Scaling Ecosystem Services to Reef Development: Effects of oyster density on nitrogen removal and reef community structure

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Effects of oyster density on nitrogen removal and reef community structure

A final report to:
National Oceanic and Atmospheric Administration’s
Chesapeake Bay Office

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Scaling Ecosystem Services to Reef Development

EFFECTS OF OYSTER DENSITY ON NITROGEN REMOVAL AND REEF COMMUNITY STRUCTURE

Award Information

Project Title: Scaling ecosystem services to reef development: Effects of oyster density on nitrogen removal and reef community structure

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Abstract

Eighteen native oyster experimental reefs (16-m² each) were restored using six oyster densities (0, 10, 25, 50, 100 and 250 adult oysters m⁻²) with three replicates of each density at each of two sites: one subtidal site in Onancock Creek, Virginia and one intertidal site in Hillcrest Oyster Sanctuary within The Nature Conservancy’s Virginia Coast Reserve. 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. Data were collected from the intertidal site during six sampling periods between April 2012 and July 2013 and from the subtidal site in April and June 2012. Relationships between reef structural parameters and functional parameters were complex and variable. As of July 2014, the intertidal 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 (*Crassostrea virginica*) 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 and by reefs of similar biomass density located in different environmental settings (e.g. subtidal versus intertidal). The ecosystem services provided by intertidal oyster reefs are expected to differ from those provided by subtidal oyster reefs as a result differences in physical conditions and the impacts those physical conditions have on reef community structure. One of the most poorly quantified, yet potentially important, ecosystem services provided by restored oyster reefs is their role in nitrogen dynamics, especially the transformation of particulate organic nitrogen (PON), dissolved organic nitrogen (DON) and dissolved inorganic nitrogen to 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 goals of this project were to assess denitrification rates for subtidal and intertidal oyster reefs within the same region, and to identify reef structural characteristics that are easily measured and could be used to reliably predict denitrification rates, provision of habitat for other species, and other ecosystem functions.

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 a manipulative experiment and science-based monitoring to quantify relationships between structural and functional habitat characteristics on replicate reefs of differing oyster density constructed both in Onancock Creek, VA (hereafter “Onancock”) and in The Nature Conservancy’s Virginia Coast Reserve (VCR) within the Hillcrest Oyster Sanctuary (hereafter “Hillcrest”). The experimental design
and sampling methods used at the two sites were kept as similar as possible to allow for direct comparisons between the two sites.

**Objective 1:**

Develop an experimental platform for identify relationships between oyster reef structural and functional characteristics, both for the proposed project and future ones.

**Methods:** Experimental reefs were constructed at Onancock and Hillcrest November 2011 (Fig 1 and 2). Prior to the start of restoration, 21 plots (16-m² each) were identified at each site and marked with stakes as potential restoration sites. At Hillcrest, all plots were in a single contiguous area on an intertidal flat. At Onancock, sufficient contiguous area of similar depth was not available to allow placement of all plot with sufficient spacing between plots. Instead, seven plots were identified within each of three areas (hereafter “blocks”) on the same side of the creek channel. In all plots at both sites, rebar stakes were placed at 0.5-m intervals throughout the plot to limit predation by rays, facilitate even distribution of oysters during reef construction and assist in identifying randomly selected subplots during future sampling events.

**Fig. 2.** Locations of subtidal study site at Onancock Creek, VA (star) and intertidal study site at Hillcrest Oyster Sanctuary (circle) in the Virginia Coast Reserve.
At both sites, 18 of the 21 plots were randomly selected to become reef plots. At Hillcrest, these plots were randomly selected from the entire set of 21 plots. At Onancock, six of the seven plots within each of the three blocks were randomly selected to become reef plots. Clean oyster shell was spread evenly across each reef plot at both sites. The remaining three plots at each site 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⁻²). At Onancock, one replicate of each oyster density was placed within each of the three blocks.

The source of oysters planted at the two sites differed. At Hillcrest, the oysters used to populate experimental plots were collected from nearby reefs within the Virginia Coast Reserve. Virginia Institute of Marine Science Eastern Shore Laboratory (VIMS-ESL) staff and volunteers (recruited by TNC) then sorted, counted and evenly distributed the appropriate number of adult oysters (≥ 50 mm shell height) across each subplot (Fig. 2). At Onancock, subtidal oyster populations were not sufficient to populate the experimental plots using local oysters. Instead, oysters grown at the Chesapeake Bay Foundation’s oyster farm in Sarah Creek, VA were used to populate these reefs.

**Fig. 1. Top:** TNC volunteers measure, count and sort oysters prior to placement on reefs at Hillcrest (photo: VIMS-ESL staff). **Middle:** VIMS-ESL staff place oysters on one of the high density reefs (photo: Frank Renshaw). **Bottom:** VIMS-ESL staff construct subtidal reefs in Onancock Creek, VA (photo: VIMS-ESL staff).
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 from each site during each sampling period. Sampling locations were selected using a stratified random design resulting in one sample from the edge of each reef, one from the central area of each reef and one from between these two areas. Each sample was collected by excavating a 0.035 m² area to a depth of 15 cm below the sediment surface (Fig. 3). 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 for each sampling period at each site. 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. These values were calculated separately for the contents of each flux tray and for each reef plot to allow assessment of which was the better predictor of biogeochemical fluxes.

<table>
<thead>
<tr>
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<td>Site selection and survey</td>
<td>Aug 2011</td>
</tr>
<tr>
<td>Reef construction</td>
<td>Nov 2011</td>
</tr>
<tr>
<td>Oyster population surveys</td>
<td>Apr 2012-Jun 2012</td>
</tr>
<tr>
<td></td>
<td>Apr 2013-Jul 2013</td>
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</table>

Fig. 3. VIMS-ESL staff and summer interns collecting (top) and cleaning (bottom) samples prior to laboratory analyses (photo: VIMS-ESL staff).
**Results:** Success in maintaining a range of oyster density treatments varied between sites, with much higher survival at Hillcrest than at Onancock. Data from Onancock Creek in April 2012 (Fig. 4) demonstrate that a reasonable range of oyster densities persisted at that time. However, sampling in June 2012 resulted in collection of a single oyster. Additional surveys revealed that <1% of the oysters placed at this site survived and sampling was terminated at this site.

![Fig. 4. Estimated oyster tissue biomass density at Onancock in April 2012. 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.](image)

After the loss of oysters at Onancock, sampling at Hillcrest was expanded. Original sampling plans for the site included collection of oyster and macrofauna data during four sampling periods and collection of denitrification data during a single sampling period. Expanded sampling plans included assessment of oysters and macrofauna during six sampling periods and assessment of denitrification rates during four sampling period. This expanded sampling allowed for both longer term evaluation of reef development at that site and assessment of seasonal patterns in denitrification rates. Oyster abundance and biomass were assessed to determine whether differences in oyster density persisted over time (Fig. 5). As expected, oyster biomass densities changed on individual reefs but, as a whole, the reef complex retained a range in biomass density across reefs.
Objective 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).

Revised monitoring program: The original science-based monitoring plan for reefs in Onancock Creek included sampling in August 2011 prior to reef construction and in April, June, August and October 2012 after reef construction. Due to the loss of >99% of oysters from the sites in Onancock Creek, sampling plans were revised to focus on
the reefs constructed at Hillcrest using funding from The Nature Conservancy (TNC) and NOAA’s Community Restoration Program. Originally, detailed biogeochemical measurements at Hillcrest were only scheduled to take place in August 2012 to allow direct comparison of rates from an intertidal site (Hillcrest) to those from a nearby subtidal site (Onancock) during the same sampling season and year. Using funds from NOAA Chesapeake Bay Office (NCBO) supplemented by additional funds from The Nature Conservancy, detailed biogeochemical measurements were carried out at Hillcrest in August and October 2012 and in April and July 2013.

Pre-construction sampling was completed at both sites 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 and June 2012 at Onancock, and in April, June, August and October 2012 and in April and July 2013 at Hillcrest. Sampling was supported by combined funding from NOAA’s Chesapeake Bay Office, NOAA’s Community Restoration fund and from The Nature Conservancy. Processing of all macrofaunal and biogeochemical samples has been completed. The table below lists the completion dates for individual tasks.

<table>
<thead>
<tr>
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<td>Pre-construction macrofauna sampling</td>
<td>Aug 2011 Aug 2011</td>
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<tr>
<td>Processing of pre-construction macrofauna samples</td>
<td>Dec 2011 Dec 2011</td>
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<tr>
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<td>Oct 2012 Apr 2013</td>
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<td></td>
<td>Jul 2013</td>
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<tr>
<td>Biogeochemical flux measurement</td>
<td>Apr 2012 Jun 2012</td>
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<td></td>
<td>Aug 2012 Oct 2012</td>
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<tr>
<td></td>
<td>Apr 2013 Jul 2013</td>
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<tr>
<td>Processing of all biogeochemical flux samples</td>
<td>Oct 2013</td>
</tr>
<tr>
<td>Processing of all macrofaunal samples</td>
<td>Oct 2013</td>
</tr>
</tbody>
</table>
| Collection and analysis of organisms for tissue and shell nutrient analyses | May 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 with 1-mm mesh and thoroughly rinsed. Each oyster and oyster shell was thoroughly scrubbed and soaked in fresh water until no additional organisms were found. All organisms retained on a sieve with 1-mm mesh were preserved for analyses (Fig. 6). 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 1.5 cm. 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 m² 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 plot.

Fig. 6. 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).
selected reef from each treatment. Location within reef was randomly selected within the central sampling area. Deployment consisted of filling the tray with existing material at the reef and embedding it flush with surrounding sediments (Fig. 7). 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 (O₂), nitrogen gas (N₂), combined nitrate and nitrite (NOₓ) 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 of Macrofaunal Analyses:** Significant relationships between oyster reef structural parameters and reef-associated macrofaunal species and/or macrofaunal functional groups were observed. For example, at Hillcrest mud crab abundance and biomass was positively correlated with oyster tissue biomass during all sampling
periods (Figs. 8 and 9). 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

![Graphs showing Mud crab abundance in relation to oyster tissue dry weight for all six sampling periods at Hillcrest. DW = dry weight.](image)

**Fig. 8.** Mud crab abundance in relation to oyster tissue dry weight for all six sampling periods at Hillcrest. DW = dry weight.
April 2012. Without additional data, it is not 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 an important component of the macrofaunal community.

**Fig. 9.** Mud crab biomass in relation to oyster tissue dry weight for all six sampling periods at Hillcrest. DW = dry weight.
The relationships between oyster biomass density and polychaete abundance and biomass at Hillcrest (Fig. 10 and 11) were also positive and significant during most sampling periods. Again, the amount of variance and slope of the relationship differed.

**Fig. 10** Polychaete abundance in relation to oyster tissue dry weight for all six sampling periods at Hillcrest. DW = dry weight.
with season and the slope of the relationship was greater in April 2013 than in April 2012. Seasonal patterns were driven primarily by the abundance and biomass of *Alitta succinea*.

![Graphs showing relationship between polychaete and oyster tissue dry weight across different months and years.](image)

*Fig. 11.* Polychaete biomass in relation to oyster tissue dry weight for all six sampling periods at Hillcrest. DW = dry weight.
Non-oyster bivalve abundance and biomass were also positively correlated with oyster biomass density in many sampling periods (Fig. 12 and 13). Lack of significant correlation between non-oyster bivalve abundance and oyster biomass in density in April 2013 was driven primarily by high recruitment of *Mytilus edulis*, a species which recruits

![Non-oyster bivalve abundance in relation to oyster tissue dry weight for all six sampling periods at Hillcrest. Note that y-axis for April 2013 differs from all other sampling periods. DW = dry weight.](image)

**Fig. 12.** Non-oyster bivalve abundance in relation to oyster tissue dry weight for all six sampling periods at Hillcrest. Note that y-axis for April 2013 differs from all other sampling periods. DW = dry weight.
to this area but rarely survives to maturity. Non-oyster bivalve biomass was most frequently dominated by *Geukensia demissa* and *Mercenaria mercenaria*.

![Fig. 13. Non-oyster bivalve biomass in relation to oyster tissue dry weight for all six sampling periods at Hillcrest. DW = dry weight.](image-url)
Results of Biogeochemical Analyses

ONANCOCK

The rates of oxygen (O$_2$) uptake and nitrogen transformation in the Onancock experiment compare favorably with the high rates observed in the upper Choptank River (Kellogg et al. 2013); the peak dark oxygen uptake rates approached 15,000 $\mu$mol m$^{-2}$ h$^{-1}$ (Figure 14), much higher than rates of sediment oxygen uptake found anywhere in the Chesapeake or Maryland Coastal Bays (Boynton and Bailey 2008). Fluxes of ammonium (NH$_4^+$) in the dark ranged from a high rates of uptake with no oysters, presumably by benthic microalgal assimilation in the dark, to very high rates of efflux (> 1,000) with high oyster biomass. These highest rates compare favorably with Choptank rates and, as with oxygen, are

![Graphs showing oxygen, ammonium, nitrate, and nitrogen gas flux rates](image)

**Fig. 14.** Relationship between Onancock nitrogen flux rates (O$_2$ demand, NH$_4^+$, NO$_x$, and N$_2$-N) during dark incubations and the biomass density of oyster tissue (measured as ash-free dry weight [AFDW] per unit area) in the incubation tray.
higher than commonly observed in Chesapeake and coastal sediments. Fluxes of nitrate and nitrite (NO\textsubscript{x}) in the dark peaked at 248 \(\mu\text{mol m}^{-1}\text{ h}^{-1}\), a very high rate of efflux compared to sediment rates. Dark rates of N\textsubscript{2}-N efflux, commonly referred to as denitrification, peaked at \(~800 \mu\text{mol m}^{-1}\text{ h}^{-1}\), much higher that rates commonly observed in coastal sediments (Joye and Anderson 2008).

Illuminated or “light” rates of oxygen uptake (Figure 15) were lower than that observed under dark conditions with the difference between dark and light rates ranging from 2,299 to 6,403 \(\mu\text{mol m}^{-1}\text{ h}^{-1}\). These differences are attributable to benthic microalgal photosynthesis and are relatively high for mid-Atlantic sediments (Chick 2009).

Interestingly, the highest rates of photosynthesis occurred with high oyster biomass,

\[ R^2 = 0.81 \text{ p = 0.015} \]
\[ R^2 = 0.92 \text{ p = 0.003} \]
\[ R^2 = 0.64 \text{ p = 0.056} \]
\[ R^2 = 0.92 \text{ p = 0.002} \]

Fig. 15. Relationship between Onancock nitrogen flux rates (O\textsubscript{2} demand, NH\textsubscript{4}\textsuperscript{+}, NO\textsubscript{x}, and N\textsubscript{2}-N) during light incubations and the biomass density of oyster tissue (measured as ash-free dry weight [AFDW] per unit area) in the incubation tray.
suggesting reef production of nutrients may stimulate benthic photosynthesis. Consistent with other studies, rates of \( \text{NH}_4^+ \) and \( \text{NO}_x \) efflux were attenuated by uptake of nitrogen for microalgal nutrition. Consistent with benthic microalgal photosynthesis effluxes of \( \text{NH}_4^+ \) were highly attenuated by illumination with decreased effluxes (or net uptake) up to 995 \( \mu \text{mol m}^{-1} \text{ h}^{-1} \). Changes in \( \text{NO}_x \) release were somewhat smaller than changes in \( \text{NH}_4^+ \), with large effects at low oyster biomass and minimal effects at high oyster biomass.

The effect of oyster biomass on biogeochemical has been examined using biomass within the trays (Figures 14, 15) and biomass determined separately at the experimental plots (Figures 16, 17). At Onancock, \( \text{O}_2 \) demand and fluxes of \( \text{NH}_4^+ \), combined nitrate and

![Graphs showing relationships between O2 flux, NH4+, NOx, and N2-N flux with oyster tissue AFDW+1](image)

**Fig. 16.** Relationship between Onancock nitrogen flux rates (\( \text{O}_2 \) demand, \( \text{NH}_4^+ \), \( \text{NO}_x \), and \( \text{N}_2-\text{N} \)) during dark incubations and the biomass density of oyster tissue (measured as ash-free dry weight [AFDW] per unit area) on the reef where the incubation tray was deployed.
nitrite (NO₃⁻) and dinitrogen gas (N₂) were positively correlated with the soft tissue biomass of oysters in incubation chambers during both dark (Fig. 14) and light incubations (Fig. 15). Two patterns were observed in the flux versus biomass data: linear increases of NH₄⁺ and NOₓ with increasing biomass, and O₂:biomass and N₂-N:biomass relationships with slope attenuation of fluxes at higher biomasses. Changes in the O₂ fluxes would be consistent with increasing rates of other terminal electron accepting processes, such as sulfate or iron reduction resulting in iron sulfide formation (Holyoke 2008). At higher rates of metabolism, O₂ uptake by microbes becomes a proportionally less important pathway. The dark slope of NOₓ efflux was ~10% of the dark NH₄⁺ efflux; the process of denitrification depends on nitrification which can be estimated as the sum of N₂-N and NOₓ effluxes when overlying water NOₓ concentrations are low (Kellogg et al.

![Graphs showing the relationship between O₂, NH₄⁺, NOₓ, and N₂-N fluxes and oyster tissue biomass.]

**Fig. 17.** Relationship between Onancock nitrogen flux rates (O₂ demand, NH₄⁺, NOₓ, and N₂-N) during light incubations and the biomass density of oyster tissue (measured as ash-free dry weight [AFDW] per unit area) on the reef where the incubation tray was deployed.
The relative importance of NH$_4^+$ to these other pathways increases with oyster biomass, suggesting that nitrification becomes less efficient at higher biomasses. Relative attenuation of nitrification relative to overall nitrogen remineralization, may account for the leveling out of the denitrification response. However, other processes such as increasing importance if dissimilatory reduction of nitrate of ammonium (DNRA; Giblin et al. 2013) could also account for attenuation of denitrification by competition for nitrate. In general, under illumination there was a decrease in most efflux rates or oxygen uptake.

The pattern of biogeochemical fluxes versus biomass changed little whether tray biomass or reef biomass was used for the analysis. Slope changes were observed in the NH$_4^+$ and NO$_x$ to biomass relationship would be consistent with enhanced placement of oysters in the trays when trays were emplaced in the substrate. Reef topographic complexity and sediment characteristics were all poor predictors of denitrification rates (Table 1).

Table 1. Results of regression analyses of measured structural parameters at Onancock 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.

<table>
<thead>
<tr>
<th>Parameters Tested</th>
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<tr>
<td></td>
<td>Dark</td>
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<tr>
<td>Incubation tray oyster tissue dry weight</td>
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<tr>
<td>Average reef oyster tissue dry weight</td>
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<tr>
<td>Incubation tray oyster tissue AFDW</td>
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<tr>
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<td>Average reef surface shell volume</td>
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<td></td>
</tr>
<tr>
<td>Reef sediment organic content</td>
<td></td>
</tr>
<tr>
<td>Reef sediment % silt + clay</td>
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HILLCREST

Overall rates of sediment oxygen exchange at Hillcrest were quite similar to Onancock, with a maximal $O_2$ uptake rates of 29,312 $\mu$mol m$^{-1}$ h$^{-1}$ in July/August 2012 in a tray with high oyster biomass (data not shown). August rates 2012 and July 2013 had the highest rates of oxygen uptake for a given oyster biomass. As at Onancock, the highest oxygen uptake rates occurred with high oyster biomass, and differences between light and dark incubations suggest high rates of benthic microalgal photosynthesis.

At Hillcrest, very high $NH_4^+$ effluxes were observed in August 2012 and October 2012, with much lower rates in April and July 2013 (Fig 18 and 19). The peak $NH_4^+$ fluxes in August 2012 exceeded 2,000 $\mu$mol m$^{-1}$ h$^{-1}$, similar to the maximal Onancock rate.

**Fig. 18.** Relationship between Hillcrest ammonium fluxes during dark incubations and the biomass density of oyster tissue (measured as ash-free dry weight [AFDW] per unit area) in the incubation tray.
Although ammonium fluxes varied widely between seasons, within season light had only a modest impact on flux rates. The median ratio of light to dark ammonium effluxes was 0.8. The strongest response between biomass and NH$_4^+$ effluxes was observed in August 2012, with the August 2012 regression slope 2.8 times that of October 2012 at Hillcrest, and 2.4 times that of the Onancock measurements.

![Graphs showing relationships between ammonium flux and oyster tissue AFDW](image)

**Fig. 19.** Relationship between Hillcrest ammonium fluxes during light incubations and the biomass density of oyster tissue (measured as ash-free dry weight [AFDW] per unit area) in the incubation tray.
Effluxes of NOx were generally modest except for several high rates in July 2013 and a dominance of NOx uptake in April 2013 (Figures 20, 21). The highest efflux rate was 401 $\mu$mol m$^{-1}$ h$^{-1}$ in July 2013 and the highest rate of NOx uptake was -103 $\mu$mol m$^{-1}$ h$^{-1}$ in April 2013. In general, the relationship between NOx and biomass was weaker than

![Graphs showing relationship between NOx flux and biomass density](image)

**Fig. 20.** Relationship between Hillcrest combined nitrate and nitrite fluxes during dark incubations and the biomass density of oyster tissue (measured as ash-free dry weight [AFDW] per unit area) in the incubation tray.
observed for NH$_4^+$ and biomass. A total of 21 of 36 NO$_x$ fluxes decreased under illumination, although in two cases in July 2013, NO$_x$ fluxes greatly increased.

**Fig. 21.** Relationship between Hillcrest combined nitrate and nitrite fluxes during light incubations and the biomass density of oyster tissue (measured as ash-free dry weight [AFDW] per unit area) in the incubation tray.
Positive fluxes of dinitrogen gas during dark incubations were recorded for oyster reefs during all sampling periods with maximum rates exceeding 1,000 $\mu$mol m$^{-1}$ h$^{-1}$. 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. 22). 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

![Graphs showing relationship between denitrification and oyster tissue AFDW](image)

**Fig. 22.** Relationship between Hillcrest denitrification rates during dark incubations and the biomass density of oyster tissue (measured as ash-free dry weight [AFDW] per unit area) in the incubation tray.
rates were generally very low. Light incubations for denitrification had rates lower, often dramatically lower, than dark rates (Figure 23). Competition with benthic microalgae for NOx and NH4+ results in light data with a high level of variability.

No significant relationships between oyster biomass density in sample trays and denitrification were found for samples incubated under light conditions (Fig. 23). Fluxes were generally positive during August 2012 and July 2013 but oyster biomass density explained less 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.

\[
\begin{align*}
\text{Aug 2012:} & \quad R^2 = 0.00, \quad p = 0.96 \\
\text{Oct 2012:} & \quad R^2 = 0.34, \quad p = 0.23 \\
\text{Apr 2013:} & \quad R^2 = 0.17, \quad p = 0.42 \\
\text{Jul 2013:} & \quad R^2 = 0.03, \quad p = 0.72
\end{align*}
\]

**Fig. 23.** Relationship between Hillcrest denitrification rates during light incubations and the biomass density of oyster tissue (measured as ash-free dry weight [AFDW] per unit area) in the incubation tray.
Comparisons of reef-level oyster biomass density to denitrification rates from dark incubations (Fig. 24) 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. 24.** Relationship between Hillcrest 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 2). 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 2.** 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.

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<tr>
<td>Incubation tray oyster tissue dry weight</td>
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<td>Average reef oyster tissue dry weight</td>
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<tr>
<td>Incubation tray oyster tissue AFDW</td>
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<tr>
<td>Average reef oyster tissue AFDW</td>
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<tr>
<td>Incubation tray oyster shell dry weight</td>
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<tr>
<td>Average reef live oyster shell dry weight</td>
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<td>Incubation tray surface shell volume</td>
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<tr>
<td>Average reef surface shell volume</td>
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<tr>
<td>Average reef complexity</td>
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<tr>
<td>Reef sediment organic content</td>
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<td>Reef sediment % silt + clay</td>
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<td>Incubation tray macroalgae dry weight</td>
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<tr>
<td>Incubation tray macroalgae AFDW</td>
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In months where rates of NH$_4^+$ efflux was high (August and October 2012), the dominant form of efflux was as NH$_4^+$. In contrast, in April 2013 and July 2013, denitrification was the dominant fate of remineralized nitrogen. The key question is whether reef age, basically the time after experimental establishment, was a key factor in the increased dominance of denitrification as the end product of nitrogen remineralization. Making such a conclusion with this data set would be speculative at best, but it is clear that understanding the trajectory of reef development is essential in estimating its long term biogeochemical impact.

**Objective 3:**

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

**Methods:** Data from both Hillcrest and Onancock were analyzed to determine whether oyster biomass density could reliably be used to predict either macrofaunal community structure or biogeochemical fluxes. Based on these analyses we concluded that, although some general predictions can be made, development of a tool for estimating functional characteristics and ecosystem services would be premature because of the relatively high variability in these relationships between seasons, years and sites.

**Results:** The existing dataset does not allow straightforward prediction of macrofaunal community structure or biogeochemical fluxes based on oyster reef structural parameters at this time. However, several generalizations can be made:

- Increasing oyster biomass density generally results in increasing macrofaunal biomass density.
- Increasing oyster biomass density generally results in increasing oxygen demand and fluxes of ammonium to the water column.
- When significant relationships exist between oyster biomass density and denitrification rates, they are generally positive.
- At the two sites studied, the presence of light had a greater impact on denitrification rates at the intertidal site than at the subtidal site.
- Data collected thus far suggest that reef ecosystem services may increase as reefs mature.

**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 estimated.
• Assuming the reefs at Hillcrest 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. For intertidal reefs, 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.

• The data gathered thus far indicate that it is feasible to identify significant relationships between oyster reef structure 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, including the specific relationships and parameters listed below.
  o Studies focusing on how relationships between structural and functional parameters change as a restored reef matures would be of particular interest.
  o Studies of seasonal variation in nitrogen dynamics on subtidal reefs in the euphotic zone to determine if strong relationships between denitrification and reef structure persist throughout the year.
  o The lack of correspondence between intertidal 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 samples that did not contain oysters.
  o Studies characterizing microbial communities and their relationship to oyster reef structural parameters could explain some of the observed variation in nitrogen dynamics.
  o Characterization of microphytobenthos associated with oyster reefs would help to elucidate the role of competition for nitrogen species and the pathways that dominate nitrogen fluxes.
  o Better characterization of the geochemical environment and processes, particularly iron and sulfate reduction, could explain some of the variance observed here between oyster reef structural characteristics and nutrient dynamics.

Literature Cited


Holyoke, R. R. 2008. Biodeposition and biogeochemical processes in shallow, mesohaline sediment of Chesapeake Bay. UMCP.


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.

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<th>Total</th>
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<tr>
<td>Volunteer Numbers</td>
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<td>Volunteer Hours</td>
<td>175</td>
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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:


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, 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.