

2010

Evaluating remote setting techniques for oyster (*Crassostrea virginica*) seed production in Louisiana

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EVALUATING REMOTE SETTING TECHNIQUES FOR OYSTER (*CRASSOSTREA*
VIRGINICA) SEED PRODUCTION IN LOUISIANA

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
Victoria Ippolito
B.S., Roger Williams University, 2008
December 2010

To my parents who have always supported and encouraged me.

To my brother and sister who I love tremendously.

To my Aunt Perky who I admire immensely.

To David who stood by me.

Acknowledgements

I thank Dr. John Supan for serving as my major advisor; his wonderful guidance and great wisdom throughout this experience has shaped my education to meet my future goals. I extend great thanks to the other members of my graduate committee, including Dr. Robert Romaine and Dr. Megan LaPeyre, as well as Dr. Ralph Portier who helped to shape my education in environmental science.

There are many people who helped me with my master's degree pursuit. I extend great thanks to the staff of Auburn University Shellfish Lab, including Dr. William Walton and Scott Rikard, for providing their facility and assistance with oyster spawning and rearing of larvae. I also thank to Dr. Michael Kaller for his help performing statistical analysis on my data. I also thank Dr. Stan Allen for his review of a manuscript before publication.

Motivatit Seafood Company was an industry cooperator with part of my research and I thank the late specifically Ernie Voisin and Jared Voisin. I extend great thanks to all members of the Louisiana oyster industry, whose participation in a research survey is greatly appreciated.

I thank all at the Louisiana Sea Grant College Program who has supported me, as well as other students who have worked at the Louisiana Sea Grant Oyster Hatchery, specifically Esther Young, Marc Stubbs, Ryan Moring and Erin Leonhardt.

Funding was provided by the Louisiana Board of Regents Support Fund, Grant No. LEQSF-(2008-11)-RD-B-03.

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List of Definitions

Barrel: One barrel of oysters is equivalent to two sacks of oysters or 6451.26 cubic inches

(Louisiana Revised Statute 2010).

Diploid: Having two sets of chromosomes.

Competent pediveligar larvae: Larvae ready to set on a substrate.

Cultch: Any hard substrate used for larval setting.

Macro-cultch: Large pieces of cultch (i.e. oyster shell, limestone, gravel) generally used for extensive (on-bottom) culture.

Micro-cultch: Very small pieces of cultch (sand size) used to produce single oysters for intensive (off-bottom) culture.

Off-bottom: The culture of oysters in the water column using floating rafts, trays mesh bags, etc., for growing single oysters with micro-cultch.

On-bottom: The benthic culture of oysters usually using macro-cultch.

Refuge size: A size to reduce natural predation of oysters.

Remote setting: The use of hatchery-produced oyster larvae for the production of oysters.

Sack: One sack of oysters is equivalent to 3225.63 cubic inches, or half of a barrel, or one and a half bushels (Louisiana Revised Statute 2010).

Seed bedding: The use of wild oyster seed for the production of oysters.

Triploid: Having three sets of chromosomes.

Abstract

Research was conducted to implement remote setting technology for the commercial-scale production of oysters on alternative cultch material in Louisiana. Genetic research has created an enhanced oyster stock for commercial cultivation. Since the Louisiana oyster industry relies on natural seed production for product, they are unable to utilize these new advances and are never guaranteed a reliable source of oyster seed. Remote setting is one way of producing a consistent supply of genetically improved oysters. Cultch material for oyster settlement is a limiting resource. Several alternatives to clamshell have been explored.

The objectives of this study are: (1) test larval setting success (penetration) using three cultch types in aerated and non-aerated treatments; (2) characterize triploid oyster seed growth and abundance among three cultch types on a commercial oyster lease; (3) characterize ploidy dilution of planted triploid oyster seed by natural diploids among three cultch types on an oyster lease; and (4) document the costs associated with seed bedding. Limestone had significantly less spat set than both whole and crushed oyster shell; spat set significantly less at bottom-depth and mid-depth in non-aerated compared to aerated treatments (objective 1). This shows the importance of aeration for spat settlement distribution among depths. Oyster growth was significantly less on limestone compared to both whole and crushed shell (objective 2). Spat on limestone were limited in two dimensional growing space. Of the fifty individuals sampled from each cultch type for percent triploidy, 2%, 4% and 8% were triploid for crushed shell, limestone and whole shell, respectively; all cultch types showed at least 92% diploid dilution (objective 3). These results are based on one site and season, therefore cannot be used to make conclusions about overall practicality for oyster production in Louisiana. The cost associated with seed bedding is \$6.00 per barrel (objective 4). Whole oyster shell had significantly more initial spat

set and growth than limestone. There was no significant difference in oyster abundance among all cultch types.

Chapter 1

Introduction

Oysters are culturally, economically and environmentally important. Oyster production provides many jobs not only for the grower, but also for the shipper, seller, shucker and many more individuals. Louisiana has led the United States in oyster production. In 2008 alone, Louisiana produced over 5 million kilograms of oysters (shucked meat), bringing in over \$38 million dock side value (NMFS 2010). Louisiana has 1.7 million acres of public oyster lease (LDWF 2010b). Oysters are also important for the environment; they create critical habitats for associated organisms, as well as acting as a natural filter (Kennedy *et al.* 1996).

The American oyster, *Crassostrea virginica*, is protandric starting as male producing sperm, that requires less energy and later changing to female producing egg, which requires more energy (Kennedy *et al.* 1996). Gametogenesis is controlled by both endogenous and exogenous factors (Kennedy *et al.* 1996). As temperature increases, oysters spawn, with the first spawn in the Gulf region usually occurring in the spring (Kennedy *et al.* 1996). Functional males have been reported able to spawn as early as six weeks post settlement (Andrews 1979). Oysters continue to “dribble” spawn for the rest of the summer months, making their glycogen reserves scarce resulting in low meat yield (Kennedy *et al.* 1996). At this time of the year, the oyster industry is less profitable as consumers usually discontinue or reduce oyster consumption due to the decrease in meat quality.

Genetic research has created an enhanced oyster stock for commercial cultivation (Que *et al.* 1997). Triploid oysters are sexually sterile, so they keep their winter glycogen reserves throughout the summer, resulting in an increase in summer meat yield. Triploid oysters have an increase in meat quality and quantity compared to diploid oysters (Allen 1988). The commercial

production of triploid oysters has been successfully implemented for the Pacific oyster, *Crassostrea gigas* (Nell 2002). Commercial-scale production of triploid American oysters, *C. virginica*, has been implemented along the east coast of the United States, but not yet in the Gulf of Mexico. The commercial-scale production of triploid *C. virginica*, during the summer months will yield a superior oyster for consumption.

Remote setting is a method of producing oysters that differs from natural oyster production. Remote setting is the production of oyster spat by setting hatchery-reared larvae onto cultch at a remote location from the hatchery; spat are then planted on-bottom (using macrocultch) or off-bottom (using microcultch). Typically, oyster larvae are purchased from a hatchery and transported to an oyster grower. The oyster grower will then set the larvae on a cultch material of their choice over 48 to 72 hours. Following the set of larvae, the spat are transferred to the nursery. The nursery phase allows the oyster spat to grow to seed or refuge size in a protected area until they are ready to be planted. Oyster seed are then planted on a lease, if extensively grown, or deployed off-bottom, if intensively grown, and harvested after they reach market-size.

Hatcheries are an integral part of the remote setting process. They supply the grower with a consistently reliable source of eyed larvae. The first successful artificial spawning of oysters occurred in 1879 by Brooks (Jones and Jones 1983, 1988). Although it was not until 1920, when oyster larvae were successfully spawned and set, that hatchery operations were refined by Wells (Henderson 1983, Jones and Jones 1983, 1988). In 1972, the first commercial application of remote setting of hatchery-reared oyster larvae was achieved by W.W. Budge of Pacific Mariculture (Jones and Jones 1988, Supan 1991). In 1978, the method of remote setting became a widespread practice when the Whiskey Creek Oyster Hatchery in Oregon was built

(Jones and Jones 1988). Numerous manuals have been written to act as a guide for the remote setting process (Henderson 1983, Jones and Jones 1983, 1988, Roland and Broadley 1990, Noshov and Chew 1991, Supan 1991, Bohn *et al.* 1995, Swartzenberg 1999). Clayton and Lorne (1984) showed how much the industry has utilized hatchery-grown larvae for remote setting from one company in 1981 to sixteen in 1984.

The process of remote setting has continued to be developed and improved for maximum efficiency. Henderson (1983) showed that larval setting success increased with increased water temperatures from 15 to 30°C and decreased with continued increased temperature up to 35°C for *Crassostrea gigas*. He also showed a similar trend with salinity. Larval setting success increased with increased salinity from 15 to 30 parts per thousand and decreased with a salinity of 35 ppt and greater (Henderson 1983), which agrees with Lund's (1972) results of optimum salinity range from 22-34 ppt. Henderson (1983) also showed that feeding had no effect on setting success from 0 to 75,000 cells/mL, but at 100,000 cells/mL setting success decreased with feeding. These results are in contrast to what Lund (1972) found, that setting success increased with increased food from 0 to 100,000 cells/mL. Larval set increased 37% by increasing food during setting from 0 to 75,000 cells/mL, although it is recommended not to feed at concentrations of 100,000 cells/mL (Roland and Broadley 1990). Lund (1972) also found that setting success increased as a result of cultch material coated with oyster tissue extract, oyster glycogen, cornstarch and algae. Henderson (1983) showed that eyed larvae can be held for up to eight days in a moist 5°C environment and still provide an acceptable set. It has become common practice for setting tanks to be aerated for an even set among cultch, although limited studies have been performed to confirm this (Jones and Jones 1983, Supan 1991, Bohn *et al.*

1995, Swartzenberg 1999). Gibbson (1988) noted that aeration enhances the set of larvae on cultch, although no data was reported.

Biofilms are an important part of spat settlement. Biofilms are formed by microbial communities attaching to the surface of a substrate. They have been thought to enhance the attachment and development of larval invertebrates to surfaces (Zobell and Allen 1935). Weiner *et al.* (1989) found that biofilms, specifically those composed of *Alteromonas colwelliana*, enhance the set of oyster spat on surfaces. Biofilms have also been shown to serve as an important cue for other invertebrates to settle (Dworjanyn and Pirozzi 2008). *Shewanella colwelliana* bacterium produces L-3-4-dihydroxyphenylalanine (L-DOPA), melanin and other melanin precursors that enhance larval settlement (Weiner *et al.* 1985, 1989).

Cultch material for oyster spat settlement has become a limiting resource in Louisiana. Lake Pontchartrain was one of the leading resources for clamshell cultch, however, due to adverse ecological impacts from hydraulic shell dredging, Lake Pontchartrain can no longer be dredged for cultch material. The lack of suitable cultch material has driven extensive research for the discovery of a new, acceptable, alternative cultch material. Alternative cultch materials must be biologically suitable, economically feasible and environmentally benign (Haywood *et al.* 1999). Biologically suitable cultch material will promote spat set, provide a hard substrate and sustain oyster survival. Economically feasible cultch material are usually found locally and have a relatively low weight to decrease transportation and maintenance cost. Alternative cultch materials should also be tested for adverse environmental side effects. Sinking rate into sediment is another consideration when choosing an acceptable alternative cultch material. Brodtmann (1991) showed that there is no significant difference in sinking rate of whole clamshell and #57 limestone on silt/clay sediment.

Several alternatives to clamshell have been explored. Limestone has been shown by many to be a suitable, alternative cultch material (Chatry *et al.* 1986, Soniat *et al.* 1991, Haywood *et al.* 1999, Soniat and Burton 2005). It has been compared to concrete, gravel, gypsum and clamshell (Soniat *et al.* 1991, Haywood *et al.* 1999). Limestone has also shown to be a superior cultch type compared to sandstone (Soniat and Burton 2005). There is some research that shows crushed concrete as a superior cultch compared to crushed limestone and crushed oyster shell (Cirino 2002). Cement board was also shown to attract more spat when compared to plexiglas, frosted glass and oyster shell (Butler 1995).

Stabilized coal ash has also been studied as an alternative cultch material for oyster spat. Coal combustion by-products can significantly benefit coastal estuarine habitats (Baker *et al.* 1991). Ash-cultched oysters grew 30 percent larger than shell-cultched oysters and had half the mortality rate of shell-cultched oysters (Price *et al.* 1991). Oysters grown on coal ash are significantly larger than shell-cultched oysters (Mueller 1990, Price *et al.* 1991) and residual metal concentration were within acceptable ranges (Mueller 1990). Coal ash was shown to be an acceptable substrate for oyster spat (Baker *et al.* 1991, Mueller 1990, Price *et al.* 1991).

Advances in cultch utilization increased the efficiency of the remote setting process. This results in an overall increase in the industries' efficiency and economic benefit. With the advent of triploid oysters as a viable commercial summertime crop, remote setting is proven even more valuable. However in Louisiana, the oyster industry historically relies on natural production, with remote setting being a rare occurrence for the production of oysters. Remote setting is becoming a more appealing option in the Louisiana oyster industry, with the advent of triploid oysters.

Remote setting has been successfully implemented for the production of oysters along the Pacific coast and the Chesapeake Bay area of the United States. Remote setting developed in the Pacific in response to low natural oyster production as a result of over harvesting, pollution, siltation, disease and predation (Jones and Jones 1983, Henderson 1983). Initially, the Pacific coast oyster industry was depended on imported seed, which became an unreliable source, however, with the development of hatcheries along the Pacific coast, remote setting continued to develop and thrived (Henderson 1983). Remote setting developed along the Chesapeake Bay area in an effort to increase oyster production and to utilize disease resistant larvae produced by hatcheries (Congrove *et al.* 2009).

In Louisiana, the oyster industry relies primarily on seed bedding to produce market oysters. Oyster farmers harvest wild oyster seed from public grounds and transport or “bed” it to their private leases, where it remains until it reaches market size. Melancon and Condrey (1992) report on the economics of this seed bedding process in Louisiana in 1988 dollars. They found that the expenses of bedding seed oysters averaged \$4.04 per Louisiana barrel (one barrel is equivalent to two sacks), with a range from \$2.52 to \$5.14, and the expenses associated with bringing a sack of oysters to market averaged \$4.77, with a range from \$2.98 to \$6.08 (Melancon and Condrey 1992). This results in a cost range from \$4.24 to \$8.65 to produce a sack of market-size oysters (Melancon and Condrey 1992).

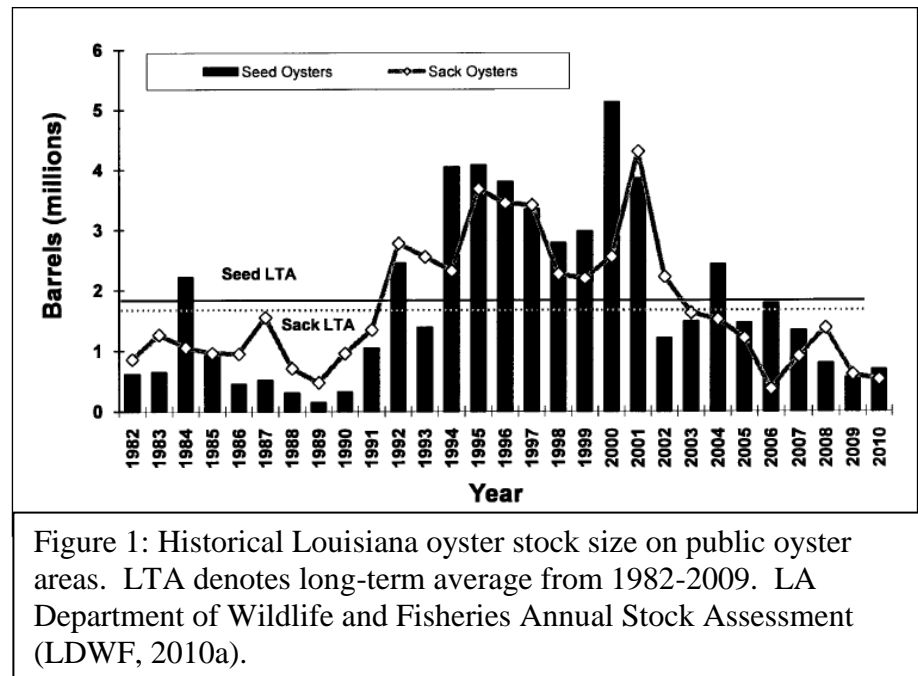
Natural oyster seed production is cyclical (Figure 1). Louisiana oyster seed production decreases with periods of drought and low Mississippi River discharge and increases with periods of high rainfall and river discharge. These influences dictate salinity regimes over the seed grounds, which in turn affect oyster predation and disease (Chatry *et al.* 1983). Due to this highly variable production of oyster seed, which is dependent on weather and environmental

factors, the oyster industry is never guaranteed a reliable source of seed. Therefore, when seed supply is lacking from the public grounds, so is farm production.

Remote setting is a process that can

increase the production of oyster seed. It has added benefits of producing oysters resistant to disease and sterile triploid oysters, which produce a superior product than natural diploids in the summer months. Supan *et al.* (1999) showed that the potential production cost of market-size oysters (200/sack) via remote setting varied from \$2.98 to \$8.66 at 5% to 10% survival respectively.

Remote setting is a viable candidate for the production of a reliable source of oysters in Louisiana. This is important since our environment is constantly changing. Models predict that global climate change is capable of impacting bivalve production (Canu *et al.* 2010). Not only does our environment change but man is also capable of having drastic effect on our natural environment. The Deepwater Horizon oil spill off the coast of Louisiana can also have an effect on Louisiana's oyster production. This provides further support for the need of a reliable source of oyster seed that remote setting is able to provide.



Chapter 2

Oyster Spat (*Crassostrea virginica*) Settle at Different Penetration Depths and Cultch Types in Aerated and Non-Aerated Treatments

In Louisiana, the oyster industry relies primarily on seed bedding as a means of producing market oysters. Oyster farmers harvest wild oyster seed from public grounds and transport or “bed” them to their private leases, where they remain until they attain market-size (Supan, 2002). If seed supply is lacking from the public grounds, so is farm production.

Remote setting is a process that can increase the production of oyster seed. Remote setting is a common practice along the west coast of the United States and is being developed on the east coast. In remote setting, hatchery-reared oyster larvae are purchased by oyster farmers, who then add the larvae to setting tanks of cultch (whole oyster shells) to produce spat-on-shell for extensive culture or micro-cultch to produce single seed for intensive culture. Producing single seed requires more time and effort than spat-on-shell as it is more labor intensive and seed must grow to a larger refuge size before being deployed in intensive systems. Remote setting has numerous benefits over seed bedding, including a consistent supply of oysters, the ability to use disease resistant strains and the ability to use triploid oysters. Despite these potential benefits of remote setting it has yet to be established in Louisiana.

It has become common practice for setting tanks (usually deep with cultch, about 122 cm) to be aerated to achieve an even set among cultch (Jones & Jones 1983, Supan 1991, Bohn *et al.* 1995, Swartzenberg 1999), although limited studies have been conducted to test this procedure. A uniform spat set on cultch is important to limit spat from excessive clustering. An uniform set makes the process efficient by utilizing each piece of cultch. Gibbons (1988) noted that aeration enhances the set of larvae on cultch, although no data were reported.

Remote setting using barges as setting tanks is being explored in Louisiana as a means of improved cultch handling to produce seed oysters. Barges have been designed to hold cultch at shallow depths of 17.8 cm. Larvae will be added to the barge of cultch to set before planted on an oyster lease. After larvae have been set on cultch the entire barge may be transported to a lease to plant the newly struck spat. This will eliminate the need for cultch handling at this stage when spat are still very vulnerable to mortality. The barge may allow for a more efficient way of transporting spatified cultch. Aeration of barges in remote locations is problematic because of electricity supply. If shallow setting tanks are used in barge deployment, is aeration necessary?

Cultch material for oyster spat settlement is a limiting resource (GSMFC 1991). Several alternatives to shell have been explored. Chatry *et al.* (1986) showed that limestone was twice as efficient at attracting spat as clamshell, and similar results were reported by Haywood *et al.* (1999). Limestone and clamshell attracted significantly more spat than concrete and gravel, with no significant difference between clamshell and limestone (Soniati *et al.*, 1991). Limestone has also been shown to attract more spat than sandstone (Soniati & Buton 2005). However, none of these studies address the penetration of larvae throughout the mass of cultch in remote setting systems.

The objectives of this study were to determine the setting success (count per volume) of three cultch types (whole oyster shell, crushed oyster shell and limestone) and determine the penetration depth (top, middle and bottom) of oyster spat in aerated and non-aerated treatments.

Materials and Methods

Cultch material used for this study was purchased from aggregate vendors or provided by an industry cooperator. Shucked oyster shells were left outdoors to age for approximately one year to allow any fouling organisms or oyster meat remaining on the shells to decompose. Half

of the shells were then crushed at Motivati Seafood Company (Houma, LA) to an approximate average size of 3 cm long by 3 cm wide. Limestone #57 was also used and all cultch material was rinsed of grit and graded on a screen size of 1.9 cm. Whole oyster shell, crushed oyster shell and limestone were then arranged in three rows in four containers 54 cm long by 37 cm wide by 50 cm high, two of which were aerated and two of which were not (Figure 2).

Consistent aeration was provided to two containers. A blower (GAST Model R2103) provided air to a 2.54 cm diameter PVC pipe manifold (Figure 2). The manifold was reduced to four 1.27 cm PVC delivery pipes, two for each of the two containers. Each pipe delivered air to one



Figure 2. This image shows the aerated and non-aerated treatments with an arrow pointing to the manifold at the connection point to the blower.

row of perforated 1.27 cm PVC pipe that were flush against the bottom of the two containers. At the end of the manifold was a valve to control air pressure to the two containers, where enough air was provided to create water movement to distribute the larvae.

Oyster larvae were produced by strip-spawning ripe diploid individuals (Allen & Bushek 1992) at the Sea Grant Bivalve Hatchery, Grand Isle, LA (29°14'12"N, 89°59'14"W). All eggs were pooled and sperm were pooled before fertilization. After fertilization, standard rearing methods were used to grow larvae to setting size (Dupuy 1977, Supan and Wilson 1993,).

Approximately 350,000 competent pediveliger larvae from the same hatchery-reared brood were added to each of the two aerated and two non-aerated containers each with the three cultch types 17.8 cm deep (depth determined by barge designs). After 96 hours, two equal samples by volume (180 mL water displacement) of each cultch type were collected from each

treatment container at three depths: top (surface), middle (8.9 cm depth) and bottom (17.8 cm depth) and placed in labeled bags. Water displacement value, of 180 mL, was chosen because it represents approximately 8 whole oyster shells. The spat were counted by cultch type, aeration treatment and depth with a dissecting scope at 10-30x. Counts were then analyzed using SAS ANCOVA test followed with a Tukey-Kramer means comparison (SAS 9.2, SAS Institute Inc., SAS Campus Drive, Cary, NC, 27513, USA). ANCOVA was used to analyze data due to a small sample size: the dependent variable was number of spat, the independent variable was substrate type and the covariate was treatment (i.e., aeration or no aeration). Since the number of observations per combination of treatment, cultch, and location was small, a power analysis (PROC GLMPOWER, SAS 9.2, SAS Institute, Inc., Cary, NC) was conducted, with the power effect size designated as large, determined by effect standard deviations (Gerow 2007).

Results

Spat set significantly less at bottom-depth ($T_{28}=3.74$, $p=0.0008$) and mid-depth ($T_{28}=4.05$, $p=0.0004$) in non-aerated treatments compared to aerated treatments (Figure 3, Table 1). Limestone had significantly less spat set than both whole ($T_{28}=-6.44$, $p<0.0001$) and crushed ($T_{28}=4.44$, $p=0.0001$) oyster shell, which did not vary significantly from each other

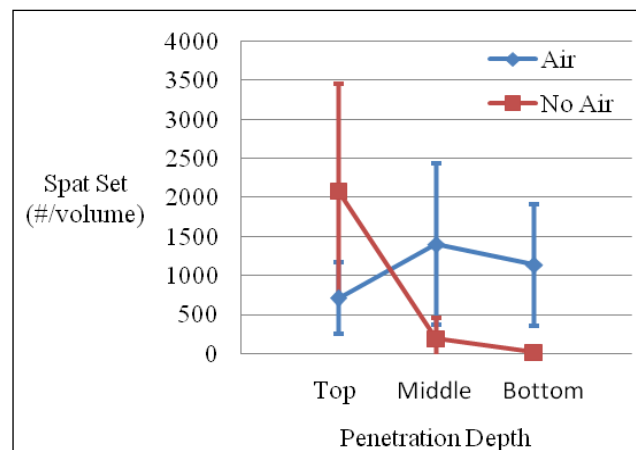


Figure 3. Effect of aerated and non-aerated treatments on spat set (count/180 mL) versus penetration depth (top 0 cm deep, middle 8.9 cm deep and bottom 17.8 cm deep) mean values with standard deviation bars.

(Table 1). Power analyses for these tests suggest a reasonable ability to discern large differences for air versus no air treatments and cultch type. The ability to discern location effect was

enhanced by the interaction of treatment type because the surface location did not vary among treatments and the interaction allowed the assessment of middle and bottom locations.

Since taking samples volumetrically is not a true representation of available surface area for spat to set, the number of pieces of cultch material that make up 180 mL water displacement

Table 1. Average spat set (standard deviation) per cultch type in aerated and non-aerated treatment at top, middle and bottom depths.				
		Whole shell	Crushed shell	Limestone
Air	Top	916 (86)	1062 (300)	155 (8)
	Middle	2380 (110)	1698 (57)	140 (13)
	Bottom	1894 (100)	1336 (23)	190 (33)
No Air	Top	3459 (285)	2343 (407)	459 (3)
	Middle	460 (337)	112 (138)	10 (13)
	Bottom	17 (3)	44 (39)	5 (6)

were counted. Water displacement was conducted five times for each cultch type to get an average number of pieces that make up 180 mL. Results showed that 180 mL water displacement is equivalent to 77 ± 21 pieces of limestone, 34 ± 9 pieces of crushed shell and 8 ± 1 pieces of whole shell. Due to the irregularity of the cultch surfaces, it is impractical to determine surface area without assumptions and estimations.

Discussion

This study showed that oyster spat penetrated cultch deeper in aerated treatments compared to non-aerated treatments. Aeration helps to mix the larvae throughout the cultch, resulting in more spat set at depth. Spat settlement decreases with depth in non-aerated treatments. This may be due to the addition of larvae at the top and minimal water movement through the cultch material. It is common commercial practice to aerate cultch material for the setting of oyster larvae in large tanks. This study shows aeration is important even in shallow cultch depths of 17.8 cm and supports Gibbons' (1988) report of an enhanced larval set on cultch due to aeration.

Overall, spat set on limestone the least and whole oyster shell the most with a whole shell, crushed shell, limestone ratio of 9.5 to 6.9 to 1.0 respectively. Shell has been accepted as the optimal cultch type for oyster farmers, although it has been shown by many that limestone is better at attracting oyster larvae compared to clam shell. A limestone, clamshell spat set ratio of 1.9 to 1.0 was shown by Haywood *et al.* (1999) and Soniat *et al.* (1991). Chatry *et al.* (1986) found a similar limestone to clamshell ratio of 2.1 to 1.0. A limestone to clam shell ratio of 1.3 to 1.0 was found by Brodtmann (1991). Cirino (2002) compared limestone to oyster shell and found a ratio of 4 to 1.0. This study found oyster shell as a superior cultch type for attracting spat compared to limestone, which may still be an acceptable alternative.

Whole oyster shell packs volumetrically different than crushed oyster shell and limestone, which pack similarly. Due to the shape of each piece, whole oyster shell packs more loosely, with large spaces allowing more water flow, possibly contributing to superior setting success. Limestone and crushed oyster shell packed similarly, although significantly more spat set on crushed shell than limestone. This suggests that there are factors other than water flow associated with setting success.

Crisp (1967) found that oyster larvae were attracted to oyster shell that retained some organic matter as opposed to shells in which all organic matter had been removed. Waterborne cues associated with oysters have also been shown to attract larvae and induce settlement (Hidu *et al.* 1978, Zimmer-Faust 1994).

Biofilms are an important part of spat settlement. Biofilms are formed by microbial communities attaching to the surface of a substrate. They have been thought to enhance the attachment and development of larval invertebrates to surfaces (Zobell and Allen 1935). Weiner *et al.* (1989) found that biofilms, specifically those composed of *Alteromonas colwelliana*,

enhance the set of oyster spat on surfaces. Biofilms have also been shown to serve as an important cue for other invertebrates to settle (Dworjanyn and Pirozzi 2008). *Shewanella colwelliana* bacterium produces L-3-4-dihydroxyphenylalanine (L-DOPA), melanin and other melanin precursors that enhance larval settlement (Weiner *et al.* 1985, 1989). This study addresses biofilm contribution to settlement by adding ambient filtered seawater at the same hour for equal exposure of all three cultch types in all four treatment containers prior to larval addition.

This study shows that aeration is important to allow spat to penetrate even shallow depths (17.8 cm) of cultch. Since aeration is important, it should be considered while designing barges for remote setting. The use of solar panels or batteries should be investigated to aerate large barges in remote locations away from electricity supply.

Chapter 3

Triploid *Crassostrea virginica* as a Viable Summertime Crop in Louisiana

Oysters are culturally, economically and environmentally important. Oyster production provides many jobs not only for the grower but also for the dealer, shipper, seller, processor and many more individuals. Louisiana has become the leading resource for oyster production in the United States. In 2008 alone, Louisiana produced over 5 million kilograms of oysters (shucked meat) bringing in over \$38 million dock side value (NMFS 2010). Louisiana has 1.7 million acres of public oyster lease (LDWF 2010b). Oysters are also important for the environment; they create critical habitats for associated organisms, as well as acting as a natural filter (Kennedy *et al.* 1996).

Genetic research has created an enhanced oyster stock for commercial cultivation (Que *et al.* 1997). Triploid oysters are sexually sterile, so they keep their winter glycogen reserves throughout the summer, resulting in an increase in summer meat yield. Triploid oysters have an increase in meat quality and quantity compared to diploid oysters (Allen 1988). The commercial production of triploid oysters has been successfully implemented for the Pacific oyster, *Crassostrea gigas* (Nell 2002). Commercial-scale production of the American oyster, *C. virginica*, has been implemented along the east coast of the United States, but not yet in the Gulf of Mexico. The commercial-scale production of triploid *C. virginica*, during the summer months, will yield a superior oyster for consumption.

Remote setting is a method of producing oysters that differs from the natural oyster population. Remote setting is the production of oyster spat by setting hatchery-reared larvae onto cultch at a remote location from the hatchery until the spat are ready to be planted on-bottom (using macrocultch) or off-bottom (using microcultch). Typically, oyster larvae are

purchased from a hatchery and transported to an oyster grower. The oyster grower will then set the larvae on a cultch material of their choice over 48 to 72 hours. Following the set of larvae, the spat are transferred to the nursery. The nursery phase allows the oyster spat to grow to seed or refuge size in a protected area until they are ready to be planted. Oyster seed are then planted on a lease, if extensively grown, or deployed off-bottom, if intensively grown, and harvested after they reach market-size.

The many advances in the field have increased the efficiency of the remote setting process. This results in an overall increase in the industries' efficiency and economic benefit. With the advent of triploid oysters as a viable commercial summertime crop, remote setting is proven even more valuable. However, in Louisiana the oyster industry continues to rely on natural production, with remote setting used rarely for the production of oysters. With the advent of triploid oysters and disease resistant strains, the use of remote setting may provide enormous benefits. Remote setting is becoming a more appealing option for the Louisiana oyster industry.

The objectives of this study are to: (1) characterize triploid oyster seed growth and abundance among three cultch types on an oyster lease; and, (3) test ploidy dilution of planted triploid oyster seed by natural diploid overstrike among three cultch types on an oyster lease (% diploid dilution).

Materials and Methods

Cleaned oyster shells were left outdoors to age for approximately one year to allow any fouling organisms or oyster meat remaining on the shells to decompose. Half of the shells were then crushed at Motivati Seafood Company (Houma, LA) to an approximate size of 3 cm long by 3 cm wide. Limestone #57 was used and all cultch material was rinsed of grit, graded and

pieces less than 1.9 cm discarded. The whole oyster shell, crushed oyster shell and limestone were then arranged in three separate tanks.

Oyster larvae were produced by crossing eggs from diploid females with sperm from tetraploid male oysters at the Auburn University Shellfish Laboratory, Dauphin Island, AL (Guo *et al.* 1996). All eggs were pooled and all sperm were pooled before fertilization. After fertilization, standard rearing methods were used to grow larvae to setting size (Loosanoff and Davis 1963).

In June 2009, approximately 671,000 competent triploid pediveligar larvae, from the same hatchery-reared brood, were added to tanks of each cultch type, containing filtered, aerated, ambient seawater. Each polyethylene tank has a diameter of 208 cm and was filled between 14 cm and 19 cm with cultch. Each tank was flooded with filtered bay water for 2 days prior to larval introduction to allow a biofilm to form. After 6 days, spatulated cultch was shoveled into burlap sacks, transported by industry cooperator and planted on an oyster lease in Moncouse Bay, Terrebonne Parish, Louisiana (N 29°14'48.2" W 90°52'28.9"). Volumetrically equal samples (180 ml water displacement) of each cultch type were collected during planting and subsequently examined for spat set with a dissecting scope at 10-30x to determine setting success. Data was analyzed using SAS ANCOVA test followed with a Tukey-Kramer means comparison (SAS 9.2, SAS Institute Inc., SAS Campus Drive, Cary, NC, 27513, USA). ANCOVA was used to analyze data due to a small sample size: the dependent variable was number of spat and the independent variable was substrate type.

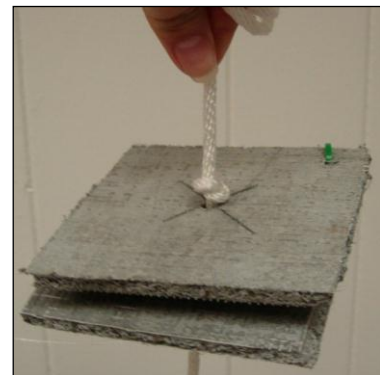


Figure 4. One pair of spat plates used to determine natural oyster production.

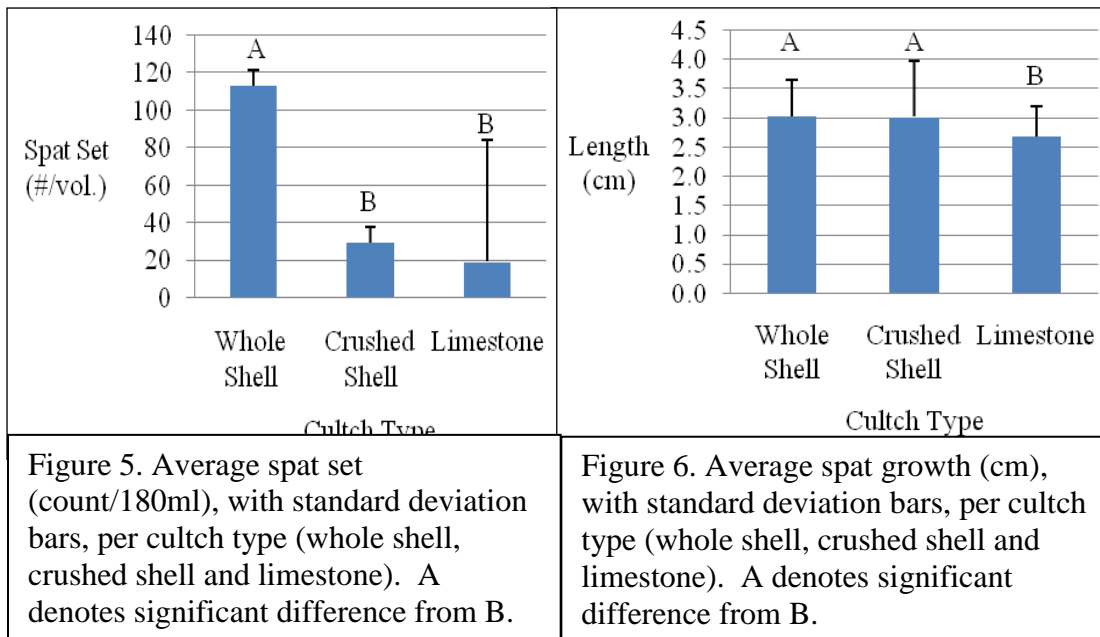
Coupled with the planting of triploid oysters in Moncleuse Bay, three sets of paired spat plates were deployed to determine natural diploid oyster set. Spat plates were made of two pieces of cement board cut in 10 cm X 10 cm squares separated by a piece of PVC pipe (approximately 1 cm thick) (Figure 4). The spat plates were deployed at time of cultch planting and changed monthly throughout the sample period. Surface and bottom salinity and water temperature were measured during monthly spat plate changes.

After the spat-cultch was planted in June of 2009, volumetrically equal samples each cultch type were collected the following September and November. Oyster abundance was measured in November 2009 and compared to initial counts obtained in June of 2009 to determine mortality and recruitment. Fifty individuals from each cultch type were measured in September and November to determine oyster growth. Spat planted in June were small to get initial growth measurements; therefore, September measurements were compared against November measurements. Oyster abundance and growth was analyzed using SAS ANCOVA test with a Tukey-Kramer means comparison (Copyright © SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA). ANCOVA was used to analyze data due to a small sample size: the dependent variable was number of spat and the independent variable was substrate type.

Samples collected in November of 2009 to determine seed growth and abundance were also used to determine ploidy dilution. Fifty individuals from each cultch type were taken and gill samples obtained using Virginia Institute of Marine Science protocol (Appendix A). The samples were labeled accordingly and processed with a flow cytometer (Partec CyFlow ® SL) using the procedures outlined by VIMS (Appendix B). Percent diploid dilution was calculated for each cultch type.

Results

Significantly more spat set on whole oyster shell compared to both crushed shell ($T_6=-3.25$, $p=0.01$) and limestone ($T_6=-4.36$, $p=0.005$) in June of 2009 (Figure 5). Oyster growth was significantly higher on both whole shell ($T_{101}=4.00$, $p=0.0001$) and crushed shell ($T_{101}=-3.83$, $p=0.0002$) compared to limestone (Figure 6). Over seventy-seven days from September to November of 2009, oyster spat on both whole and crushed shell grew 30 mm and spat on limestone grew 27 mm. No significant difference in oyster abundance was found among the three cultch types in November 2009. Of the fifty individuals sampled from each cultch type, 2% were triploid for the crushed shell 4% for limestone and 8% for whole shell. Substantial spat set was shown on deployed spat plates in October, with 63 individual oysters estimated per square meter, and November, with 9 individuals per square meter.



Discussion

Cirino (2002) reported that oyster spat on oyster shell grew significantly more than spat on limestone. In this study, spat on limestone were limited in two dimensional growing space, possibly contributing to significantly more growth on whole and crushed oyster shell compared

to limestone. Oyster growth has been shown by Kingsley-Smith *et al.* (2009) to be site dependent, however, this study and Cirino (2002) showed oyster growth to be significantly greater on oyster shell compared to limestone.

Natural diploid oyster production in October and November resulted in a great amount of diploid dilution. Diploid dilution was shown to be 92%, 96% and 98% for whole shell, limestone and crushed shell respectively. This large amount of diploid dilution may also have resulted in no significant difference in oyster abundance in November of 2009, although there was significantly more spat on whole shell compared to crushed shell and limestone when the cultch was planted in June.

The large amount of diploid dilution among all cultch types shows a need to address this problem. Wild diploid overset can reduce the value of a triploid oyster crop by reducing its overall summertime meat yield; it is important to have confidence upon harvest that you are harvesting oysters you planted.

This may be accomplished by appropriate farm management and/or culture methods. Planting triploid oysters, in locations and times of the year that have low natural diploid recruitment such as low salinity areas, may help to maximize overall summertime meat yield. This can be done by conducting preliminary data on natural diploid oyster set over time at specific locations. Growing triploid oysters to a larger refuge size in a nursery area before planting may also help to increase confidence upon harvest that natural diploids are those that set atop cultured triploids (Nosho and Chew 1991). Intensive culture is another means of producing triploid oysters, where oysters are grown in equipment such as floating rafts, trays or modules, as is done on the Pacific coast for the culture of cultchless oysters (Nosho and Chew 1991).

This data is site and season specific. Since only one site and season were selected, due to industry cooperator and cultch material availability, it is not possible to make conclusions about overall practicality of extensive culture of triploid oysters in Louisiana.

Chapter 4

Cost Assessment of Seed Bedding in Louisiana

Oysters are an important part of the Louisiana coastal economy. Oyster production provides many jobs not only for the grower but also for the dealer, shipper, seller, processor and many more individuals. Louisiana has become the leading state for oyster production in the United States. In 2008 alone, Louisiana produced over 5 million kilograms of oysters (shucked meat), with a dockside value of over \$38 million (NMFS 2010). Louisiana has 1.7 million acres of public oyster lease (LDWF 2010b).

Natural oyster seed production is cyclical (Figure 1). Louisiana oyster seed production decreases with periods of drought and low Mississippi River discharge and increases with periods of high rainfall and river discharge. Due to this highly variable production of oyster seed, which is dependent on weather and environmental factors, the oyster industry is never guaranteed a reliable source of seed.

In Louisiana, the oyster industry relies primarily on seed bedding to produce market oysters. Oyster farmers harvest wild oyster seed from public grounds and transport or “bed” it to their private leases, where it remains until it reaches market size. Melancon and Condrey (1992) report on the economics of this seed bedding process in Louisiana in 1988 dollars. They found that the expenses of bedding seed oysters averaged \$4.04 per Louisiana barrel (one barrel is equivalent to two sacks), with a range from \$2.52 to \$5.14 (Melancon and Condrey 1992).

Remote setting is a process which can increase the production of oyster seed utilizing hatchery technology. It has added benefits of producing disease resistant oysters and sterile triploid oysters, which produce a superior product than natural diploids in the summer months.

The goal of this research is to document the costs to Louisiana oyster farmers associated with seed bedding as a means of oyster production.

Materials and Methods

To document the costs of seed bedding, surveys were mailed out to select members of the Louisiana Oyster Dealers and Growers Association and the Oyster Commodity Group Members of the Louisiana Farm Bureau Federation (Appendix C).

This survey asked questions to gather data about investment costs, fixed costs and production costs of seed bedding, as well as other relevant information, such as cultch planting. Investment costs questions were formulated to gather information about vessel operations, as well as dredges, using both multiple choice and “fill in the blank” styles. Production cost questions gathered data about the costs of bedding seed per boat load and annual costs of maintenance. Production cost questions were all fill in the blank style. Fixed cost questions gathered data about the annual costs of leases, licenses, insurance and dockage for vessels; these questions were both multiple choice and fill in the blank style. Other relevant information was gathered about planting seed and cultch planting. One theoretical question (#33) was asked about buying seed as a means of measuring the potential of remote setting.

Surveys were mailed initially using Louisiana Oyster Dealers and Growers Association envelopes and addressed to specific individual members rather than anonymous addressees (e.g. “dear neighbor” or “dear business owner”). Included with the survey was a cover letter (Appendix D) encouraging members to participate, as well as a stamped envelope for the survey’s return. On day 8 of the survey period, another replacement survey was mailed out to the same individuals with a stamped envelope for return and a cover letter (Appendix E) thanking individuals who had already replied.

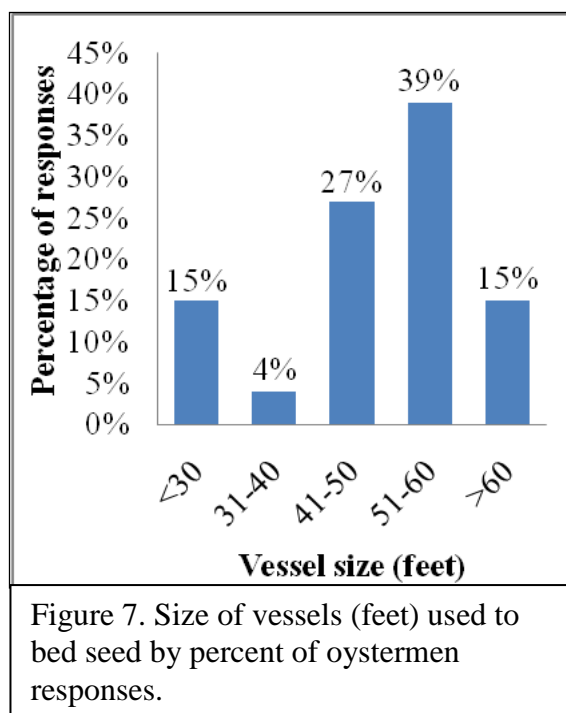
The survey was created using suggestions from Dillman *et al.* (2009) about formatting and question style (ie. multiple choice, fill in the blank etc.) as well as knowledge about the Louisiana oystermen being surveyed.

Results

Survey mailings had a 29% return; 15 of the 52 individuals surveyed responded. Two of the responses gathered no data about seed bedding, since recipients did not bed seed. Therefore, all data is based on the replies from 13 individuals (n=13). Not all participants answered every question; therefore, all answers are based off the 13 participants unless noted otherwise.

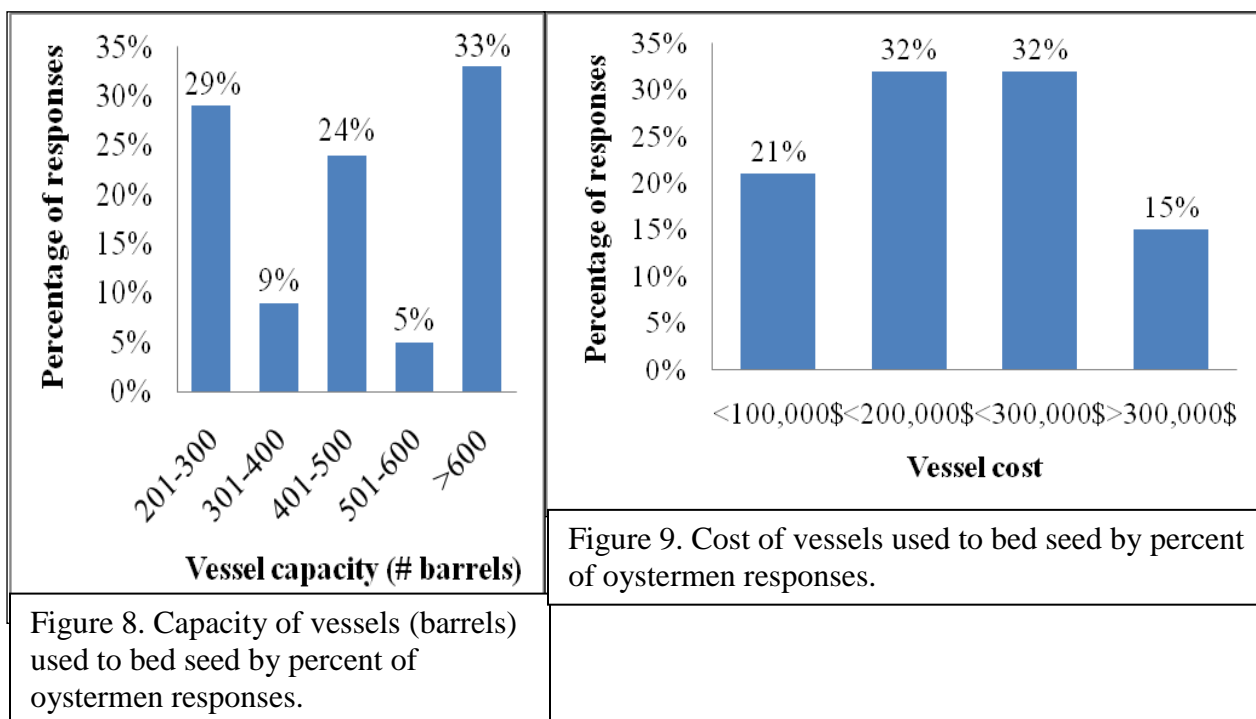
Investment Costs

Investment cost questions gathered information about vessels. Sixty two percent of responding oystermen use one vessel to bed seed, 15% use 2 vessels, 15% use 3 vessels and 8% use 20 vessels (i.e.: one respondent). Vessel size varied from 39% of responded oystermen using 51-60 foot vessels to 4% using 31-40 foot vessels (Figure 7). Vessel capacity shows 33% of vessels used by surveyed oystermen have a capacity of



more than 600 barrels of oysters and 29% have a capacity of 201 to 300 barrels of oysters (Figure 8). The majority of the vessels, 73%, were built by the oystermen, while only 27% were purchased. Vessels were acquired from 1965 to 2008, with most vessels acquired in the mid 1980s. Most oystermen, 68%, expect their vessels to last over 21 or more years, with 24% expecting to last from 11 to 15 years and 9% from 16 to 20 years. It cost 32% of participants less

than \$200,000 to build or buy their vessel and 32% <\$300,000 to build or buy their vessels (Figure 9).



Dredge Costs

Information about oyster dredges was also gathered. All oystermen surveyed use two oyster dredges per vessel to harvest seed oysters, which were purchased within the last three years. On average, dredges cost $\$1,000 \pm \407 (n=12). Oyster dredges are purchased each year by 54% of the participants, every three years by 23% and every 2 years by 23%. Dredge repairs cost an average of $\$1,011 \pm \782 (n=11) annually. High dredge repair costs may result from oystermen owning more than two dredges per vessel, so when some dredges are being repaired they are ensured two working dredges per vessel.

Production, Annual Variable and Annual Fixed Costs

There are many expenses associated with seed bedding. Production costs per boat load, averaged

\$1,216, and include labor (n=9), groceries and galley supplies (n=11), vessel fuel (n=11), oil and grease (n=11) and propane and ice (n=10) (Table 2). Annual variable costs average \$22,385 and include vessel, equipment and engine maintenance, as well as dredge repairs (n=11) and poles to mark lease boundaries (Table 2). Annual fixed costs averaged \$19,855 and include lease costs (n=10), licenses (n=10), insurance (n=10), crop insurance (n=8) and dockage for vessels (n=9) (Table 2).

Other Information

The majority, 92%, of oystermen plant seed from both public and private leases, with public: private percentage ratios from 20:80 to 95:5. Fifty-six percent of oystermen plant seed from September to December (Figure 10). On average, 38 ± 18 (n=11) bedding trips are made per year by

Table 2. Expenses associated with seed bedding. Data from 13 participants.

Costs	Average	SD
Annual variable costs		
dredge repairs*	1,011	782
vessel maintenance	9,577	5,972
equipment maintenance	5,538	3,388
engine maintenance	2,682	1,901
poles to mark lease boundary	3,577	3,141
Total	22,385	
Annual fixed costs		
lease costs**	3,530	1,616
licenses *	845	527
Insurance**	11,625	5,437
crop insurance*****	2,844	2,389
dock or slip***	1,011	284
Total	19,855	
Production costs per boat load		
Labor***	598	356
Groceries and galley supplies*	157	150
vessel fuel*	411	243
oil and grease*	43	37
propane and ice**	7	9
Total	1,216	

*denotes n=11 **denotes n=10 ***denotes n=9
*****denotes n=8

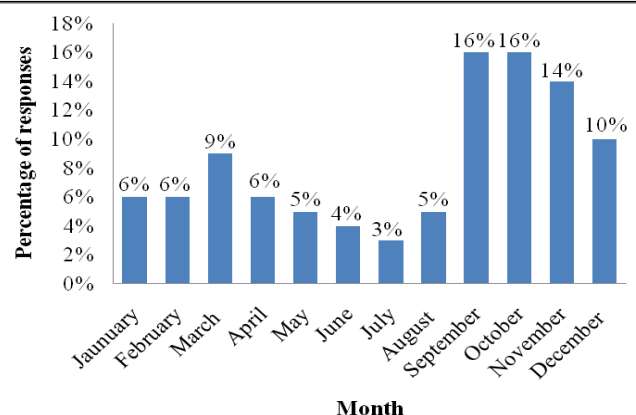


Figure 10. Percent of responded surveyed oystermen that plant seed each month.

an oysterman. Oystermen use an average of 9 ± 3 (n=10) leases per year to bed seed. Oystermen travel an average of 30 ± 39 (n=9) miles to bed seed. Estimates of seed verses cultch percentage per vessel load are made during each trip by 67% of surveyed oystermen (n=12); these estimates are made

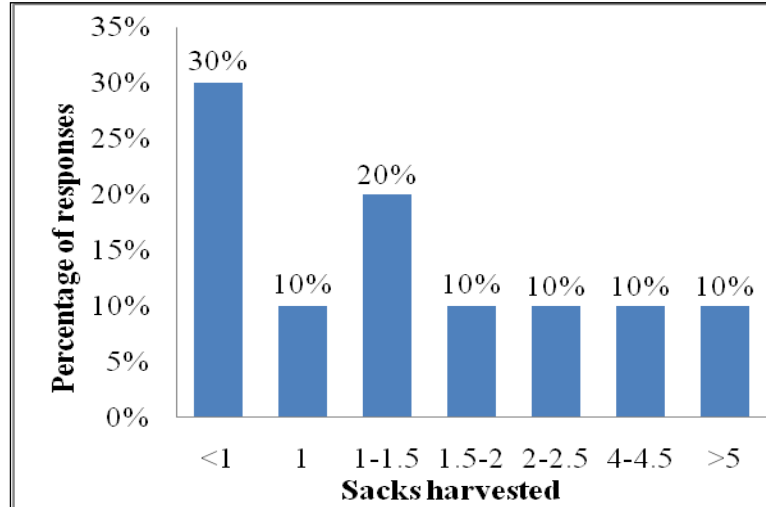


Figure 11. Average number of sacks harvested per sack bedded by percentage of surveyed oystermen

33% of the time by counting a subsample of oysters by volume, while 67% are made by guessing (n=12). The survey revealed that between 50-80% of a vessel load is seed (n=10). The average return from bedding a load of seed varies from <1 sack harvested per sack bedded to >5 sacks harvested per sack bedded, with 30% of the respondents answered <1 sacks harvested per one sack bedded (n=10) (Figure 11).

Cultch Planting

Of the 13 individual survey responses, 10 individuals plant cultch. All 10 individuals plant cultch themselves, while 2 also hire a contractor. The most prevalent type of cultch used is crushed concrete, followed by oyster shell and limestone; gravel, rock, brick and clamshell are also used as cultch. Annually, 30% of surveyed oystermen plant 2001-3000 cubic yards of cultch (n=10) (Figure 12). The cost of planting cultch averages $\$32 \pm \19 per cubic yard (n=5). The average return on investment from planting cultch showed 30% harvest >5 sacks per cubic yard cultch planted (n=7) (Figure 13).

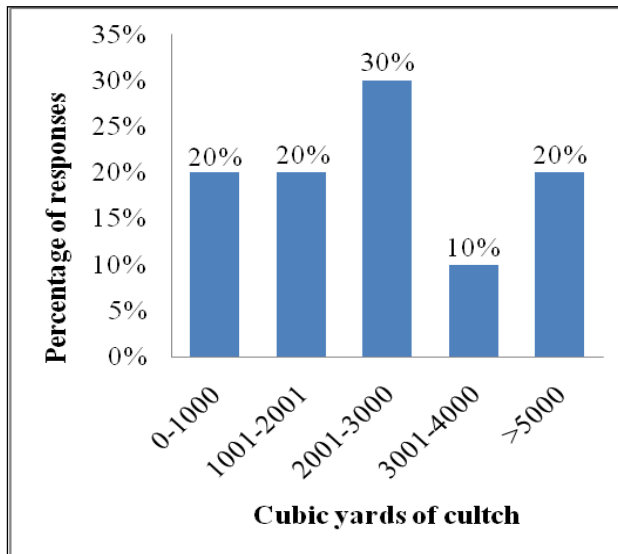


Figure 12. Cubic yards of cultch planted annually by percent of oystermen responses.

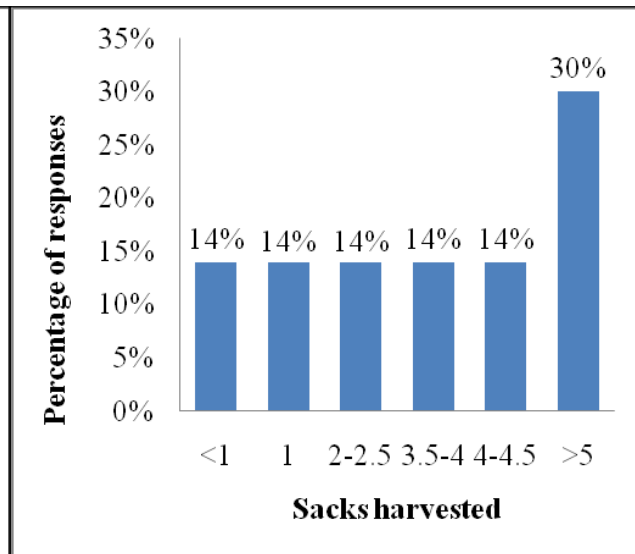


Figure 13. Average number of sacks harvested per cubic yard of cultch planted.

A theoretical question about buying seed was asked. The results showed the worth of a boatload of spat-on-shell averages $\$3,083 \pm 1,917$ ($n=6$). In order to get the worth of a barrel of spat-on-shell to compare to seed production costs, the average worth of \$3,083 of a boatload of spat-on-shell was divided by the median vessel capacity of 400 barrels. This results in an average value of \$7.70 for a barrel of spat-on-shell.

Costs

In order to determine the cost of seed bedding per barrel, first annual variable costs and annual fixed costs are added giving an average total cost of \$42,240. This was then divided by 38, the average number of bedding trips per year, for a value of \$1,112, which was then added to the average production cost per load of \$1,216 for a total

Table 3. Calculation to determine cost of seed bedding per barrel.	
	Average
Annual costs	\$22,385
Annual fixed costs	\$19,855
Sum	\$42,240
Bedding trips/year	38
Quotient	\$1,112
Production cost/ boat load	\$1,216
Sum	\$2,328
Vessel capacity (barrels)	400
Quotient (cost of seed bedding/ barrel)	\$6.00

average cost per boat load of \$2,328. Vessel capacity will affect the cost of seed bedding per barrel. This study determined that vessel capacity ranged from 201 to over 600 barrels. The median of 400 barrels was used to divide by the average total cost, resulting in an average cost of \$6.00 per barrel to bed seed in Louisiana (Table 3).

Data in the literature was brought up to present dollar value by multiplication of an inflation factor. Melancon and Condrey (1992) data in 1988 dollars was multiplied by the inflation factor of 1.7476 (DNR 2010). This gives an average cost of seed bedding of \$7.06 per barrel, with a range from \$4.40 to \$8.98.

Discussion

Overall, this survey had acceptable return of 29%. Dillman *et al.* (2009) noted an acceptable 8% return on mail-in surveys to businesses. The Louisiana Department of Wildlife and Fisheries' mail-in survey sent to individuals with hunting licenses had a 25% response (LDWF 2010b). Survey return numbers could be attributed to the fact that they were mailed to specific individuals rather than anonymous addressees (e.g. "dear neighbor" or "dear business owner"). This helps to personalize the survey, in turn making individuals more likely to participate. Surveys were also mailed in Louisiana Oyster Dealers and Growers Association envelopes, which are familiar to most recipients and provide further encouragement for completion of the survey. These factors also made it more likely that recipients would open the envelope instead of simply discarding.

This survey documents the costs associated with seed bedding, as well as additional information about bedding and cultch planting. This data showed that the cost of seed bedding per barrel is \$6.00. This data is less than Melancon and Condrey (1992) reported value of \$7.06 per barrel, adjusted for inflation. Although higher, this may be due to inflation factor error.

Since the inflation factor is for the entire United States; Louisiana or the Gulf region inflation may not be as high. This survey also showed the worth of a purchased barrel of spat-on-shell is \$7.70, greater than both seed bedding production costs. This information also shows that remote setting may be an acceptable alternative for production of oysters in Louisiana. Future analyses can be compared to the cost of seed bedding when new technologies are implemented for the production of oysters. A comparison of new versus old production methods is important to determine economic change.

As a result of this research an accounting tool for the documentation of seed bedding was created as a Microsoft Excel spreadsheet (Appendix F). This tool will aid in recording costs associated with seed bedding and standardize documentation.

If seed could be produced via remote setting for purchase by oyster farmers, this may be an acceptable alternative to seed bedding. Such production of seed would guarantee farmers a reliable source when natural production is low. It can also produce an oyster resistant to disease and sterile triploids, therefore producing a superior product than what nature can provide to them.

Chapter 5

Summary and Conclusion

Genetic research has created an enhanced oyster stock for commercial cultivation (Que *et al.* 1997). Since the Louisiana oyster industry relies on natural seed production for product, they are unable to fully utilize these stocks. With the process of remote setting this can be implemented by the Louisiana oyster industry.

This thesis provides information about the potential viability of remote setting in Louisiana, as well as documenting the costs of seed bedding, to later compare with new means of oyster production in Louisiana.

Spat settlement is an important part of the remote setting process and an optimal cultch type to maximize spat set is important. Spat set significantly more on both whole and crushed oyster shell compared to limestone, which did not differ significantly from each other. Whole oyster shell packs more loosely than both crushed shell and limestone, allowing more water flow through whole shell, possibly contributing to superior setting success. Limestone and crushed oyster shell packed similarly, although significantly more spat set on crushed shell than limestone. This suggests that there are factors other than water flow associated with setting success. Crisp (1967) found that oyster larvae were attracted to oyster shell that retained some organic matter as opposed to shells in which all organic matter had been removed. Waterborne cues associated with oysters have also been shown to attract larvae and induce settlement (Hidu *et al.* 1978, Zimmer-Faust 1994).

Oyster growth was shown to be greater on whole and crushed oyster shell compared to limestone. This is due to the increased surface areas of both whole and crushed shell over limestone, allowing spat to grow on the surface in two dimensions as opposed to setting on a

smaller piece of cultch and then having to grow in three dimensions. Through this study and Cirino (2002), growth is shown to be significantly greater on oyster shell compared to limestone.

Wild diploid oversight can reduce the value of a triploid oyster crop by reducing its overall summertime meat yield; it is important to have confidence upon harvest that you are harvesting oysters you planted. In order to avoid harvesting an unintended product, it is important that leases used to grow triploid oysters have minimal natural diploid set. This is most likely accomplished by planting triploids in locations and times of the year that have of low natural recruitment, such as low salinity areas. A large amount of diploid oversight on a triploid-planted lease can drastically reduce the percent triploids harvested.

The cost of seed bedding per barrel is \$6.00. This data is less than the Melancon and Condrey (1992) reported value of \$7.06 per barrel adjusted for inflation. The worth of a purchased barrel of spat-on-shell was shown to be \$7.70, greater than both seed bedding production costs.

As a result of this research, an accounting tool for the documentation of seed bedding was created as a Microsoft Excel spreadsheet (Appendix F). This tool will aid in recording costs associated with seed bedding and standardize documentation. This information shows that remote setting may be an acceptable alternative for production of oysters in Louisiana. This information should be extremely useful in the future when new technologies are implemented for the production of oysters. A comparison of new versus old technologies for oyster production is important to determine if the change was economically superior.

Remote setting remains to be an acceptable alternative to oyster production in Louisiana. With the implementation of remote setting in Louisiana, the oyster industry may be able to prosper.

Future research is important for the Louisiana oyster industry. Continued evaluation of new technologies is needed to compare to traditional seed bedding and ensure that the changes made were beneficial to the oyster industry.

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Appendix A

VIMS Gill Sample Procedure for RCM Analysis, *Crassostrea* spp

Gill Sampling Procedure for FCM Analysis, *Crassostrea* spp.¹

Preparation

1. Materials needed
 - Shucking knife
 - Surgical scissors and/or forceps
 - Small beaker of 70-95% ethanol
 - Alcohol lamp
 - 1.5ml Microcentrifuge tubes
 - Microcentrifuge rack
 - Dapi/dmsa solution
2. Sample Tube Staging
 - Line up the needed number of microcentrifuge tubes on a micro centrifuge rack (one tube per gill sample to be taken). We recommend using first and last rows of the rack only.
 - Label tubes if necessary.
 - Fill each tube with ~1.0 ml DAPI/DMSO.
 - Label the rack with sample information (e.g. site collected, species, date, tissue type, etc.).
 - Place the rack on ice and keep on ice throughout the sampling process.
3. Animal staging
 - Label top of tray with same code used to label the micro centrifuge rack.
 - Spray and brush mud, etc. off oysters with cold tap water.
 - Line up animals on tray. We recommend keeping the number of animals per row consistent; this helps if a mistake is made.

Sampling

1. Shuck the first animal.
 - Insert the shucking knife into the hinge joint and pop the upper shell.
 - Slide the knife along the right side of the top shell, cutting the adductor muscle.
 - If the hinge joint is too strong, insert knife directly into the right side of the oyster, prying as you go. As soon as knife slides in easily, cut adductor muscle.
2. Take gill snippet
 - Fold up the upper mantle piece to expose the gill folds.
 - Using forceps remove a SMALL (~2-3mm square) piece of gill tissue and place in the designated microcentrifuge tube.
 - Securely cap the tube and shake tissue to bottom of tube.
 - Before moving on to the next animal, sterilize the forceps. We recommend dipping the forceps in 95% ethanol and then flaming them. Allow the forceps to cool or dip in beaker of cold water before using again.
3. Preserving samples
 - Samples should be flowed immediately or frozen at -80°C to be flowed at a later date. *Note: Gill must be frozen in the DAPI/DMSO solution. To ensure this we recommend initially freezing the tubes in their rack. Once DAPI/DMSO solution has frozen, tubes may be placed in a bag.*

¹ Developed by the Aquaculture Genetics and Breeding Technology Center, Virginia Institute of Marine Science, Gloucester Pt., VA

Appendix B

VIMS Flow Cytometry Analysis Protocol Sample Preparation and Processing

Flow Cytometry Analysis Protocol¹ Sample Preparation & Processing

Pre – Preparation

1. Sample Tube Thawing:

Note: It is important to keep samples cold and or frozen until processed.

Work quickly and on ice. Store sets of tubes to be processed the same day in labeled racks at -20 C and rotate to fridge (to thaw) as you go.

Do not store samples long term at -20C.

Determine how many samples you will process in the time available.

We advise setting up ~ 4 sets of 32 samples for ½ day work (n=128).

- Remove samples from -80°C freezer and place onto labeled microcentrifuge tube rack in first and last rows only (32/rack).

2. Labeling:

Choose a labeling system. We recommend processing 32 samples at a time.

- Set up 2 rows of microcentrifuge sample tubes (as described above) on a rack and likewise 2 rows of flow tubes on their rack.
- Designate the same code for each set with a piece of tape in the bottom right hand corner (ex. A, B, C....)
- Determine sample direction and be consistent.

Recommendation:

Arrange flow cytometer tubes in **mirror image** of samples on the micro centrifuge rack.

3. Sample Tube Staging:

- Place first 2 racks of labeled sample tubes in fridge to thaw.
- Place the remaining sets of sample tubes at -20 C and rotate to fridge as you go.

4. Flow Tube Staging:

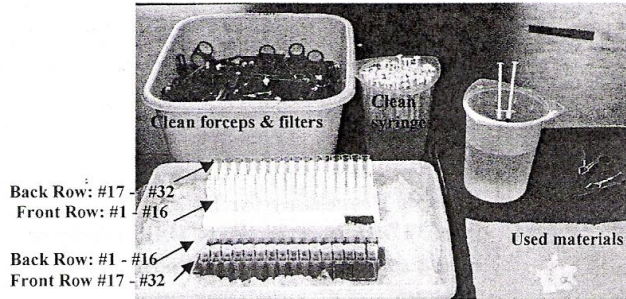
- Place the labeled flow tube racks in the -20 °C freezer until needed.
(Not mandatory - keeps samples colder while prepping)

5. Filter Preparation:

- Cut 23 micron Nytex into 15 X 15 mm squares.
- Fold fiber squares twice and clip to blue Nalgene Polypropylene Scissors
Type forceps on one side forming a secure funnel.

6. Syringe Staging:

- Clean syringes are kept in a beaker
- Set up a separate beaker of hot water for used syringes



¹ Developed by the Aquaculture Genetics and Breeding Technology Center, Virginia Institute of Marine Science, Gloucester Pt., VA

Sample Processing for FCM

Note: Work in order from 1-32. Never reuse syringes or filters. Be very careful of liquid transfer (splashing, spilling, bubbles...) that can cross contaminate your samples

1. Comfortable setup:
 - Remove first sample rack from fridge and corresponding flow tube rack from freezer
 - Arrange racks on ice with sample rack (label front right corner) in front and flow rack (label front right corner) behind
2. Mix and Separate Clumps (to avoid doublet readings)
 - *If samples have been pelleted previously or did not go through freezing and thawing, vortex for 15 seconds*
 - Place 1cc syringe needle in sample and aspirate at least 3 times pulling 0.4cc sample (or more) each time.
3. Filter out cell material that will clog machine:
 - Draw out ~0.5 cc DAPI/sample mixture
 - Place tip of 23 μ m Nytex cone inside the flow tube and slowly empty syringe into filter
4. Set aside used materials before moving on to the next sample:
 - Draw hot water from “used syringe beaker” into syringe and leave for cleaning more thoroughly later.
 - Dispose of DAPI soaked Nytex in trash.
 - Place forceps aside to rinse thoroughly with hot water later.
5. Repeat steps 1-4 on remaining samples.

What to look out for to prevent cross-contamination during sample processing:

1. *NEVER TOUCH the DAPI dispensing pipet to the sample tubes*
2. *Do not aspirate too quickly, this brings more air than sample into the syringe makes bubbles.*
3. *Release the sample into the nytex cone carefully and at a speed to prevent overflowing.*
4. *Be sure to rinse the syringes, forceps, and tube stands thoroughly with hot water and the flow tubes with hot water and soap.*
5. *When handling any of the tubes, be sure to place them back into the appropriate rack with care to prevent splashing of sample.*



Appendix C
Louisiana Oyster Industry Survey
Seed Bedding Practices and Cost



Introduction

In Louisiana, the oyster industry relies primarily on seed bedding as a means of producing market oysters. Remote setting is another way of producing seed oysters, where a farmer will purchase oyster larvae from a hatchery and set them on cultch in setting tanks and then bed the spatulated cultch to a lease for grow-out until it reaches market-size. Research is being conducted to compare the cost of seed bedding to the cost of remote setting, as well as the cost of planting cultch to catch wild spat. This survey will be used to understand the costs of seed bedding and planting cultch as part of this research.

All information provided will be held in the strictest of confidence and only used when summarized with all those who provide information. The identities of participants will not be disclosed.

This study has been approved by the LSU Institutional Review Board (IRB). For questions concerning participant rights, please contact the IRB chair, Dr. Robert C. Mathews, 578-8692, or irb@lsu.edu.

Please complete this survey to the best of your ability. If you have any questions please contact Victoria Ippolito via phone or e-mail. Please feel free to include any relevant additional information. Your help is greatly appreciated.

Survey

Instructions: Please circle the appropriate answer(s) or fill in the blank(s).

1. Do you bed oyster seed and/or plant cultch (shell, limestone, concrete exc.)? (if no, skip to question 34)? Yes No
- a. If yes, do you bed oyster seed annually? Yes No

Investment Cost Questions

2. How many vessels do you use to bed seed?
- a. 1
- b. 2
- c. 3
- d. 4
- e. Other (include number) _____.
3. What size is (are) your vessel(s) you use for bedding seed?
- | Vessel 1 | Vessel 2 | Vessel 3 | Vessel 4 |
|---------------------------|----------|----------|----------|
| a. Less than 30 feet long | a | a | a |
| b. 31-40 feet long | b | b | b |

- | | | | |
|----------------------|---|---|---|
| c. 41-50 feet long | c | c | c |
| d. 51-60 feet long | d | d | d |
| e. Over 60 feet long | e | e | e |

4. What is your vessel capacity (number of barrels planted per trip) (1barrel= 2sacks)?

- | Vessel 1 | Vessel 2 | Vessel 3 | Vessel 4 |
|------------------|----------|----------|----------|
| a. Less than 100 | a | a | a |
| b. 101-200 | b | b | b |
| c. 201-300 | c | c | c |
| d. 301-400 | d | d | d |
| e. 401-500 | e | e | e |
| f. 501-600 | f | f | f |
| g. Over 600 | g | g | g |

5. What year did you acquire your vessel (s)?

Vessel 1 _____ Vessel 2 _____ Vessel 3 _____ Vessel 4 _____

6. Did you purchase your vessel(s) or build it?

- | Vessel 1 | Vessel 2 | Vessel 3 | Vessel 4 |
|-------------|----------|----------|----------|
| a. Purchase | a. | a. | a. |
| b. Build | b. | b. | b. |

7. How many more years do you expect your vessel(s) to last?

- | Vessel 1 | Vessel 2 | Vessel 3 | Vessel 4 |
|------------------|----------|----------|----------|
| a. 1-5years | a | a | a |
| b. 6-10 years | b | b | b |
| c. 11-15 years | c | c | c |
| d. 16-20 years | d | d | d |
| e. Over 21 years | e | e | e |

8. How much did your vessel(s) cost to buy/build?

- | Vessel 1 | Vessel 2 | Vessel 3 | Vessel 4 |
|--------------------|----------|----------|----------|
| a. Under \$50,000 | a | a | a |
| b. Under \$100,000 | b | b | b |
| c. Under \$200,000 | c | c | c |
| d. Under \$300,000 | d | d | d |
| e. Over \$300,000 | e | e | e |

9. How many oyster dredges are used to harvest seed oysters? _____

- a. What year did you purchase them? _____
- b. How much did each cost? \$ _____

10. How often do you purchase dredges?

- a. Each year
- b. Every two years
- c. Every three years
- d. Every four years
- e. Every five years
- f. Over five years

11. How much do you spend on dredge repairs annually? \$ _____

Production Cost Questions

12. What are the costs associated with the following **per boat load**?

- a. Labor \$ _____
- b. Groceries and galley supplies \$ _____
- c. Vessel fuel \$ _____
- d. Oil and grease \$ _____
- e. Ice \$ _____
- f. Propane (for cooking) \$ _____

13. What are the costs of vessel maintenance annually? \$ _____

14. What are the costs of equipment maintenance annually? \$ _____

15. What is the cost of engine maintenance annually? \$ _____

16. What are the costs of poles to mark lease boundaries annually? \$ _____

Fixed Cost Questions

17. What are your annual lease costs (e.g. rent, surveys, etc.)? \$ _____

18. What are your annual vessel, harvester and gear licenses costs? \$ _____

19. What is your annual insurance cost? \$ _____

20. Do you have crop insurance? Yes No

a. If yes, how what is the annual cost? \$ _____

21. Do you rent a dock or slip for you vessel(s)? Yes No

a. If yes, how much is it annually?

Vessel 1 \$ _____ Vessel 2 \$ _____ Vessel 3 \$ _____ Vessel 4 \$ _____

Other Questions

22. Do you plant seed from public leases, private leases or both?

- a. Public
- b. Private
- c. Both

i. If both what approximate percentage from each (eg. 60% public, 40% private)?

_____ % Public _____ % Private

23. What months do you plant seed (circle all that apply)?

- a. January
- b. February
- c. March
- d. April
- e. May
- f. June
- g. July
- h. August
- i. September
- j. October
- k. November
- l. December

24. How many bedding trips do you make per year? _____

- a. To how many different leases? _____
- b. What is the greatest distance traveled to bed seed? (from harvest area) _____
- c. What is the least distance traveled to bed seed? (from harvest area) _____

25. When bedding, what are the approximate percentages of seed and cultch?

_____ %seed _____ % cultch

- a. How do you make these estimates (circle one)?
 - i. Count number of oysters by volume (bucket or sack measure)
 - ii. Other _____
 - iii. guess
- b. Do you make these estimates during each trip? Yes No

26. What is the **AVERAGE** return from bedding a load of seed?

- a. Less than 1 sack harvested per sack bedded.
- b. 1 sack harvested per sack bedded
- c. 1 to 1½ sacks harvested per sack bedded
- d. 1½ to 2 sacks harvested per sack bedded
- e. 2 to 2½ sacks harvested per sack bedded
- f. 2½ to 3 sacks harvested per sack bedded
- g. 3 to 3½ sacks harvested per sack bedded
- h. 3½ to 4 sacks harvested per sack bedded
- i. 4 to 4½ sacks harvested per sack bedded
- j. 4½ to 5 sacks harvested per sack bedded
- k. Over 5 sacks harvested per sack bedded

27. Do you plant cultch?(if no, skip to question 33)

Yes No

28. Do you plant cultch yourself or hire a contractor to plant cultch?
- Yourself
 - Contractor
29. How many cubic yards of cultch do you plant annually?
- 0-1000
 - 1001-2000
 - 2001-3000
 - 3001-4000
 - 4001-5000
 - More than 5000
30. How much does cultch planting cost per cubic yard (1.35 tons of #57 limestone = 1 cubic yard)? \$ _____
31. What type(s) of cultch do you plant? (circle all that apply)
- Oyster shell
 - Limestone
 - Crushed concrete
 - Gravel
 - Other _____
32. What is the **AVERAGE** return on investment from planting cultch?
- Less than 1 sack harvested per cubic yard
 - 1 sack harvested per cubic yard
 - 1 to 1½ sacks harvested per cubic yard
 - 1½ to 2 sacks harvested per cubic yard
 - 2 to 2½ sacks harvested per cubic yard
 - 2½ to 3 sacks harvested per cubic yard
 - 3 to 3½ sacks harvested per cubic yard
 - 3½ to 4 sacks harvested per cubic yard
 - 4 to 4½ sacks harvested per cubic yard
 - 4½ to 5 sacks harvested per cubic yard
 - Over 5 sacks harvested per cubic yard

Buying seed

33. If you could drive your boat to a local dock (e.g., Hopedale, Empire, or Bayou Dularge, LA) and load it fully (taking about two hours) with spat-on-shell, which would result in about 3 market-sized oysters per shell upon harvest, eliminating the need for an initial harvest and its costs, how much would that be worth to you per boat load?
- \$ _____

34. Would you like to receive a copy of the overall report when completed? Yes No
a. If yes, please contact Vicki Ippolito or John Supan via e-mail or telephone.

If you have any questions about the survey

Please contact:

Vicki Ippolito
Graduate Research Assistant
Louisiana Sea Grant College Program
Vippol1@lsu.edu
732-513-0549

John Supan, Ph.D.
Associate Research Professor
Louisiana Sea Grant College Program
jsupan@lsu.edu
985-264-3239

Thank you for completing this survey. Your information is greatly appreciated!

Appendix D
Survey Cover Letter for Initial Mailing



November 16, 2010

NOTICE TO ALL MEMBERS

The Louisiana Oyster Dealers and Growers Association is proud to sponsor the enclosed survey from the Louisiana Sea Grant College Program. This anonymous survey is part of important research being conducted to document our current oyster farming methods, which will help lead us to the future. I encourage all of you to take a moment to fill out the survey and return it with the enclosed stamped envelope by October 21, 2010.

Gratefully,

John Tesvich
President

Appendix E
Survey Cover Letter for Second Mailing



November 16, 2010

NOTICE TO ALL MEMBERS

The Louisiana Oyster Dealers and Growers Association would like to thank you for completing the Louisiana Sea Grant College Program survey. This anonymous survey is part of important research being conducted to document our current oyster farming methods, which will help lead us to the future. If you have yet to complete it, enclosed is another copy. Please take a moment to fill out the survey and return it in the enclosed stamped envelope by October 21, 2010.

Gratefully,

John Tesvich
President

Appendix F
Microsoft Excel Spreadsheet for Estimating Seed Bedding Costs

	A	B
1	Date:	
2		Production costs/day
3	Labor	
4	Groceries, galley supplies	
5	vessel fuel	
6	oil and grease	
7	propane and ice	
8	Total	=SUM(B3:B7)
9	bedding trips /day	
10	Production cost/ trip	=B8/B9
11		
12		Annual Costs
13	dredge purchases	
14	dredge repairs	
15	vessel maintenance	
16	equipment maintenance	
17	engine maintenance	
18	poles to mark lease boundaries	
19	lease costs	
20	licenses	
21	insurance (i.e. vessel)	
22	crop insurance	
23	vessel dockage	
24	Total	=SUM(B13:B23)
25	Bedding trips per year	
26	Annual cost/ bedding trips	=B24/B25
27		
28	Total costs/trip	=SUM(B26,B10)
29	Vessel capacity (barrels)	
30	Cost /barrel	=B28/B29

Vita

Victoria Ippolito was born in April 1986 and grew up in Manasquan, New Jersey. In June 2004, she graduated from Manasquan High School and attended Southampton College of Long Island University, where she majored in marine biology. While at Southampton College Victoria traveled to The Republic of Vanuatu for an intersession class entitled Tropical Marine Biology. There she conducted research on the giant clam entitled “Diversity and Depth Distribution of Mollusca: Pelecypoda: Tridacnidae.”

In 2005, Victoria transferred to Roger Williams University in Rhode Island due to the closure of Southampton College. While at Roger Williams University, Victoria joined the swim team and conducted research with her advisor, Dr. Dale Leavitt, entitled “A Complete Energy Budget for the Giant Clam (*Tridacna crocea*).” During this time she traveled to Belize for an intersession class and also became rescue SCUBA certified. Victoria graduated sigma cum laude from Roger Williams University, with a Bachelor of Science degree.

Upon graduation from Roger Williams University Victoria 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 a major in fisheries science and a minor in environmental science.