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Seasonal settlement of oysters, *Crassostrea virginica*, on fresh and fossil shells at Robinson Preserve, Manatee County, Florida

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Introduction

In addition to their historic commercial importance, oysters provide important ecological services to estuaries. Oysters are capable of filtering suspended particulates (seston) from large volumes of water as they feed. Filtration (clearance) rates vary seasonally with temperature, but rates of 5 l h^{-1} g^{-1} dry tissue weight are typical (Newell, 1988). This results in the reduction of particle concentrations by 30%-45%; chlorophyll-a concentrations by as much as 90%; and nitrogen removal up to 25% of daily load (Carmichael et al. 2012). Some of the cleared particulate matter is ingested and assimilated into tissue and shell growth; some is excreted (primarily as ammonium) and is available for primary production. The remainder ends up on the bottom as biodeposits (feces and pseudofeces). The process of transferring nutrients from the water column to the sediment, or benthic-pelagic coupling, benefits both the water column (pelagic) and bottom (benthic) communities in several ways. First, nutrients contained in biodeposits can be removed from the system by burial in sediments. Second, nitrogen in biodeposits can be microbially denitrified to N_2 gas and permanently removed from the system (Newell et al. 2002, Kellogg et al. 2013). Third, assimilation of nutrients into shell and tissue of bivalves (secondary production) effectively removes nitrogen and phosphorus from the water column (Newell 2004, Higgins et al. 2011, Carmichael et al. 2012, Kellogg et al. 2013, Reitsma et al. 2016). Fourth, filter feeding exerts “top-down” grazer control on phytoplankton, reducing water column turbidity and increasing the depth to which photosynthetically active radiation penetrates and seagrass can grow (Newell 2004, Newell & Koch 2004). In addition, oyster reefs create three dimensional structures that serve as habitat and nursery areas for many species of fish and invertebrates (Harding & Mann 2001, Stunz et al. 2010) and protect shorelines from erosion (Piazza et al. 2005, Scyphers et al. 2011).

Unfortunately, oyster populations throughout the world have declined to the point of functional extinction in many areas. Beck et al. (2011) examined oyster abundances in 144 bays throughout the world and concluded that oyster reef ecosystems have been reduced by 85% globally. This decline has multiple causes that include overharvesting; disease; shoreline alteration (dredging and filling); changes in freshwater inflows; and increased loadings of sediments, nutrients and toxins. The loss of oyster biomass in U.S. estuaries has resulted in an 85% decrease in the historic filtration capacity and its associated ecological services (zu Ermgassen et al. 2012).

Attempts to enhance oyster reefs were initially undertaken to augment commercial fisheries. Since about 1990, however, a growing effort to restore oyster habitat for its ecological function has been undertaken by federal, state, and non-governmental agencies (George et al. 2014, Baggett et al. 2015). Both take advantage of the natural reproductive cycle of living oysters inhabiting the region by adding clean substrate in the desired location[1]. Oysters undergo an annual reproductive cycle in which adults produce gametes (either eggs or sperm) and then spawn, releasing gametes into the water column. The timing of gamete production and spawning are controlled by environmental factors such as temperature and food availability. Fertilization of eggs is external, and oysters spend the first 2-4 weeks of life as planktonic larvae. The length of this planktonic larval stage is highly variable, depending primarily on temperature and food availability. When larval development is complete, they "settle" to the bottom to begin their benthic life stage. Oysters require something hard to attach to when they settle. Juvenile oysters that have just settled and attached to a substrate are called "spat" (Figure 1). They then undergo metamorphosis and grow into adult oysters.

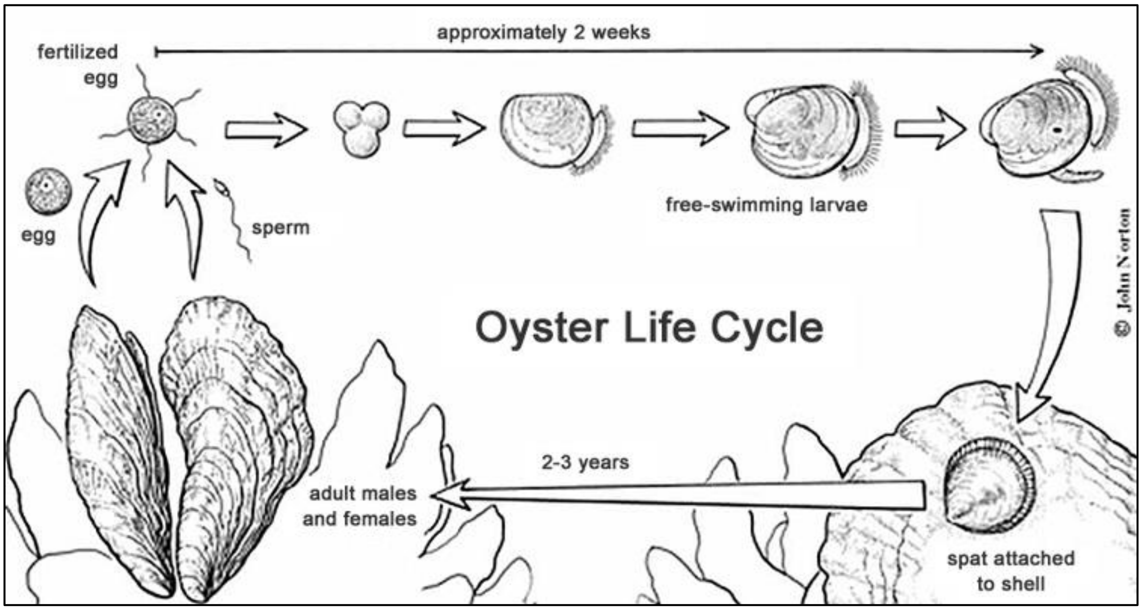

Figure 1. Diagram of the life cycle of the eastern oyster, *Crassostrea virginica*.

The most common approach for oyster habitat restoration is to create "reefs" of substrate material, such as limestone rock, concrete or oyster shell (fresh or fossil), and that will attract the natural settlement of oysters. There are still many questions regarding the most cost-effective ways to create new oyster habitat, including the best substrate for attracting oyster settlement, the best locations (including tidal height) to place the substrate and the best time of year to initiate reef construction.

The research described here was designed to answer questions that may help restoration biologists maximize their efforts in future restoration projects throughout Tampa Bay. The two major questions being asked were:

[1] Despite the large loss of oyster reef habitat compared to historic levels, oysters are commonly found attached to mangrove prop roots, seawalls and bridge and dock pilings (see Drexler et al. 2014). Thus in many estuaries, including Tampa Bay, oysters are primarily substrate limited.

1. How does fresh shell obtained from restaurants perform as a settlement substrate compared to the more commonly used substrate, fossil shell? This will help determine the relative cost effectiveness of each material.
2. What time of year does most oyster settlement occur? Settlement patterns are related to the time of spawning. Having this information will enable biologists to time the planting of shell to maximize the success of habitat restoration efforts.

Methods and Materials

Fresh shell was obtained from local seafood restaurants as part of the Gulf Coast Oyster Recycling and Renewal Program (GCORR). Fossil shell (obtained from a local limestone quarry) was provided by the Manatee County Parks and Natural Resources Department. Beginning on June 1, 2017 and monthly thereafter, ten fossil shells and ten fresh shells of similar size were placed in separate mesh bags placed in the water at Robinson Preserve, near a recently restored oyster reef (27°30'45.67"N; 82°40'19.59"W).

Bags containing the shell (Figure 2) were hung under the pier (Figure 3) so the shells were in the mid-intertidal zone, where oyster survival is greatest. At the end of each month, exposed shells were removed and replaced by clean shells. Exposed shells were examined using a dissecting microscope and all recently settled oysters (spat) observed on both sides of each shell were counted (Figure 4).

Proportions of total oyster spat settling on fresh and fossil shells and on inner and outer surfaces of both types of shell were compared statistically using Z tests (MedCalc Statistical Software version 18.5).

Figure 2. Fresh (left) and fossil (right) shells in mesh bags.

Figure 3. Shells in bags hung below the pier at Robinson Preserve

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Figure 4. Fresh oyster shell with a single oyster spat (circled). Recently settled barnacles are also visible

On each sampling date, ten live oysters were collected from the surrounding shoreline, and water temperature and salinity were determined using a thermometer and refractometer, respectively. Each oyster was measured (shell height, mm) and then opened for determination of condition index (Lucas & Beninger 1985). Tissues and shells of all oysters were dried to a constant weight at 60 °C, and condition index (CI) was calculated as:

$$CI = [\text{tissue dry weight (g)} / \text{shell dry weight (g)}] \times 100$$

Condition index is a simple way to estimate reproductive activity throughout the year. As oysters produce gametes (eggs and sperm), the CI typically increases. When oysters release those gametes (spawn), CI typically decreases.

Results

Mean shell height of live oysters collected at the study site ranged from 59-74 mm and were capable of reproducing. Water temperature at the study site ranged from a maximum of 31.5 °C in June 2017 to a minimum of 14.2 °C in December 2017; salinity was generally above 30 psu (maximum of 35 psu in April 2018) except in July, August and September 2017 and May 2018, when salinity as low as 15 psu was recorded in conjunction with rainfall events (Figure 5).

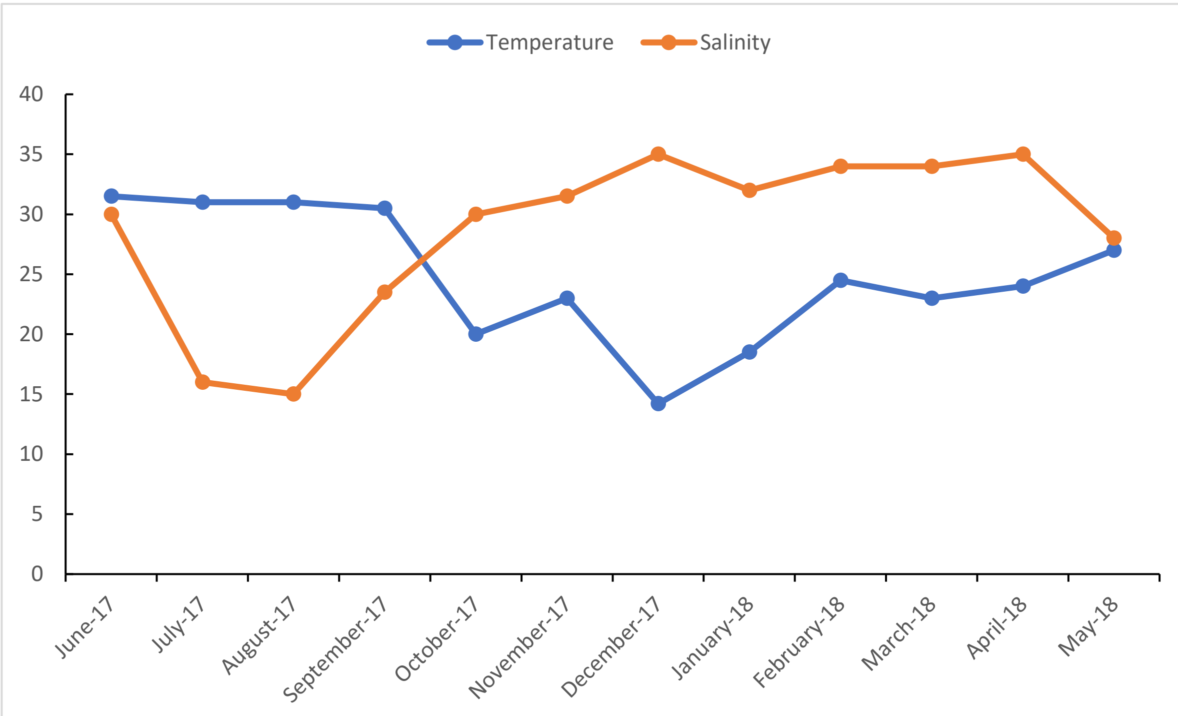

Figure 5. Water temperature (°C) and salinity (psu) at the Robinson Preserve study site from June 2017 through May 2018.

Mean condition index was over 3.5 in both June and July, but decreased in August and September before increasing again in October through January 2018, when a maximum value of 6.9 was recorded. Condition index declined steadily from January through May 2018 (Figure 6).

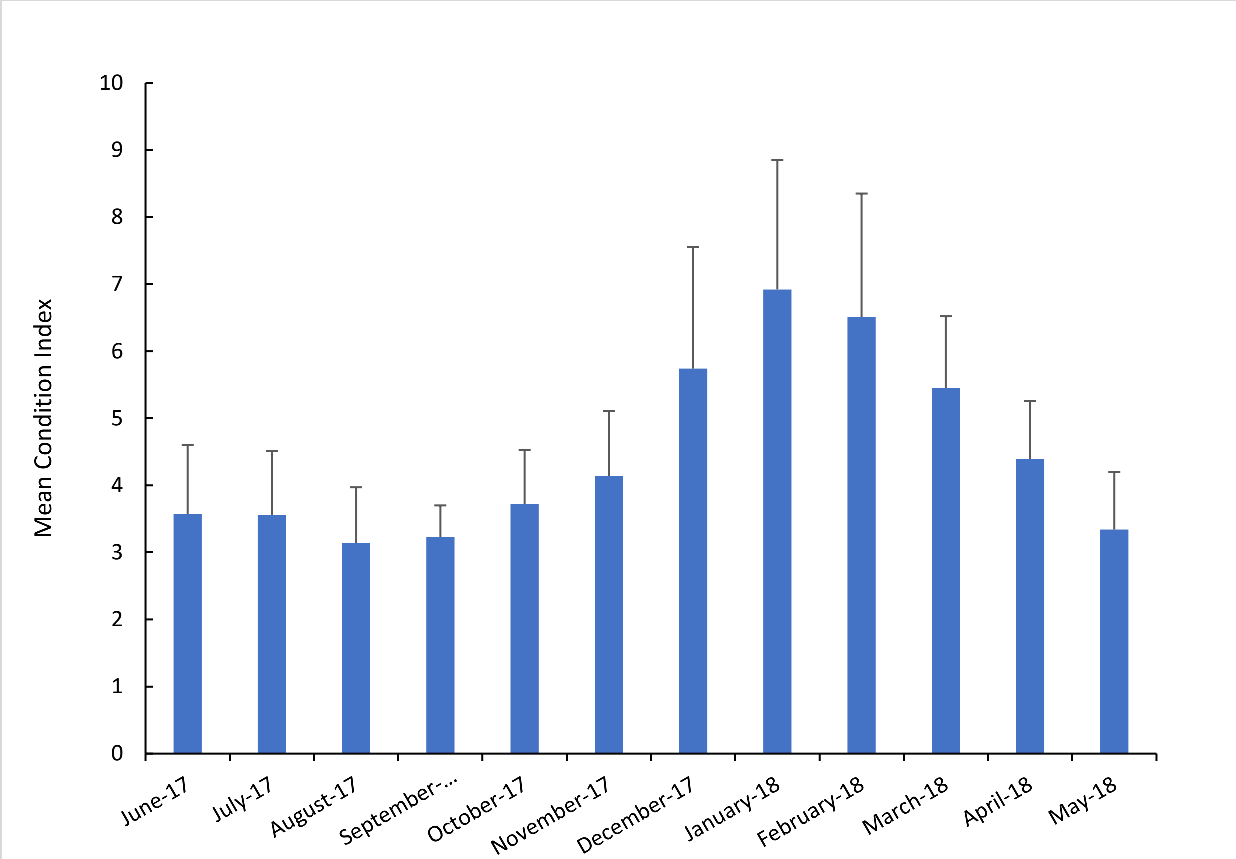

Figure 6. Mean (+1 SD) condition index of oysters collected at Robinson Preserve from June 2017 through May 2018 (n=10).

In all months that settlement occurred, fresh shells had more oyster spat than fossil shells (Figure 7). A total of 742 oyster spat was observed throughout the yearlong study; 409 (55%) settled on fresh shell and 333 (45%) settled on fossil shell; these proportions are significantly different ($P \leq 0.001$). The number of spat that settled on fresh shell was 23% greater than the number of spat that settled on fossil shell.

A considerably greater number of spat settled on the inner shell surface of both fresh and fossil shells compared to the outer shell surface. For fresh shells, 297 of the 409 spat (73%) were found on the inner shell surface and for fossil shells, 237 of the 333 spat (71%) settled on the inner shell surface. Both differences were statistically significant ($P \leq 0.001$).

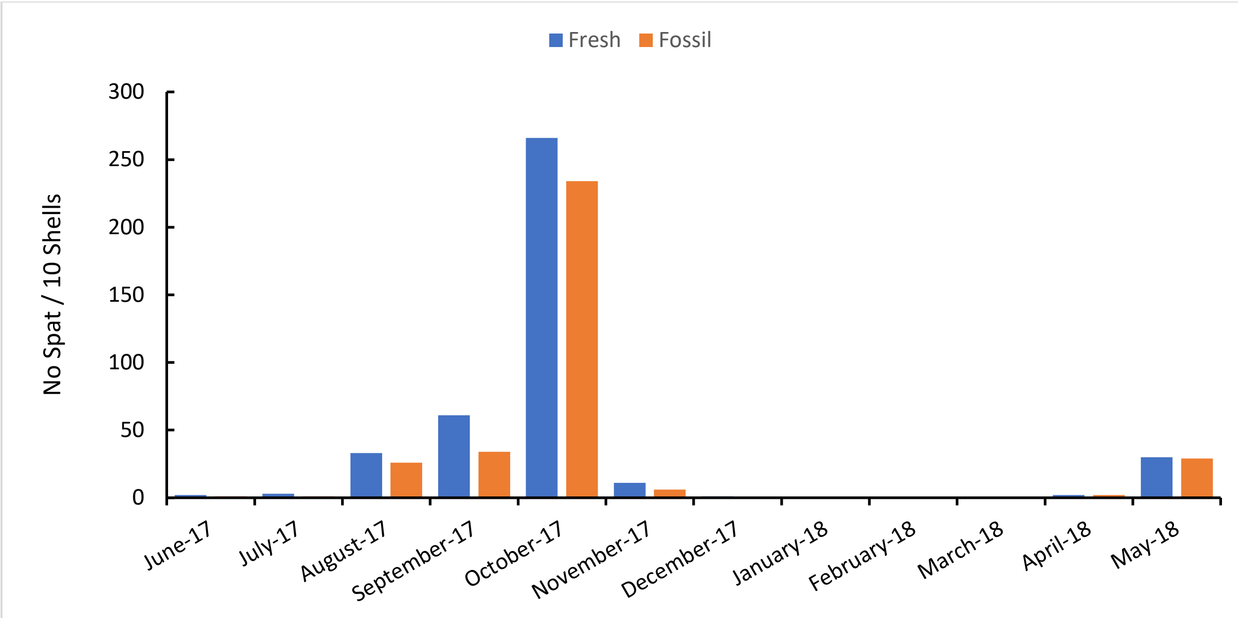

Figure 7. Number of spat on ten shells (fresh and fossil) from June 2017 through May 2018.

The seasonal pattern of oyster settlement was similar for both types of shell (Figure 7). Total settlement (all shells) was low in June (n=3), July (n=4), but increased in August 2017 (n=59). The total number of spat on all shells increased to 95 in September and reached a maximum in October 2017, when 500 spat were counted on all shells; one fossil shell from October 2017 had 48 spat. A significantly greater ($P \leq 0.001$) number of spat settled in October 2017 than any other month. Settlement decreased in November (n=17) and December 2017 (n=1). No settlement occurred in January, February and March 2018. Settlement resumed in April and May 2018, when 4 and 59 spat were counted, respectively.

Discussion

This study found that fresh shells are significantly superior to fossil shells as a substrate for settling oysters, attracting 23% more spat than fossil shells. This finding supports the general conclusion that oyster shell is the most desirable material for reef restoration. The problem is that the supply of fresh oyster shell is limited and therefore costly. This has led to research examining the effectiveness of potential alternative (and more economical) materials as substrate for oyster settlement.

In Chesapeake Bay, Haven et al. (1987) planted equal volumes of oyster shell and fragmented slate at two locations in the James River; after one year, there were 4-5 times more oyster spat on the shell than on the slate. Similarly, Mann et al. (1990) compared oyster settlement on oyster shell, tire chips (shredded tires) and expanded shale (natural shale heated to induce expansion) at four reefs in the James River and found that oyster shell attracted significantly greater oyster settlement (64%) than either tire chips (22%) or expanded shale (14%). At

Fisherman's Island, near the mouth of Chesapeake Bay, reefs were constructed of surf clam (*Spisula solidissima*) shell, oyster shell and stabilized coal ash at three tidal heights; it was found that even though settlement was similar on all three reef types, post-settlement mortality was lowest on the reefs constructed of oyster shell and oyster abundance was highest in the intertidal zone on all reefs (O'Beirn et al. 2000). In Louisiana, significantly more oysters settled on limestone than clam shell (*Rangia cuneata*); limestone, clam shell and concrete attracted significantly more spat than gravel (Soniat et al. 1991). This was reinforced by Haywood & Soniat (1992) who found that clam shell (*R. cuneata*) attracted significantly fewer spat then cement-stabilized gypsum and Mexican limestone. Similarly, Soniat & Burton (2000) found that limestone attracted significantly more oyster settlement than sandstone. More recently, George et al. (2014) compared oyster settlement in St. Charles Bay, Texas on concrete, porcelain, limestone, river rock and oyster shell and concluded that spat recruitment density was similar on all materials. Overall, oyster shell was found to be the optimal substrate for settlement and survival, but limestone appears to be a reasonable alternative. Thus, to create a new reef or enhance an existing reef, it would be more cost effective to construct the base of the reef using limestone or concrete and then place oyster shells on top (within the intertidal zone) to maximize settlement. The reason that oyster shells are the best material for attracting settling oyster larvae may have to do with species-specific chemical cues sensed by oyster larvae when they are competent to settle and undergo metamorphosis (Crisp 1967, Bonar et al. 1990). This would explain why shells of other bivalves, *Rangia cuneata* and *Spisula solidissima*, do not perform as well as oyster shells. Accordingly, fossil oyster shell, although not previously evaluated as a substrate material, and not as good as fresh oyster shell (this study), is likely better than other non-shell materials such as limestone and concrete.

Oyster settlement was significantly greater on the inner shell surface than the outer shell surface of both species. This occurred in spite of the fact there was no attempt to orient the shells used in this study in any particular way; they were simply dropped into the bags. This would suggest that oyster settlement is influenced by factors other than chemical cues alone. Recent research has suggested that the concavity of the inner shell surface allows water movement great enough to maintain larval supply but not so great as to inhibit searching and attachment behaviors of oyster larvae (Johnson 2017).

At Robinson Preserve, 88% of all settlement occurred in three months, August, September and October 2017. This means that most spawning activity occurred between July and September 2017. This is supported by the condition index data. Oysters at Robinson Preserve had the lowest condition index in August and September 2017, suggesting that spawning occurred primarily in late summer. In 2017, high rainfall in July and August caused a reduction in salinity that may have inhibited settlement. Additional oyster settlement was observed in May 2018, indicating a second, spring spawning event in April 2018.

C. virginica ranges from Labrador to Florida along the east coast of North America and throughout the Gulf of Mexico. Along this range, the timing of gonadal development, maturation and spawning varies in response to genetically determined environmental cues. The general trend is that with decreasing latitude, spawning occurs later in the year and over a longer period of time (Barber et al. 1991). In Florida, Hayes & Menzel (1981) found that newly set oysters spawn in late September or early October, before the end of their first year. They also noted that larger, older oysters may spawn a second time, earlier in the year. In Tampa Bay, it was determined that oysters are reproductively active from April through September, with settlement occurring from May through October, with a peak in July (Drexler et al. 2014). The difference in peak settlement occurring in October (this study) and July (Drexler et al. 2014) could be due to slight environmental differences between locations or interannual variations. This points out the importance of knowing the reproductive cycle and time of peak settlement in any location where oyster restoration is contemplated.

It was observed that numerous other species competed with oysters for space on both fresh and fossil shell. Fouling by mussels, barnacles and polychaetes was especially heavy during the summer months. This suggests that restoration efforts will experience the greatest success if clean substrate (any material) is deployed just prior to the time of maximum settlement.

Conclusions and Recommendations

The success of oyster reef restoration will depend on both the material used to provide substrate for settling oysters and the time of year when the reef is constructed. Fresh shell is the obvious optimal substrate material. Shell is usually put into mesh bags that are moved to the restoration site and stacked in the intertidal zone to form the new reef. The supply of fresh shell in Manatee County, although currently limited, will be increasing due to the efforts of the Gulf Coast Oyster Recycle and Renewal (GCORR) program, which collects shells from local seafood restaurants. For larger projects, crushed limestone, concrete or fossil shell (depending on cost and availability) should be used for the reef foundation, with the fresh shell placed on top where it will attract the greatest number of settling oysters.

Based on the results reported here, restoration efforts will be maximized when fresh shell is planted (mid-intertidal zone) as close to the end of September as possible. If shell is planted before this date, shell surfaces will become colonized by other, competing organisms (e.g., barnacles, mussels, polychaetes), rather than oysters. If shell is planted after this date, there will be very few oyster larvae available to settle.

Further research should be undertaken to determine the extent of interannual and site-specific variation in oyster reproductive cycles and peak settlement periods throughout the Tampa Bay region.

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References

Baggett, L.P., S.P. Powers, R.D. Brumbaugh, L.D. Coen, B.M. DeAngelis, J.K. Greene, B.T. Hancock, S.M. Morlock, B.L. Allen, D.L. Breitburg, D. Bushek, J.H. Grabowski, R.E. Grizzel, E.D. Grosholz, M.K. LaPeyre, M.W. Luckenbach, K.A. McGraw, M.F. Piehler, S.R. Westby and P.S.E. zu Ermgassen, 2015. Guidelines for evaluating performance of oyster habitat restoration. Restor.Ecol. 23: 737-745.

Barber, B.J., S.E Ford and R. N. Wargo, 1991. Genetic variation in the timing of gonadal maturation and spawning of the eastern oyster, *Crassostrea virginica* (Gmelin). Biol. Bull. 181: 216-221.

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

Bonar, D.B., S.L. Coon, M. Walch, R.M. Weiner, M. Ronald and W. Fitt, 1990. Control of oyster settlement and metamorphosis by endogenous and exogenous chemical cues. Bull. Mar. Sci. 46: 484-498.

Carmichael, R.H., W. Walton & H. Clark, 2012. Bivalve-enhanced nitrogen removal from coastal estuaries. Can. J. Fish. Aquat. Sci. 69: 1131-1149.

Crisp, D.J., 1967. Chemical factors inducing settlement in *Crassostrea virginica* (Gmelin). J. Anim. Ecol. 36: 329-335.

Drexler, M., M.L. Parker, S.P. Geiger, W.S. Arnold and P. Hallock, 2014. Biological assessment of easten oysters (*Crassostrea virginica*) inhabiting reef, mangrove, seawall and restoration substrates. Estuar. Coast. 37: 962-972.

George, L.M., K. De Santiago, T.A. Palmer and J.B. Pollack, 2014. Oyster reef restoration: effect of alternative substrates on oyster recruitment and nekton habitat use. J. Coast Conserv. DOI 10.1007/s11852-014-0351-y.

Harding, J.M. and R. Mann, 2001. Oyster reefs as fish habitat: opportunistic use of restored reefs by transient fishes. J. Shellfish Res. 20: 951-959.

Haven, D.S., J.M. Zeigler, J.T. Dealteris and J.P. Whitcomb, 1987. Comparative attachment, growth and mortalities of oyster (*Crassostrea virginica*) spat on slate and oyster shell in the James River, Virginia. J. Shellfish Res. 6: 45-48.

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

Haywood, E.L. and T.M. Soniat, 1992. The use of cement-stabilized gypsum as cultch for the eastern oyster, *Crassostrea virginica* (Gmelin, 1791). J. Shellfish Res. 11: 417-419.

Higgins, C.B., K. Stephenson & B.L. Brown, 2011. Nutrient bioassimilation capacity of aquacultured oysters: Quantification of an ecosystem service. J. Environ. Qual. 40: 271-277.

Kellogg, M.L., J.C. Cornwell, M.S. Owens & K.T. Paynter, 2013. Denitrification and nutrient assimilation on a restored oyster reef. Mar. Ecol. Prog. Ser. 480: 1-19.

Johnson, K.B., 2017. Laboratory settlement of the eastern oyster *Crassostrea virginica* influenced by substratum concavity, orientation, and tertiary arrangement. J. Shellfish Res. 36: 315-324.

Lucas, A. and P.G. Beninger, 1985. The use of physiological condition indices in marine bivalve aquaculture. Aquaculture 44: 187–200.

Mann, R., B.J. Barber, J.P. Whitcomb and K.S. Walker, 1990. Settlement of oysters, *Crassostrea virginica* (Gmelin, 1791), on oyster shell, expanded shale and tire chips in the James River, Virginia. J. Shellfish Res. 9: 173:175.

Newell, R.I.E., 1988. Ecological changes in Chesapeake Bay: Are they the result of overharvesting the American oyster, *Crassostrea virginica*? Pages 536-546, in Lynch, M.P. and E.C. Krome, eds. Understanding the Estuary: Advances in Chesapeake Bay Research. Chesapeake Research Consortium Publication 129.

Newell, R.I.E., 2004. Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve mollusks: A review. J. Shellfish Res. 23: 51-61.

Newell, R.I.E. & E.W. Koch, 2004. Modeling seagrass density and distribution in response to changes in turbidity stemming from bivalve filtration and seagrass sediment stabilization. Estuar. Coast. 27: 793-806.

Newell, R.I.E., J.C. Cornwall and M.S. Owens, 2002. Influence of simulated bivalve biodeposition and microphytobenthos on sediment nitrogen dynamics: A laboratory study. Limnol. Oceanogr. 47: 1367-1379.

O'Beirn, F.X., M.W. Luckenbach, J.A. Nestlerode and G.M. Coates, 2000. Toward design criteria in constructed oyster reefs: oyster recruitment as a function of substrate type and tidal height. J. Shellfish Res. 19: 387-395.

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

Reitsma, J., D.C. Murphy, A.F Archer & R.H. York, 2016. Nitrogen extraction potential of wild and cultured bivalves harvested from nearshore waters of Cape Cod, USA. Mar. Poll. Bull. 116:175-181.

Scyphers, S.B., S.P. Powers, K.L. Heck and D. Byron, 2011. Oyster reefs as natural breakwaters mitigate shoreline loss and facilitate fisheries. PLoS ONE 6: e22396. Doi: 101371/journal.pone.0022396.

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

Soniat, T.M., R.C. Broadhurst and E.L. Haywood, 1991. Alternatives to clamshell as cultch for oysters, and the use of gypsum for the production of cultchless oysters. J. Shellfish Res. 10: 405-410.

Stunz, G.W., T.J. Minello and L. Rozas, 2010. Relative value of oyster reef as habitat for estuarine nekton in Galveston Bay, Texas. Mar. Ecol. Prog. Ser. 406: 147-159.

Zu Ermgassen, P.S., M.D. Spalding, R.E. Grizzle and R.D. Brumbaugh, 2012. Quantifying the loss of a marine ecosystem service: filtration by the eastern oyster in US estuaries. Estuaries and Coasts: DOI 10.1007/s12237-012-9559-y.