Review of SMAST Report

Context of review

The following is a review of the methods used for experiments and the analyses of resulting data provided in the SMAST report to the Town of Orleans entitled "Lonnies Pond Shellfish Demonstration Project Year 1 Monitoring Summer/Fall 2016 Oyster Deployment" and responses to questions submitted to the same group. Instances in which details are drawn from the AECOM Memorandum to George Meservey and Michael Domenic dated 02/16/17 are cited as "AECOM". It is understood that the work completed thus far was a pilot study which was conducted with the intent of collecting the data needed to inform a broader scale assessment of nitrogen dynamics in Lonnie's Pond. It is further understood that the SMAST group is adapting their experimental design based upon the results of Year 1 data. This review was conducted without knowledge of budgetary constraints upon Year 2 sampling and it is recognized that it is likely not feasible to incorporate all suggestions. However, it is hoped that at least some of these suggestions can be incorporated into future sampling plans to better estimate the potential for nitrogen removal associated with oysters in Lonnie's Pond.

The following review was conducted with the intended use of the resulting data constantly in mind. An underlying assumption of the use of oysters to meet TMDL requirements is that consistent relationships can be found between a measurable oyster metric and nitrogen removal. For nitrogen removal associated with oyster harvest, the amount of nitrogen removed involves fairly straightforward measurements that estimate the biomass of oysters removed and the nutrient content of those oysters. However, these measurements do not include all of the potential effects of oysters on nitrogen cycling. As noted in the report, oysters filter phytoplankton and other particulates from the water column and produce biodeposits that settle and alter nitrogen cycling in underlying sediments. Oyster biodeposition can have positive or negative impacts on total nitrogen removal. In systems where denitrification in the sediments (defined for this review as the net flux of nitrogen gas from the sediments to the water column) is limited by the supply organic material to the bottom, oysters can significantly enhance denitrification. In contrast, if the sediments receiving the biodeposits are already anoxic or if they are driven towards anoxic or hypoxic conditions by the addition of biodeposits, oysters may have either no effect on denitrification rates or negative effects. Thus, the net effect of adding oysters to the system will be the sum of the nitrogen removed by harvest, which is always a positive number, and the net effect on denitrification, which may be a positive or a negative number. Although burial of nitrogen in the sediments is a third factor that should be considered, very few if any reliable numbers for this have ever been collected and, for the purposes of the present review, burial is assumed to be negligible.

Before the impacts of oysters on sediment nitrogen cycling can be incorporated into efforts to meet TMDL requirements, it is important that the data collected can be used to develop predictions of nitrogen removal that are reliable either in their mean, median or minimum value. If mean values are used, the variance associated with the mean needs to be relatively low. Median values are less susceptible to outliers and are more useful in some situations. In situations where there is consistently high variance, then minimum values may be most appropriate.

In addition to collecting quality data, it is important that the oyster practices and locations used be as representative as possible of the practices that will be used once crediting is approved. Of particular concern is the scale of the oyster addition both in total area and in the biomass of oysters per unit area.

Review of Study Design and Methods Used to Measure Nitrogen Fluxes

As noted by the SMAST group, the Year 1 study was a pilot study conducted with the goal of informing Year 2 measurements. The design used was appropriate for gaining a better understanding of the system in terms of the scale and variance of nitrogen and oxygen fluxes and defining the area of sediments likely influenced by oysters.

Collection of sediment cores followed by "batch-style" incubations in the laboratory is currently the best available method for measuring net denitrification rates. This method preserves the structure of the sediments the cores, a key requirement for collecting data that represent field conditions. Below is a list of suggestions for changes to the current methods and/or for additional data analyses that could suggest additional changes to methods are needed.

1) The first flux measurements were collected after oysters were deployed. As the SMAST group notes, more data are needed to determine the rates of nitrogen fluxes in the absence of oysters. A standard approach for these types of studies is to use a Before-After-Control-Impact (BACI) design. This type of study design is particularly well suited to studies of relatively large-scale impacts that cannot be replicated due to logistic, financial, or moral constraints. Use of this type of design in Year 2 would allow direct assessment of fluxes before and after the installation of oysters. If the impacts of oyster biodeposition are likely to persist into the coming field season as suggested by the SMAST group, then a new site should be selected and utilized for these studies.
2) The AECOM memo states that "A key constraint in the operation and maintenance routine was meeting the requirement of no disturbance to the bottom sediment in order for SMAST to accurately determine denitrification rates". This practice and the resulting denitrification measurements should be considered in the context of likely scenarios for the maintenance of the final project. If there are no scenarios in which maintenance would be achieved without disturbance of the sediments, then denitrification rates should be measured in the presence of this disturbance. If it is unclear whether there are any scenarios in which maintenance of the final project would be conducted without disturbing sediments, then both maintenance regimes (i.e. with and without sediment disturbance) should be incorporated into the study design.
3) The SMAST group notes that all samples taken in Year 1 were likely within the area of impact of oyster biodeposition. Their plan to expand the sampling area is well-founded. Again, use of a BACI design is recommended.

4) Based on the data provided in the report and in response to questions, it is unclear the extent to which laboratory incubations replicate field conditions. The following are some considerations that should be taken into account in Year 2 studies:
   a) All incubations were conducted in the dark. Based on light data provided in Table IV.1 of the report and upon additional information from SMAST that the water depth across the site as low tide ranges from 0 to 1.3 m, it is necessary to consider the potential impact of light on nitrogen cycling. Incubations should be run under light and dark conditions. Laboratory light levels supplied during incubations should be measured to demonstrate that sufficient light was supplied to approximate field conditions. Light incubations are needed because it is possible for benthic algae to outcompete denitrifiers for available nitrate thereby reducing rates of denitrification. Alternately, denitrification could increase in the light if sediment nitrification is enhanced by benthic oxygen production.
   b) Oxygen concentrations as well as fluxes need to be explicitly accounted for in the field and in the laboratory and sampling adjusted appropriately. Most studies of denitrification associated with oysters to date have studied field sites where bottom waters and pore water in surface sediments do not approach hypoxic or anoxic conditions. Standard procedures for these studies are to raise oxygen concentrations in the overlying water column in the cores to saturation prior to the start of incubation and then run those incubations until oxygen levels reach a preset percentage of saturation (usually somewhere in the range of 50-80% saturation). Fluxes are then calculated using a linear regression of concentration against time. When the fit of the regression lines has an R^2 of greater than 70-80%, the calculated flux is generally considered to be a reliable estimate. Cores for which data and associated regression do not meet these criteria are generally rejected. As oxygen concentration approach hypoxic conditions, nitrogen cycling rates tend to change and fitting linear regression of concentration versus time can be inappropriate. However, these standard procedures may be inappropriate for replicating conditions in Lonnie's Pond. The following adjustments to sampling should be considered:
      i) Data from sondes deployed in Lonnie's Pond in August suggest that conditions frequently approach hypoxic and/or anoxic conditions. In Year 2, sondes should be placed as close as possible to the sediment surface to collect field data on oxygen concentrations for use in extrapolating laboratory measurements.
      ii) Respiration rates in many of the cores collected in August were very high. The oxygen concentrations in water samples collected from cores in Year 1 should be closely examined to determine whether conditions in the cores ever approached hypoxic or anoxic conditions. If oxygen concentration in cores dropped below 50% saturation, data should be closely re-examined to see if it looks like nitrogen fluxes changed at any point during the time course. Denitrification rates are generally expected to decrease once oxygen levels are below ~2 mg L^{-1}. No indication was given as to whether oxygen concentrations in cores were measured in real time during incubations. Incorporation of oxygen probes into cores can be extremely helpful in determining the appropriate time intervals between samples. Because of the field conditions in Lonnie's pond, it is likely desirable to collect samples a minimum of

four times before oxygen saturation reaches 50% to calculate fluxes for oxic conditions. Additional samples should be collected to estimate fluxes as oxygen concentrations approach the lowest levels recorded in the pond. Close examination of the data from Year 1 should allow determination of whether there is a single change in slope and thus only four additional time points are needed or whether there are multiple changes in slope and more points are needed. Once Year 2 data have been collected, the sonde data should be used to appropriately extrapolate laboratory flux rates based on measured oxygen conditions in the pond.

c) Comments on details of incubation procedures
   i) Using filtered water can help to eliminate the need to correct for the water column but in some cases it can break up cells and actually make water column respiration higher. If filtered water is used again in Year 2, data demonstrating negligible respiration are needed to address this potential concern.
   ii) Use of HCl to reduce pH can cause degassing of the samples and is not generally considered an appropriate method for preserving samples for N_2/Ar analyses. Preservation using 50% saturated $HgCl_2$ is one appropriate method for preservation. Zinc chloride has also been suggested as an appropriate preservative.

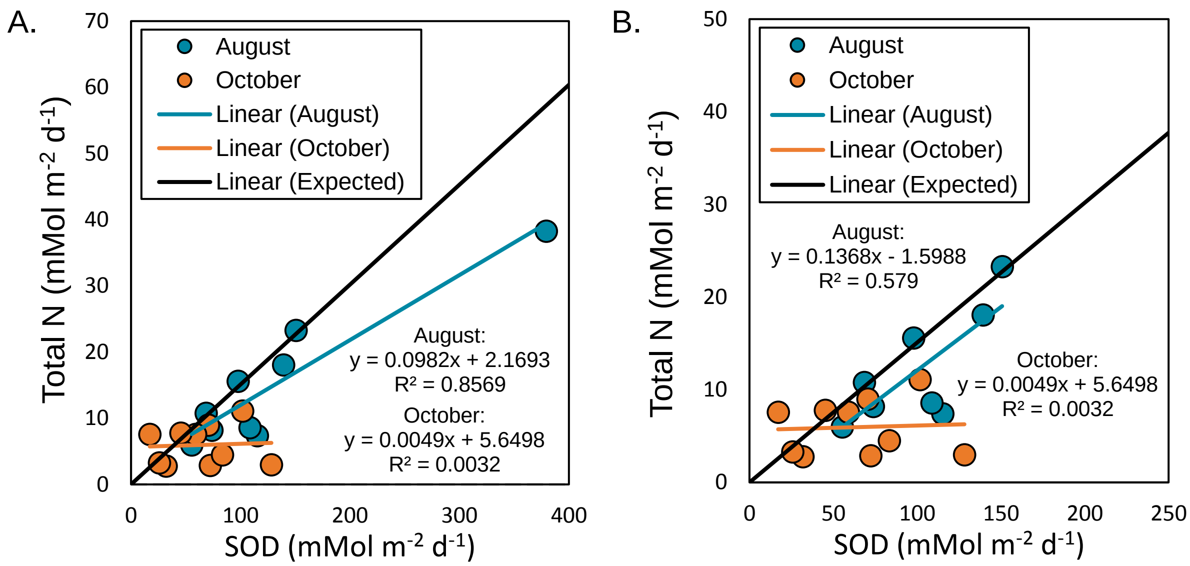
Review of Data and Interpretation

It is recognized that the data collected thus far were collected as part of a pilot study. As such, replication is limited which complicates interpretation and analyses of results. The review below is intended to provide insights into what these data may say about the potential for oysters to enhance denitrification in the system and, hopefully, to help guide Year 2 sampling plans to address any potential issues identified.

Relationship between SOD and Total N:

Interpretation of the data collected must be consistent with its intended use. One of the primary goals of this project is to determine the potential use of oysters to remove nitrogen from Lonnie's Pond. Underlying the experimental design and interpretation is the assumption that oyster biodeposition increases the amount of nitrogen remineralized in the sediments and that these rates of remineralization are accurately reflected in flux measurements. This assumption can easily be checked by comparing sediment oxygen demand (SOD) to the sum of the nitrogen fluxes (Total N). If biodeposits derived from filtration of phytoplankton are the primary source of organic material and those biodeposits decompose under oxic conditions, then a ratio of SOD to Total N of approximately 6.625 is expected. Data that deviate significantly from this ratio suggest that the nitrogen cycling in the system is poorly described by the fluxes measured and there are a likely other processes that are important to nitrogen cycling in the system.

When SOD and Total N fluxes from core incubations by the SMAST group (Table VII.1. in the SMAST report) are graphed (Fig. 1A), data from August show a reasonable approximation of the expected ratio. If the one data point with exceptionally high respiration rates is removed from analyses, the agreement with the expected ratio improves further (Figure 1B). Thus, in August,



the benthic decomposition of phytoplankton-derived material appears to account for the majority of nitrogen-related processes. The same is not true in October (Fig. 1). For these samples, there appears to be no relationship between SOD and Total N. Points falling far below the "expected"

Figure 1. Comparison of sediment oxygen demand (SOD) to total nitrogen flux (Total N) for all data (A) and for the same data without the August point with the highest SOD (B). The black line is the expected ratio of SOD to Total N of 6.625. Data are taken from Table VII.1 of the SMAST report.

line suggest that there is nitrogen "missing" from these flux measurements. Additional data are needed to determine the fate of the missing nitrogen. Possible explanations include uptake of nitrogen by benthic algae. In the absence of additional information about the mechanism of this loss, data from these cores should be used with caution.

Relationship between SOD and N_2-N Production:

Some previous studies have suggested that sediment oxygen demand could be used as a proxy for denitrification rates associated with oyster reefs or oyster aquaculture (e.g. Humphries et al. 2016) and relationships identified to date have been linear and positive. However, other studies (e.g. Murphy et al. 2016) have demonstrated that excessive loading with organic matter like biodeposits can ultimately lead to anoxic conditions that shut down denitrification. Thus, it is important to understand the relationship between SOD and nitrogen gas fluxes (N_2-N), the variance in that relationship, and the circumstances under which there is no longer a linear relationship between SOD and N_2-N production. Extrapolation of N_2-N fluxes from SOD would require identifying a tight and consistent relationship between these measures.

When SOD and N_2-N fluxes from core incubations by the SMAST group (Table VII.1. in the SMAST report) are graphed (Fig. 2A), data from August and October show little or no correlation between sediment oxygen demand and fluxes of N_2-N. If the one data point with exceptionally high respiration rates is removed from analyses, the relationship between SOD and N_2-N flux in August improves but is still relatively weak (Figure 2B). As the group from SMAST notes, these data suggest that a much more extensive sampling program would be required to understand whether a reliable relationship between SOD and N_2-N fluxes can be found. Preliminary measurements suggest that variance could be high enough that substitution of measurements of SOD for N_2-N flux may not be a viable option. The high variance in the N_2-N dataset for similar SOD values suggests that a conservative approach which develops a minimum estimate of N_2-N flux rather than a mean or median may be appropriate.

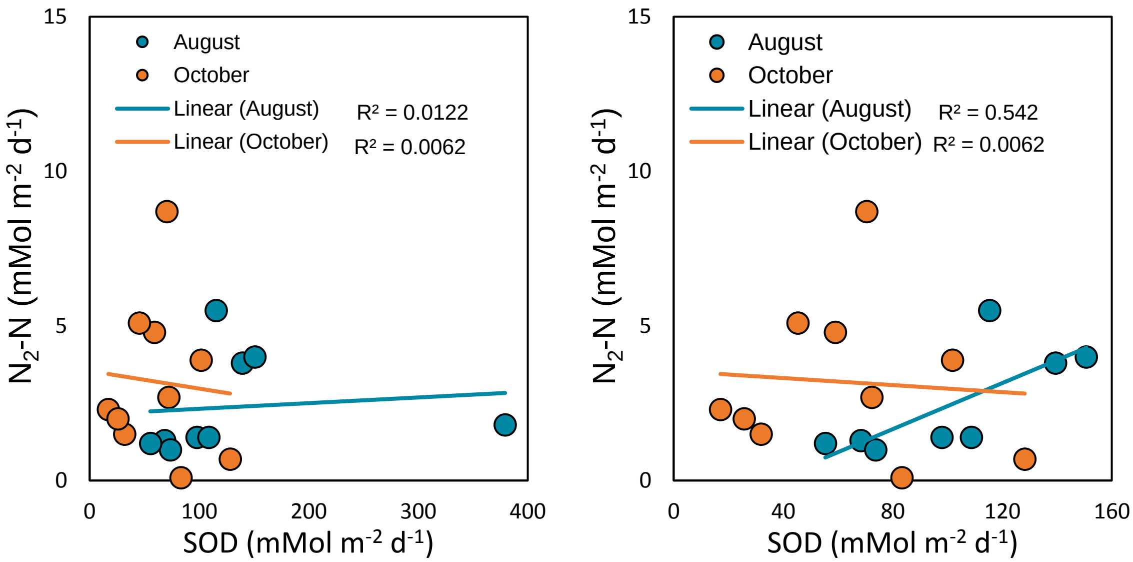


Figure 2. Comparison of sediment oxygen demand to flux of N_2-N.

Fluxes Inside and Outside Oyster Areas:

As noted by the SMAST group, pilot studies collected limited data that may not be entirely representative of the impacts of oysters on nitrogen removal in Lonnie's Pond. In particular, they note that the areas sampled outside the oyster impact area may have in fact been enriched with biodeposits and that samples should be collected farther away in future. Data are insufficient to run statistical analyses and determine if patterns are significant at this time due to low sample numbers. Keeping these limitations in mind, data collected to date suggest that the effect of oysters on sediments may change with season (Fig. 3). In both August and October, patterns in SOD and ammonium fluxes (NH_4) match expectations with higher values at the oyster sites than at the non-oyster sites within season and with higher values in August than in October. Patterns in nitrate (NO_3) and N_2-N fluxes are not as consistent with expectations. NO_3 fluxes are highly variable and suggest net uptake from the water column in October. Patterns in N_2-N fluxes suggest that the oyster sites may have lower denitrification rates than the non-oyster sites in August but higher denitrification rates in October.

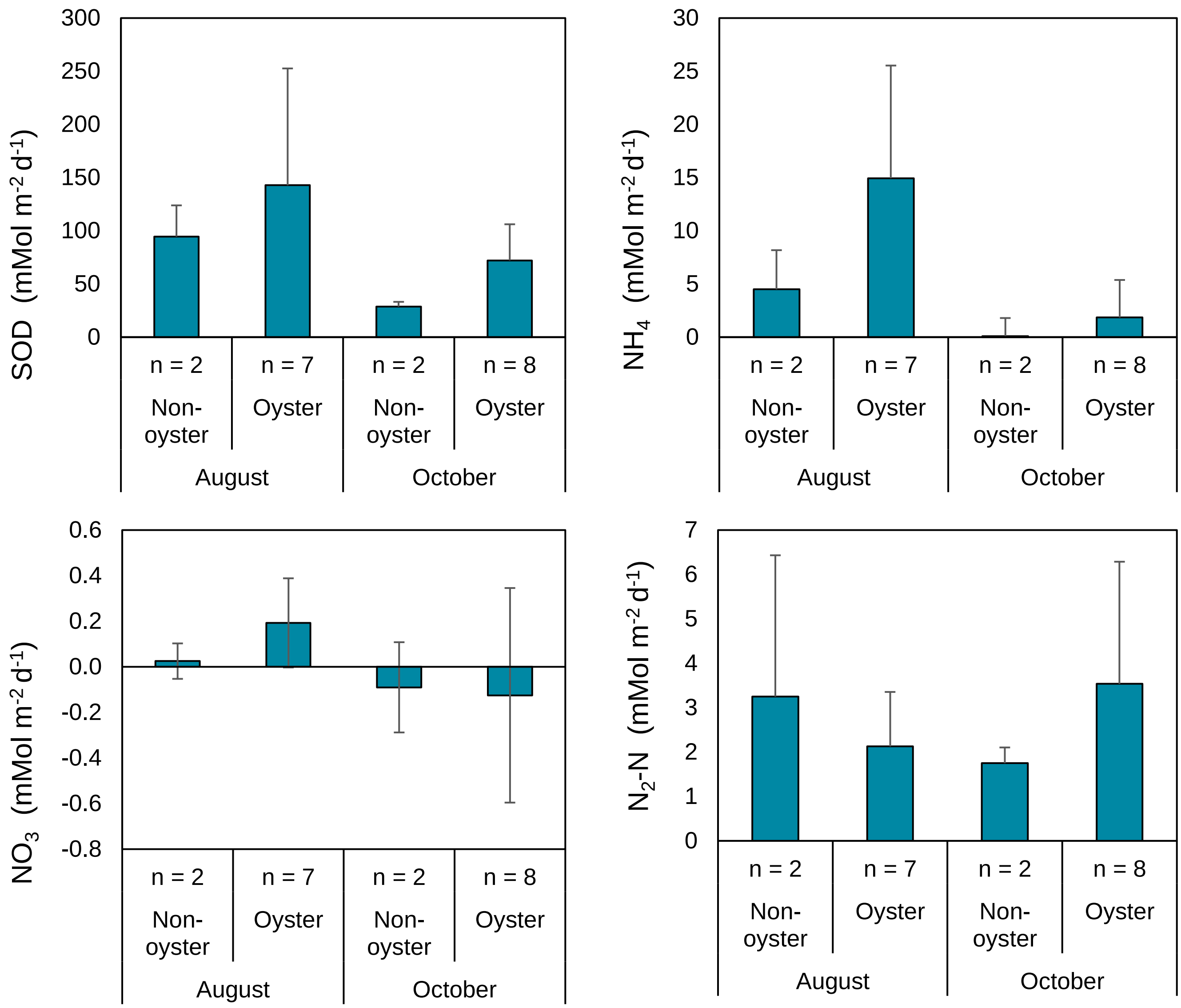


Figure 3. Average fluxes measured in areas farthest from the oyster site compared to areas impacted by oysters and/or oyster biodeposits based upon the map given in Figure VI.9. of the SMAST report. Error bars represent standard deviation and n indicates the number of cores included in analyses.

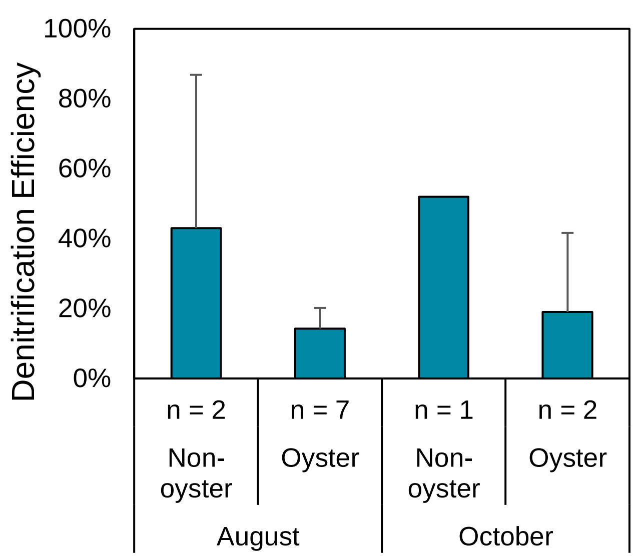
Because measured denitrification rates are available, they are the most appropriate means for estimating the net effect of oysters on denitrification in this system. This can be calculated by subtracting the mean at the non-oyster site from the mean at the oyster site within each season. Mean rates of denitrification in August were 2.13 mMol m^{-2} d^{-1} in the oyster areas and 3.25 mMol m^{-2} d^{-1} in the non-oyster areas. Thus, on average, denitrification in the oyster areas was reduced by 1.12 mMol m^{-2} d^{-1}. In October, mean denitrification rates were 3.54 mMol m^{-2} d^{-1} in the oyster areas and 1.75 mMol m^{-2} d^{-1} in the non-oyster areas yielding an increase in denitrification in the oyster areas of 1.79 mMol m^{-2} d^{-1}. In considering the net effect of oysters on nitrogen cycling in the system, it is important to remember that any negative impacts on denitrification can potentially be offset by nitrogen assimilated into the oysters. Thus, understanding of the net effect of oysters on the system at any point in time will require adding the effects of assimilation of nitrogen into oysters to the effects of oysters, positive or negative, on denitrification.

Negative Fluxes and Denitrification Efficiencies:

In a simple system where oysters are the primary control on nitrogen cycling, fluxes of nitrogen are generally expected to be out of the sediments (i.e. positive values) rather than into the sediments from the water column (i.e. negative values). Negative values can occur in a number of situations including when benthic algae play a significant role in nitrogen cycling in the system. An additional complication of negative values is that they limit or negate the utility of calculating denitrification efficiency. If oysters are the primary control on nitrogen cycling, then denitrification efficiency (reported as a percent) can be calculated as the N_2-N flux divided by the sum of the NH_4, NO_x and N_2-N fluxes and multiplied by 100. The resulting value then represents the proportion of the total nitrogen remineralized that was converted to N_2 gas and removed from the system. However, fluxes from the water column into the sediments cannot be used to make simple, direct comparisons because some of the nitrogen cycled is not coming from the biodeposits.

The majority of fluxes in October included negative values. A number of processes could account for this, including luxury uptake of nitrogen by benthic algae. However, without additional data, no conclusions can be made at this time. Using only data from cores that have no uptake of nitrogen from the water column to compare denitrification efficiencies suggests that denitrification efficiency may be lower in areas with oysters than areas without oysters (Fig. 4).

Figure 4. Average denitrification efficiency in areas farthest from the oyster site compared to areas impacted by oysters and/or oyster biodeposits based upon the map given in Figure VI.9. of the SMAST report. Error bars represent standard deviation and n indicates the number of cores included in the analyses. All cores with negative fluxes of any form of nitrogen were excluded from these calculations.



Primary Recommendations for Year 2

Many recommendations have been made in the review above. Below is a summary of key recommendations for the coming year:

1) Use a BACI experimental design in Year 2. Care should be taken to insure that the control sites are sufficiently far away from the impact site that there is no effect of oysters on these sites. Sampling prior to the addition of oysters should demonstrate no significant differences between the control site and the site where oysters will be added.
2) Deploy a sonde in the center of the oyster area in a manner that allows collection of quality data on oxygen concentrations as close to the sediment surface as possible. Especially with increased oyster biomass in Year 2, it will be important to demonstrate that oyster biodeposition is not resulting in anoxic conditions on the bottom.
3) Incorporate light incubations and adjust incubation techniques per recommendations above. If at all possible, use oxygen probes to provide real-time data on oxygen concentrations in cores so that sampling points can be spaced appropriately.
4) Investigate factors that might explain “missing” nitrogen and account for them appropriately in Year 2 incubations.
5) Use only measured rates of denitrification to estimate impacts of oysters on nitrogen removal in Lonnie’s Pond.

Review of AECOM Report

Context of review

The following is a review of the AECOM report provided by Thomas Parece to George Meservey and Michael Domenica on February 16, 2017. This review covers only Section 8, Subsections C-E. It is understood that the calculations made were undertaken when the only data available to support those calculations were data from the pilot study by the SMAST group in Lonnie's Pond. It is further understood that the Year 2 flux sampling program will be more extensive than the Year 1 program and will provide additional data for estimating denitrification rates and other fluxes in Lonnie's Pond.

Review of Section 8.C.

This section starts with the sentence "It is believed that denitrification enhancement will be proportional to the amount of biodeposition". This statement implies a positive linear relationship between the amount of organic matter added to the sediments and denitrification. However, as the SMAST authors note, too much organic material can lead to anoxic conditions. Thus, although it is possible that biodeposition will increase denitrification up to a point, it is important to understand that eventually the sediments can become overloaded and denitrification rates will decline and ultimately reach zero. Thus, assuming that denitrification increases proportionally with biodeposition is not appropriate.

If we assume that there is a positive linear relationship between biodeposition and sediment oxygen demand, then we can examine the SMAST data for a significant positive relationship between SOD and denitrification (Fig. 2 in Review of SMAST Report). As noted above, a consistent positive linear relationship between SOD and N_2-N fluxes has not been documented for Lonnie's Pond. When SOD and N_2-N fluxes from core incubations by the SMAST group (Table VII.1. in the SMAST report) are graphed (Fig. 2A), data from August and October show little or no correlation between sediment oxygen demand and fluxes of N_2-N. If the one data point with exceptionally high respiration rates is removed from analyses, the relationship between SOD and N_2-N flux in August improves but is still relatively weak (Figure 2B). Thus, the data collected so far from Lonnie's Pond confirm that calculation of expected denitrification rates based upon models of biodeposition is inappropriate.

Review of Section 8.D.

According to the methods described in this section, "the amount of projected denitrification for a full-scale system was estimated to be the product of the function in Figure 35, the total amount of nitrogen taken out of the water by growth of tissue and shell and the enhanced denitrification factor of 67 percent." As noted above, data collected thus far do not suggest that there is a positive linear relationship between biodeposition and denitrification. Both for initial estimates using preliminary data and future estimates, removal of nitrogen via denitrification should be based measured fluxes of N_2-N in Lonnie's Pond.

Review of Section 8.E.

As noted above, the methods used to estimate nitrogen removal via denitrification in Lonnie's Pond are not appropriate. Because of this, all conclusions and calculations in this section that rely up those estimates are inaccurate and may significantly overestimate nitrogen removal. In the absence of consistent patterns of denitrification in relation to oyster biomass density, all estimates of nitrogen removal via denitrification should be based upon measured rates.