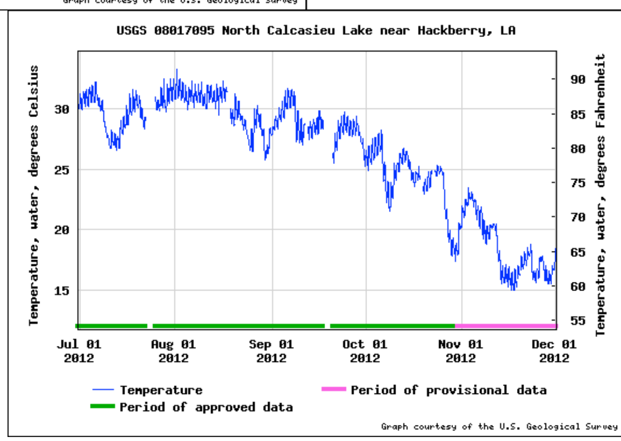
California Bay January 22, 2013

Cov Parm	Group	Estimate	Standard Error	Z Value	Pr > Z	Alpha	Lower	Upper
Plot (TreatedUntreated)	TreatedUntreated T	0	-	-	-	-	-	-
Plot (TreatedUntreated)	TreatedUntreated U	0	-	-	-	-	-	-
Residual		0.4861	0.1719	2.83	0.0023	0.05	0.2696	1.1260

Appendix F
Salinity, Specific Conductance and Temperature at the United States Geological Survey Station North Calcasieu Lake near Hackberry Bay, LA (Supplemented for Calcasieu Lake).







00480, Salinity, water, unfiltered, parts per thousand,
YEAR Monthly mean in ppth (Calculation Period: 2002-11-01 -> 2012-08-31)

Period-of-record for statistical calculation restricted by user

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2002											3.37	4.63
2003	10.25	8.75		8.47	14.05	13.62	9.87	18.91		14.11	18.14	
2004	9.55	2.42	5.41	10.65	3.15			16.94	19.56	18.95	11.24	6.64
2005	10.83	4.99	7.29	13.32	17.57	19.73	16.98	18.96				21.51
2006	21.20	16.60				17.75		13.31	21.80			
2007								11.67	11.38			
2008	14.36	5.37	7.96	9.56	10.35	10.76	13.41	15.58				
2009										13.42	6.66	6.50
2010	7.01	4.47	8.23	12.42	19.83	17.42	14.23	16.31	20.29	20.43	21.69	
2011	21.47	19.18			16.27		18.44	21.17	24.85	26.38	25.37	24.19
2012	11.93	3.13	3.09	5.06	11.84	17.44		14.37				
Mean of monthly Salinity	13.3	8.1	6.4	9.9	13.3	16.1	14.6	16.4	19.6	18.7	14.4	12.7

**** No Incomplete data have been used for statistical calculation**

Appendix F Continued.

00010, Temperature, water, degrees Celsius,
YEAR Monthly mean in deg C (Calculation Period: 2002-11-01 -> 2012-09-30)

Period-of-record for statistical calculation restricted by user

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2002											17.08	13.68
2003	11.59			21.96	26.85	29.30	30.02	30.31		23.79	20.33	
2004	13.29	11.95	19.45	22.53	25.13			29.67	28.35	26.07	20.04	13.55
2005	14.35	14.36	16.88	21.54	25.85	29.98	30.55	31.36				13.23
2006	15.03	14.10				28.84	29.83	31.09	28.28			
2007								30.88	28.93	24.87	19.17	16.19
2008	11.95	15.60	18.26	22.92	25.97	29.31	30.23	29.48				
2009			18.77	20.72	26.20	29.65	30.45	30.67	27.93	23.93	18.00	12.14
2010	10.75	10.64	15.15	22.22	27.05	30.64	30.36	31.29	29.15	24.01	18.67	
2011	11.35	13.56			25.56	29.82	30.71	31.85	27.52	23.43	18.46	13.78
2012	15.46	15.43	20.63	23.96	27.38	29.46		29.99	28.53			
Mean of monthly Temperature, water	13.0	13.7	18.2	22.3	26.2	29.6	30.3	30.7	28.4	24.3	18.8	13.8

**** No Incomplete data have been used for statistical calculation**

00095, Specific conductance, water, unfiltered, microsiemens per centimeter at 25 degrees Celsius,
YEAR Monthly mean in uS/cm @25C (Calculation Period: 2002-11-01 -> 2012-08-31)

Period-of-record for statistical calculation restricted by user

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2002											5,833	8,201
2003	17,260	14,880		14,580	23,250	22,550	16,800	30,420		23,350	29,360	
2004	16,200	4,476	9,586	18,000	5,573			27,580	31,450	30,590	18,790	11,500
2005	18,290	8,779	12,640	22,140	28,530	31,720	27,650	30,580				34,240
2006	33,830	27,070				28,730		22,070	34,700			
2007								19,510	19,130			
2008	23,610	9,538	13,770	16,330	17,570	18,210	22,280	25,550				
2009										22,210	11,640	11,300
2010	12,210	7,927	14,180	20,770	31,820	28,300	23,520	26,610	32,490	32,720	34,560	
2011	34,210	30,890			26,590		29,810	33,750	39,060	41,200	39,780	38,120
2012	19,880	5,722	5,581	8,945	19,810	28,340		23,700				
Mean of monthly Specific cond at 25C	21,900	13,700	11,200	16,800	21,900	26,300	24,000	26,600	31,400	30,000	23,300	20,700

**** No Incomplete data have been used for statistical calculation**

Vita

Erin Leonhardt grew up in Brewster, Massachusetts. In 2005, she graduated from Nauset Regional High School, and attended Roger Williams University, where she majored in marine biology. While at Roger Williams University, she worked for the Rhode Island Oyster Gardening for Restoration and Enhancement (RI-OGRE) program and swam for the RWU Swimming and Diving team, where she was team captain senior year. While working for RI-OGRE, she trained oyster gardening volunteers and educated volunteers about the importance of oysters in the marine environment. Erin also studied abroad at the Bermuda Institute of Marine Sciences. Erin graduated from Roger Williams University, with a Bachelor of Science degree.

After graduation, Erin served as a member of AmeriCorps Cape Cod from 2009 to 2010. As a member, she managed Cape Cod's natural resources, coordinated community outreach programs, educated elementary and middle school students about the marine environment and assisted the Cape Cod Cooperative Extension on documenting the county's shellfish propagation program.

Upon graduation from AmeriCorps, Erin was recruited by Dr. John Supan to attend Louisiana State University. She has achieved the degree of Master of Science from the school of Renewable Natural Resources with an Area of Concentration in Fisheries and Aquaculture.