Quantification of the potential spawning contribution from oyster (*Crassostrea virginica*) restoration projects: a comparative study among restoration sites and substrates in coastal Alabama

by

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Abstract

Although numerous ecosystem services of oyster reef restoration have been documented, the contribution of larvae has not been previously quantified. In this study, we selected 5 restoration sites in coastal Alabama (Billy Goat Hole, Alabama Port, Coffee Island, Point aux Pins and Little Bay) with 6 different substrates materials (Reef Balls™, Reef Blocks™, bagged oyster shell, loose oyster shell and 2 types of Wave attenuation restoration materials (WARMs). In 2011, we sampled the oysters monthly during the presumed reproductive season (June to November), dividing the oysters into 2 size classes ($\geq$ 30 & < 75 mm shell height, and $\geq$ 75 mm), with up to 30 oysters collected per size class per sampling. Oysters were sexed by microscopic examination of the gonad, and the number of eggs per female was estimated using a modified method of Cox & Mann (1992). The result suggests that spawning primarily occurred from June to September 2011. And in both sizes oysters there are two observable spawning peaks, one is in June or July and the other one is in September. The sex ratio shows a dramatic decline of female oyster in October and November. Among all the conservation materials, WADs in Billy Goat Hole has the highest potential egg production per female in legal size oyster, while Reef Balls™ in Alabama Port is the highest in sub-legal size oysters. There is no significant different ($p<0.05$) among different sites for the potential egg production per female. For the potential spawning contribution per square meter and potential spawning contribution, WADs in Billy Goat Hole is the highest as well, and the significant difference ($p<0.05$) existing among different sites.

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Introduction

The eastern oyster, *Crassostrea virginica*, is an important fishery in the United States. In 2010, 8,517.6 metric tons of eastern oysters were harvested in the US, with a market value of $84.9 million. In Alabama in 2011, 5,762.8 metric tons of eastern oysters were landed, with a farm-gate value over $1,321,960. The oyster, however, is not only an economically important species; it also provides significant ecological functions in estuarine ecosystems, including creation of habitat, clearance of the water column, reduction of eutrophication and protection from coastal erosion. Populations of natural oysters, however, have been threatened by overharvesting (Newell 1988, Dame 1993, Rothschild et al. 1994, Jackson et al. 2001, Beck et al. 2009), habitat loss (Rothschild et al. 1994, Hargis et al. 1999, Smith et al. 2005), disease (Andrews 1988, 1996, Burreson et al.1996), predation (e.g., Soniat et al. 2004) and environmental degradation (Frankenberg 1995, Rothschild et al. 1994). The decline of these oyster populations have also led to declines in oyster fisheries and the ecological functions that they provide. These declines have led to a tremendous interest in oyster restoration efforts, with numerous oyster restoration projects funded, constructed, completed and planned.

These projects have, in turn, spurred a large number of studies to evaluate the effectiveness of these projects, including the ecological functions and ecosystem services provided by restored oyster reefs. Though there have been a number of studies done (summarized below) that have demonstrated that oyster restoration can enhance the abundance of certain marine species, reduce the particulate organic carbon and chl-a, and increase the abundance of submerged aquatic

vegetation, there has been quantification of the spawning contribution from oyster restoration projects. This ecological function has been presumed and often estimated based on rough estimates of oyster reproductive potential from the scientific literature. Additionally, the presumed spawning contribution is often noted as a valuable ecosystem service to commercial and recreational fisheries adjacent to oyster restoration projects. Despite the potential flaws in the estimation and the importance of this service, there appears to be no rigorous quantification of potential spawning contribution from oyster restoration projects.

Here I use the case study of oyster restoration in the coastal waters of Alabama as a first attempt at quantifying potential spawning contribution from different oyster restoration projects. This will allow an index to evaluate the magnitude and variability of the spawning contribution from at least these particular restoration projects, and provide a baseline for comparison among oyster restoration projects elsewhere. Furthermore, within Alabama, this work could be used to compare different restoration materials and different years. Collectively, this work and other studies like this could improve the planning of future restoration projects and gives a more complete value of the benefits derived from oyster restoration.

Ecosystem Services Provided by Oysters

Habitat Services

The Eastern oyster acts as an ecosystem engineer that creates and modifies the habitat (e.g., Lenihan et al. 1998). The extensive irregular surfaces of a reef provide 50 times the surface area of a similar sized flat bottom (Henderson et al. 2003). At large spatial scales oyster reefs can persist for thousands of years in the same general location (Gutiérrez et al. 1999, Commito et al. 2001, Hertweck et al. 2002, Smith et al. 2003, Stone et al. 2005), providing nursery and refuge habitats for estuarine and marine species. Researchers have found that oyster reef habitat could enhance estuarine biodiversity (Wells 1961, Luckenbach et al. 2005, Brumbaugh et al. 2006). The shell matrix creates stable habitat for mobile and sessile fauna (Wells 1961, Zimmermen et al. 1989, Kennedy 1996, Rodney et al. 2006) and vertebrate (Lenihan et. al. 1998, Henderson et al. 2003, Brumbaugh et al. 2006) and marine and estuarine invertebrate organisms (Wells 1961,

Zimmerman et al. 1989, Henderson et al. 2003), including economically valuable species (Atlantic State Marine Fisheries Commission 2007). Researchers have observed that oyster reefs have been shown to support marine species like finfish, flounder, menhaden, herring, anchovies, spadefish, striped bass, cobia, croaker, silver perch, spot, speckled trout, Spanish mackerel, pinfish, butter fish, harvest fish, blue crab, stone crab, penaeid shrimp, black drum, red drum, spotted sea trout, and several species of mullet (Zimmerman et al. 1989, Swann 2008. Bahr et al. 1981, Breitburg 1999, Coen et al. 1999, Lenihan et al. 2001, Atlantic States Marine Fisheries Commission 2007).

Water Quality Maintenance

The Eastern oyster contributes to the maintenance of water quality. Natural populations of bivalves are known to reduce total suspended solids through filter feeding (Cloern 1982, Officer et al. 1982, Soto et al. 1999, Nelson et al. 2004, Cerco et al. 2005, Grabowski et al. 2007), recycle and remove organic nutrients in the water column (Doering et al. 1986, Rice 1999) and reduce turbidity (e.g., Dame. 1996, Grabowski et al. 2007). The nutrients that eastern oysters consume from the water column deposit as feces and pseudofeces, which can provide food resource for benthic invertebrates and bacteria feeding at the sediment water interface (e.g., Newell 1988). Thus, oysters link the pelagic and benthic food webs (Newell 2004, Newell et al 2005). Also, oyster reefs are able to control phytoplankton growing by both reducing the suspended material in water column and controlling the rate that nutrients are exchanged between the sediments and overlying water (e.g., Ulanowicz et al. 1992, Haamer et al. 2000, Newell 2004, Nelson et al. 2004, Brumbaugh et al. 2006, Grizzle et al. 2006, Peabody et al. 2008). For example, Cerrato et al. (2004) found that oysters could suppress blooms of “brown tides” that occurred along the mid-Atlantic coast. Also oyster reefs could benefit submerged aquatic vegetation (SAV) by reducing the water turbidity (Brumbaugh et al. 2006). Therefore, oyster reefs could help reduce the estuarine eutrophication (e.g., Baird et al. 1989, Jackson et al. 2001, Pietros et al. 2003, Kemp et al. 2005, Newell et al. 2005).

Shoreline Protection and Sediment Stabilization

Oyster reefs provide protective influence on shoreline habitat (e.g., Meyer et al. 1997, Piazza et al. 2005, Atlantic States Marine Fisheries Commission 2007, Borsjea et al. 2011, Scyphers et al. 2011). Oyster reefs can dissipate wave energy and alter tidal creek hydrodynamics and increase sediment grain size, which stabilizes bottom sediment, therefore reducing the erosion of the shoreline (Newell 1988, Meyer et al. 1997). Also, the ability to stabilize the sediment could further reduce the turbidity of the water column (Peabody et al.

2008). Furthermore, oyster reefs help disrupt water flow and increase mixing in the water column, which could potentially reduce the development of hypoxic conditions (Atlantic States Marine Fisheries Commission 2007). Compared to concrete breakwaters, living ecologically functional oyster reefs provide a more aesthetically pleasing and ecologically sound solution to coastal erosion problems (Meryer et al.1996, Marsh et al. 2002).

Restoration of Eastern Oyster Populations

Populations of Eastern oysters have experienced dramatic declines during the 20th century along the east coast of America (Hargis et al. 1988, Rothschild et al. 1994, Coen et al. 2000, Jackson et al. 2001, Kirby 2004, Lotze et al. 2006, Beck et al. 2009) with a concurrent decline in oyster-dependent ecosystem services, fisheries and coastal economies. Overharvesting (Newell 1988, Dame 1993, Rothschild et al. 1994, Jackson et al. 2001, Beck et al. 2009), habitat loss (Rothschild et al. 1994, Hargis et al. 1999, Smith et al. 2005), disease (Andrews 1988, 1996, Burreson et al.1996), predation (e.g., Soniat et al. 2004) and environmental degradation (Frankenberg 1995, Rothschild et al. 1994) are the primary factors responsible for these population declines. Such decline has dramatically changed the ecology of coastal waters as well as oyster fisheries, and the absence of the ecological functions provided by oysters has threatened the health of ecosystems. For example, Newell(1988) proposed that Chesapeake Bay had changed from a benthic-based ecosystem to a more pelagic ecosystem, and attributed this change to the long-term depletion of more than 99% of the standing stock of oysters. In response to this and similar declines, a large number of oyster restoration projects have been implemented in the United States. Specifically, the recent commercial harvest of the eastern oyster in Alabama has declined in recent years: for example, the 2011 harvest was 313,310 pounds, relative to peaks within the last 20 years such as the 1992 harvest of 1,201,799 pounds. This surge in restoration projects has led to a tremendous amount of research into the effectiveness and impacts of these projects, which are reviewed below briefly.

Review of Restoration Projects

Enhancement of fisheries

Enhance oyster population

One of the most direct contributions of restoration projects is the enhancement of the oyster population, both locally at the restoration site and regionally. In 2008, Swann (2008) conducted an oyster population survey on a recently installed restoration site in Billy Goat Hole, Dauphin Island. The restoration materials were Wave attenuation devices, or WADs^{TM}, and were installed in November 2006. The result of the survey showed that the population on the WADs^{TM} was 205 oysters/M^2, which exceeds the densities of Alabama’s most productive oyster reefs which are located 4 km north at Cedar point (150 oysters/M^2) (Alabama Department of Conservation and Natural Resources, Marine Resources Division, personal communication).

Also in Alabama, the populations of oysters at restoration sites at Alabama Port and Coffee Island have been well monitored by Dauphin Island Sea Lab personnel (2011). All the restoration materials were installed in the spring of 2010. The data showed that during the year 2010 to 2011, the abundance of live oyster at Coffee Island increased slightly from September 2010 to January 2011. And from January 2011 to April 2011, the abundance of live oysters had significantly increased and reached about twice the abundance of the previous population.

Coen (2008) sampled the oyster density in five restoration sites in South Carolina**.** Those restoration sites were built from 2002 to 2006. The density results show the mean densities of recruits and total oysters at all five sites were higher than the respective mean density from 45 reefs constructed by South Carolina Department of Natural Resources throughout the state.

Not all restoration sites show positive growth. In Maryland's portion of Chesapeake Bay, restoration projects aimed at increasing the oyster population 10-fold from 1994 to 2010. Jordan (2002) surveyed the biomass of the oyster population in this restoration sites. The result of this survey showed the population biomass of the oyster had decreased 3-fold from 1986 to 2001. There were also important spatial differences in population structure. In 2007, the Maryland Department of Nature Resources monitored the oyster populations in the same area. The report of this survey indicated that the population of oysters in the year 2007 was below the oyster population of 1994. But in some of the sites, 5 to 100- fold increases in site-specific sanctuary oyster populations had been observed. Most of these restoration sites were low-salinity sites. At the high-salinity sites, the population of these sites seemed restricted by Dermo and MSX disease. This finding was put forward to explain why the restoration project did not increase the oyster population by the hoped for 10-fold increase.

Enhance the biomass of other marine and estuarine species

In Chesapeake Bay, Rodney et al. (2006) observed the density of macrofauna was an order of magnitude higher on restored reefs, epifaunal density was more than twice as high on restored reefs and sessile macro faunal density was two orders of magnitude higher on restored reefs. Three out of the five dominant taxonomic groups were much more abundant on restored plots. Mean amphipod density was 20 times higher on restored plots and densities of xanthid crabs and demersal fish were both four times greater on restored plots. Two out of four functional feeding groups: suspension feeders and carnivore/omnivores were more abundant on restored plots.

Harding (2010) sampled the population of blue crab though May to October during 2006 and 2007 at Shell Bar oyster reef in Great Wicomico River, VA. Approximately 9 million cultchless oysters were planted on Shell Bar as part of the oyster restoration project between May and October in 2006. The result suggested that the annual average weekly CPUE (catch per unit effort) of year (13.42 crabs per pot; SE = 1.57) and the maximum CPUE (32.06 crabs per pot) was twice as high in 2006 as it was in 2007. This observed inter-annual differences in crab CPUEs may be the result of an enhanced forage base for the oyster restoration planted in the year 2006.

Along the coast of Alabama, Gregails (2009) sample three reefs locating in Cedar Point, Sand Reef and Bon Secour Bay. In this study he observed there were substantial differences in reef community among the three sites and, compared with unstructured bottoms, increased abundance of several species of small demersal fishes and sessile invertebrates. In another study, Scyphers and Powers (2011) found that blue crab, penaeid and caridean shrimp, and juvenile silver perch were more abundant near oyster reefs than mudflat controls. Also spotted sea trout, drum and flounder were substantially enhanced by oyster reefs at Alabama Port. For the newly built restoration sites, Alabama Port and Coffee Island, seine data showed more fish were caught at both sites during post-restoration sampling events compared to pre-restoration sampling. Also gillnet data at both sites showed both similarities in species richness across treatments for both sampling periods and an increase in species richness from the pre to post-restoration sampling period (Alabama Economic recovery and Ecological restoration Project Post-Restoration Monitoring Report, 2011)

Model has been developed to estimate the fisheries production of the oyster reef as well. For example, Peterson et al. (2003) quantified the enhancement of abundance of fishes and large mobile crustaceans on oyster reefs in the southeastern United States by model. In this study, the density of each species by size class on restoring oyster reef and sedimentary bottom was compared as a means of estimating the degree to which restoration of oyster reef could augment abundances of associated species. The result suggested that each 10 M^2 of restored oyster reef in the region is expected to yield an additional 2.6 kg yr^{-1} of production of fish and large mobile crustaceans for the functional lifetime of the reef. A reef lasting 20 to 30 years would have been expected to augment fish and large mobile crustaceans production by a cumulative amount of 38 to 50 kg 10 M^{-2}.

Enhancement of Water Quality

Oyster reefs could also affect water quality through the water filtering ecosystem service. Brumbaugh (2006) used Lynnhaven River, a small tidal tributary in the southern Chesapeake Bay, as a model for both evaluating the efficacy of restoration approaches and developing scaling arguments for the larger Chesapeake Bay-wide restoration effort. Oyster biomass was used to calculate the filtration capacity. The results showed that the restored oyster reefs were able to filter a volume equivalent to the river's volume every 63.5 days.

Water Quality Measurement

Particulate organic carbon, ammonia, and total suspended solids are the most common water quality parameters. Particulate organic carbon (POC) is a pertinent signal to investigate in a marsh estuarine ecosystem because it is the major source of food to the microorganisms.

Ammonia is a product of metabolism in many animals, including oysters. It could increase the eutrophication of the estuarine. Total suspended solids could indicate the turbidity of the water column, which could impact the light penetration, which could further impact the establishment of aquatic vegetation.

Control of the biomass of phytoplankton is also a very important ecological service that oyster reefs provide. One of the most common ways to estimate phytoplankton biomass is through monitoring the abundance of Chlorophyll a (Chl-a).

Dame (1984) suggested that oyster reefs worked as estuarine materials processor in the system. In this study, Dame used a flow-through plastic tunnel to monitor the water quality measurements. The result suggested that oyster reefs were probably a sink for particulate organic carbon (POC) and this attenuated the POC signal in the water column passing the oyster reef as well. Chl-a decreased as the water passed through the reef, indicating that phytoplankton was taken up as it passing over the reef. Ammonia was always higher in the output than in the input water; the oyster reef is a source for ammonia and is an amplifier of then ammonia signal in the creek.

In Hewletts Creek, in southeastern of North Carolina, Cressman (2003) examined the effects of intertidal oyster reefs on Chl-a, fecal coliform bacteria and total suspended solids concentration under field conditions in a tidal creek estuary. Samples were taken upstream and downstream of each reef and also from a control area. The results differed between summer and spring. In summer, Chl-a showed a consistent and statistically significant decrease by 10% to 25% as water moved through the reef. Though not significant, the count of fecal coliform also decreased from upstream to downstream. In the spring, Chl-a decreases were less frequent than in summer, but significant fecal coliform decreases were more frequent. Despite the differences between seasons, the results show the reduction in both Chl-a and fecal coliform after passing through the oyster reefs.

Also in Hewletts Creek, Nelson (2004) examined the effects of small-scale oyster additions on sediment loading, Chl-a, nutrient concentrations and flow in small tidal creeks. This experiment started in September 2000 and lasted to June 2001. All the data were measured upstream and downstream of each created reef and from a control non-reef area adjacent. This study has the similar result as Cressman (2003). The mean total suspended solid (TSS) concentrations of downstream of reef began to reduce right after the first month of placement. Although not statistically significant, TSS concentrations downstream of the reefs were less than upstream concentrations for five out of nine and five out of seven post-reef sampling months for the upland and the lower creek sites, respectively. Chl-a concentrations did not differ significantly after the initial reef placement (2x3 m), but were reduced substantially after reef enlargement (3x4 m) in one of the experimental creeks. Ammonium concentration downstream of the reef placement was significantly increased.

Modeling is another way to analyze the potential for oyster reefs to affect water quality. Based on their spatially detailed Chesapeake Bay model, Cerco and Noel (2005) concluded that the ability of nitrogen removal via oyster restoration was significant. In 1992, Ulanowicz developed a quasi-equilibrium, mass action model of the exchanges transpiring in the Chesapeake Bay mesohaline ecosystem to evaluate the potential benefits of oyster restoration reef in Chesapeake Bay. The output from the model suggested that increasing oyster abundance would decrease particulate organic carbon, phytoplankton productivity and the stock of pelagic microbes, ctenophores, medusa, and particulate organic carbon. Similar conclusions were reached by Gerritsen et al. (1994) and Brumbaugh et al. (2000). All of these results suggest that the oyster reefs have the ability to remove seston from the water column, and reduce suspended sediment, detritus, and particulate nutrients.

Some studies, however, have reached different conclusions. Plutchak (2010) monitored the ammonium, Chl-a, and metabolic response variables in response to oyster reefs placed in tidal creeks around Dauphin Island, Alabama. The results indicated that the oyster reefs did not seem to reduce water column particulates or have an impact on phytoplankton or microphytobenthic biomass or productivity. The author noted that the young age of the restoration site and the low density of the oysters could be the reason for this result.

Phytoplankton Control

The ecosystem service of controlling phytoplankton biomass has been studied as well.

Newell (1988) pointed out oyster reefs could reduce the nutrients in the water column, and this would affect the growth of phytoplankton. He suggested that the increase in zooplankton biomass with decreased oyster abundance was support for this hypothesis.

Fulford et al. (2007) developed a filtration model to estimate the effect of bivalve restoration on the rate of phytoplankton removal over a range of spatial and temporal scales in Chesapeake Bay. They found out that the current (2007) scope and scale of restoration sites was unlikely to result in significant bay-wide reductions in phytoplankton biomass. This could be attributed to the low biomass oysters at restoration sites and several spatial and temporal mismatches between oyster and phytoplankton biomass that limited the ecosystem benefit of oyster restoration. This study, however, found the enhancement of submerged aquatic vegetation in Chesapeake Bay. In another study (Fulford et al. 2010), a network simulation model was developed to address the difficulty of analyzing the impact from multiple stressors. This model was intended specifically to examine ecosystem-level responses to management; this model was applied to allow a comparison of nutrient load reduction and restoration of highly reduced stocks of Eastern oyster in Chesapeake Bay. The model showed the different pattern of the oyster reef controlling the phytoplankton in spring and reducing the secondary pelagic production of spring and summer. The model suggested that the biomass phytoplankton production could be reduced if the nutrient inputs could be reduced by 50%. The model also predicted that low levels of oyster restoration would have no effect in the spring but in summer the oyster restoration would result in a reduction in phytoplankton standing stocks. In the model, low levels of oyster did not have significant effects on the phytoplankton due to the size-selective filtration and also the seasonality of the heavy grazing on the phytoplankton was ahead of the maximum top-down control of oysters. The model suggested that in order to have efficient control over the phytoplankton, the abundance of oysters at restoration sites would need to be increased as much as 25-fold present biomass and as much as 50-fold to achieve the nutrient reduction of 50% in the whole bay.

Shoreline Stabilization

The reef restoration also has the ability to stabilize the sediment. The result of Hargis et al. (1999)'s study suggested that restoration reefs tend to redistribute the sediment. Also the result of the latest report from Dauphin Island Sea Lab (Alabama Economic Recovery and Ecological Restoration Project Post-Restoration Monitoring Report 2011) was that the restoration sites in Coffee Island and Alabama Port tend to build more stable sediment than the controlled areas.

Submerged aquatic vegetation (SAV) could increase the benthic dissolved oxygen, stabilize the sediment and build up benthic ecosystem as well. Cerco and Noel (2007) developed a model to estimate the abundance of the SAV as a function of oyster abundance. They found that the SAV biomass increased from 33% to more than 100% in some sites due to installed of the oyster restoration sites. Laboratory studies (Newell et al. 2002) confirmed the enhancement of microphytobenthios and denitrification through oyster restoration in Chesapeake Bay.

Estimates of the potential spawning contribution of restored oysters

Despite the extensive analysis and documentation of ecosystem services provided by oyster restoration projects, there appears to be no quantitative, field-based assessment of the potential spawning contribution from restored oyster populations. This potentially significant ecosystem service is typically simply presumed and calculated based on average published values of oyster fecundity.

Oyster reproduction biology

The reproductive system of Eastern oyster has been well studied. The reproductive organ of the oyster is gonad that located between the digestive gland and the mantle. The germinal epithelium usually develops into oocytes and spermatocytes by the end of the first year (Galtsoff 1964) when the oyster becomes sexually mature. During the dormant phase, the gonad cannot be distinguished grossly from the surrounding vesicular connective tissue. In the spawning season, however, the sex of the oyster can be determined by the presence of egg or sperm. Additionally, the Eastern oyster is protandric. According to Andrews (1979), 90% of the oysters smaller than

35 mm shell height were male in the James River, Virginia. The proportion of functional females in each size class increased with size.

Quantitative method to estimate the fecundity of oyster

A number of quantitative methods have been developed to estimate the spawning potential of oysters, including direct counts of eggs released by individual oysters in a laboratory setting (Galstoff 1930, with improvements by Davis et al. 1956), histological examination of the gonads, including determination of gonad/body ratios (Lannan et al. 1980), and enzyme-linked immunosorbent assays, or ELISA (Choi et al. 1993). Each of these methods, however, has important limitations or constraints. A direct count of eggs released by individual oyster is a simple, low cost method to estimate the fecundity of oysters. Stimulating the oysters to spawn in the lab poses logistical difficulties, especially when multiple sites or treatments are being compared. Histological analysis of gonadal condition is a high-cost and time-demanding.(Hopkins 1931, Hopkins et al. 1953, Loosanoff 1969, Hayes et al. 1981, Gauthier et al. 1989). Even though ELISA (Choi et al. 1993) is extremely accurate, the cost for the equipment and analysis is high.

For this study, we used the Cox and Mann (1992) approach. In this method, the soft tissues of a single whole oyster are macerated in a blender and sieved, retaining the tissue on the screen and allowing the eggs to pass through. The eggs are retained in solution, allowing a determination of eggs per ml. Although this method does not yield the actual number of eggs released during spawning, it does provide an estimate of spawning potential. It also has the advantages of being relatively inexpensive and simple enough to allow sampling of multiple populations over time and/or space.

This project is the first to quantify the potential spawning contribution from oyster restoration, as one of the potential ecosystem services provided by restoration. This study will provide first estimates of the magnitude and variability of the spawning contribution from eastern oyster restoration projects in coastal Alabama. These data can provide a baseline for comparison among oyster restoration projects elsewhere. Furthermore, within Alabama, this work could be used to compare different restoration materials and different years. Collectively, this work and other studies like this could improve the planning of future restoration projects and gives a more complete value of the benefits derived from oyster restoration.

Materials and Methods

Site Selection

Five fully completed oyster restoration sites were selected along the Alabama coast: Billy Goat Hole, Alabama Port, Coffee Island, Little Bay and Point Aux Pines (Fig. 1, Table 1). Across the five restoration sites, a total of five different restoration materials were used (Fig. 2): Reef Balls™, Reef Blocks™, bagged oyster shell, wave attenuation restoration materials (WARMs)_, including wave attenuation™ (WADs™) and wave attenuation units (WAUs), and loose oyster shell. All sites were within 100 m of the mean high tide line and were in water no more than 1.5 m deep. At two sites (Alabama Port and Coffee Island), three restoration materials had been arranged in a formal replicated experimental design (with appropriate non-restored controls) to rigorously compare the performance of these materials in a separate study (Figs. 3 & 4.).

Sampling Date

In the Gulf of Mexico, prior work suggested that oysters begin to spawn when the water temperature reaches 25°C (Wallace 2001, Hayes et al. 1981). According to the National Oceanographic Data Center, in 2011 the water temperature in Dauphin Island reached 25°C in June 2011, and dropped below this consistently in October. Therefore, we collected oysters once a month from each site (including each sampling location) beginning in June and concluding in November 2011 (7 sample periods total).

Figure 1. Locations of the five oyster restoration sites sampled along the coast of Alabama, June - November 2011.

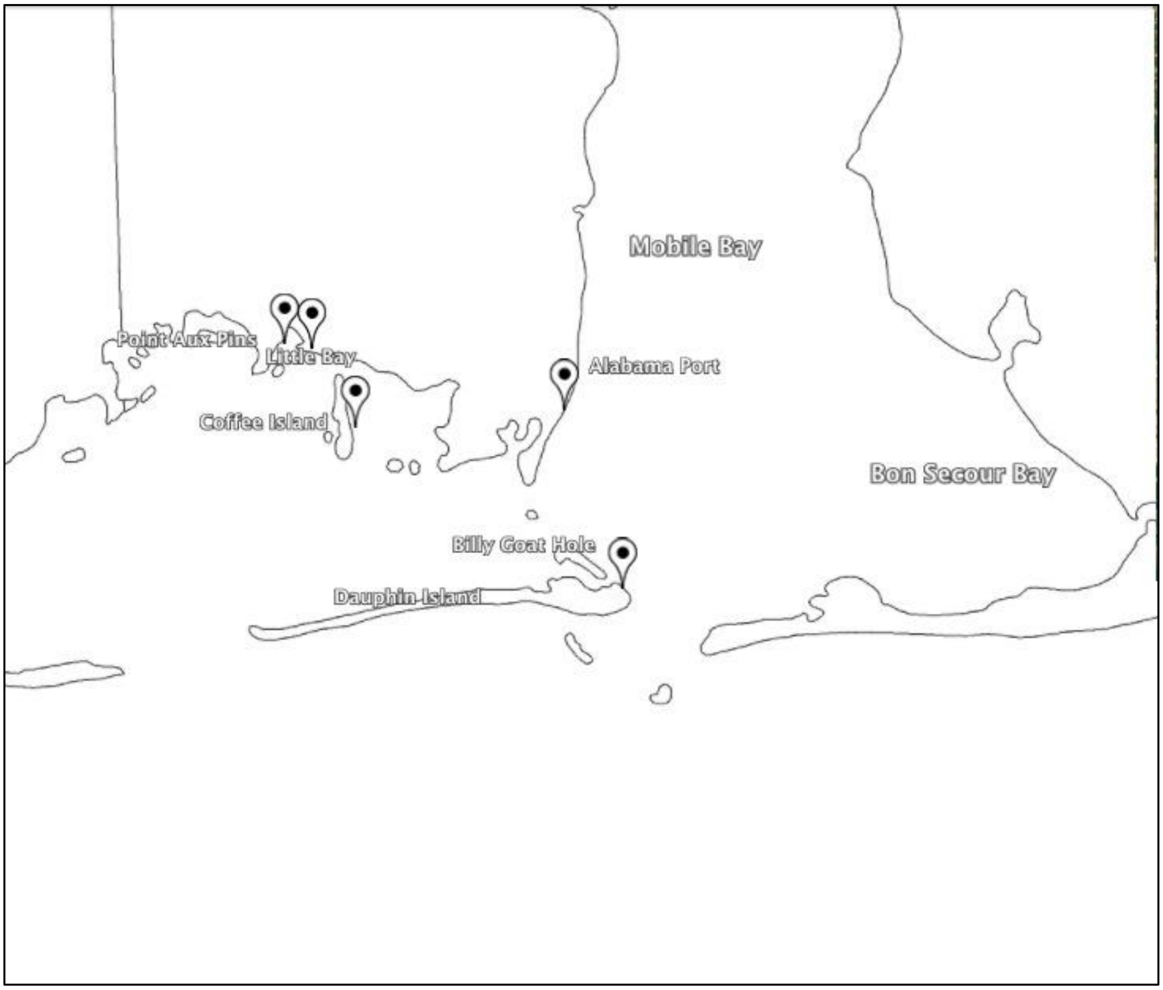


Table 1. Descriptions of the five oyster restoration sites (and five restoration materials) installed in coastal Alabama waters.

Site	Restoration Materials	Date of Installation	Date of Completion	Surface Area M^2
Coffee Island	Reef Blocks™	April 2010	November 2010	278
	Shell Bag			2,200
	Reef Balls™			2,376
Alabama Port	Reef Blocks™	March 2010	March 2011	139
	Shell Bag			1,100
	Reef Balls™			1,188
Little Bay	WADs™	January 2010	April 2010	3,557
Point Aux Pins	Loose Shell	September 2009	April 2011	1,500
Billy Goat Hole	WAUs	April 2005	April 2005	520

Figure 2. Five different restoration materials used at the Alabama oyster restoration sites sampled in this study: A. Reef Balls™; B. Reef Block; C. Bagged oyster shell; D. Wave attenuation restoration materials (including WADs™ and WAUs); and, E. Loose oyster shell.

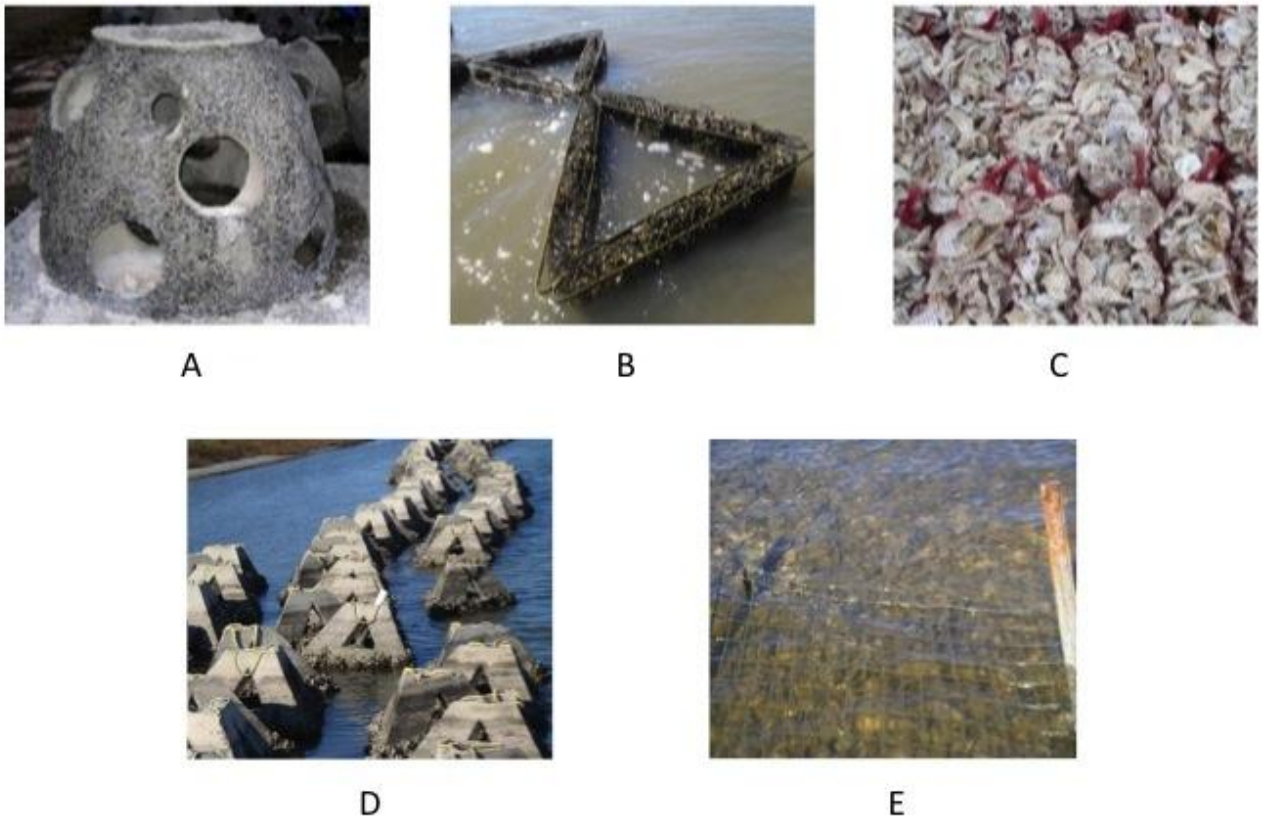


Figure 3. Arrangement of restoration materials and other experimental treatments at the oyster restoration site at Alabama Port, AL. (Dauphin Island Sea Lab, 2011)

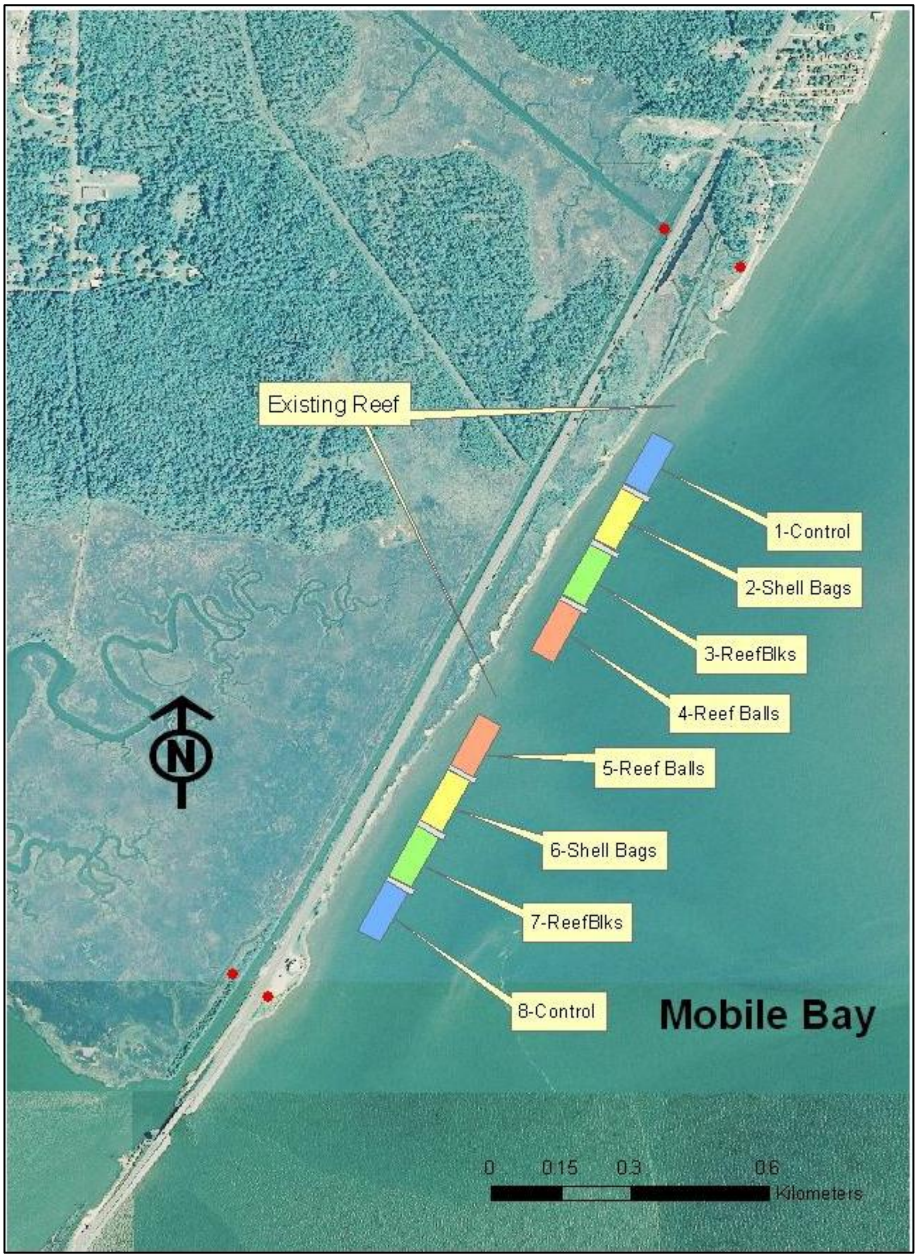
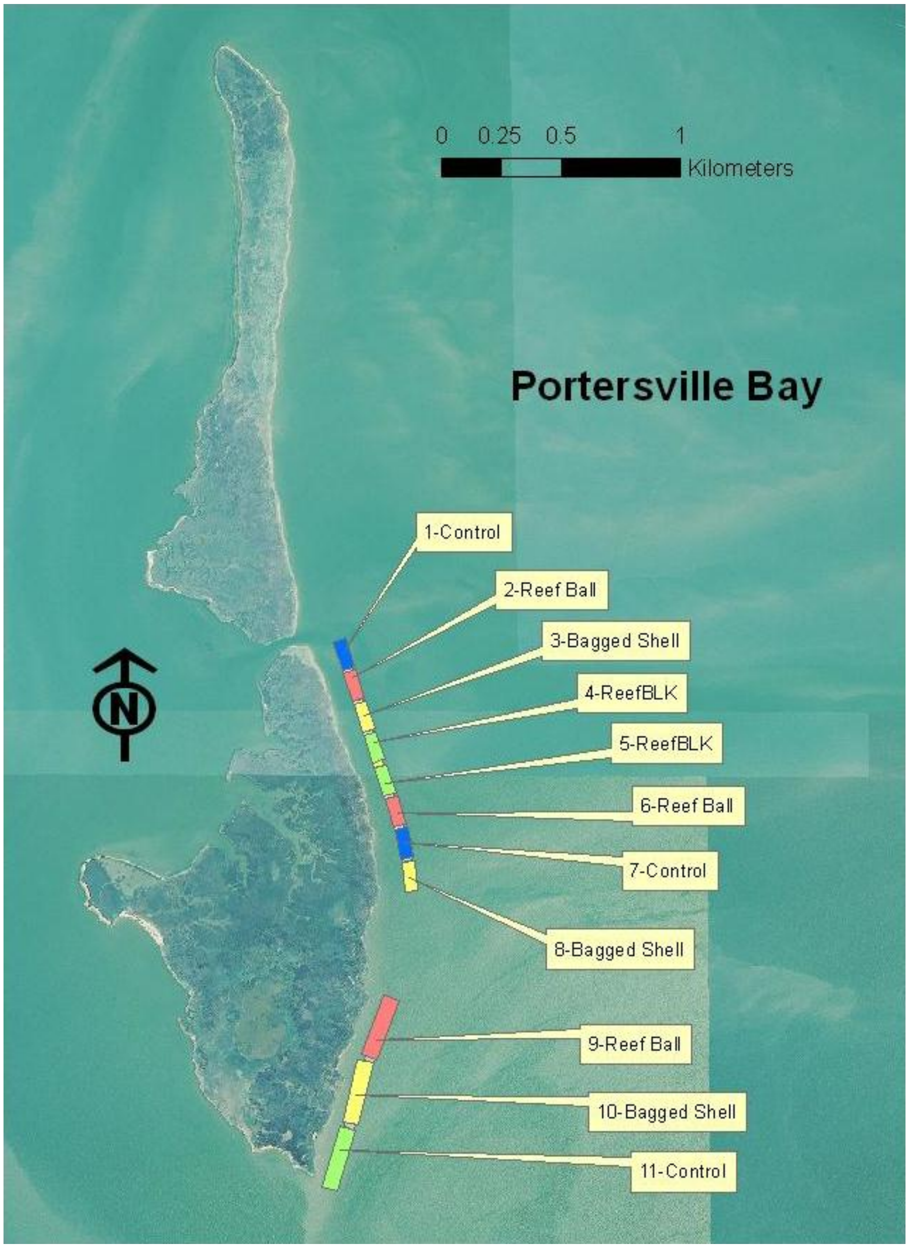


Figure 4. Arrangement of restoration materials and other experimental treatments at the oyster restoration site at Coffee Island, AL. (Dauphin Island Sea Lab, 2011)



Sample Collection

Although *C. virginica* has been found to be capable of becoming sexually mature within four months in some warm area such as Gulf of Mexico (Wallace 2001), the smaller sizes are predominantly male. Andrews (1979) reported that 90% of the oyster population < 35 mm (shell height) was male in the James River, Virginia. Even in oysters that were 50-70 mm shell height, Burkenroad (1931) found a majority of males. Given the focus on egg production, we sampled oysters ≥ 30 mm (shell height). Furthermore, sampled oysters were grouped into two size categories: "sub-legal" (30-75 mm) and "legal" (> 75 mm). At each sampling, up to 30 oysters of each size category were haphazardly collected from each restoration materials at each site. In some size categories at some sites, less than 30 oysters were found total. In those cases, we collected as many as could be found within a reasonable period of time (i.e., ~1 hr). Given these limitations on numbers of oysters per sample within a size category in some cases, the average fecundity per female was estimated only if ≥ 5 oysters were collected. Similarly, condition index was determined only if ≥ 15 oysters were collected. In cases where we found <5 oysters in a sample, only the maximum potential fecundity was determined for the sample. All oysters were collected by hand. For bagged oyster shell and Reef Blocks™, at least 3 different units were opened for sampling during any sample period.

Physical Condition

For each size category of each sample collection from each sample location and time, we measured shell height, shell width, and shell length of all collected oysters using Mitutoyo calipers (± 0.01 mm). If at least fifteen oysters had been collected in a sample for a given size category, five oysters were selected haphazardly for determination of condition index using the methods of Abbe & Sanders (1988). All oysters that were used for the condition index were cleaned of fouling and any commensal organisms and washed with tap water. After drying at room temperature, individual whole wet weights were measured with a Mettler Toledo scale (± 0.0001 g). Oysters were then shucked and the shells and soft tissues were separated. The shells were allowed to dry at room temperature for 48 hrs (± 2 hr), and then weighed to obtain individual dry shell weights. Soft tissues were placed in a VWR aluminum dish and dried at 80°C in Fisher Isoptmp drying oven for 48 hrs (± 2 hrs) and then weighed to obtain individual dry tissue weights.

With these data, we calculated condition index (CI) using Abbe & Sanders (1988) equation: CI = [dry tissue weight (g)/whole wet weigh(g)-dry shell weight(g)] x 100. Shell cavity volume, in turn, was estimated as the difference between the whole wet weight of the oyster (g) and the weight of the dry shell (g).

Quantitative Estimates of Egg Production

For each oyster not used for condition index (above), we shucked the oysters and used a scalpel to extract gonadal material from the body. The gonadal material was examined microscopically (Meiji Techno 10x) for the presence of identifiable eggs or sperm. This allowed determination of the sex ratio for each sample, and identified females for subsequent enumeration of egg production.

To determine the potential fecundity of females, we used a modified Cox & Mann (1992) methodology (By use low speed instead of median, also we change the mesh size of sieve from 90 µ, 53 µ to 100 µ and 50 µ). In this method, the whole body of each female oyster is removed and blended for 30 seconds at low speed in a commercial blender (Waring) with 150 ml of 1 µ-filtered seawater (brought to 20 psu by dilution or addition of artificial sea salts, as appropriate). After blending, the resultant homogenate was sieved sequentially on 100 µ and 50 µ mesh. The blender and sieves were then rinsed with an additional 750 ml of identically filtered seawater. Afterwards, filtered seawater was added to bring the volume to 1 L as necessary. From this mixture, 10-1,000 µl (depending on the concentration of eggs) triplicate sub-samples were removed; 1,000 µl samples with more than 200 eggs were diluted by a factor of 10 to 100. All eggs in each triplicate sample were then counted on a Sedgwick-Rafter cell. This was repeated for each identifiable female oyster in every sample.

Density of Oysters

In this study, size-specific oyster densities at all the restoration sites except the WADs™ in Billy Goat Hole were monitored by researchers at the Dauphin Island Sea Lab (DISL), Dauphin Island, AL. Briefly, the methods used at each site are reviewed here. In loose oyster shell (only Point aux Pins), the density survey was conducted twice in 2011 (April and November). During the survey, the whole site was divided into four equal parts and within each part, 18 samples were taken. For each sample, all the live oysters in a 0.25 m^2 quadrat were measured and the density of each size class was determined.

The density survey of Alabama Port was done in August 2011 and the density survey of the Coffee Island was conducted in July 2011 (where each site had three restoration materials). For Reef Blocks™ (Alabama Port and Coffee Island), five gallons of shell were collected from each of 6 locations on each reef unit (two on Coffee Island and two on Alabama Port.). All the sampled oysters were counted and measured. For the bagged oyster shell, the same method was applied. Five gallons of shell were haphazardly collected from 6 locations on each Shell bag complex (two on Coffee Island, two on Alabama Port). For the Reef Balls™ treatments, total surface counts were performed by removing two Reef Balls™ reefs each from three locations on each reef complex (where both Alabama Port and Coffee Island have two experimental Reef Balls™ complexes) and counting and measuring all the collected live oysters.

For determination of densities of live oysters on WARMs (WAUs in Little Bay and WADs™ in Billy Goat Hole), the area available for oyster settlement was determined as the surface area between the substrate and the mean high water line for each sampled WARMs (with any openings discounted from the total). Though oysters were observed inside the WARMs, these were determined to be prohibitively difficult to sample and were excluded from the analysis; thus any measures of oyster abundance for sites with WARMs are a conservative underestimate. Due to the low density of oysters on the WAUs in Little Bay, all the oysters on one wall of a single unit were counted and measured, with counts converted to densities.

We conducted the survey of the WADs™ in Billy Goat Hole in December 2011. The 182 WADs™ were divided into three roughly equal zones. Within each zone, six WADs™ were randomly selected, three from the offshore line and three from the inshore line. At each WAD™, one wall was haphazardly selected for sample collection. For each sample collection, a 0.0625 m^2 (0.25m each side) frame was haphazardly placed on the wall between the substrate and high water mark and all oysters within the frame were collected, including any oysters whose umbos were within or under the frame.

All collected samples were kept cool and transported back to the laboratory. At the lab, in each sample all live oysters were counted (including those damaged in the collection). Of the live oysters within a sample, up to 25 oysters were haphazardly selected for measurement. Selected oysters were measured for shell height (to the nearest 0.1 mm), and placed into one of three categories: spat < 30 mm; sub-legal ≥ 30 mm and < 75 mm, and legal≥ 75 mm. The abundances of each size class have divided by the estimated area, to yield size-class specific densities.

Data Analysis

For quantitative egg counts, each of the triplicate counts was multiplied by the total dilution factor, and then averaged to yield the average potential fecundity of each sampled female. To test the hypothesis that month of sampling would affect oyster metrics, an ANOVA was run with month as the fixed factor. Response variable were average potential fecundity, condition index, sex ratio, etc.. Data were rank-transformed to meet the assumptions of the ANOVA.

Once this analysis was performed, it was determined that the last two months (October and November) were significantly different with little to no reproductive activity. Thus, these months were excluded from further analysis. The months (June – September) were then treated as replicates for each site (with sampling times where <5 oysters were collected excluded from the analysis), allowing calculations of the egg production per female, potential spawning contribution per square meter and the potential spawning contribution of each site. The restoration sites were used as the replicates to determine the shell height, sex ratio, condition index and average fecundity per female of these four months. As a response variable of potential ecological interest, maximum potential fecundity was calculated by the maximum fecundity per female that can be found each month and each site.

Finally, to allow comparison across restoration sites, a number of response variables were combined with the estimates of size-class specific densities and the total restoration area. For example, the total potential spawning contribution of oysters at a site was determined by multiplying the size-class specific average per capita egg production by the size-class specific density and sex ratio. This figure was multiplied by the total area available for settlement at the restoration site. This, in turn, was added to the total for the other size category to give a total potential spawning contribution of each site in 2011. We also ran ANOVA to test the effect of restoration site on the potential spawning contribution per square and potential spawning contribution.

Results

Density

Densities and abundances of oysters varied greatly among restoration materials and between size categories (Table 2). Densities of legal size oysters were generally low on the two most recent restoration sites (Coffee Island, Alabama Port), while Billy Goat Hole supported the densest and largest population of legal oysters. There were very dense populations of sub-legal oysters on the Reef Blocks™ and bagged shell at Coffee Island (Table 2). Conversely, no oysters of either size were found on the Reef Balls™s at Coffee Island.

Oyster shell height as a function of site, restoration material and month

Generally, on any restoration materials at any given site, over time there were no large differences among shell heights of the oysters (Figs. 5, 6 & 7). However, a slight increase in shell height across the sampling period was observed in the sub-legal oysters at Coffee Island bagged shell, Alabama Port bagged shell and among the legal size and sub-legal size oysters at the Alabama Port Reef Balls™. The results of the site-specific ANOVA tests (testing the effect of sampling month on average shell height) are in the Appendix. In addition, the legal oysters at Billy Goat Hole had substantially larger average shell height than any other site.

Table 2. Abundances of two size categories of oysters at five oyster restoration sites (and five restoration materials) installed in coastal Alabama, based on population surveys conducted in 2011. All surveys were conducted by personnel at Dauphin Island Sea Lab with the exception of Billy Goat Hole, which was surveyed in November 2011 by the author.

Site	Restoration Materials	Density of Legal Oysters/M^2 (±SEM)	Density of Sub-Legal Oysters/M^2 (±SEM)	Estimated Abundance of Legal Oysters	Estimated Abundance of Sub-Legal Oysters	Estimated Total Abundance of Oysters
Coffee Island	Reef Blocks™	0	102.13	0	28,392	28,392
	Shell Bag	0	214.67	0	472,274	472,274
	Reef Balls™	0	0	0	0	0
Alabama Port	Reef Blocks™	0.38	37.22	53	5,174	5,226
	Shell Bag	1.35	4.79	1,485	5,269	6,754
	Reef Balls™	0.44	21.69	522	25,768	26,290
Little Bay	WAUs	0	1.08(±0.35)	0	3,842 (±1,245)	3,842 (±1,245)
Point Aux Pins	Loose Shell	0.08(±0.05)	14.63(±2.03)	120 (±75)	21,945 (±3,045)	22,065 (±3,120)
Billy Goat Hole	WADs™	38.35(±3.98)	43.87(±4.55)	19,942 (±2,069)	22,812 (±2,366)	42,754 (±4,435)

Figure 5. Average shell height of legal and sub-legal oysters (mm + SEM) sampled from the Coffee Island (Alabama) restoration site as a function of sample month (June-November 2011). A. Reef Blocks ™ restoration materials. B. Bagged shell restoration materials. NF indicates that ≤ 5 oysters were found on the restoration substrate material during the sampling period. Lower case letters for the sub-legal size oysters indicate significant differences.

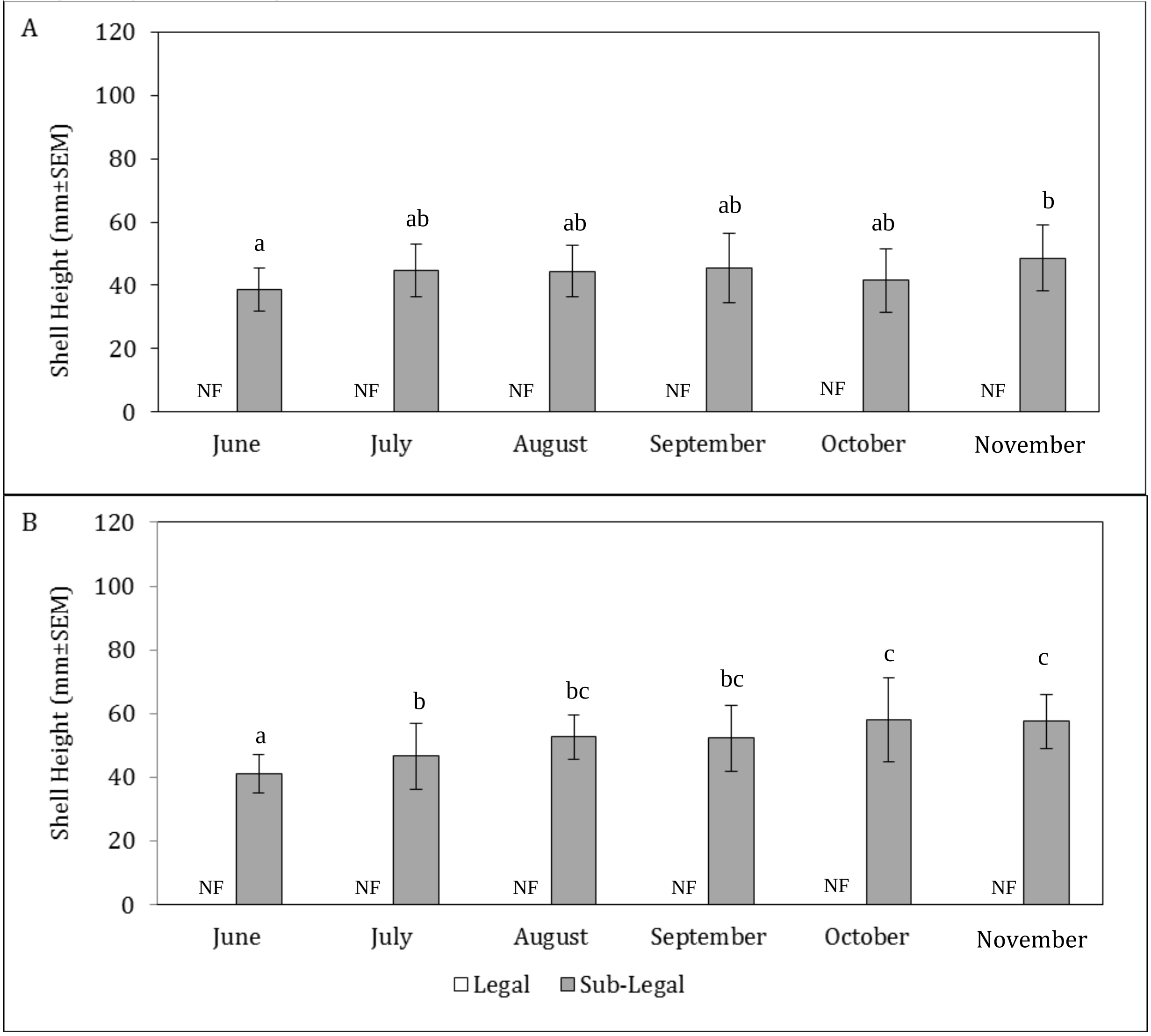


Figure 6. Average shell height of legal and sub-legal oysters (mm + SEM) sampled from the Alabama Port (Alabama) restoration site as a function of sample month (June-November 2011). A. Reef Blocks ™ restoration materials. B. Bagged shell restoration materials. C. Reef Balls™ restoration material. Note that no legal size oysters were found on the Reef Blocks. NF indicates that $\leq$ 5 oysters were found on the restoration substrate material during the sampling period. Capital letters for the legal-size oysters and lower case letters for the sub-legal size oysters indicate significant differences.

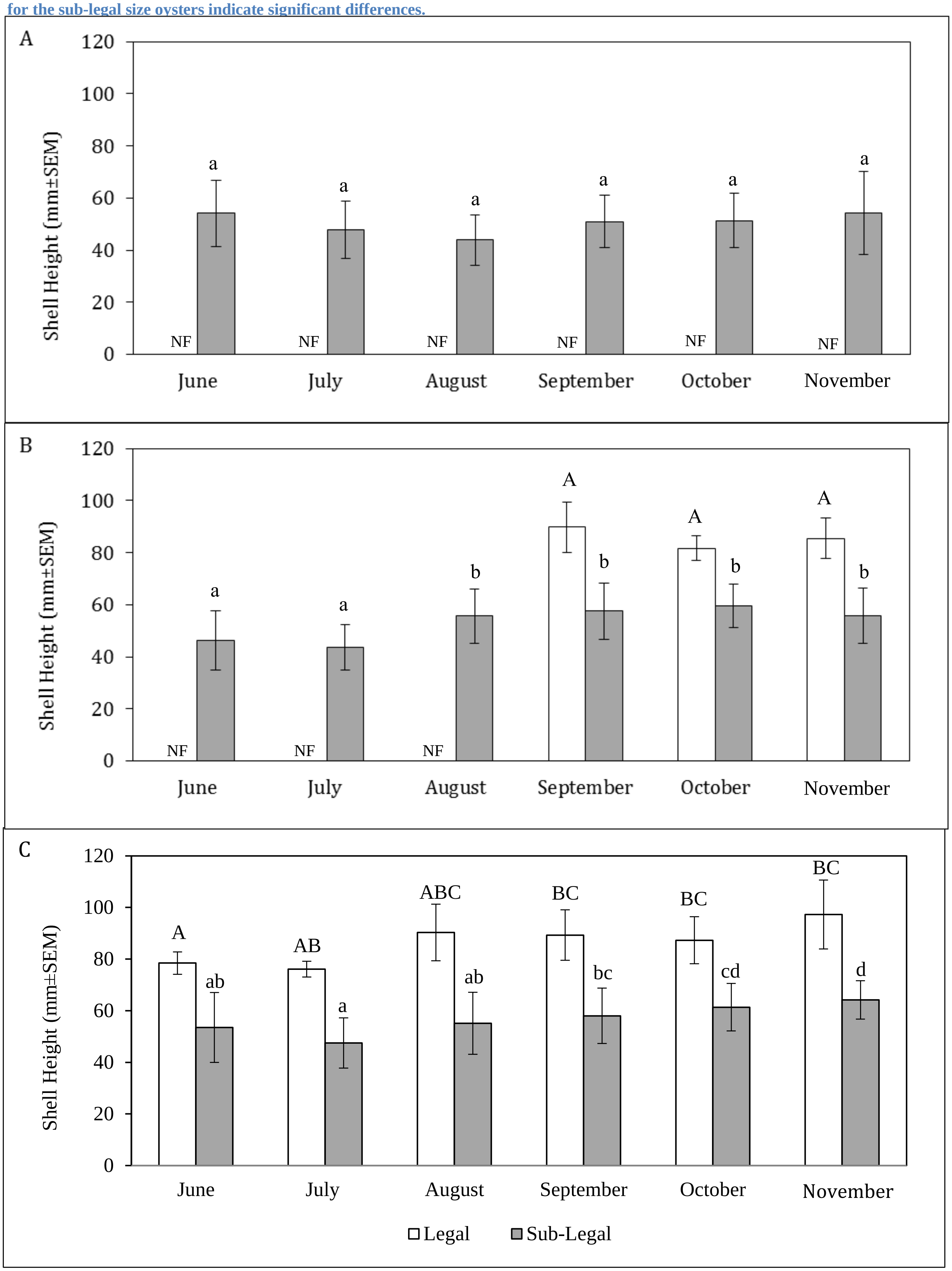
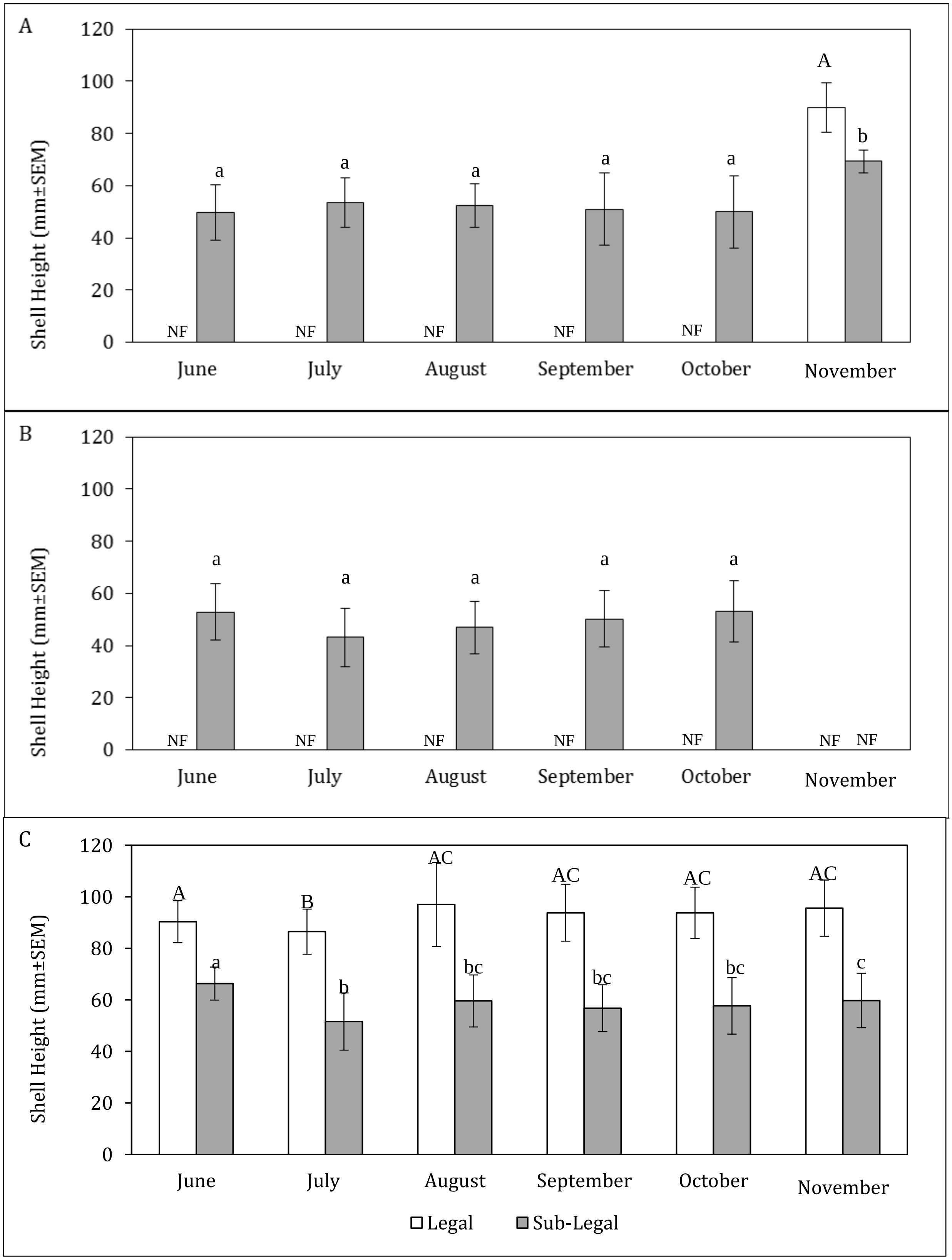


Figure 7. Average shell height of legal and sub-legal oysters (mm + SEM) sampled from three different restoration sites in coastal Alabama as a function of sample month (June-November 2011). A. Wave Attenuation Units (WAUs) in Little Bay. B. Loose shell restoration materials, Point aux Pines. C. WADs^{TM} in Billy Goat Hole. NF indicates that ≤ 5 oysters were found on the restoration substrate material during the sampling period. Capital letters for the legal-size oysters and lower case letters for the sub-legal size oysters indicate significant differences.



Sex Ratio

In both legal and sub-legal oysters (Fig.8), the percent female oysters at the restoration sites was relatively consistent across the spawning season (June-September). Among legal oysters, there population was predominantly female during the spawning season. Among sub-legal oysters, however, the population was predominantly male. In both size categories, there was a large increase in the percent of indeterminate oysters beginning in October and further increasing in November.

Across the sites, the sex ratio varied in both legal and sub-legal size oysters (Fig. 9), but there was no significant different among sites in both sizes oysters (ANOVA, legal: p=0.067; sub-legal: p=0.066).

Condition Index

The condition index of oysters at the restoration sites (Fig. 10) were lowest during the middle of the spawning season. Legal size oysters were lowest in July, while sub-legal size oysters were lowest in August. Condition index for both size categories increased slightly after these lows. Overall, the legal size oysters had a higher condition index than the sub-legal oysters through the season.

Figure 8. Sex ratio of oysters sampled from oyster restoration sites in coastal Alabama (with all sites combined) from June to November 2011. A. Legal sized oysters. B. Sub-legal sized oysters.

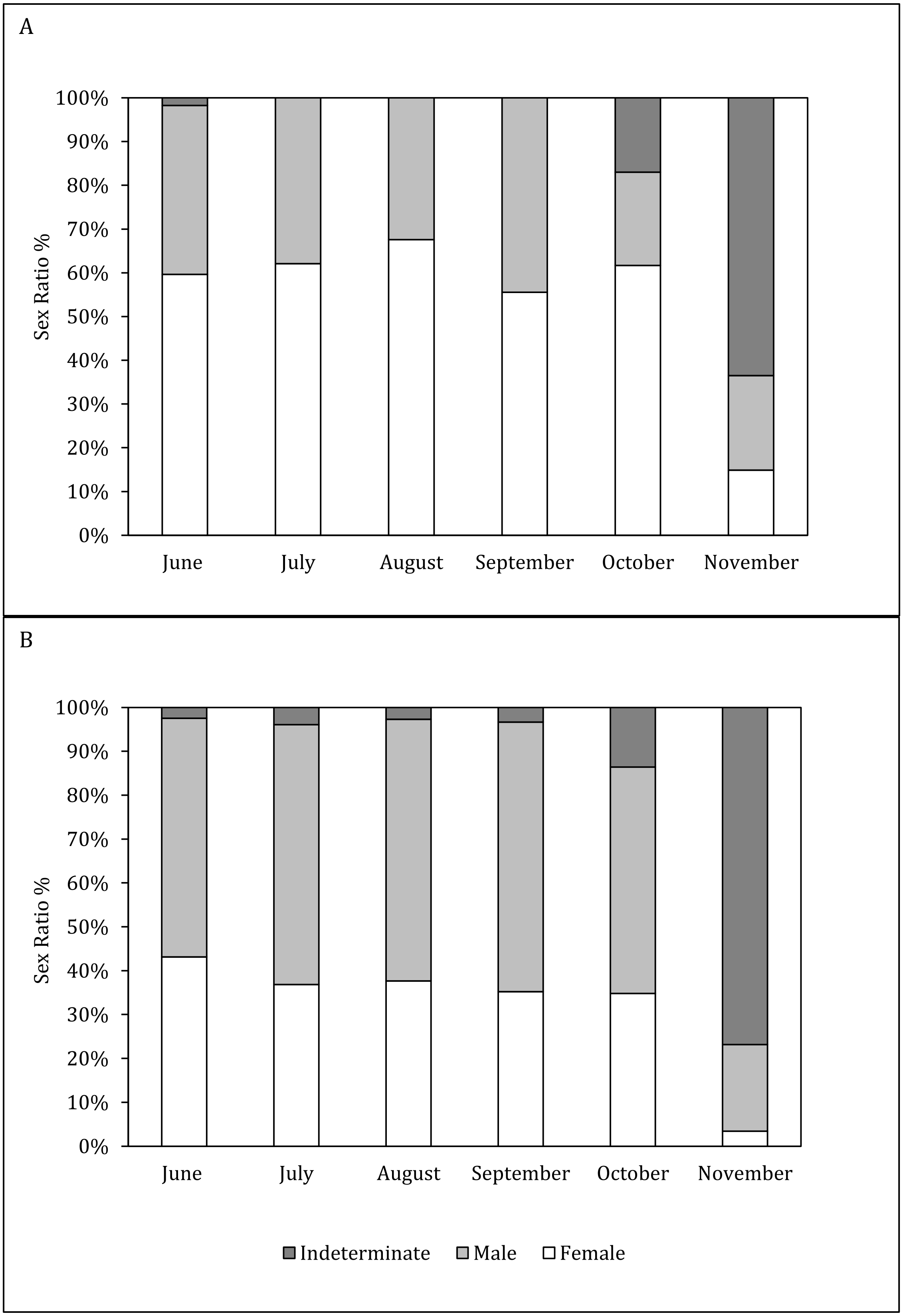


Figure 9. Sex ratio of oyster sampled from each oyster restoration sites in costal Alabama from June to November 2011 (with all months combined). A. Legal sized oyster. B. Sub-legal sized oyster.

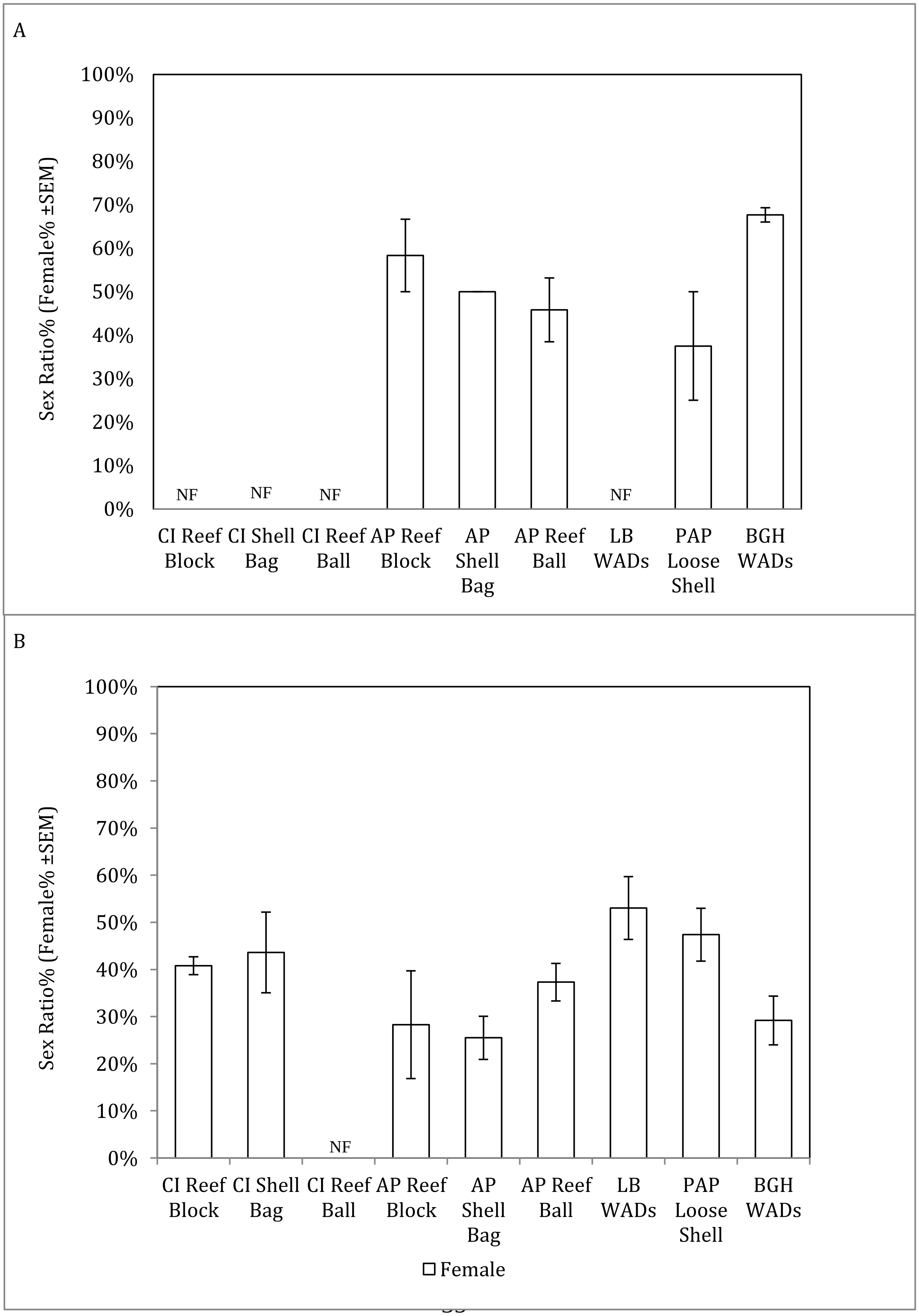
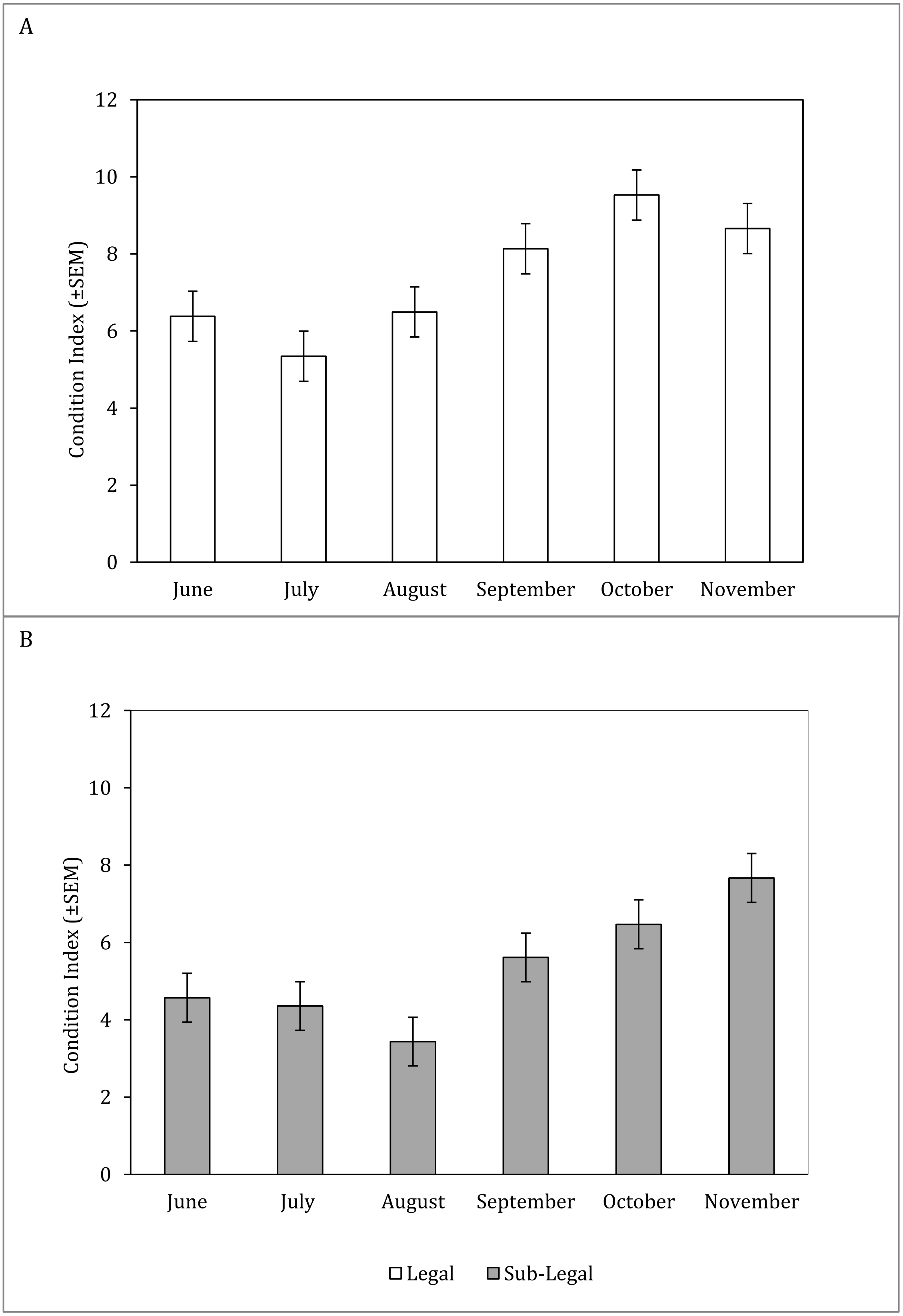


Figure 10. Condition index of oysters (+ SEM) sampled from oyster restoration sites in coastal Alabama (with all sites combined) from June to November 2011. A. Legal sized oysters. B. Sub-legal sized oysters.



Average Monthly Egg Production per Female Oyster

For both oyster sizes, the monthly peak of average egg production per female occurred between June and September (Fig. 11). Among legal size oysters, the peak was in July and was approximately 2.5 million eggs per female. Among sub-legal size oysters, the highest average egg production per female was in September but was similar to June and July. Also, average egg production per female in October and November was much lower than the other four months. Based on an ANOVA of the ranked values, there was a significant effect of month ($p < 0.05$); based on Tukey post-hoc pairwise comparisons, the average egg production per female per month was significantly lower in October and November than the other four months.

Maximum Potential Egg Production per Female

Another potentially ecologically important measure of fecundity is the maximum monthly potential egg production per female (maximum value observed among all the sampled females for any given site and restoration material from June through November). Among the legal size oysters (Table 3), the trend was the same as the average monthly potential egg production per female: the highest count occurred in July (over 20 million eggs) with values decreasing after that. In the sub-legal size class, the maximum egg production was more consistent but substantially lower (with values around 3 million from June through September). For both size classes, these values fell sharply after September.

Comparing restoration materials (Table 4), the highest value for the maximum monthly potential egg production was found among the legal size oysters in Billy Goat Hole. The highest maximum potential egg production among sub-legal size oysters came from Alabama Port Reef Blocks™ (3,770,000). Among legal size oysters, there was substantial variation among the sites;

among the sub-legal oysters, these values were more consistent. Based on these results and those for average egg production per female and condition index, we decided to use June through September as the months to represent the 2011 spawning season for subsequent analyses.

Average Potential Fecundity per Female by Month

Figure 11. Average egg production per female oysters (± SEM) sampled from oyster restoration sites in coastal Alabama (with all sites combined) from June to November 2011. A. Legal sized oysters. B. Sub-legal sized oysters. Note the difference in scales on the y-axes. Untransformed data are presented but significant differences are based on the ANOVA of rank-transformed data. Different letters indicate significant differences

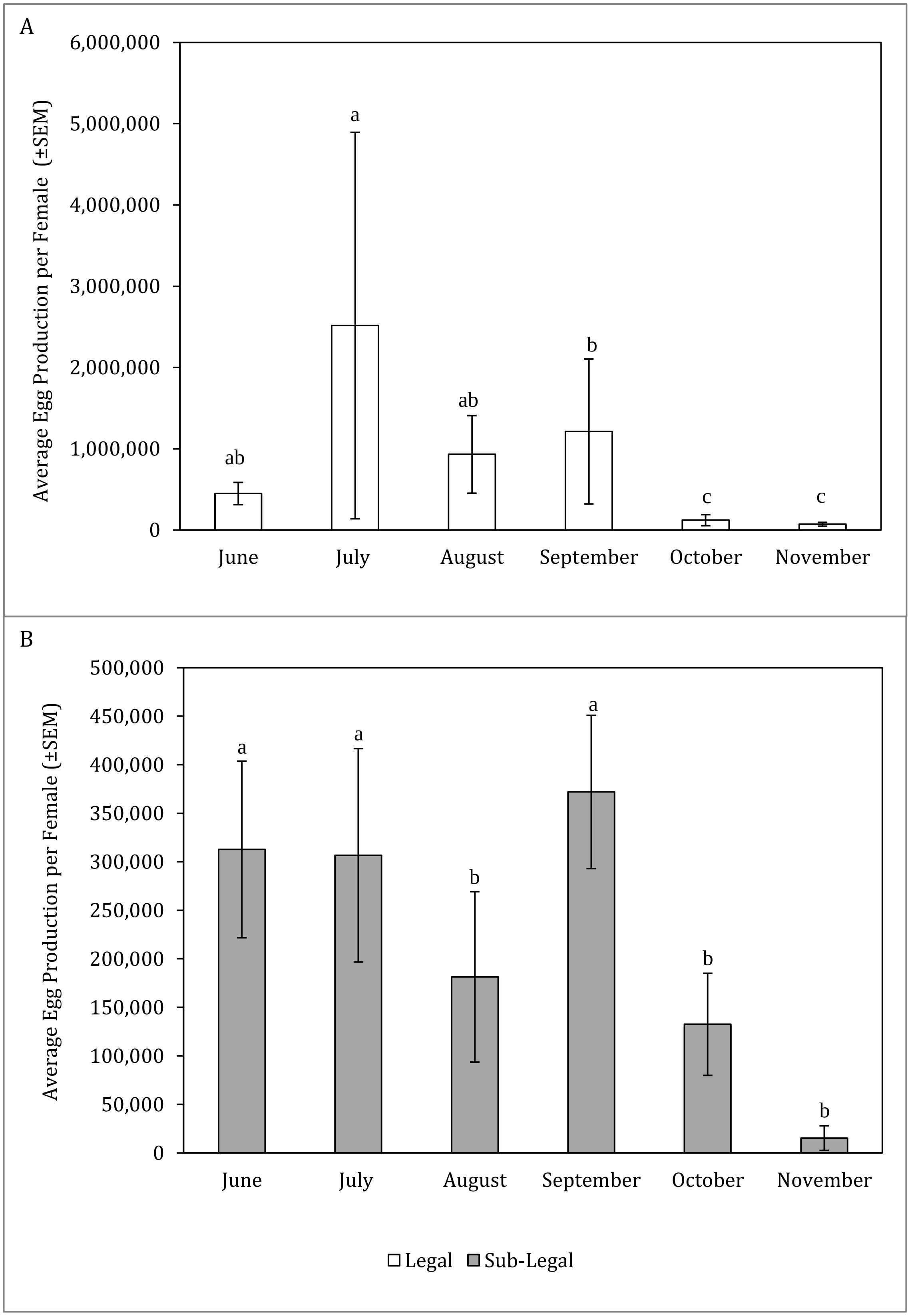


Table 3. Maximum egg production per female oyster at restoration sites in coastal Alabama as a function of sample month (June to November, 2011).

Month	Maximum Egg Production per Female Legal Oyster	Maximum Egg Production per Female Sub-Legal Oyster
June	3,613,333	3,770,000
July	20,033,333	2,706,667
August	7,966,667	3,353,333
September	6,533,333	3,506,667
October	876,667	1,543,333
November	956,667	50,333

Table 4. Maximum egg production per female oysters at restoration sites in coastal Alabama as a function of restoration site and materials. Oysters were sampled monthly from June to November, 2011.

Site	Restoration Materials	Maximum Egg Production per Female of Legal Oyster	Maximum Egg Production per Female of Sub-Legal Oyster
Coffee Island	Reef Blocks™	200,000	1,800,000
	Shell Bag	536,667	2,390,000
	Reef Balls™	0	0
Alabama Port	Reef Blocks™	730,000	3,770,000
	Shell Bag	123,333	3,353,333
	Reef Balls™	1,486,667	3,506,667
Little Bay	WAUs	6,533,333	2,300,000
Point Aux Pins	Loose Shell	840,000	1,600,000
Billy Goat Hole	WADs™	20,033,333	1,790,000

Average Egg Production per Female Oyster among Sites

Average egg production per female varied among sites during the spawning season, defined as June to September (Fig. 12). Notably, no live oysters of the specified categories were found at the following sites during any sampling period: legal size oysters at the Coffee Island Reef Blocks™, shell bags, and Point Aux Pines (loose shell) and sub-legal size oysters at Coffee Island Reef Balls™. Among all the legal oysters (Fig. 12), the WAD's in Billy Goat Hole had the largest average egg production per female (nearly 2 million/female), with all the other sites at substantially lower levels. Point Aux Pins loose shell had the second largest egg count and Reef Blocks™ in Alabama Port ranked third. Among the sub-legal oysters, Alabama Port Reef Balls™ had the highest egg count per female (just over 0.5 million/female), followed closely by the Alabama Port Reef Blocks™. While the legal-sized oysters tended to have higher egg counts than sub-legal sized oysters, at some sites there was no apparent difference between size categories (e.g., Alabama Port Reef Balls™). The variance among legal size oysters was higher than amongst the sub-legal size oysters. Due to the high amount of variation, egg production per female did not significantly differ among the different sampling sites for either size class (ANOVA, legal size oyster: p=0.093; sub-legal size oyster: 0.412).

Relationship between Average Egg Production and Shell Height

To quantify the effect of shell height upon egg production, I conducted a linear regression. The potential egg production was log transformed. There was a very weak positive relationship between egg production and shell height (Fig. 13).

Figure 12. Average egg production per female oyster (+ SEM) as a function of site & restoration materials. Values from June through September 2011 were averaged. A. Legal sized oysters. B. Sub-legal sized oysters. Note the difference in scales on the y-axes. NF indicates that ≤ 5 oysters were found on the restoration substrate material during the sampling period. Capital letters for the legal-size oysters and lower case letters for the sub-legal size oysters indicate significant differences.

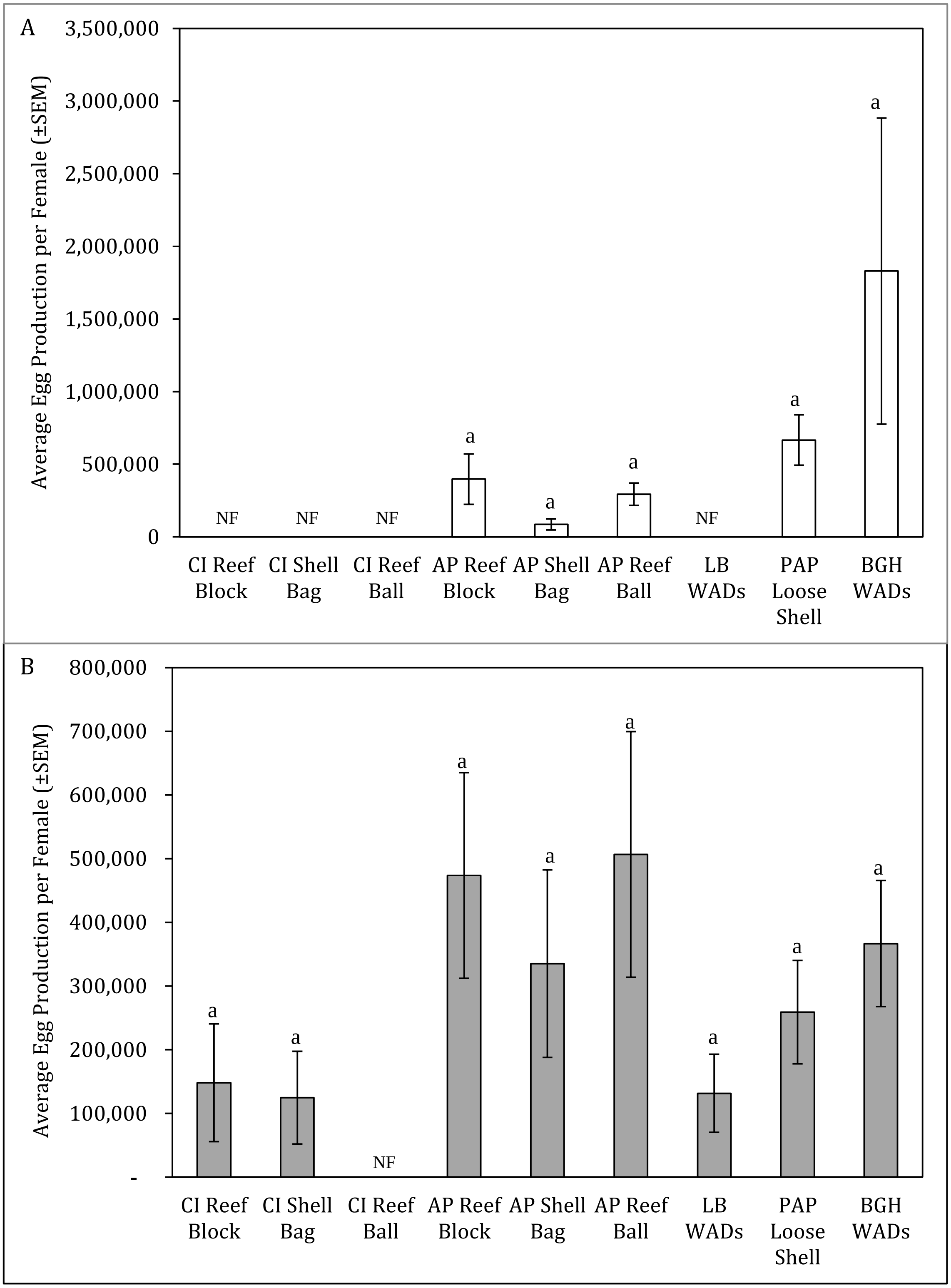
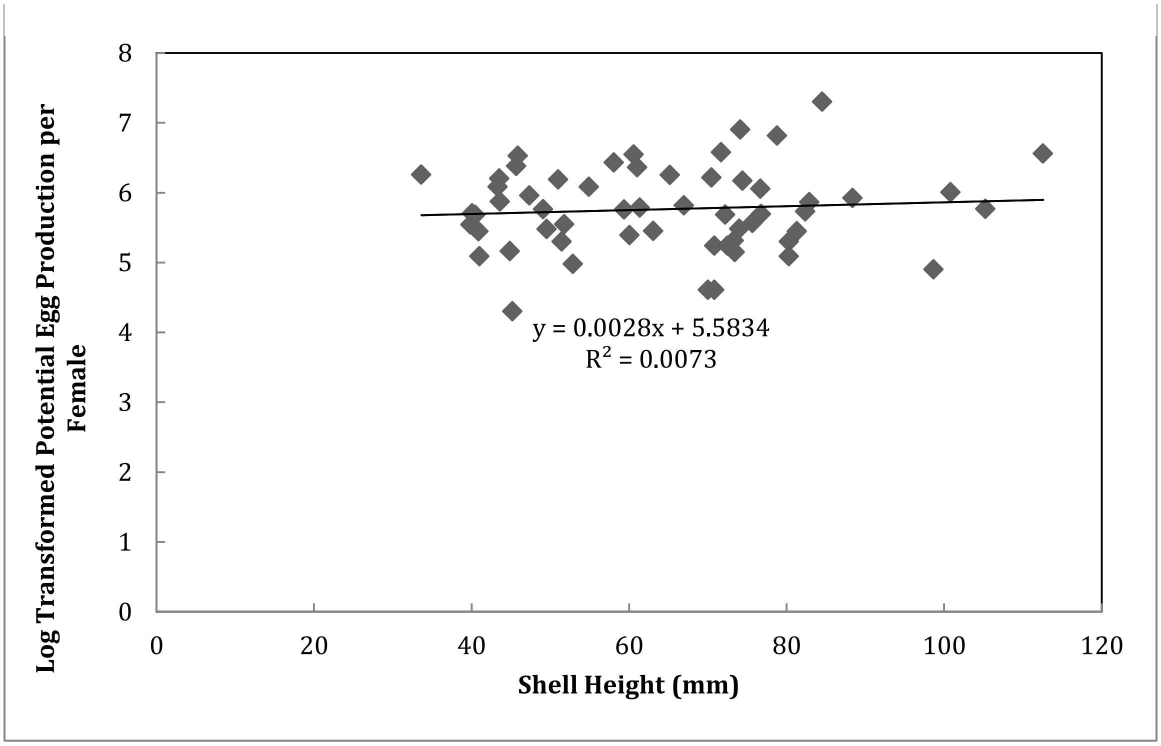


Figure 13. Relationship between log transformed potential egg production per female oyster and shell height (mm) analyzing only the oysters with the respective maximum egg production for each month at each site from June to September 2011.



Potential Spawning Contribution per Square Meter

To determine the average potential spawning contribution per square meter of restoration materials, for each of the four months during the spawning season, we multiplied the average egg production per female by the appropriate month's sex ratio and the size-specific oyster density (determined once in 2011 for each site). These four months of values were averaged to yield the potential spawning contribution (PSC) of each size category for each restoration site. The PSC per square meter of legal and sub-legal oysters were added together to represent the PSC for each sites.

There was a very large range of PSC per square meter among the different restoration sites and restoration materials (Fig. 14), ranging from less than 70,000/M^2 (WAUS on Little Bay) to over 53 million/M^2 (on the WAD™'s at Billy Goat Hole). Oysters on Coffee Island shell bags ranked second for PSC per square meter at over 12.5 million/M^2. Most of the other sites averaged around two million. The PSC per square meter in the WADs™ at Billy Goat Hole was significantly greater than all the other sampling sites except bagged shell at Coffee Island (ANOVA, p=0.015).

Total Monthly Potential Spawning Contribution from Restoration Sites

To determine the total monthly PSC for each restoration site, the PSC per square meter (above) was multiplied by the area of each of the restoration materials (Table 1). Similar to the PSC per square meter, Billy Goat Hole (Fig. 15) had the highest monthly PSC (over 27.5 billion eggs). Oysters on the bagged shell at Coffee Island were again second, with a monthly PSC of almost 14 billion. The range of total monthly PSC counts was very large (Fig. 15), with Billy Goat Hole only significantly larger than the Point aux Pines loose shell, the Alabama Port Reef

Balls™ and the Alabama Port Reef Blocks™ (ANOVA, p=0.023) with all other sites not differing significantly. Adding all the monthly PSC together from all the restoration sites in this study, the overall total monthly PSC in 2011 exceeded 50 billion eggs per month of the spawning season.

Total Potential Spawning Contribution from Restoration Sites over the Entire Spawning Season

The total PSC of the entire spawning season (June to September) from the restoration site was determined by adding the total PSC of each spawning month for each restoration material together to create an overall sum for each restoration project (Fig. 16). The trend of the total PSC over the entire spawning season is similar to the total PSC. It ranged from a high from the WADs in Billy Goat Hole (about 110 billion) to essentially zero from the Reef Balls on Coffee Island. Summing all the restoration sites together over the year, the potential spawning contribution over the entire 2011 spawning season exceeded 200 billion eggs.

Figure 14. The potential spawning contribution (PSC) of oysters per square meter of restoration (+ SD) at restoration sites in coastal Alabama in 2011. PSC is calculated as described in the text. NF indicates that < 5 oysters were found on the restoration substrate material during the sampling period. Capital letters for the legal-size oysters and lower case letters for the sub-legal size oysters indicate significant differences.

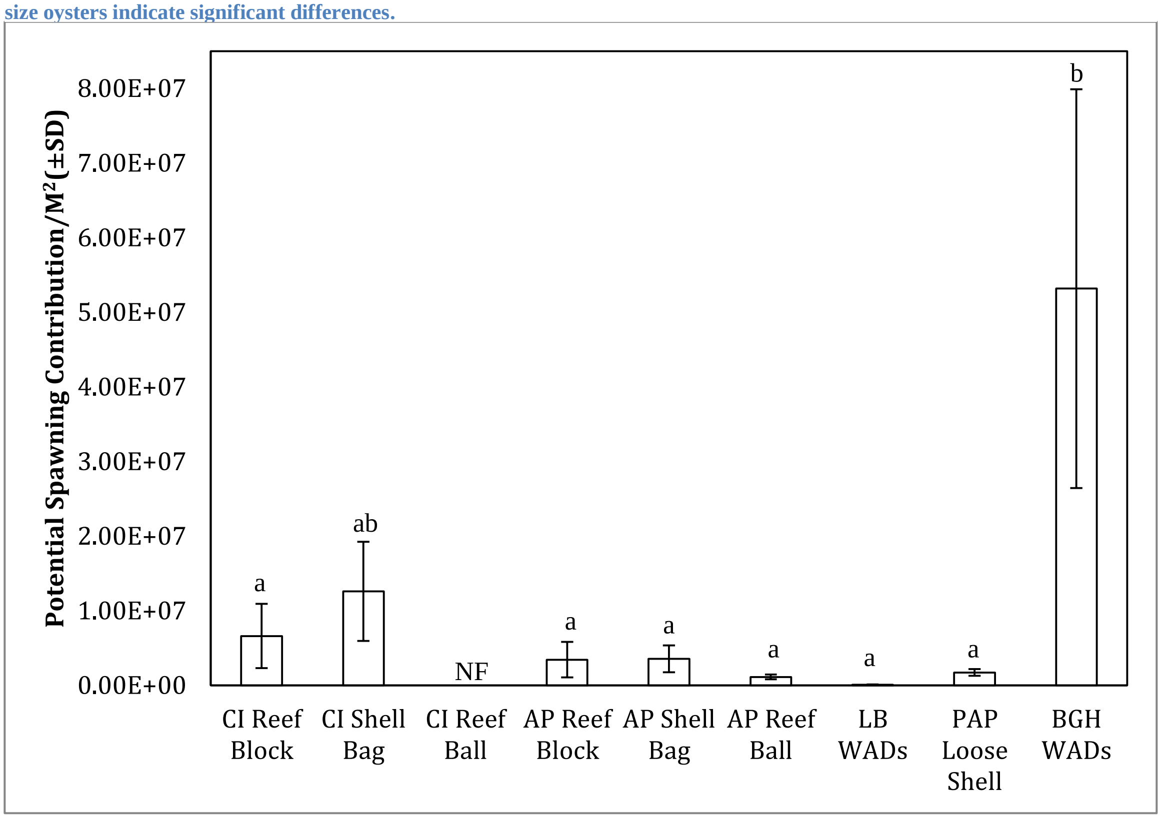


Figure 15. The total potential spawning contribution (PSC) of oysters (+ SD) at restoration sites in coastal Alabama in 2011. PSC is calculated as described in the text. NF indicates that < 5 oysters were found on the restoration substrate material during the sampling period.

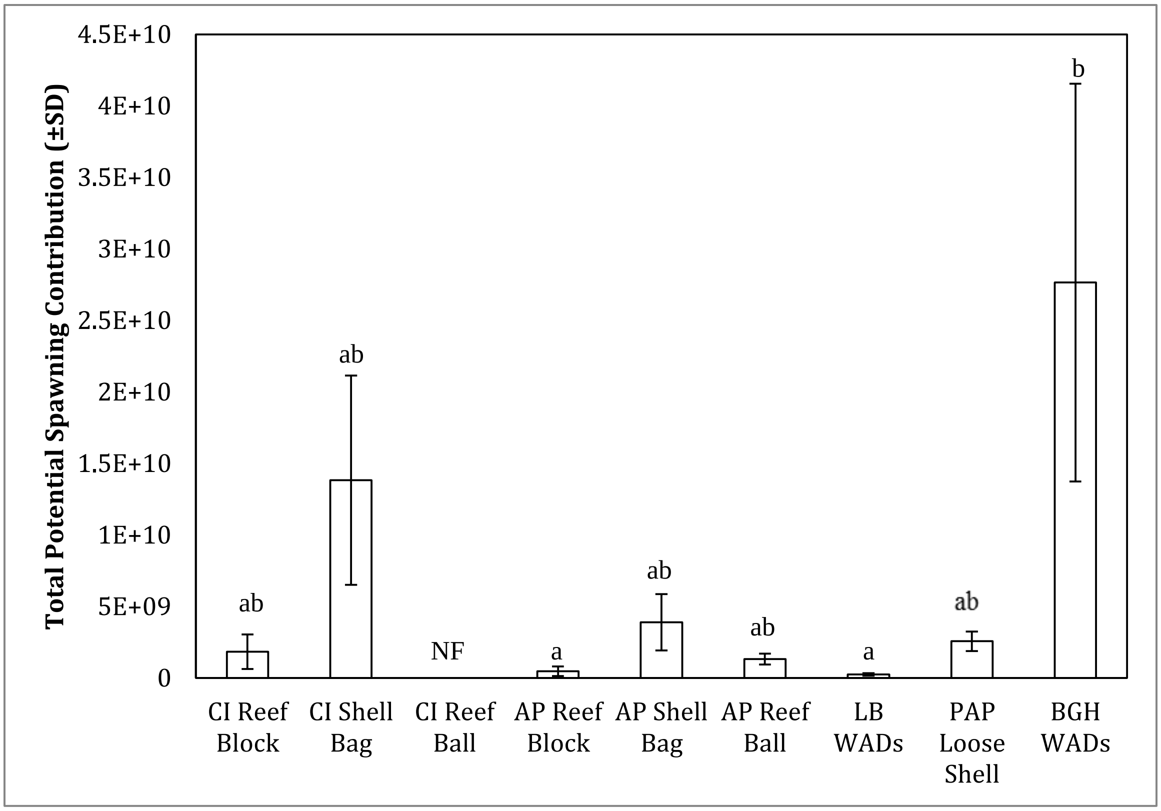
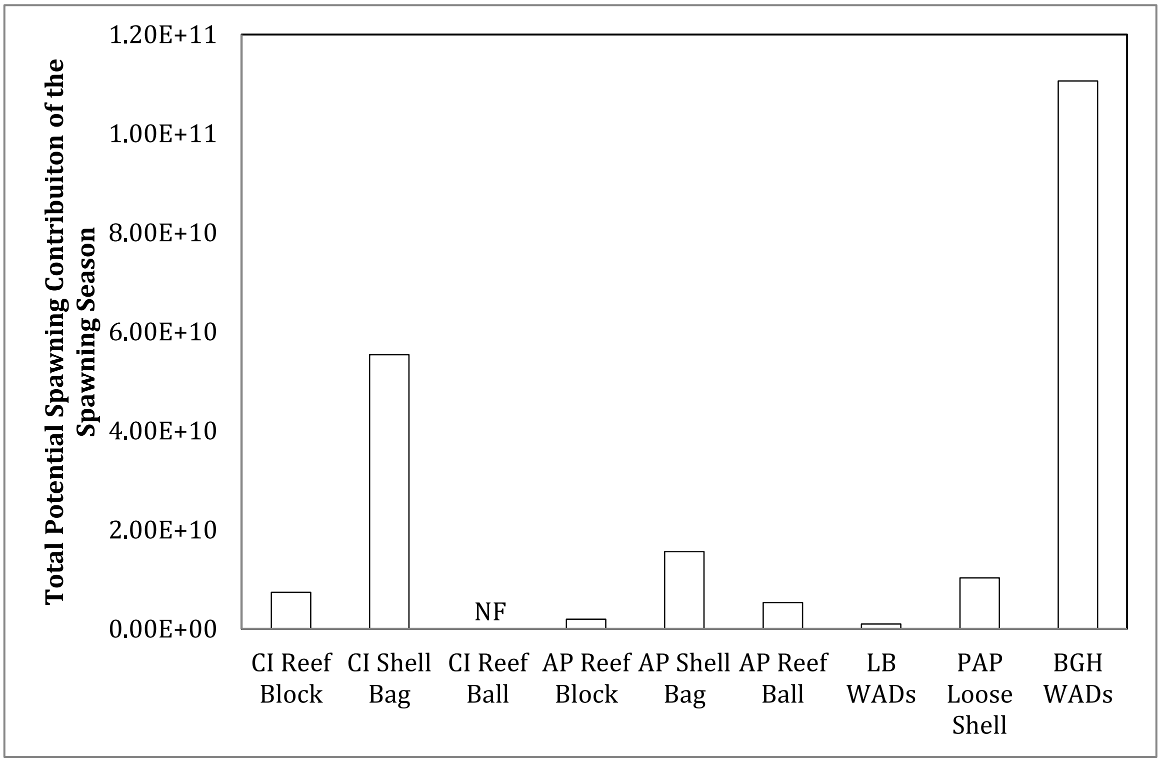


Figure 16. The total potential spawning contribution (PSC) of oysters (+ SD) over the entire spawning season at restoration sites in coastal Alabama in 2011. PSC is calculated as described in the text. NF indicates that < 5 oysters were found on the restoration substrate material during the sampling period.



Estimation of Costs of Potential Spawning Contribution

The estimated cost per million potential eggs at the various oyster restoration sites (Table 5.) is based on estimates of total potential spawning contribution and each project's reported design & installation costs. Of course, these costs are based only on the estimate of total PSC and do not include other project benefits (e.g., shoreline erosion, habitat creation, etc.). For this metric, the cost of Little Bay is far above the other projects ($12,276/million potential eggs). In contrast, the cost for the Billy Goat Hole project was only $2/million potential eggs. All the other projects' costs ranged from tens to hundreds of dollars per million potential eggs.

Table 5. The estimated cost per million potential eggs of oysters at restoration sites in coastal Alabama in 2011 based on estimates of egg production and restoration project design & installation costs.

Site	Restoration Materials	Cost per Million Potential Eggs ($US)
Coffee Island	Reef Blocks™	169
	Shell Bag	18
	Reef Balls™	-
Alabama Port	Reef Blocks™	327
	Shell Bag	65
	Reef Balls™	110
Little Bay	WAUs	12,276
Point Aux Pins	Loose Shell	31
Billy Goat Hole	WADs™	2

Discussion

1. Quantification of potential spawning contributions provided by oyster restoration

Potential spawning contribution (PSC) from oyster restoration projects is one of the most critical ecological functions that, until this current study, had not been quantified. Over the span (six months) and geographic range (coastal waters of Alabama), the results provide a first estimate of the magnitude and variability in this service. Specifically, in this study, the highest total PSC was over 25 billion potential eggs (from Billy Goat Hole), while other restoration sites contributed essentially no eggs.

Additionally, we also determined the PSC per square meter, and again noted a large range among the sampled sites; PSC per square meter ranged from essentially zero up to over 5 million potential eggs produced per square meter. These are the first estimates of this ecosystem service that we can document, and provide a baseline for comparison for future studies. PSC per square meter, monthly PSC and total PSC all provide indices for the spawning contribution from various restoration materials.

2. Factors affecting potential spawning contribution

There are a number of factors that could significantly influence PSC, which are addressed below.

a. Surface area of the restoration materials

By comparing the total monthly PSC and PSC per square meter, it is clear that the surface area of the restoration materials is an important factor. For PSC per square meter, the Billy Goat

Hole WADs™ had a significantly greater value than every other site except the bagged shell in Coffee

Island (Fig.14). In contrast, for total monthly PSC, Billy Goat Hole WADs™ only had values greater than two restoration sites, Alabama Port Reef Block and WAUs in Little Bay (Fig.15), which can be attributed to the larger surface areas of the other restoration projects. Large surface area alone, however, is not correlated with monthly PSC. Among all the restoration materials, WAUs in Little Bay had the biggest surface area, but had some of the lowest monthly PSC. It is noteworthy that Reef Block provided relatively low amounts of surface area for oyster settlement.

b. Size-specific oyster density

Size-specific oyster density is another factor that impacts the PSC. The PSC per square meter is calculated by multiplying the potential egg production by the size-specific oyster density and the sex ratio. The highest density of legal sized oyster was found in WADs™ in Billy Goat Hole, while bagged shell at Coffee Island had the highest density of sub-legal oysters (Table 1). During this study, no legal size oysters (and their corresponding higher potential egg production) were found on any Coffee Island restoration materials (Fig.15); however, the relatively high density of sub-legal oysters on Reef Blocks and Reef Balls at Coffee Island yielded a relatively high PSC per square meter.

Notably, the densities of oysters of different restoration sites were quite uneven, both in terms of abundance and distribution across size categories. For example, the population structure in Billy Goat Hole was quite mature; both sizes of oysters were present and the density of sub-legal was slightly higher than legal. The absence of the legal size oysters at Coffee Island was likely simply a consequence of the relatively new installation of restoration materials. At some restoration sites installed earlier (Little Bay WAUs and Point Aux Pins loose shell), oyster densities were relatively low, suggesting ongoing low recruitment and/or survival, possibly from predation by oyster drills. These low densities, in turn, brought down estimates of monthly PSC.

c. Size-specific sex ratios in the oysters

Although there is no significant difference among restoration sites in terms of the sex ratio for either size category (ANOVA; legal p=0.067; sub-legal, p=0.066), there was a large range among different restoration materials (Fig.9). Hypothetically, though, differences in sex ratios could affect PSC. In this study, legal size oysters had a higher average female sex ratio than sub-legal size oysters, further raising the relative contribution to PSC by the legal size oysters.

d. Oyster size-structure;

Prior work has shown that egg production increases as the dry weight increases (Thompson et. al, 1996), so the expectation was that legal size oysters would contribute to higher potential egg production than sub-legal size oysters. Thompson et al. (1996) analyzed the data collected from Cox and Mann (1998), and found a strong positive relationship p. In this study, we quantified the effect of shell height upon egg production by a linear regression, with a very weak positive relationship (Figs.14, 16). Despite this weak relationship, the average egg production per legal size female oyster was much higher than average egg production per sub-legal size female oyster (Fig.13). Thus, the sub-population size distribution could be one of the factors that impacted the PSC.

Additionally, the sex ratio of legal size oyster tended to be a higher proportion female than sub-legal size oysters. During the spawning season, over 50% of the legal size oysters (average shell height 85.4 mm ± 1.79 were female, while the female ratio was under 50% in the sub-legal size oysters (average shell height 51.9 mm ± 0.97. Needler (1932) suggested that eastern oysters tend to be male in their early life stage, and tend to change sex and become female as they age. This trend was confirmed in this study.

e. Length of time since installation of restoration materials

While there is no clear association between monthly PSC and the length of time since installation of restoration materials (Fig. 12), it is worth noting that the most productive site in terms of PSC was also the oldest installation. The very low monthly PSC of the second oldest restoration site (Point aux Pines loose shell), however, illustrates that length of time since installation is not a predictor of PSC. Rather, we suggest that length of time since installation is necessary but not sufficient for establishment of and growth of oysters, leading to a sub-population of legal sized oysters at a site. Notably, though, even relatively young sites with a good recruitment of sub-legal size oysters were observed to contribute a relatively large PSC.

f. Physical environmental factors

The physical environmental factors at restoration sites could impact PSC as well. Among all the factors, temperature is the primary determining factor of the beginning and length of the spawning season, affecting the development of the gonad as well as the growth of the oyster (Thompson et al., 1996). Salinity is also a factor that may affect the growth and energetics of oysters; variations in salinity or prolonged periods of low salinity would be expected to interfere with feeding.

In this study, the water temperature along the coast Alabama generally stays above 10°C all year round and at or over 25 °C during the spawning season, and was expected to be favorable to oyster reproduction. The salinity of the restoration sites during the study ranged from 10 ppt to 24 ppt, both spatially and temporally. Despite the oyster's wide salinity tolerance, the lower values could have decreased the average potential egg production (Mann et al. 1994).

g. Biological environmental factors

During the collection period, oyster drill (*Stramonita haemastoma*), one of the most common predators of the eastern oysters at Alabama, was found at the sites in Little Bay, Point Aux Pins and Coffee Island. Of course, predation by the oyster drill could lead to decreased oyster densities, which in turn would decrease PSC. Also, predation pressure could also stress oysters, causing diversion of energy to shell thickening and/or disrupting feeding; this could also lead to decreased PSC.

3. Determination of the spawning season

For the purposes of this study, we wanted to ensure that measures of reproductive output (egg production, PSC, etc.) were derived from the period of peak spawning. While it was clear that oysters were reproducing in October and November, there was a sharp distinction between June through September and October and November. Temperature is known to be one of the determining factors for the spawning of the oyster, *C. virginica*; water temperatures need to reach the critical minimum and also have a rapid rise to stimulate spawning (Nelson, 1931, Gutsell 1924). In the Gulf of Mexico, the water temperature required for spawning is 25°C and the spawning will continue until the temperature drops back below this critical value (Wallace, 2001). According to NOAA national oceanographic data center (2011), the average monthly water temperature around Dauphin Island (30° 15' N, 88° 4.5' W) during June to September in the year 2011 maintains above 25°C, and in October, the average temperature dropped below 25°C, which corresponds with the data we collected from the field during sampling in 2011. Also, two qualitatively rapid temperature changes during the spawning season were observed: the first occurred through May to June, and the second occurred in September through October (Fig.11).

The analysis of potential egg production as a function of month further supported this definition of the 2011 spawning season as June through September. The potential egg production per female of legal size oysters in October and November were significantly lower (ANOVA on rank-transformed data, $p<0.05$) than the other four months. Furthermore, potential egg production among sub-legal oysters in October and November was significantly less than all the other months except August. Based on the other data and to keep a continuous spawning season, we included August in the definition of the spawning season. The monthly maximum egg production per female of both size oysters also dropped after September. There was also a dramatic increase in the indeterminate classification of oyster sex in October and November for both oyster size categories, also the condition index of both size oyster reaches lowest during June to September and then raise up on October further suggesting that the primary spawning season concluded in September in 2011.

Finally, it should be noted that, based on water temperatures in 2011, spawning could have occurred between May and June. Due to logistical difficulties, this study did not begin until

June. Future study of the PSC should attempt to sample during the entire potential spawning season.

4. Oyster egg production comparisons

The egg production of oysters studied in this project was much lower than the estimates of Cox and Mann (1992), which have been previously recognized as lower than prior estimates (Kennedy. et. al, 1992). To explain this discrepancy, we suggest that any of the following factors (or combination thereof) could have affected the results of this study.

First, there were minor alterations of the methods from those of Cox and Mann (1992); the modifications were, however, conservative. The slurry was sieved over a larger mesh (100um instead of 90um), and eggs were retained on a finer mesh (50um instead of 53um). Therefore, we do not suspect that there was a methodological bias towards lower estimates.

Second, there could have been significant differences in the quality of the environment between the studies. For example, the food quality and quantity could have varied among study sites and impacted the oyster fecundity. If food was relatively low at our study sites, this could have resulted in smaller body weights and less energy that could have been spent on the spawning which could theoretically result in less potential egg production. Similarly, the salinity could have impacted fecundity. In the extensive study of Mann et al. (1994) on the oysters in James River, they estimated the fecundity at two sites for three years. The result showed a gradual reduction and this reduction was correlated salinity. We do not have the data to determine how the local salinities compare to other assessments of fecundity, but it could be one factor to be considered.

Third, predation pressure from oyster drills could also potentially reduce oyster egg production, either by inducing greater energy expenditures on shell thickening (and thus less to reproductive effort) or by interference with feeding by the oysters. The oyster drills was very common at the Little Bay, Point Aux Pins and Coffee Island sites during the study period.

Finally, though the age of the restoration project did not appear to be correlated with PSC, it did certainly affect the oyster size structure. With a prevalence of sub-legal size oysters sampled in this study (and their lower egg production), it is possible that the egg production estimates in this study were biased towards lower values; the size structure in some of the newer restoration sites could underestimate the potential egg production.

5. Conclusions and recommendations

Based on this study, oyster restoration project managers now have a measure of a previously presumed ecosystem service, the potential spawning contribution (PSC). In areas where oyster populations have declined (Hargis et al. 1988, Rothschild et al. 1994, Coen et al. 2000, Jackson et al. 2001, Kirby 2004, Lotze et al. 2006, Beck et al. 2009), this ecosystem service could be especially important. This service could help establish new sub-populations or maintain current sub-populations. For example, base on the currents of Mobile Bay, it has been suggested that locally the eastern oyster experiences recruitment limitation in the southeastern portion (Kim et al. 2012). Oyster restoration projects could help the establishment of the eastern oyster population in this recruitment area, based on the spawning contribution documented in this study. This, in turn, allows the other ecosystem services that are commonly recognized as provided by oysters, and provides a rationale for how oyster restoration can contribute to commercial and recreational oyster fisheries.

For oyster restoration project managers looking to maximize PSC, the following guidelines are recommended:

1. Select a site that maximizes oyster recruitment and survival.

Not surprisingly, oyster density is a very important factor in PSC. Sites that maximize recruitment and subsequent survival should be identified and selected.

2. Select restoration materials with greater surface area.

Greater surface area allows for increased areas for oyster recruitment and could support larger oyster populations. From the result of this study, the bagged shell seemed have the biggest surface area per unit.

3. Select the most cost-effective materials.

In this study, the type of restoration materials did not seem to affect significantly egg production or PSC. If PSC were the only ecosystem service being sought as an outcome, the cost per million potential eggs (Table 5) provides guidance on the most cost-effective choices. Of course, oyster restoration project managers are typically looking to maximize a suite of ecosystem benefits, and these could very well vary by restoration material. For example, the WARMs and Reef Ball could provide more refuge for the marine species than other restoration materials for the large interstitial space they each create. And loose shell is less expensive than other restoration materials. The manger should also consider the bottom type of potential sites to determine the most appropriate restoration materials. Also it should be noted that there were confounding factors in this study, with no formal experimental test of restoration materials. Additional study would be required to determine cost-effectiveness.

In summary, the aim of this study was to quantify for the first time the potential spawning contribution (PSC) of the eastern oyster from restoration sites. This provides a measure of an additional ecosystem service provided by oyster restoration efforts and provides a baseline for future studies.

4. Future Work

Although this project is the first to quantify the PSC of oysters in restoration projects, some of the sites in this project (e.g., Alabama Port and Coffee Island) had just been installed, and had not acquired fully developed populations. Further monitoring over time is suggested to quantify the changes over time in the respective potential spawning contributions of the sites. Additionally, it is recommended that similar work be conducted in other regions to compare with the results of this study.

Furthermore, this study was limited to studying PSC among oyster restoration projects. It is recommended that similar studies be done of the PSC of natural reefs, to provide context for the values obtained in this study and others like it. Without the estimates of typical natural PSC, it is very difficult to judge the importance of the PSC of oyster restoration projects. Similarly, such studies could be performed on private oyster beds and oyster farms to allow for comparisons among the different groups.

Appendix

Table 5. The ANOVA Result of Shell Height of sub-legal oyster in Reef Blocks™ Coffee Island of each month.

Tukey's Honestly-Significant-Difference Test					
Collection Month	Collection Month	Difference	p-value	95% Confidence Interval	
				Lower	Upper
June	July	-5.417	0.351	-13.196	2.361
June	August	-5.158	0.352	-12.567	2.252
June	September	-7.529	0.064	-15.308	0.249
June	October	-1.225	0.998	-9.376	6.925
June	**November**	**-10.058**	**0.001**	**-17.392**	**-2.725**
July	August	0.26	1	-7.59	8.11
July	September	-2.112	0.978	-10.311	6.087
July	October	4.192	0.729	-4.361	12.745
July	November	-4.641	0.532	-12.419	3.137
August	September	-2.372	0.956	-10.222	5.478
August	October	3.932	0.749	-4.287	12.152
August	November	-4.901	0.411	-12.31	2.509
September	October	6.304	0.287	-2.249	14.857
September	November	-2.529	0.94	-10.307	5.249
October	**November**	**-8.833**	**0.025**	**-16.984**	**-0.682**

Table 6. The ANOVA Result of Shell Height of sub-legal oyster in Bagged Shell Coffee Island of each month.

Tukey's Honestly-Significant-Difference Test					
Collection Month	Collection Month	Difference	p-value	95% Confidence Interval	
				Lower	Upper
June	**July**	**-8.862**	**0.018**	**-16.778**	**-0.945**
June	**August**	**-12.267**	**0**	**-20.266**	**-4.269**
June	**September**	**-11.996**	**0**	**-19.912**	**-4.08**
June	**October**	**-18.592**	**0**	**-26.171**	**-11.012**
June	**November**	**-17.064**	**0**	**-24.98**	**-9.148**
July	August	-3.406	0.831	-11.404	4.593
July	September	-3.134	0.87	-11.051	4.782
July	**October**	**-9.73**	**0.003**	**-17.309**	**-2.151**
July	**November**	**-8.202**	**0.037**	**-16.119**	**-0.286**
August	September	0.271	1	-7.727	8.27
August	October	-6.324	0.174	-13.989	1.341
August	November	-4.797	0.526	-12.795	3.202
September	October	-6.596	0.13	-14.175	0.984
September	November	-5.068	0.45	-12.984	2.848
October	November	1.528	0.993	-6.052	9.107

Table 7. The ANOVA test of Shell Height of the sub-legal oyster in Bagged Shell in Alabama Port of each month.

Tukey's Honestly-Significant-Difference Test (A)					
Collection Month	Collection Month	Difference	p-value	95% Confidence Interval	
				Lower	Upper
June	July	4.750	0611	-3.810	13.310
June	**August**	**-10.835**	**0.006**	**-19.421**	**-2.248**
June	**September**	**-10.634**	**0.004**	**-18.73**	**-2.539**
June	**October**	**-12.572**	**0**	**-20.667**	**-4.477**
June	**November**	**-10.501**	**0.004**	**-18.597**	**-2.406**
July	**August**	**-15.585**	**0.000**	**-24.608**	**-6.561**
July	**September**	**-15.384**	**0.000**	**-23.945**	**-6.824**
July	**October**	**-17.322**	**0.000**	**-25.882**	**-8.762**
July	**November**	**-15.251**	**0.000**	**-23.811**	**-6.691**
August	September	0.2	1	-8.386	8.787
August	October	-1.737	0.98	-10.324	6.849
August	November	0.333	1	-8.253	8.92
September	October	-1.938	0.964	-10.033	6.158
September	November	0.133	1	-7.962	8.229
October	November	2.071	0.954	-6.025	10.166

Table 8. The ANOVA Result of Shell Height of oyster in Reef Balls™ of Alabama Port of each month. A. Legal Size. B. Sub-legal Size.

Tukey's Honestly-Significant-Difference Test (A)					
Collection Month	Collection Month	Difference	p-value	95% Confidence Interval	
				Lower	Upper
June	July	2.337	0.999	-14.96	19.633
June	August	-11.842	0.052	-23.746	0.063
June	**September**	**-12.481**	**0.021**	**-23.705**	**-1.258**
June	October	-11.051	0.098	-23.253	1.15
June	**November**	**-20.326**	**0**	**-31.214**	**-9.438**
July	August	-14.178	0.192	-31.924	3.568
July	September	-14.818	0.135	-32.114	2.479
July	October	-13.388	0.257	-31.334	4.559
July	**November**	**-22.663**	**0.003**	**-39.744**	**-5.581**
August	September	-0.64	1	-12.544	11.265
August	October	0.79	1	-12.04	13.621
August	November	-8.484	0.276	-20.073	3.105
September	October	1.43	0.999	-10.771	13.631
September	November	-7.845	0.293	-18.733	3.044
October	November	-9.275	0.214	-21.168	2.619

Tukey's Honestly-Significant-Difference Test (B)					
Collection Month	Collection Month	Difference	p-value	95% Confidence Interval	
				Lower	Upper
June	July	5.016	0.561	-3.614	13.645
June	August	-3.314	0.879	-11.848	5.219
June	September	-5.535	0.527	-14.774	3.704
June	**October**	**-9.166**	**0.027**	**-17.7**	**-0.633**
June	**November**	**-12.855**	**0**	**-21.388**	**-4.321**
July	August	-8.33	0.089	-17.335	0.675
July	**September**	**-10.551**	**0.023**	**-20.227**	**-0.874**
July	**October**	**-14.182**	**0**	**-23.187**	**-5.177**
July	**November**	**-17.87**	**0**	**-26.876**	**-8.865**
August	September	-2.221	0.986	-11.812	7.37
August	October	-5.852	0.42	-14.765	3.061
August	**November**	**-9.54**	**0.028**	**-18.453**	**-0.628**
September	October	-3.631	0.89	-13.222	5.96
September	November	-7.319	0.25	-16.91	2.271
October	November	-3.688	0.847	-12.601	5.224

Table 9. The ANOVA test of Shell Height of the sub-legal oyster in WAUs in Little Bay of each month.

Tukey's Honestly-Significant-Difference Test (A)					
Collection Month	Collection Month	Difference	p-value	95% Confidence Interval	
				Lower	Upper
June	July	-3.694	0.771	-11.635	4.246
June	August	-4.431	0.605	-12.371	3.51
June	September	-4.147	0.682	-12.174	3.88
June	October	-2.705	0.927	-10.646	5.235
June	**November**	**-21.005**	**0**	**-28.945**	**-13.065**
July	August	-0.736	1	-8.898	7.426
July	September	-0.453	1	-8.699	7.794
July	October	0.989	0.999	-7.173	9.151
July	**November**	**-17.311**	**0**	**-25.473**	**-9.149**
August	September	0.284	1	-7.963	8.53
August	October	1.725	0.991	-6.437	9.887
August	**November**	**-16.574**	**0**	**-24.736**	**-8.412**
September	October	1.442	0.996	-6.805	9.688
September	**November**	**-16.858**	**0**	**-25.104**	**-8.611**
October	**November**	**-18.3**	**0**	**-26.462**	**-10.138**

Table 10. The ANOVA test of Shell Height of the sub-legal oyster in Loose Shell in Point Aux Pins of each month.

Tukey's Honestly-Significant-Difference Test					
Collection Month	Collection Month	Difference	p-value	95% Confidence Interval	
				Lower	Upper
June	July	8.816	0.058	-0.182	17.814
June	August	6.091	0.464	-3.705	15.887
June	September	0.477	1	-8.29	9.245
June	October	-1.542	0.998	-12.075	8.991
June	November	8.869	0.849	-13.172	30.909
July	August	-2.725	0.971	-12.97	7.519
July	September	-8.339	0.103	-17.605	0.928
July	October	-10.358	0.075	-21.31	0.594
July	November	0.052	1	-22.191	22.296
August	September	-5.613	0.583	-15.656	4.43
August	October	-7.633	0.401	-19.249	3.983
August	November	2.778	0.999	-19.8	25.356
September	October	-2.019	0.994	-12.783	8.744
September	November	8.391	0.879	-13.76	30.543
October	November	10.411	0.771	-12.497	33.319

Table 11. The ANOVA Result of Shell Height of oyster in Billy Goat Hole of each month. A. Legal Size. B. Sub-legal Size

Tukey's Honestly-Significant-Difference Test (A)					
Collection Month	Collection Month	Difference	p-value	95% Confidence Interval	
				Lower	Upper
June	July	5.014	0.565	-3.645	13.673
June	August	-7.452	0.139	-16.112	1.207
June	September	-4.267	0.725	-12.926	4.393
June	October	-6.81	0.219	-15.47	1.849
June	November	-5.969	0.363	-14.628	2.691
July	**August**	**-12.466**	**0.001**	**-21.511**	**-3.422**
July	**September**	**-9.281**	**0.04**	**-18.325**	**-0.236**
July	**October**	**-11.824**	**0.003**	**-20.869**	**-2.78**
July	**November**	**-10.983**	**0.007**	**-20.027**	**-1.938**
August	September	3.186	0.917	-5.859	12.23
August	October	0.642	1	-8.402	9.686
August	November	1.484	0.997	-7.561	10.528
September	October	-2.544	0.967	-11.588	6.501
September	November	-1.702	0.995	-10.746	7.342
October	November	0.842	1	-8.203	9.886

Tukey's Honestly-Significant-Difference Test (B)					
Collection Month	Collection Month	Difference	p-value	95% Confidence Interval	
				Lower	Upper
June	**July**	**16.14**	**0**	**7.944**	**24.335**
June	**August**	**8.267**	**0.047**	**0.071**	**16.463**
June	**September**	**9.085**	**0.02**	**0.889**	**17.281**
June	**October**	**9.381**	**0.014**	**1.185**	**17.577**
June	November	7.119	0.131	-1.077	15.315
July	August	-7.873	0.068	-16.069	0.323
July	September	-7.055	0.138	-15.251	1.141
July	October	-6.759	0.174	-14.955	1.437
July	**November**	**-9.021**	**0.021**	**-17.217**	**-0.825**
August	September	0.818	1	-7.378	9.014
August	October	1.114	0.999	-7.082	9.31
August	November	-1.148	0.999	-9.344	7.048
September	October	0.296	1	-7.9	8.492
September	November	-1.966	0.984	-10.162	6.23
October	November	-2.262	0.97	-10.458	5.934

Table 12. The ANOVA Result Legal size oyster of the Average Potential Fecundity of Each Month. A Legal Size. B. Sub-legal Size

Tukey's Honestly-Significant-Difference Test (A)					
Collection Month	Collection Month	Difference	p-value	95% Confidence Interval	
				Lower	Upper
June	July	-64.504545	0.399626	-162.226393	33.217302
June	August	-15.744444	0.993778	-98.401598	66.912710
June	September	66.200000	0.996703	-6.650253	139.050253
June	October	**197.929167**	**0.000004**	**122.003723**	**273.854611**
June	November	**207.313636**	**0.000004**	**109.591789**	**305.035484**
July	August	48.760101	0.766801	-57.341787	154.861989
July	September	**130.704545**	**0.002713**	**-32.050783**	**229.358308**
July	October	**262.433712**	**0.000004**	**161.487793**	**363.379632**
July	November	**271.818182**	**0.000004**	**153.602426**	**390.033938**
August	September	81.944444	0.058964	-1.812410	165.701299
August	October	**213.673611**	**0.000004**	**127.228690**	**300.159969**
August	November	**223.058081**	**0.000004**	**116.956193**	**329.159969**
September	October	**131.729167**	**0.000041**	**54.607973**	**208.850361**
September	November	**141.113636**	**0.000904**	**42.459874**	**239.767399**
October	November	9.384470	0.999805	-91.561450	110.330389

Tukey's Honestly-Significant-Difference Test (B)					
Colleciton Month	Collection Month	Difference	p-value	95% Confidence Interval	
				Lower	Upper
June	July	21.485353	0.891491	-35.471010	78.444806
June	August	**78.657762**	**0.001002**	**22.241143**	**135.074380**
June	September	9.893827	0.996703	-48.251646	68.029300
June	October	**87.838655**	**0.000180**	**30.595811**	**145.081499**
June	November	**176.827160**	**0.004654**	**36.024196**	**317.630125**
July	August	57.172409	0.078995	-3.594900	117.939718
July	September	-11.591525	0.995002	-73.967218	50.784168
July	October	**66.353302**	**0.025850**	**4.818156**	**127.888448**
July	November	**155.341808**	**0.023440**	**12.739894**	**297.943722**
August	September	**-68.763934**	**0.019236**	**-130.644324**	**-6.883545**
August	October	9.180893	0.998169	-51.852129	70.213915
August	November	98.169399	0.362770	-44.216561	240.555359
September	October	**77.944828**	**0.005242**	**15.310245**	**140.579410**
September	November	**166.933333**	**0.011422**	**23.853556**	**310.013111**
October	November	88.988506	0.480770	-53.726840	231.703851

Table 13. The ANOVA Result of Legal size oyster of the Potential spawning contribution per square meter of each site.

Tukey's Honestly-Significant-Difference Test

Collection Site	Collection Site	Difference	p-Value	95% Confidence Interval	
				Lower	Upper
AP Bagged Shell	AP Reef Balls™	2,426,764.540	1.000	-44,020,643.539	48,874,172.620
AP Bagged Shell	AP Reef Blocks™	118,001.721	1.000	-46,329,406.359	46,565,409.800
AP Bagged Shell	**BGH WADs™**	**-49,620,768.422**	**0.030**	**-96,068,176.501**	**-3,173,360.342**
AP Bagged Shell	CI Bagged Shell	-9,032,459.872	0.998	-55,479,867.952	37,414,948.208
AP Bagged Shell	CI Reef Blocks™	-3,063,856.218	1.000	-49,511,264.298	43,383,551.861
AP Bagged Shell	LB WAUs	3,472,462.993	1.000	-42,974,945.087	49,919,871.073
AP Bagged Shell	PAP Loose Shell	1,828,533.806	1.000	-44,618,874.274	48,275,941.886
AP Reef Balls™	AP Reef Blocks™	-2,308,762.820	1.000	-48,756,170.900	44,138,645.260
AP Reef Balls™	**BGH WADs™**	**-52,047,532.962**	**0.021**	**-98,494,941.042**	**-5,600,124.882**
AP Reef Balls™	CI Bagged Shell	-11,459,224.412	0.990	-57,906,632.492	34,988,183.668
AP Reef Balls™	CI Reef Blocks™	-5,490,620.759	1.000	-51,938,028.838	40,956,787.321
AP Reef Balls™	LB WAUs	1,045,698.452	1.000	-45,401,709.627	47,493,106.532
AP Reef Balls™	PAP Loose Shell	-598,230.735	1.000	-47,045,638.814	45,849,177.345
AP Reef Blocks™	**BGH WADs™**	**-49,738,770.142**	**0.030**	**-96,186,178.222**	**-3,291,362.063**
AP Reef Blocks™	CI Bagged Shell	-9,150,461.592	0.998	-55,597,869.672	37,296,946.487
AP Reef Blocks™	CI Reef Blocks™	-3,181,857.939	1.000	-49,629,266.019	43,265,550.141
AP Reef Blocks™	LB WAUs	3,354,461.272	1.000	-43,092,946.808	49,801,869.352
AP Reef Blocks™	PAP Loose Shell	1,710,532.085	1.000	-44,736,875.995	48,157,940.165
BGH WADs™	CI Bagged Shell	40,588,308.550	0.118	-5,859,099.530	87,035,716.630
BGH WADs™	**CI Reef Blocks™**	**46,556,912.203**	**0.049**	**109,504.124**	**93,004,320.283**
BGH WADs™	**LB WAUs**	**53,093,231.415**	**0.017**	**6,645,823.335**	**99,540,639.494**
BGH WADs™	**PAP Loose Shell**	**51,449,302.228**	**0.023**	**5,001,894.148**	**97,896,710.307**
CI Bagged Shell	CI Reef Blocks™	5,968,603.653	1.000	-40,478,804.426	52,416,011.733
CI Bagged Shell	LB WAUs	12,504,922.865	0.984	-33,942,485.215	58,952,330.944
CI Bagged Shell	PAP Loose Shell	10,860,993.678	0.993	-35,586,414.402	57,308,401.757
CI Reef Blocks™	LB WAUs	6,536,319.211	1.000	-39,911,088.869	52,983,727.291
CI Reef Blocks™	PAP Loose Shell	4,892,390.024	1.000	-41,555,018.056	51,339,798.104
LB WAUs	PAP Loose Shell	-1,643,929.187	1.000	-48,091,337.267	44,803,478.893

Table 14. The ANOVA Result of Legal size oyster of the Potential spawning contribution of each site.

Tukey's Honestly-Significant-Difference Test

Collection Site	Collection Site	Difference	p-Value	95% Confidence Interval	
				Lower	Upper
AP Bagged Shell	AP Reef Balls™	2.571E+009	1.000	-2.375E+010	2.890E+010
AP Bagged Shell	AP Reef Blocks™	3.417E+009	1.000	-2.291E+010	2.974E+010
AP Bagged Shell	BGH WADs™	-2.375E+010	0.098	-5.008E+010	2.576E+009
AP Bagged Shell	CI Bagged Shell	-9.936E+009	0.908	-3.626E+010	1.639E+010
AP Bagged Shell	CI Reef Blocks™	2.051E+009	1.000	-2.427E+010	2.838E+010
AP Bagged Shell	LB WAUs	3.655E+009	1.000	-2.267E+010	2.998E+010
AP Bagged Shell	PAP Loose Shell	1.327E+009	1.000	-2.500E+010	2.765E+010
AP Reef Balls™	AP Reef Blocks™	8.455E+008	1.000	-2.548E+010	2.717E+010
AP Reef Balls™	BGH WADs™	-2.632E+010	0.050	-5.265E+010	4,613,657.511
AP Reef Balls™	CI Bagged Shell	-1.251E+010	0.761	-3.883E+010	1.382E+010
AP Reef Balls™	CI Reef Blocks™	-5.200E+008	1.000	-2.685E+010	2.581E+010
AP Reef Balls™	LB WAUs	1.084E+009	1.000	-2.524E+010	2.741E+010
AP Reef Balls™	PAP Loose Shell	-1.244E+009	1.000	-2.757E+010	2.508E+010
AP Reef Blocks™	**BGH WADs™**	**-2.717E+010**	**0.040**	**-5.349E+010**	**-8.409E+008**
AP Reef Blocks™	CI Bagged Shell	-1.335E+010	0.699	-3.968E+010	1.297E+010
AP Reef Blocks™	CI Reef Blocks™	-1.366E+009	1.000	-2.769E+010	2.496E+010
AP Reef Blocks™	LB WAUs	2.385E+008	1.000	-2.609E+010	2.656E+010
AP Reef Blocks™	PAP Loose Shell	-2.090E+009	1.000	-2.842E+010	2.424E+010
BGH WADs™	CI Bagged Shell	1.381E+010	0.664	-1.251E+010	4.014E+010
BGH WADs™	CI Reef Blocks™	2.580E+010	0.058	-5.246E+008	5.213E+010
BGH WADs™	**LB WAUs**	**2.741E+010**	**0.037**	**1.079E+009**	**5.373E+010**
BGH WADs™	PAP Loose Shell	2.508E+010	0.070	-1.249E+009	5.140E+010
CI Bagged Shell	CI Reef Blocks™	1.199E+010	0.796	-1.434E+010	3.831E+010
CI Bagged Shell	LB WAUs	1.359E+010	0.681	-1.273E+010	3.992E+010
CI Bagged Shell	PAP Loose Shell	1.126E+010	0.841	-1.506E+010	3.759E+010
CI Reef Blocks™	LB WAUs	1.604E+009	1.000	-2.472E+010	2.793E+010
CI Reef Blocks™	PAP Loose Shell	-7.240E+008	1.000	-2.705E+010	2.560E+010
LB WAUs	PAP Loose Shell	-2.328E+009	1.000	-2.865E+010	2.400E+010

Figure 17. Average egg production per female legal size oysters (± SEM) sampled from Alabama Port Reef Block from June to November 2011. A. Reef Block B. Bagged Shell. C. Reef Balls™.

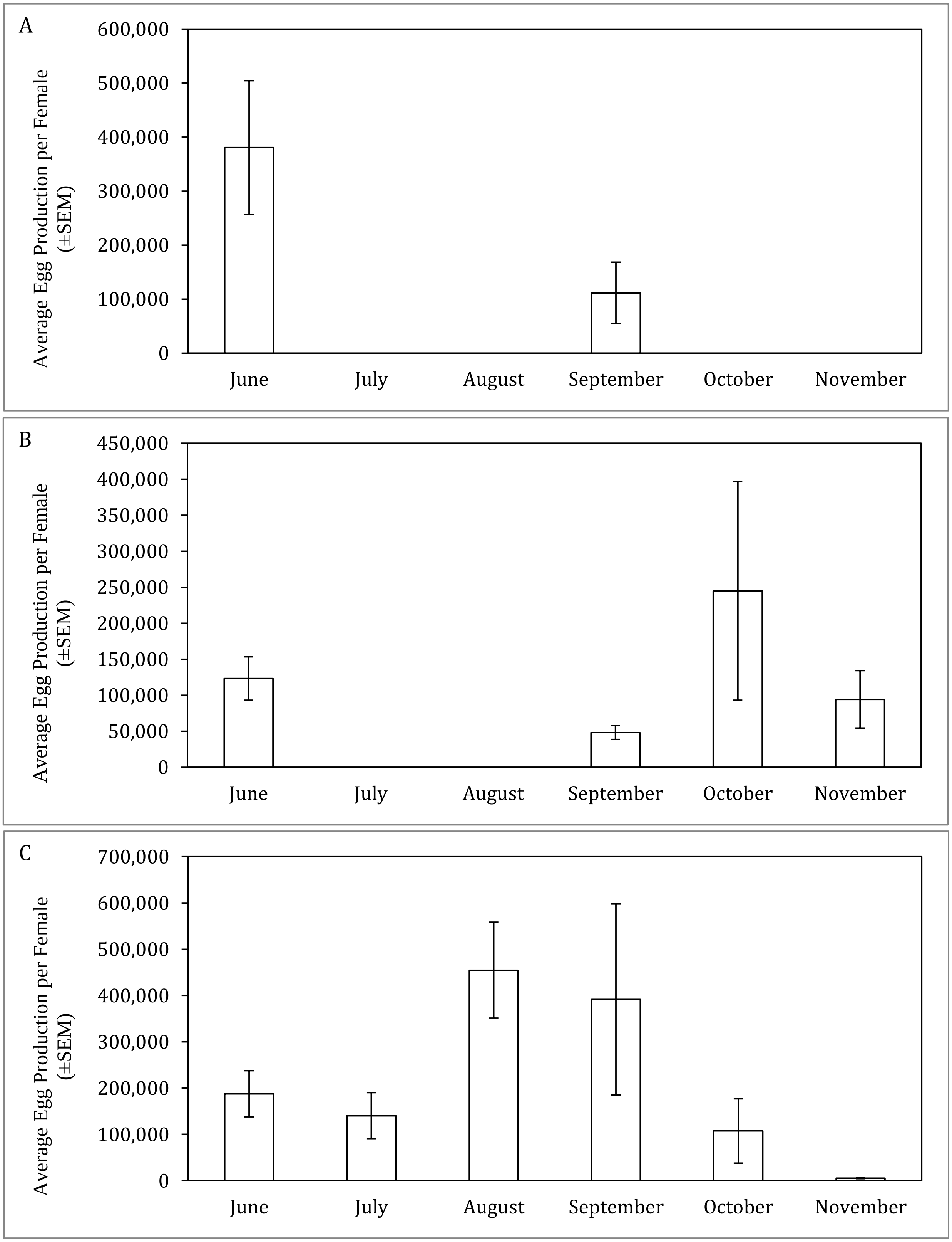


Figure 18. Average egg production per female legal size oysters (± SEM) sampled in restoration reef in Alabama from June to November 2011. A. WAUs in Little Bay. B. WADs^{TM} in Billy Goat Hole.

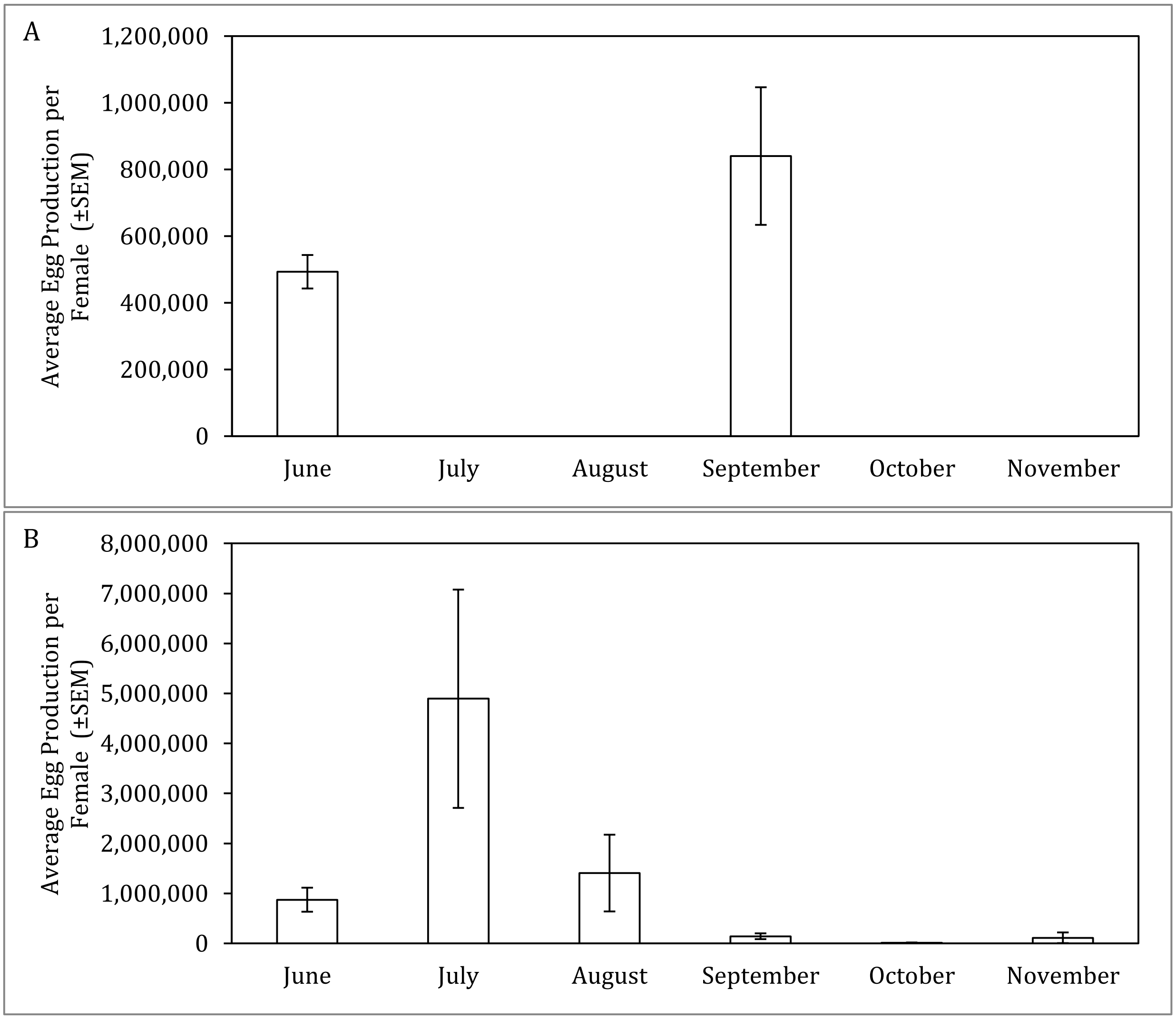


Figure 19. Average egg production per female sub-legal size oysters (± SEM) sampled from Coffee Island from June to November 2011. A. Reef Block. B. Bagged Shell.

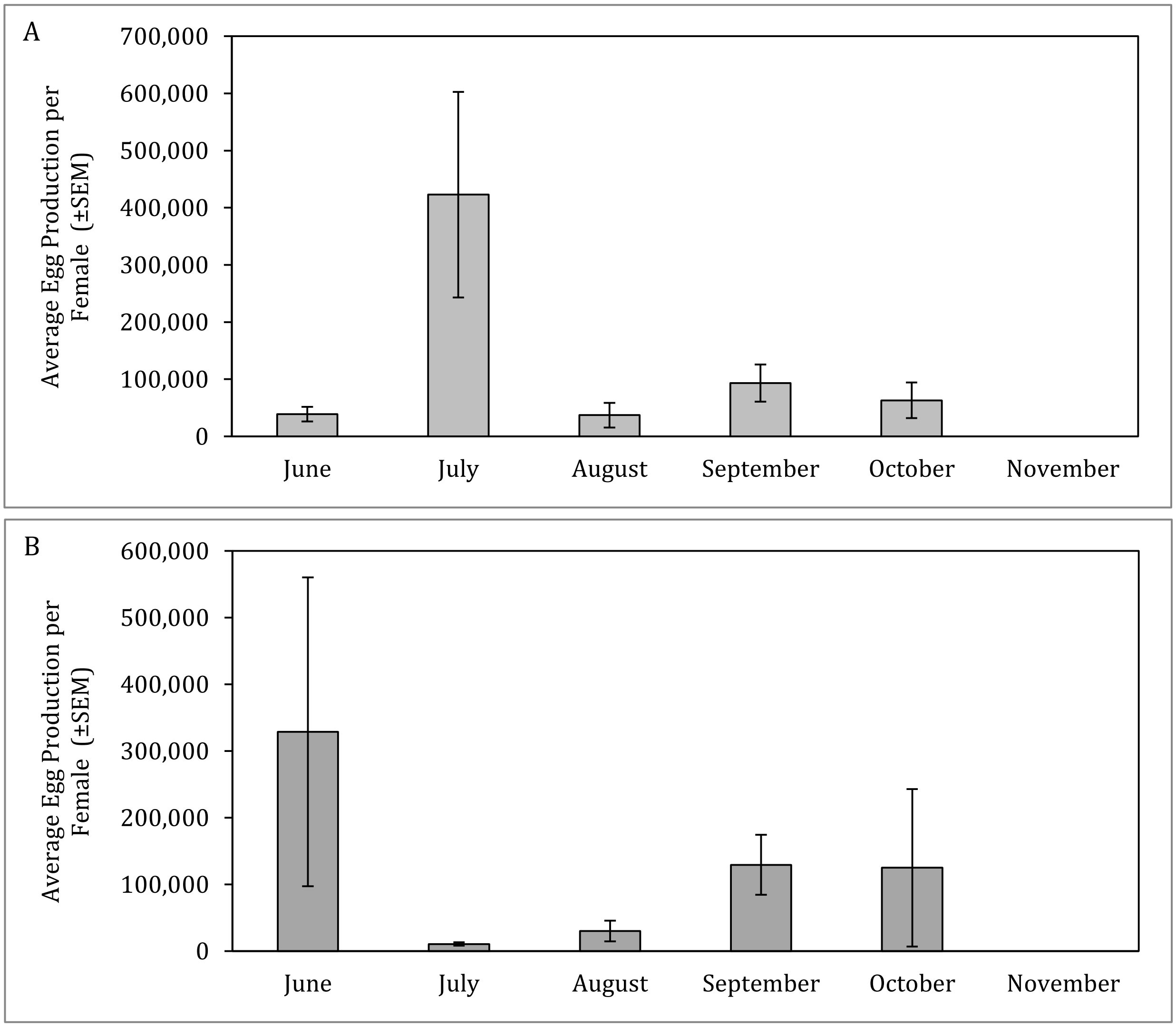


Figure 20. Average egg production per female sub-legal size oysters (+ SEM) sampled from Alabama from June to November 2011. A. Reef Block. B. Bagged Shell. C. Reef Balls™.

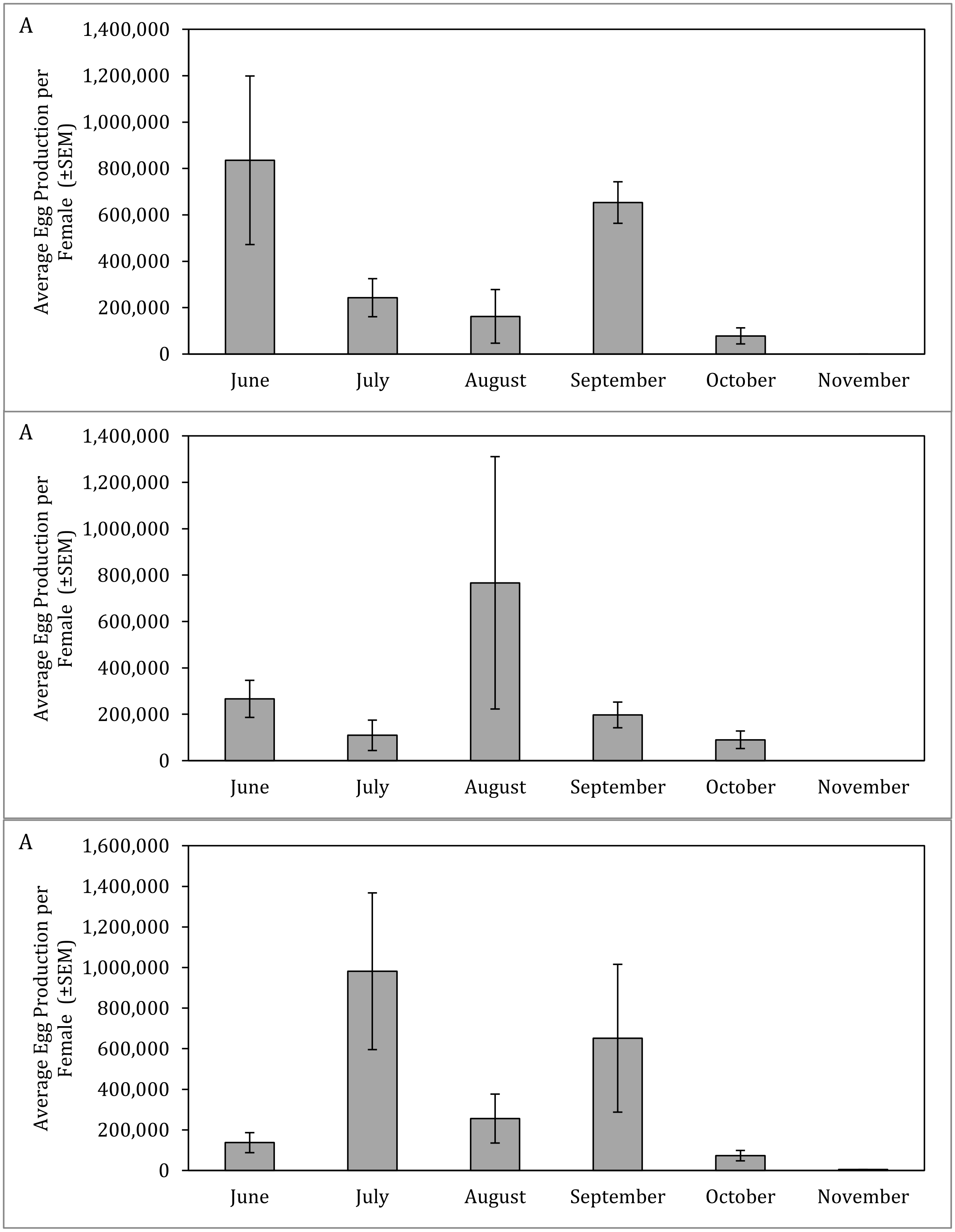
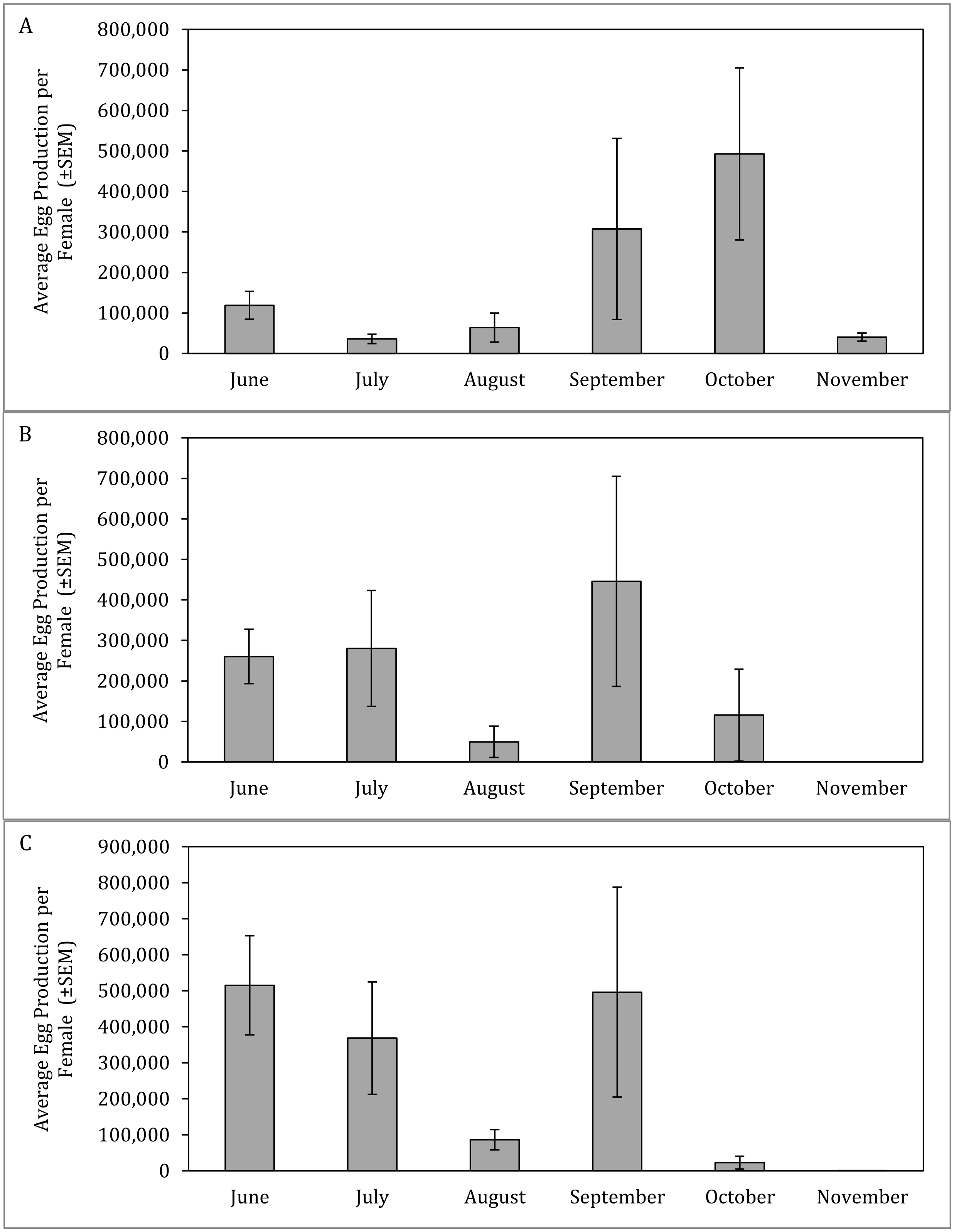


Figure 21. Average egg production per female sub-legal size oysters (± SEM) sampled from restoration sites from June to November 2011. A. WAUs in Little Bay. B. Loose Shell in Point Aux Pins. C. WADs^{TM} in Billy Goat Hole.



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