

INTEGRATED ASSESSMENT OF OYSTER REEF ECOSYSTEM SERVICES



6/17/2016

Quantifying Denitrification Rates and Nutrient Fluxes

A final report to:
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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

Measurements of nutrient exchange were made in restored oyster reefs and creek sediments in 2014 and 2015 in Harris Creek, Maryland, USA. Rates of ammonium, nitrate and di-nitrogen fluxes were much higher in reef environments than in sediments, and rates of oxygen uptake reflected high inputs of biodeposits. The rate of denitrification was related to oyster biomass and oyster numbers. The shallow nature of the restoration allows light to reach the bottom and benthic microalgal photosynthesis affects the net nutrient exchange with the bottom. After several years, oyster restoration has increased denitrification in Harris Creek, though observations in mature upper Choptank restored reefs are higher. The trajectory of increase of the nutrient ecosystem services is positive and will be followed over time.

Rationale

The depletion of Chesapeake Bay oyster habitat by over harvest, poor water quality and disease has reduced oyster populations to a small fraction of the original population (Kemp et al. 2005). 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 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.

Estimates of denitrification in oyster reefs has generally involved simulations of the processing of pseudofeces and feces (Newell et al. 2002) or sampling of sediment in the vicinity of oyster reefs (Piehler and Smyth 2011, Smyth 2013). Other estimates of oyster nutrient or respiratory processes have been made using benthic “tunnels” (Dame et al. 1989), but no measurement of denitrification was made. Absent from the literature are denitrification measurements in oyster reef “communities”; recently, we have made the first measurements that have included the reef community (Kellogg et al. 2011, Kellogg et al. 2013).

Project Narrative

The approach to making the measurements of denitrification and nutrient fluxes were modeled after our successful work upstream in the Choptank River. Our chief goal was to obtain representative data from multiple planted reefs to determine a measure of the nutrient-related ecosystem service values of the Harris Creek Oyster Sanctuary. These measurement are designed to examine the trajectory of these young reefs with regard to the process of denitrification. Issues with identification of controls and locating representative reefs for study, not only for biogeochemistry but for biological function, resulted in a slow start to the main part of the project.

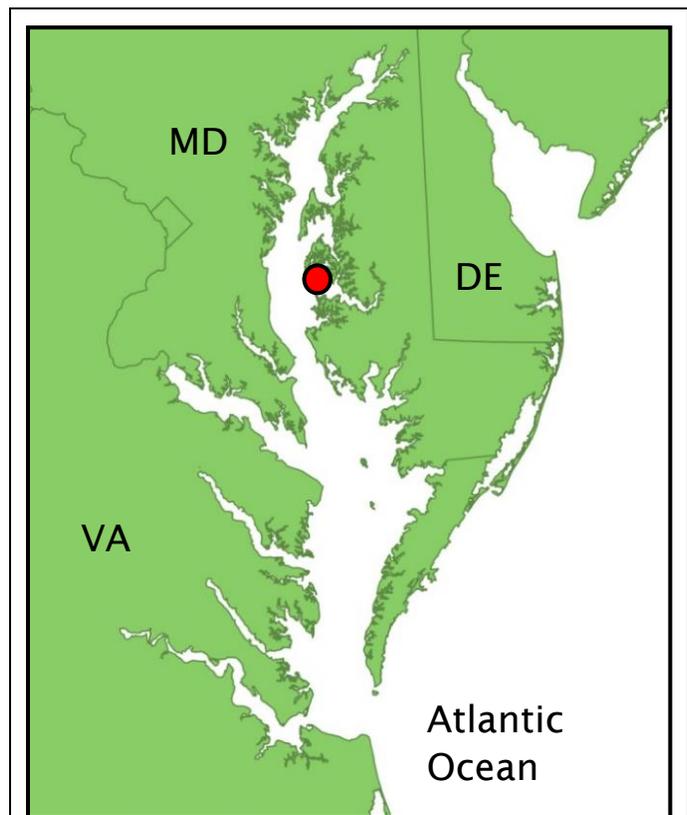


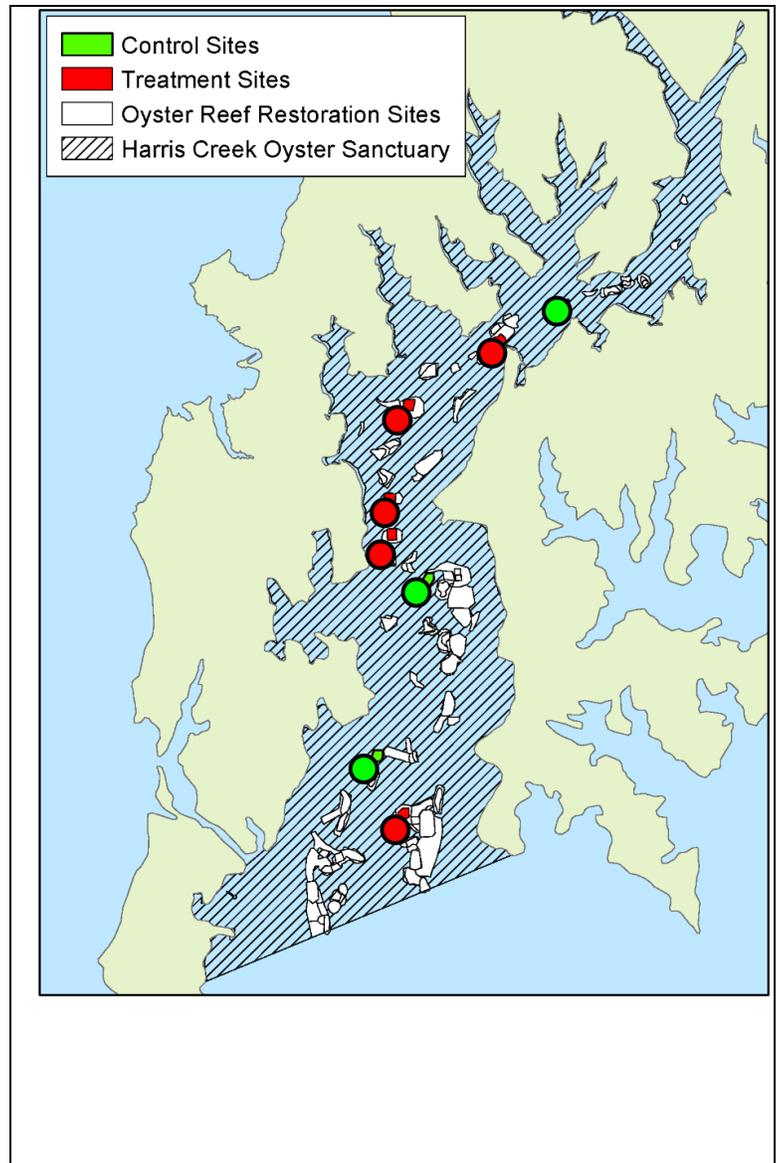
Figure 1. Location of Harris Creek Oyster Sanctuary in the Maryland portion of Chesapeake Bay.

In this report we show the results of 2014 and 2015 rate measurements in oyster reefs and in non-restored sedimentary environments in Harris Creek. The results of two projects funded by the Maryland Sea Grant REU program are also shown and the key results from two summer internships are illustrated.

Methods

Study sites and experimental design: Oyster trays were collected on 6 occasions to examine seasonal patterns of sediment-water exchange. A total of 8 trays were usually collected for experiments. Dates included October 16, 2014 (only 4 trays), May 13, 2015, June 1, 2015, July 27, 2016, Oct 27, 2015 and December 15, 2015. Reef sites in Harris Creek (Figure 2) included Rabbit Island East, Little Neck, Walnut, Lodges, Seth’s Point, Mill Point and Change.

Field sampling: To deploy trays, divers placed materials from a 0.1-m² 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.

Biogeochemical flux measurement:

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 (Fig. 3) 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 $<0.005 \text{ mg L}^{-1}$ (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.



Figure 3. Collection of water samples by Mike Owens (UMCES) under light conditions in incubation facility at Horn Point Laboratory.

Core incubations were carried out on three occasions (Sept 15, 2014, May 12, 2015, June 26, 2015) at six general areas of Harris Creek. At each area, 7 cm id cores were collected at shallow (1 m) and deeper (2 m) locations. Samples were incubated at Horn Point Laboratory at field temperatures and the incubation procedures followed our standard protocols (Cornwell et al. 2014, Owens and Cornwell 2016). In previous work, we have compared such cores to our oyster trays using sediment in each and have note no differences.

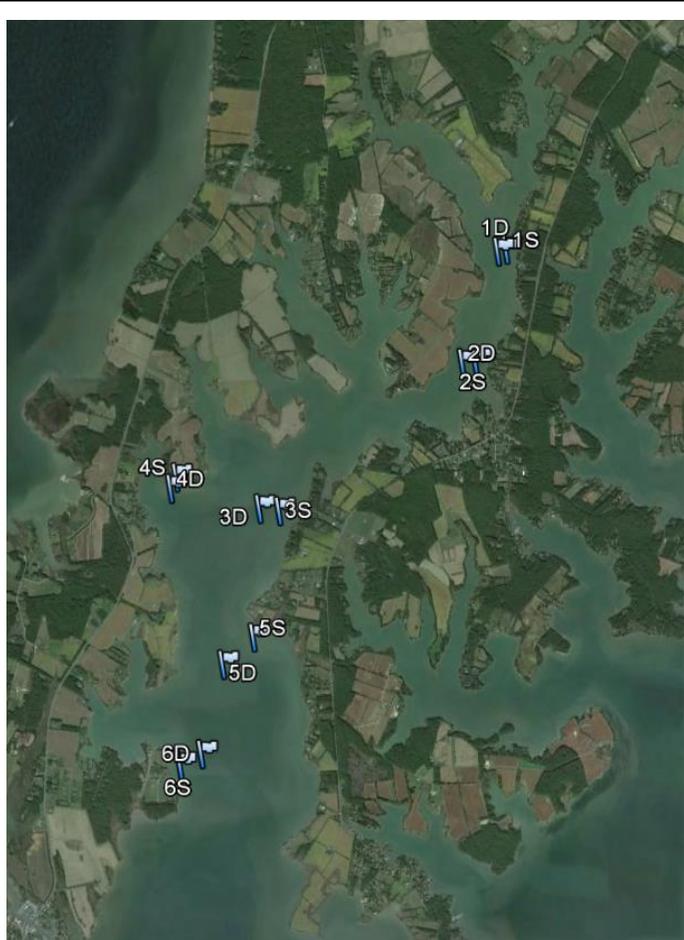


Figure 4. Location of sediment sites used for background rates of sediment nutrient processing.

Results and Discussion

The core incubation and oyster tray incubations were successful and in general provided data similar to that observed in Virginia experimental plots (Kellogg unpublished). The incubations had a large range of rates and oyster biomass (Table 1).

Table 1. Summary of all oyster reef and sediment measurements.

Measure	Unit	Mean	Std. Dev.	Std. Error	Maximum	Minimum	Median
Oyster Reef							
Dry Oyster Mass	g m ⁻²	229	214	33.9	703	0	177
Dark O ₂	μmol m ⁻² h ⁻¹	-11227	7649	1241	-1057	-26589	-8811
Dark N ₂ -N	μmol m ⁻² h ⁻¹	200	190	33.0	676	0	150
Dark NH ₄ ⁺	μmol m ⁻² h ⁻¹	957	1010	162	3460	-221	683
Dark NO _x ⁻	μmol m ⁻² h ⁻¹	429	510	88	1726	-146	218
Dark SRP	μmol m ⁻² h ⁻¹	13	30	5	117.9	-25	0
Light O ₂	μmol m ⁻² h ⁻¹	-9091	6643	1050	-84	-21354	-6538
Light N ₂ -N	μmol m ⁻² h ⁻¹	235	245	41	997	-51.6	162
Light NH ₄ ⁺	μmol m ⁻² h ⁻¹	774	966	157	3470	-592	474
Light NO _x ⁻	μmol m ⁻² h ⁻¹	416	585	99	2134	-212	227
Light SRP	μmol m ⁻² h ⁻¹	10	40	6	141	-76	0
Sediment							
Dark O ₂	μmol m ⁻² h ⁻¹	-1534	870	145	-383	-3560	-1483
Dark N ₂ -N	μmol m ⁻² h ⁻¹	54	54	9	298	-11	41
Dark NH ₄ ⁺	μmol m ⁻² h ⁻¹	87	118	20	527	-13	39
Dark NO _x ⁻	μmol m ⁻² h ⁻¹	12	58	10	234	-83	0
Dark SRP	μmol m ⁻² h ⁻¹	-9	14	2	0	-41	0
Light O ₂	μmol m ⁻² h ⁻¹	53	906	151	3406	-2231	-48
Light N ₂ -N	μmol m ⁻² h ⁻¹	14	44	8	123	--73	11
Light NH ₄ ⁺	μmol m ⁻² h ⁻¹	2	100	17	388	-307	0
Light NO _x ⁻	μmol m ⁻² h ⁻¹	9	58	10	179	-92	0
Light SRP	μmol m ⁻² h ⁻¹	-1	6	1	15	-20	0

Core Incubation Data

Sediment oxygen fluxes showed considerable variability over time, with median rates in the dark ranging from < -750 to $> -2000 \mu\text{mol m}^{-2} \text{h}^{-1}$ (Figure 5). Rates directed into the sediment are expressed at negative values, thus the higher rates show an uptake of $\sim 2,000 \mu\text{mol m}^{-2} \text{h}^{-1}$. Under illumination, rates of uptake decreased and in some instances oxygen fluxes were positive. Rates of benthic photosynthesis were relatively high, with median May and June 2015 rates in excess of $2,000 \mu\text{mol m}^{-2} \text{h}^{-1}$. The relatively shallow water conditions in Harris Creek allow sufficient light for photosynthesis at the sediment-water interface. Overall the dark uptake rates are similar to those observed in other Chesapeake Bay shallow environments (Boynton and Bailey 2008), with photosynthetic rates similar to those upstream in the Choptank River (Chick 2009).

Dark denitrification rates ($\text{N}_2\text{-N}$ flux) were generally low, partly a reflection of the competition for ammonium and nitrate between 1) nitrifying and denitrifying bacteria and 2) benthic algae. In general, rates decreased with illumination illustrating the effect of benthic algae on coupled nitrification-denitrification (Risgaard-Petersen 2003). Overall rates are similar to those observed in La Trappe Creek, a small tributary of the Choptank River (Holyoke 2008).

An examination of the combined N flux data from three dates showed that, in general, $\text{N}_2\text{-N}$ and NH_4^+ fluxes decreased in the light (Figure 6). Overall both dark and light NO_x^- median flux rates were very low (Table 1). Only dark $\text{N}_2\text{-N}$ and dark NH_4^+ fluxes were important contributors of nitrogen to the water column.

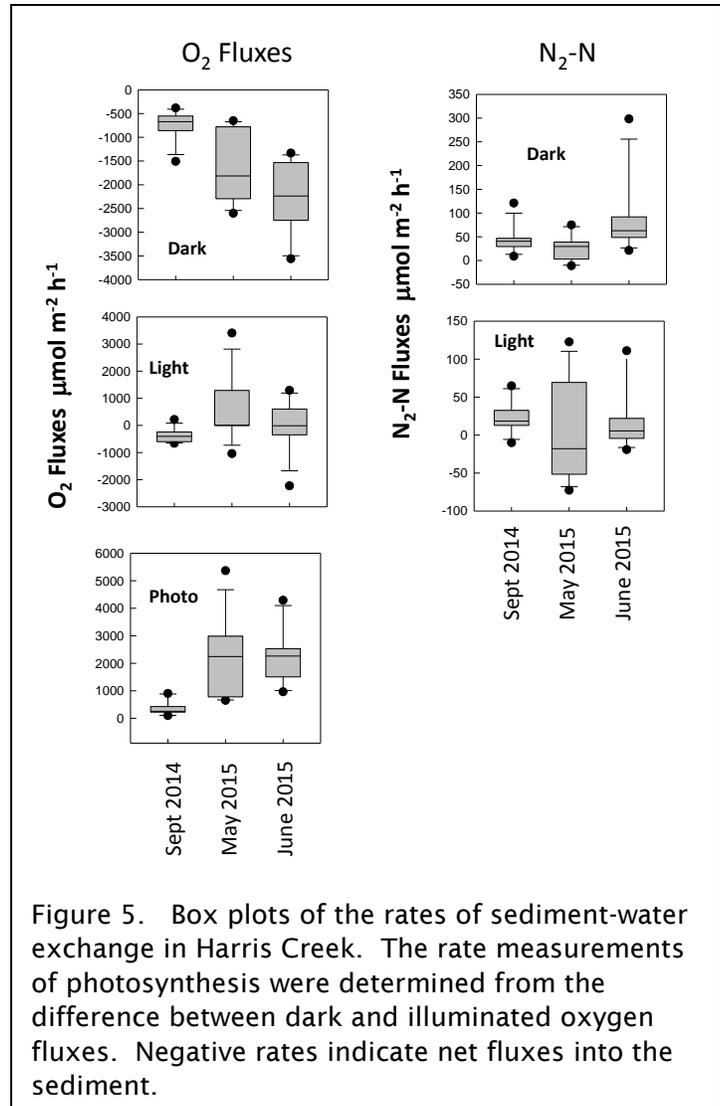
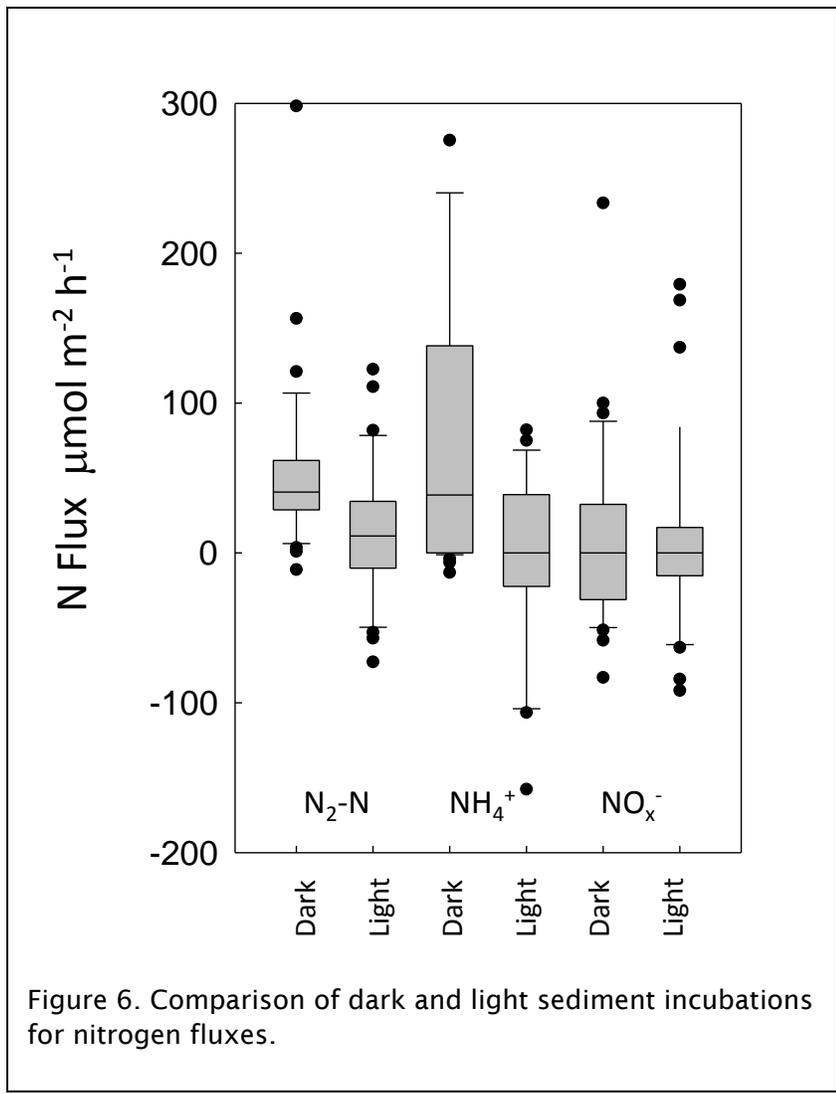


Figure 5. Box plots of the rates of sediment-water exchange in Harris Creek. The rate measurements of photosynthesis were determined from the difference between dark and illuminated oxygen fluxes. Negative rates indicate net fluxes into the sediment.



Oyster Reef Incubation Data

Denitrification rates in the oyster community were higher than those observed in the sediments (Figure 7), with warm season rates of $\sim 300 \mu\text{mol m}^{-2} \text{h}^{-1}$, with lower rates in late fall/early winter. On average, mean dark rates are ~ 4 times those of sediments and light rates are 17 times those of the sediments. These ratios compare well with the mean oyster community dark oxygen uptake rates which were 7 times those of sediments (Table 1). Overall the rates were lower than the highest rates observed in the upper Choptank (Kellogg et al. 2013), but similar to early spring and late fall rates from that site. The highest O_2 uptake rates at Harris Creek were $\sim 50\%$ of the highest rates from that study.

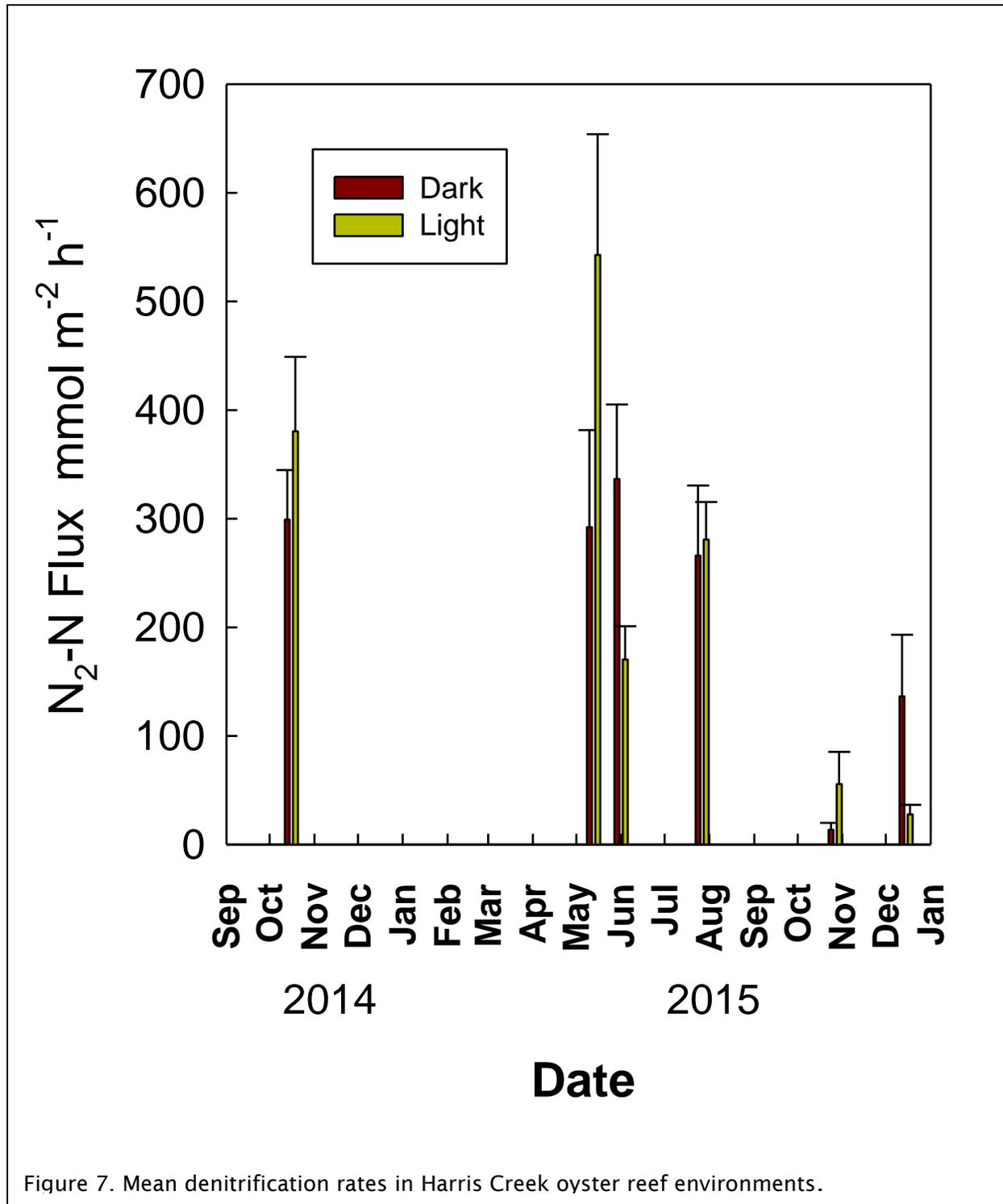
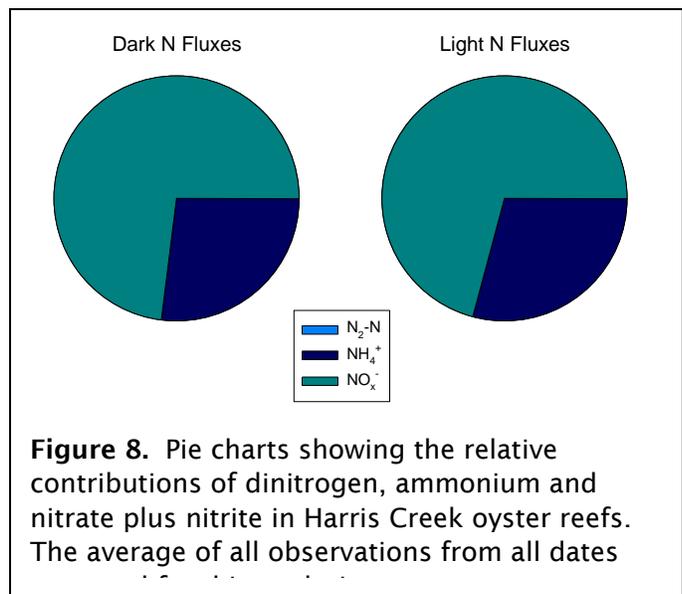
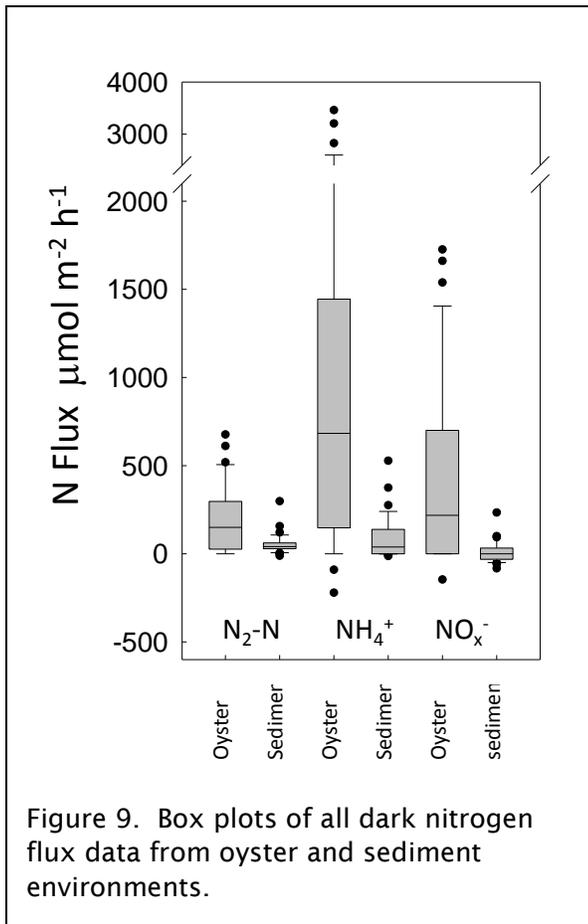


Figure 7. Mean denitrification rates in Harris Creek oyster reef environments.

The remineralization of organic nitrogen from biodeposits results in the production of ammonium, which can be microbially transformed to N_2 or NO_x^- . An examination of the mean proportions of N efflux shows that ammonium is the dominant nitrogen form leaving the oyster community (Figure 8). The flux of N_2 -N is a relatively small part of the overall balance, averaging 14% and 16% of the total inorganic N flux for dark and light incubations respectively. The proportion of N_2 -N flux in the upper Choptank ranged from 15-25%, somewhat higher than in



Harris Creek (Kellogg et al. 2013).



A much higher rate of nitrogen processing occurs in Harris Creek oyster reefs relative to Harris Creek subtidal sediments (Figure 9). These results mirror those from the Kellogg et al. (2013) study. The box plots (Fig. 9) reinforce the observation that in oyster reefs the efflux of ammonium is a dominant biogeochemical feature. The efflux of nitrate plus nitrite (NO_x^-) from the reef environments is also very high, particularly relative to the sediment environments.

A major difference between the Harris Creek study and our previous work in the upper Choptank River is a much shallower water depth and the presence of light at the sediment surface. Benthic photosynthesis was likely the dominant mode of algal photosynthesis prior to the eutrophication of the Chesapeake Bay (Cooper and Brush 1993, Cooper 1995) and in the Choptank River may be the source of ~10% of organic production (Chick 2009). Rates of algal photosynthesis in Harris Creek sediments and Harris Creek oyster reefs are similar (Figure

10), with rates of ~2,000 $mmol\ m^{-2}\ h^{-1}$ of oxygen production. Oyster filtration of phytoplankton may well enhance light penetration in Harris Creek and help restore a better balance between benthic and water column algal production.

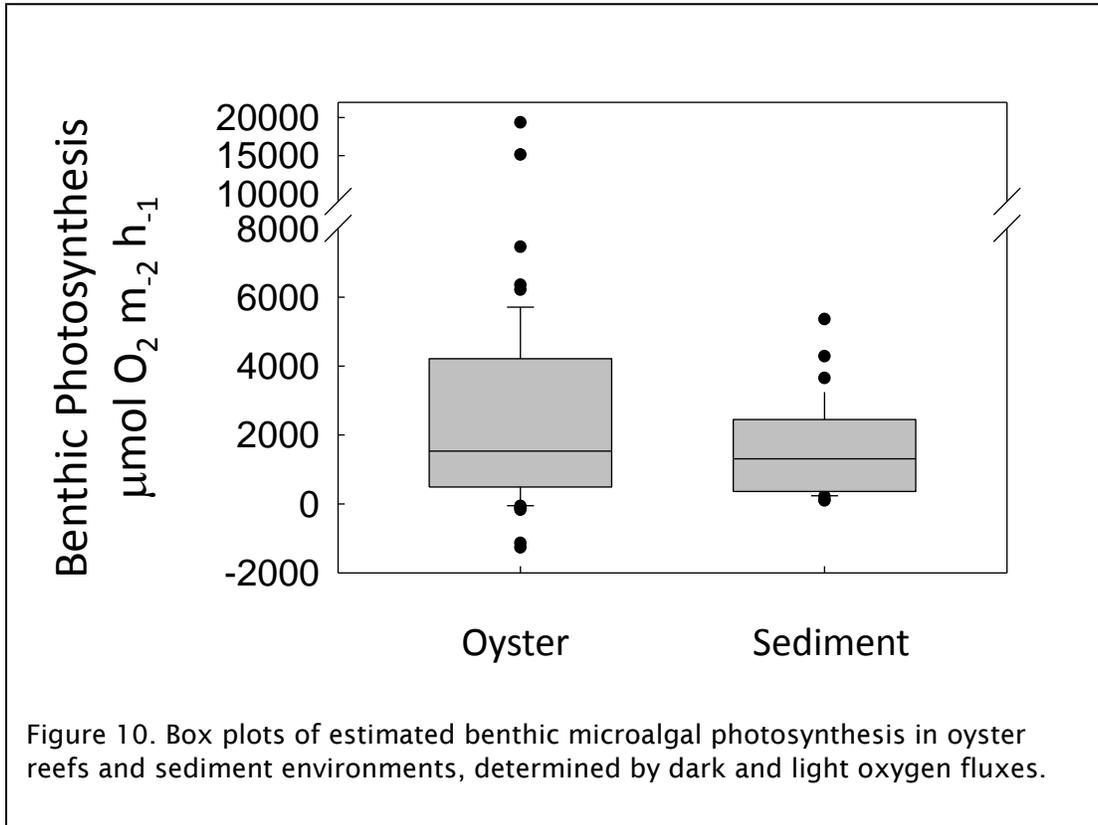
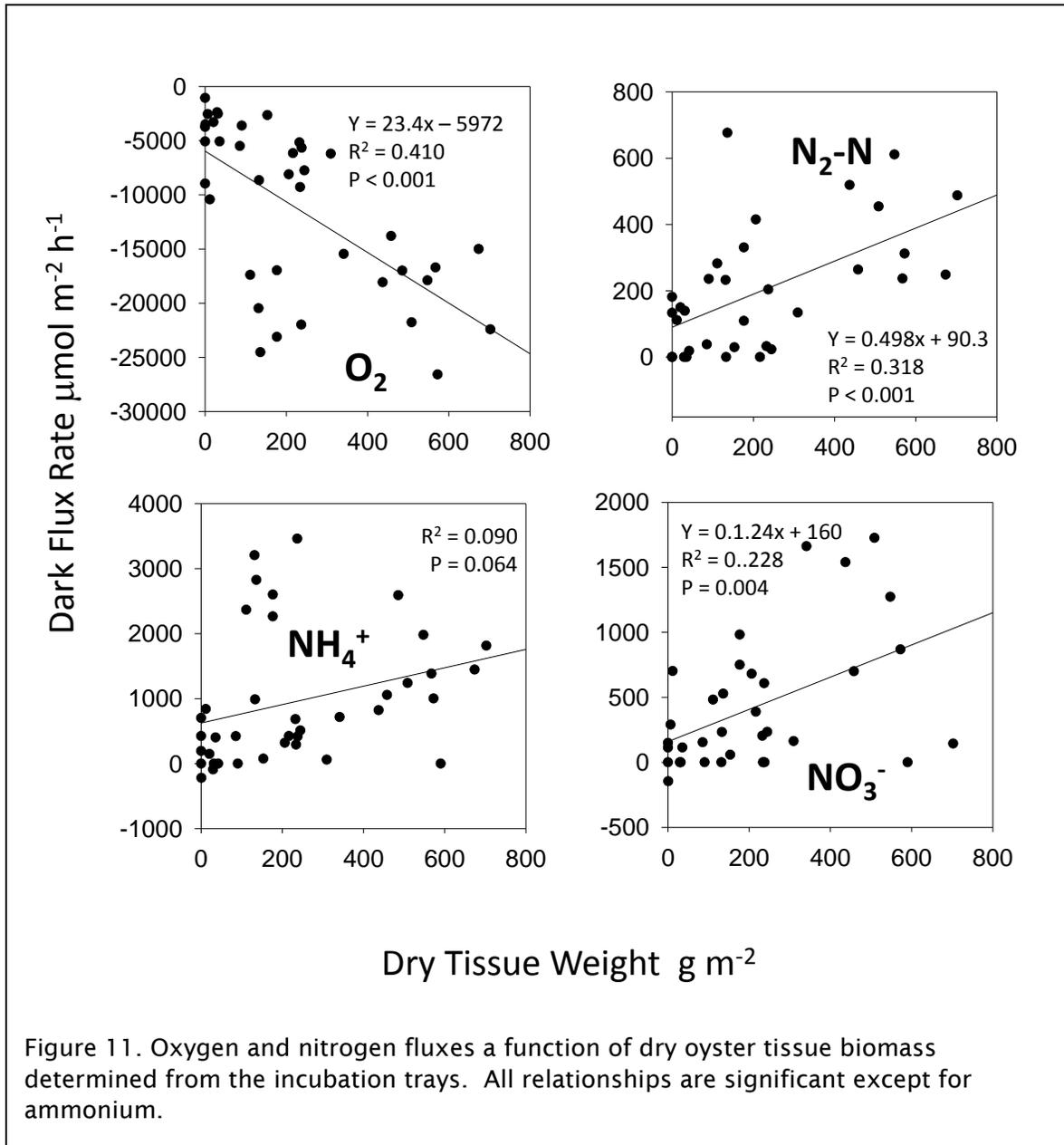
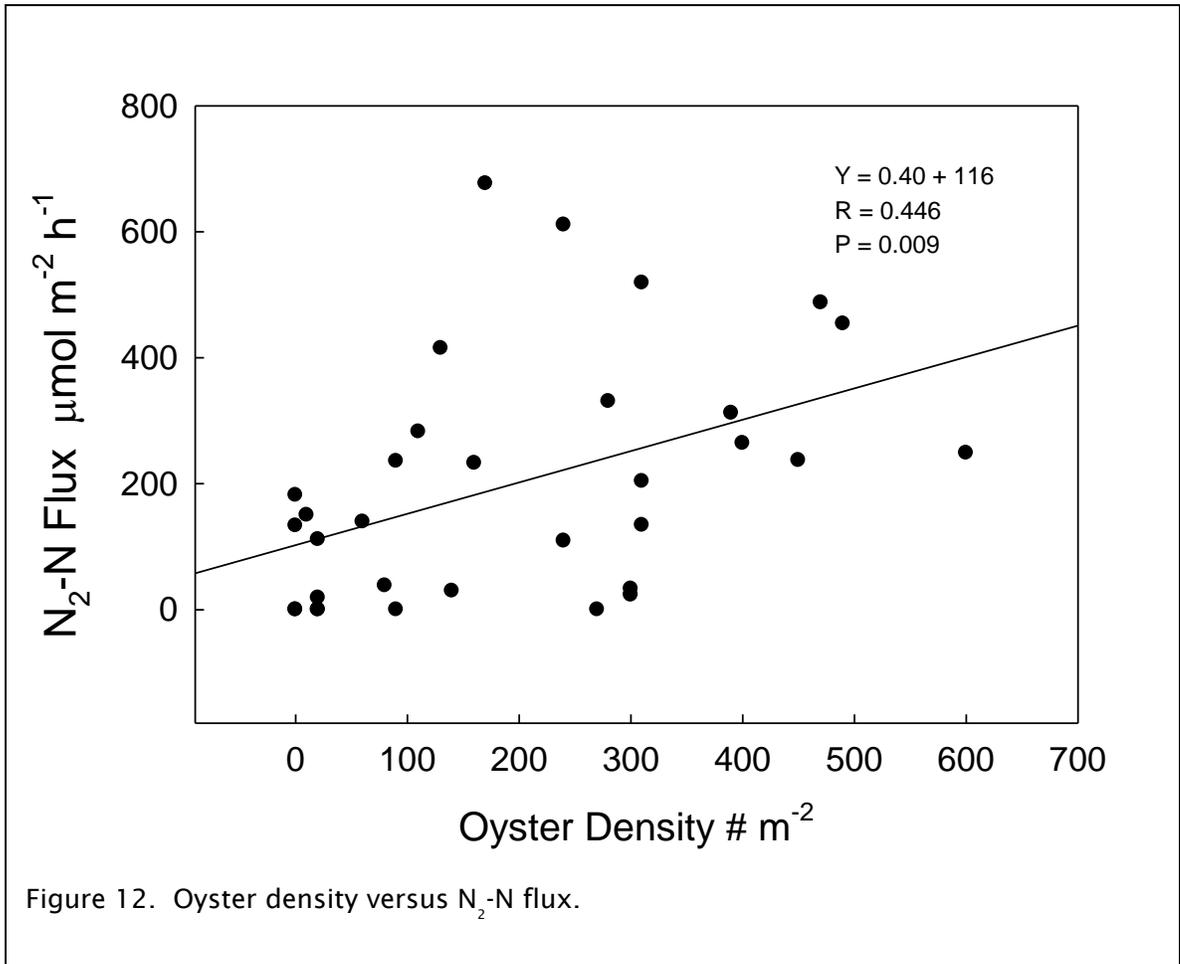


Figure 10. Box plots of estimated benthic microalgal photosynthesis in oyster reefs and sediment environments, determined by dark and light oxygen fluxes.

From the perspective of using oyster restoration for nutrient-related ecosystem services, it would be valuable to be able to assign a water quality value to oyster biomass or numbers. Oyster filtration fuels the metabolism of oxygen and nitrogen in an oyster reef and one might expect biomass might be a reasonable predictor. For oxygen, $\text{N}_2\text{-N}$ and NOx- , dry tissue weight on an areal basis provides a highly significant relationship (Figure 11), with a much poorer relationship between tissue weight and ammonium. These regressions are for all seasonal data and further examination of seasonal difference may provide more insight. An even better relationship would be from oyster numbers to denitrification; Figure 12 shows a significant relationship. These data suggest that it may be possible to assign denitrification numbers based on population. More data is needed over a longer period of time to confirm this.





Examples of the work of our Sea Grant interns is shown in Figures 13 and 14. The senior project of Ms. Anna McClain was designed to describe the CO₂ system in our incubations, particularly the flux of alkalinity. While alkalinity fluxes are not amenable to our short-term incubations, a strong relationship was found between dissolved inorganic and oxygen fluxes. The work of Zach Nickerson (Figure 14) showed a clear production of N₂ from oyster shell, suggesting that macrofaunal and microbial communities in the shell may be an important part of oyster reef denitrification.

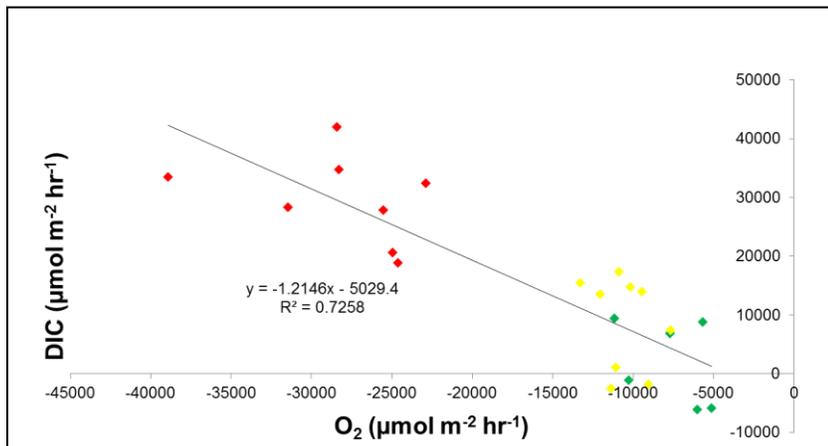


Figure 13. Oxygen versus DIC flux relationships in Harris Creek Oyster Sanctuary reefs, from the REU project of Anna McClain. This work also constituted her senior project at St. Mary’s College (MD).

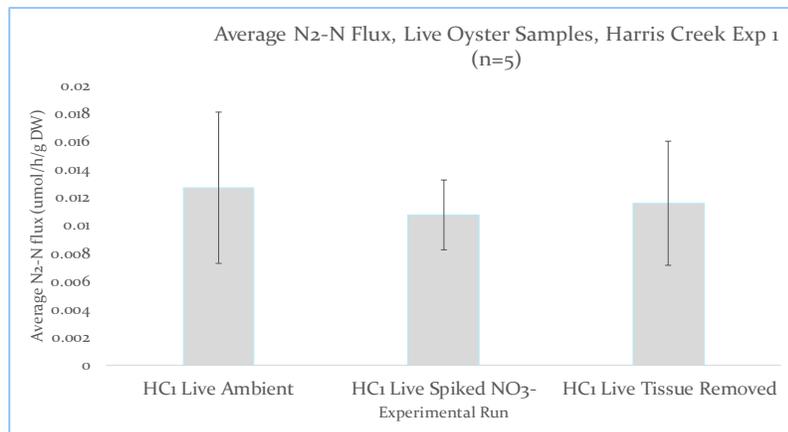


Figure 14. Rates of N₂ production from live oysters and oysters with the living tissue excised. These experiments were the REU project of Zach Nickerson and point toward shell, not sediment, as the loci of denitrification.

Summary and Conclusions

The first full year of observation of biogeochemical processes in Harris Creek have yielded a number of useful observations and have provided insights into this still-developing ecosystem. The key take home points are:

- The Harris Creek oyster sanctuary is a work in progress. Our main point of contrast is the work of Kellogg et al. (2013) in the upper Choptank
- A key feature of this restoration is the shallow nature of the oyster reefs. They are shallow enough that benthic photosynthesis can occur and having light at the reef surface may have a large biogeochemical impact (Newell et al. 2005).
- Our experimental data suggest that sediments are not the only source of denitrification and that oyster shell may harbor the communities that carry this process out.
- Denitrification rates in oyster reefs are greatly enhanced over Harris Creek sediments.
- Understanding the controls of the balance between ammonium, nitrate and di-nitrogen fluxes has large implications for the water quality value of oyster reefs. In order to apply our knowledge of N cycling processes to other reefs and ecosystems, we need develop a basic knowledge of the environmental controls of the balance of N cycling processes.

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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:

Dissemination

Preliminary results of these studies have been presented at the following national and international meetings:

Nickerson, Z.L., J.C. Cornwell and M. S. Owens. (2015). Locating the source of denitrification in a restored oyster reef. ASLO 2015 – Granada.

Cornwell, J.C. and M.S. Owens. Environmental controls on sediment-water exchange of nutrients. ASLO 2015 – Granada

Kellogg ML, Paynter KT, Cornwell JC, Ross PG, Owens MS, Handschy AV, Dreyer JC, Luckenbach MW (2014) Integrated assessment of oyster reef ecosystem services: Harris Creek, MD. 16th International Conference on Shellfish Restoration, Charleston, SC

Cornwell JC, Owens MS, Kellogg ML (2015) Feedback processes in Chesapeake Bay: did oysters matter in the past, do they matter now? Coastal and Estuarine Research Federation's 23rd Biennial Conference, Portland, Oregon

McClain A, Cornwell JC, Owens MS, Kellogg ML (2015) Carbonate Chemistry in Experiment Incubations of Restored Chesapeake Bay Oyster Communities. Coastal and Estuarine Research Federation's 23rd Biennial Conference, Portland, Oregon

Kellogg ML (2015) Measuring the benefits of oyster reef restoration: Quantifying denitrification rates and other ecosystem services. NC State Center for Marine Sciences and Technology, Morehead City, NC

Kellogg ML, Cornwell JC, Owens MS, Ross PG, Dreyer JC, Paynter KT, Luckenbach MW (2015) Integrated assessment of ecosystem services provided by tributary-scale oyster reef restoration in Chesapeake Bay. Coastal and Estuarine Research Federation's 23rd Biennial Conference, Portland, Oregon

Cornwell JC, Owens MS, Kellogg ML, Gao, Y (2015) Chesapeake Bay sediment nitrogen cycling: oysters, anoxia, and cyanobacteria. Seminar, Virginia Commonwealth University, Richmond, VA

Cornwell, JC Chesapeake Bay sediment nitrogen cycling: oysters anoxia and cyanobacteria. Seminar, Virginia Institute of Marine Science, Gloucester Point, VA

Cornwell, J.C. (2015). Influence of Chesapeake Bay sediment and oyster communities on Chesapeake Bay nitrogen cycling. Seminar, Salisbury University, Salisbury, MD

Cornwell JC, Owens MS, Kellogg ML (2016) Integrated assessment of oyster reef ecosystem services: Quantifying denitrification rates and nutrient fluxes. NOAA Chesapeake Bay Office Principal Investigator Meeting, Fredericksburg, MD

Collaborative Activities

Oyster BMP Expert Panel: Cornwell and Kellogg, Cornwell – Chair

NSF Coastal SEES – Oyster Futures. NSF-funded project head by Elizabeth North, seeks common ground on Chesapeake Bay oysters management. Cornwell part includes basic research on denitrification. First stakeholder workshop February 26-27

NOAA Ocean Acidification – new project lead by Jeremy Testa (CBL), with PI's at Horn Point (Kemp, Li), Oregon State University (Waldbusser), and University of Delaware (Cai)

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