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## Linking structural and functional characteristics of restored oyster reefs : A Restoration Project in the Virginia Coast Reserve

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# LINKING STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF RESTORED OYSTER REEFS



6/30/2014

A Restoration Project in the Virginia Coast Reserve

A final report to:  
The Nature Conservancy  
and  
National Oceanic and Atmospheric Administration's  
Community Restoration Program

Prepared by:  
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# Linking structural and functional characteristics of restored oyster reefs

## A RESTORATION PROJECT IN THE VIRGINIA COAST RESERVE

### Award Information

**Project Title:** Linking Structural and Functional Characteristics of Restored Oyster Reefs: A Restoration Project in the Virginia Coast Reserve

**Principal Investigators:**

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Paige G. Ross, Virginia Institute of Marine Science  
Bowdoin Lusk, The Nature Conservancy

**Award Number:** GMT-VIMS-070111

**Award Period:** 01-Jul-2011 to 31-May-2014

**Grantee Org.:** Virginia Institute of Marine Science

**Contact Person:** Lisa Kellogg  
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804-684-7706

### Restored Area Information

**Habitat Type:** Oyster Reef

**Acres Restored:** 0.071

**Location:** 37° 17.032' N -75° 54.241' W

**River Basin:** Hillcrest Oyster Sanctuary, Virginia Coast Reserve

**Geographic Id.:** Virginia Coastal Bays

**Species Benefiting:**

Eastern Oyster, *Crassostrea virginica*  
Oyster reef-associated organisms

## Abstract

Eighteen native oyster reefs (16-m<sup>2</sup> each) were restored using six oyster densities (0, 10, 25, 50, 100 and 250 adult oysters m<sup>-2</sup>) with three replicates of each density at an intertidal site in The Nature Conservancy's Virginia Coast Reserve. Reef construction was successful and continues to provide a range of oyster biomass densities useful for exploring relationships between oyster reef structural and functional parameters. Between April 2012 and July 2013, a science-based monitoring program explored quantitative relationships between structural and functional characteristics of these restored reefs. Structural parameters examined included oyster abundance, oyster size/biomass, surface shell volume, reef topographic complexity and sediment characteristics. Functional parameters included denitrification rates and macrofaunal abundance and biomass. Relationships between reef structural parameters and functional parameters were complex and variable. As of July 2014, these reefs continue to serve as a platform for continued studies of the relationships between reef structural and functional characteristics.

## Rationale

Efforts to restore viable oyster reefs and expand oyster populations in Chesapeake Bay and elsewhere have been increasingly motivated by the desire to enhance ecological functions and attendant ecosystem services. Though it is widely appreciated that these services and functions can include enhanced secondary production, biodiversity, benthic-pelagic coupling and water quality, few oyster restoration projects have actually quantified these functional characteristics. For most restoration projects, directly measuring ecological functions is too costly to include as part of routine monitoring programs. As a result, the success of these projects has been defined solely on the basis of structural metrics (often the density of market-sized oysters). While appropriate for a project targeting fisheries enhancement, this approach fails to capture the ecosystem services provided by restored reefs. Tools are needed that allow estimation of ecological function and related ecosystem services based upon structural reef parameters that are easily measured.

Scaling ecosystem services to structural parameters requires rigorous, quantitative, post-restoration monitoring of ecological functions and ecosystem services provided by reefs of differing oyster abundance and biomass density. One of the most poorly quantified, yet potentially important, ecosystem services provided by restored oyster reefs is their capacity to transform dissolved inorganic nitrogen into nitrogen gas via denitrification, thereby preventing its use by phytoplankton to fuel their growth. However, accurate measurement of denitrification rates is a complex and expensive undertaking. The primary goal of this project was to identify reef structural characteristics that are easily measured and could be used to reliably predict denitrification rates. Relationships between reef structure and the associated macrofaunal community were also explored.

## Project Narrative

Our overarching goal was to develop a tool for estimating the ecosystem services provided by restored oyster reefs based on easily measured structural parameters. To achieve this goal we used science-based monitoring to quantify relationships between structural and functional habitat characteristics on replicate reefs of differing oyster density constructed in the The Nature Conservancy's Virginia Coast Reserve (VCR).

### Goal 1:

Construct 18 oyster reefs (16 m<sup>2</sup> each) of varying initial oyster densities (0, 10, 25, 50, 100 and 250 adult oysters m<sup>-2</sup>) to serve as a platform for identifying relationships between oyster reef structural and functional characteristics, both for the proposed project and future ones.

**Completed Tasks:** All tasks required to achieve this goal were complete as of November 2011. Prior to the start of restoration, 21 plots (16-m<sup>2</sup> each) were identified and marked with stakes as potential restoration sites within the intertidal zone in the Hillcrest Oyster Sanctuary (all natural oyster reefs in this region are intertidal). Eighteen of these plots were randomly selected to become reef plots and 16 bushels of clean oyster shell were spread evenly across each. The remaining three plots served as unmanipulated reference sites. Each reef plot was randomly assigned an oyster density treatment (0, 10, 25, 50, 100 or 250 adult oysters m<sup>-2</sup>). Volunteers recruited by TNC and the Virginia Institute of Marine Science Eastern Shore Laboratory (VIMS-ESL) staff sorted, counted and evenly distributed the appropriate number of adult oysters ( $\geq 50$  mm shell height) across each subplot (Fig. 1). All oysters placed on these reefs were collected from within the VCR. Plots have rebar stakes at 0.5-m intervals throughout the plot to limit predation by rays.



**Fig. 1.** *Top:* TNC volunteers measure, count and sort oysters prior to placement on reefs (photo: VIMS-ESL staff). *Bottom:* VIMS-ESL staff place oysters on one of the high density reefs (photo: Frank Renshaw).



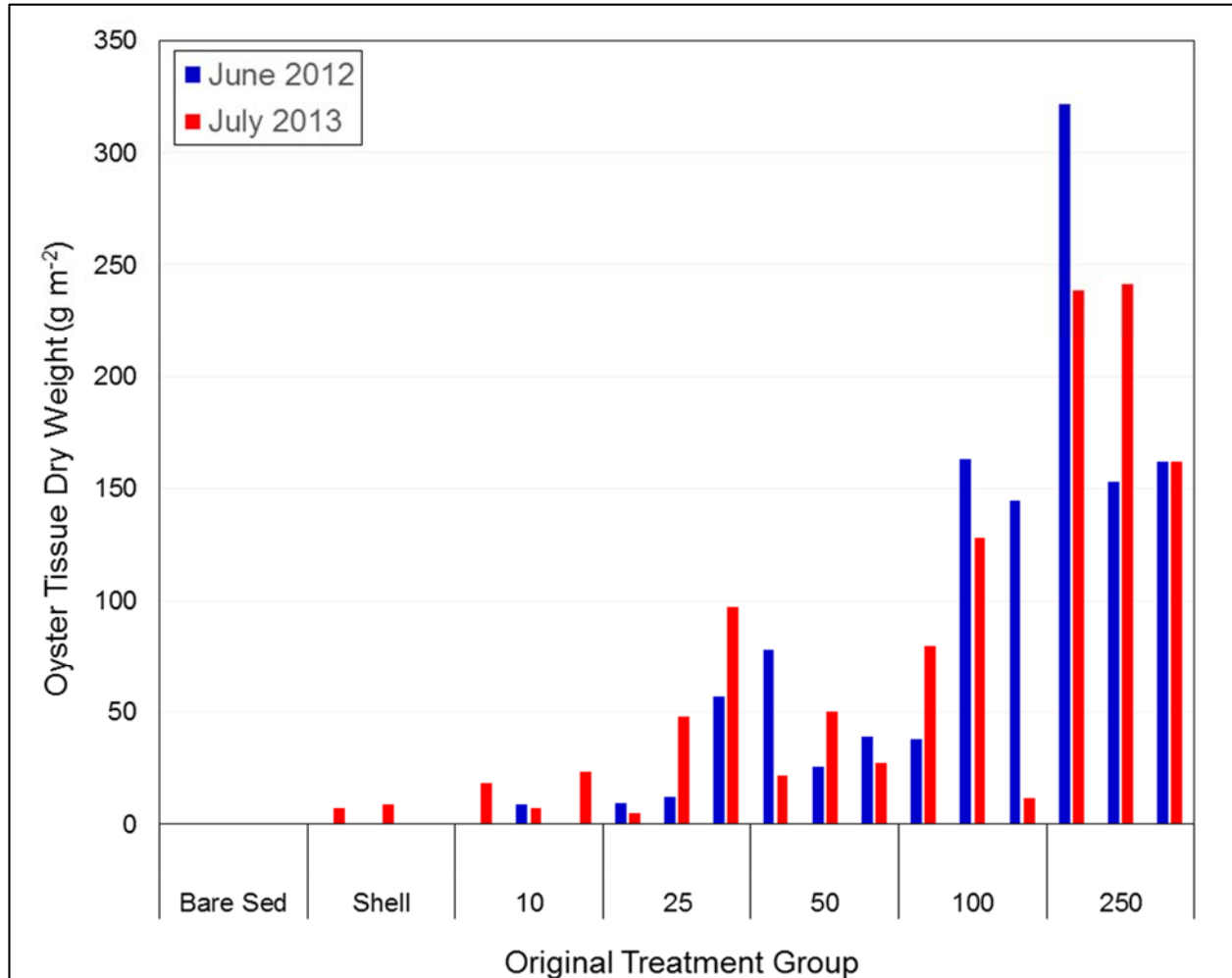
Task	Completion Date
Site selection and survey	Aug 2011
Reef construction	Nov 2011
Oyster population surveys	Apr 2012 Jun 2012 Aug 2012 Oct 2012 Apr 2013 Jul 2013

**Methods:** Oyster abundance and biomass density were determined by collecting three replicate samples from each individual reef and control plot during each sampling period for a total of 63 samples. Sampling sites were selected using a stratified random design resulting in one sample from the edge of each reef, one from the central area and one from between these areas. Each sample was collected by excavating a 0.035 m<sup>2</sup> area to a depth of 15 cm below the sediment surface (Fig. 2). All material was placed in a fine mesh bag and returned to the laboratory where all oysters ≥15mm were counted and measured. To develop length to biomass and shell mass regressions, the dry weight, ash-free dry weight and shell weight was determined for a subset of oysters during each sampling period. Length to biomass and shell mass regressions were then used to calculate oyster tissue dry weight, ash-free dry weight and shell weight per unit area.



**Fig. 2.** VIMS-ESL staff and summer interns collecting (top) and cleaning (bottom) samples prior to laboratory analyses (photo: VIMS-ESL staff).

**Results:** Oyster abundance and biomass were assessed during each of six sampling periods to determine whether differences in oyster density persisted over time (Fig. 3). As expected, oyster biomass densities changed on individual reefs but, as a whole, the reef complex has retained a range in biomass density across reefs.



**Fig. 3.** Estimated oyster tissue biomass density for each reef in June 2012 and July 2013 grouped by original treatment. July 2013 is the most recent date for samples collected as part of the present study. Comparison was made to June 2012 data rather than April 2012 data to avoid differences in tissue biomass due to spawning state. Bare Sed = unmanipulated control plots, Shell = plots to which shell was added but no adult oysters, numbers represent the original densities of adult oysters per square meter planted on each reef.

**Recommendations:** Oyster abundance and biomass density vary over time on oyster reefs. The ability to identify relationships between oyster abundance or biomass and oyster reef function will rely heavily upon gathering accurate data on the oyster population each time oyster reef function is measured.

**Goal 2:**

Employ science-based monitoring of constructed reefs to determine quantitative relationships between structural parameters (e.g. oyster tissue biomass density, surface shell volume, sediment characteristics, reef topographic complexity) and functional characteristics (e.g. denitrification rate and macrofaunal community structure).

**Completed Tasks:** The original science-based monitoring plan for these reefs included sampling in August 2011 prior to reef construction and in April, June, August and October 2012 after reef construction. Additional funding from TNC and the NOAA Chesapeake Bay Office (NCBO) expanded this sampling plan to include additional sampling periods in April and June 2013. Pre-construction sampling was completed in August 2011 and included measurement of biogeochemical fluxes and assessment of the abundance and biomass of macrofauna. Because no oysters or oyster shell were found on the surface of our plots prior to construction, we did not assess other reef metrics. Processing of all pre-construction samples was completed in December 2011.

Post-construction macrofaunal sampling was conducted in April, June, August and October 2012 and in April and July 2013. Funding from another source is supporting an additional sampling period in June/July 2014. Processing of samples collected in 2012 and 2013 is complete. Incubations to assess biogeochemical fluxes were conducted in August and October 2012 and in April and July 2013. The table below lists the completion dates for individual tasks.

Task	Completion Date
Pre-construction flux sampling	Aug 2011
Pre-construction macrofauna sampling	Aug 2011
Processing of pre-construction macrofauna samples	Dec 2011
Post-construction macrofaunal sampling	Apr 2012 Jun 2012 Aug 2012 Oct 2012 Apr 2013 Jul 2013
Biogeochemical flux measurement	Aug 2012 Oct 2012 Apr 2013 Jul 2013
Processing of all biogeochemical flux samples	Oct 2013
Processing of all macrofaunal samples	Oct 2013
Collection and analysis of organisms for tissue and shell nutrient analyses	May 2014
Data analysis	Jun 2014



**Methods:** Macrofauna analyses were conducted on all samples collected to assess the oyster population on each reef. Each sample was placed on a sieve series and thoroughly rinsed. All organisms retained on a sieve with 1-mm mesh were preserved for analyses. Analyses consisted of counting and identifying each organism to the lowest practical taxonomic level (usually species). For each sample from each reef during each sampling period, both dry weight and ash-free dry weight were determined for major macrofaunal groups. For species with unknown nitrogen (N) and phosphorus (P) content, samples were analyzed to determine percent N and P content.

Additional data collected from each reef included the percentage of organic material in the sediments, the percentage of sediments composed of silt and clay and the topographic complexity of the reef. Sediment samples were collected from the surface of each plot to a depth of 1.5cm. In the laboratory, sediment organic content was determined by loss on ignition and grain size distribution was determined by sieving. Topographic complexity was measured by conforming a chain to the surface of the reef and dividing by the linear distance covered.

Biogeochemical fluxes were determined by incubating 0.11 m<sup>2</sup> sections of intact reef in the laboratory and directly measuring changes in concentration in the overlying water column in a manner very similar to that of Kellogg et al. (2013). One month prior to each sampling period, an incubation tray was deployed on a minimum of one randomly selected reef from each treatment. Location within reef was randomly selected within the central area. Deployment consisted of filling the tray with existing material at the reef and



**Fig. 4.** *Top:* VIMS-ESL summer intern collects macrofauna from a sieve for preservation. *Bottom:* VIMS-ESL staff use dissecting microscopes to help identify and count organisms in preserved samples (photos: VIMS-ESL staff).

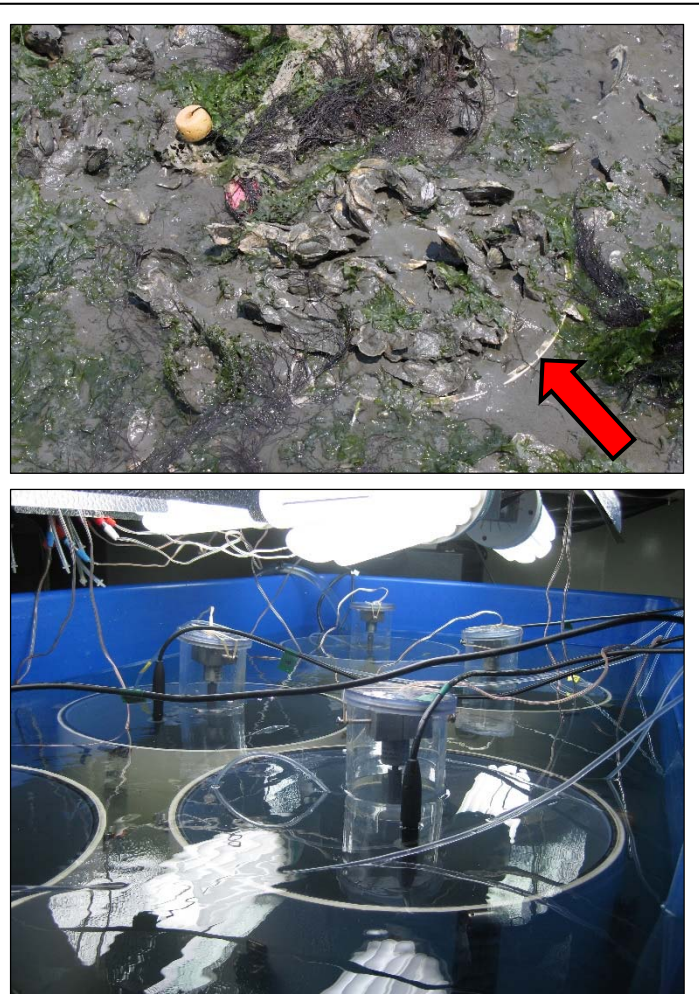
embedding it flush with surrounding sediments (Fig. 5). Incubation trays were then allowed to equilibrate for one month prior to collection from the field.

Trays were collected when water depth over the site was a minimum of 0.5 meters allowing underwater placement of a water-tight field lid prior to lifting the tray from the reef. Trays were collected, transported in to a nearby dock, submerged in tanks of water to reduce temperature variations and transported back to the lab as quickly as possible. At the laboratory, trays were placed in a water bath and supplied with oxygen to return dissolved oxygen levels to saturation prior to the start of incubations. During incubations, chambers were sealed with a gas-tight lid and samples were collected at intervals determined by the rate of oxygen consumption as monitored by oxygen probes in each chamber. All incubations included a seawater control. During all sampling periods, each incubation tray was incubated under both dark and light conditions.

All water samples were analyzed for concentrations of oxygen ( $O_2$ ), nitrogen gas ( $N_2$ ), combined nitrate and nitrite ( $NO_x$ ) and soluble reactive phosphorus (SRP). Fluxes of each analyte were determined based on

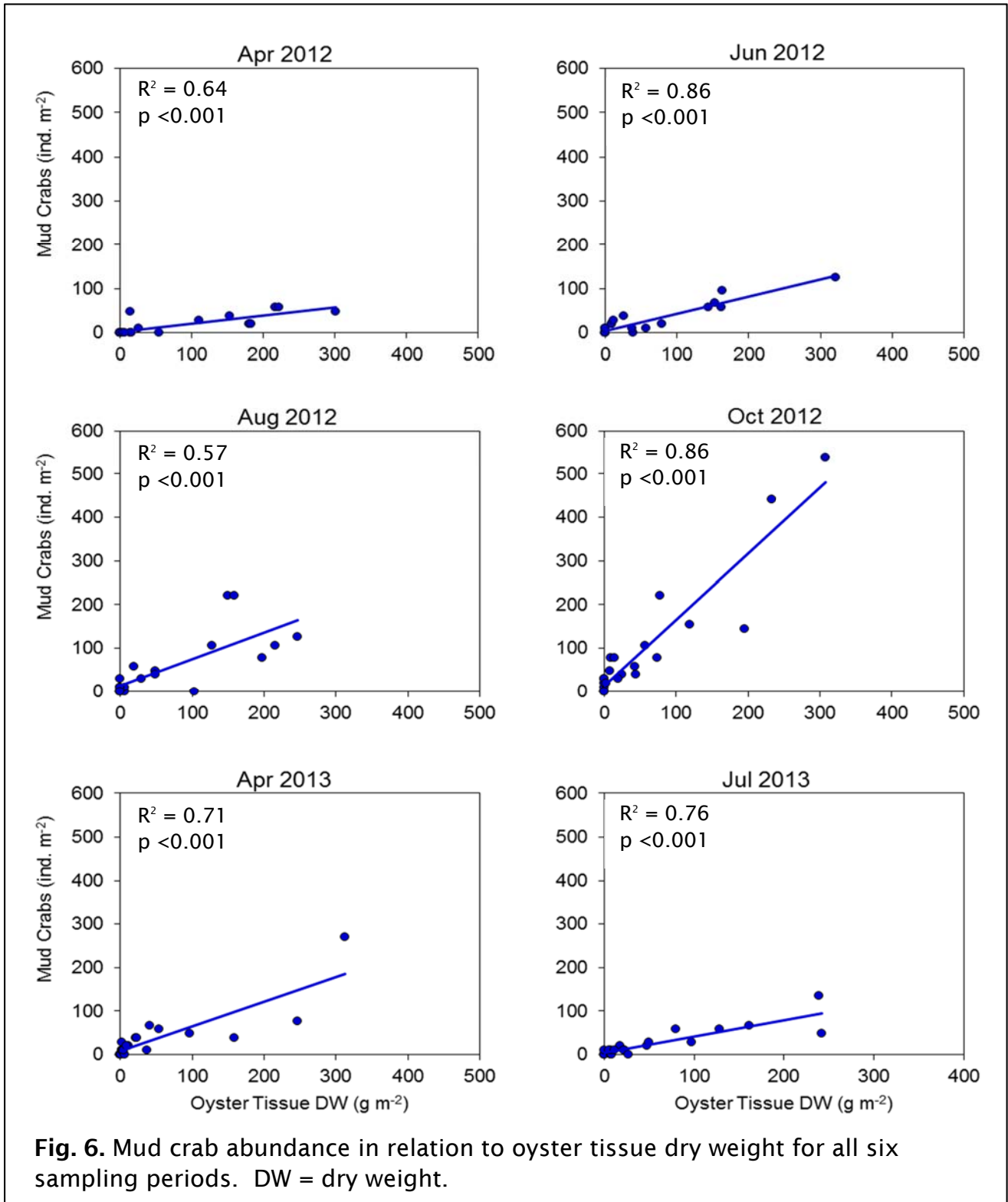
changes in concentration over time. In instances where there was a significant flux in the control chamber, this was subtracted from all other chambers.

**Results:** Macrofaunal analyses found significant relationships between oyster reef structural parameters and reef-associated macrofaunal species and/or macrofaunal functional groups. For example, mud crab abundance was positively correlated with oyster tissue biomass during all sampling periods (Fig. 6). Although this relationship was always significant, the slope of the relationship and the amount of variance explained by the relationship varied with season and year. In April 2013, the slope of the relationship is greater than that for April 2012. Without additional data, it is not



**Fig. 5.** *Top:* Incubation tray three days after deployment. Red arrow points to edge of embedded tray. *Bottom:* Chambers in a water bath during a light incubation. (photos: VIMS-ESL staff).

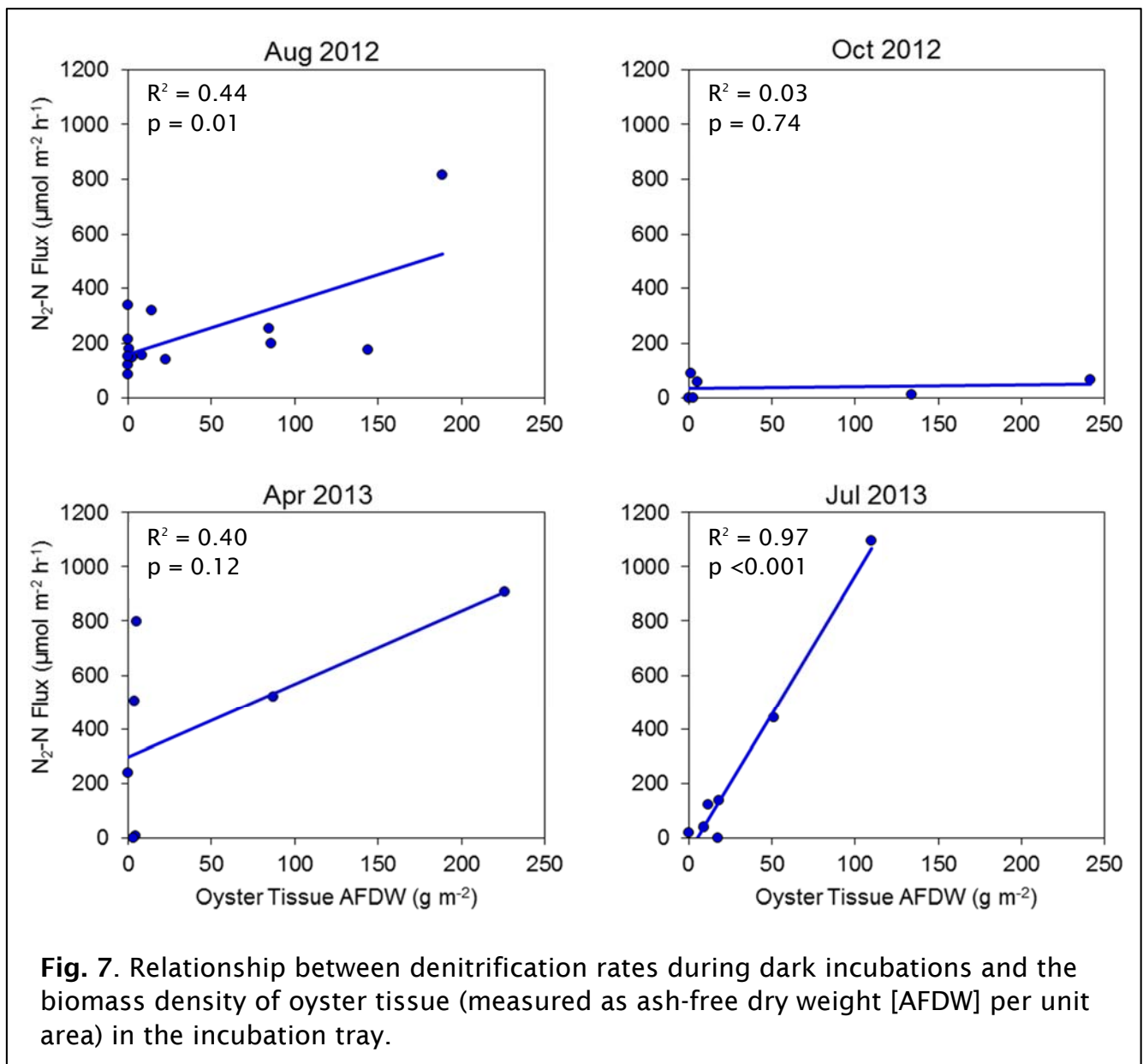
possible to determine whether this change in slope results from increasing reef maturity or from interannual variability. Regardless, the data indicate that it is feasible to identify relationships between reef structural characteristics and macrofaunal community structure.



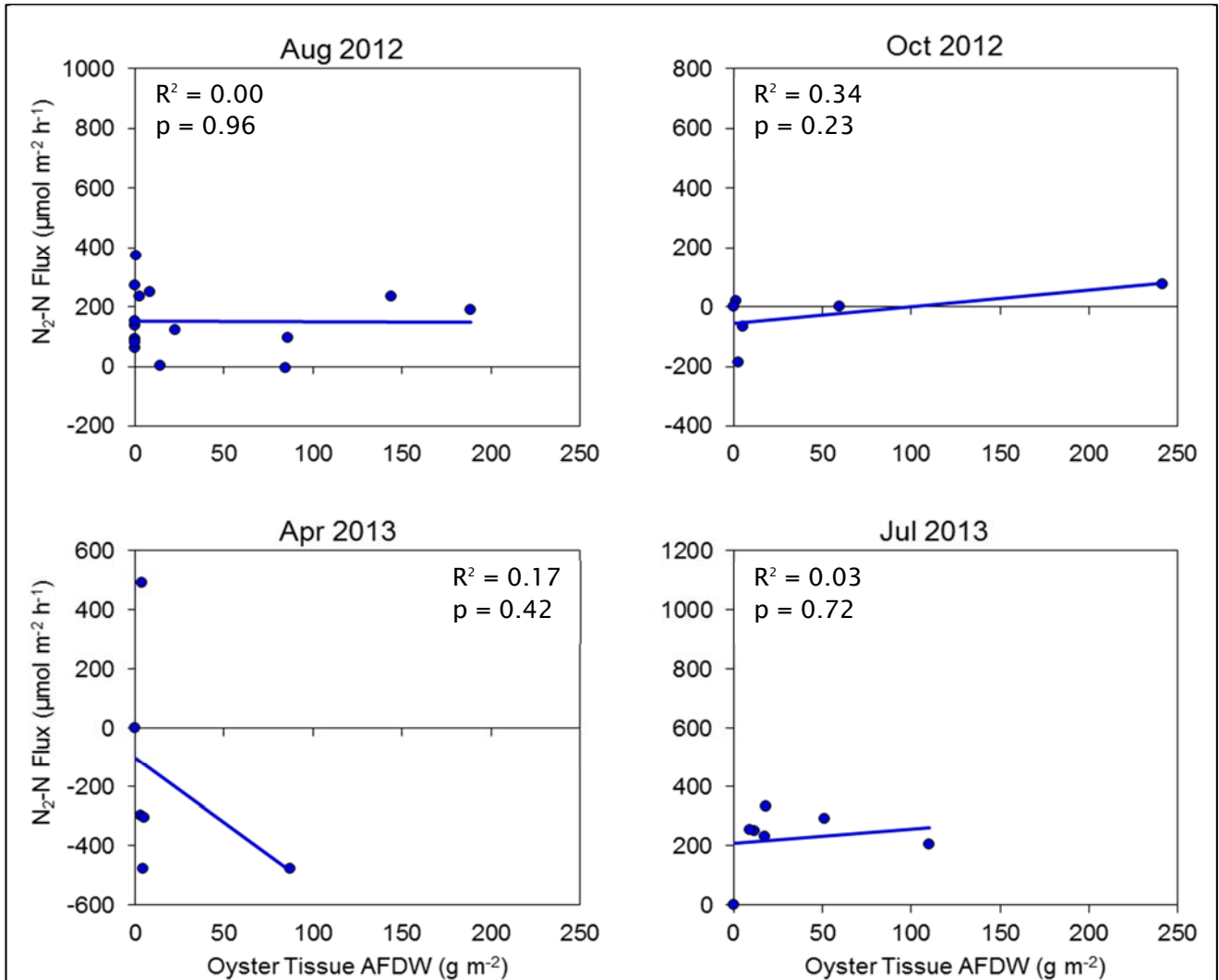
**Fig. 6.** Mud crab abundance in relation to oyster tissue dry weight for all six sampling periods. DW = dry weight.



Positive fluxes of nitrogen gas during dark incubations were recorded for oyster reefs during all sampling periods. With the exception of October 2012 which had relatively low rates across all biomass densities, the single highest denitrification rate was associated with the sample containing the greatest oyster biomass (Fig. 7). However, the degree of correlation between oyster tissue biomass in the sample and denitrification rates during dark incubations varied widely with season. Significant relationships between oyster tissue biomass and denitrification rate were observed in August 2012 and July 2013. Although the April 2013 sample with the highest biomass had the highest denitrification rate, the relationship between oyster tissue biomass and denitrification was not significant. In October 2012, denitrification rates were generally low and oyster biomass density was a poor predictor of denitrification rate.



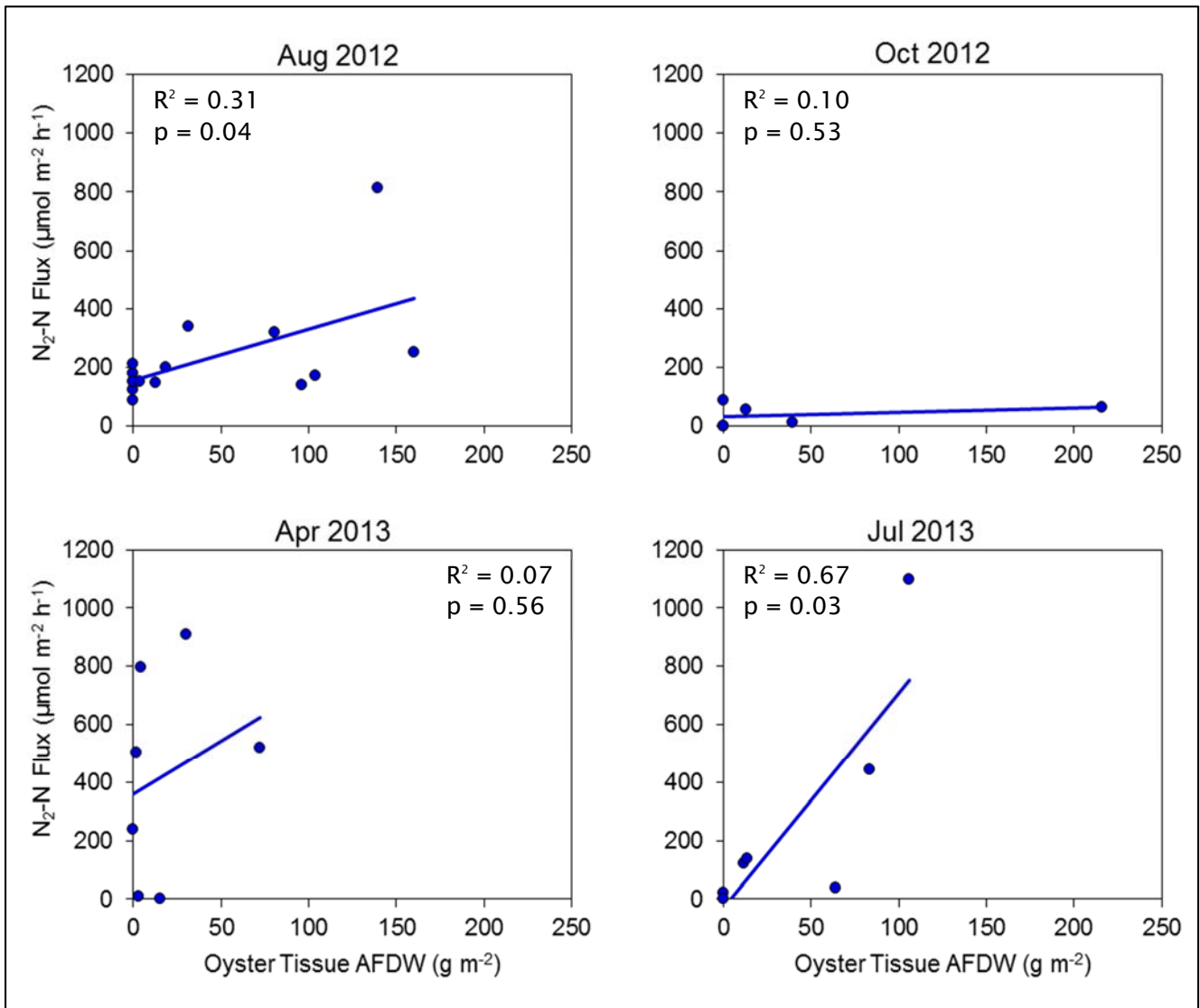
No significant relationships between oyster biomass density in sample trays and denitrification were found for samples incubated under light conditions (Fig. 8). Fluxes were generally positive during August 2012 and July 2013 but oyster biomass density explained less than 5% of the variance in denitrification rates. In both October 2012 and April 2013, both positive and negative fluxes were measured. Such fluxes are often observed in photosynthetic sediments when oxygen bubbles form, or from nitrogen fixation associated with sulfate reduction.



**Fig. 8.** Relationship between denitrification rates during light incubations and the ash-free dry weight (AFDW) of oyster tissue in the incubation tray. The maximum and minimum values on the y-axes differ from Fig. 6 but the range of values is the same allowing for direct comparisons of the slopes of regression lines between graphs.



Comparisons of reef-level oyster biomass density to denitrification rates from dark incubations (Fig. 9) demonstrated significant positive relationships in August 2012 and July 2013. However, these relationships explained less of the variance in denitrification rates than explained by the oyster biomass density in the incubation tray. As observed for the biomass in incubation trays, there was not significant relationship between reef-level oyster biomass density and denitrification rates.



**Fig. 9.** Relationship between denitrification rates during dark incubations and the average ash-free dry weight (AFDW) of oyster tissue on the reef where the incubation tray was deployed. All axes are the same as Fig. 6 allowing for direct comparison between graphs.

Denitrification rates under dark conditions generally corresponded best to measurements directly related to the oysters contained in the incubation tray and/or the oysters on the surrounding reef (Table 1). However, these relationships were only significant during the August 2012 and July 2013 sampling periods with no significant relationships found in October 2012 or April 2013. With the exception of measures of macroalgal biomass in October 2012, oyster reef structural characteristics were not significantly correlated with denitrification rates under light conditions.

**Table 1.** Results of linear regression analyses of measured structural parameters in relation to denitrification rates for samples incubated in the dark (D) and in the light (L) demonstrating both positive (+) and negative (-) relationships. AFDW = ash-free dry weight; NA = data not available.

Parameters Tested	Aug 2012		Oct 2012		Apr 2013		Jul 2013	
	D	L	D	L	D	L	D	L
Incubation tray oyster tissue dry weight	+						+	
Average reef oyster tissue dry weight	+						+	
Incubation tray oyster tissue AFDW	+						+	
Average reef oyster tissue AFDW	+						+	
Incubation tray oyster shell dry weight	+						+	
Average reef live oyster shell dry weight	+						+	
Incubation tray surface shell volume	+						+	
Average reef surface shell volume							+	
Average reef complexity								
Reef sediment organic content							+	
Reef sediment % silt + clay	+							
Incubation tray macroalgae dry weight				-				
Incubation tray macroalgae AFDW	NA	NA		-				

**Recommendations:** The data gathered thus far indicate that it is feasible to identify significant relationships between oyster reef structural and some functional parameters. However, these relationships vary depending upon the functional parameter of interest, season, year and likely reef maturity. Thus we recommend continued studies focusing on further elucidating these relationships. Studies that focused on how these relationships change as a restored reef matures would be of particular interest. The lack of correspondence between reef structural parameters and denitrification rates under light conditions also warrants further study, especially in light of data from July 2013 demonstrating fluxes in the light for samples containing oysters but not for the sample that did not contain oysters.

### Goal 3:

Based upon our monitoring data, develop a tool for estimating habitat functional characteristics and ecosystem services using measured values for structural characteristics.

**Completed Tasks:** We have analyzed all data from this project but development of a tool for estimating functional characteristics and ecosystem services was precluded by seasonal and interannual variability in relationships between restored reef ecosystem structure and function and, in some cases, inability to identify significant relationships between structural and functional characteristics.

**Results:** The existing dataset does not allow straightforward prediction of denitrification rates based on oyster reef structural parameters at this time. Relationships between denitrification and oyster biomass varied widely between seasons and between light conditions. However, the strong relationship between oyster biomass density and denitrification rates observed in the July 2013 dataset suggests the possibility that as these reef mature, the degree of correlation between oyster reef structural and functional metrics may increase. Although reef structural characteristics are more easily related to macrofaunal community structure, the degree to which these relationships vary as a function of reef age versus interannual variability is unclear.

**Recommendations:** Assuming the reefs at this site continue to provide a range of oyster biomass density, we recommend additional sampling as the reefs mature to determine the roles of interannual variation versus reef age/maturity in determining relationships between oyster reef structural and functional characteristics. We also recommend expanding the suite of variables studied in an effort to find structural characteristics that have significant relationships to spring and fall denitrification rates as well as denitrification rates when light is available.

## Monitoring and Maintenance Activities

Post-construction monitoring was conducted in April, June, August and October 2012 and in April and July 2013. Monitoring will continue with funds from another source June /July 2014. In March 2013, fouled PVC marker stakes at the site were replaced. To date, no other maintenance has been required.

## Community Involvement

Eight community volunteers participated in reef construction on October 25 and 26, 2011, contributing a total of 46.5 hours of time. From October 31, 2012 to November 5, 2012 eighteen volunteers contributed 128.5 hours of time while assisting with the processing of macrofauna samples. In all, 26 community volunteers contributed a total of 175 hours of time to the project. This exceeds our original goal of 160 hours, and at \$13/hour, represents a match value of \$2,275.

	<b>Total</b>
Volunteer Numbers	26
Volunteer Hours	175

## Outreach Activities

Data from or information about this project have been presented at a variety of meetings attended by resource managers, restoration practitioners and researchers. Presentations to date include:

Kellogg ML (2013) Oysters, reef restoration and water quality: A Chesapeake Bay perspective, 12th Annual Ronald C. Baird Sea Grant Science Symposium, Warwick, RI.

Kellogg ML, Cornwell JC, Owens MS, Luckenbach MW (2013) Denitrification and nutrient assimilation associated with oyster reefs, 22nd Biennial Conf. of the Coastal and Estuarine Research Fed., San Diego, CA.

Luckenbach MW, Kellogg ML (2013) Shellfish and water quality: Searching for policy options in Chesapeake Bay clean-up, 22nd Biennial Conference of the Coastal and Estuarine Research Federation, San Diego, CA.

Kellogg ML, Cornwell JC, Owens MS, Luckenbach MW (2013) Quantifying oyster reef ecosystem services: Denitrification and nutrient assimilation, SER 2013 World Conf. on Ecological Restoration, Madison, WI.

- Kellogg, ML, J.C. Cornwell JC, Owens MS (2013) Quantifying nitrogen removal and nutrient sequestration capacity of subtidal and intertidal oyster reefs. Workshop on Quantifying Nitrogen Removal by Oysters, Wachapreague, VA.
- Kellogg, ML (2012) Bivalve impacts on water quality: Positives, negatives and unknowns. Delaware Center for the Inland Bays Scientific and Technical Advisory Committee, Lewes, DE.
- Kellogg ML, Cornwell JC, Owens MS, Luckenbach MW (2012) Scaling ecosystem services to reef development: Effects of oyster density on nitrogen removal and biodiversity. Chesapeake Bay Program Sustainable Fisheries Goal Implementation Team Meeting, Annapolis, MD.
- Kellogg ML, Cornwell JC, Owens MS, Luckenbach MW (2012) Quantifying nitrogen removal and nutrient sequestration capacity of subtidal and intertidal oyster reefs. 41st Benthic Ecology Meeting, Norfolk, VA.

## Literature Cited

- Kellogg ML, Cornwell JC, Owens MS, Paynter KT (2013) Denitrification and nutrient assimilation on a restored oyster reef. *Marine Ecology Progress Series* 480: 1-19.



## Project Expenditures

	Budget Categories	Total Budgeted Grant Fund	Total Budgeted Match	Grant Funds Expended this Report	Grant Funds Expended Cumulative	Match Funds Expended this Report	Match Funds Expended Cumulative	Match Fund Source
a	Personnel	27,127	26,982	4,632.36	36,460.96	0.00	29,124.36	VIMS
b	Fringe	10,851	9,745	842.49	7,972.71	0.00	8,858.76	VIMS
c	Travel	2,619	0	1,058.26	3,778.76	0	-	
d	Equipment	0	0	-	-	0	-	
e	Supplies	4,600	0	7.10	2,848.90	0	-	
f	Contractual	47,560	31,211	5,226.69	42,328.94	0	-	UMCES and TNC
g	Other	2,625	2,080	139.20	1,844.20	10,545.88	35,695.06	UMCES and TNC
h	Total Direct Costs	95,382	70,018	11,906.10	95,234.47	10,545.88	73,678.18	
i	Indirect Costs	19,915	37,894	1,345.31	19,915.00	20,474.91	38,474.79	VIMS
j	Totals	115,297	107,912	13,251.41	115,149.47	31,020.79	112,152.97	

The above budget reflects additional funds have been redirected to this project from an NCBO sponsored project with a similar experimental design as well as additional funds awarded by TNC for sampling in June 2013. Budgeted matching funds include contractual services provided by UMCES and volunteer hours provided by TNC.

The undersigned verifies that the descriptions of activities and expenditures in this report are accurate to the best of my knowledge; and that the activities were conducted in agreement with the grant contract. I certify that matching fund levels established in the grant contract have been met.

Grantee Signature: -----

Grantee Name: Mary Lisa Kellogg-----