Introduction: In coastal areas with moderate to high astronomical tides and high salinities, oyster reefs typically occur as intertidal structures often fringing shorelines. In these areas, completely subtidal oysters occur along the lower reaches of the intertidal reefs and appear to be sustained through physical forces moving intertidal oysters to the subtidal reef margins. Biological stressors in high salinity waters, such as predation, bioerosion and disease, are proposed to prevent subtidal reef formation, but as levels of biological stressors decline with increasing intertidal elevation, reef accretion is favored. One significant gap in our knowledge regarding oyster reef formation in high salinity coastal waters is at what point in the tide range (= mean seawater inundation period) does the transition from reef accretion to reef deterioration occur? The APNEP funded project, “Evaluating Restoration Success for Newly Constructed Oyster Reefs Spanning a Critical Intertidal Elevation,” was designed to answer this important question by building replicate sets of oyster-shell reefs precisely positioned on substrate at 2 intertidal elevations (middle and low), one marginally subtidal elevation and one completely subtidal elevation in the Middle Marsh region of the Rachel Carson National Estuarine Research Reserve near Beaufort, North Carolina (Fig. 1).
Figure 2 shows the conceptual model being tested by this project, with tide-related values specific to Middle Marsh and test substrate elevations determined through a preliminary examination in fall 2009 by project Co-PIs of oyster reefs constructed by UNC graduate student, Jonathon Grabowski, in 1997 and 2000. The main object of this 10-month APNEP-funded project was to construct in each of 4 zones 8 experimental reefs (4 small 60 bushel reefs and 4 large 300 bushel reefs) precisely positioned at each of the 4 substrate elevations shown in Figure 2. Reef construction occurred in March and April 2011. This was optimal deployment timing because the reefs were completed before the late-spring/early-summer oyster recruitment period but not constructed too far in advance of the major oyster recruitment season, which could have resulted in some reefs being heavily colonized by oyster competitors and predators. Data from immediate post-construction elevation surveys will provide the baseline for each reef against which to detect future reef accretion or deterioration. Extensive biological surveys will begin once oyster recruitment has occurred. These near-term, post-construction physical and biological surveys are supported by other agencies and investigator resources. Future monitoring of reef sizes and community structure will be supported by multiple state and federal programs, as we expect these reefs to become important long-term experimental stations. Data from these reefs will aid the development of best restoration models and practices for oyster reefs in intertidal settings and better define their range of ecosystem services and habitat value.

**Methods:** To test our “critical depth zone” hypothesis for intertidal oyster reef formation, 4 zones bordering Middle Marsh were chosen for the building of replicate oyster reefs. Within each of these zones, large sand/mud flats were present that spanned the necessary depth range, had sufficient areal coverage at each test depth, and appeared to be relatively stable over decadal time frames (as determined from Google Earth images). In June 2010, Goggle Earth imagery and initial field inspections were used to choose 4 potential reef building zones along sand/mud flats on the southern end of Middle Marsh. This information was used to draft a Coastal Area Management Act (CAMA) Major Development Permit, which is required by the North Carolina Division of Coastal Management for oyster reef construction activity in North Carolina. The CAMA permit (#89-10) for the project was issued on August 18, 2010. Between July 2010 and February 2011, the project team used a Trimble-based GPS system (Fig. 3) to produce course substrate elevation maps of the 4 proposed reef building zones. These elevation surveys produced medium-scale (1s to 10s of m) elevation maps from which specific reef building plots where chosen that met the exact elevation criteria for our test of the “critical substrate
Elevation profiles for 1 of these 4 proposed reef building areas proved to be too steep, thus another sand/mud flat on the NW corner of Middle Marsh was mapped and determined to be a suitable alternative 4th reef building zone. CAMA approval for reef building in the new zone was obtained through a “Minor Modification” amendment to CAMA permit #89-10. Using the Trimble GPS system (Fig. 3), high resolution (0.5-1.0 cm horizontal and vertical) mapping identified specific plots within each zone to build 1 small (3m x 5m) and 1 large (10m x 15m) oyster-shell reef (15 cm deep) at each of 4 intertidal to subtidal substrate depths: -0.5, -0.6, -0.75 and -0.90 m (relative to the NAVD88 datum) (Fig. 4).

Figure 3. Left: Tony Rodriguez acquiring Trimble-based substate elevations while Joel Fodrie inspects a reef constructed by IMS graduate student Jon Grabowski in 1997. Right: Tony Rodriguez using the Trimble GPS system.

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Figure 4. Reef locations, sizes and substrate depths for the 4 test zones in the Middle Marsh region of the Rachel Carson National Estuarine Research Reserve.
Oyster shell for reef construction was eventually obtained from two sources. We acquired ~40% of our shell from a Virginia supplier but the low quality of the material (too much associated dirt and debris) compelled us to switch to higher quality Gulf of Mexico (GOM) shell. However, shellfish harvest closures in the GOM following the massive release of oil from the Deepwater Horizon gusher created a nationwide shortage of oyster shell and delayed our getting GOM shell until oyster harvest resumed there in the fall of 2010. By October 2010, ~10,000 bushels of oyster shell had been delivered to IMS (Fig. 5). This delay and the need to allow the recently harvested shell to “cure” (i.e. to kill off shell-associated flora and fauna through exposure to heat/freezing, dessication, and rainfall) for at least six months before moving it into North Carolina waters necessitated requesting a 4-month no-cost extension for the project to allow completion of reef construction.

Figure 5. Left: Curing oyster shell on the IMS campus purchased for reef construction. Right: Project team members left to right, Tony Rodriguez, Sara Coleman, Joel Fodrie and Niels Lindquist.

The project’s 3m x 5m reefs were constructed in March 2011 by an “all-hands-on-deck” method that involved (1) filling bushel baskets with oyster shell at the IMS campus, (2) trucking the shell to an staging area at the east end of Taylor’s Creek, which is near the northwest corner of Middle Marsh, (3) transferring baskets of oyster shell from trucks to small boats, (4) boating the shell to each small reef building plot, (5) dumping the shell on site, (6) forming each shell pile to the desired dimensions and (7) marking each reef with identifying floats and posts (Fig. 6). Additionally, a 30-cm high chicken wire fence was formed around each of the small reefs to prevent oyster shell from being moved from the plots by currents and wind waves before the reef had the opportunity to settle and consolidate. It soon became apparent, however, that shell loss from the reefs was not an issue under normal conditions and that the reefs were quickly becoming well consolidated and even more resistant to physical disturbances. Overall, several weeks of hard work yielded 16 beautifully shaped 3m x 5m x 0.15m reefs (e.g. Fig. 6F) and thus the start of our long-term experiment.

The project’s large reefs (Fig. 7) were constructed with major assistance from the Resource Enhancement Section of the North Carolina Division of Marine Fisheries (DMF). DMF provided for only the cost of fuel a barge capable of transporting up to 500 bushels of oyster shell per trip, dump trucks for moving oyster shell from IMS to a shell staging area at the Harkers Island harbor, front-end loaders for moving shell, and personnel to operate the heavy equipment and barge. Over a week-long period in April 2010, a 2-person IMS team worked with DMF barge Captain Thomas Hardin and mate Michael Jordan to place 300 bushels of oyster shell on each of the 16 pre-selected plots. Over the following 2 weeks, teams of IMS personnel and commercial fisherman Adam Tyler worked to aggregate the shell
and shape the reefs to a standard size of 7m x 9m x 0.15m. This was a smaller footprint than originally proposed but was necessitated by the large amount of dirt and debris delivered along with some loads of shell. Thus, in subsequent years, we will be following the trajectory of reef development for 60 and 300 bushel oyster reefs constructed at 4 intertidal to subtidal substrate elevations (see Fig. 2).

Figure 6. A – digging into the IMS oyster shell pile for small reef building. B – some of the small reef building crew. C – moving baskets of shell from trucks to boats. D – fisherman Adam Tyler and IMS student Sara Coleman taking shell to a reef building site. E – unloading 60 bushels of oyster shell on a small reef plot. F – completed “shaped” small oyster reef.

1 Special Thanks to the Boathouse at Front Street Village marina in Beaufort, North Carolina for allowing our use of their docks at no costs for boat dockage and as a shore-to-boat transfer area for the small reef construction

Results: In April 2011, construction of the proposed 32 oyster shell reefs was completed – (i) shell was deployed and shaped to the desired dimensions, (ii) a float and a corner pole were deployed to identify each reef and alert boaters to their presence, and (iii) 4 large (3-inch diameter) PVC posts were erected at the corners of each of the 4 test zones, each bearing a sign informing the public that the newly constructed reefs are research sanctuaries and that shellfish harvest from the reefs is prohibited. All other legal fishing activities are permitted within these zones. We expect that the APNEP reefs will become the focus of a great deal of hook-and-line fishing and flounder gigging activity.
Preliminary visual examination of the reefs has shown substantial differences among the treatment reefs in the amount of sedimentation and colonization by algae and colonial invertebrates (Fig. 8). The deeper reefs appear to have sandy sediment filling in some space between shells, but abundant exposed shell is still available for oyster recruitment on most deep reefs (compare Fig. 8C and 8D). Further, the shallower reefs are relatively free of colonizing algae and invertebrates (Fig. 8C), while the deeper reefs, especially the deepest treatment reefs (-0.9 m, NAVD 88, Fig. 2) that are always submerged, have a high cover of filamentous algae and colonial hydroids and bryozoans. Not unexpectedly, these observations confirm higher rates of biologically processes among reefs constructed at the lower substrate elevations. Future monitoring will examine how multiple physical and biological processes interact at the different substrate elevations (= submergence times) determine rates of reef accretion or decline, and thus begin the process of quantitative parameterization of our conceptual model of oyster reef development in high salinity coastal waters. Data from the APNEP reefs will aid the development of best
restoration models and practices for oyster reefs in intertidal settings and better define their range of ecosystem services and habitat value.

Figure 8. Comparison of (A) a high intertidal reef (prior to final shaping) and (B) a low intertidal reef that remains submerged over all but the most extreme tide cycles. C – high intertidal reef at high tide 2 months after shell deployment – note the absence of algal cover. D – low intertidal reef 2 months after shell deployment – note the high cover of filamentous algae and small colonial invertebrates, mostly hydroids and bryozoans. E – the predicted future condition of the higher intertidal reefs. F – the predicted future condition of low intertidal reefs. Foundations of reefs shown in panels E and F were constructed by UNC graduate student Jonathon Grabowski in 1997.

Synergies: The Middle Marsh region of the Rachel Carson National Estuarine Research Reserve has been a magnet for estuarine studies for decades, including investigations of multiple aspects of oyster reef ecology such as the dissertation research of Jonathon Grabowski that lead to the establishment between 1997 and 2000 of multiple replicates oyster reefs across multiple landscape settings. The present-day patterns of success and decline among these reefs underpinned the present APNEP oyster reef building project (#3154) testing the “critical substrate elevation” hypothesis. During the APNEP grant period, additional research conducted with other federal and state dollars examined a wide variety of processes and structural elements of the Grabowski reefs and nearby natural reefs, including:
• Coring natural and constructed reefs to examine long-term changes in reef condition and accretion rates,
• Impacts of seasonal macroalgae blooms on natural and constructed reefs,
• Faunal surveys of natural and constructed reefs across the intertidal gradient.

Additional concurrent and newly funded oyster projects that integrate with this APNEP project include:
• Fodrie’s crab pots to oyster reef project funded by both NC Sea Grant and APNEP,
• Lindquist et al.’s survey of oyster-shell boring sponges in NC oyster reef habitats funded by the NC Sea Grant Fisheries Resource Grant program,
• Lindquist, Eggleston (NCSU) and Tyler’s (commercial fisherman) examination of the suitability and possible advantages of using non-carbonate substrates for oyster reef foundations in high salinity waters funded by the NC Sea Grant Fisheries Resource Grant program.

These projects and future research on the structural and functional evolution of the new APNEP reefs in Middle Marsh should be exceptionally informative for oyster reef restoration efforts, both within and beyond the Albemarle-Pamlico estuarine system.

Collaborating Institutions and Personnel:
**UNC Chapel Hill Institute of Marine Sciences**
  - **Faculty:** Niels Lindquist, Joel Fodrie, Tony Rodriguez
  - **Graduate Students:** Sara Coleman, Ethan Theuerkauf
  - **Technicians:** Abigail Poray, Chris Baille, Sam Fuller, Erin Voigt, Joe Purifoy, Glenn Safrit

**NC Division of Marine Fisheries, Resource Enhancement Section**
  - Craig Hardy, Clay Caroon, Michael Jordan, Thomas Hardin, Gretchen Lauriat, Tom Piner, and Joe Carraway

**Commercial Fisherman**
  - Adam Tyler (license # F290312); David “Clammerhead” Cessna (license # F302711)

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