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Examining the Sustainability of Restored Sub-Tidal Oyster Reefs in Coastal Louisiana

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EXAMINING THE SUSTAINABILITY OF RESTORED SUB-TIDAL OYSTER REEFS IN COASTAL LOUISIANA

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Abstract

Climate related alterations and anthropogenic disturbance threaten the ecological integrity and sustainability of coastal estuaries. Many activities seek to restore and sustain these at-risk areas with the goal of restoring systems to historic patterns of succession and community development; however long-term monitoring of restoration projects remains limited. Additionally, restoration efforts aim to achieve certain success thresholds, however, these thresholds are often vague, absent, or inconsistent, and receive little long-term analyses following restoration. A key coastal engineer, the eastern oyster (*Crassostrea virginica*), provides multiple ecosystem services, but recent population decline has prompted investment in restoration. Restoration activities include cultch planting, reef enhancement, incorporation into living shorelines, and hatchery construction for oyster seed. This work examines restoration trajectories and project sustainability for two oyster restoration projects in coastal Louisiana. Here I quantify on-reef oyster density and demography, adjacent shoreline movement, and water filtration services for a living shoreline project in Sister Lake Louisiana 11 years post-construction (2009 – 2020), as well as examine the outcomes of oyster restoration projects implemented across Louisiana as part of the early restoration funding from the Deepwater Horizon Oil Spill. Restored reefs persisted through time but decreased in integrity and robustness 2-3 years post-restoration. Declines were likely influenced by sub-optimal water quality (i.e., salinity) and heavy localized harvest. Despite these declines, reefs still provided crucial ecosystem services (i.e., water filtration) to estuarine habitats. While success thresholds vary between restoration projects, I suggest restoration success thresholds may incorporate three different performance criteria (achievement of target densities; provision of ecosystem services; achievement of harvest quotas). For these criteria to be met and detected, incorporation of long-term monitoring and adaptative management are crucial. This work provides critical information for managers on the outcomes of oyster restoration projects and explains specific variables which influence project sustainability.

Chapter 1. Introduction

Coastal impacts from climate change, sea-level rise, shallow and deep subsidence, natural disasters, disconnection from alluvial sediments, and anthropogenic activities have all altered Louisiana coastal marshes and estuaries. Between 2004 -2008, the Louisiana coast lost more than 770 km^2 of land to hurricanes Katrina, Rita, Gustave, and Ike (CPRA 2017). Shortly afterwards, the Deepwater Horizon Oil Spill (2010) resulted in the largest accidental oil spill in U.S. history resulting in damage to as much as 1770 km of linear Gulf coastal marsh (NAS 2017). Along the Louisiana coast several large-scale restoration plans including the Louisiana Coastal Master Plan with funds from the RESTORE ACT and Natural Resource Damage Assessment, have been proposed to focus explicitly on coastal habitat and gulf fisheries restoration. Understanding how these efforts influence the ecological integrity of coastal systems, including Louisiana estuaries, is critical to ensure sound investments of restoration funds.

Ecological restoration seeks to return natural systems to historic patters of structure and function; however, it is still unclear whether restoration activities can successfully reverse degradation (Lindig-Cisneros et al. 2003). For this to occur, restoration efforts must aim to create self-supporting ecosystems that remain resilient to perturbation without further enhancement (Urbanska et al. 1997; Ruiz-Jaen and Aide 2005). This process is often time intensive and the procession in which ecosystem services or historic patterns of structure and function become noticeable differs. Additionally, restoration projects vary widely in their determination of success or completion. The Society of Ecological Restoration International (SER 2004) recommends nine attributes every restored ecosystem should display for restoration to be noted as successful: (1) similar diversity and community structure in comparison with reference sites; (2) presence of indigenous species; (3) presence of functional groups necessary for long-term stability; (4) capacity of the physical environment to sustain reproducing populations; (5) normal functioning of biotic and abiotic processes; (6) integration with the landscape; (7) elimination of potential threats; (8) resilience to natural disturbances; and (9) self-sustainability (SER 2004; Ruiz-Jaen and Aide 2005). Knowledge of how these attributes fare in the long-term (> 10 years) post-restoration is critical to inform management as well as provide a better understanding on restoration trajectories.

Monitoring of restoration effectiveness provides information to support adaptative management and helps to inform future management decisions. Unfortunately, long-term monitoring is rare for many restoration projects, especially those located in temporally dynamic environments such as coastal estuaries. Adaptive management serves as a solution, in which, targeted monitoring efforts aid in the identification of high priority uncertainties, thereby improving restoration actions over the long-term (NAS 2017). The flexible process allows for the adjustment of planning, construction, and operation throughout the lifetime of a restoration project, ensuring maximum benefit from funds and management decisions (NAS 2017). For coastal systems, projects including living shoreline creation or oyster reef restoration through cultch plantings provide excellent opportunities to examine long-term success and restoration trajectories.

The Louisiana Coastal Master Plan (CPRA 2017) outlines several coastal restoration activities where long-term monitoring could be incorporated, ultimately providing additional information to project managers on future outcomes, site selection, and restoration trajectories common for the selected area. Living shorelines serve as one example, usually taking the form of a constructed oyster reef, that provide protection to adjacent shorelines from erosive wave energy while also maintaining critical habitat for nekton and other estuarine species (Bilkovic et al. 2017). Although once implemented, living shorelines receive little long-term monitoring. While short-term (i.e., < 3years) provision of services such as reduced erosion in high wave exposure settings and increased habitat heterogeneity (Coen et al 2007; La Peyre et al. 2014) have been observed following living shoreline construction, little is known about long-term ecosystem services or environmental alterations associated with living shorelines. Sustainability of living shorelines is dependent on the continued recruitment and survival of the eastern oyster (*Crassostrea virginica*), which inhabit and build the reef.

In Louisiana, eastern oysters are most abundant along the southeastern and south-central portions of the coast from Breton and Chandeleur Sound to the Atchafalaya and Vermilion bays (Nelson et al. 1992; Hijuelos et al. 2016). Oysters are considered ecosystem engineers (Coen and Humphries 2017) as they create their own habitat while also providing other ecosystem level services, including enhanced water quality, essential fish habitat (EFH), and shoreline protection (GMFMC 1998; VanderKooy 2012; La Peyre et al. 2014). Apart from the ecological value oysters contribute to Louisiana estuaries, eastern oyster landings make up most of the volume and real dockside value for all oysters harvested in the Gulf (Banks et al. 2016). The fishery is the fifth largest (by volume) and fourth most valuable fishery in Louisiana, with landings totaling 12 million pounds and $64 million in dockside value during 2014 (Banks et al. 2016). Despite these services, more than 85% of oyster reef habitat has been lost worldwide, as estimated from current and historic oyster abundances (Beck et al. 2011). Although not as heavily impacted as the Atlantic coast, the Gulf coast is experiencing historic declines due to freshwater influxes, heavy periods of harvest, and climate related changes (Coen et al. 2007; LDWF 2015). Oyster reefs influence water flow, consolidate and stabilize sediments, and provide high economic value to coastal communities, and are being restored and incorporated into large-scale restoration projects along the eroding Louisiana coast (DWH NRDA 2012; CPRA 2017).

Oyster reef restoration through localized cultch plantings serves as one of the main approaches used to restore or enhance oyster populations across the Gulf. Goals for these projects include creating suitable substrate for larvae attachment and ensuring subsequent growth into self-sustaining reefs (DWH NRDA 2012). However, ecological restoration proves difficult for organisms such as the eastern oyster, as they are sessile and support a valuable fishery, making them vulnerable to intense harvest and dependent on overlying water quality, including salinity and temperature, for continued survival, reproduction and growth (Kennedy et al. 1996). Oyster reef restoration efforts in Louisiana usually involve the placement of cultch material (limestone or concrete) on to historic oyster grounds near or included in areas available for public harvest. These cultch sites are then generally monitored 2-3 years post-construction, or until project success thresholds are met. In public harvest areas in Louisiana, success criteria of a density of 20 seed size oysters (25-75 mm shell height) per meter squared is usually used; for cultch sites built for restoration and not harvest, a total oyster density of 25 ind. m^{-2} is often used, when defined (Baggett et al. 2015). However, restoration projects vary greatly in the determination of success thresholds, making large-scale assessments of restoration performance difficult, especially for dynamic systems such as coastal estuaries or subtidal reefs. In some cases, economics are used to determine success. For example, Louisiana Department of Wildlife and Fisheries calculated a benefit-cost ratio for all cultch planting projects to be as high as 20 to 1 (Banks et al. 2016). One cultch plant in St. Bernard Parish, Louisiana cost $1.4 million to construct in 2011 and produced $14 million in oyster landings during a 5-day harvest in 2015 (Banks et al. 2016). While this investment proved economically worthwhile in the short-term, data on the longevity and performance criteria of these created reefs are lacking, and the consequences to overall reef resources remains unclear.

This work explores the sustainability and restoration trajectories of two oyster restoration projects in coastal Louisiana. Here I address questions exploring how restoration projects define success, what influences success (i.e., salinity and harvest), and what is needed to ensure long-term success? Chapter 2 examines 6 oyster reefs in Sister Lake, Louisiana, constructed as living shorelines in 2009, that differ in wave exposure intensity. These reefs were monitored in 2009 – 2011 (La Peyre et al. 2014) providing data on short-term post-construction oyster density, population demographics, adjacent shoreline movement, and water filtration services. I revisit these sites in 2019 and 2020, to collect the same data, comparing reef structure and services, 10- and 11-years post-construction (2009 -2020). In Chapter 3, I examine the outcomes of the oyster restoration projects in Louisiana implemented as part of the early restoration funding from the Deepwater Horizon Oil Spill (DWH NRDA 2015). Specifically, the chapter examines total oyster density and population demographics from 2013 through 2019 for 6 sites restored with this funding. These sites were established to restore oyster populations impacted by the Deepwater Horizon oil spill, and to support future oyster production. I examine project outcomes over time, examining how salinity and possible harvest openings impacted long-term reef sustainability.

Chapter 2. Examining a created living shoreline 11 years post-construction: oyster reef sustainability and provision of ecosystem services

2.1. Abstract

Coastal degradation due to climate change and anthropogenic disturbance threatens the sustainability of coastal estuaries worldwide. Many activities seek to restore and sustain these at-risk areas; however, long-term monitoring of restoration projects remains limited. A key coastal engineer, the eastern oyster *(Crassostrea virginica)* provides multiple ecosystem services, but recent population decline has prompted investment in restoration. Here, I document trends in oyster density and oyster size class distribution, and calculate their contribution to water filtration, and adjacent shoreline protection over an 11-year period after construction of the reefs. Created experimental reefs were sampled years 1-3 post-construction and revisited in years 10 and 11 post-construction. Overall, reefs persisted over the 11 year timeframe with oysters of all sizes present at all sites in years 10 and 11. Total oyster density in years 10-11 was less than 5% of the mean density reported for years 1-3 post-restoration (yr. 1: ~2500 ind. m^{-2}, yr. 11: ~100 ind. m^{-2}), however, market oyster density (shell height $\geq$ 75mm) was similar in years 10 and 11 to year 3 post-restoration (~10-40 ind. m^{-2}). Lack of smaller oysters in years 10 and 11 likely reflected the impact of multiple extended low salinity events after 2012, including the record-breaking low salinity in 2019, which may limit recruitment, and increase mortality of smaller oysters. Reefs provided potential filtration capacity ranging from ~300 L hr^{-1} m^{-2} in years 10 and 11, compared to ~5000 L hr^{-1} m^{-2} in years 1-3. Shoreline erosion at both reef and control sites immediately post-construction, and 10 years later, remained high (~1m y^{-1}) indicating no shoreline protection impacts. This study demonstrated a potential for restored reef sustainability; despite decreased oyster density 10 years post-construction, reefs sustained reproductive sized oysters, and provided water quality filtration services.

2.2. Introduction

Climate change and anthropogenic activities impact coastal habitats, including marshes, mangroves, and shellfish reefs. Numerous studies have documented declines in biodiversity, water quality, flood abatement, and carbon sequestration as a result of coastal degradation and subsequent population declines for estuarine species (Coen and Humphries 2017; Van Zomeren et al. 2019). Along the Gulf coast of the United States, the vast majority of Louisiana's ~12,000 km^2 wetlands are eroding at a rate of about 75 km^2 annually due to combined effects of shallow and deep subsidence, sediment depletion, sea level rise, erosive energies, and anthropogenic interference (CPRA 2017). Similarly, oyster resources in this region have declined by over 50% and were recently identified as moderately to highly vulnerable to future environmental changes (Beck et al. 2011; Reece et al. 2018). In response, significant resources have been committed to restore coastal ecosystems, including oyster reefs, and the ecological services they provide. However, few studies have documented the long-term success of restoration in terms of ecological recovery (McGranahan et al. 2007; Arkema et al. 2013; Bukvic et al. 2020).

Coastal restoration projects seek to restore ecosystem structure and function, with the goal of incorporating natural processes to reduce erosion, while building sustainable habitat for estuarine and coastal organisms. One approach, living shorelines, promotes the inclusion of ecosystems such as marshes, mangroves, or inter- or sub-tidal oyster reefs into coastal protection strategies (Bilkovic et al. 2017). Living shorelines provide shoreline protection, improved connectivity between intertidal environments, and enhancement of other ecosystem services, while also serving as an alternative to artificial shoreline armoring such as bulkheads and sills (Coen et al. 2007; Currin et al. 2010). Living shorelines have been implemented across the east and Gulf coasts of the United States as techniques to increase biodiversity and enhance erosion control for areas of relatively low – wave and wind energy (Bilkovic et al. 2017; Morris et al. 2019). Despite these applications, uncertainty remains concerning the efficacy and sustainability of living shorelines as their success is dependent on both environmental conditions and timing of construction (Morris et al. 2019).

Living shorelines along the U.S. Gulf coast often take the form of an artificial or restored intertidal oyster reef, dependent on the recruitment and growth of the native eastern oyster *(Crassostrea virginica)*. Oysters and the reefs they build provide both valuable economic and ecological services including water filtration, nekton habitat, shoreline stabilization, and commercial harvest (Eastern Oyster Biological Review 2007; Grabowski et al. 2012). In coastal Louisiana, oyster-based living shorelines have been built since the early 2000s, are listed in Louisiana's Coastal Master Plan (CPRA 2017) and have been identified as a key approach for oyster restoration in regional planning documents (DWH NRDA 2015). Assessments of their effectiveness, however, remain limited and indicate mixed success, with outcomes dependent on favorable water conditions for oyster growth and survival (i.e., salinity and temperature), or relative exposure to wave energy (La Peyre et al. 2014, 2015, Soniat et al. 2013). For example, La Peyre et al. (2015) demonstrated that living shorelines composed of oysters reduced shoreline erosion most effectively in locations experiencing higher wave energy as compared to those located in low energy environments. Most studies, however, have only examined short-term impacts of living shorelines on adjacent shorelines, and fail to consider longevity of the created living shoreline, or how development over time may impact future shoreline protection services (see La Peyre et al. 2017).

Oyster reef-based living shorelines ultimately remain dependent on the recruitment, growth and survival of *C. virginica*. Oysters are highly vulnerable to both changing biotic and abiotic factors as they are sessile for most of their life. Temperature and salinity serve as the two main abiotic drivers for reef persistence (Coen and Humphries 2017) with salinity affecting oyster growth, mortality, reproduction, predation, and disease tolerance (Shumway 1996; La Peyre et al. 2015). Oysters can tolerate wide salinity ranges for short periods of time however, habitat suitability is greatest with an annual mean range of 10-15 (Soniat et al. 2013). Oysters are also sensitive to water movement, with cessation of feeding activity under high currents, or energy exposure (Casas et al. 2015; La Peyre et al. 2015). Therefore, the persistence and sustainability of natural and created oyster reefs are highly dependent on environmental conditions, including salinity, temperature, and tidal or wave energies (Meyer et al. 1997; La Peyre et al. 2014, 2015; Coen and Humphries 2017).

Within estuaries, environmental conditions vary significantly throughout and across years, often complicating assessments of living shoreline success, and specifically, reef sustainability. Further, with estuarine conditions changing over the long-term from coastal restoration (i.e., shoreline stabilization, ridge restoration, marsh creation, sediment diversion, etc.), and from climate change, tracking restored project outcomes over extended time frames is increasingly important. Sea surface temperatures in the northern Gulf of Mexico have risen by 4°C in the last 30 years (Fodrie et al. 2010), which could affect bivalve physiological responses and reef sustainability (Casas et al. 2018b). For restoration projects to remain successful and effective, management will need to document and account for these environmental changes over longer periods of time.

Here I quantify on-reef oyster density and demography, adjacent shoreline movement, and water filtration services on 6 oyster reefs in Sister Lake, Louisiana, in 2019 and 2020. These reefs were constructed as living shorelines in 2009 and differ in wave exposure settings. Initial assessment of the reefs (La Peyre et al. 2014) found reef sustainability and water quality increased linearly over time (3 years), however, for continued persistence, suitable substrate for oyster recruitment, and adequate salinity and temperature must be maintained. With data collected in the 3 years immediately following reef creation, along with data from re-visitation in 2019 and 2020, I examine changes in oyster density, population demography, potential water filtration capacity, and the impact of these reefs on adjacent shoreline and marsh characteristics. I hypothesize that reef density and potential filtration will vary over time due to fluctuations in water quality, and reefed shorelines will have higher shoreline productivity and lower erosion compared to shorelines not bordered by an oyster reef.

2.3. Materials and Methods

2.3.1. Study Site

Sister Lake (also referred to as Caillou Lake), located in Terrebonne Parish, Louisiana (29º 14' 11.09N, 90º 55' 15.48W) is a primarily open-water, brackish system, with water level influenced predominantly by southeastern winds and a mean tidal range of 0.3 ± 0.03 m (La Peyre et al. 2014). Annual mean (± SE) salinity, temperature, and gauge height in Sister Lake from 2009 – 2020 was 10.5 ± 0.3, 23.0 ± 0.4 °C, and 1.3 ± 0.01 m respectively (LDWF/USGS 07381349- Caillou Lake southwest of Dulac, LA, U.S.A.). Annual mean salinity and temperature ranged from 7-14 and 22-24°C respectively during 2009- 2020. These conditions are favorable habitat for *Crassostrea virginica*, with Sister Lake historically supporting one of Louisiana’s most productive public oyster grounds (LDWF 2016a). Sister Lake has benefitted from recent restoration funding, with 358 acres of cultch planted (DWH NDRA 2012) and has been identified within state planning documents (CPRA 2017) as a suitable location for the creation of fringing oyster reefs to reduce shoreline erosion. Vegetative assemblages along Sister Lake shorelines are predominately composed of *Spartina alterniflora* and *Juncus roemerianus* with *Spartina patens*, *Batis maritima*, and *Distichlis spicata* interspersed throughout. Typical for Louisiana brackish marsh environments, soils are composed of 13 -22 % organic matter with bulk density ranging between 0.3 - 0.4 g cm^{-3} (Coastwide Reference Monitoring System (CRMS); CRMS0383, CRMS4455).

2.3.2. Study Design

In March 2009, six experimental intertidal oyster reefs (25 m x 1 m x 0.7 m, length x width x height) were constructed parallel to, and 5-10 m away from the adjacent shoreline using clean, dry oyster shell. Three reefs were located along shorelines identified as having a lower exposure from dominant wave energies, based on their orientation with respect to dominant winds, and fetch; three were located along shorelines identified as having a higher exposure from dominant wave energies (see La Peyre et al. 2014) *(Figure 1)*. For each experimental reef and adjacent shoreline, a reference bottom and shoreline were established 50 m away. Monitoring of oyster reef sustainability, shoreline vegetation, soils, and shoreline movement, was conducted in late summer (August/September) in 2009, 2010, 2011; La Peyre et al. 2014). In 2019 and 2020, all sites were revisited, replicating the late summer sampling events, as described below, to quantify reef sustainability and filtration capacity, shoreline vegetation and soil characteristics, and shoreline movement 10- and 11-years post-reef creation.

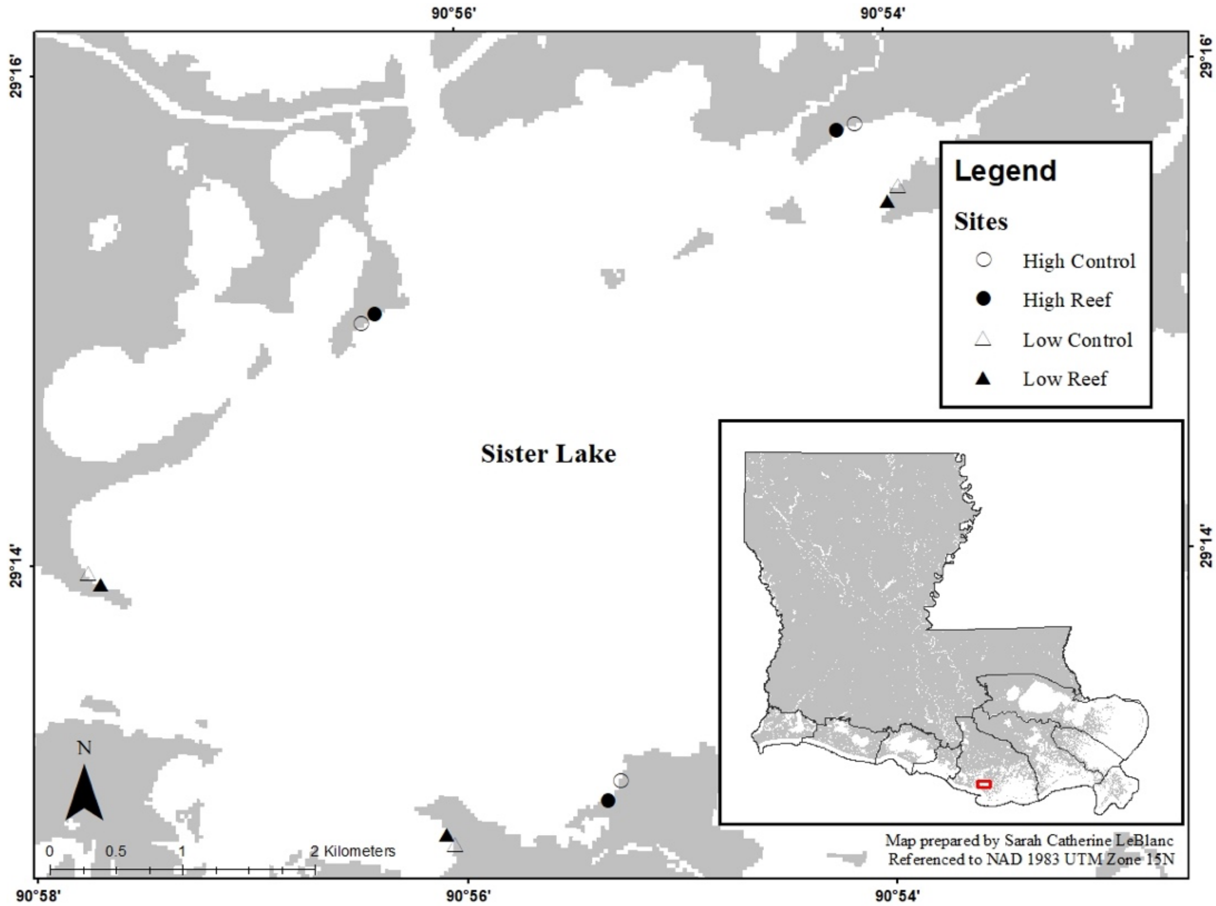


Figure 2.1. Location of study sites within Sister Lake, Louisiana. In 2009, six experimental reefs were created with paired reference bottom and shoreline along the northern, southern and western edges of Sister Lake. Reefs are located in areas with low [stars; Low Control (open symbols) and Low Reef (filled symbols)] and high [circles; High Control (open symbols) and High Reef (filled symbols)] exposure based on fetch and dominant winds.

2.3.3. Reef sustainability and filtration

Three 0.25 x 0.25 m (0.0625 m^2) quadrat samples were taken at each reef and control water-bottom site (12 sites x 3 quadrats x 5 sampling periods = 180 samples), with a weighted quadrat and buoy attached haphazardly thrown from the boat over the sample area. Reef material was removed by hand, from the taphonomically active zone (depth of 10 cm), placed in a mesh bag, stored on ice, and returned to the lab at Louisiana State University (LSU) for processing. In the lab, total volume (L) was estimated by water displacement, and reef material was separated into categories of live oysters, dead oysters, and shell hash within 72 hours of collection. Density and shell height (SH $\pm$ 0.1 mm) of live oysters was measured. Data were used to quantify total reef volume (L m^{-2}) (2019 and 2020 only), total live oyster density (ind. m^{-2}), and live oyster density by size class (spat < 25 mm, seed 25 $\leq$ SH < 75 mm, market $\geq$ 75 mm).

Reef oyster density by oyster size class was used to estimate potential filtration services provided by each reef over time. Shell heights (SH $\pm$ 0.1 mm) were converted to dry tissue weight (DW; g) with the regression equation derived from Sister Lake oysters (La Peyre et al. 2014).

$$DW = 0.0004*(SH)^{1.9217} \qquad \textit{(Eqn. 1)}$$

Potential filtration rate was estimated with the temperature corrected equation provided by Cerco and Noel (2005), originally based on Riisgard (1988), that calculates filtration rate based on regressions of filtration rate (L h^{-1}) on temperature:

$$\text{DW: Filtration rate (L } h^{-1}) = 6.79DW^{0.73}e^{(-0.15(\text{temperature}-27))2} \qquad \textit{(Eqn. 2)}$$

The mean 11-year temperature (23.0° ± 0.1°C) for Sister Lake was used to make the adjustment. Bivalve filtration is also affected by salinity, with lower rates occurring below 7.5 (Newell and Langdon 1996; Dame 2012). The mean salinity in Sister Lake over the 11-year period (10.5 $\pm$ 0.1) was above 7.5, and thus the equation was applied without adjusting for salinity. Daily fluctuations for both variables can result in either higher or lower rates of filtration, and thus, the rates presented provide only a comparison between years, assuming a temperature of 23.0°C, and salinity above 7.5.

2.3.4. Shoreline characteristics

Vegetation and soil characteristics on shorelines adjacent to the reefs were quantified in triplicate 1 m^2 plots located haphazardly within 5 m of the marsh edge at each site (12 sites x 3 replicates x 5 years = 180 samples). Vegetation and soil characteristics determined for all five years include species-specific percent cover (%), total above ground vegetation (g m^{-2}), soil bulk density (g m^{-2}), and soil percent organic matter (%), following protocols listed below (2.4.1, 2.4.2).

In 2019, total (live and dead) belowground vegetation (gdw m^{-2}), soil extractable nutrients (NO_x, NH_4^+, PO_4^{3-}), and soil shear strength were quantified to examine long-term effects of adjacent reefs on these vegetation and soil properties. During both sampling events of 2019 and 2020, soil shear strength (kpa) was quantified with a shear vane (Geotechnics Geovane #2285) in each of the 1 m^2 plots within (15 cm) the root zone, however only 2020 data is reported due to instrument failure in 2019 (Lin et al. 2016).

2.3.4.1. Vegetation

Species-specific percent cover was estimated by dividing the 1 m^2 plot into four equal sections and recording the species-specific percent cover in each section. The mean species-specific percent cover of all four sections was then used for analyses. Stem height (cm) of dominant vegetation (either *S. alterniflora* or *J. roemerianus*) was measured prior to destructive sampling. One stem in each of the four corners and one in the middle of the 1 m^2 plot was measured from the sediment surface to the tallest point on the stem. One haphazardly placed 0.25 m x 0.25 m quadrat was then placed inside of the 1 m^2 plot and destructively sampled for live and dead aboveground biomass (g m^{-2}). All stems within the 0.0625 m^2 quadrat were cut at the marsh surface, placed in a labelled bag and returned to LSU for processing. In the lab, samples were identified at the species level, and then live and dead stems were separated based on the presence of photosynthetic tissue and dried to a constant weight at 60°C. Dry weight (g) of live and dead material for each species was recorded.

In 2019 only, total belowground biomass (g m^{-2}) was measured in the destructively sampled quadrat. Belowground biomass was collected by taking one auger core (6.35 cm dia. x 30 cm depth), placing it in a labelled bag on ice, and processing in the laboratory. In the laboratory, the 30 cm core was rinsed free of sediment, sorted into live and dead biomass following procedures detailed in Hill and Roberts (2017), and then dried to a constant weight at 80ºC.

2.3.4.2. Soil Properties

Within the 0.0625 m^2 quadrat, one sediment core (6.7 cm dia. x 15 cm depth) was collected for measurement of bulk density (g m^{-3}) and organic matter (%) . Cores were spilt into three 5 cm increments (0-5 cm, 5-10 cm, and 10-15 cm), placed in plastic labelled bags and returned to the lab at LSU for processing. In the lab, homogenized subsamples (~10 g) were weighed wet, dried to a constant mass at 60ºC, and reweighed to determine moisture content (%) (2019-2020 only). Samples were then placed in a muffle furnace at 400°C for 4 hours to determine organic content by mass loss on ignition (Marton and Roberts 2014). Bulk density was calculated as the dry mass of the core divided by core volume (g m^{-3}).

In 2019 only, a separate surficial sediment (0-5 cm) core (6.7 cm diameter) was taken to quantify extractable nutrients (NO_x, NH_4^+, PO_4^{3-}). Approximately 2 grams of soil were added to each of two 50-mL centrifuge tubes, one tube for extractable dissolved inorganic nitrogen (DIN), and the other for dissolved inorganic phosphorus (DIP) (Schutte et al. 2020). A total of 30 mL of 2 N KCL was added to the DIN tube and shaken at 250 rpm for 2 hrs. The DIN tube was then centrifuged at 3000 rpm for 10min, filtered (0.2 μm), and stored frozen until analysis. Similarly, 30 mL of 0.5 M $NaHCO_3$ was added to the DIP tube and shaken for 16hrs at 250 rpm centrifuged at 3000 rpm for 10min, filtered (0.2 μm), and stored frozen until analysis. NO_x was analyzed using Cu-Cd reduction followed by azo colorimetry with a Lachet Instruments QuickChem ® FIA + 8000 series automated Ion analyzer with an ASX-400 series XYZ Autosampler (APHA 1992; Schutte et al. 2020). Samples were analyzed for dissolved NH_4^+(with phenate cloroimetry) and PO_4^3 (with ascorbic acid reduction method) on a Shimadzu UV-1800 Spectrophotometer (APHA 1992). Standard curves were prepared by diluting NO_3^-, NH_4^+, and PO_4^3 stock solutions (Hach, Loveland CO) and yielded r^2 values of > 0.99 for each analyte.

2.3.5. Shoreline stability

Shoreline movement from 2005 – 2019 was analyzed using aerial imagery from Google Earth Pro (7.3.3.7699). Each experimental shoreline was delineated with the Google Earth polyline tool at highest practical resolution and imported into ArcGIS (10.8.1). Shoreline location was determined by the presence of vegetation. To be consistent between years, analyses were conducted with winter images (November– January). The Digital Shoreline Analysis System (DSAS) was used to quantify net shoreline change (m yr^{-1}), for “pre-reef” (2005-2010), “early reef” (2010-2014), and “late reef” (2014-2019) periods.

2.3.6. Statistical analyses

Data were analyzed with two-way ANOVAs (R 3.6.3; R Foundation for Statistical Computing, 2020). Data met the basic assumptions for the model. The ANOVA test is appropriate for this dataset, as multiple means are being compared. An alpha value of 0.05 was used to determine significance. For oyster density and filtration, a two-way ANOVA with factors being exposure (high, low) and year (2009, 2010, 2011, 2019, 2020) was run on total, spat, seed and market sized density, and filtration rate, examining the single and interactive effects. For shoreline characteristics collected for all five years (percent cover (%), total above ground vegetation (g m^{-2}), soil bulk density (g m^{-2}), and soil percent organic matter (%)), a two-way ANOVA including treatment (reef, control), and year was run separately for each exposure (high, low). For parameters collected only in 2019 (belowground biomass, nutrients) or 2020 (soil shear strength), data were analyzed with a one-way ANOVA (factor: treatment) by exposure. A two-way ANOVA by exposure (high or low) examined shoreline erosion (m y^{-1}) by treatment (reef, control) and time period (pre-reef, early-reef, and late reef). Where significant differences occurred, a post-ANOVA Tukey test was used.

2.4. Results

2.4.1. Water quality and reef sustainability

Mean annual salinity in 2009 (10.4 ± 0.3), 2010 (9.8 ± 0.2), and 2011 (13.2 ± 0.4) was greater compared to 2019 (6.9 ± 0.2; $F = 50.2$, $p < 0.00001$) *(Figure 2a)*. In contrast, mean annual water temperature for 2019 (23.0°C ± 0.3) and 2020 (23.0°C ± 0.3) were not statistically different from 2009 (23.0°C ± 0.4), 2010 (22.0°C ± 0.4), or 2011(23.9°C ± 0.4) *(Figure 2b)*.

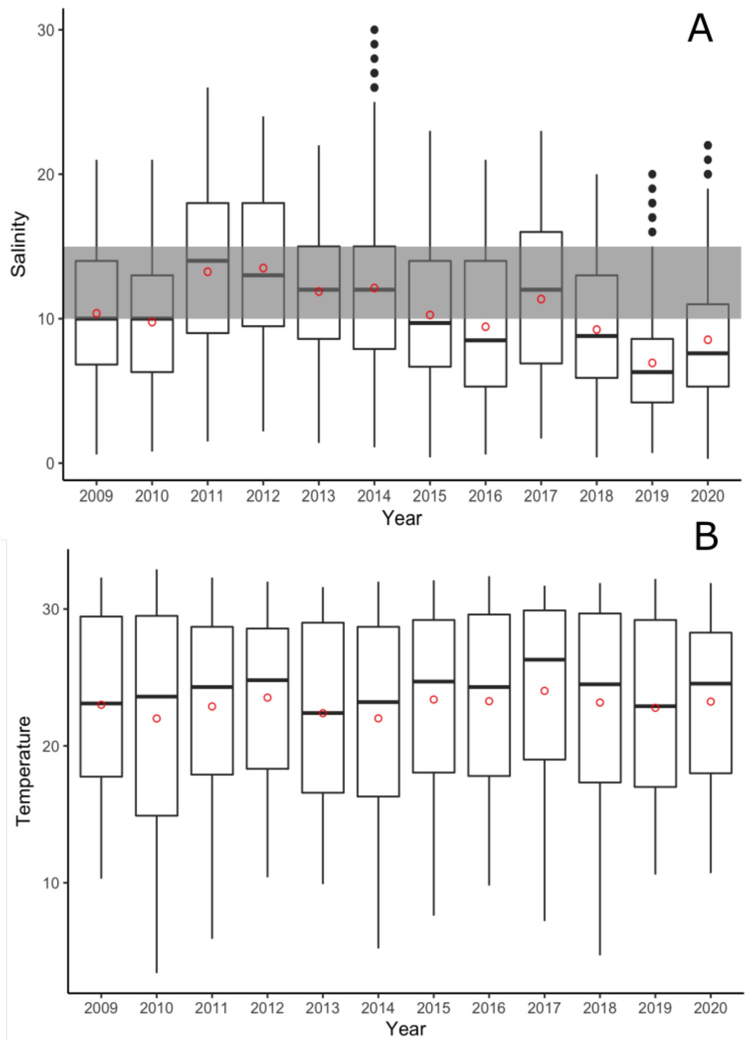


Figure 2.2. A: Box and whisker plot showing mean annual salinity for 2009 – 2020. Open red circles denote mean, shaded region denotes annual mean salinity range where oyster habitat suitability has a value of 1 (see Soniat et al. 2013) B: Box and whisker plot showing mean annual temperature for 2009 – 2020.

Total reef volume ranged from 426 to 439 g L^{-1} m^{-2} in 2019 -2020 and did not vary by year ($F = 0.006$, $p > 0.05$), exposure ($F = 0$, $p > 0.05$), or in the interaction of year and exposure ($F = 0.579$, $p > 0.05$). Although, total oyster density did not differ in the interaction of year by exposure ($F = 0.479$, $p > 0.05$) or the single effect of exposure ($F = 0.580$, $p > 0.05$), however, year was statistically different ($F = 9.824$, $p < 0.00001$) *(Figure 3a),* with total oyster density being significantly higher during the initial sampling periods of 2009 (2216 ind. $m^{-2} \pm 605$), and 2010 (1205 ind. $m^{-2} \pm 191$) compared to 2019 (49 ind. $m^{-2} \pm 18$) and 2020 (101 ind. $m^{-2} \pm 42$) *(Figure 3A)*. Density of spat and seed size oysters did not differ by exposure (Spat: $F = 0.671$, $p > 0.05$; Seed: $F = 0.456$, $p > 0.05$) or in the interaction of year by exposure (Spat: $F= 0.411$, $p > 0.05$; Seed: $F = 0.575$, $p > 0.05$). However, there was a significant effect of year on both spat ($F = 9.592$, $p < 0.00001$) and seed sized oysters ($F = 18.558$, $p < 0.00001$). Mean spat density was only significantly higher in 2009, compared to remaining years *(Table 1),* whereas seed density in 2009, 2010, and 2011 were all significantly higher compared to 2019 and 2020 *(Table 1)*. In contrast, density of market sized oysters differed significantly for the interaction of year by exposure ($F = 4.41$, $p = 0.002$). Market size oysters were not collected on any reefs in 2009, and only on low exposure reefs in 2010. In addition, market density in 2011 was significantly higher than all other year and exposure combinations *(Table 1)*.

Table 2.1. Oyster densities by size class (spat, SH < 25 mm; seed, 25 ≤ SH > 75 mm; market, SH ≥ 75 mm) and reef exposure for all created reefs (mean ± SE) from 2009,2010,2011,2019, and 2020. Superscript letters denote significant statistical differences between years and exposure (2-way ANOVA, alpha value = 0.05) within each oyster size class.

Year	**Exposure**	**Spat Ind. m^{-2}**	**Seed Ind. m^{-2}**	**Market Ind. m^{-2}**	**Total Reef Volume g L^{-1} m^{-2}**
2009	High	1881 ± 821^{a}	766 ± 294^{a}	0 ± 0^{a}	-
	Low	1258 ± 277^{a}	474 ± 88^{a}	0 ± 0^{a}	-
2010	High	638 ± 247^{b}	587 ± 124^{a}	0 ± 0^{a}	-
	Low	565 ± 94^{b}	618 ± 59^{a}	3 ± 1^{b}	-
2011	High	324 ± 112^{b}	830 ± 129^{a}	23 ± 6^{b}	-
	Low	197 ± 43^{b}	834 ± 129^{a}	59 ± 10^{c}	-
2019	High	14 ± 8^{b}	2 ± 2^{b}	7 ± 4^{b}	253 ± 122^{a}
	Low	46 ± 20^{b}	16 ± 14^{b}	12 ± 6^{b}	208 ± 60^{a}
2020	High	46 ± 31^{b}	62 ± 36^{b}	4 ± 2^{b}	192 ± 84^{a}
	Low	55 ± 34^{b}	28 ± 17^{b}	7 ± 7^{b}	237 ± 115^{a}

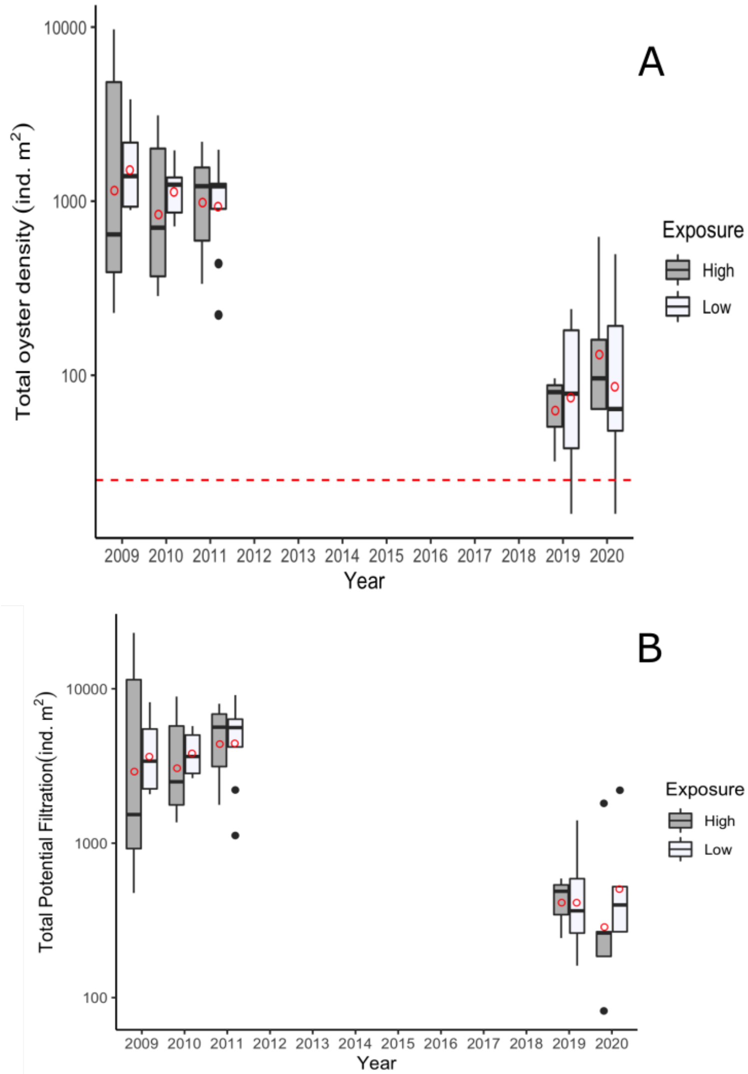


Figure 2.3. A: Box and whisker plot showing oyster density (ind. m^{-2}) over time (2009 – 2020). No data were collected in years 2012-2018. B: Box and whisker plot showing annual mean for total potential filtration between 2009 – 2020. No data were collected in years 2012-2018. Open red circles denote mean, horizontal black lines denote median. Letters on each plot signify a statistical difference between years (Two-way ANOVA, alpha = 0.05).

Total estimated reef filtration potential varied from an estimated low of approximately 250 L h^{-1} m^{-2} (2019) to over 5000 L h^{-1} m^{-2} (2009) *(Figure 3B)*. Seed sized oysters contributed over 70% of filtration capacity across all years *(Table 2)*. Filtration potential contributed by spat and seed were highly correlated to total filtration potential, therefore, only results for market and total oysters are reported. Total oyster filtration potential did not differ for the interactive effects of year by exposure ($F = 0.603$, $p > 0.05$), or in the single effect of exposure ($F = 0.257$, $p > 0.05$). However, total filtration did differ significantly by year ($F = 12.605$, $p < 0.0005$), with 2009, 2010, and 2011 all differing and being higher compared to 2019, and 2020. Total potential reef filtration was greatest in 2009 (5382 ± 1421 L h^{-1} m^{-2}) and lowest in 2019 (252 ± 86 L h^{-1} m^{2}) *(Figure 3B)*. In contrast, there was a significant interactive effect of year by exposure for market oyster filtration potential ($F = 5.47$, $p = 0.0006$). In 2011, high and low exposure sites differed significantly from one another with low exposure sites having higher rates of filtration for market sized oysters compared to high exposure sites, as well as from all other year and exposure combinations *(Table 2).*

Table 2.2. Filtration potential contributed by each size class (spat, seed, market) by exposure (High, Low) and year (2009, 2010, 2011, 2019, 2020). Superscript letters denote significant statistical differences between years and exposure for market sized oysters.

		Potential filtration rate ($L\ h^{-1}\ m^{-2}$)		
Year	**Exposure**	**Spat**	**Seed**	**Market**
2009	High	2568 ± 1120	3915 ± 1505	0 ± 0^{a}
	Low	1718 ± 402	2425 ± 475	0 ± 0^{a}
2010	High	871 ± 337	3004 ± 634	0 ± 0^{a}
	Low	771 ± 129	3160 ± 304	28 ± 15^{b}
2011	High	443 ± 153	4245 ± 660	232 ± 63^{c}
	Low	269 ± 59	4266 ± 660	593 ± 101^{d}
2019	High	2 ± 2	73 ± 42	72 ± 39^{b}
	Low	22 ± 19	227 ± 103	107 ± 54^{b}
2020	High	75 ± 47	145 ± 89	69 ± 69^{b}
	Low	63 ± 42	318 ± 182	25 ± 17^{b}

2.4.2. Shoreline characteristics

2.4.2.1. Vegetation

Percent cover ranged from 29 ± 2 % to 57 ± 3 % *(Table 3)* and differed by year for both high ($F = 9.099$, $p < 0.0004$) and low ($F = 12.907$, $p < 0.00003$) exposure settings, but did not differ in treatment for either exposure (High: $F = 2.586$, $p > 0.05$; Low: $F = 2.68$, $p > 0.05$). In contrast, percent cover did differ in the interaction between treatment and year, but only for low exposure settings ($F = 4.802$, $p = 0.0016$). Apart from 2011 in low exposure sites, all reefed sites were similar throughout the years. The year 2019 was the only year to differ in treatment with control sites having a higher percent cover compared to reefed sites *(Table 3)*. For high exposure sites, percent cover only differed by year, with 2011 having significantly less cover than all other years *(Table 3).* Total aboveground biomass did not differ by treatment (High: $F = 2.55$, $p > 0.05$; Low: $F = 1.34$, $p > 0.05$) or in the interaction of year by treatment for high ($F = 1.45$, $p > 0.05$) or low ($F = 0.364$, $p > 0.05$) exposure settings. However, year had a significant effect in both high ($F = 4.671$, $p = 0.00516$) and low ($F = 2.946$, $p = 0.041$) exposure settings. For high exposure sites, aboveground vegetation biomass was similar in 2010, 2019 and 2020, all having higher biomass than in 2009 and 2011, which were similar to one another *(Table 3).* In low exposure sites, aboveground biomass in 2010 was similar to all years, except 2011, which had a significantly lower biomass *(Table 3).* Belowground biomass did not differ by exposure in low exposure settings ($F = 0.479$, $p = 0.499$), but was significant for high exposure settings ($F = 5.085$, $p = 0.0385$) during 2019, with control sites having a greater biomass than reefed sites *(Table 3)*.

Table 2.3. Reported means ± SE for percent cover (%), aboveground biomass (g m^{-2}) 2009, 2010, 2011, 2019, and 2020, and belowground biomass for 2019 only. Letters denote significance between years.

	Year	**Treatment**	**Percent cover % m^{-2}**	**Above Ground Biomass g m^{-2}**	**Belowground Biomass g m^{-2}**
High Exposure	2009	Reef	53 ± 9[a]	520 ± 162[a]	-
		Control	44 ± 7[a]	812 ± 141[a]	-
	2010	Reef	66 ± 9[a]	1254 ± 180[b]	-
		Control	57 ± 4[a]	1119 ± 192[b]	-
	2011	Reef	28 ± 8[b]	712 ± 134[a]	-
		Control	23 ± 4[b]	785 ± 135[a]	-
	2019	Reef	48 ± 4[a]	760 ± 123[ab]	1285 ± 188[a]
		Control	47 ± 6[a]	1233 ± 162[ab]	2307 ± 413[b]
	2020	Reef	52 ± 2[a]	1704 ± 206[ab]	-
		Control	45 ± 2[a]	1340 ± 107[ab]	-
Low Exposure	2009	Reef	41 ± 5[b]	1127 ± 128[ab]	-
		Control	33 ± 5[b]	829 ± 205[ab]	-
	2010	Reef	51 ± 3[ab]	1324 ± 149[b]	-
		Control	53 ± 3[ab]	1297 ± 169[b]	-
	2011	Reef	33 ± 4[c]	946 ± 131[a]	-
		Control	31 ± 2[c]	737 ± 62[a]	-
	2019	Reef	44 ± 3[a]	1096 ± 190[ab]	1019 ± 130[a]
		Control	71 ± 7[b]	1089 ± 226[ab]	878 ± 157[a]
	2020	Reef	46 ± 3[b]	1339 ± 196[ab]	-
		Control	48 ± 4[b]	1676 ± 300[ab]	-

2.4.2.2. Soils

Soils were characteristic of Louisiana estuarine environments with organic matter ranging from 17 to 30 % and bulk density from 0.2 to 0.4 g m^{-2} *(Table 4)*. Soil bulk density and percent organic matter (%) were highly correlated, therefore, only results for bulk density are discussed below. Bulk density (g m^{-2}) did not differ in both the single effects of year (High: $F = 0.927$, $p > 0.05$; Low: $F = 2.44$, $p > 0.05$) or treatment (High: $F = 0.287$, $p > 0.05$; Low: $F = 0.004$, $p > 0.05$) or the interactive effects between year and treatment (High: $F = 0.321$, $p > 0.05$; Low: $F = 0.363$, $p > 0.05$). Additionally, moisture content (%) did not differ between years (High: $F = 0.010$, $p > 0.05$; Low: $F = 0.088$, $p > 0.05$), treatment (High: $F = 0.131$, $p > 0.05$; Low: $F = 2.711$, $p > 0.05$), or the interactive effects of year and treatment (High: $F = 0.045$, $p > 0.05$; Low: $F = 0.002$, $p > 0.05$) for each exposure setting. Concentrations for soil extractable nutrients (NO_3^-, PO_4^{2+}, NH_4^+) were also insignificant by treatment for each exposure setting during 2019 *(Table 4).* Similarly, soil shear strength did not differ by treatment for both high ($F = 1.39$, $p > 0.05$) and low ($F = 1.41$, $p = 0.301$) exposure settings. Interestingly, settings of high exposure saw generally greater shear strengths for control sites, while settings of low exposure saw greater shear strengths for reefed sites *(Table 4)*.

Table 2.4. Reported means ± SE for soil bulk density (g m^{-2}), soil moisture content (%), soil organic matter (%), soil extractable NO_{3-}, NH_{4+} and PO_4^{2+} and soil shear strength (kpa m^{-2}) in the adjacent marsh of reef and control high and low exposure oyster reef sites during the years 2009, 2010, 2011, 2019, and 2020

	Year	Treatment	Bulk Density g m^{-2}	Moisture Content %	Organic Matter %	NO_3^- uM dwg^{-1}	PO_4^{2+} uM dwg^{-1}	NH_4^+ uM dwg^{-1}	Shear Strength kpa m^{-2}
High Exposure	2009	Reef	0.4 ± 0.1	-	22 ± 5	-	-	-	-
		Control	0.4 ± 0.1	-	24 ± 5	-	-	-	-
	2010	Reef	0.4 ± 0.1	-	24 ± 6	-	-	-	-
		Control	0.4 ± 0.1	-	24 ± 5	-	-	-	-
	2011	Reef	0.3 ± 0.1	-	15 ± 4	-	-	-	-
		Control	0.4 ± 0.1	-	19 ± 4	-	-	-	-
	2019	Reef	0.3 ± 0	65 ±1.9	23 ± 3	0.09 ± 0.02	13 ± 2.3	10.8 ± 0.7	-
		Control	0.4 ± 0.1	63 ±2.5	19 ± 4	0.10 ± 0.04	10.6 ± 1.6	12.9 ± 0.8	-
	2020	Reef	0.3 ± 0	64 ±1.7	18 ± 3	-	-	-	8.87 ±1.09
		Control	0.3 ± 0.1	64 ±3.2	20 ± 4	-	-	-	14.09 ± 2.22
Low Exposure	2009	Reef	0.2 ± 0	-	27 ± 4	-	-	-	-
		Control	0.3 ± 0.1	-	31 ± 6	-	-	-	-
	2010	Reef	0.3 ± 0	-	24 ± 3	-	-	-	-
		Control	0.3 ± 0	-	30 ± 6	-	-	-	-
	2011	Reef	0.2 ± 0	-	22 ± 2	-	-	-	-
		Control	0.2 ± 0	-	24 ± 3	-	-	-	-
	2019	Reef	0.2 ± 0	71± 2.1	27 ± 3	0.11 ± 0.02	10.7 ± 1.8	11.1 ± 1.8	-
		Control	0.2 ± 0	76 ±1.4	35 ± 5	0.07 ± 0.02	11.1 ± 1.6	10.3 ± 0.4	-
	2020	Reef	0.2 ± 0	72 ±1.9	27 ± 3	-	-	-	10.38 ± 1.46
		Control	0.2 ± 0	77 ±1.6	33 ± 5	-	-	-	7.73 ± 1.69

2.4.3. Shoreline stability

Shoreline movement of high exposure shorelines did not differ significantly by treatment ($F = 1.556$, $p > 0.05$), year ($F = 3.008$, $p > 0.05$), or the interaction of year by treatment ($F = 1.170$, $p > 0.05$). In contrast, shoreline movement of low exposure shorelines differed significantly for the interaction of year and treatment ($F = 8.631$, $p = 0.000266$). In general, mean estimated shoreline movement for control and reefed sites were similar during the pre-reef (2005-2010) and late reef (2014 – 2019) periods. However, during the pre-reef period (2005-2010), reefed sites had significantly higher shoreline movement as compared to control sites. Mean shoreline movement for the early reef period (2010 -2014) (~ -0.78 m y^{-1}) was statically lower than pre- and late- reef periods, however, treatment was not significant *(Figure 4)*.

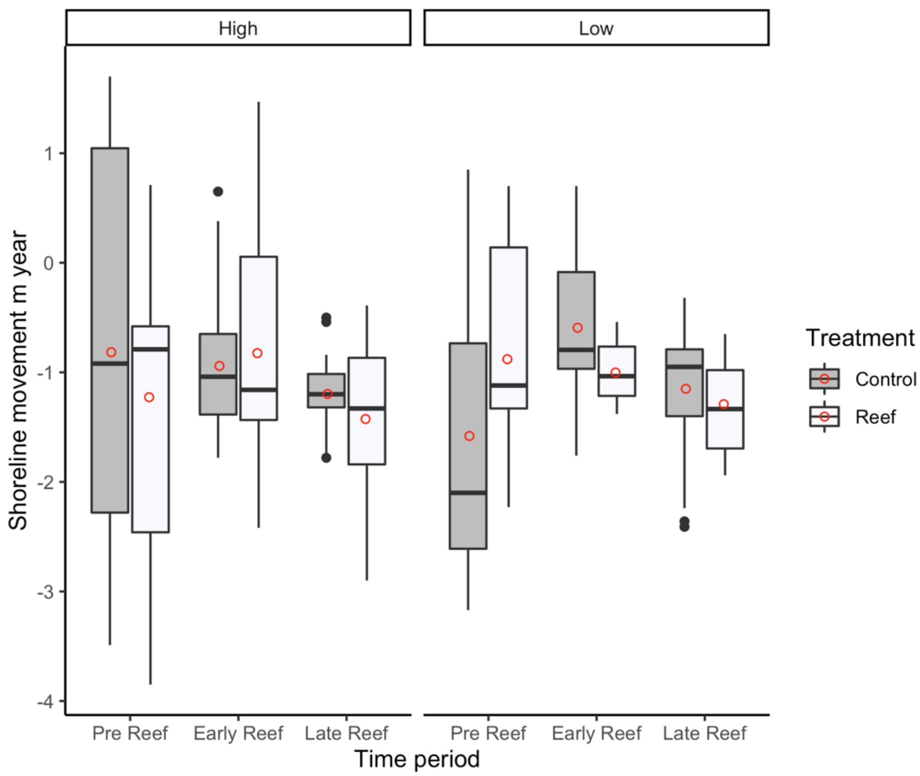


Figure 2.4. Box and whisker plot (median, quartiles) of shoreline movement (m y^{-1}) by treatment. Negative shoreline movement indicates shoreline erosion. Time period represents pre-reef (2005-2010), early reef (2010-2014) and late reef (2014-2019) periods. Letters denote significance for the interaction of time period by treatment for sites of low exposure. Reefs were constructed in 2009

2.5. Discussion

Continued development and sustainability of restored oyster reefs depends on maintenance of suitable environmental conditions (i.e., salinity and temperature) and the physical characteristics of a site through time. In this case, the restored oyster reefs persisted over 11 years, although oyster density and filtration potential declined significantly during the period of study. Sessile organisms such as the *C. virginica* have a broad tolerance to environmental variation although prolonged (i.e., > 30 days) or repeated exposure to unfavorable conditions often leads to high mortality and loss of ecosystem services (Du et al. 2021; Soniat et al. 2013; La Peyre et al. 2013). In this instance, repeated and extended exposure to low salinity from 2016-2020 may have resulted in the decrease of total oyster density in Sister Lake, Louisiana with over 40% of this time period reporting salinity less than 8 (*Supplemental Table 1*). While market sized oysters persisted throughout the period of study, total oyster density decreased by 96 % from initial densities in 2009 compared to 2020, although filtration services were still evident.

Despite this decrease in oyster density, reefs still matched success criteria suggested for some oyster restoration projects. Success criteria are often vague and varied for reef restoration as some agencies including the Louisiana Department of Wildlife and Fisheries (LDWF) define success as 20 seed-sized oysters m^{-2}, while others relate success to a total oyster density of at least 25 ind. m^{-2} (Baggett et al. 2015). All years met the thresholds of at least 25 ind. m^{-2} except for the 2019 high exposure reefs which had a mean total density of 23 ± 12 ind. m^{-2}. Additionally, 2019 was the only year to not surpass 20-seed sized oysters m^{-2}, with a mean seed density of 2 ± 2 ind. m^{-2} in high exposure settings and 16 ± 14 ind. m^{-2} in low exposure settings. In 2020, 11 years post-reef construction, these reefs would be considered successful restoration projects by both LDWF and Baggett et al. 2015 performance standards.

The effects of salinity and temperature on oyster reproduction, mortality and physiology have been well documented (La Peyre et al. 2013, Soniat et al. 2013; Lavuad et al. 2017; Lowe et al. 2017; Casas et al. 2018b), with physiological effects present after prolonged exposure to salinity between the range of 3-7 (Powell et al. 1992; Cerco and Noel 2005; Fulford et al. 2007) and overall habitat suitability falling below 1 for mean annual salinities < 10 (Soniat et al. 2013). Mean annual salinity in Sister Lake remained above 10 from 2009 through 2015. However, during the 2016 to 2020 period, only one year (2017) maintained a mean or median salinity above 10, while all other years had mean annual salinities less than 10. Unfavorable water quality conditions reduce larvae survival and often lead to poor recruitment. Reef sustainability depends also on reproduction, with optimal spawning season (May- September) salinities greater than 10 (Soniat et al. 2013). Mean salinity during spawning months followed a similar trend to annual mean salinities, with mean spawning season salinities < 8 in later years *(See supplemental figure 1).* Combined, these salinity data suggest less than optimal conditions to support oyster reproduction, or survival during the 2016-2020 period. Although data were only collected in the last two years of this period, the low spat and seed density numbers recorded (< 10% of 2009 – 2011 densities), suggest that these reefs experienced limited recruitment and survival during 2016-2020 years. In contrast, market oyster density was highest in 2011, 2019, and 2020, which suggests reef persistence through the survival of larger oysters, providing potential reproductive capacity for future generations, when conditions become more conducive to reproduction. Adult oysters are known to survive extended exposure to less-than-optimal salinity, allowing reef sustainability to occur through smaller annual recruitment classes.

Filter feeders, like *C. virginica,* contribute numerous ecosystem services to estuarine habitat, including local improvements in water quality through filtration (Cerco and Noel 2005; Coen et al. 2007). However, lack of sustainable populations and favorable environmental conditions results in decreased filtration potential, feeding time, and clearance rates (Casas et al. 2018a) For example, a 90-95% reduction in eastern oyster clearance rate has been noted after continuous exposure to salinities of 6 and 3 (Casas et al. 2018a); because clearance rates also represent feeding by oysters, such a reduction will result in reduced growth, and energy available for reproduction (Lavaud et al. 2017). Reduced total filtration potential calculated in this study between 2009 (5000 L h^{-1} m^{-2}) and 2019 (250 L h^{-1} m^{-2}), was calculated based on a 10-year mean salinity, thus reduced filtration reflected only the decrease in oyster densities and change in oyster size distribution. If annual salinities were also used to calculate each year, the filtration provided in the lower salinity years (i.e., 2019) would be further reduced. Although limited, these reefs still provide a valuable service through their filtration activities.

As reported in La Peyre et al. (2014), reefs had little impact on productivity of adjacent shorelines. Vegetative characteristics including percent cover and above and belowground biomass did not generally differ between treatments, with only percent cover varying between reef and non-reef shorelines in low exposure locations. Additionally, soil parameters were similar across treatments and years. The lack of impacts may be due to the low oyster density and volume of reefs at these sites which were not sufficient to produce a measurable impact. Shellfish reefs have been hypothesized to provide nutrient and sediment subsidies to adjacent salt marsh habitat through their filtration activities which can concentrate material. However, evidence to support this remains limited across studies (Chowdury et al. 2019).

This lack of effect on shoreline productivity, soil characteristics, and reef decline likely contributed to the failure in preventing shoreline movement. La Peyre et al. (2014) found no significant differences in shoreline movement between marshes adjacent to created reefs and non-reefed sites over the first three years post-reef creation. This lack of benefit to shoreline movement has persisted through at least 10 years post-creation indicating that there is not a delay in detection for benefits to adjacent marsh shorelines. Failure to slow landward erosion could be due to an ineffective reef height, footprint, and/or oyster density (Morris et al. 2018). In addition, shoreline retreat could likely be occurring from a combination of subsidence and erosive forces. If subsidence contributes more to net shoreline loss than erosive energies, then fringing oyster reefs would not prove useful to slow shoreline retreat. While reefs failed to impact large-scale shoreline movement, many studies have demonstrated oyster reefs' ability in trapping sediments and contributing to local sedimentation (Meyer et al. 1997; Coen et al. 2007; Chowdhury et al. 2019). Past studies have linked reef presence to wave attenuation, with lower wave energies measured behind the reef compared to the front of the reef (Meyer et al. 1997; Chauvin 2017; Morris et al. 2018), however, this has not always translated to reduced shoreline erosion (i.e. Morris et al. 2018). In this case, the lack of measurable impact on shoreline productivity, the low relief and reef density resulting in minimal hard structure, and the high subsidence in this region, likely resulted in limited shoreline protection benefits.

These findings demonstrate that restoration projects and their impacts are not static and change over time. For managers assessing restoration impacts and planning future projects, understanding how these restoration efforts might fare over time and adapt to changing conditions is critical for long-term success. Ecological restoration seeks to restore natural systems' structure and function, often following an ecological trajectory, whereby ecosystems approach historic patterns of succession and community development over some time period (Odum 1969; Gann and Lamb 2006; La Peyre et al. 2014). Unfortunately, these trajectories are generally not well described for marine environments, and subsequently not accounted for in coastal management. Temporally dynamic ecosystems, such as estuaries, make long-term assessments difficult, however, given the challenges and dire state of many coastal resources, management cannot wait for complete certainty on restoration decisions (NAS 2017). Adaptive management serves as a solution, in which, targeted monitoring efforts aid in the identification of high priority uncertainties, thereby improving restorative actions (NAS 2017). The flexible process allows for the adjustment of planning, construction, and operation during the lifetime of a restorative project, thereby ensuring the maximum benefit from restoration funds and decisions (NAS 2017).

2.6. Conclusion

Revisitation of the reefs 10-11 years post construction found them intact, but not as robust as 3 years post construction. Reef density and subsequently filtration potential decreased through time, however, reef presence remained through the survival of market sized oysters. Extended exposure to low salinity from 2016 to 2020 may have resulted in the decrease of total oyster density in Sister Lake, with over 40% of this time period reporting salinities < 8.
There was no evidence of sustained benefits of restored reefs on marsh vegetation, soil properties, or reduction in shoreline movement; likely a factor of reef size, width, and location (Morris et al. 2018). While still present, reefs were greatly reduced in total density and efficiency of ecosystem services, with total oyster densities decreasing by 96% from initial densities in 2009. However, all reefs met some density threshold throughout the period of study, but in varying years and exposure settings. While peak densities were not sustainable in the long-term, the presence of suitable cultch material and optimal salinity requirements (La Peyre et al. 2015), could allow reefs to rebound and grow. These restoration trajectories will however go unnoticed without the incorporation of long-term monitoring or adaptive management.

Chapter 3. Examining effects of salinity and harvest on six restored oyster reefs in coastal Louisiana

3.1. Abstract

Declines in eastern oyster (*Crassostrea virginica*) populations have prompted significant restoration activities with goals of both restoring oyster resources for production and enhancing reef development to support associated ecosystem services. Restoration of oysters remains difficult, as they are sessile for most of their life, depending largely on dynamic overlying water quality and local harvest regulations for continued reef survival. In Louisiana, $15M was allocated to early oyster restoration for damages assessed from the Deepwater Horizon oil spill. This funding was used to restore 6 historic reefs across several estuaries. Examining annual oyster density and monthly salinity data collected by Louisiana Department of Wildlife and Fisheries as part of their annual restoration and long-term monitoring program, this work explored the trajectory of restored oyster reefs in coastal Louisiana from 2013-2019. Specifically, we examined changes in total oyster and size class density across years and compared changes to annual mean salinity, annual spawning season mean salinity, and minimum monthly salinities. At all sites, total oyster density increased immediately following restoration, with all size classes reported at each site within 2 years of restoration. Mean spat, seed, and market density generally decreased after 2016 with total oyster densities falling below 25 ind. m^{-2} for remaining years at all but two sites. This decline may be related to sub-optimal mean annual salinity in 2016 (i.e., < 10), exposure to sub-optimal monthly mean salinities (i.e., < 6) for multiple months, or the opening of several sites for harvest starting in 2016. Identifying outcomes of restoration funding is important to inform future efforts and requires careful consideration of both environmental and management impacts.

3.2. Introduction

The eastern oyster (*Crassostrea virginica*) provides critical ecological and economic services to estuarine environments, including shoreline protection, water filtration, habitat heterogeneity, and local biodiversity (Meyer et al. 1997; Coen et al. 2007; Coen and Humphries 2017). Despite their contribution to coastal services, 85% of oyster reefs have been lost worldwide based off estimates from historical accounts and current monitoring (Beck et al. 2011). While not considered as imperiled as the Atlantic Coast, significant declines in *C. virginica* populations have been documented across the Gulf Coast, as a result of anthropogenic activities, changes in water quality, and over harvest (La Peyre et al. 2003; Beck et al. 2011; Furlong 2012; Baggett et al. 2015). These declines were further exasperated by the Deepwater Horizon Oil spill (2010), resulting in the closure of nearly 37% of federal fishing waters throughout the Gulf of Mexico (Upton 2011). Damage to oyster beds and public fishing waters along with the continual declines in oyster landings prompted significant restoration activities, with goals of both restoring oyster resources for production, and enhancing reef development to support critical ecosystem services (Natural Resource Damage Assessment 2012; RESTORE ACT; Louisiana Costal Master Plan 2014).

Specifically, along the Louisiana Gulf Coast, efforts to restore oyster populations and to compensate the public for injury to oysters from the Deepwater Horizon Oil Spill (2010), included the allocation of $15M to the execution of the Louisiana Oyster Cultch Project (DWH NRDA 2015). The project consisted of the placement of over 5 km^2 of oyster cultch material across six regions and 4 Louisiana Coastal Study Areas (CSA), along with the construction of an oyster hatchery facility (DWH NRDA, 2015). These restoration activities were selected based on the identified damages from the oil spill, which estimated a loss of 4-8.3 billion subtidal oysters from direct mortality and a lack of reproduction, with the most pronounced loss occurring on southeast Louisiana reefs (DWH NRDA 2015). The restoration plan set specific goals for the project including creating suitable substrate for larvae attachment and subsequent growth into self-sustaining reefs (DWH NRDA 2015). In Louisiana, this involved the placement of material to create six separate reefs located across historic oyster grounds.

Restoration of organisms such as the eastern oyster, which are sessile for most of their lives, depends largely on selecting a location with appropriate water quality conditions. Temperature and salinity are the two main abiotic factors driving oyster survival, reproduction, and larval recruitment. Spawning in the Gulf of Mexico usually occurs when salinities are above 10 and water temperatures exceed 20°C (May – September) (Soniat et al. 2015; Hijuelos et al. 2016). Larvae spend 2-3 weeks in the water column before settling on appropriate hard substrate. Once settled, oysters grow, reproduce and die in their settled location. Although oysters are osmoconformers and can tolerate a wide range of salinities, predation, disease, or osmotic failure can all increase within certain salinity ranges (Soniat et al. 2013; Casas et al. 2015; Casas et al. 2018b). Predation rates by stenohaline organisms such as the oyster drill (*Thais haemastoma*) or the protozoan parasite (*Perkinsus marinus*) usually increases with warmer waters and higher salinities (>15) (Barnes et al. 2007; Hijuelos et al. 2016). Critical to long-term survival of the reef is ensuring overlying water conditions remain within the tolerance ranges of oysters. Despite their ability to close their shells, oysters thrive at relatively narrow zones of intermediate salinities (10-15; see Soniat et al. 2013), and location of restoration projects within these zones are often key to their success. However, Louisiana estuaries face increasingly variable changes in salinity due to numerous ecological and anthropogenic factors, including changes in climate and freshwater influxes from freshwater or sediment diversions. These changes have impacted key oyster areas over the last few decades and are predicted to impact these areas in the future (Soniat et al. 2013; Wang et al. 2017). Specifically, changes in freshwater influxes affect oyster survival and recruitment, by lowering estuarine salinities, sometimes beyond oyster tolerance ranges (Soniat et al. 2013; Wang et al. 2017; Lavaud et al. 2021).

Adaptive management, with the use of information from restoration monitoring, helps to address uncertainties such as changing water conditions (i.e. salinity and temperature), and improve restoration actions (NAS 2017). The flexible process allows for the adjustment of planning, construction, and operation throughout the lifetime of a restoration project, thereby ensuring the maximum benefit from restoration funds and decisions (NAS 2017). For reef restoration, this includes incorporation of long-term monitoring both on biological (i.e. oyster density and size distribution) and physical aspects (i.e., salinity, temperature, and presence of substrate) of the project.

This work examined the outcomes of the oyster restoration projects in Louisiana implemented as part of the early restoration funding from the Deepwater Horizon Oil Spill (DWH NRDA 2015). Specifically, this work examined total oyster density and population demographics from 2012 through 2019 for all six sites restored in the Louisiana Oyster Cultch Project. Oyster densities were examined in relation to three key salinity variables from the oyster habitat suitability index used in the Louisiana Coastal Master Plan (CPRA 2017), and include annual mean salinity, mean spawning salinity and monthly mean salinity. These variables, along with availability of cultch are used to identify suitable oyster habitats (CPRA 2017). Given the significant time and resources invested in oyster restoration, and the continued focus on coastal restoration in the state of Louisiana, understanding the outcome of specific restoration activities can critically inform future restoration.

3.3. Methods

3.3.1. Study area

As part of the Louisiana Oyster Cultch Project, phase 1 Deepwater Horizon early restoration project, six cultch plants were created between June 2012 and May 2013 *(Figure 5; Table 5).* These sites were located on Louisiana's publicly managed oyster seed grounds. Cultch plants consisted of the placement of limestone or concrete material sub-tidally by blowing cultch material off barges.

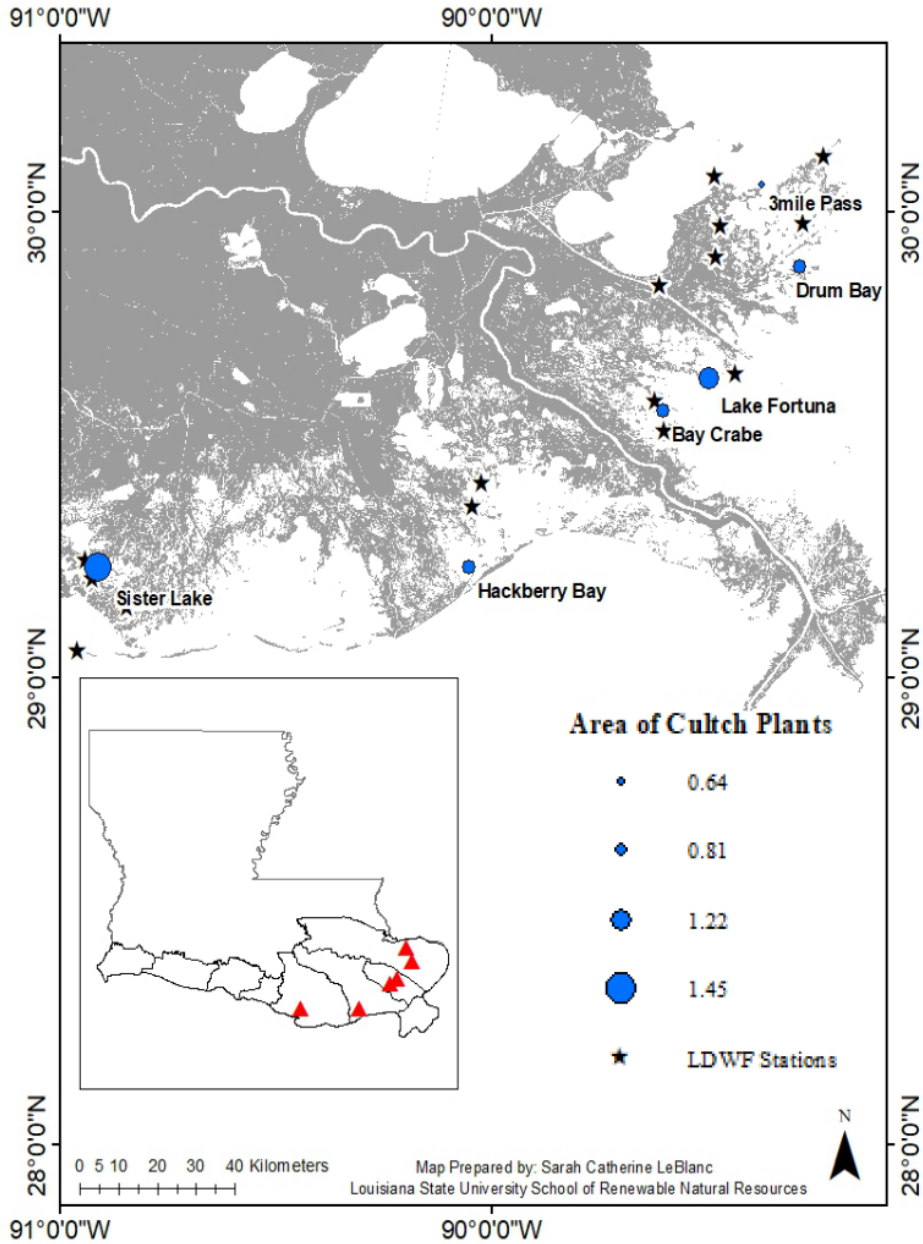


Figure 3.1. Location and area restored (Km^2) for sites constructed by Louisiana Department of Wildlife and Fisheries as part of the Louisiana Oyster Cultch project across 4 coastal study areas. Sites spanned three Louisiana basins (Ponchartrain, Terrebonne, and Barataria) and were all part of Louisiana's publicly managed oyster seed grounds and reservations. Red triangles denote cultch plants in the locator map.

Table 3.1. Sites restored by Louisiana Department of Wildlife and Fisheries as part of the Louisiana Oyster cultch project throughout 4 coastal study areas (CSA). Sites were restored throughout 2013. A total area of 5.75 km^2 was restored between all six sites, with summer quadrat sampling beginning in late 2013.

Site	CSA	Date Constructed	Cultch Material	Area of Cultch Plant km^2
Sister Lake	CSA 5W	6/2/2012	Limestone	1.45
Hackberry Bay	CSA 3	5/21/2012	Limestone	0.81
Bay Crabe	CSA 1S	10/6/2012	Limestone	0.81
Lake Fortuna	CSA 1S	11/19/2012	Concrete	1.22
Drum Bay	CSA 1N	6/2/2013	Limestone	0.81
3-Mile Pass	CSA 1N	5/9/2013	Limestone	0.64

3.3.2. Oyster Indices

Data for analyses were obtained from Louisiana Department of Wildlife and Fisheries (LDWF) who conducted annual summer quadrat sampling through 2019 for all 6 sites, excluding Bay Crabe, which turned to mud by 2016. Lake Fortuna was the only site to undergo an enhancement which involved adding more cultch to the site in 2018. Additionally, Sister Lake and Hackberry Bay did not undergo quadrat sampling in 2015. During each sampling event, LDWF biologists haphazardly placed a square-meter aluminum quadrat on top of the reef and collected by hand, live and dead oysters, reef associated organisms, and exposed reef material from the upper portion of the substrate (LDWF 2014). Five (5) replicates were collected for each site. However, this methodology was altered when sampling recent cultch plants, whereby 5 haphazardly placed quarter-meter squared samples were chosen by a random grid selection placed over the cultch plant. Samples were processed immediately by recording the number of live and dead oysters and recording oyster shell height (SH) by 5-mm increments. Based on SH, oysters were separated into three size classes; spat (< 25mm SH, seed (25mm $\leq$ SH < 75mm), and market ($\geq$ 75 mm SH). In 2014, data were entered in NOAA DIVER (https://www.gulfspillrestoration.noaa.gov/project?id=5) for all sites, as required by the funding program, and data were retrieved from this portal. All other data were obtained from the Louisiana Department of Wildlife and Fisheries. All 2013, 2015-2019 data for Bay Crabe, Lake Fortuna, Drum Bay, and 3-Mile Pass were made available by LDWF as raw field data sheets. Data were entered into an excel spreadsheet. Sister Lake and Hackberry Bay 2013, 2016-2019 data were available from the LDWF long-term monitoring database *(Table 6)*. Data from all sources were compiled into one database, and oyster density by size class standardized as individuals m^{-2}

Table 3.2. Description of data sources for each of the six restored sites. Data used were either from LDWF field data sheets (LDWF-F), LDWF long-term monitoring database (LDWF-D), or DIVER (https://www.gulfspillrestoration.noaa.gov/project?id=5) N/A indicates that no data were collected. Quadrat size used for each sampling is indicated (0.25 or 1 m^2).

Site	2013	2014	2015	2016	2017	2018	2019
Sister Lake	LDWF-D 1m^2	NOAA Diver 0.25m^2	N/A	LDWF-D 1m^2	LDWF-D 1m^2	LDWF-D 1m^2	LDWF-D 1m^2
Hackberry Bay	LDWF-D 1m^2	NOAA Diver 0.25m^2	N/A	LDWF-D 1m^2	LDWF-D 1m^2	LDWF-D 1m^2	LDWF-D 1m^2
Bay Crabe	N/A	NOAA Diver 0.25m^2	LDWF-F 1m^2	N/A	N/A	N/A	N/A
Lake Fortuna	LDWF-F 1m^2	NOAA Diver 0.25m^2	LDWF-F 1m^2	LDWF-F 1m^2	LDWF-F 1m^2	*Enhancement*	LDWF-F 1m^2
Drum Bay	LDWF-F 0.25m	NOAA Diver 0.25m^2	LDWF-F 0.25m	LDWF-F 1m^2	LDWF-F 1m^2	LDWF-F 1m^2	LDWF-F 1m^2
3-mile pass	LDWF-F 0.25m	NOAA Diver 0.25m^2	LDWF-F 0.25m	LDWF-F 1m^2	LDWF-F 1m^2	LDWF-F 1m^2	LDWF-F 1m^2

3.3.3. Salinity

Salinity trends over a 7-year period (2013-2019) were quantified with data obtained from LDWF long-term monitoring stations located near each site. LDWF samples bottom salinity approximately monthly. Mean annual salinity, mean spawning salinity (May-Sept) and number of months reporting salinity below 2 were calculated with monthly data from the nearest two - three monitoring stations for each of the six project sites. These indices were selected as they represent three of the five variables used to determine oyster habitat suitability within Louisiana's Coastal Master Plan (CPRA 2017).

3.3.4. Analyses

Reef density was calculated for each site with available data *(Table 5)* from the LDWF summer quadrat surveys. Mean total oyster density (ME $\pm$ SE), along with mean spat, seed, and market-size oyster density were calculated and reported by site and year.

3.4. Results

3.4.1. Salinity

Across all sites from 2013-2019, annual mean salinity ranged from 7.9 ± 0.4 (2019) to a high of 15.5 ± 0.5 (2014). Except for 2019, overall annual mean salinity was between 10 and 15 for all years. However, sites varied between years where annual mean salinity was between 10-15 *(Figure 6).* Drum Bay reported the highest annual mean salinities, with all years reporting an annual mean greater than 10, while Bay Crabe remained the freshest with all but two years reporting a mean annual salinity less than 10. Hackberry Bay was below 10 for 2016-2019, while Sister Lake was below 10 in 2016, 2018 and 2019 with Lake Fortuna and 3-Mile Pass only falling below 10 in 2019 *(Figure 6, Table 7)*.

Mean salinity during the May-September spawning season ranged from a low of 5.2 ± 1.3 (Bay Crabe) to a high of 14.3 ± 0.8 (Drum Bay), with all sites apart from Drum Bay reporting mean spawning season salinities below 18 for all years 2013-2019 *(Table 7)*.

Monthly mean salinities were ≤ 2 for over 2 months at Bay Crabe in every year except for 2014, at Hackberry Bay in 2016-2019, and 3-Mile Pass in 2019 *(Table 7).* Additionally, Bay Crabe was exposed to salinities ≤ 2 for an estimated 30% of the time throughout the 7-year period *(Table 7).*

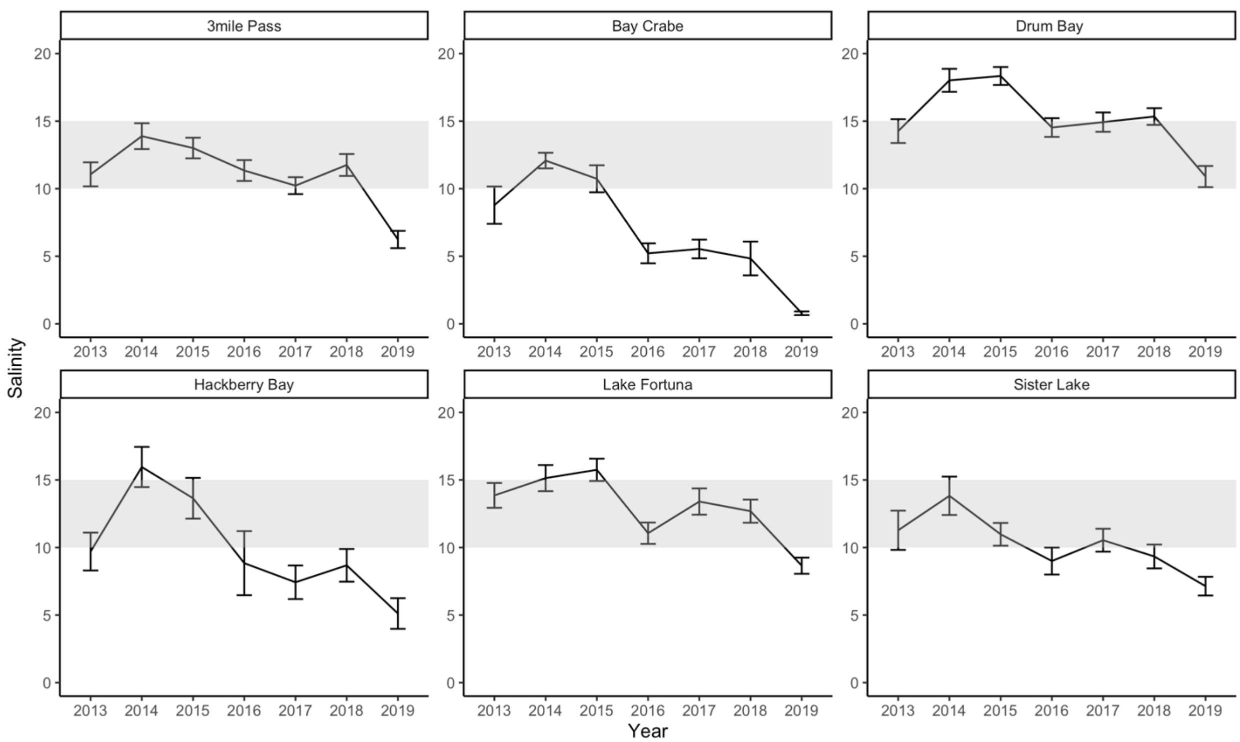


Figure 3.2. Annual mean salinity (ME ± SE) for all sites from 2013 – 2019. Shaded region denotes optimal salinity range (10-15; HSI value = 1) (Soniat et al. 2013).

Table 3.3. Mean ± SE annual salinity, mean± SE spawning salinity (May-Sept), and percentage of 12-month year where salinity was < 2 based on monthly means (i.e. killing salinity, see Soniat et al. 2013) by site and year.

Site	Year	Annual Mean Salinity	Mean Spawn Salinity	% of year $\leq$ 2
3mile Pass	2013	11.1 ± 0.9	12.4 ± 2.6	0.0
	2014	13.9 ± 1.0	12.5 ± 2.5	0.0
	2015	13.0 ± 0.8	12.4 ± 2.2	0.0
	2016	11.3 ± 0.8	11.7 ± 1.8	0.0
	2017	10.2 ± 0.6	8.6 ± 1.5	0.0
	2018	11.8 ± 0.8	11.9 ± 2.0	0.0
	2019	6.2 ± 0.6	4.7 ± 1.2	25.0
Bay Crabe	2013	8.8 ± 1.4	6.7 ± 0.0	8.3
	2014	12.1 ± 0.6	11.6 ± 0.0	0.0
	2015	10.7 ± 1.0	6.4 ± 0.0	8.3
	2016	5.2 ± 0.7	5.7 ± 0.0	41.7
	2017	5.5 ± 0.7	2.0 ± 0.9	16.7
	2018	4.8 ± 1.2	6.3 ± 0.0	50.0
	2019	0.8 ± 0.1	0.7 ± 0.0	91.7
Drum Bay	2013	14.3 ± 0.9	14.0 ± 2.0	0.0
	2014	18.0 ± 0.9	14.4 ± 2.2	0.0
	2015	18.3 ± 0.7	17.3 ± 1.4	0.0
	2016	14.5 ± 0.7	15.2 ± 1.6	0.0
	2017	14.9 ± 0.7	12.6 ± 2.0	0.0
	2018	15.3 ± 0.6	15.8 ± 1.5	0.0
	2019	10.9 ± 0.8	9.6 ± 2.5	0.0
Hackberry Bay	2013	9.7 ± 1.4	9.3 ± 0.0	0.0
	2014	16.0 ± 1.5	14.9 ± 0.0	0.0
	2015	13.6 ± 1.5	11.7 ± 0.0	0.0
	2016	8.8 ± 2.4	3.4 ± 1.0	8.3
	2017	7.4 ± 1.2	4.6 ± 0.5	8.3
	2018	8.7 ± 1.2	11.4 ± 0.7	8.3
	2019	5.1 ± 1.1	6.1 ± 1.1	41.7
Lake Fortuna	2013	13.9 ± 0.9	11.7 ± 2.3	0.0
	2014	15.1 ± 1.0	13.0 ± 2.9	0.0
	2015	15.8 ± 0.8	13.0 ± 3.0	0.0
	2016	11.1 ± 0.8	10.4 ± 2.9	0.0
	2017	13.4 ± 1.0	10.5 ± 2.8	0.0
	2018	12.7 ± 0.9	12.3 ± 2.9	0.0
	2019	8.7 ± 0.6	7.3 ± 2.1	0.0
Sister Lake	2013	11.3 ± 1.5	12.0 ± 2.8	0.0
	2014	13.8 ± 1.4	11.5 ± 0.9	0.0
	2015	11.0 ± 0.8	7.9 ± 2.2	0.0
	2016	9.0 ± 1.0	8.4 ± 1.1	8.3
	2017	10.5 ± 0.8	6.4 ± 1.4	0.0
	2018	9.3 ± 0.9	9.4 ± 1.4	0.0
	2019	7.1 ± 0.7	8.4 ± 1.2	0.0

3.4.2. Reef Density

Total oyster densities ranged from a low of 0 (Bay Crabe, Lake Fortuna, 2019), to a high of 206 ± 149.8 ind. m^{-2} (3-Mile Pass, 2015). Generally, following restoration in 2013, total oyster density increased for the first 2-3 years, with all sites declining by 2016, and not recovering by 2019 *(Figure 7)*. Bay Crabe was the least productive site following restoration, turning to mud in early 2016.

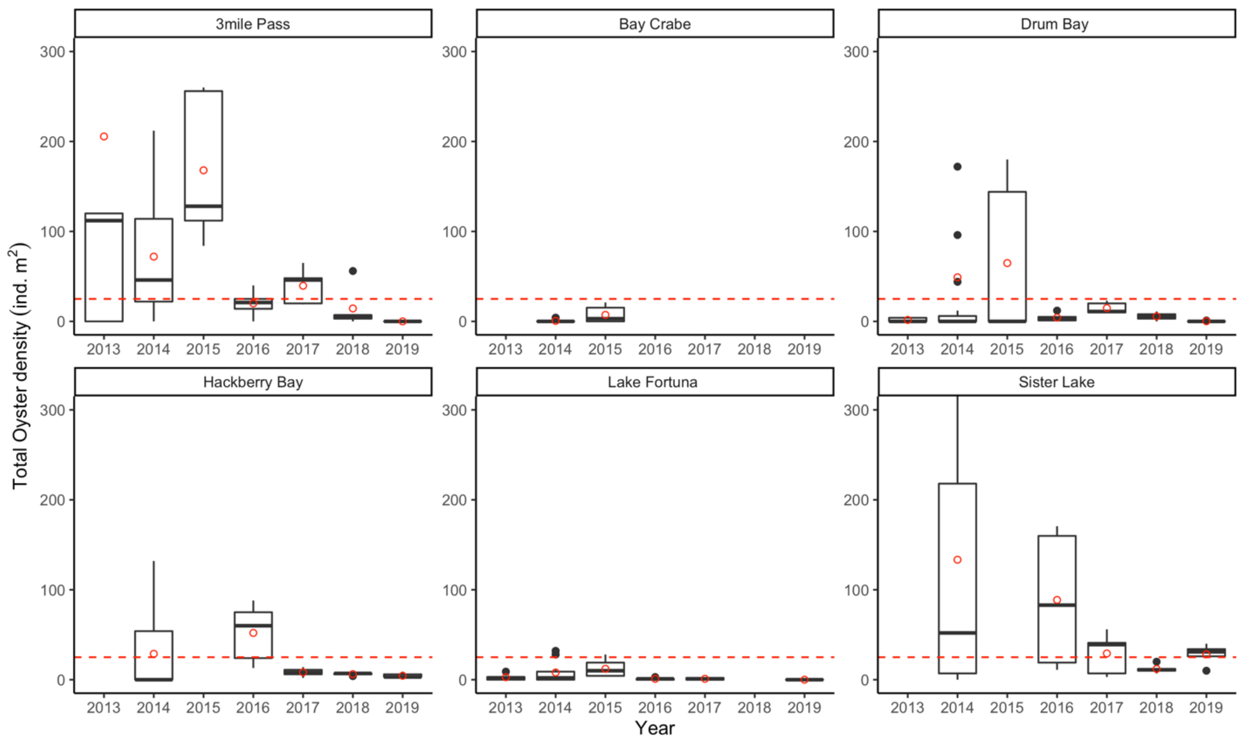


Figure 3.3. Box and whisker plot showing long-term changes in total oyster density (ind. m^{-2}) for all sites across years 2013-2019. Black lines denote medians, red open circle denotes mean. Red dashed line indicates project success threshold suggested for restoration of 25 ind. m^{-2} (Baggett et al. 2015). Data were not collected at sites and years missing boxplots.

Congruent with the trend of declining total densities, mean spat density generally decreased for all sites throughout the 7-year monitoring effort. 3-Mile Pass had the highest spat density, with a peak immediately following restoration in 2013 (204 ind. m^{-2} ± 150). Apart from 3-Mile Pass, spat density generally peaked around years 3 and 4 post-restoration (2015, 2016) *(Figure 8)*. However, following the peak, spat density drastically declined throughout the remainder of project monitoring. Bay Crabe was again the least productive site, with mean densities of 0 ind. m^{-2} during years 2014 and 2015.

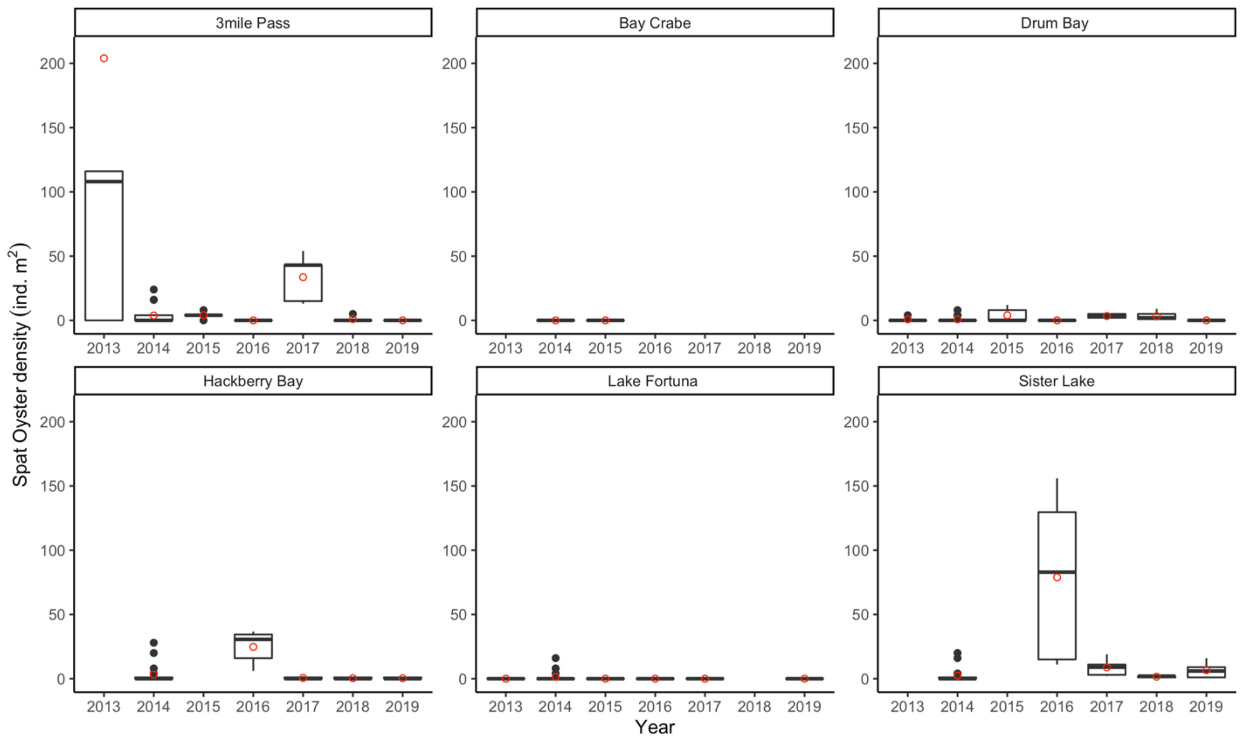


Figure 3.4. Box and whisker plot showing long-term changes in spat (< 25 mm SH) density (ind. m^{-2}) for all sites across years 2013-2019. Black lines denote medians, red open circles denote mean. Missing plots indicates site and year where data was not collected.

Seed sized oyster density ranged from 0 to a high of 154 ind. m^{-2} $\pm$ 33 (2015, 3-Mile Pass). Density of seed size oysters showed a similar trend to total density with decreasing densities after 2016 for all sites, except for Sister Lake *(Figure 9)*.

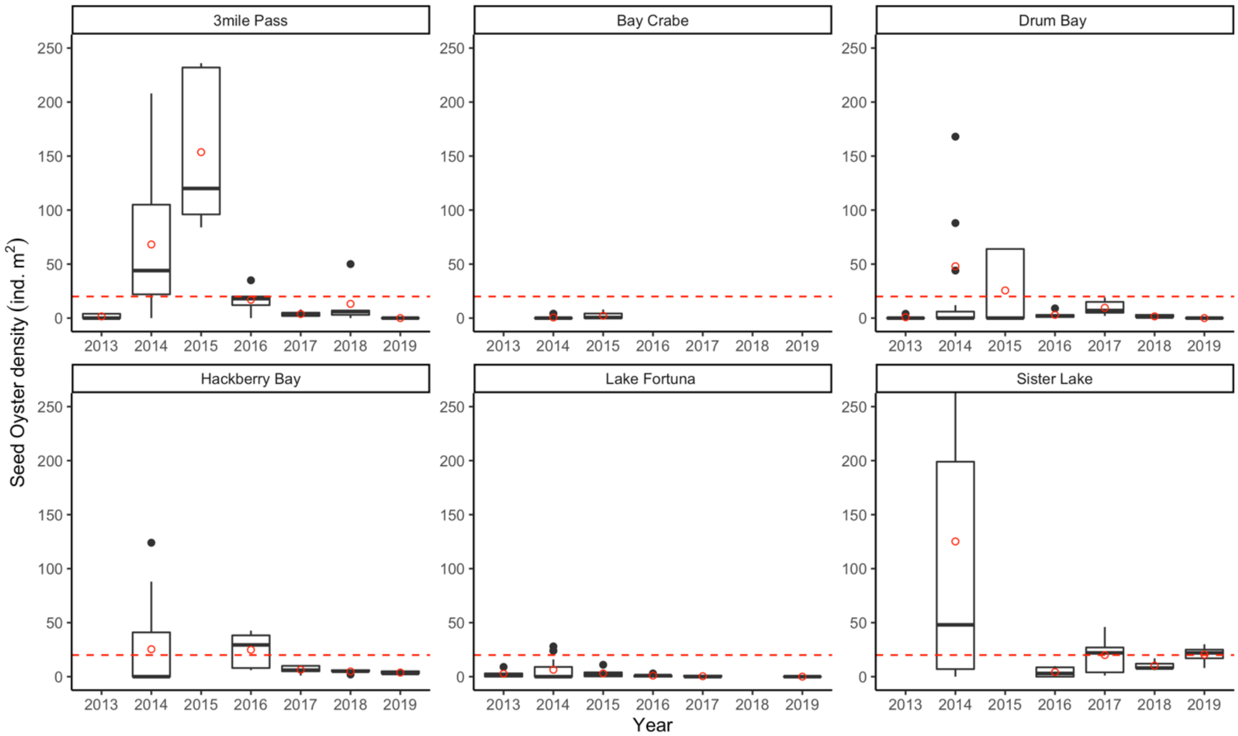


Figure 3.5. Box and whisker plot showing long-term changes in seed (25 mm ≤ SH > 75 mm) density (ind. m^{-2}) for all sites across years 2013-2019. Black lines denote medians, red open circle denotes the mean, red dashed line indicates LDWF threshold indicated for project success (20 seed oysters m^{-2}; NRDA DWH 2012). Data were not collected at sites and years missing boxplots.

Market sized oysters generally reached peak densities for all sites 2-3 years post-restoration (2014, 2015) *(Figure 10)*. This result is congruent with the peak in spat densities and indicates a successful period of reproduction from adult sized oysters and recruitment of oyster larvae. In addition, following peak densities in 2014 and 2015, market densities decreased for all sites for the remainder of project monitoring. The highest density reported for market sized oysters occurred on Drum Bay in 2015 (35 ind. m^{-2} ± 22).

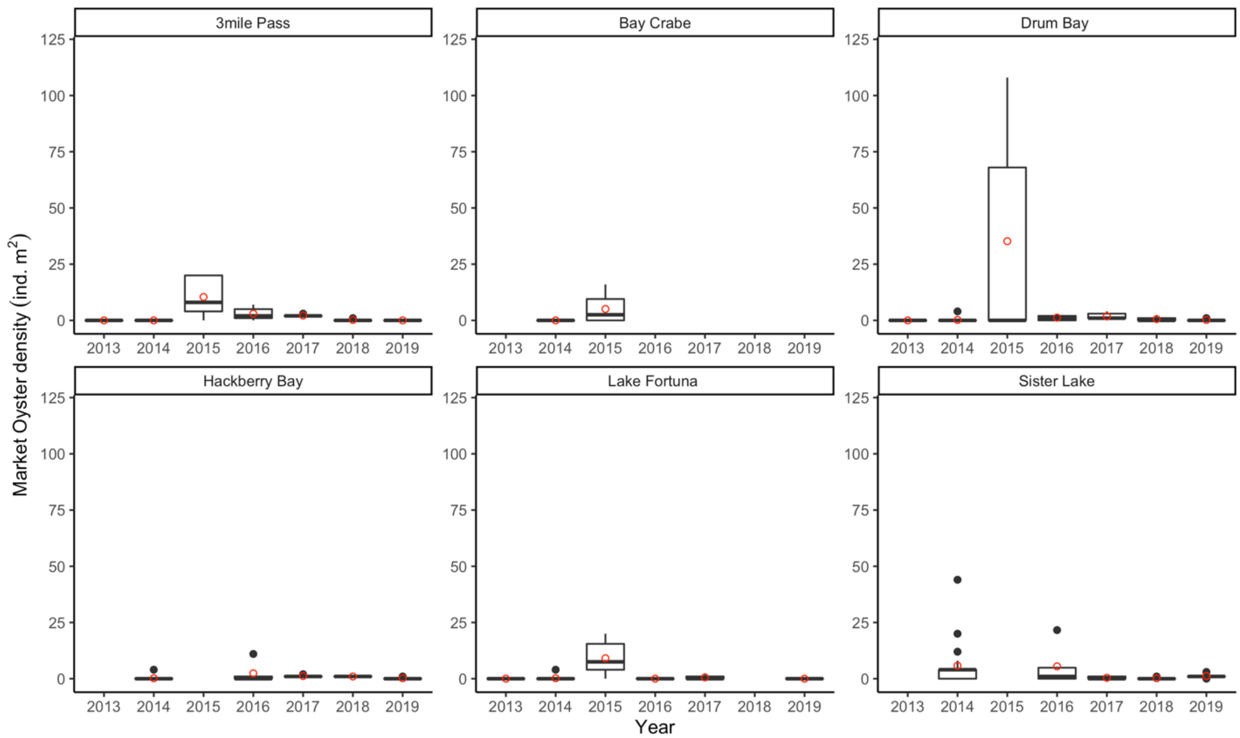


Figure 3.6. Box and whisker plot showing long-term changes in market (SH ≥ 75 mm) density for all sites across years 2013-2019. Black lines denote medians, red open circle denotes the mean. Data were not collected at sites and years missing boxplots.

3.5. Discussion

Assessing restoration success and applying adaptive management relies on documentation of restoration activities and outcomes. Data to support such assessments are rarely available for extended periods of time but can be highly useful. Six individual oyster reef restoration projects completed as part of the DWH NRDA early restoration projects provided a rare opportunity to examine long-term restoration outcomes. Across most of the sites, oyster density peaked around 3-4 years post construction but declined through the last few years (2016 – 2019) *(Figure 7)*. This decline was likely affected by declining salinities and sub-optimal conditions for oyster habitat suitability, including a historic freshwater inflow into Louisiana estuaries in 2019, as well as periods of heavy localized harvest (*Supplemental Table 2*). Determining project success may require long-term views of project sustainability as oyster reefs and the populations they support may reflect cyclical patterns in overlying water conditions, which could be influenced by climatic patterns, including ENSO which has been shown to influence oyster resources, largely through salinity impacts (Soniat et al. 2006). Additionally, the presence of harvest should be monitored as an influence controlling long-term reef sustainability.

Performance metrics and success criterion for oyster restoration are often vague, absent, or inconsistent amongst restoration projects (Kennedy et al. 2011; La Peyre et al. 2014). This lack of specific goals or metrics refers both to concrete goals to achieve on established metrics, but also the timing and longevity for achieving and maintaining these goals. In assessing oyster restoration success, past studies have used thresholds including the minimal requirement of the presence of cultch material, or live oysters (i.e., La Peyre et al. 2014). This approach assumes reefs with oysters have the potential to support future recruitment, and provide ecosystem services, but those with only cultch material may offer a subset of services and the potential for oyster recruitment in the future. Others have suggested more specific oyster densities, such as 25 ind. m^{-2} (Baggett et al. 2015) or 20 seed-sized oysters m^{-2} (NRDA DWH 2012) as thresholds for determining restoration success. Additionally, Baggett et al. (2015), proposed the use of universal restoration metrics to measure project success and sustainability, which included the monitoring of structural attributes (vertical relief of oyster reefs, oyster density, and spatial footprint over time). This approach indicates that several metrics can be used to assess restoration outcomes but require greater investment in monitoring. In the work reported here, oyster density and oyster size frequency were the only metrics collected for these specific restoration projects and thus restoration could only be measured by these metrics.

Successful restoration was defined for this work as reaching an average density of 20 seed sized oysters m^{-2} (NRDA DWH 2012). Bay Crabe and Lake Fortuna never met this criterion throughout the duration of project monitoring, while remaining sites differed in which years this goal was achieved. Both 3-Mile Pass and Drum Bay contributed a mean seed density above 20 ind. m^{-2} during 2014 (68 ind. m^{-2} ± 14; 48 ind. m^{-2} ± 33) and 2015 (154 ind. m^{-2} ± 33; 26 ind. m^{-2} ± 16), but not 2016-2019. Hackberry Bay met the threshold during both the 2014 (25 ind. m^{-2} ± 9) and 2016 (25 ind. m^{-2} ± 8) sampling periods, while Sister Lake reached or surpassed the threshold in 2014 (125 ind. m^{-2} ± 33), 2017 (20 ind. m^{-2} ± 8), and 2019 (20 ind. m^{-2} ± 4). Alternate measures of success suggested in the literature (i.e., Baggett et al. 2015), such as total oyster density greater than 25 ind. m^{-2} was similarly only met at a few sites over a few years *(Figure 7)*, while the lowest bar, presence of live oysters, was met for all sites but in varying years, with 2019 having the lowest density across all sites. This variation in timing, and number of years that these density targets were met bring into question how to determine success and whether there should be a specific temporal requirement for achievement and/or maintenance of these target thresholds. Further, the presence of any oyster could be used to indicate the potential for reef recruitment, and survival in future years, and aside from Bay Crabe, all reefs supported at least a few oysters throughout this study.

Oyster reef development and sustainability rely on overlaying water quality and recruitment of oyster spat from surrounding reefs. In this region, salinity is often an overriding variable impacting oyster reef sustainability and is the primary variable driving habitat suitability indices (Soniat et al. 2013) as well as many oyster growth models (Lavaud et al. 2017, Wang et al. 2017, Lavaud et al. 2021). As all reefs did receive recruitment, as evidenced by 2014 oyster densities above 0, other factors, such as salinity may help explain reef trends over the 7 years of data. Three key variables in the Louisiana oyster habitat suitability index (HSI) relate to salinity and provide some insight into the results. Specifically, annual mean salinity, minimum monthly salinity, and mean spawning season salinity at each site drive the HSI when oyster cultch is present. Mean annual salinity defines the range over which adult oysters survive and thrive and for this region, a range of 10-15 indicates suitable habitat (HSI=1.0), with suitability dropping off rapidly to 0 when salinity reaches 5. Minimum monthly salinities below 2 are considered “killing salinities”, and have been used to explain high mortality on reefs exposed to such a salinity during one month only. Lastly, to ensure reef persistence, salinities greater than 10 during May through September are considered a minimal requirement for spawning to occur, with ideal spawning salinities generally considered to be above 18 (Soniat et al. 2013).

The majority of sites were in or very close to the optimal annual mean salinity of 10-15 range until 2019 *(Figure 6),* where salinity dropped below 10 for all sites, except Drum Bay. Higher levels of salinity in 2014-2015 could have likely led to increased disease or predation in Drum Bay (Shumway 1996; La Peyre et al. 2013; Casas et al. 2015), whereas the lower salinities reported for other sites and across varying years likely contributed to decreased reproduction and larval survival and reproduction (Soniat et al. 2006; Soniat et al. 2013). Minimum monthly salinities also likely contributed to reef decline, with Bay Crabe reporting mean salinities ≤ 2 for a portion of each year, except 2013 and 2014, and 2019 reporting the highest percentage of the year below optimal conditions (~90%) *(Table 7)*. However, as sites only had monthly discrete samples available, it's unclear if these results are truly representative of the mean monthly salinity, or only represent low salinity for a few days out of the month. Although not available for each site, the USGS continuous daily data recorded in Sister Lake, provides a comparison to the LDWF data, and reports similar annual trends, with 2019 being an historically fresh year *(Supplemental Figure 1).*

Harvest of restored oyster reefs has also been linked to decrease restoration success and reef longevity, with some suggesting that harvest and restoration are not compatible (Lipcius et al. 2008; Puckett and Eggleston 2012; Lipcius et al. 2015). Over the last 10 years, the LDWF management area (CSA 1N) which includes 3-Mile Pass and Drum Bay has experienced heavy localized harvest, high mortality events, tropical events, and damage associated with the Deepwater Horizon oil spill. Similar pressures have resulted in a reported 87% decrease in total available oyster stock for the management unit which includes Bay Crabe and Lake Fortuna (CSA 1S). In contrast, the Sister Lake management unit (CSA 5W) was one of the more successful fishing areas, with 88% of available seed and 85% of available market sized oysters reported to come from the Sister Lake cultch plant in 2016 (LDWF 2016a). Despite being closed to public harvest, heavy periods of illegal harvest are thought to have occurred on the cultch plant during the 2014/2015 oyster season (LDWF 2015) which may partially explain the decrease in total oyster density from 2014 to 2016. Similarly, available seed-sized oysters present on the Hackberry Bay cultch plant attributed to the combined stock increase over the 10-year average in its management area (CSA 3) for the 2014/2015 season (LDWF 2015).

In 2016 all restored cultch plants opened to public harvest and this is credited with a 13% increase in total oyster landings from the previous 2014/2015 season across all LDWF Coastal Study Areas (LDWF 2016a). Total oyster harvest estimates in 2017 reported a 68% decrease from the previous season, potentially from harvest and lower salinity in 2016 across several of the sites. While harvest limits mandating only market sized oysters, and a limit of 50 sacks per day were implemented statewide for 2016 and 2017 harvest, LDWF did report high instances of undersized harvest and removal of cultch material for the 2016 oyster season (LDWF 2016a). This resulted in a complete closure in 2017 of areas containing Bay Crabe and Lake Fortuna due to drastic declines in seed-sized oyster density. Areas of 3-Mile Pass, Drum Bay and Hackberry Bay were closed in 2018. Declines in total oyster density on these restoration cultch plants, likely reflect their opening to harvest in 2016 *(Supplemental Table 2).*

Although some restoration projects identify metrics to monitor, rarely, if at all, do any indicate when these targets should be achieved, and whether they should be maintained. Ecological restoration seeks to restore natural systems' structure and function, often following an ecological trajectory, whereby ecosystems approach historic patterns of succession and community development over some time period (Odum 1969; Gann and Lamb 2006; La Peyre et al. 2014). Unfortunately, these trajectories are generally not well described for marine environments, and subsequently not accounted for in coastal management. However, they could provide valuable information to inform restoration activities. In this analysis, project success thresholds seemed to have been met within 2-3 years post-construction all sites but in varying years, however, given the dynamic nature of estuarine environments, and when working with a harvestable species, restoration success may become dependent on periodic enhancements, informed by long-term monitoring.

While project success thresholds were met for a period of project monitoring at many of the sites, it is unclear, based on the trends documented in oyster density how these restored reefs will fare over the long-term. As the last year of monitoring (2019) represented a historic low salinity year in Louisiana estuaries, further monitoring would provide valuable insight into reef recovery and resilience. The need for universal performance criterion that define both the metric target and the temporal trend would benefit restoration monitoring and inform adaptive management. While individual target oyster densities may differ across restoration projects, the presence of suitable cultch material or live oysters may also still provide viable metrics for the evaluation of project performance, as oysters live in metapopulations, and thriving reefs change depending on season and overlying water quality. Furthermore, with the presence of viable cultch material and live oysters, reefs still have the chance to recruit larvae and persist.

3.6. Conclusion

This work examined the long-term trajectory of restored oyster reefs in coastal Louisiana from 2012-2019, with analysis of reef density, size-density distribution and the habitat suitability index of salinity. Reef density peaked around 3-4 years post construction but continued to decline throughout the study period. This finding was congruent with the declining trend of salinity across all sites, with 2019 being the freshest year, as well as heavy periods of localized harvest, which combined, likely contributed to the reduced densities across all sites. While the cultch plants clearly added to oyster reefs within the region and provided increased resources for harvest 3-4 years post-restoration, it remains unclear how many of these reefs will be sustainable in the future, supporting either more harvest, or further oyster habitat. Data indicates 4/6 restored reefs persisted and met some threshold target at least once during the period of record.
A combination of harvest management, cyclical patterns of salinity across sites, and consideration of other management actions, including added cultch over time may be critical in determining future reef sustainability. Although, without the adoption of long-term monitoring these variables cannot be fully understood or examined.

Chapter 4. Conclusions

Coastal land loss, degradation of estuarine environments, and declines in population density for estuarine species, have prompted the allocation of numerous funds and creation of several restoration projects across coastal Louisiana with the goal of restoring these systems to historic patterns of structure and community development. Restoration efforts aim to achieve success thresholds; however, these thresholds and performance metrics are often vague, absent, or inconsistent, and receive little long-term analyses after completion. The creation of living shorelines and oyster cultch plantings are two examples of restoration projects which could benefit from long-term monitoring, adaptive management, and knowledge on variables affecting project success, as they both revolve around the survival and growth of the eastern oyster *(Crassostrea virginica).* Restoration projects focused on oyster reefs remain difficult, as oysters are sessile for most of their life, depending largely on overlying water quality (i.e., salinity) and harvest regulations for continued reef survival.

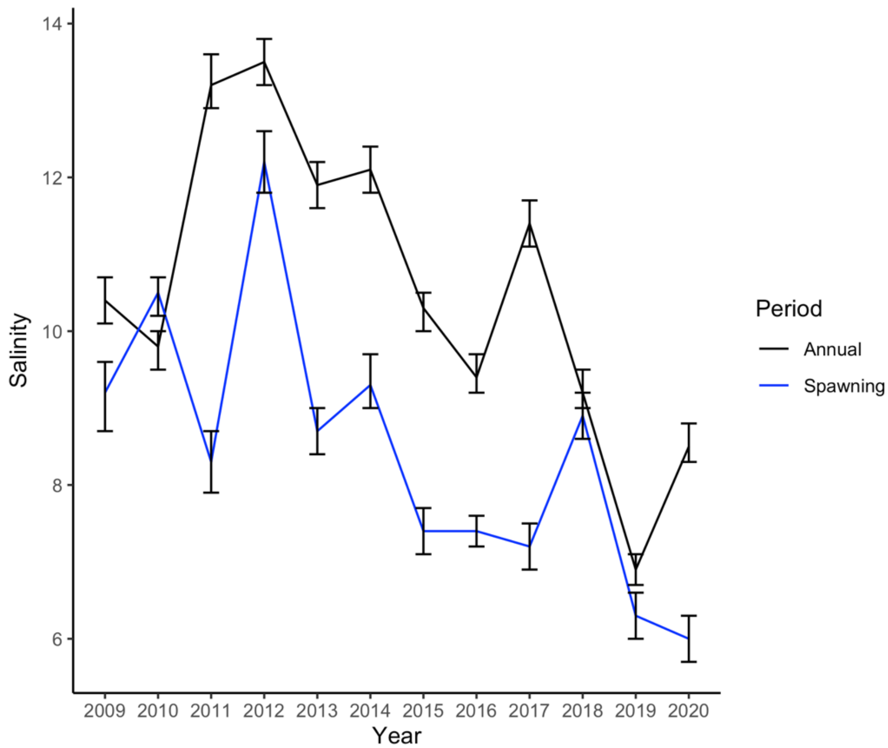
In Chapter 2 we examined 6 constructed oyster reefs in Sister Lake Louisiana, 10- and 11- years post-construction, and found them intact, but not as robust as 3 years post construction. Reef density and subsequently filtration potential decreased through time, however, reef presence remained through the survival of market sized oysters. No evidence was found to indicate these reefs enhanced adjacent marsh productivity, or reduced shoreline erosion. However, despite decreases, reef presence still provided ecological services (i.e. water filtration).

Chapter 3 quantified a few of the influences on reef sustainability, namely salinity and harvest, for 6 restored reefs located throughout coastal Louisiana. Reef density peaked around 3-4 years post-construction but continued to decline throughout the study period. This finding was congruent with the declining trend of salinity across all sites, with 2019 being the freshest years, as well as heavy periods of localized harvest in 2016, 2017, and 2018. For Louisiana, harvest and restoration co-exist due to the high economic value associated with Louisiana seafood, however, for restoration to remain successful, clear thresholds for success must be defined, as well as the incorporation of long-term monitoring.

Collectively, this work demonstrated that despite declines and loss of integrity over the long-term, restored oyster reefs may still provide services in relation to estuarine habitat, water quality, support a viable fishery, and aid in small-scale sediment trapping. In both instances however, reefs were more robust, as measured by oyster reef density, within the first 3 years of construction or restoration, declining in later years. Declines were likely influenced by sub-optimal water quality (i.e. salinity), heavy localized harvest, or a combination of both. Continued monitoring provides the avenue needed to assess these temporal changes as well as determine project success. Based on this data, a monitoring effort which revisits restored sites at least once year, preferably during the months of November – January, would provide an adequate assessment of reef sustainability, as it would indicate previous season's spawning success, recruitment success, and overall reef persistence.

Restoration success is vaguely defined across restoration projects or in many cases absent, however, for restoration funds to be used efficiently, explicit metrics for success are needed. This work explored success criteria based on target densities, provision of ecosystem services, and support of a viable fishery. All 6 restored reefs as part of the Louisiana Oyster Cultch project, determined success based on a target density of 20 seed-sized oysters m^{-2}, however, other projects have defined success in terms of total density (25 ind. m^{-2}; Baggett et al. 2015). For both studies, a target density of 20 seed sized oysters m^{-2} or 25 ind. m^{-2} did not affect the conclusion that reefs declined following peaks 2-3 post restoration. Despite these decreases, the provision of ecosystem services such as enhanced water quality was still able to persist through the service of filtration, however, it was clear that higher oyster densities also corresponded to higher rates of filtration. Shoreline protection was not affected by oyster density, even during years of peak performance. This service may be independent of oyster density and influenced more heavily by reef height, footprint, or relief. It was clear that even at low densities, reefs provided crucial services to estuarine habitat, although, with the influence of harvest, reefs continued to lose integrity and robustness, thereby leading to further decline. As was evident with the opening of restored cultch plants, harvest regulations can often influence the definition of restoration success. Restoration success based on target densities, provision of ecosystem services, and harvest impacts are all viable options for assessing project performance. Although, without the inclusion of long-term monitoring and adaptive management, failure to meet success thresholds may go unnoticed. With adaptive management, which may include continued monitoring, reef enhancement, and assessment of ecosystem service provision, restored oyster reefs and living shorelines could potentially continue to grow and meet restoration goals and performance criteria.

Appendix. Supplemental Data



Supplemental Figure 1. Annual mean and spawning (May – September) salinity for Sister Lake from 2009 – 2020. Data were obtained from USGS continuous data recorder using daily recordings (LDWF/USGS 07381349- Caillou Lake southwest of Dulac, LA, U.SA.).

Supplemental Table 1. Annual mean salinity (ME, spawning salinity, and percentage of 12-month year where mean salinity was < 10 for Sister Lake, Louisiana from 2009-2020. Data obtained from USGS daily recordings (LDWF/USGS 07381349- Caillou Lake southwest of Dulac, LA, U.SA.)

Year	**Annual Mean Salinity**	**Spawning Salinity**	**% of year < 10**
2009	10.4 ± 0.3	9.2 ± 0.4	25
2010	9.8 ± 0.2	10.5 ± 0.3	50
2011	13.2 ± 0.3	8.3 ± 0.4	25
2012	13.5 ± 0.3	12.2 ± 0.4	33
2013	11.9 ± 0.3	8.7 ± 0.3	33
2014	12.1 ± 0.3	9.3 ± 0.3	33
2015	10.3 ± 0.3	7.4 ± 0.3	42
2016	9.4 ± 0.3	7.4 ± 0.2	58
2017	11.4 ± 0.3	7.2 ± 0.3	42
2018	9.2 ± 0.2	8.9 ± 0.3	58
2019	6.9 ± 0.2	6.3 ± 0.3	92
2020	8.5 ± 0.3	6.0 ± 0.3	67

Supplemental Table 2. Seed, Market, and Total Harvest estimates on Louisiana public oyster grounds containing cultch plants from 2015-2018. Data was obtained from fisheries dependent surveys and taken from LDWF stock assessments (LDWF 2015, 2016, 2017, 2018). 1 barrel = 2 sacks. CSA 1N includes 3mile pass and Drum Bay; CSA 1S includes Bay Crabe and Lake Fortuna; CSA 5w includes Sister Lake; and CSA 3 includes Hackberry Bay.

CSA	**Year**	**Seed Oysters (barrels)**	**Market Oysters (barrels)**	**Total (barrels)**
CSA 1N	2015	111,434	16,372	119,620
	2016	79,133	86,972	122,619
	2017	32,265	18,072	41,301
	2018	25,260	17,872	34,196
CSA 1S	2015	14,500	8,351	18,676
	2016	11,628	7,323	15,289
	2017	0	0	0
	2018	0	0	0
CSA 3	2015	0	0	0
	2016	9,359	2,038	10,387
	2017	890	1,420	1,600
	2018	0	0	0
CSA 5W	2015	1175	3,837	3,094
	2016	38,308	65,147	70,882
	2017	2,670	18,865	12,103
	2018	6,410	25,290	19,055

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Vita

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