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Integrated assessment of oyster reef ecosystem services: Quantifying denitrification rates and nutrient fluxes

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INTEGRATED ASSESSMENT OF OYSTER REEF ECOSYSTEM SERVICES

7/15/2019

Quantifying Denitrification Rates and Nutrient Fluxes

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

Prepared by:
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Integrated assessment of oyster reef ecosystem services

QUANTIFYING DENITRIFICATION RATES AND NUTRIENT FLUXES

Award Information

Project Title: Integrated assessment of oyster reef ecosystem services: quantifying denitrification rates and nutrient fluxes

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Abstract

Fluxes of N_2-N (denitrification), dissolved ammonium, nitrate plus nitrite, and dissolved oxygen were determined at the 350 acre oyster restoration project at Harris Creek, Maryland. The *ex situ* incubation approach involved adding oyster communities to embedded trays for ~1 month, incubating the trays under dark and light conditions for 1-2 hour time courses for gas and solute sampling, and determination of the rates of gas and solute exchange for 136 individual reef tray incubations. Reef exchange rates were compared to rates of sediment-water exchange in core incubations throughout Harris Creek and in reef-adjacent environments.

Rates of sediment nutrient exchange, denitrification and oxygen exchange were variable, but higher rates of denitrification were generally associated with higher amounts of oyster biomass and higher temperatures (e.g. the warm season); the effects of light on reef denitrification rates were not discernable when the whole data set was examined. Two separate experiments clearly showed that incubations of reef cores alone resulted in underestimates of denitrification; incubations of oyster clumps alone showed that a considerable proportion of the denitrification was associated with the oyster community.

Under warm summer conditions, the total denitrification estimate for oyster biomass < 75 g DW m^{-2} was 57 lbs $acre^{-1}$ y^{-1}, increasing to 160 lbs $acre^{-1}$ y^{-1} for biomass > 225 g DW m^{-2}. Inclusion of denitrification rates for colder conditions has the potential to increase these rates up to 25-50%. Overall, the restoration of oysters at Harris Creek has resulted in a conservative estimate of N removal of ~20,000 lbs N y^{-1}.

This NCBO project resulted in one Ph.D. dissertation, one undergraduate thesis, numerous scientific presentations, and two published papers (thus far). The results of this work have informed the USEPA Bay Program Oyster BMP panel and are being used in the development of an oyster denitrification BMP. In addition, the results have been used in the NSF Coastal SEES Program – Oyster Futures. Cooperative work with a multi-investigator NOAA Ocean Acidification Program resulted in new reef vibracoring protocols and assisted a Ph.D. student at Oregon State University.

Rationale

Eutrophication of estuarine and coastal ecosystems is pervasive worldwide and presents perhaps the largest challenge to the health of any of these ecosystems (Bricker et al. 2008). There are a numerous effects of nutrient over-enrichment and the depletion of light and dissolved oxygen, including deterioration of benthic communities (Diaz and Rosenberg 1995), loss of submerged aquatic grasses (Orth and Moore 1984), occurrence of harmful algae (O'Neil et al. 2012), loss of biodiversity (Chang et al. 2012), and a shift in the food web towards microbial processes (Jonas 1990, Hewson et al. 2014). Multi-decadal and multi-century changes in the ecology of the Chesapeake Bay have been well documented (Cooper 1995, Kemp et al. 2005), with deleterious effects on the use of the Bay for recreation and fisheries. The loss of Chesapeake Bay oyster habitat has been a major casualty of the changes in Chesapeake Bay, and the interaction of eutrophication/nutrient cycling and the oyster community is the primary subject of this proposal.

The depletion of Chesapeake Bay oyster habitat by overharvest, poor water quality and disease has reduced oyster populations to a small fraction of the original population (Kemp et al. 2005, Wilberg et al. 2011). With the loss of oyster reef acreage there has been a simultaneous loss of nutrient sequestration and biogeochemical nutrient removal (Newell et al. 2005). While nutrient sequestration into oysters, both natural and aquaculture-reared, provides a net benefit via removal of nitrogen in harvested biomass, the processing of feces and pseudofeces can lead to net removal of nitrogen (N) via conversion of nitrate (NO_3^-) to N_2 gas. The production of N_2 gas in estuaries is generally attributed to microbial denitrification (Cornwell et al. 1999), although other pathways such as ANAMMOX (Rich et al. 2008) may have a minor importance.

In this study, our goal was to improve the understanding of the N removal capability of oyster restoration by measuring nutrient recycling and denitrification rates associated with oyster reef community development. To reach this goal, this project 1) developed denitrification data sets in areas with ongoing oyster restoration, 2) examined the oyster community relative to non-restored areas suitable for restoration and to soft sediment environments, and 3) provided data suited to modeling and analysis that may lead to a better predictive capability regarding oyster N-based ecosystem services (Kellogg et al. 2018).

Project Objectives

- Quantify the relationship between denitrification rates and oyster biomass density at small (0.1 m^2), intermediate (1.0 m^2) and reef scales.
- Quantify changes in denitrification rates in relation to oyster biomass density
- Assess seasonal patterns in denitrification rates and nutrient fluxes
- Estimate annual rates of denitrification for reefs of varying oyster biomass density
- Work with NCBO in both communicating research results to better inform management decisions and providing an assessment of the utility of oyster restoration in achieving Chesapeake Bay water quality goals.

What's In This Report

The funding for this project represent work that followed earlier NCBO-funded research on Harris Creek (NA13NMF4570210), work that was completed in 2016 (Cornwell et al. 2016b). **Because we have developed further understanding not only from the new data in this project, as well as from re-examination and interpretation of our 2014-2015 measurements, this report utilizes that earlier data set in many sections**. In addition, Harris Creek work supported by our National Science Foundation and NGO partners is incorporated here, with much of the interpretation of that work in conjunction with this NCBO work. Throughout this report, we will identify that earlier work by citing the final report from that work.

Project Narrative

Project Sampling Schedule

All biogeochemical sampling that UMCES and VIMS carried out in Harris Creek is identified in Tables 1 and 2. The previously-funded field research is shown in Table 1, with sampling in 2014 and 2015. Sampling in 2016 and 2017 included 1) whole community fluxes ("tray fluxes"), 2) incubations of sediment cores radiating away from a reef (halo cores), 3) experiments in which we incubated the whole community, then incubated the oysters from the trays separately (Jackson et al. 2018) and 4) water column measurements to assess evidence for benthic regeneration of nutrients (Jackson 2019). Figure 1 shows photographs of field and laboratory activities.

Table 1. Sampling prior to current NCBO Project. The biomass manipulations in 2015 were supported by The Nature Conservancy and the Oyster Recovery Program.

Dates	Sites	# obs	Notes
Tray Fluxes 2014			
October 16		4 trays 6 cores	Kellogg, M.L., J.C. Cornwell and M. S. Owens. Submitted. Measurement of biogeochemical fluxes in oyster reef environments. Submitted to Marine Ecology Progress Series.
Core Incubations 2014			
September 15	Transect through creek	12 cores	
Tray Fluxes 2015			
May 13	Rabbit Island East, Little Neck, Walnut, Lodges, Seth's Point, Mill Point, Eagle Point, Change	8 trays	Seasonal fluxes, one tray per reef.
June 1		8 trays	
July 27		8 trays	
Oct 27		8 trays	
December 15		8 trays	
Core Incubations 2015			
May 12	Transect through creek		
June 26	Transect through creek		
Biomass Manipulation 2015			
July 6	Lodges	8 trays	Biomass manipulation
July 7	Seth's Point	8 trays	
July 8	Mill Point	5 trays	

Table 2. Sampling dates in current NCBO project. In 2017, the tray-oyster separate incubations, oyster-only incubations, and water column sampling were supported in part by the National Science Foundation-funded Oyster Futures program.

Shallow/Deep Tray Fluxes 2016			
June 28/29	Walnut Creek	16 trays	
Sept 27/28	Walnut Creek	16 trays	
Halo Cores 2016			
July 6	Walnut Creek	12 cores	
September 21	Walnut Creek	12 cores	
Tray Fluxes 2017			
June 5/6	Walnut Creek	18 trays	
August 21/22	Walnut Creek	18 trays	
Tray-Oyster Separate Incubation 2017			
June 5/6	Walnut Creek		Jackson, M., M. S. Owens, J. C. Cornwell, and M. L. Kellogg. 2018. Comparison of methods for determining biogeochemical fluxes from a restored oyster reef. Plos One 13: e0209799.
August6 21/22	Walnut Creek		
Oyster-Only Incubations 2017			
August 10			Jackson, M. L. 2019. Characterization of oyster-associated biogeochemical processes in oyster restoration and aquaculture. Ph.D. Thesis. University of Maryland Center for Environmental Science.
September 20			
Water Column Sampling/Physical Measurements 2017			
June 9			Jackson, M. L. 2019. Characterization of oyster-associated biogeochemical processes in oyster restoration and aquaculture. Ph.D. Thesis. University of Maryland Center for Environmental Science.
August 16			

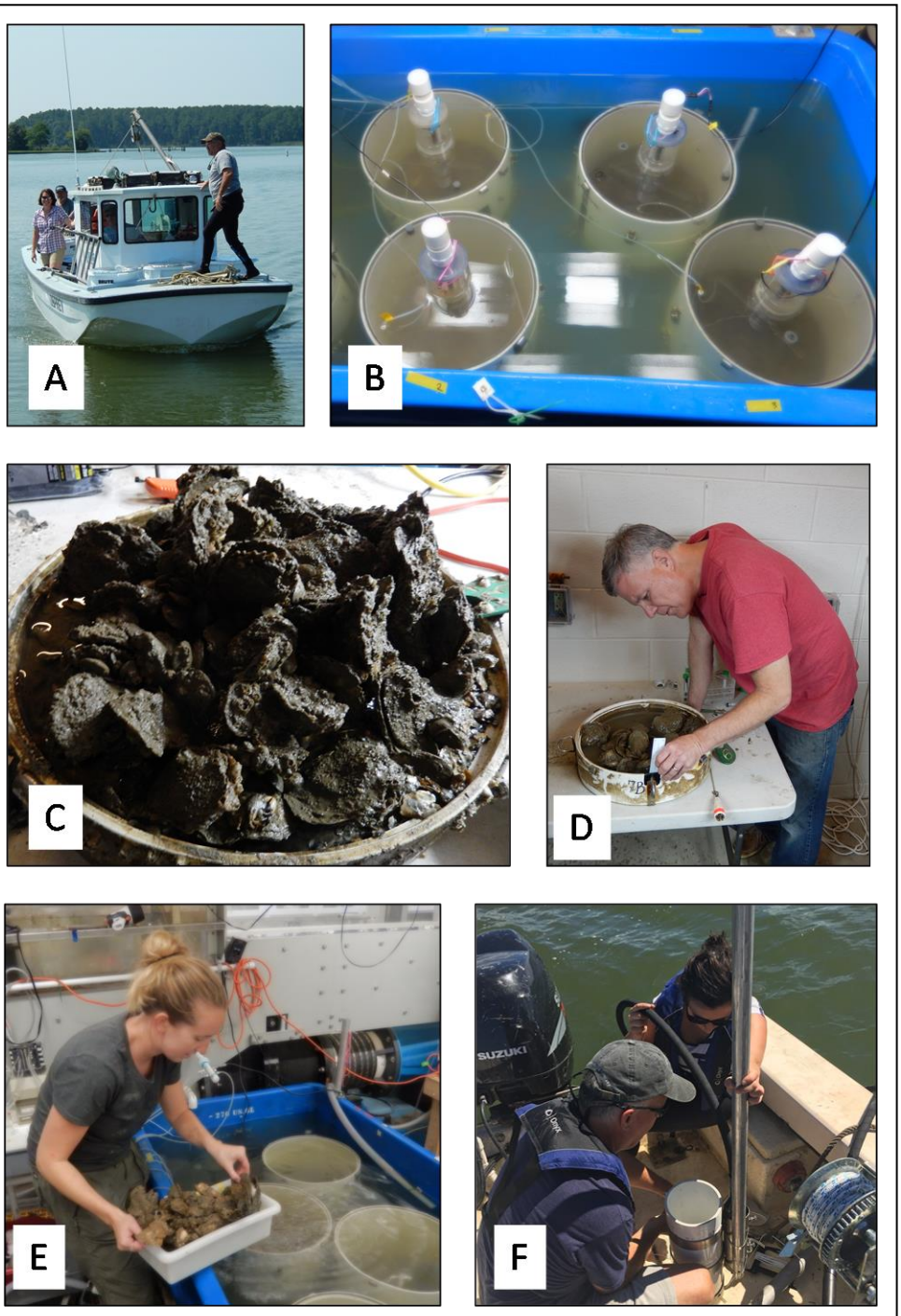

Figure 1. Field and laboratory activities including VIMS dive team returning oyster trays in plastic drums to shore (A), incubating oysters in a HPL laboratory (B), oysters from an incubated tray, August 2017 (C), measuring height of sediment and oyster community (D), returning oysters to apparatus for oyster-only incubation i 2017 (E), and vibracoring Harris Creek reef sediments for ocean acidification project (Giménez 2018) in 2017 (F).

Methods

Field Sampling

TRAY DEPLOYMENT

Sampling locations were identified using GPS and trays were successfully deployed and recovered in almost all cases. Sites are shown in Figure 2. Divers placed materials from a 0.1-m^2 area of the substratum into the sampling tray (38 cm diameter x 9 cm depth) and then re-embedded the materials in their original position, flush with the surrounding substratum. Since these methods result in initial disturbance of the sediment-water interface, trays were left in the field to re-equilibrate for over a month prior to sampling, a time period shown to be sufficient in our previous studies. At the time of retrieval, sampling trays were capped using the incubation chamber midsection and transport lid which allowed collection of the sample along with a portion of the overlying water column (see Kellogg et al. 2013 for details of incubation chamber design and collection methods). Immediately after collection, samples were placed in containers on the boat that were filled with water from the sampling site. Each sample was aerated from the time it came onboard the boat until arriving at the incubation facility at Horn Point Lab. Once samples arrived at the lab, the transport lid was removed, the upper section of the chamber attached, and the incubation chambers covered with a 500-µm mesh lid to prevent mobile macrofauna from escaping. The incubation chamber then was held in a tank of unfiltered seawater with temperature matched to field conditions. Samples were bubbled with air for ≥1 h in the dark to bring dissolved oxygen levels to saturation.

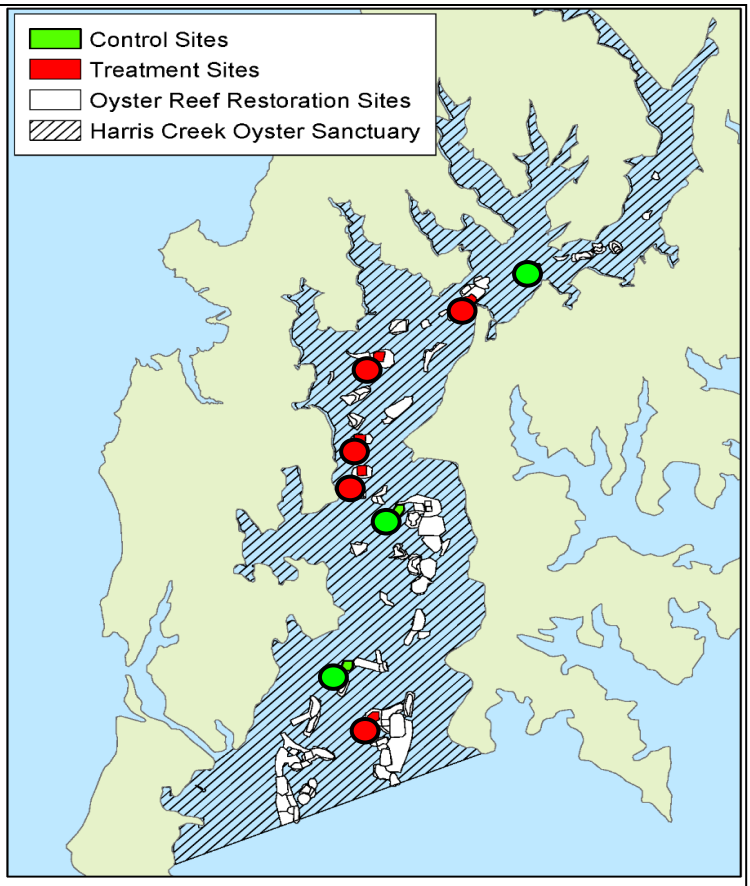

Figure 2. Location of Harris Creek sampling sites (for oyster incubations).

SEDIMENT CORE COLLECTION

Cores were collected in 2.5" inner diameter acrylic tubes using a pole coring device (Cornwell et al. 2014) that collects undisturbed cores in water depths < 3.5 m. Cores were capped on the bottom with an o-ring plate, a rubber stopper was used to seal the surface, and cores were kept in a cooler at near-ambient temperatures until placed in an environmental chamber for incubation. At each site, temperature and salinity from a YSI sonde were recorded, photosynthetically active radiation was measured just below the water surface and at 1 m, and water for incubation was collected in 20 L carboys.

WATER COLUMN FLUX METHODS

We collected discrete water sample profiles and current profiles to understand nutrient dynamics over a restored oyster reef and to estimate fluxes above the reef using the gradient flux approach. These estimates were compared to fluxes measured from enclosure experiments taken within the same week. The gradient approach was applied *in situ* over a restored reef in June 2017 and at a site adjacent to the restored reef in August 2017.

The currents close to the bed were measured with an Aquadopp Profiler (2 MHz ADP, Nortek) with sampling above the blank at 0.13 m and subsequent measurements every minute at 0.03 m intervals. Gradients in nutrients and gases were directly assessed from water samples. Water sampling occurred during two 3-h experiments conducted once in June and August following tray incubation experiments several days prior to water column measurements. A tripod was placed on the reef facing the main direction of the flow with tubing attached at 5 discrete heights (0.2, 0.4, 0.8, 1.2, and 1.6 m) in June and 6 heights (0.2, 0.4, 0.6, 0.8, 1.1, and 1.5 m) in August above the pads on the tripod. Solutes (NH_4^+, NO_x^-, SRP) and dissolved gases (O_2, N_2, Ar, DIC – dissolved inorganic carbon) were collected from each depth approximately every 30 minutes.

The calculation approach used a gradient modeling approach that used mean gradients in momentum and chemical parameters in the bottom boundary layer to estimate fluxes. The details of this research are located in Jackson's dissertation (Jackson 2019) with a publication planned in conjunction with Dr. Larry Sanford, a physical oceanographer with a cooperative research program funded by NCBO in Harris Creek.

Biogeochemical flux measurements

OYSTER TRAY INCUBATIONS

Biogeochemical fluxes in each chamber were measured first under dark, then under light conditions with a one-hour period of aeration between incubations to bring dissolved oxygen levels to saturation. During light incubations, overhead broad-

spectrum lights sufficient for photosynthesis were supplied. Other than lighting, all methods for incubations, sample collection, and sample analyses were identical for light and dark incubations.

Water samples were collected periodically during both light and dark incubations and analyzed to determine net fluxes O_2, N_2, NH_4, NO_x, and SRP. Concentrations of N_2 and O_2 were determined using membrane inlet mass spectrometry, a high-precision rapid method for analyzing concentrations of dissolved gases (Kana et al. 1994, Kana and Weiss 2004). Concentrations of SRP were determined using colorimetric analysis with a detection limit of <0.005 mg L^{-1} (Parsons et al. 1984). Concentrations of NH_4 were determined using phenol/hypochlorite colorimetry (Parsons et al. 1984). Concentrations of NO_x were determined colorimetrically using vanadium reduction (Garcia-Robledo et al. 2014). Fluxes of all analytes were determined as the slope of a linear regression fitted to plots of analyte concentration versus time. To remove the influence of water column processes, slopes of regression lines were adjusted using data from the seawater blank.

SEDIMENT-ONLY INCUBATIONS

Sediments were incubated at the temperature observed in the field, using a temperature-controlled environmental chamber. This program supported a methods paper (Owens and Cornwell 2016) that outlines in great detail the measurement of sediment water exchange and sediment measurements followed the dark – illuminated sequence of incubations, similar to the measurement of community fluxes. A video showing the methods is available at:

http://www.jove.com/video/54098/the-benthic-exchange-o2-n2-dissolved-nutrients-using-small-core

OYSTER-ONLY INCUBATIONS

These experiments were carried out immediately after oyster tray incubations. A subset of these samples (4 samples in June and 6 samples in August) was selected for additional study based upon whether the sample had at least one oyster over ~75 mm visible on the surface sediment. For each tray selected, the live oysters and oyster clumps were carefully removed from each tray, placed in clean and empty incubation chambers, aerated for ~1 h, and incubated in the dark. Incubations were carried out in the same manner as the tray incubations. More details on these incubations are published elsewhere (Jackson et al. 2018, Jackson 2019).

OYSTER BIOMASS

Details on these measurements are available from Kellogg et al's 2019 NCBO report on the Harris Creek benthic community. Oysters were photographed in place, counted, and the tissue excised and dried to determine tissue biomass (g DW).

Results and Discussion

Denitrification Rate Overview

All project denitrification rates are presented both as a histogram (Figure 3) and as a box plot (Figure 4). These rates exceed average annual rates in the Patuxent River subestuary (Boynton et al. 2008), with annual sediment rates of 32 μmol m^{-2} h^{-1} in the lower estuary. Spring and summer sediment denitrification rates in the Potomac subestuary were 54±47 and 153±97 μmol m^{-2} h^{-1} respectively in that nitrate-enriched system. Background sediment rates in Harris Creek were ~ 25% of reef rates in the dark and 15% of reef rates in the light (Cornwell et al. 2016b). Overall the reef denitrification rates were somewhat lower than observed in the upper Choptank study (Kellogg et al. 2013), higher than observed in reef-adjacent sediments in North Carolina (Piehler and Smyth 2011), and lower than New England rates (Humphries et al. 2016).

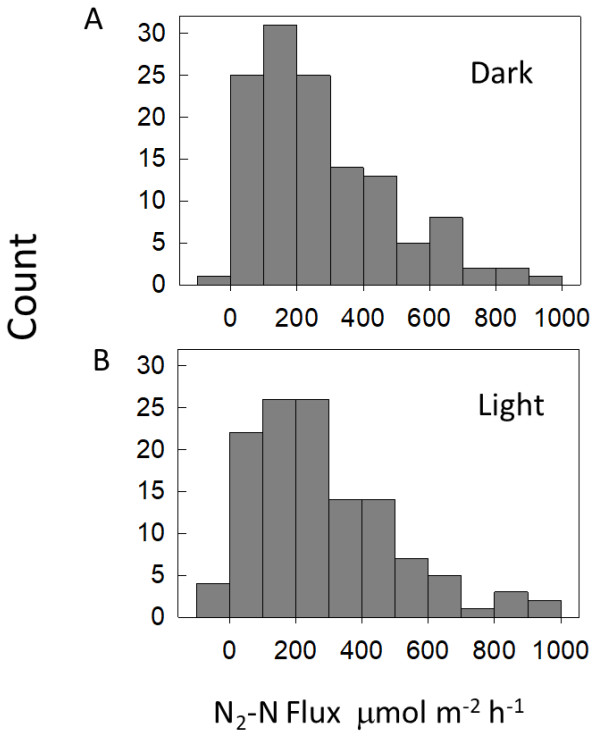

Figure 3. Histogram of all 2015-2017 dark and illumined fluxes of N_2-N (denitrification).

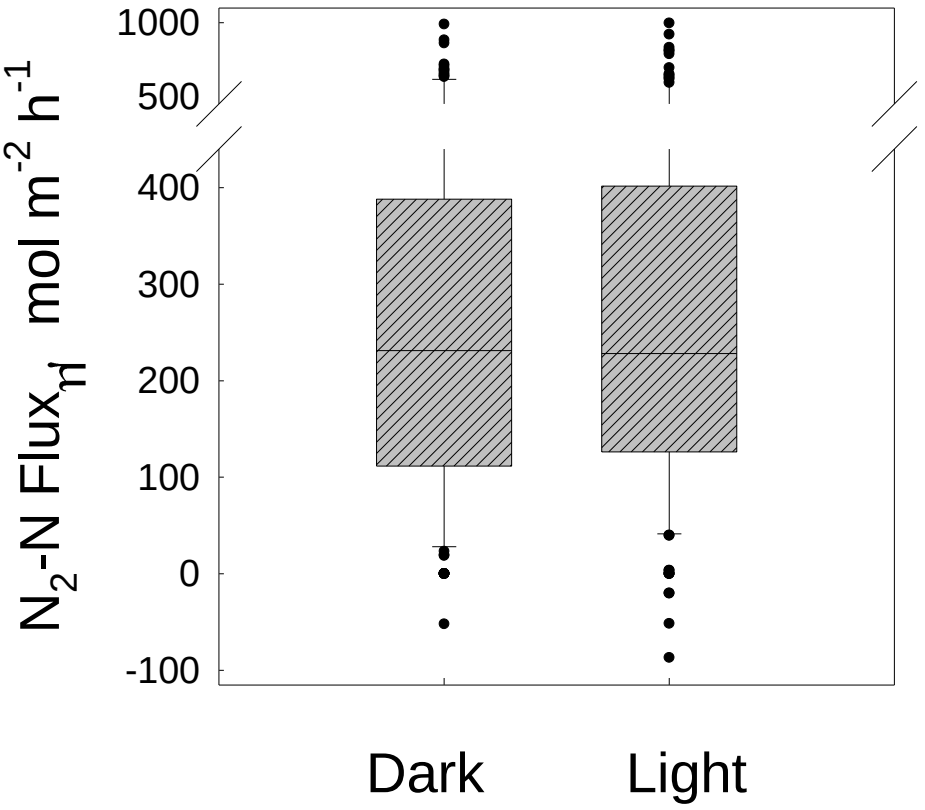

Figure 4. Box plot of all 2015-2017 N_2-N flux data. The median dark and light rates, shown as the line in the middle of each box are 231 and 228 μmol m^{-2} h^{-1} respectively. Mean dark and light rates are 269±213 and 276±217 μmol m^{-2} h^{-1} respectively.

Using all data, we observe a significant relationship between denitrification rates and sediment oxygen demand (Figure 5). The strong relationship has important implications for estimating the efficiency of denitrification and shows the potential that oxygen fluxes could potentially be a proxy for denitrification.

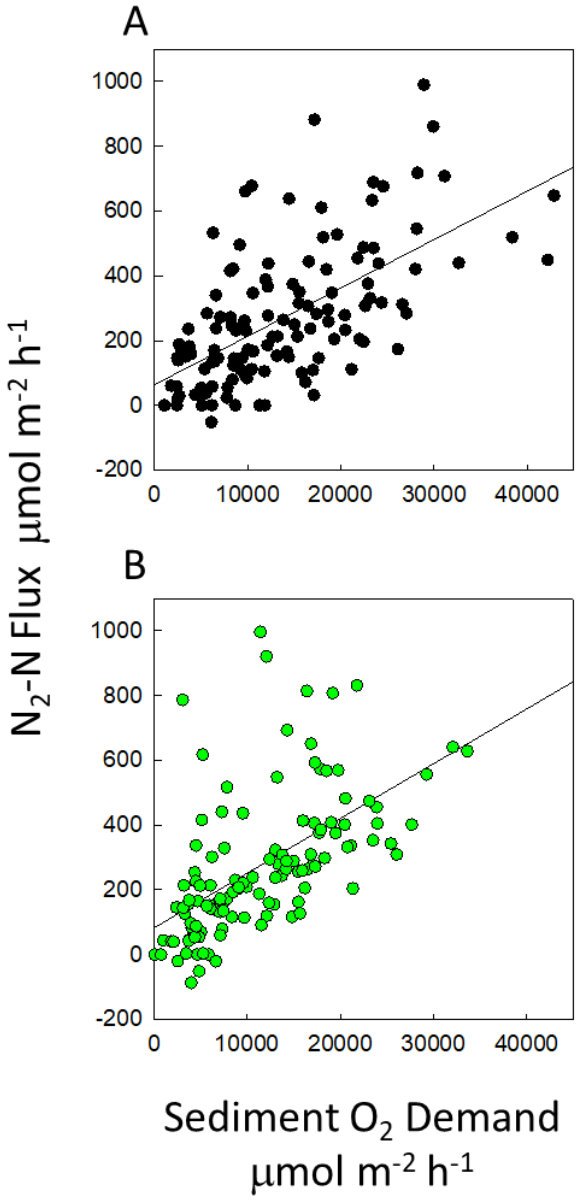

Figure 5. Plot of sediment oxygen demand (the inverse of oxygen flux) and flux of N_2-N. The upper panel plots dark only data and the bottom panel shows the illuminated data. Both relationships are significant (slope $P < 0.001$), with dark and light R^2 of 0.380 and 0.320, with dark and light slopes of 0.0149±0.0017 and 0.0169±0.0022 (slope ± std. error) respectively.

Effects of Illumination

The main effect of illumination is to provide light to the sediment surface where photosynthetic organisms produce oxygen, remove nutrients, and build up biomass (Sundback et al. 1991, MacIntyre et al. 1996, Semcheski et al. 2016). These benthic microalgae alter nitrogen cycling pathways and often intercept remineralized ammonium that would otherwise support coupled nitrification-denitrification (Risgaard-Petersen 2003). Most denitrification work in oyster reefs has not considered potential effects of illumination (Smyth et al. 2015) or found difficulty with incubation under light conditions (Humphries et al. 2016). This work is the first to explicitly consider illumination as a factor in oyster reef denitrification studies.

We estimated the daily rate of denitrification in two ways. The dark rate (μmol m^{-2} h^{-1}) was multiplied by 24 hours to get a daily rate (mmol m^{-2} d^{-1}); this approach was consistent with that the approach used for most literature rates. In addition, we did a more detailed estimate, in which the dark rates times the dark hours was added to the light rate times the light hours, with day length for the region obtained for each date from the US Naval Observatory website.

In Figure 6, we observe that for reef environments, 53% of the rates that considered both dark and light incubations were > the daily rates from dark alone incubations. The line presented in Figure 6 is the 1:1 line; a regression through zero yields a slope of 1.02. From these data, it appears that any interpretation of dark versus dark + light data would not change our estimates of reef denitrification.

The sediments have a somewhat different proportional response to illumination. In the estimate of daily rates, the dark rates exceeded the illuminated rates 78% of the time and the slope of a regression line was 1.26; the difference in these two estimations of denitrification indicated that without the use of the illuminated rate in the calculation, the dark rates will over-estimate the daily rate (Risgaard-Petersen 2003).

These data suggest that the illumination of reef communities does not yield appreciably different daily rate of denitrification. More data from other reefs and from sites with different light attenuation are needed to make this a generalization beyond Harris Creek. Consistent with the benthic microalgal literature, sediment incubations require a dark and an illuminated incubation.

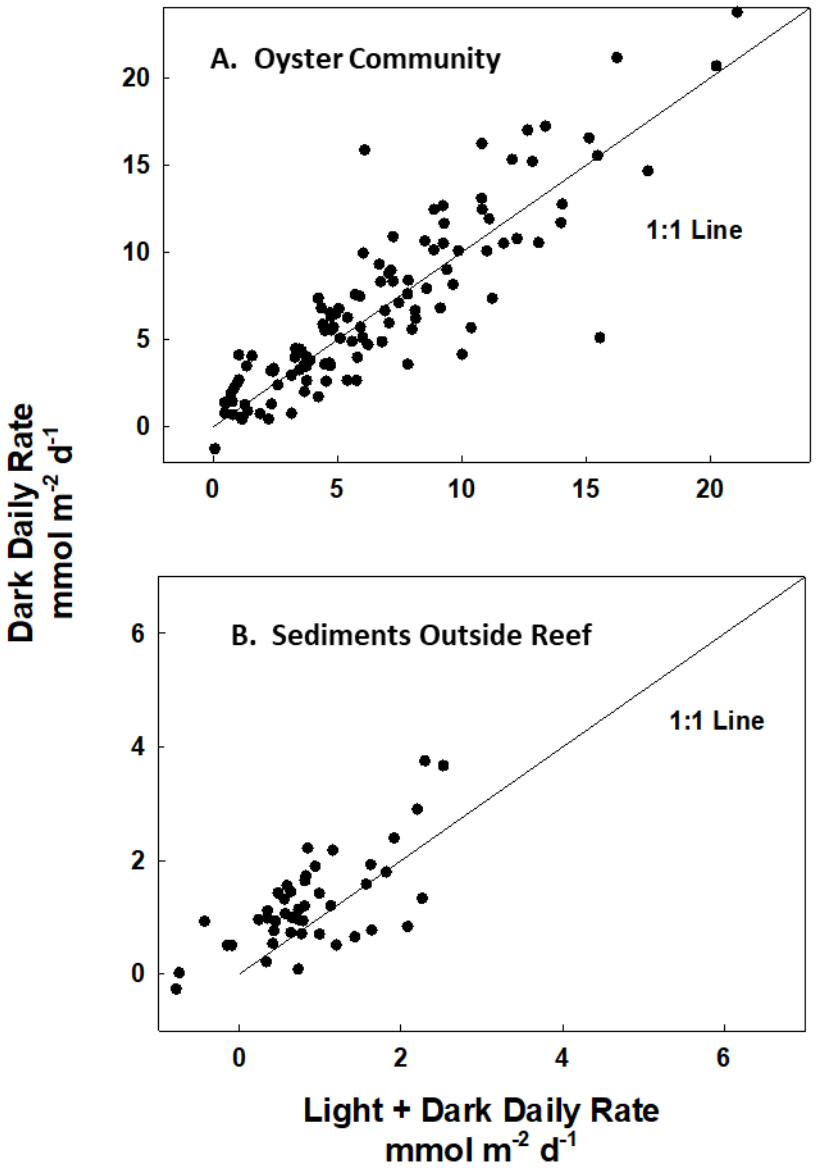

Figure 6. Plot of oyster community and sediment fluxes calculated as the sum of dark and illuminated rates (x axis) and the values if the dark rate was extrapolated to 24 hours. Data in the upper left quadrant of each graph indicates dark-alone data would result in over-estimation of denitrification rates. For sediments, 2/3 of observations suggest that dark incubations over-predict daily denitrification, while 16% of the data suggest dark incubation-only under-predict denitrification. Both plots show significant correlation with $P < 0.001$ and R^2 of .763 and .643 for reef and sediments respectively.

Biomass-Related Sediment-Water Exchange Overview

The data from all years were parsed into three oyster tissue biomass classes based on tissue dry weight (DW) per square meter: low (<75 g DW m^{-2}), medium (75 - 225 g DW m^{-2}), and high (> 225 g DW m^{-2}). Mean oyster tissue biomass in these categories varied by year and ranged from 16-37 g DW m^{-2} (low), 111-158 g DW m^{-2} (medium) and 349-370 g DW m^{-2} (high; Figure 7). Note that for all years, the means for the low biomass category fall between the threshold (15 g DW m-2) and target (50 g DW m-2) restoration biomass categories identified by the Oyster Metrics Working Group (OMWG 2011).

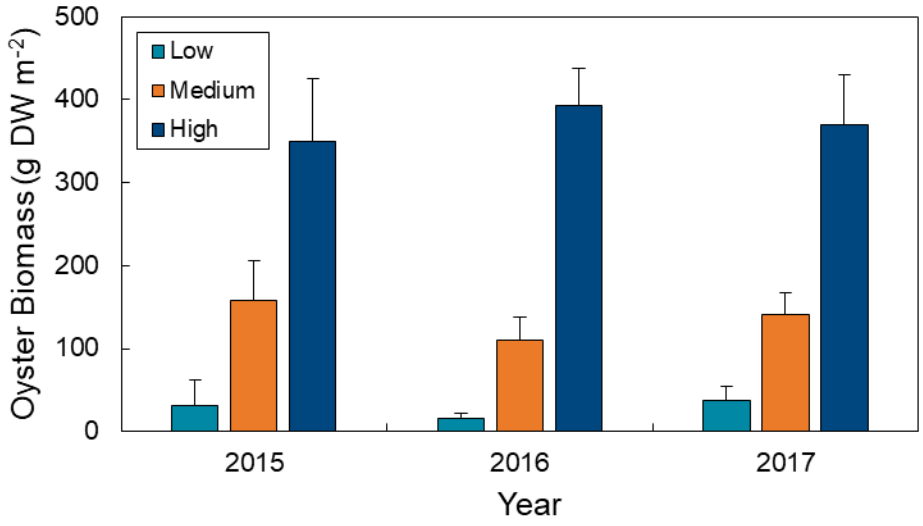

Figure 7. Biomass class definition for denitrification analysis. The data used here are from summer tray incubations used for the determination of oyster biomass and do not include the accumulation of shell and organisms other than oysters.

Using the categorization of low, medium and high biomass, we can examine the mean rates of reef biogeochemical fluxes for each year and under light and dark incubation conditions. Relative to sediments, oxygen uptake rates in reefs are generally much higher than in sediments, and the data from Harris Creek is consistent with other reef studies (Kellogg et al. 2013, Humphries et al. 2016, Volaric et al. 2018). In all cases in Harris Creek (Figure 8), the average rate of oxygen uptake decreased when the sediments were illuminated, suggesting that as in sediment environments (Sundback et al. 1991, Semcheski et al. 2016), illuminated reef environments have benthic

microalgal photosynthesis. Increasing oyster biomass had a positive impact on oxygen uptake, with higher biomass having higher rates of uptake in all years. In 2017, the oxygen uptake in low biomass was ~1/3 of the rate under the high biomass condition. Given the observation that high biomass is ~10 times that of low biomass, the increase in oxygen uptake is clearly not a linear function of biomass.

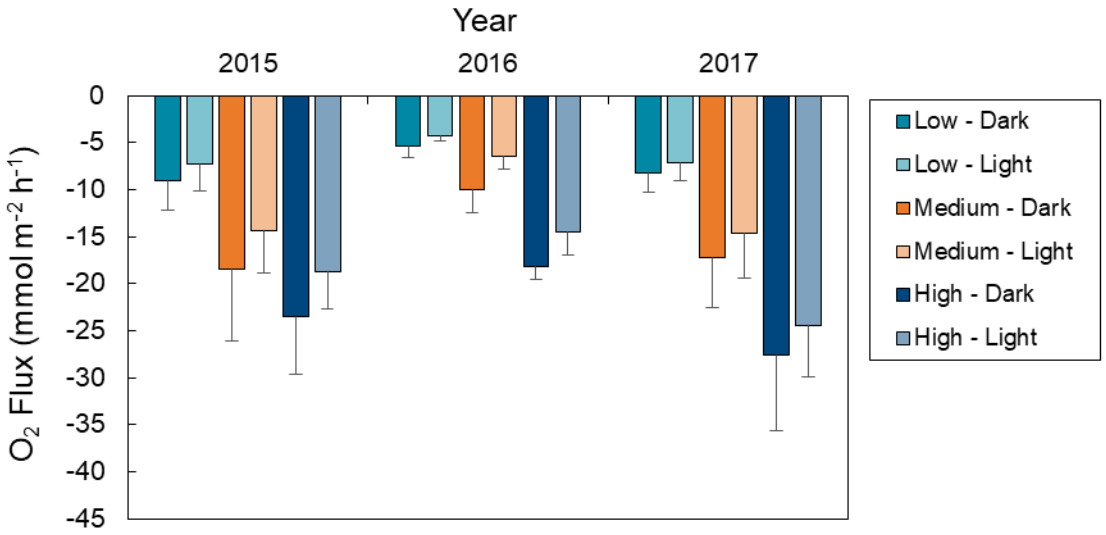

Figure 8. Sediment-water exchange of dissolved oxygen for the years 2015-2017. The biomass classes in Figure 7 are used to define low, medium and high biomass. Averages (± std. dev.) of both dark and light incubations for all May-October incubations are presented. Negative rates indicate uptake of oxygen within the incubation apparatus.

In Chesapeake Bay shallow water sediments, NH_4^+ fluxes are generally directed from the sediment to the water column, with median effluxes < 0.2 mmol m^{-2} h^{-1} (Boynton and Bailey 2008), a small fraction of the rates in Harris Creek reefs. Sediment surveys within Harris Creek (Cornwell et al. 2016b) showed average dark effluxes of 0.09±0.12 and light effluxes of 0.002±0.10 mmol NH_4^+ m^{-2} h^{-1}. These effluxes are consistent with data from the Choptank River (Kellogg et al. 2013). Decreased NH_4^+ efflux rates under illumination, particularly noticeable in 2015 and 2016, are consistent with the "interception" of remineralized N by algae at the reef-water interface. The consistent ~3 fold increase in NH_4^+ efflux observed from low to high biomass follows that of oxygen fluxes and is less than that of the biomass increase.

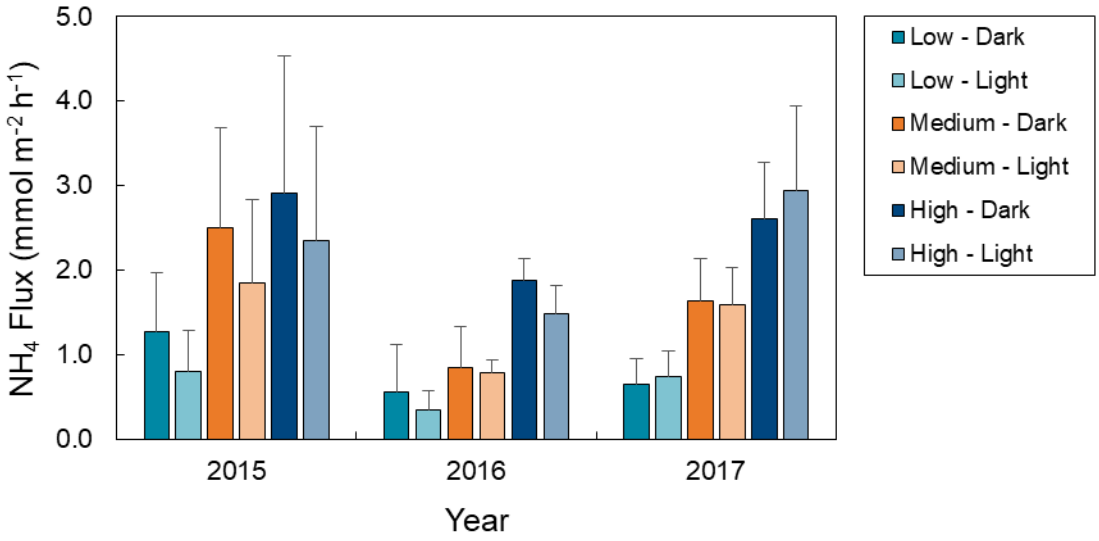

Figure 9. Sediment-water efflux of NH_4^+ from whole community incubations. Averages (± std. dev.) of both dark and light incubations for all May-October incubations are presented.

As observed in our previous Choptank work (Kellogg et al. 2013), NO_x^- was generally an efflux from sediment, with average rates of exchange up to 1 mmol m^{-2} h^{-1} in high boimass incubations (Figure 10). These rates are much higher than observed in Chesapeake sediments (Boynton and Bailey 2008), which typically had NO_x^- uptake or low rates of efflux. The effects of illumination are not clear from this aggregated data, with a large proportional decrease in nitrate uptake in the light for low biomass in 2015, with an increase with light under medium biomass conditions. Efflux was negligible under low biomass in 2016 and quite high for all biomass categories in 2017.

Nitrate effluxes from reef communities result from high rates of nitrification (Kellogg et al. 2013), likely in biofilms on oysters and oyster shell (Ray et al. 2019). Nitrification requires both a source of ammonium and oxygen, with high rates of biodeposit N remineralization and oyster excretion providing abundant ammonium. Increased NO_x^- efflux results from both increased rates of remineralization and possibly more shell surface area. Nitrate is the key substrate for denitrification (Cornwell et al. 1999).

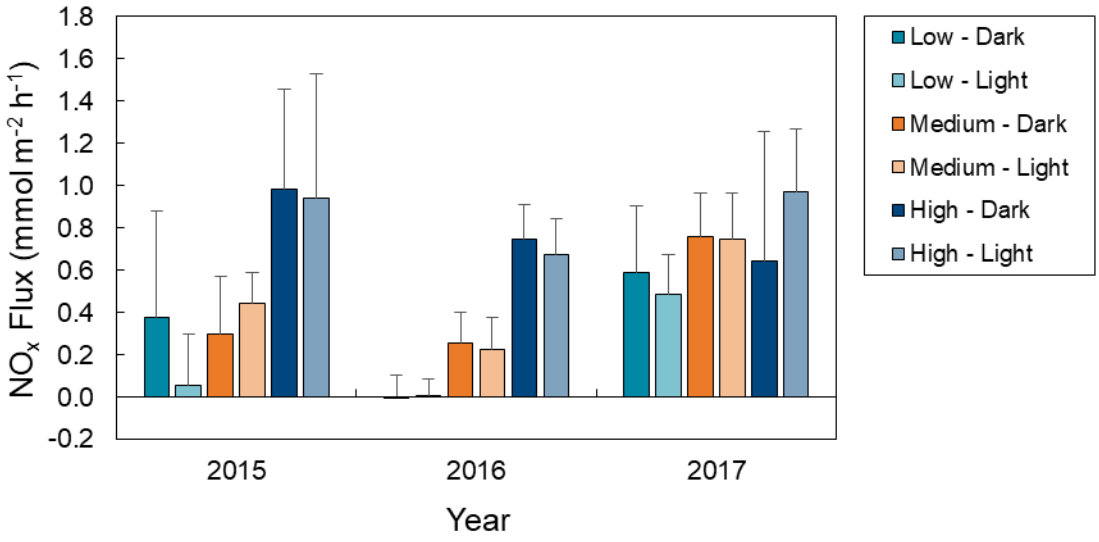

Figure 10. Sediment-water efflux of NO_x^- from whole community incubations. Averages (± std. dev.) of both dark and light incubations for all May-October incubations are presented.

The biomass effect on oyster reef N_2-N exchange (denitrification) is muted relative to the effects of biomass on fluxes of O_2, NH_4^+ and NO_x^- (Figure 11), with no consistent effect of illumination. As with other N fluxes and O_2 fluxes, 2016 data were somewhat lower the other years, but differences were not significant. Rates of denitrification above 0.2 mmol m^{-2} h^{-1} are not typical of Chesapeake Bay sediments (Francis et al. 2013, Testa et al. 2013, Cornwell et al. 2016a), with deep water Choptank River sediments exceeding 0.15 mmol m^{-2} h^{-1} only in the spring and fall (Owens 2009), with diminished rates in the summer because of oxygen limitation of nitrification (Kemp et al. 2005). Translation of these rates to daily rates relevant to nutrient management may be found later in this report.

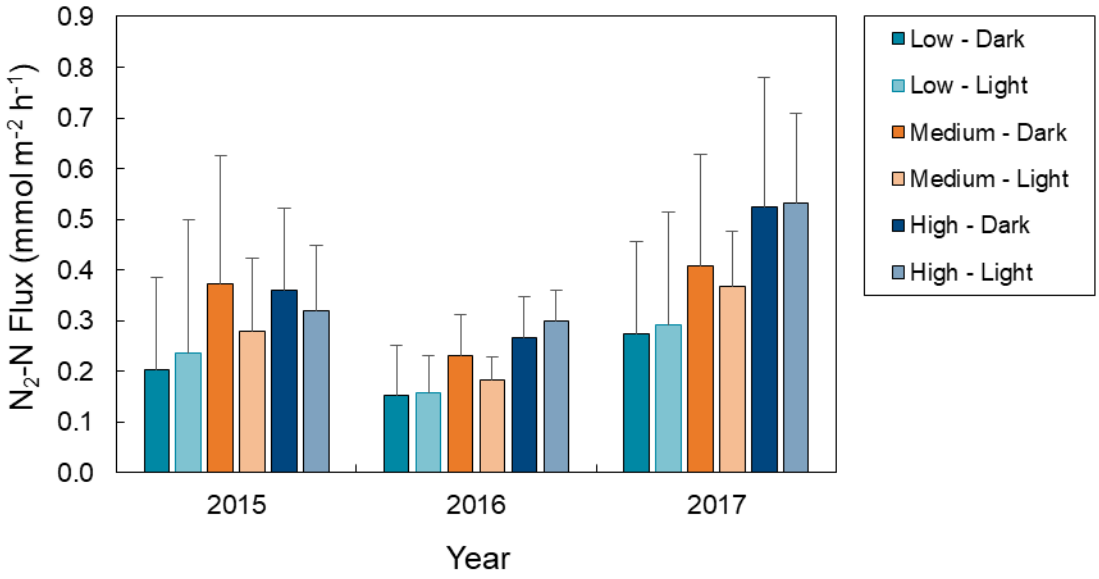

Figure 11. Sediment-water efflux of N_2-N- from whole community incubations. Averages (± std. dev.) of both dark and light incubations for all May-October incubations are presented.

Seasonal Patterns of Denitrification

The 2015 data from our 2016 report (Cornwell et al. 2016b) are the best representation of seasonal data for an intact oyster reef (Figure 12). Denitrification was relatively high in May through October, with a large decrease in rates in late October 2016 and December 2016, though average dark rates in December 2015 were relatively high (> 100 μmol m^{-2} h^{-1}). The argument for mainly using "warm season" data for assessment purposes is supported by higher rates observed during that period.

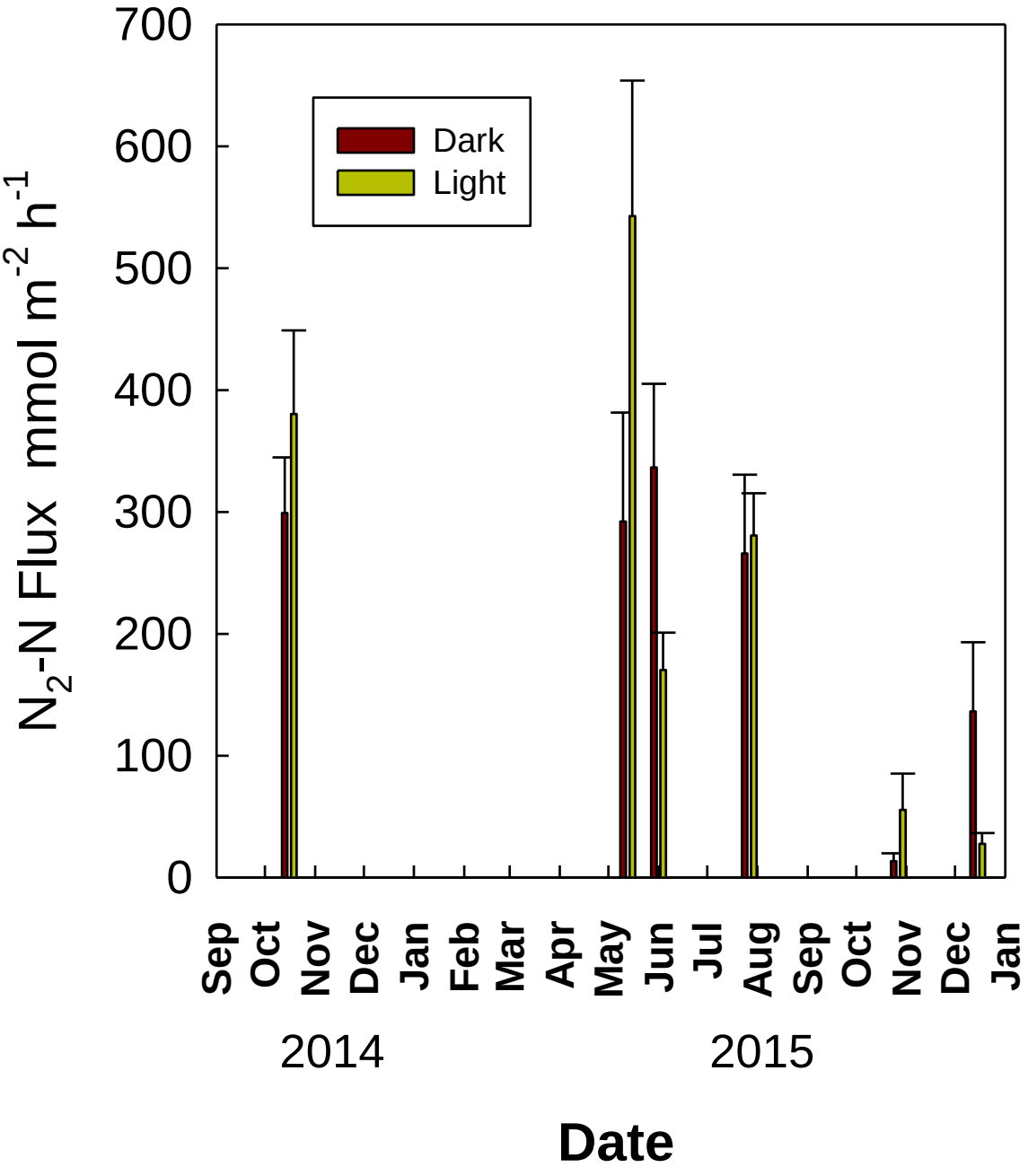

Figure 12. Seasonal fluxes of denitrification (data from multiple reefs) as presented in Cornwell et al. (2016b).

Sediment Incubations vs Whole Community Incubations

Although most studies measure biogeochemical fluxes by enclosing a sample within an incubation chamber and assessing changes in analyte concentration over time. Incubation approaches vary in the type of sample enclosed (e.g. oyster reef sediments (Piehler and Smyth 2011) vs. intact segments of oyster reef (Kellogg et al. 2013), the size and type of chamber used (e.g. 0.0032 m^2 cores (Smyth et al. 2013) vs. 0.1m flux trays (Kellogg et al. 2013), whether incubations were conducted in the field (Humphries et al. 2016) or in the laboratory (Piehler and Smyth 2011), whether the incubation chamber was sealed (a.k.a. "batch"; Kellogg et al. (2013)) or had water passing through (a.k.a. "flow-through" (Piehler and Smyth 2011). The methods used to assess denitrification included changes in N_2 gas concentrations via N_2:Ar ratios in the overlying water column (Kellogg et al. 2013) or ^{15}N tracer approaches such as isotopic pairing (Smyth et al. 2018) techniques). Despite the wide variety of methods used to assess biogeochemical fluxes in oyster reef environments, direct assessments of the effects of incubation approach on resulting flux rates are lacking. We report here the results of two comparisons of whole community versus sediment-only or oyster-only incubations. The first uses comparison of cores from within the reef to the whole community (Kellogg et al. submitted) and the second uses whole community incubations compared to sequential incubation of oyster clumps from the initial community incubation (Jackson et al. 2018).

CORES VERSUS WHOLE COMMUNITY INCUBATIONS

To directly compare two commonly used sampling methods (Figure 13), we incubated cores containing oyster reef sediments (0.0038m^2) and trays (0.1m^2) containing intact segments of oyster reef and measured fluxes of oxygen (O_2), ammonium (NH_4^+), combined nitrate and nitrite (NO_x), dinitrogen gas (N_2) and soluble reactive phosphorus (SRP). Our experiments demonstrate that inclusion of a representative sample of the oyster reef habitat in the incubation chamber is required for accurate biogeochemical flux measurements in oyster reef environments. This work has been reviewed for publication and revisions are currently being made (Kellogg et al. submitted).

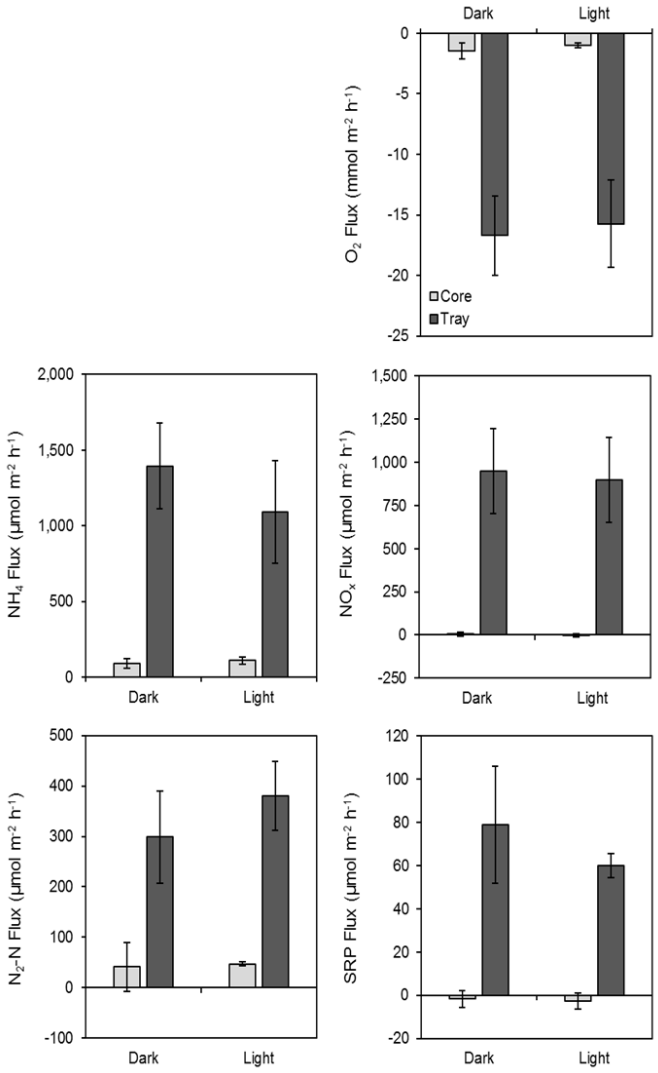

Figure 13. Comparison of fluxes from tray and core samples collected from a restored oyster reef in Harris Creek, MD (Kellogg et al. submitted). Note the difference in units reported for oxygen and nitrogen fluxes.

OYSTER CLUMPS – LOCATING DENITRIFICATION

In contrast to the core incubations, the data from trays and from the oysters removed from the same trays (Figure 14) indicated that a large proportion of the whole community denitrification "moved" with the oysters into the second incubation (Figure 15). The occurrence of denitrification bacteria (Arfken et al. 2017) and activity in oysters alone (Caffrey et al. 2016, Ray et al. 2019) observed in other studies are consistent with these observations of oyster-associated denitrification.

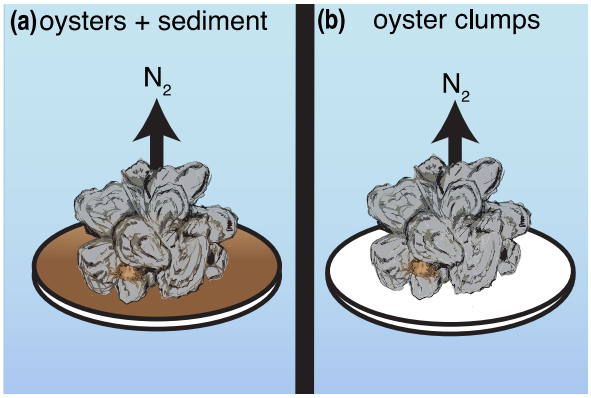

Figure 14. Diagram of whole community (a) versus oyster clump (b) incubations (Jackson et al. 2018).

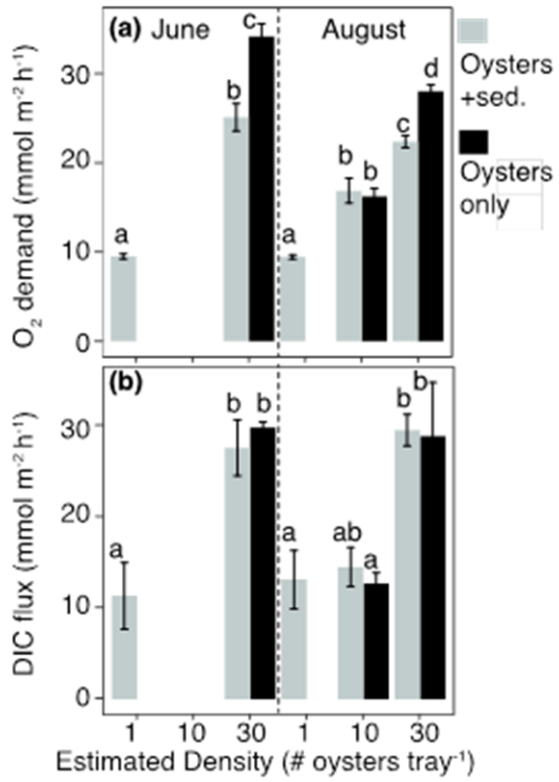

Figure 15. June and August fluxes of (a) oxygen demand and (b) DIC flux. Error bars represent one standard error (n=3). Letters are used to indicate significant differences between levels within each main effect from a 1-way ANOVA followed by Tukey's post hoc comparison (α=0.05). Bars that share a letter are not significantly different.

The implication of these results is that the best incubation approach for a valid assessment of oyster reef denitrification includes both sediments and the living community associated with oysters and their shells. Cores alone likely underestimate reef denitrification. From the perspective of crediting nitrogen removal in a reef environment, cores would provide an extremely low estimate, but nevertheless would ensure that the estimate is conservative. Oysters alone would generally provide a higher estimate than cores, but our data is likely too limited to suggest that this is a useful approach. Thus, a community incubation approach is the most efficacious way to estimate denitrification in an oyster reef.

Denitrification Efficiency

Denitrification efficiency is the proportion of the nitrogen remineralized in a reef that is converted to N_2. Alternative fates include fluxes of ammonium, nitrate plus nitrite, and nitrous oxide. This concept has been useful for site to site comparisons in sediment environments (Berelson et al. 1998, Eyre and Ferguson 2009, Gao et al. 2014) and this calculation has been made previously in our work in the upper Choptank (Kellogg et al. 2013) in which denitrification efficiency ranged from 15±2% in summer to 25±7% in spring.

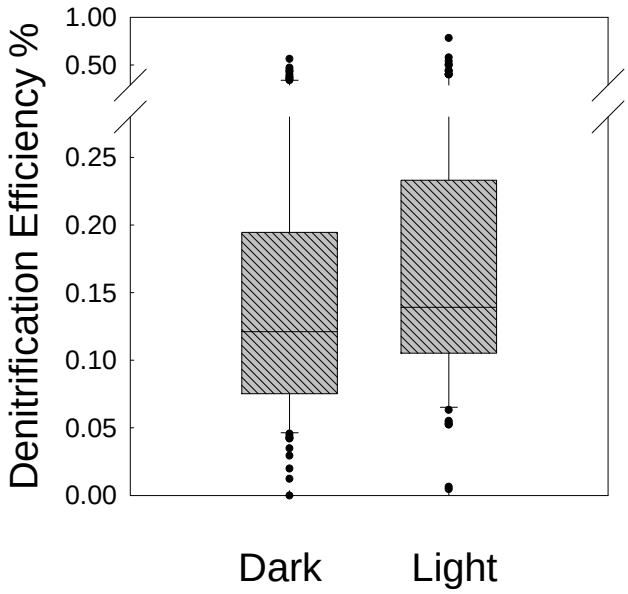

Figure 16. Box plots of denitrification efficiency in Harris Creek warm season (May – October) incubations. Median efficiencies were 12.1% and 13.9% for dark and illuminate incubations.

In this calculation, we estimate the total nitrogen remineralization from the oxygen flux, first assuming that oxygen flux is equivalent to DIC flux as observed in our earlier work (Kellogg et al. 2013). Similarly, our observations that nitrogen remineralization follows the Redfield ratio (Kellogg et al. 2013), we divided the oxygen flux by 6.625 to estimate total N remineralization. By dividing the observed denitrification rate by the estimated remineralization, we can estimate efficiency (Figure 16). Harris Creek denitrification efficiency for warm season data averaged 15.4±11.2 % and 18.7±19.3 % for dark and illuminated incubations. If we use the slopes of the oxygen versus N_2-N regression from Figure 5, we can calculate dark denitrification efficiency of 9.9±1.1 % and an illuminated efficiency of 11.2±1.5% (mean ± std. error), slightly lower than the averages using the means of individual points. Overall, an efficiency ≥ 10% is valid for Harris Creek oyster communities.

In this application, denitrification efficiency allows a comparison to other sites. However, the moderate range of efficiency suggests that this concept may prove useful in estimating denitrification for nitrogen ecosystem services. If these data prove robust, i.e. they are similar across many sites, it may be possible to estimate denitrification from oxygen fluxes, bypassing the need for a more detailed biogeochemical analysis.

Water Column Evidence for Oyster Reef Biogeochemical Fluxes

The measurement of nutrient exchange across the reef-water interface is most typically measured by encapsulating part of the oyster reef, either the whole community *in situ* (Humphries et al. 2016) or *ex situ* (Kellogg et al. 2013), oysters without sediment (Jackson 2019, Ray et al. 2019), or sediment without oysters (Piehler and Smyth 2011, Kellogg et al. submitted). It is appealing to consider if there is a sufficient water column biogeochemical signature that would obviate the need for encapsulation. For oxygen, such measurements are possible via eddy correlation (Volaric et al. 2018), but the high rate of sampling required for this technique are not currently amenable to the measurement of nutrient and N_2 fluxes.

As part of her dissertation (Jackson 2019) and in conjunction with Larry Sanford (UMCES), Jackson deployed current meters and measured gradients of nutrients and gases above the reef to estimate vertical fluxes (Figure 17). This work requires a combination of boundary layer physics, chemical measurement and modeling to estimate such fluxes. The level of detail required for this analysis is included in her dissertation, and her chapter abstract is included here:

Studies focused on quantifying the nutrient ecosystem services in oysters reefs and oysters clusters have been shown to effectively remove nitrogen through denitrification. Complex community structure and spatial variability in oyster reef environments make it difficult to measure biogeochemical fluxes over intact reefs while accounting for all relevant environmental variables that potentially influence nitrogen cycling processes. Although enclosures or chamber experiments can include oysters and other reef associated organisms to directly measure their impacts on nutrient cycling, it is difficult to replicate natural circulation and other sources of large-scale variability within an enclosed experimental chamber. This work couples nutrient and gas concentration data from the water column with physical measurements to provide a noninvasive measure of chemical gradients and biogeochemical fluxes over a restored oyster reef. This study compared oyster reef biogeochemical fluxes measured using benthic chambers (in situ equilibration followed by ex situ incubation) to an in situ vertical gradient approach. A pumping system and current meter were deployed to collect a sequence of depth profiles to estimate the fluxes of di-nitrogen (N_2), dissolved inorganic carbon (DIC), oxygen (O_2), and nutrients from the oyster reef. While biogeochemical rates varied considerably, benthic chambers provided better constrained results than the vertical gradient approach. Above the oyster reef, time series and ensemble-averaged normalized profiles reveal that oxygen was removed at the sediment-water interface, whereas DIC, NH_4^+, N_2, and SRP were produced at the bottom. The gradient approach produced O_2, DIC, and NH_4^+ flux estimates that were in the same direction and order of magnitude as benthic chamber flux estimates. Observations from this work reveal

how these contrasting methods fit into our current toolbox for understanding how oysters modify biogeochemical cycles.

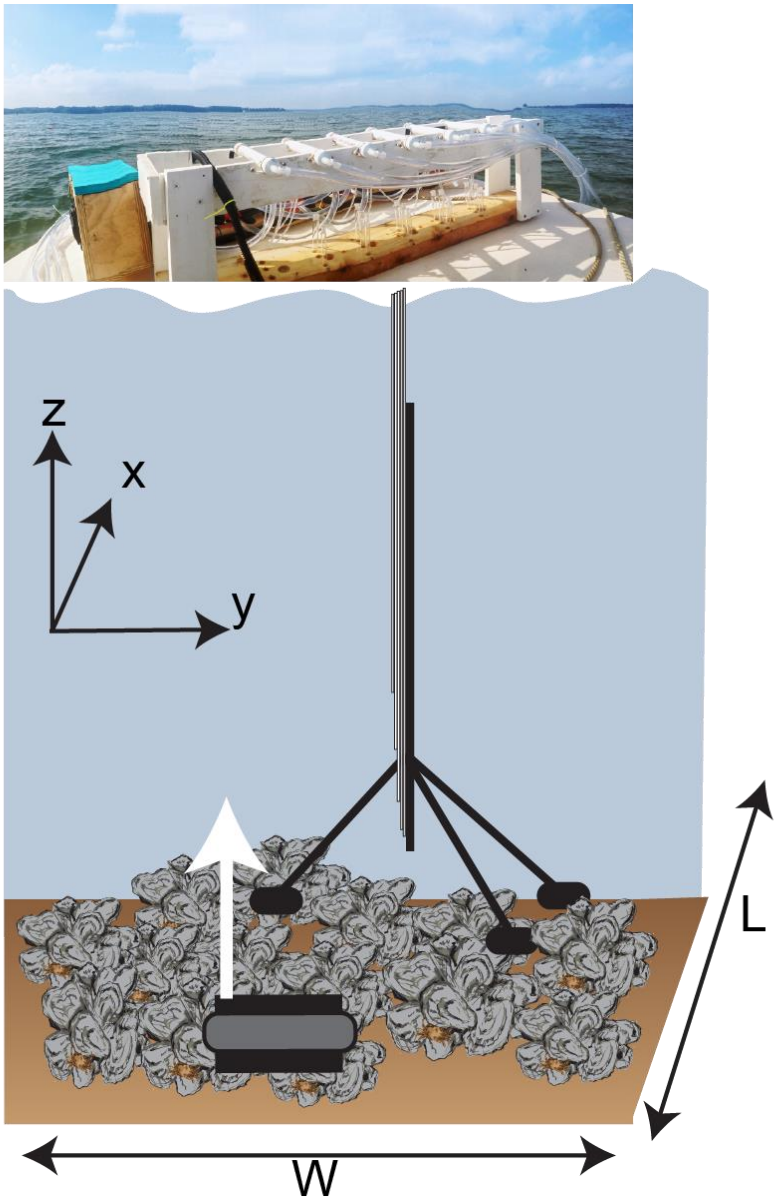

Figure 17. A schematic drawing of the gradient approach and sampling manifold on the boat. The ADP is shown in front of the sampling tripod frame to measure hydrodynamics. The dominant flow is in the x direction. Sampling tubes were connected to the tripod at various depths, 5 discrete heights (0.2, 0.4, 0.8, 1.2, and 1.6 m) in June and 6 heights (0.2, 0.4, 0.6, 0.8, 1.1, and 1.5 m) in August. Each sampling tube was connected to a separate diaphragm pump on the boat, which were distributed through tubing on the manifold (top).

Denitrification Rates For Watershed Implementation Plans – Harris Creek Rates Are Useful for Planning Purposes

Background

In 2019, we addressed the question of how much nitrogen could be removed from Harris Creek reefs – with a goal of providing data in a useful from (e.g. lbs of N per acre of restored reef). The challenge in deriving a useful number is how to aggregate all of the data to make the best estimation possible. The observed differences in denitrification at different biomass levels presented our biggest challenge. The low, medium and high biomass classification proved very useful for this analysis. This information was delivered to Sean Corson (NCBO), head of the Fisheries Goal Implementation Team (GIT) for potential inclusion of NOAA-supported restoration sites in a watershed implementation plan (WIP). The Water Quality Goal Implementation Team supported inclusion of these data for planning purposes.

The version of the document that is presented shows a relatively complex calculation approach to derive a conservative, defensible number for planning. The document was authored by Cornwell, Michael Owens (UMCES), Lisa Kellogg (VIMS), and Julie Reichert-Nguyen (ORP). We have added a short section showing that a simple data aggregation gives a similar nitrogen yield.

Rationale and Approach

The restoration of oyster communities has a net positive benefit with regard to nitrogen removal via microbial denitrification (Newell et al. 2005, Kellogg et al. 2013, Humphries et al. 2016). While many studies suggest denitrification may be assessed with reef-adjacent sediments (Smyth et al. 2015), other studies, including published work at Harris Creek (MD), suggest that the best measurement of oyster-related biogeochemical fluxes require consideration of the whole community (Caffrey et al. 2016, Jackson et al. 2018).

Recently, private oyster aquaculture practices related to assimilated nitrogen (N) and phosphorus (P) in the tissue of harvested oysters were approved as best management practices (BMPs) by the Chesapeake Bay Program (CBP) Partnership (Cornwell et al. 2016c). These oyster BMPs are now available to help jurisdictions meet their N and P reduction goals outlined in the Chesapeake Bay TMDL. With the option of oyster tissue being credited by the USEPA Chesapeake Bay Program, both Maryland and Virginia governments are now working towards implementation of oyster BMPs. In summer 2019 the Oyster BMP Expert Panel will submit a new report that suggests that denitrification and assimilation of nitrogen and phosphorus in oyster biomass associated with oyster reef restoration are viable best management practices. Approval will be considered by fall 2019.

With watershed implementation plans being developed in summer 2019, the urgent need for information on reef denitrification has been identified. This report is a section being incorporated into a much larger data and analysis report of denitrification in Harris Creek. To advance the use of this data the goal of this report is to:

- Provide a synopsis of the data developed via NOAA and other funding in Harris Creek
- **Provide a defensible and conservative areal rate of enhanced denitrification related to N reduction from oyster reef restoration suitable for planning of watershed implementation plans.**

Overall, the estimate of 57 lbs N per acre per year (based on an eligible crediting timeframe of 184 days from measured values) is recommended for planning purposes. This estimate can be applied toward various oyster reef restoration projects in Maryland and Virginia, but only for planning purposes. It should not be used for crediting purposes, since site-specific estimates are needed to address variability (Oyster BMP Expert Panel in draft).

Data Sources

All samples were collected from "seed only" reefs on which the only restoration activity was the planting of spat on shell directly on the bottom. Although we assume here that similar rates occur on reefs restored with a shell or stone base beneath the spat on shell, direct measurements are needed to determine whether this is an appropriate assumption. However, for planning purposes, the N reduction estimates presented in this document can be used in these situations. The NCBO-funded program made measurements in 135 incubation trays encompassing all seasons; this analysis uses spring, summer and fall measurements from 2015-2017 (n = 121).

Calculation Approach

To calculate net enhancement of denitrification associated with oyster reef restoration, we subtracted mean measured seasonal fluxes for sediments (i.e. background denitrification rates) from those for restored oyster reefs. Given the seasonal variability in denitrification rates, it is not recommended to extrapolate the hourly seasonal rates to the full annual timeframe of 365 days without data from all seasons (Spring, Summer, Fall, and Winter). The dataset for the planning estimate only captures the timeframe from May-October; therefore, the seasonal net hourly rates were scaled up to a total of 184 days (May 1 thru October 31) to represent the annual net denitrification enhancement using appropriate information on number of days and average day length. This estimate is conservative because it assumes no enhanced denitrification for any other days of the year.

The steps in our calculations were as follows:

1. Assign an oyster tissue biomass category based on dry weight (DW) to each flux value. Categories used were low (<75 g DW m^{-2}), medium (75 - 225 g DW m^{-2}), and high (> 225 g DW m^{-2}; Figure 1) based on summer data (June-August).
2. Calculate average seasonal (Spring: May, Summer: June-August, Fall: September-October) reef denitrification rates (µmol N m^{-2} h^{-1}) within each biomass category using Harris Creek data collected in 2015-2017. For seasons in which data were collected in multiple years, means were calculated within each year (Table 3) and then these values were averaged across years (Table 4). All seasons and years included data from both dark and illuminated fluxes.
3. Calculate average seasonal (Spring: May, Summer: June-August, Fall: September-October) sediment denitrification rates (µmol N m^{-2} h^{-1}) using Harris Creek data collected in 2014-2016. For seasons in which data were collected in multiple years, means were calculated within each year (Table 1) and then these values were averaged across years. All seasons and years included data from both dark and illuminated fluxes.
4. For each *season x biomass x light* level combination, subtract seasonal average sediment rates from reef rates from Table 3 to determine the dark and light enhancement of denitrification in µmol N m^{-2} h^{-1} (Table 4).
5. Extrapolate to daily rates (µmol N m^{-2} d^{-1}) by multiplying the resulting values from step 4 by the appropriate average number of daytime and nighttime hours based on data for 2016 from the United States Naval Observatory (http://aa.usno.navy.mil/data/docs/Dur_OneYear.php/) and summing the totals for each season (Table 2).
6. Calculate the net denitrification enhancement during the eligible crediting timeframe based on measured values (May-October; 184 days) by multiplying the daily rates from Step 5 by the eligible crediting days in the season and summing the results to get an estimate in µmol N m^{-2} 184 d^{-1} for each oyster tissue biomass category that can be used to represent the annual nitrogen reduction per year (Table 4).
7. To convert the enhanced net denitrification rate from µmol N m^{-2} y^{-1} to lbs N $acre^{-1}$ y^{-1}, divide by 1,000,000 micromoles to convert to moles, multiply by 14.0067 to convert moles to grams (molecular weight of N equals 14.0067 g mol^{-1}), divide by 453.592 to convert grams (g) into pounds (lbs), and lastly multiply by 4046.86 to convert square meters (m^2) to acres (Table 4).

Results

The data from all years were parsed into three oyster tissue biomass classes based on tissue dry weight (DW) per square meter: low (<75 g DW m^{-2}), medium (75 - 225 g DW m^{-2}), and high (> 225 g DW m^{-2}). Mean oyster tissue biomass in these categories varied by year and ranged from 16-37 g DW m^{-2} (low), 111-158 g DW m^{-2} (medium)

and 349-370 g DW m^{-2} (high; Figure 7). Note that for all years, the means for the **low** biomass category fall between the threshold (15 g DW m^{-2}) and target (50 g DW m^{-2}) restoration biomass categories identified by the Oyster Metrics Working Group (2011).

Table 3. Seasonally explicit estimation of denitrification rates (μmol m^{-2} h^{-1}) for the different biomass categories (low: <75 g DW m^{-2}; medium: 75 - 225 g DW m^{-2}; high: > 225 g DW m^{-2}) within year and across years for light and dark incubations. The timeframe of measured values included three seasons (Spring: May, Summer: June-August, Fall: September-October). Shaded upper left corner indicates seasonal averages that included more than one year. The diminishment of sediment denitrification rates with illumination is commonly observed in shallow water sediments (Risgaard-Petersen 2003).

			Dark Reef Denitrification Rates				Light Reef Denitrification Rates			
Oyster Tissue Biomass Category	Sampling Season	Sampling Year	n	Average N_2-N Flux witin Year (μmol m^{-2} h^{-1})	StdDev of Average N_2-N Flux within Year (μmol m^{-2} h^{-1})	Seasonal Average N_2-N Flux Across Years (μmol m^{-2} h^{-1})	n	Average of N_2-N Flux (μmol m^{-2} h^{-1})	StdDev of N2-N Flux (μmol m^{-2} h^{-1})	Seasonal Average N_2-N Flux Across Years (μmol m^{-2} h^{-1})
Low	Spring	2015	2	84	93	84	2	142	102	142
	Summer	2015	16	202	182	210	12	235	265	227
		2016	6	152	99		6	156	73	
		2017	12	275	182		12	290	224	
	Fall	2015	2	0	0	42	2	1	75	38
		2016	7	84	87		7	74	75	
Medium	Summer	2015	6	373	252	336	7	278	144	276
		2016	6	230	83		6	182	46	
		2017	12	407	222		12	368	109	
	Fall	2015	4	18	21	96	3	83	79	89
		2016	6	175	113		6	95	120	
High	Spring	2015	4	396	184	396	6	676	225	676
	Summer	2015	14	361	162	384	15	320	128	384
		2016	6	267	81		6	299	61	
		2017	12	525	254		12	532	178	
	Fall	2015	1	23		122	1	82		137
		2016	5	221	165		5	192	80	
Sediment (Background)	Spring	2015	10	26	24	26	12	2	65	2
	Summer	2015	12	88	74	88	11	17	37	17
	Fall	2014	12	43	27	55	12	23	20	38
		2016	12	66	36		12	54	39	

For restored reefs with low biomass, we observe average dark rates of 84, 210 and 42 μmol m^{-2} h^{-1} for spring, summer and fall conditions; illuminated rates were 142, 227 and 38 μmol N m^{-2} h^{-1} in spring, summer and fall (Table 1). While the sediment rates are much lower than reef rates (Table 3), they are nevertheless an important correction to reef rates. Of particular note are the observations that 1) diminished sediment denitrification in the light has an important effect on this calculation and 2) the annual benefit for enhanced denitrification is dominated by summer rates, and 3) there is a positive relationship between oyster biomass and denitrification rates but the slope of the relationship tends to be less than one.

Table 4. Calculation spreadsheet to determine the net denitrification reef enhancement in lbs per acre per year for the oyster tissue biomass categories described earlier. The enhanced dark and light denitrification reef rates are the corresponding areal reef rates minus the rates in Harris Creek sediments from Table 1 (the average was used for seasons with more than one measurement across years). The daily denitrification reef enhancement is calculated by multiplying the enhanced dark and light denitrification rates by their corresponding mean hours per day and summing the results. The seasonal net denitrification reef enhancement in lbs per acre per year (based on eligible crediting days of 184) is calculated by multiplying the daily rate by the eligible crediting days and dividing by 1,000,000 micromoles to convert to moles, multiplying by 14.0067 to convert moles to grams (molecular weight of N equals 14.0067 g mol-1), dividing by 453.592 to convert grams (g) into pounds (lbs), and lastly multiplying by 4046.86 to convert square meters (m2) to acres. The sum of the seasonal net denitrification enhancement rates determines the annual total net denitrification reef enhancement (lbs N $acre^{-1}$ y^{-1}) of the oyster tissue biomass categories for nitrogen reduction planning purposes. The means for the low biomass category fall between the threshold (15 g DW m^{-2}) and target (50 g DW m^{-2}) restoration biomass categories identified by the Oyster Metrics Working Group (2011).

	Enhanced Dark Denitrification Reef Rate ($\mu mol\ m^{-2}\ h^{-1}$)			**Enhanced Light Denitrification Reef Rate ($\mu mol\ m^{-2}\ h^{-1}$)**		
Oyster Tissue Biomass Category	***Spring***	***Summer***	***Fall***	***Spring***	***Summer***	***Fall***
Low	58	122	-13	140	210	0
Medium		248	41		259	51
High	370	296	67	674	367	99
Mean hours per day	9.7	9.7	12.2	14.3	14.3	11.8
	Daily Denitrification Reef Enhancement ($\mu mol\ m^{-2}\ d^{-1}$)			**Denitrification Reef Enhancement during Measured Timeframe ($\mu mol\ m^{-2}$ 184 d^{-1})**		
Oyster Tissue Biomass Category	***Spring***	***Summer***	***Fall***	***Sum of Season x Eligible Crediting Days***		
Low	2,558	4,183	-160	454,425		
Medium		6,112	1,096	629,202		
High	13,218	8,115	1,980	1,277,154		
Eligible Crediting Days	31	92	61	184		
	Net Denitrification Reef Enhancement ($lbs\ acre^{-1}\ y^{-1}$)					
Oyster Tissue Biomass Category	***Spring***	***Summer***	***Fall***	***Annual Total Based on 184 Eligible Crediting Days***		
Low	10	48	-1	**57**		
Medium		70	8	79		
High	51	93	15	160		

If we follow the usual approach of subtracting background sediment denitrification from reef rates (i.e. Kellogg et al. 2013), we can estimate rates of enhanced denitrification for the three biomass classes (Table 4). The summation of the average spring, summer and fall data yields an enhanced denitrification rate of 57 (low biomass), 79 (medium biomass), and 160 (high biomass) lbs N acre^{-1} y^{-1} (Table 4). While the medium and high biomass categories results are also presented; it is not expected that these would be used for planning purposes unless there are oyster tissue biomass data from the site demonstrating average levels above 75 g m^{-2}.

A Simplified Approach

Using the 3 biomass classes and taking warm season averages (Figure 18), we observed that although biomass increases 10 fold between classes, denitrification rates only change by ~3 fold. Sediment denitrification rates are relatively low. If we follow the usual approach of subtracting background sediment denitrification from reef rates (i.e. Kellogg et al. 2013), rates of enhanced denitrification for the three biomass classes may be estimated. When we subtract the sediment rate from the low biomass rate, we obtain a net denitrification of 57 lbs acre^{-1} y^{-1}, identical to the temporally explicit rate.

Conclusions

The most conservative estimate for nitrogen removal via denitrification comes from the low biomass estimate of 57 lbs N acre^{-1} y^{-1} (based on 184-day timeframe of measured values). This rate is a conservative estimate because it assumes negligible denitrification enhancement from November through April and low rates of off-site transport of biodeposits which might be denitrified in other Harris Creek environments. These rates are lower than those based on modeling efforts (Kellogg et al. 2018); the estimate of 206 lbs N acre^{-1} y^{-1} from the model

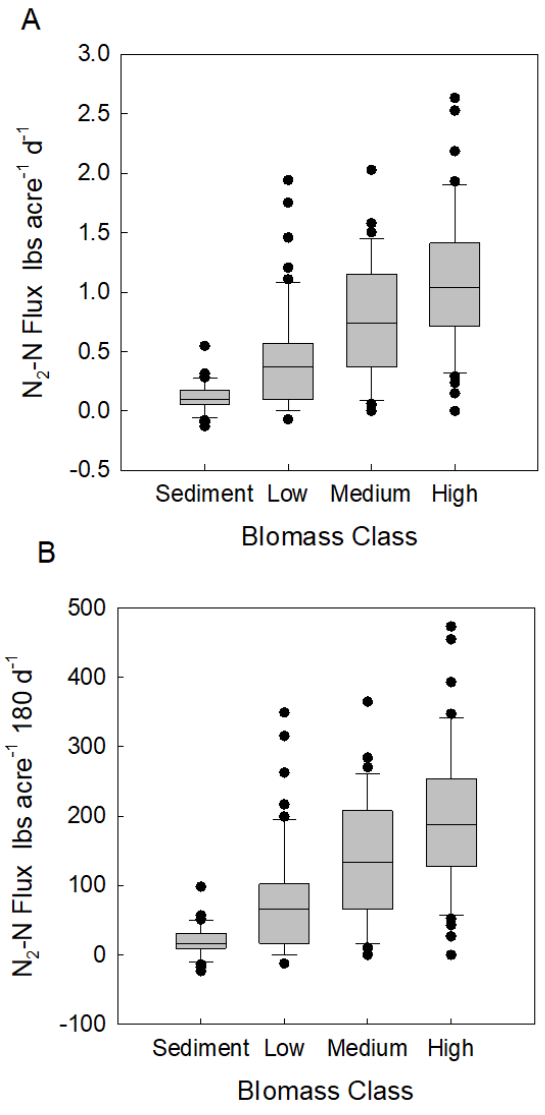

Figure 18. Box plots of all warm season N_2-N flux data from 2015-2017 in Harris Creek on a lbs acre^{-1} d^{-1} basis. Biomass categories are shown in Figure 7.

includes the whole year, not just the warm months of May thru October.

For the purposes of using this data for a preliminary estimate of enhanced denitrification in watershed implementation plans, we suggest the best available knowledge at this time yields an annual rate of 57 lbs N acre^{-1} y^{-1}. This is based on an aggregation of data from different reefs in Harris Creek and is based on the most detailed study of restored reef environments that has been carried out up to this point in time. Biomass changes are likely to have an effect on the trajectory of reef denitrification, but the current estimate is appropriate for extrapolation to the whole Harris Creek restoration area, and is appropriate as a starting point for other restoration sites.

Summary and Conclusions

Assessment of Denitrification in Harris Creek

The previous section provides one of the first defensible areal nitrogen removal numbers available for coastal waters. The approach used here was more rigorous that almost all other studies, with two sets of experiments suggesting that assessments that do not consider the whole benthic community may considerably underestimate nitrogen removal by microbial denitrification. The minimum number generated here, 57 lbs N acre^{-1} y^{-1} would translate to an annual N removal of ~20,000 lbs of N in the 350 acres of restoration in Harris Creek. Living shoreline estimates of N removal via denitrification are on the same order (85 lbs acre^{-1} y^{-1}) as oyster restoration (Beck et al. 2017). Higher oyster biomass would yield higher rates of denitrification.

Our results show that:

- Most warm weather measurements are similar, our winter measurements showed a large diminishment of denitrification.
- For oyster reefs, the effect of illumination is small relative to the high rates of biogeochemical transformation. Illumination is more important for sediment incubations.
- The oyster community, animals plus sediment, is responsible for denitrification. Excising either sediments or animals from measurement experiments is not advisable.

Implications for Management

The idea that oyster restoration or aquaculture can provide valuable ecosystem services has become more established in the last two decades. Benefits include increased habitat diversity, improved fish populations, improved water clarity that is beneficial to submerged aquatic vegetation, feeding habitat, and even changing physics and salinity (Coen et al. 2007, Ermgassen et al. 2013, Humphries and La

Peyre 2015, Kaplan et al. 2016, Sharma et al. 2016). The idea that the eastern oyster can provide important ecosystem benefits has also been popularized, with suggestions that restoration can result in important amounts of nitrogen removal through microbial denitrification (Newell et al. 2002, Piehler and Smyth 2011, Kellogg et al. 2013, Humphries et al. 2016, DePiper et al. 2017) and that aquaculture can provide similar ecosystem services through harvest of oyster and associated tissue-nitrogen (Higgins et al. 2011, Carmichael et al. 2012, Kellogg et al. 2014, Rose et al. 2014, Reitsma et al. 2017, Thompson 2017). While the strategy of using bivalves for nutrient mitigation may have few advantages at the scale of the whole Chesapeake Bay (Cerco and Noel 2007, Land 2014), within tributaries and embayments valuable water quality improvements are possible with enhanced oyster biomass (Kellogg et al. 2013). Moreover, while oysters alone may not provide sufficient nutrient reduction to fully alleviate eutrophication, they can provide another tool in the BMP toolbox. As we come to fuller utilization of more traditional agricultural and wastewater BMP's, the need for innovative strategies to meet water quality goals becomes more important.

The biogeochemical data and analysis of denitrification in Harris Creek represents the most comprehensive assessment to date, not just in Maryland, but in the world. Understanding the effects of oyster biomass, light, benthic community and restoration practices on nitrogen removal is key to incorporating oysters into ecosystem models and for estimation of nitrogen ecosystem services. The numbers generated here provide broad guidance for coastal managers to compare the resources required for oyster restoration to one more valuable ecosystem service. While direct, quantitative extrapolation of these results to all Chesapeake restoration projects is not defensible at this juncture, these results should be encouraging for the quantification of nitrogen removal in future oyster restoration projects.

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Thompson, S. 2017. Mapping Future Scenarios of Oyster Farming in Waquoit Bay, Massachusetts to Decrease Nitrogen Loading.

Volaric, M. P., P. Berg, and M. A. Reidenbach. 2018. Oxygen metabolism of intertidal oyster reefs measured by aquatic eddy covariance. Marine Ecology Progress Series **599**:75-91.

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 do not include the 13 presentations from the 2014-2016 project.

Presentations

Cornwell, J.C. Wetland and oyster biogeochemical research. Hampton Roads Planning District Commission, Water Quality Technical Meeting. March 2017

Melanie Jackson, Michael S. Owens, Lawrence P. Sanford, M. Lisa Kellogg, and Jeffrey C. Cornwell. 2018 Ocean Sciences. Portland, OR. Comparison of Two Flux Measurement Approaches for the Determination of Nutrient fluxes Over a Restored Oyster Reef, Chesapeake Bay (USA)

Melanie Jackson, Michael S. Owens, Jeffrey C. Cornwell, M. Lisa Kellogg. A confirmation that oyster clumps perform the majority of nutrient fluxes on restored oyster reefs. 2017 CERF. Providence, RI.

Cornwell, J.C., Owens, M. Jackson, M. and Kellogg, M.L. Estimating “Enhanced” Denitrification Through The Addition of Oysters: A Holistic View of The Problem. 2017 CERF Meeting, Rhode Island.

Kellogg ML, Cornwell JC, Owens MS, Ross PG, Paynter KT, Luckenbach MW, Dreyer JC, Pant M, Turner C, Birch A, Smith E. (2017) Ecosystem services provided by tributary-scale oyster reef restoration in Chesapeake Bay. 24th Biennial Conference of the Coastal and Estuarine Research Federation, Providence, Rhode Island.

Jackson, M.L. 2018. Oyster-associated denitrification: between a rock and another hard place (i.e. shell). 2018. Atlantic Estuarine Research Society, April 2018, Rehoboth Beach DE.

Owens, M.S., J.C. Cornwell, M. Jackson and M.L. Kellogg. 2018. Poster Presentation: Denitrification in restored oyster reefs. Atlantic Estuarine Research Society, April 2018, Rehoboth Beach DE.

Kellogg, M.L., J.C. Cornwell, P.G. Ross, K.T. Paynter and M.W. Luckenback. Brush. 2018. Quantifying the benefits of tributary-scale reef restoration. Oral Presentation, Chesapeake Research and Modeling Symposium. June 2018. Annapolis, MD.

Cornwell, J.C., J. Reichert-Nguyen and W. Slacum. Oyster BMP panel update: reduction effectiveness strategies of oyster bmps. Fisheries Goal Implementation Team (GIT), Newport News VA. December 2018.

Collaborative Activities

Oyster BMP Expert Panel: Cornwell and Kellogg, Cornwell – Chair. This panel has been examining whether nitrogen removal ecosystem services merit recommendation as a best management practice. USEPA has approved the removal of oyster tissue as a BMP for nitrogen. https://coastalscience.noaa.gov/news/coastal-pollution/nutrient-crediting-oyster-aquaculture-chesapeake-bay/ . We are completing work on denitrification and oyster biomass crediting for restoration.

NSF Coastal SEES – Oyster Futures. NSF-funded project head by Elizabeth North, seeks common ground on Chesapeake Bay oysters management (Cornwell co-investigator). This work has been completed (OysterFutures 2018) and the report is available - https://oysterfutures.files.wordpress.com/2018/05/oysterfutures_stakeholder_recommendations_report_14may2018.pdf. Melanie Jackson's Ph.D. dissertation, funded by NSF and with NOAA-funded logistics) used Harris Creek for two chapters of her dissertation.

NOAA Ocean Acidification – a project lead by Jeremy Testa (CBL), with PI's at Horn Point (Kemp, Li), Oregon State University (Waldbusser), and University of Delaware (Cai). Oyster work at Harris Creek and was part of a Ph.D. thesis at Oregon State University (Iria Giménez).

Nature Conservancy and Oyster Recovery Partnership-funded research on model estimation of nutrient-related benefits of oyster reef restoration (Kellogg et al. 2018).

UMCES Oyster Team - The Effectiveness of Locations of Oyster Sanctuaries, Public Fishery Areas and Aquaculture Areas in Maryland. Lead by former UMCES President Donald Boesch

Education-Related Activities

Graduate Students

Jackson, Melanie. L. 2019. Ph.D. thesis. Characterization of oyster-associated biogeochemical processes in oyster restoration and aquaculture. University of Maryland Center for Environmental Science. Her work included work on individual oyster denitrification and water column evidence for oyster reef biogeochemical processes ((Jackson 2019).

Undergraduate Students

McClain, Anna. 2016. Carbonate chemistry in a restored oyster reef in the Chesapeake Bay. St. Mary's College of Maryland. Undergraduate thesis, she was an undergraduate chemistry major and her work was part of the NSF-supported Maryland Sea Grant Research Experience for Undergraduates program. She continued this work after her REU and incorporated it into her undergraduate thesis.

Journal Publications

Kellogg, M.L., J.C. Cornwell and M. S. Owens. Submitted. Measurement of biogeochemical fluxes in oyster reef environments. Submitted to Marine Ecology Progress Series. Currenlty being revised.

Jackson, M., M. S. Owens, J. C. Cornwell, and M. L. Kellogg. 2018. Comparison of methods for determining biogeochemical fluxes from a restored oyster reef. Plos One 13:e0209799. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0209799

Owens, M. S., and J. C. Cornwell. 2016. The Benthic Exchange of O-2, N-2 and Dissolved Nutrients Using Small Core Incubations. Jove-Journal of Visualized Experiments. https://www.jove.com/video/54098/the-benthic-exchange-o2-n2-dissolved-nutrients-using-small-core

Over the next 18 months, we expect to submit papers on 1) seasonal patterns of reef denitrification, 2) correlative studies between denitrification and the composition of the benthic community, and 3) a paper on the use of oysters as a BMP.