



The School for Marine Science and Technology

University of Massachusetts Dartmouth



Technical Report

DRAFT FINAL

Lonnies Pond Shellfish Demonstration Project Year 2 Monitoring Summer/Fall 2017 Oyster Deployment

To:

Town of Orleans, MA
Water Quality and Wastewater Planning

From:

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Section I. Introduction

Background:

Based on the findings of the Massachusetts Estuaries Project (MEP 2002 – 2017, conducted by SMAST-CSP), it is clear that estuarine water and habitat quality in southeastern Massachusetts estuaries is impaired by nitrogen enrichment. As a result, towns across southeastern Massachusetts are now seeking innovative approaches for lowering estuarine nitrogen levels as these natural systems are integral to the character and quality of life in communities across the southeastern Massachusetts region and citizens want to achieve the MEP set nitrogen thresholds for restoration of their estuarine resources. While traditional sewage treatment is part of the solution for most communities, so too are non-traditional approaches to nitrogen management that have multiple benefits to the community. However, the nitrogen removal efficiency of these non-traditional approaches is still being quantified so that they may be formally considered and credited with nitrogen removal in town specific nitrogen remediation plans.

An *in situ* water quality management approach that is gaining momentum in many communities across southeastern MA (e.g. Westport, Falmouth, Mashpee) is the use of shellfish, particularly oysters, to increase water clarity and remove nitrogen, while also supporting recreational shellfishing and the local economy. CSP has been at the forefront of investigating the use of oyster deployments/reefs as an in-estuary means to improve nitrogen related water quality. CSP scientists have begun quantifying the mass removal of water column nitrogen and quantifying the additional removal via associated enhancement of sediment denitrification ($\text{NO}_3 \rightarrow \text{N}_2$) to gauge the actual nitrogen removal efficiencies of oyster deployments. Note that oysters are being considered due to their high filtration rates, rapid growth, commercial value and ability to thrive in nutrient rich, warm, shallow waters over a range of estuarine salinities. While oysters are well suited to enhancing water quality for the listed reasons, other filter feeders can be used as well.

In this context, many Massachusetts communities have begun oyster propagation. However, almost none have quantified the integrated nitrogen removal through filtration, deposition and sediment denitrification and harvest. Similarly, water quality improvements associated with oyster deployments have not generally been accurately assessed. As a result, it is difficult to compare the cost/benefit of using shellfish compared to other nitrogen management approaches (e.g. traditional WW treatment, PRBs, floating wetlands, enhanced natural attenuation). In order for Orleans and other towns across the region to be able to implement this soft solution as part of their habitat restoration strategy, it is critical to accurately assess the role oyster filtration plays in estuarine nutrient cycling. This quantitative understanding of the net effect of oyster filtration on nutrient conditions in an estuary will facilitate incorporation of shellfish culture into management and regulatory processes.

The present investigation focuses on quantifying the nitrogen processing and removal by the oyster/sediment complex within Lonnie's Pond, a terminal salt pond tributary to Upper Pleasant

Bay, Orleans, MA. The pond was selected due to its high level of nitrogen enrichment, its physical structure, its suitability for oyster culture and appropriateness for measuring nitrogen removal rates. The results are aimed at providing quantitative information to the Town of Orleans as it seeks to implement new nitrogen removal approaches and considers the use of shellfish for remediation of nitrogen related impairment of its coastal resources.

The Nitrogen Loading Problem:

Surface and groundwater flows are pathways for the transfer of land-sourced nutrients to coastal waters. Fluxes of primary ecosystem structuring nutrients, nitrogen and phosphorus, differ significantly as a result of their hydrologic transport pathway (i.e. streams versus groundwater). In sandy glacial outwash aquifers, such as in the watershed to the greater Pleasant Bay system and Lonnie's Pond at a smaller scale, phosphorus is highly retained during groundwater transport as a result of sorption to aquifer minerals (Weiskel and Howes 1992). Since even Cape Cod "rivers" are primarily groundwater fed, watersheds tend to release little phosphorus to coastal waters. In contrast, nitrogen, primarily as plant available nitrate, is readily transported through oxygenated groundwater systems on Cape Cod (DeSimone and Howes 1996, Weiskel and Howes 1992, Smith et al. 1991). The result is that terrestrial inputs to coastal waters tend to be higher in plant available nitrogen than phosphorus (relative to plant growth requirements). However, coastal estuaries tend to have algal growth limited by nitrogen availability, due to their flooding with low nitrogen coastal waters (Ryther and Dunstan 1971). Tidal reaches within Pleasant Bay and Lonnie's Pond more specifically, follow this general pattern, where the primary nutrient of eutrophication is nitrogen.

Nutrient related water quality decline, specifically due to excess nitrogen inputs, represents one of the most serious threats to the ecological health of the nearshore coastal waters. Coastal embayments, because of their enclosed basins, shallow waters and large shoreline area, are generally the first indicators of nutrient pollution from terrestrial sources. By nature, these systems are highly productive environments, but nutrient over-enrichment of these systems worldwide is resulting in the loss of their aesthetic, economic and commercially valuable attributes.

Each embayment system maintains a capacity to assimilate (process) watershed nitrogen inputs without degradation. As loading increases, a point is reached at which the capacity (termed assimilative capacity) is exceeded and nutrient related water quality degradation occurs. Protection and restoration of coastal embayments from nitrogen overloading has resulted in a focus on determining the assimilative capacity of these aquatic systems for nitrogen as well as identifying both soft and hard solutions for managing nutrient loads to impaired estuarine systems. While this effort is ongoing to varying degrees of effectiveness across the United States (e.g. USEPA TMDL studies), southeastern Massachusetts has been the site of intensive efforts in this area and specifically Pleasant Bay.

Project Need:

Since nitrogen removal is needed to meet estuarine specific TMDLs and restore water and habitat quality in nitrogen impaired estuarine systems, and since traditional approaches are quite costly to communities, less expensive more flexible non-traditional alternatives are needed and are now being considered on a site by site basis by Towns across the southeastern Massachusetts region. The Town of Orleans is investigating a number of these "soft" solutions, including shellfish aquaculture, specifically with oysters. In late 2015, the Town of Orleans initiated a multi-year oyster demonstration project within Lonnie's Pond, a eutrophic saltwater basin tributary to Pleasant Bay, in order to test the oyster filtration approach for improving water quality. A small oyster aquaculture system was established in Lonnie's Pond in summer 2016 (year 1) to determine: (1) the ability to grow oysters in this basin, (2) oyster survival, (3) the incorporation of nitrogen into oyster tissue and shell, (4) oyster filtration and biodeposition rates and, (5) the fate of nitrogen deposited by oysters to bottom sediments. The initial year 1 results of the multi-year investigation were presented to the Town of Orleans in January 2017 along with conclusions and recommendations for improving the study as the project proceeded into year 2 which captured summer 2017 conditions.

Results presented herein summarize field work completed during the summer/fall 2017 growing season and updates the 2016 data to include spring 2017 "carry over" effects. The focus is mainly on the effect of the oyster deployment on water column particulates (particulate nitrogen and chlorophyll), the amount of nitrogen deposited to the sediments by oysters and the degree to which denitrification is enhanced over background as a result of the oyster deployment. The amount that denitrification is enhanced plus the amount of nitrogen removed by oyster harvest accounts for the total nitrogen removal due to the oyster deployment. While there is a very small potential for nitrogen removal through enhanced sediment burial, the bulk of removal is through enhanced denitrification plus harvest. It is this oyster mediated N removal value that is the critical number for developing nitrogen management plans and designing larger deployments to reach specified nitrogen loading reductions required for restoration under MassDEP/USEPA TMDLs.¹

Section II. Background Water Quality Monitoring in Lonnie's Pond

Sampling Program:

A sampling program was implemented in Lonnie's Pond to establish both a pond-wide water quality benchmark and to quantify nitrogen removal by the pilot oyster culture deployment undertaken during the 2016 and 2017 growing seasons. Eight (8) water quality sampling locations were monitored in 2016 (LP1-LP4; M5-M8), building upon pre-existing database of water quality from the same monitoring stations. To better evaluate water column constituent gradients within the pond and how these may be affected by the presence of oyster culture, 6 more sampling locations (LP9-LP13) were added for the 2017 season (Figure II.1). In addition, samples and flow measurements were collected at the outflow from the cranberry bog

¹ The oyster survival and growth analysis and nitrogen removed by harvest was conducted by Science Wares Inc. and is presented in a separate companion report.

upgradient of Lonnie's Pond and the herring run (discharge from Pilgrim Lake) when sufficient flow was available for sampling. It should be noted that during the oyster deployment period, flow from the cranberry bogs was episodic during both summer 2016 and 2017, however, there was consistently measurable flow in the herring creek discharging into Lonnie's Pond from Pilgrim Lake (summarized in Section VIII). Water sampling occurred bi-weekly during mid-ebb tide conditions and usually in the early morning. Samples were collected at the surface, bottom, and at mid-water column where sufficient depth allowed. Samples were analyzed for: temperature, salinity, total nitrogen (nitrate + nitrite, ammonia, dissolved organic nitrogen, particulate organic nitrogen), chlorophyll-a (Chl-a), pheophytin-*a*, orthophosphate, dissolved oxygen, transparency (secchi depth), and alkalinity. Samples were collected according to protocols outlined for the MEP and which are followed by all other water quality monitoring undertaken by the SMAST-Coastal Systems Program across the southeastern Massachusetts region. Weather, tide-status, and results of water quality monitoring were documented. Quality Assurance samples (field duplicates) were collected (5%-10% of total number of samples collected) with the goal of gaining acceptance of study results by MassDEP and USEPA. Dissolved oxygen and temperature profiles (surface to bottom at 0.5m-1.0m increments) were completed at each sampling location using a YSI-55 handheld DO meter and following protocols developed for the MEP. Winkler samples were collected in triplicate at the DO/CHLA moorings at the sensor depth.

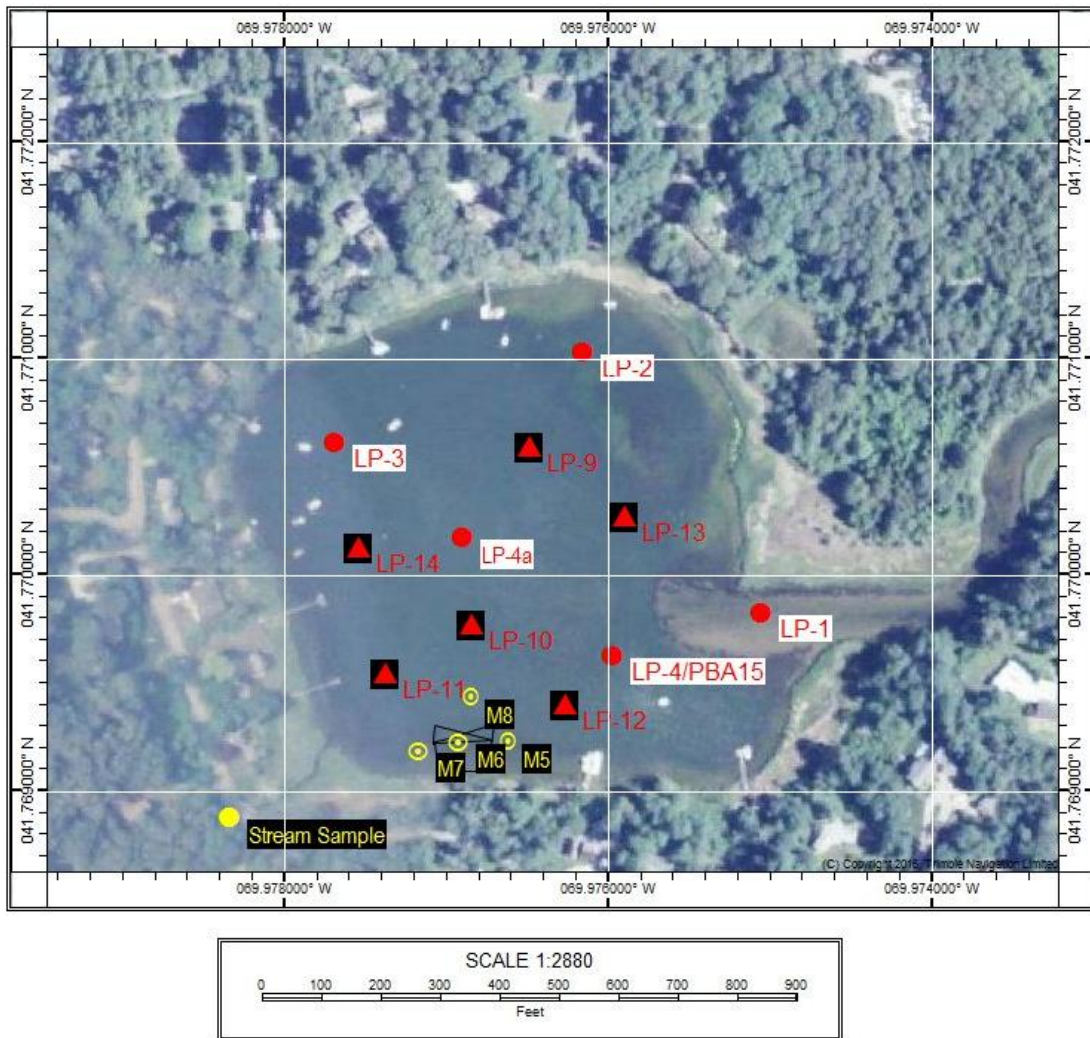


Figure II.1. Locations of water quality stations. Red dots denote stations established in 2016. Red triangles denote water quality stations added in 2017. LP4a was occupied during June 2016 until a permanent buoy was deployed by the harbormaster; hereafter LP4 was used to compliment previous water quality studies. Water quality stations LP5, LP6, LP7 and LP8 were at the mooring locations denoted on the map as M5-M8.

[Brief Description of Findings and Conclusions:](#)

The spatial distribution of major water quality constituents was variable over time. During the 2017 sampling period, there was evidence of higher concentrations of constituents along the northern edge of the pond (April samplings), however, spatial gradients (Figures II.2, II.3, II.4, II.5, top panel) were only established after deployment of the oysters (April onward). These gradients were most apparent during periods of high chlorophyll concentrations during which filtration by the oysters was enhanced. During periods of relatively low chlorophyll concentrations, gradients were small or nonexistent. This pattern differs from the 2016

sampling, during which there were drought conditions, when no evidence of point sources (e.g. stream input loads) or large scale gradients was observed (Figures II.2, II.3, II.4, II.5, bottom panel). In both 2016 (year 1) and 2017 (year 2), there was a clear pond-wide temporal trend with higher levels in mid-summer for each constituent (PON, Chlorophyll-*a*, bioactive N²). This temporal pattern is consistent with more eutrophic conditions in estuaries in the warmer summer months, with poorest water quality typically in July through mid-September and specifically evidenced by the large August phytoplankton bloom recorded by the time-series chlorophyll sensors (see Section IV). It should be noted that while there were blooms in both years, the chlorophyll-*a* levels in 2016 were significantly higher with a longer bloom in 2016 than 2017. It is possible that this could be related to the drought in 2016, but the specific mechanism is not well defined at this point.

Within the generally homogeneous distribution of the major constituents across Lonnie's Pond, there appeared to be lower levels of each constituent within the region of the oyster deployment. Although the magnitude of the constituents varied over time, each of the oyster influenced stations (M5-M8, Figure II.1) usually had lower concentrations than each of the far field stations (stations LP1-LP4, Figure II.1). Focusing on the period when the water quality constituent levels were consistently high (July 18-October 6), the spatial pattern is more clearly seen (Figure II.5) during both 2016 and 2017 field seasons. The distribution map of average water column constituent levels indicates lower levels of chlorophyll-*a* and PON are associated with the oyster deployment area compared to the area represented by the far field stations. Stations LP-1,2,3 and 4 showed consistently higher levels, station M8 intermediate levels and the stations directly associated with the oyster deployment showed lowest levels of each constituent. Even comparing the outboard station M8 to the nearby stations (M5, M6, M7) directly within the area of oyster influence, the influence of oyster filtration in removing particulate matter from estuarine waters was apparent (especially for station M6 in the middle of the oyster deployment area and in both 2016 and 2017). However, bioactive nitrogen for the stations within and adjacent to the oyster area was higher in 2017 due to increased dissolved inorganic nitrogen (NH₄ and NO₃). With the higher number of oysters deployed in 2017 the removal of PON by filtration and subsequent release of pseudofeces, labile fecal pellets and ingestion/digestion of filtered material likely results in increases in NH₄ and possibly nitrate (after nitrification) and lowering of water column PON and chlorophyll *a*.

The water quality surveys were mainly to establish temporal changes in water quality throughout the pond to establish the benchmark (setting) for the overall nitrogen cycling measurements. The surveys indicated that Lonnie's Pond tends to be relatively well mixed horizontally as seen in the spatial distribution of chlorophyll-*a* and particulate nitrogen. However, the results did indicate that in the region of the oyster deployment, particulate levels, particularly chlorophyll, are reduced, consistent with the filtering effects of the oysters. This "hole" in the particulate field indicates the high volumes filtered by the oysters as water flows through the deployed bags in minutes not hours. Overall, the results show that the increased

² Bioactive nitrogen is ammonium+nitrate/nitrite+particulate nitrogen, the most biologically active portions of the total nitrogen (TN) pool.

oyster deployment in 2017 (year 2) generated a lowering of key eutrophication indicators associated with the oysters held within the southern region of Lonnie's Pond as compared to 2016, in part due to the very high phytoplankton levels. Based upon these results, the expanded oyster deployment further improved water quality within this eutrophic estuarine basin.

A clear trend in the spatial distribution of PON and Chlorophyll-*a* was seen only after the oyster deployment (Figures II.6 and II.7), but none was observed with regards to Bioactive N (Figure II.8). The oysters appear to create a sink for PON and Chlorophyll-*a* in surface waters which extends out as far as 100m, thus the water clearing ability of oyster filtration may extend much further than the oyster impact area on the sediments. No such relationship could be seen in NH₄ and NO_x concentrations which are the precursors to chlorophyll and PON. It appears that the transformation of organic to inorganic forms after filtration by the oysters is lessening the drop in Bioactive N levels associated with the deployment. Interestingly, the oyster deployment area represented a local minimum for chlorophyll in 2016 and in 2017 (Figure II.5), although the average summer chlorophyll levels in 2016 were 2-3 times that of 2017. This suggests that filtration is leading to increased organic matter deposition to the sediments in the area of the oysters.

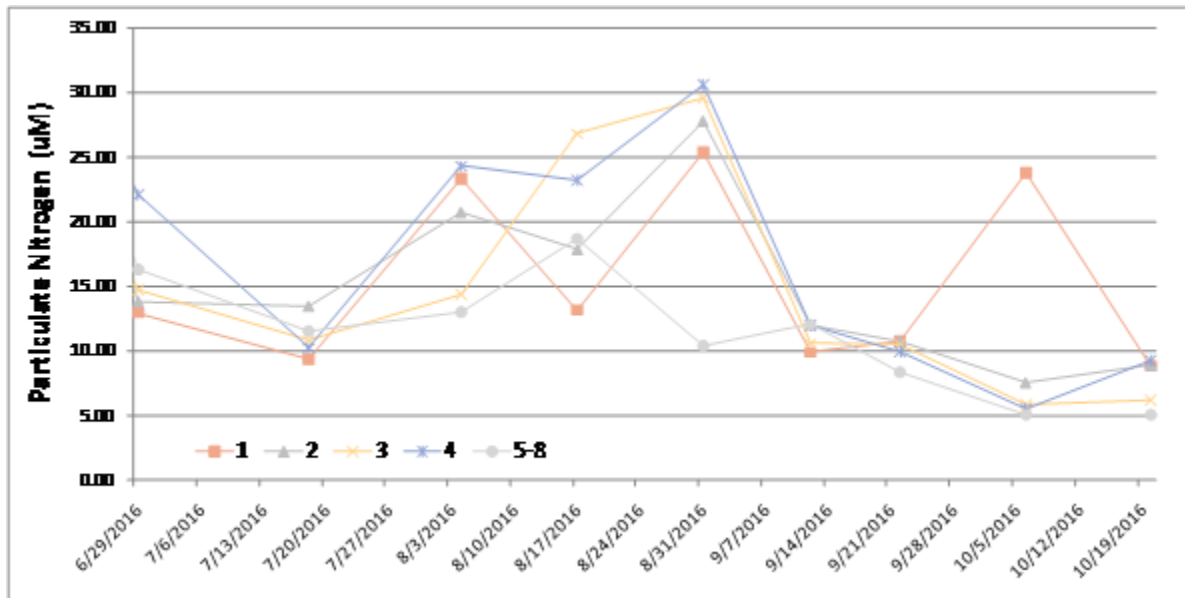
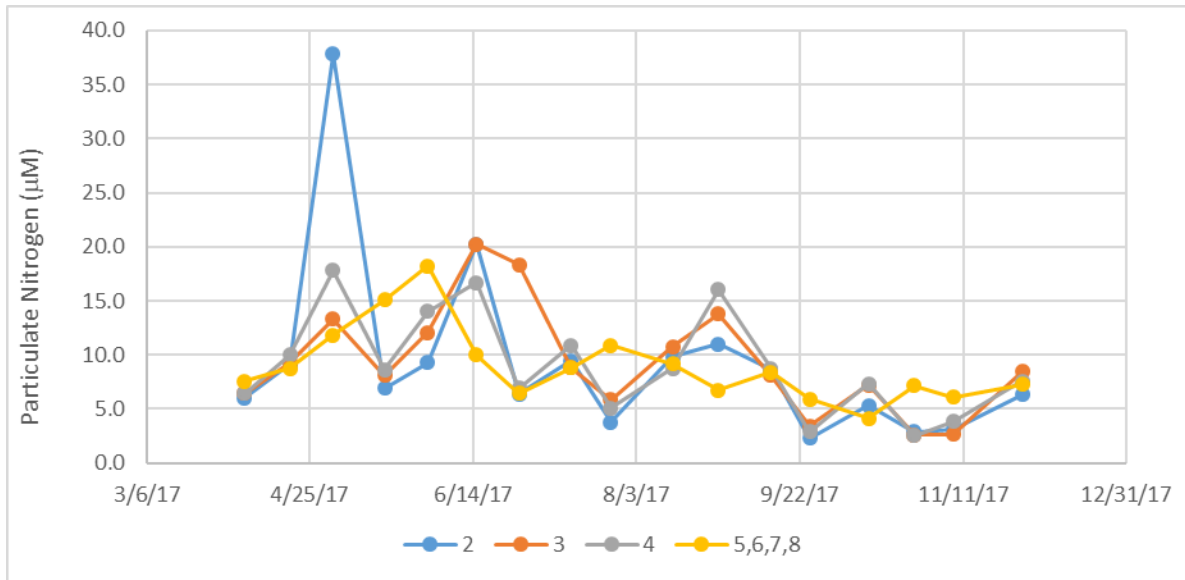


Figure II.2. Top Panel: 2017 time-series of mixed layer average particulate nitrogen at each individual sampling station and the average of the stations (5-8) associated with the oyster deployment. Bottom Panel: 2016 data shown for comparison. Station numbers refer to locations in station map above.

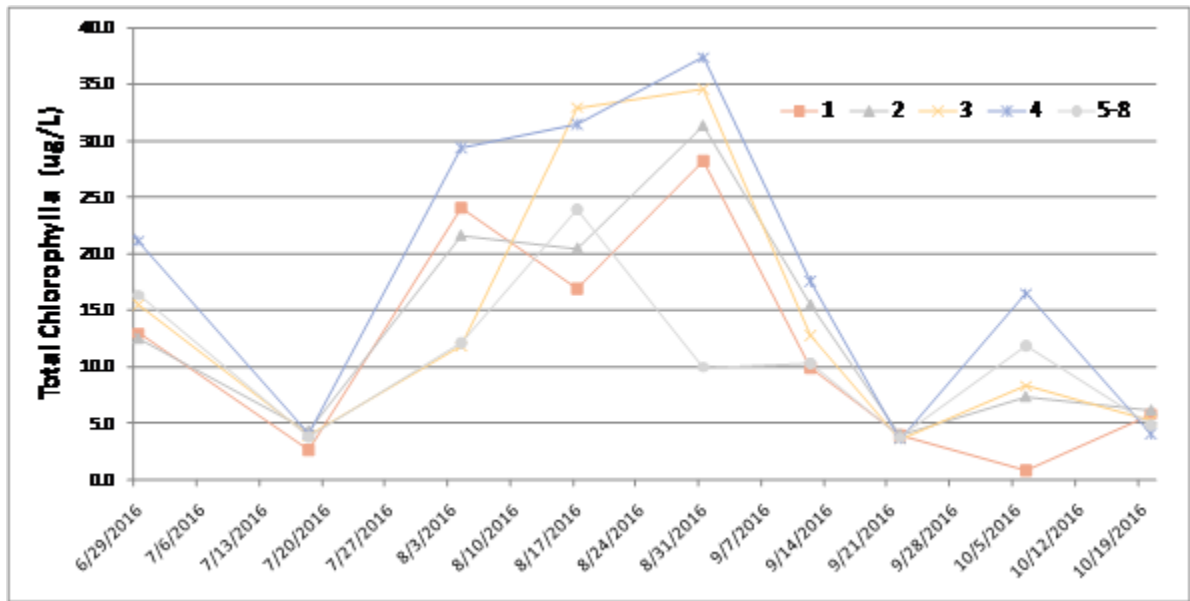
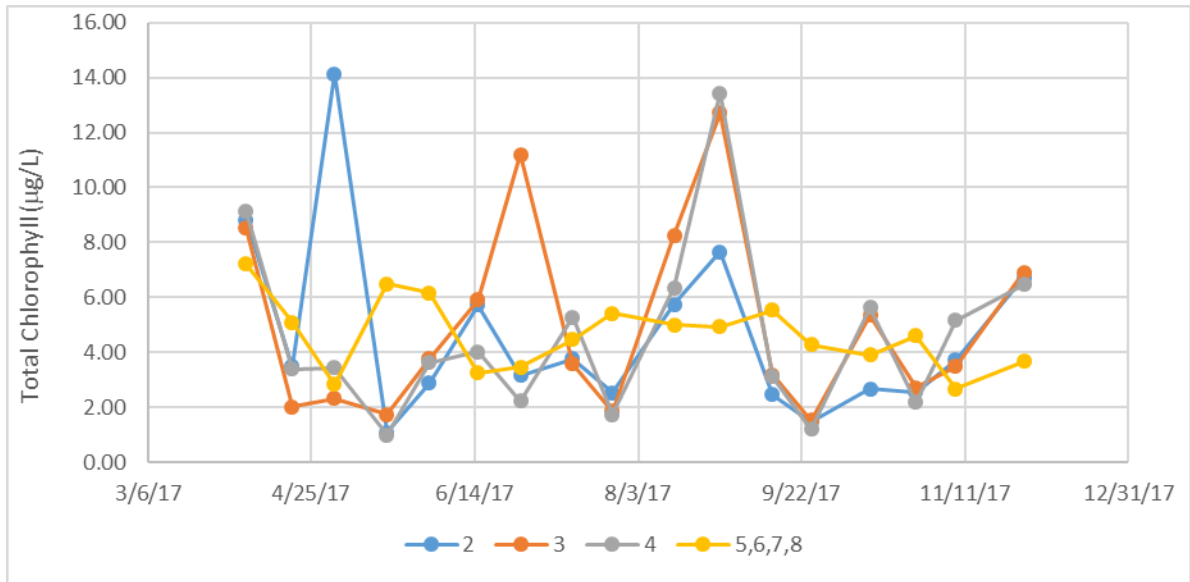


Figure II.3. Top Panel: 2017 time-series of mixed layer average total chlorophyll a at each individual sampling station and the average of the stations (5-8) associated with the oyster deployment. Bottom Panel: 2016 data shown for comparison. Station numbers refer to locations in station map above.

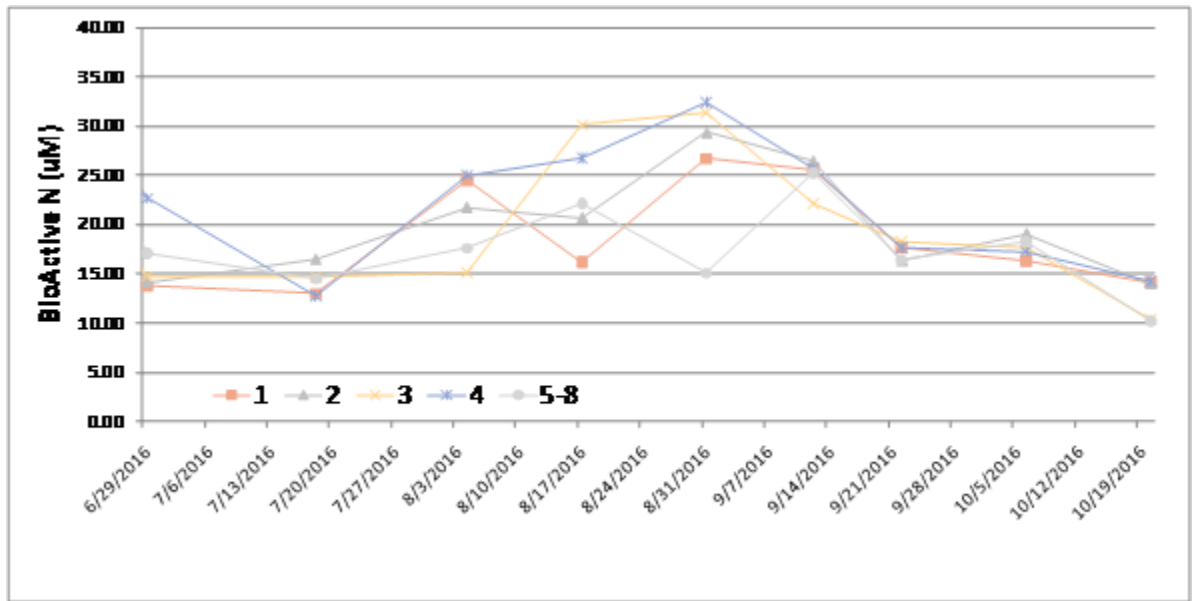
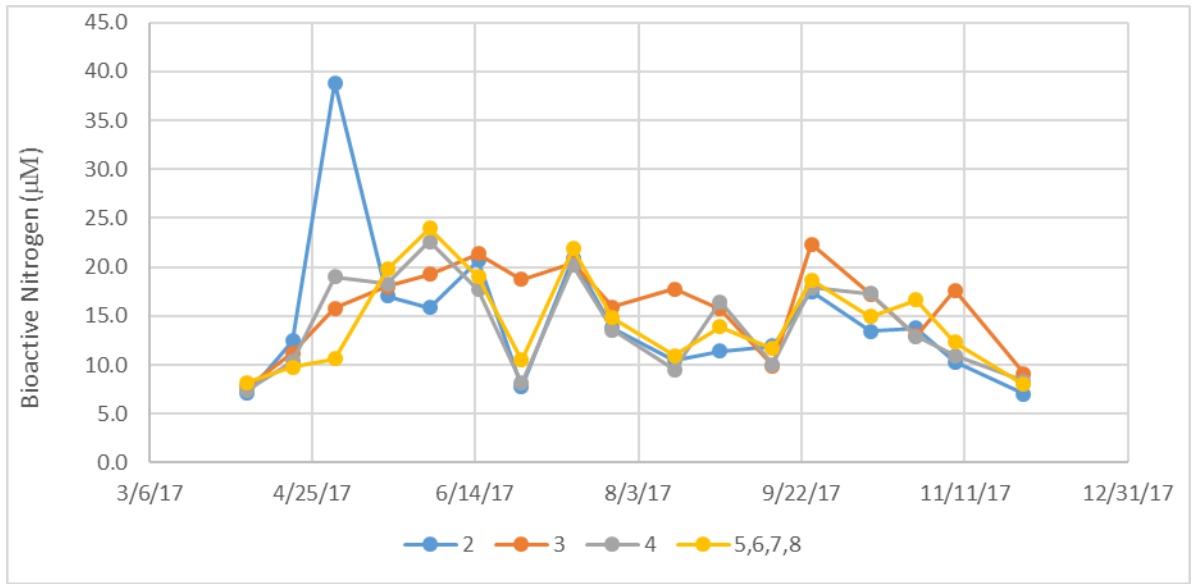


Figure II.4. Top Panel: 2017 time-series of mixed layer average bioactive nitrogen concentration at each individual sampling station and the average of the stations (5-8) associated with the oyster deployment. Bottom Panel: 2016 data shown for comparison. Station numbers refer to locations in station map above.

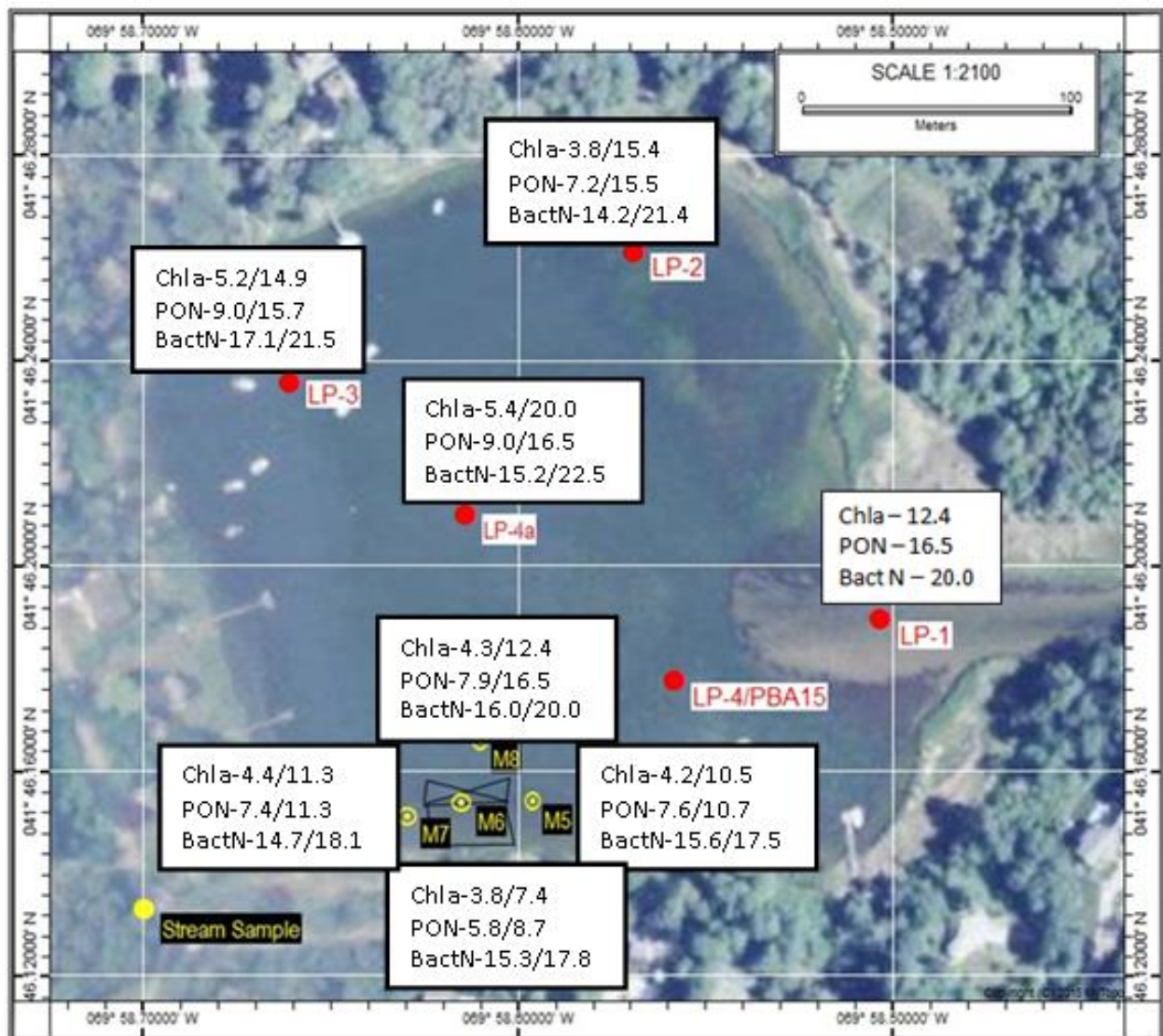


Figure II.5. Mixed layer average concentrations of total chlorophyll a ($\mu\text{g L}^{-1}$), particulate organic nitrogen (μM), and bioactive nitrogen (μM). Values represent average of samples collected from July 18 through October 6, 2016. Note the lower concentrations of each constituent in the region of the oyster deployment (stations 5,6,7,8) versus farther away (stations 1,2,3,4). 2017 values are shown first with 2016 values shown for reference (2017/2016). Values presented for station LP-1 are only from 2016.

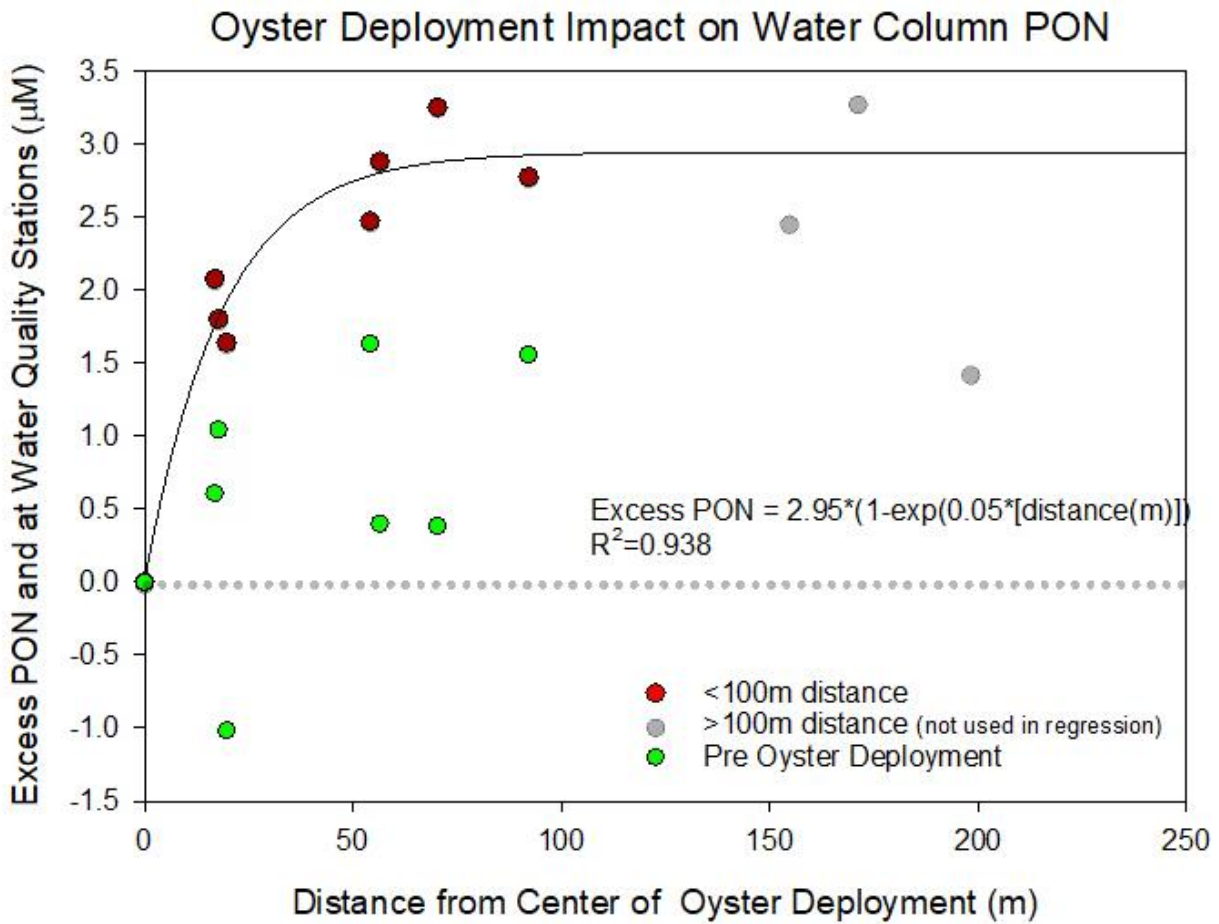


Figure II.6. Mixed layer average concentrations of particulate organic nitrogen (μM) in excess of that observed at M6 prior to oyster deployment (April–June 2017) and following oyster deployment (July–October). Note the concentrations of excess PON within 100m of oyster deployment increase exponentially with distance only after oysters were deployed.

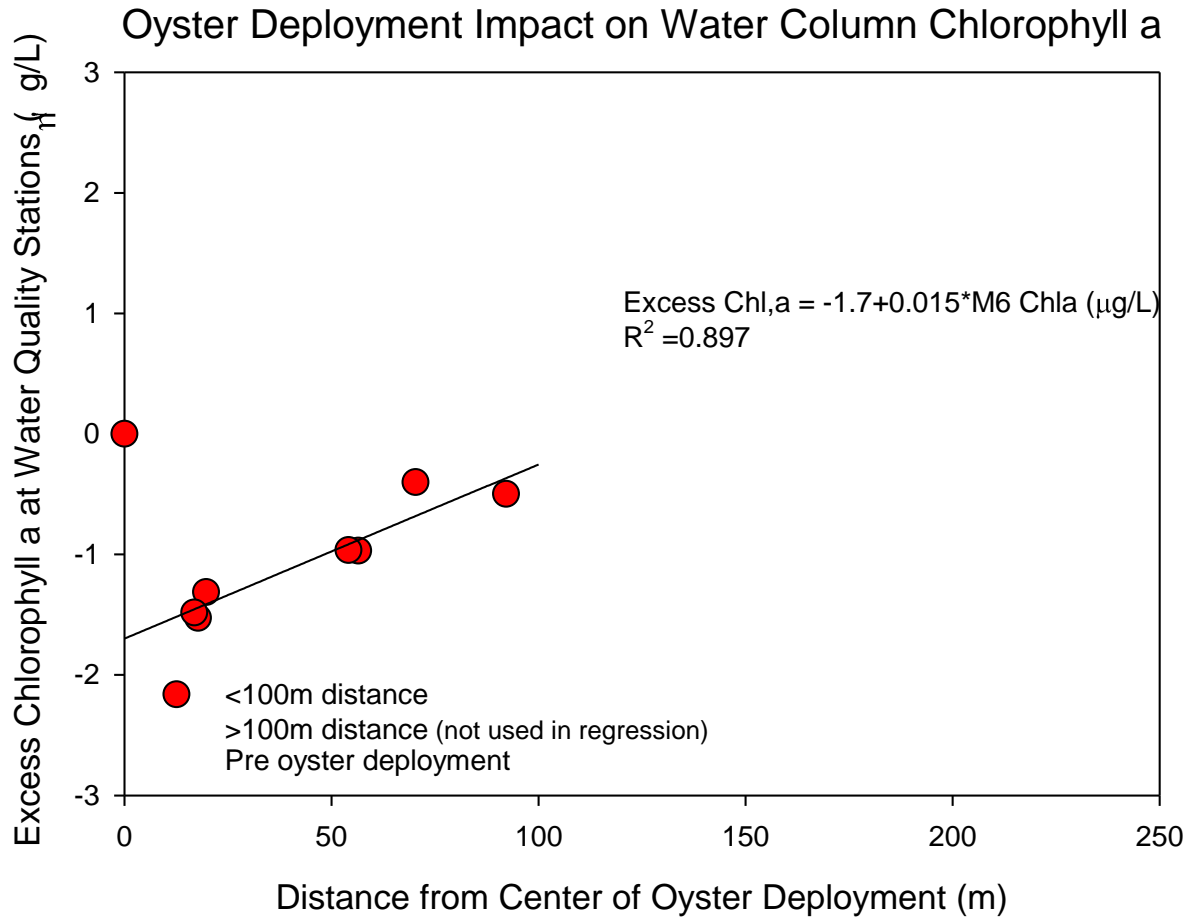


Figure II.7. Mixed layer average concentrations of chlorophyll a ($\mu\text{g/L}$) in excess of that observed at M6 prior to oyster deployment (April –June 2017) and following oyster deployment (July–October). Note the concentrations of excess Chl a within 100m of oyster deployment increase linearly with distance from the oyster site only after oysters were deployed and that the oysters create a local chlorophyll minimum.

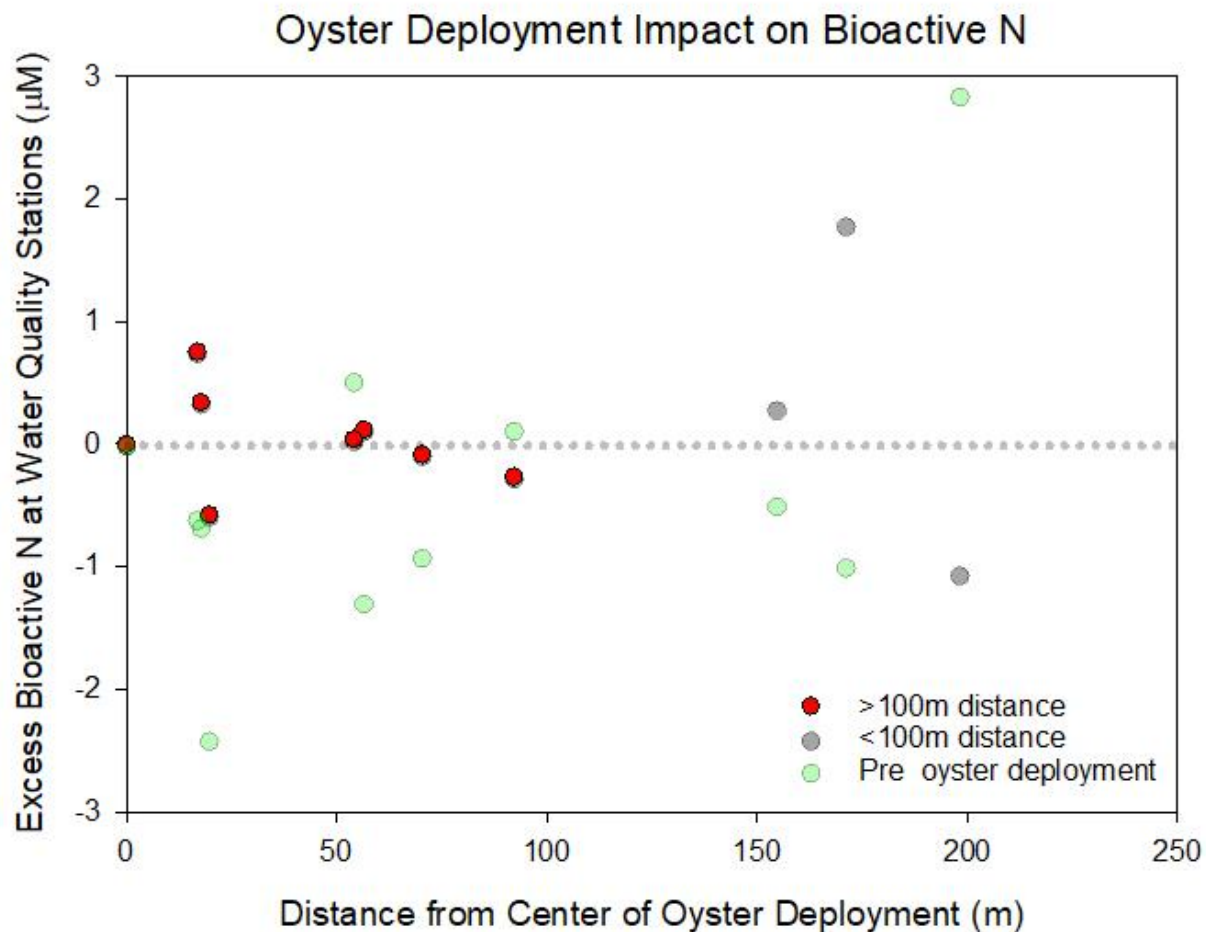


Figure II.8. Mixed layer average concentrations of bioactive N (μM) in excess of that observed at M6 prior to oyster deployment (April –June 2017) and following oyster deployment (July–October). Note that unlike PON and chlorophyll there is no obvious trend with increasing distance from the oyster deployment area, possibly due to it being a composite parameter.

[Recommendations based on water quality sampling results:](#)

Sampling should commence earlier in the season to capture any gradients not due to the activities of the deployed oysters. Sampling should focus on the oyster deployment area versus stations farther away, although it may be possible to sample station LP-2 less frequently or not at all. Lastly, it is important to monitor the surface water inflows (herring creek and cranberry bog) during subsequent oyster deployments as these sources of flow and load may figure more prominently under more average or above average precipitation conditions (summer 2016 was considered a particularly dry summer).

Section III. Time Varying Water Quality Assessment Post Oyster Deployment (2016)

Approach:

Water column surveys were conducted as water flowed through the oyster deployment area during complete tidal cycles on 4 dates in Summer 2016 (August 10 and 24, September 13, and October 12). This type of time varying survey (commonly referred to as a tidal flux survey) was not undertaken during the summer 2017 oyster growing season. During the 2016 surveys, samples were collected at nominal hourly intervals over consecutive flooding and ebbing tides. The sample sites included the long-term Lonnie's Pond water quality station (PB-15) as background, and 2 sites associated with the oyster deployment itself, one to the west and one to the east. Samples were collected at 0.3 m depth at about 1 m from the edge of the aquaculture footprint. Samples were assayed in a similar manner as for the background water quality surveys (see Section II). In parallel with the water column sampling, an acoustic doppler current profiler (ADCP) was deployed to measure current direction through the aquaculture area relative to the sampling points. The concept was to quantify changes in water column constituents, most importantly particulate organic nitrogen, total chlorophyll-*a* and bioactive N, although the complete suite of nitrogen components, orthophosphate and dissolved oxygen were assessed.

Results:

Although 4 sampling events were conducted during the 2016 sampling season (year 1), the first event (August 10, 2016) was halted midway through the survey due to an extreme rain event which resulted in aberrant water column conditions and un-interpretable flow through the oyster area. Fortunately, the other 3 events yielded usable/interpretable results.

For the three subsequent valid events, the flow through the aquaculture area was NE to SW (8/24 and 9/13) and W to E (10/12) with generally low velocities. Given uncertainties in the amount of contact that a parcel of water had with the aquaculture bags and resulting turbulence, it was not possible to calculate rates of particulate removal. However, it was possible to examine removals on a concentration basis from up-gradient to down-gradient. Since the samples were collected in parallel at the eastern and western sampling points, a paired analysis was possible. It should be noted that on each date, there was a constant wind direction. The constant wind effect resulted in a water flow direction that also did not change between flooding and ebbing tides even as water entered and left the basin (i.e. the circulation was mainly wind driven). Changes in the paired samples over flooding and ebbing tide showed no significant differences in any of the water column constituents in water flooding through the aquaculture system on any of the 3 dates. In contrast, for each survey, constituents showed higher values on the ebb tide in the water before it passed through the aquaculture system than after it had passed through (Table III.1). On ebbing tides over the 3 events, average reductions of 19%-37% in PON, 23%-48% in total chlorophyll-*a*, 12%-20% in bioactive nitrogen were observed. In the October survey, flows approximated the long axis of the aquaculture system and the highest particulate removals were seen (37% in PON, 48% in total chlorophyll-

a). This result likely is related to the water parcels moving along the full length of the oysters in this event, maximizing the opportunity for filtration. This helps to explain the lower observed uptake when water passes obliquely through the system. It should be noted that variation in observed uptake is due to the position of the sampling points relative to the flow direction, as measured uptake will vary with flow direction even if oysters are taking up the same mass in each survey. Nonetheless the results clearly indicate that the oysters filtered out sufficient amounts of particles to significantly lower PON and chlorophyll-*a* levels, which was also seen in the time-series measurements (Section IV). Equally important, the observed reduction in bioactive N indicates that the removal of PON resulted in a net N loss to the water column of 12%-20%, as only about half of the PON removed was returned as regenerated nitrogen from the sediments or excretion nitrogen by the oysters.

Conclusions:

The oyster deployment in Lonnie's Pond removed significant amounts of PON and total chlorophyll-*a* in ebbing waters. Particulate nitrogen removal resulted in a net lowering of water column bioactive nitrogen as the amount filtered out was not returned via oyster excretion or sediment regenerated nitrogen. Bioactive N levels declined by 12%-20% during passage through the aquaculture systems. Observed removals are conservative estimates due to the oblique patterns of flow through the oyster area in the surveys, which biases the uptake estimate low in these experiments.

Year 1 Recommendations (2016):

The tidal surveys yielded useful results, but were confounded by the flow pattern at the specific location in Lonnie's Pond. While the flow pattern was not ideal, the results are unequivocal and understandable, which increases their value. Future surveys should include a real-time determination of flow direction and associated adjustment of sampling points. Moving sampling points to maximize the contact of a parcel of water with the oyster system would greatly improve accuracy and should support rate estimates. However, it is clear that placement of future demonstration deployments should account for flow direction if these types of measurements are to be undertaken. None-the-less, the tidal surveys clearly indicated that particulate removal is sufficiently rapid with the result that there are quantifiable changes in water quality during individual tidal cycles. This supports the contention that shellfish should be able to make a positive change in Lonnie's Pond water quality during the critical management period.

Table III.1. Water column constituents up gradient and down gradient stations during tidal flow through the aquaculture system in Lonnie's Pond, Summer 2016. Highlighted pairs of means have significantly different levels after passing through the oyster deployment area.

Title	Flow Direction	PON (μM)			T-pig ($\mu\text{g/L}$)			TN (μM)			BioChl a (μM)		
		East	West	PB-15	East	West	PB-15	East	West	PB-15	East	West	PB-15
Survey 8/24/2016													
Flood	E to W	15.31	15.80	19.50	22.9	21.3	25.6	47.68	49.29	50.83	16.50	18.07	20.14
Ebb	E to W	11.05	8.94	15.10	12.6	9.8	19.1	47.22	44.16	48.19	14.89	13.05	16.12
Survey 9/13/2016													
Flood	E to W	8.32	11.93	11.29	7.04	8.08	7.43	51.71	52.55	52.40	22.11	24.38	22.38
Ebb	E to W	13.17	8.86	19.16	9.90	7.03	14.33	50.72	46.20	47.05	22.60	18.09	24.00
Survey 10/12/2016													
Flood	W to E	8.57	7.95	11.67	7.8	8.0	14.5	47.7	43.9	43.5	23.1	20.9	23.3
Ebb	W to E	7.03	11.21	9.19	3.0	5.8	5.3	42.2	49.4	47.2	20.2	23.0	20.8
		statistical significant $p < 0.05$											

Highlighted pairs of means are statistically significant (paired T-test). The flow did not go down the long axis of the oyster deployment, but did pass through the bags on the ebbing tide either in a East to West (events 1 and 2) or West to East (event 3) fashion. Removal can be seen as lower values at the downgradient station.

Section IV. 2016 and 2017 Time-Series Dissolved Oxygen (DO)/Chl-a Moorings (High Frequency Sampling)

Time-series Mooring Deployment and Sampling:

SMASST scientists conducted continuous monitoring of key water column parameters, dissolved oxygen and chlorophyll-a, at 15 minute intervals to assess the impact of oyster aquaculture on the ambient water column. This high frequency autonomous monitoring was completed in parallel with the traditional water grab sampling effort. The bottom moored sondes also collected measurements of temperature and tide height/depth. The mooring program undertaken in Lonnie's Pond during the 2016 and 2017 field seasons followed the same protocols and procedures developed for the Massachusetts Estuaries Project (MEP) analysis of the Pleasant Bay Estuarine System and for the Towns of Falmouth and Mashpee Oyster Demonstration Projects. The consistency of protocols allows cross comparability of data sets collected in years past and from other estuarine locations where oyster deployments have been undertaken.

A total of five YSI-6600 moored sondes were deployed at four locations in both 2016 and 2017. Moored sondes were deployed on the bottom (30 cm above the sediment surface) within the footprint of the oyster deployment area as well as to the east and west of the oyster deployment (Figure IV.1). A fourth mooring was placed to the north of the oyster deployment footprint in approximately 2.5 m of water to monitor water quality outside the influence of the oysters. Two sondes were deployed at this fourth location: 1) at the surface and 2) 30 cm above the bottom. While the moorings provided good oxygen and chlorophyll-a data during the 2016

deployment period, oxygen sensors in 2017 suffered periodic failure of their Teflon membranes. Chlorophyll-a records in 2017 and 2016 provided adequate data for comparison of the respective oyster deployment periods. It remains unclear how the sensors were damaged in situ in 2017, however, thicker membranes and better protection that does not interfere with water movement around the sensors was instituted in the 2018 field season. Bi-weekly oxygen values obtained via winkler titration were collected for sonde calibration. These data are presented as instantaneous measurements for each of the mooring locations. In addition, continuous air equilibration values were calculated from salinity and temperature data.

The moorings were maintained April – November in both 2016 and 2017 to provide information during three critical periods: 1) prior to the deployment of the oysters, 2) when the oysters were most actively filtering pond water (i.e. maximum “oyster effect”) and the pond water quality is lowest, and 3) through the autumn months when oyster activity was decreased by colder water temperatures. Moorings were calibrated bi-weekly by collection of samples at the specific depth and location of each sensor. At the time of calibration, each sonde was inspected, cleaned and then downloaded. Sondes were then returned to the moorings and secured. Calibration sampling included triplicate Winkler samples for dissolved oxygen (DO) determination as well as collection of whole water for chlorophyll extraction. The sondes recorded DO, Chl-*a* (via fluorescence), salinity, and water temperature and depth at 15-minute intervals.

Time-series Sensor and Sonde Data Results and Discussion:

The dissolved oxygen (DO) records (Figures IV.1a, IV.2, IV.4, IV.6 and IV.8) all show large diurnal variation in response to water column and sediment respiration at night and photosynthesis during the daylight hours. The levels of oxygen depletion (to 2 mg/L) and large diurnal changes indicate a system that has been organically enriched due to nitrogen enrichment and impairment was seen in the summer of both 2016 to 2017. Close examination revealed lower oxygen minima in 2016 than 2017, even with consideration of the diurnal variability. In 2016 oxygen minima at most sites was ~1 mg/L or even showed periodic anoxia, where as in 2017 oxygen levels generally were above 2 mg/L or even 3 mg/L. These findings were consistent with the measured chlorophyll levels which showed a clear inter-annual difference, with 2016 supporting 2-3 times the phytoplankton biomass than observed in 2017.

A general comparison of oxygen conditions in both 2016 and 2017 was achieved by comparing the overlapping time series data and oxygen levels from the field calibration samples in both years. Calculated air equilibration values which represent the expected DO concentrations of a well-mixed water column in the absence of any biological perturbations were also assessed. The results of the comparison of the available 2016 and 2017 Winkler DO data show generally depressed oxygen concentrations at M8 (north of oysters) due to sensors being rapidly fouled and proximate to the Pond’s natural pycnocline for the surface and bottom sensors, respectively. Moorings M5 and M7, located to the east and west of the oyster deployments show slight positive and negative deviations with the exception of a short period beginning June 1 and ending June 15. The extreme excursions from air equilibration were not accompanied by any dramatic change in chlorophyll concentration (e.g. a phytoplankton

bloom) and may have been the result of drift macroalgae periodically interacting with the moorings. The most complete DO record was at M6 in the middle of the oyster deployment. Diurnal excursions in DO were large (up to 10 mg/L) and oxygen minima reached 3 mg/L, however close examination of the records show these low levels were not persistent; the events did not last for more than 1 hour early in the morning.

The continuous chlorophyll records are of generally high quality and confirm conclusions from the bi-weekly sampling results. Phytoplankton concentrations in Lonnie's Pond were significantly lower in 2017 than in 2016. Overall temperatures in the pond (from sonde records) were also slightly lower in 2017 than in 2016 and while there are many other environmental factors (rainfall) which can affect phytoplankton biomass, the observations are potentially due to the larger biomass of oysters acting to improve water quality in 2017 possibly coupled with a larger regional meteorological effect. It was noted that in summer 2017 there were also generally lower chlorophyll levels in Pleasant Bay than in 2016 (Orleans Water Quality Monitoring Program).

Unlike the elevated chlorophyll values seen in 2016, the highest chlorophyll concentrations in 2017 were observed within the oyster deployment area (M6). The continuous record displays consistently higher chlorophyll concentrations in the middle of the deployment area than those observed at moorings either to the east or west. This pattern suggests that filtration of plankton by oysters and resulting deposition was concentrating chlorophyll (biodeposits are high in pheophytin a) and releasing it to the water column where it settled beneath the oyster arrays. This concentration and deposition within the oyster deployment area is seen in the surficial sediments and the stimulation of sediment remineralization of organic matter within the water column and surface sediments. Interestingly, chlorophyll levels at M5 and M6 rarely exceed those observed at nearby monitoring locations suggesting that deposition is mainly within the oyster deployment area.

In some locations of oyster culture and aquaculture, concerns have been raised about potential benthic habitat decline resulting from the concentration of feces on the sediment surface which can lead to increased sediment respiration and by extension depressed dissolved oxygen concentrations within the deployment area. While dissolved oxygen concentrations were observed to be below saturation values, the lowest concentrations observed within the oyster deployment area were also observed at far field stations such as LP2 and LP3, hence attribution to oyster culture is not possible. The apparent increased phytoplankton production within the oyster deployment area warrants continued monitoring, but as long as that increased production remains restricted to the deployment area (balanced by increased oyster filtration) it could be a positive outcome as recycled N is removed by oyster assimilation.



Figure IV.1. Aerial photograph showing the initial 2016 oyster deployment (floating bags) relative to mooring locations at the edges of the oyster deployment footprint (LP5 {bottom}, LP7 {bottom}, LP8 {surface+bottom}). The area defined by the green line is where the first year class oysters (2016) were redeployed in 2017 after over-wintering. The area defined by the red line is where oyster seed was deployed in 2017. The calculated 2016 impact area is denoted by the gray line.

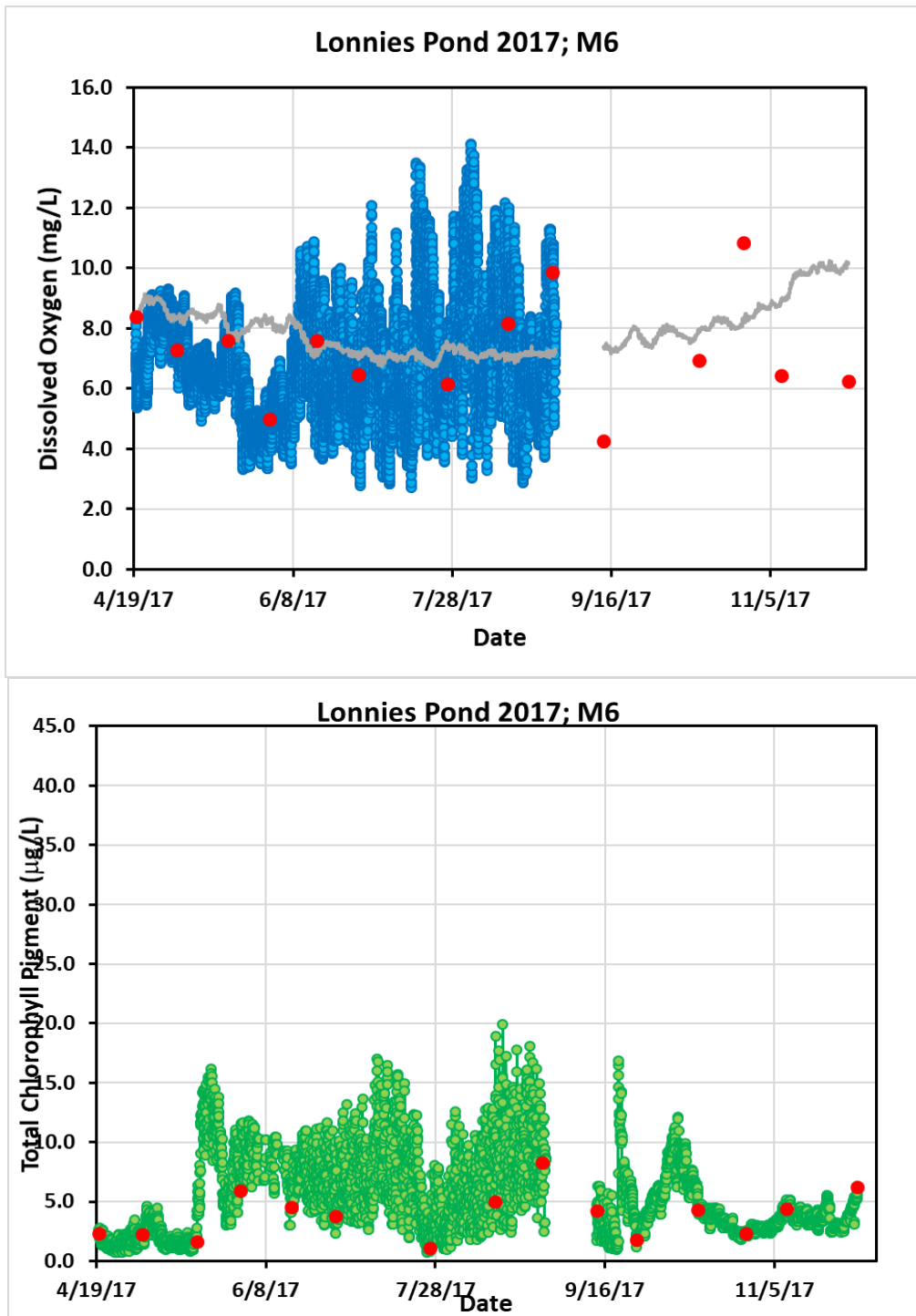


Figure IV.1a Time series dissolved oxygen at Lonnie's Pond Mooring M6 located in the center of the oyster deployment footprint. Red markers indicate Winkler titration calibration points. The gray line represents the air saturation value (top). Time series chlorophyll measurements at Lonnie's Pond Mooring M6 located in the of the oyster deployment footprint. Red markers indicate chlorophyll extraction calibration points.

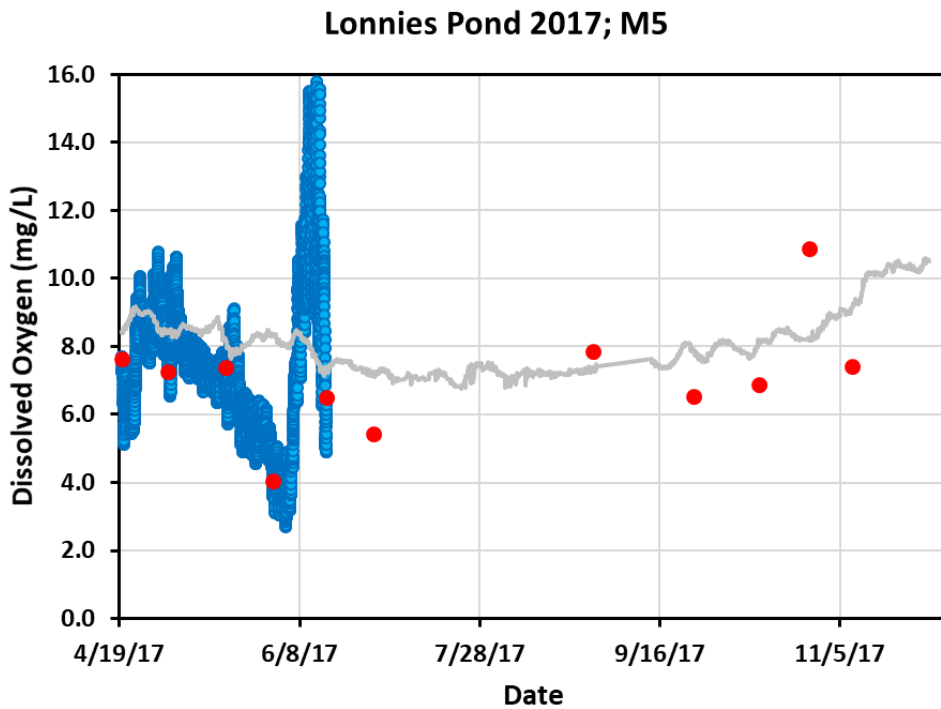
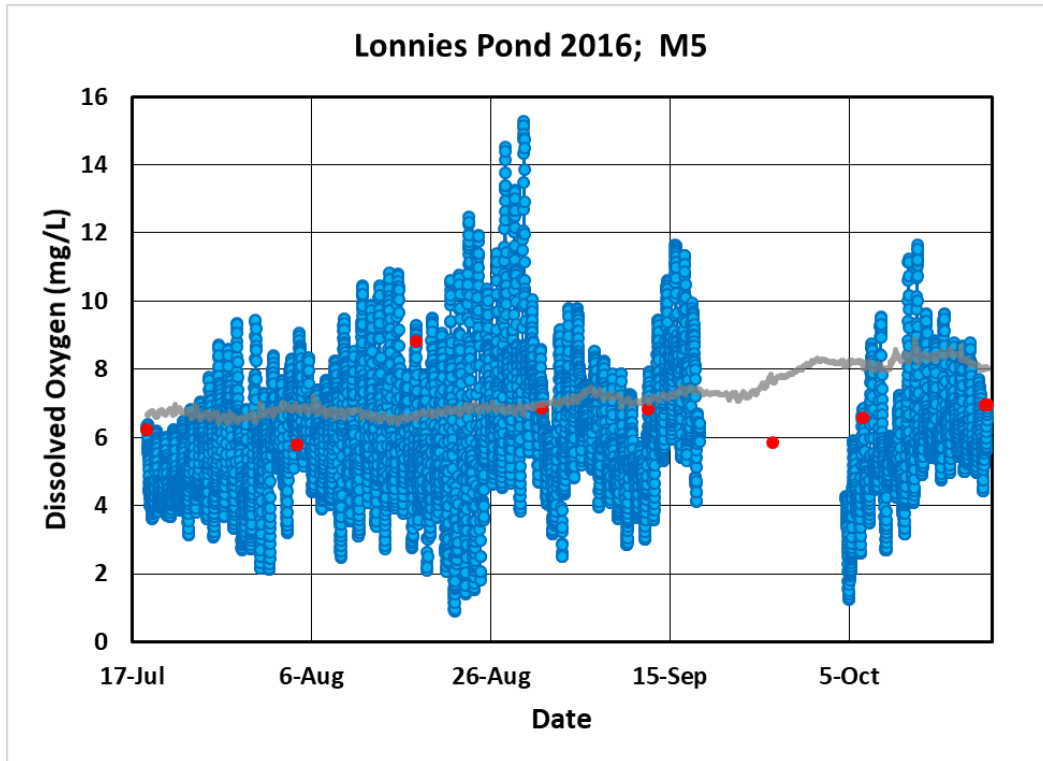


Figure IV.2. Time series dissolved oxygen at Lonnie's Pond Mooring M5 located due east of the oyster deployment footprint. Red markers indicate Winkler titration calibration points. The gray line represents the air saturation value (top panel 2016 for comparison).

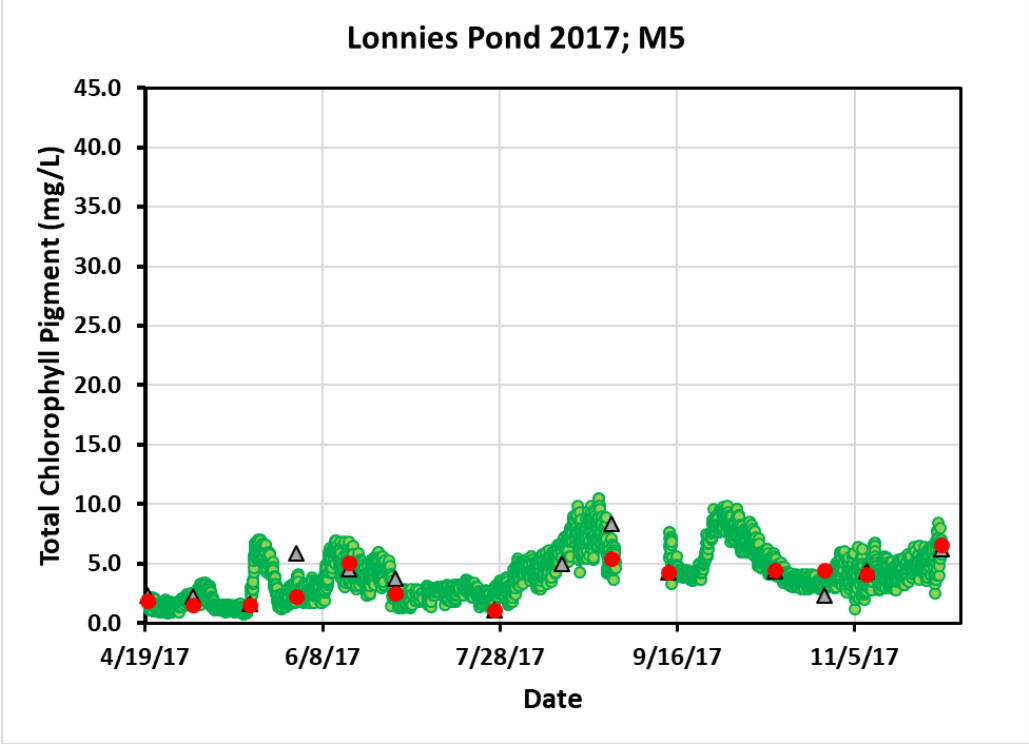
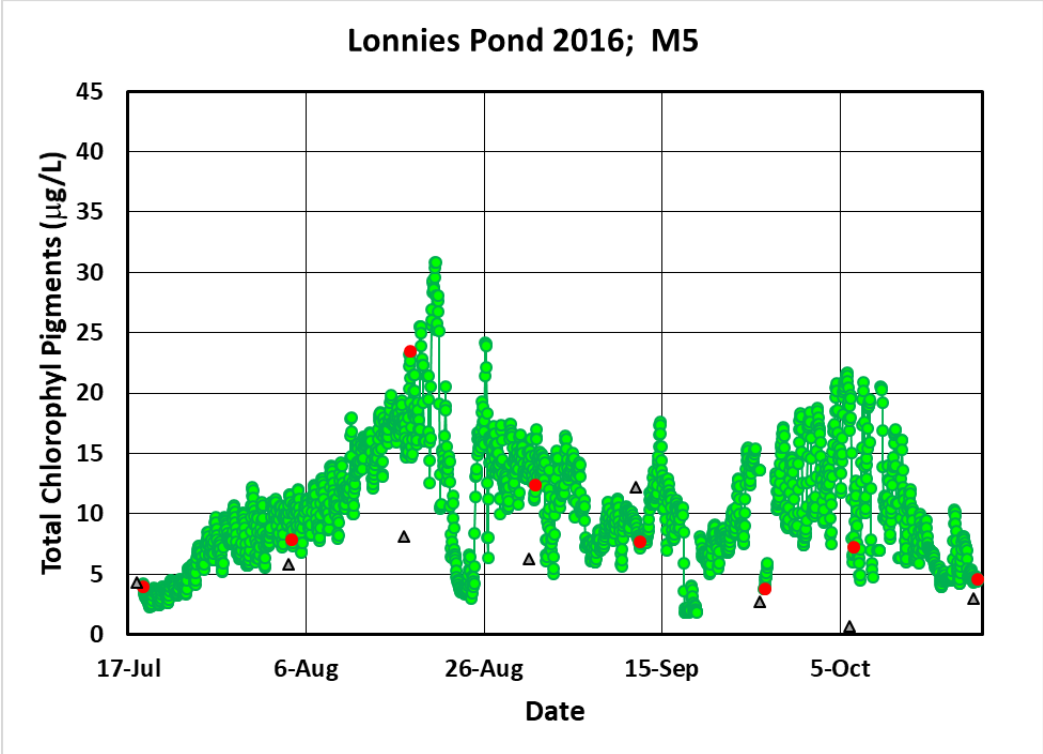


Figure IV.3. Time series chlorophyll measurements at Lonnies Pond Mooring M5 located due east of the oyster deployment footprint. Red markers indicate chlorophyll extraction calibration points. Green triangles indicate chlorophyll extractions conducted at Mooring M6 located in the middle of the deployment footprint (top panel 2016 for comparison).

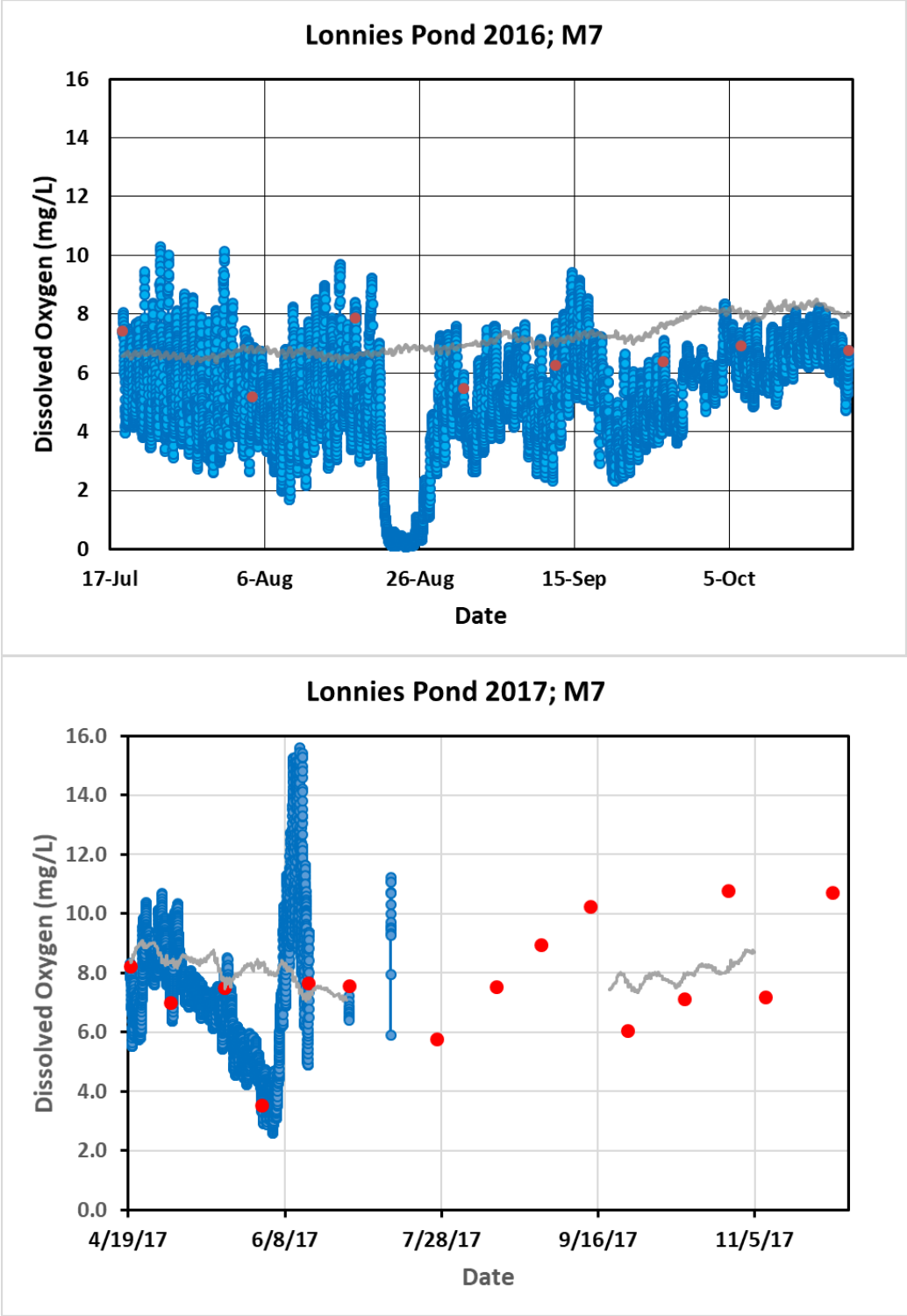


Figure IV.4. Time series dissolved oxygen at Lonnie's Pond Mooring M7 located due west of the oyster deployment footprint. Red markers indicate Winkler titration calibration points. The gray line represents the air saturation value (top panel 2016 for comparison).

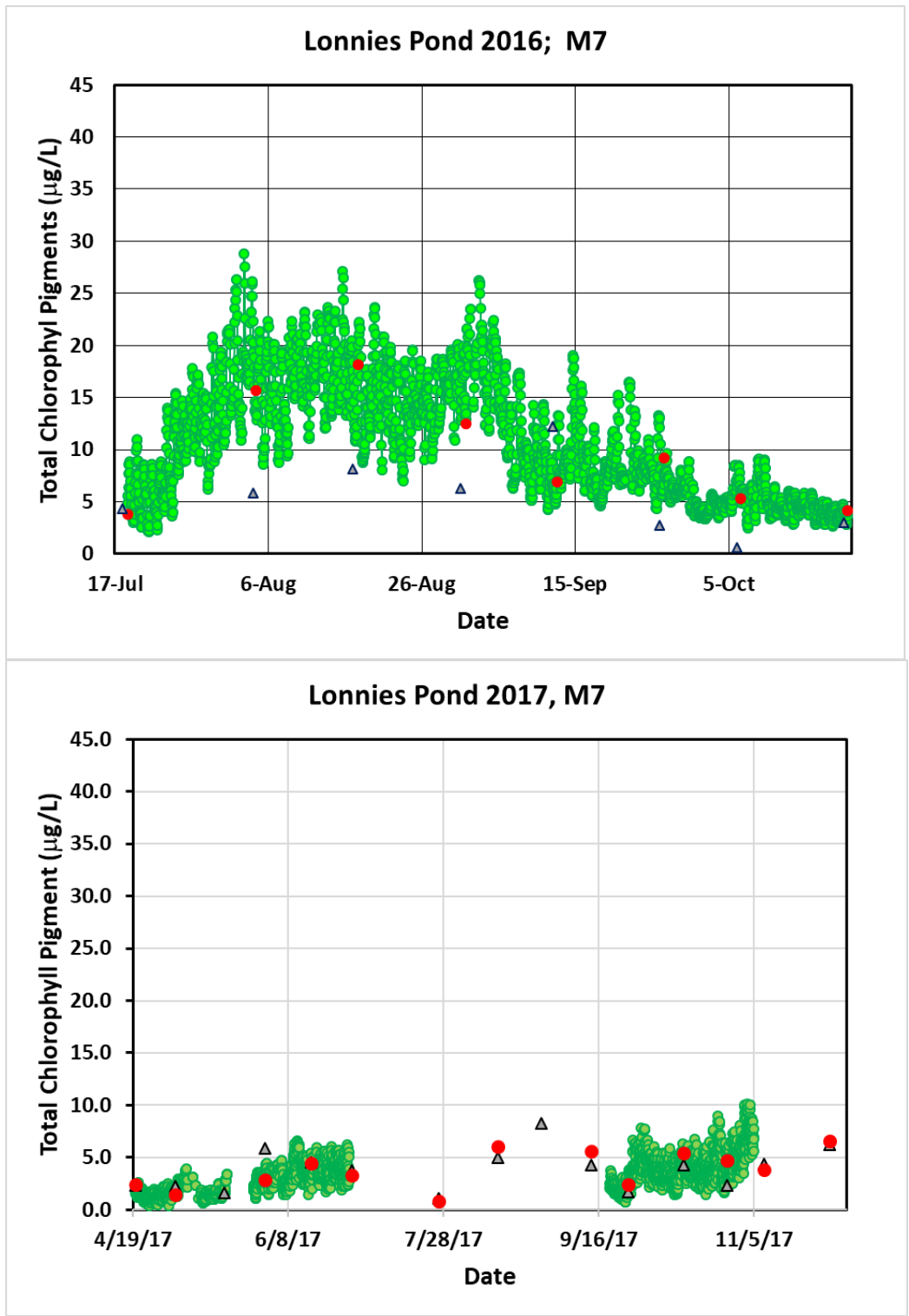


Figure IV.5. Time series chlorophyll measurements at Lonnie's Pond Mooring M7 located due west of the oyster deployment footprint. Red markers indicate chlorophyll extraction calibration points. Green triangles indicate chlorophyll extractions conducted at Mooring M6 located in the middle of the deployment footprint (top panel 2016 for comparison).

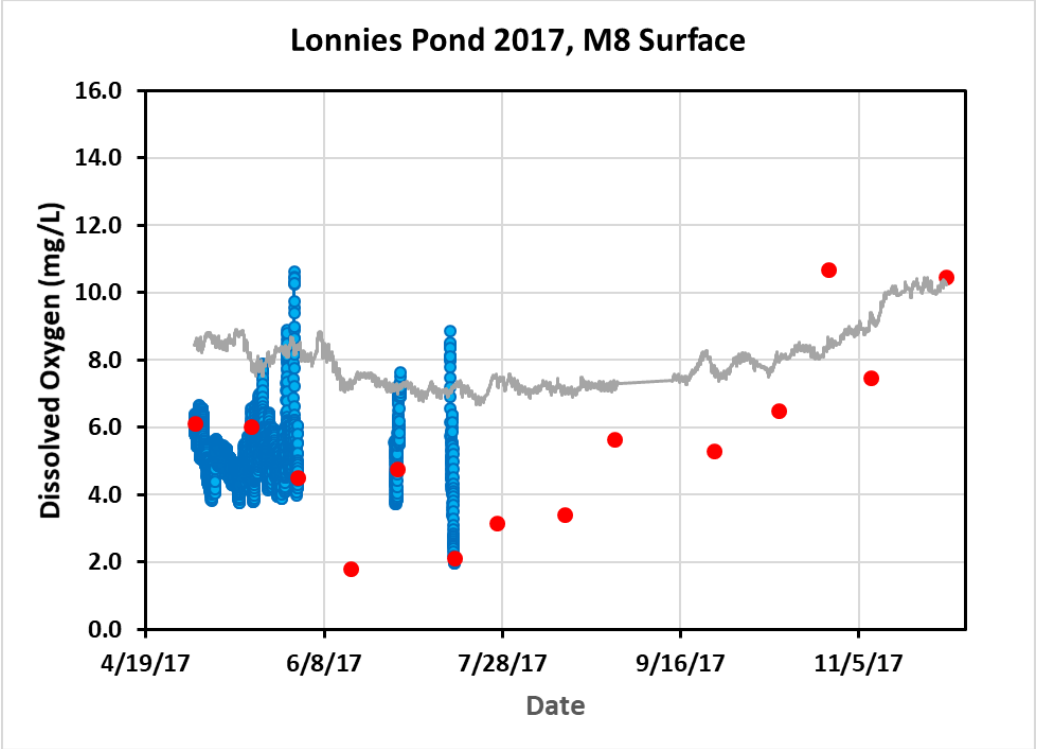
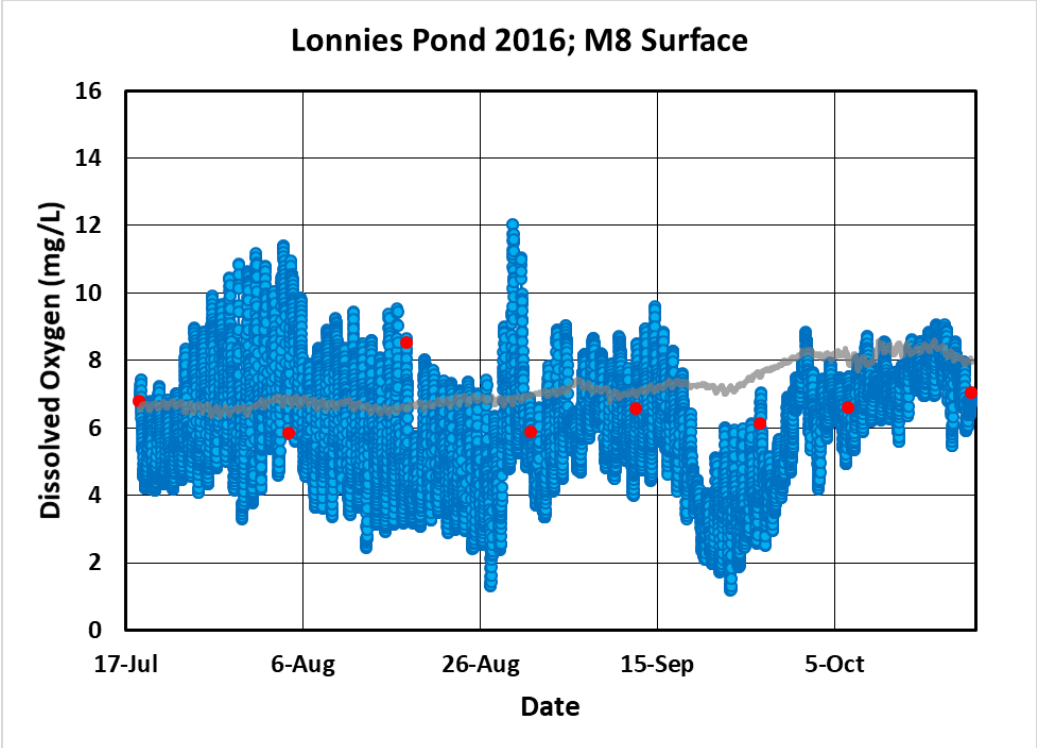


Figure IV.6. Time series dissolved oxygen at Lonnies Pond Mooring M8 (Surface) located due north of the oyster deployment footprint. Red markers indicate Winkler titration calibration points. The gray line represents the air saturation value (top panel 2016 for comparison).

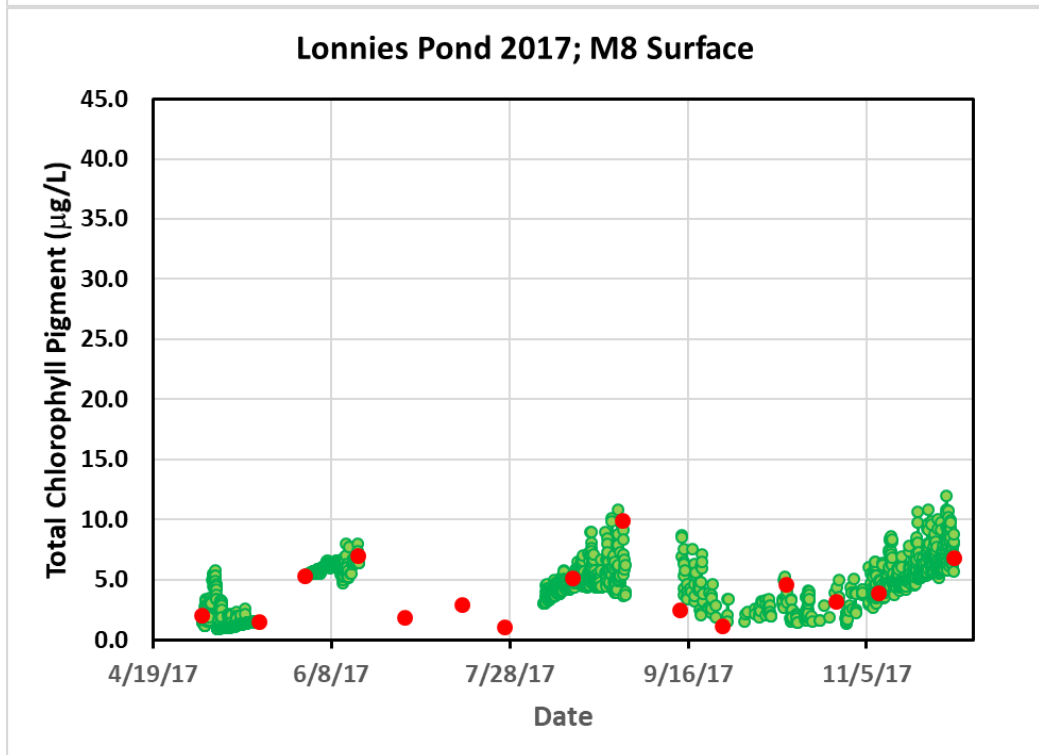
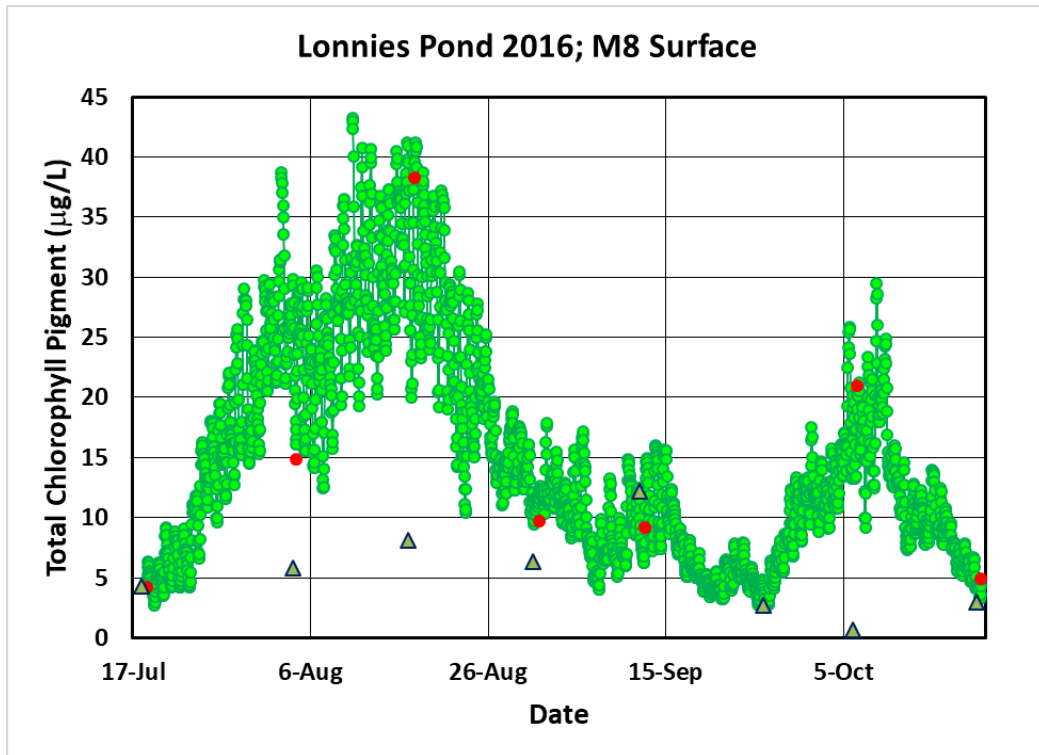


Figure IV.7. Time series chlorophyll measurements at Lonnies Pond Mooring M8 (Surface) located due north of the oyster deployment footprint. Red markers indicate chlorophyll extraction calibration points. Green triangles indicate chlorophyll extractions conducted at Mooring M6 located in the middle of the deployment footprint (top panel 2016 for comparison).

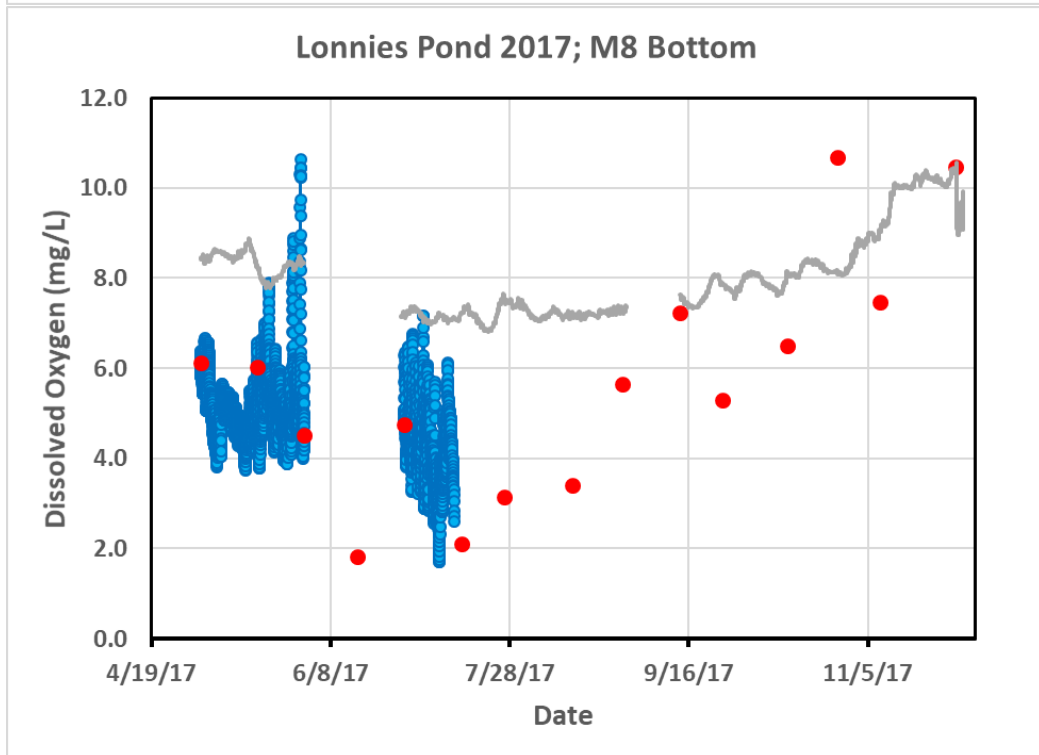
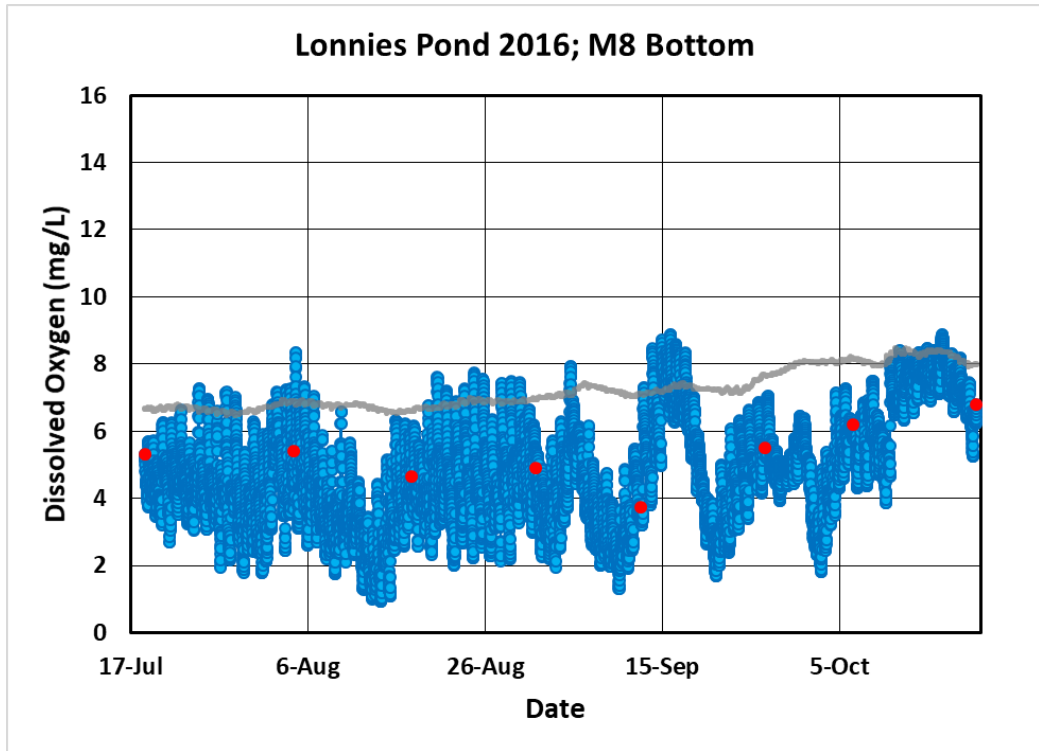


Figure IV.8. Time series dissolved oxygen at Lonnies Pond Mooring M8 (Bottom) located due north of the oyster deployment footprint. Red markers indicate Winkler titration calibration points. The gray line represents the air saturation value (top panel 2016 for comparison).

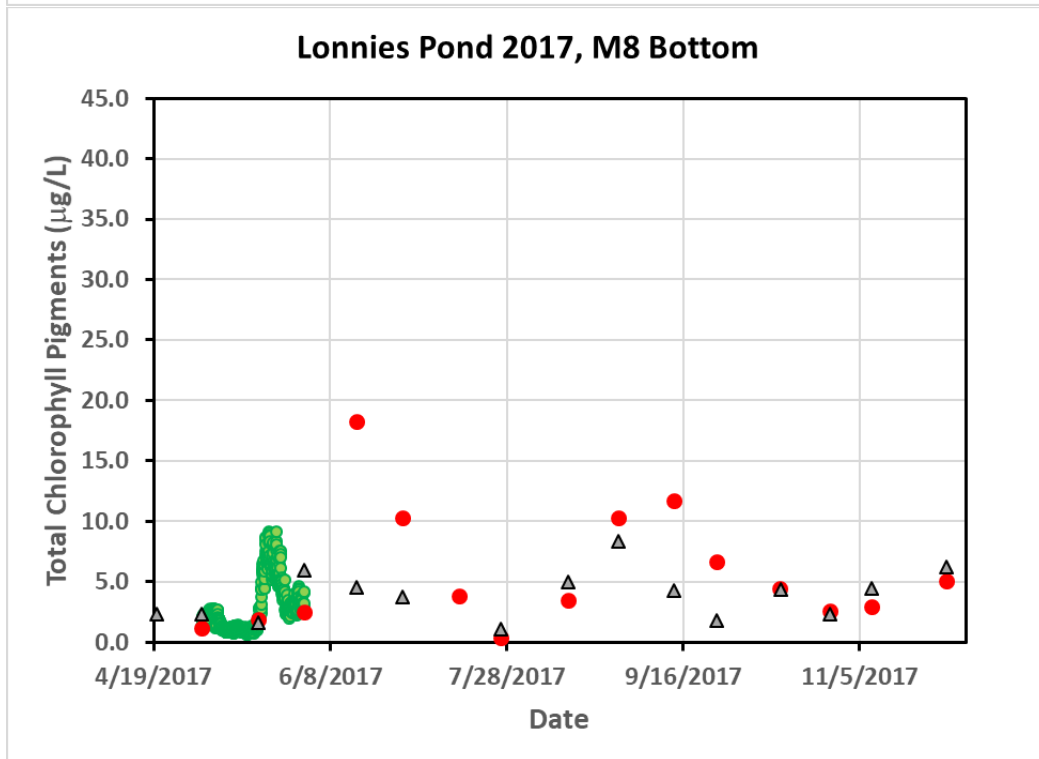
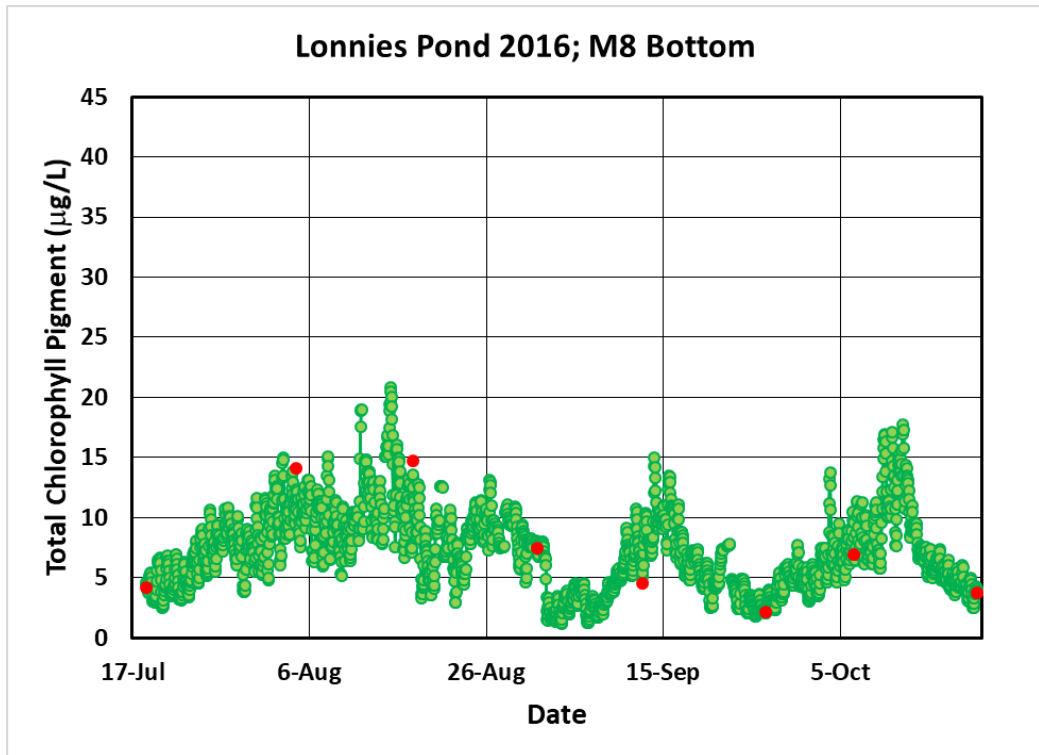


Figure IV.9. Time series chlorophyll measurements at Lonnies Pond Mooring M8 (bottom) located due east of the oyster deployment footprint. Red markers indicate chlorophyll extraction calibration points. Green triangles indicate chlorophyll extractions conducted at Mooring M6 located in the middle of the deployment footprint (top panel 2016 for comparison).

Light pendants were used to measure light intensity in units of $\mu\text{E}/\text{m}^2/\text{s}$ every 10 minutes and profiles were used to calculate the percent surface irradiance and the light extinction coefficient (k) within the water column associated with the oyster deployment in 2016. The light extinction coefficient, k was calculated using the Beer-Lambert Law which describes the logarithmic decay of light through a medium; the larger the light extinction coefficient, the more rapid the loss of light through the water column and represents a greater degree of turbidity. In contrast, small light extinction coefficients reflect greater light transmission through the water column and less turbidity. The mean daily light intensity was calculated using only the active photoperiod. In addition to the chlorophyll a samples collected for water quality and sonde calibration, total suspended solids (TSS), samples were collected to determine whether inorganic water column constituents could be responsible for changes in water clarity.

The average light characteristics, TSS, and chlorophyll concentrations are shown below (Table IV.1). Stations 5, 6, and 7 were within the oyster area and the bottom TSS values mirror the chlorophyll concentrations of these stations; however, the shallow water and variable shading caused by the moving bags makes light interpretation difficult. The highest light penetration and coinciding lowest light extinction coefficient was located at the site furthest away from the oysters, Lonnie 8, while also having the lowest chlorophyll concentration (Table IV.1). Note that station Lonnie 8 had additional surface samples collected. In contrast, when looking at only the data from stations in the oyster area, Lonnie 5, 6, 7, the lowest chlorophyll and lowest TSS ($10.05 \mu\text{g}/\text{L}$, $9.33 \text{ mg}/\text{L}$ respectively) were found directly in the middle of the oysters, Lonnie 6. The chemical data represented specially as chlorophyll concentration and total suspended solids show these parameters are reduced within the oyster area. It appears that bag shading reduced the utility of the light records, so they were not deployed in 2017. The physical and chemical data support the data from the sondes; however, it was clear that the light data was influenced bag shading. Analysis of wind, rain, and tidal stage did not provide insight into what other factors might be influencing the light data.

Table IV.1: Results from the continuous light profiles measurements from Lonnie Pond showing the mean light intensity, penetration, and the light extinction coefficients along with the total suspended solids (TSS) and total chlorophyll pigments at each station.

Station	Depth	Light Intensity ($\mu\text{E}/\text{m}^2/\text{s}$)	Mean Penetration	Light Extinction Coefficient, k	TSS (mg/L)	Total Chlorophyll Pigments ($\mu\text{g}/\text{L}$)
Lonnie5 (east)	Surface	249.74				
Lonnie5 (east)	Bottom	88.00	35.21%	1.66	10.99	11.13
Lonnie6 (oysters)	Surface	216.94				
Lonnie6 (oysters)	Bottom	44.42	20.74%	2.41	9.33	10.05
Lonnie7 (west)	Surface	156.47				

Lonnies7 (west)	Bottom	42.95	33.28%	2.39	15.64	15.54
Lonnies8 (mid)	Surface	191.06			10.39	16.62
Lonnies8 (mid)	Bottom	95.53	55.33%	0.92	16.57	9.00

Section V. Particle Capture and Biodeposit Production by Oysters

Oysters, as well as other sessile filter feeders (e.g., barnacles, sponges), increase water column clarity by filtering out particulates which are later released in biodeposition (Newell et al. 2005). The suspended particulate matter consists of photosynthesizing microscopic organisms (phytoplankton), dead particulate organic matter (detritus), and bacteria, which typically colonize the phytoplankton and detritus (Newell et al. 2002). Oysters selectively digest nitrogen-rich particles and reject the less-nutritious and inorganic particles as pseudofeces (Newell et al. 2004; Newell and Jordan 1983). Nutrients from captured foods may be assimilated into biomass (See Figure V.1) (Higgins et al. 2011). The particulates passing through the digestive system are finally deposited as feces and the rejected material deposited as pseudofeces which together are termed biodepositon.

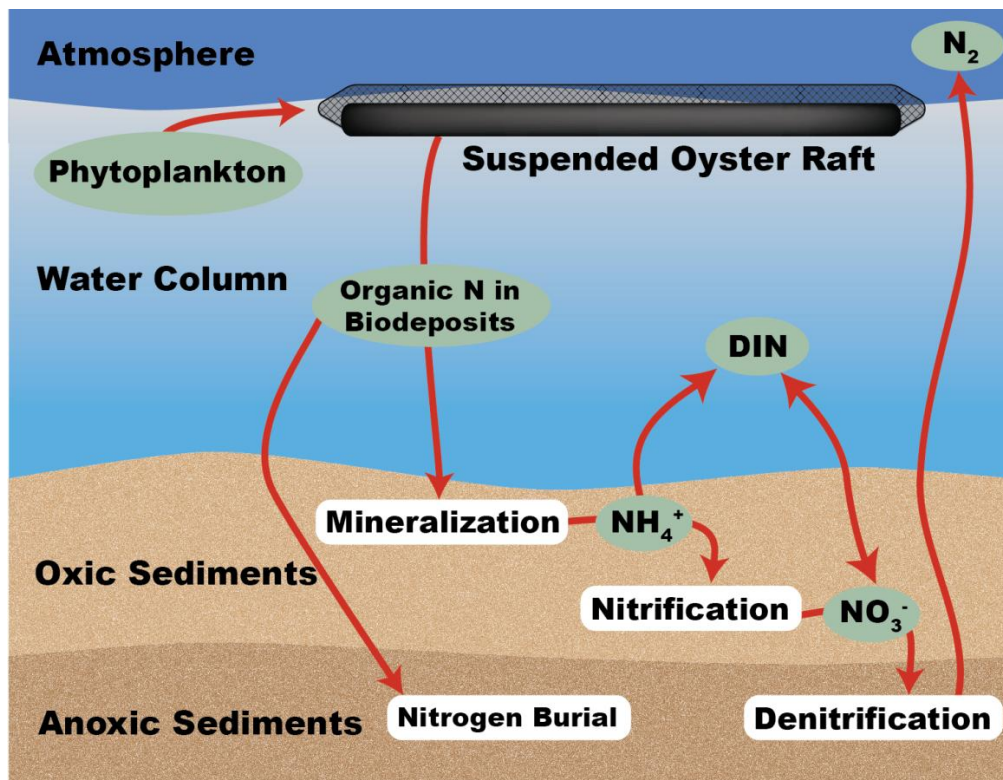


Figure V.1– Diagram of oyster’s role in the marine nitrogen cycle in a shallow water estuary with oxygenated (oxic) and non-oxygenated (anoxic) sediments. White rectangles indicate microbial processes; green ovals indicate nitrogen species (Diagram adapted from Kellogg et al 2013).

Particle Capture and Biodeposition by Oysters

In situ biodeposit traps were used to determine individual oyster biodeposition rates during the 2016 and 2017 oyster growing seasons. Oysters used in the biodeposit traps were allowed to acclimate to the environment for a period of three to five days before measurement. The traps were operated for 24 hours to span a full day/night cycle and two tidal cycles. The oysters were positioned in sets of four on a rectangular PVC platform (30 cm x 20 cm; 4 mm thickness). Eight collection containers were installed beneath the platform which are capped with funnels fitted on each side of the oysters to capture and direct feces and pseudofeces into the collection containers (Figure V.2). To account for ambient particle settling into the collection containers, a control apparatus (with oyster shells) was deployed alongside the treatment group. Additionally, small mesh plastic screens were positioned over the biodeposit trap to prevent shrimp and small fish from entering and disturbing or consuming settled biodeposits. The traps were deployed once in October 2016 and on five dates during the 2017 field season. Oysters used in the biodeposit traps were second year oysters from the Lonnie's Pond aquaculture oyster population.



Figure V.2. Biodeposit trap deployed in Lonnie's Pond, photo by E. Karplus.

Ambient surface water temperature measurements and samples were collected upon trap deployment and removal. These surface water samples were processed for total suspended solids (TSS), particulate organic carbon and nitrogen, total chlorophyll-*a* and salinity. TSS filters were analyzed for particulate organic carbon and nitrogen using a Perkin Elmer 2400 elemental analyzer.

Biodeposit traps collect feces and pseudofeces deposited by each oyster. The collection containers were returned to the laboratory where the biodeposits were given time to settle in the pre-weighed containers, at which point the over lying water layer was siphoned away. Biodeposit samples were weighed, dried to a constant weight, and then processed for particulate organic carbon and nitrogen content. Oysters deployed in the biodeposit traps were collected to measure shell length, whole oyster weight, wet tissue / shell weight, and dry tissue / shell weight. Biodeposition rates were calculated based on the collected mass of feces and pseudofeces minus the mass of ambient particulates collected in the control traps and the deployment duration. The biodeposition rates were standardized to a 1-gram (dry tissue weight) of associated oysters based on the following allometric relationship:

$$Y_s = Y_e (1/W_e)^b,$$

where Y_s and Y_e are the standardized and measured biodeposition rates, respectively, W_e is the dry tissue weight of the measured oyster, and b is the allometric exponent 0.58 (Cranford et al. 2011).

By standardizing the individual biodeposition rates it was possible to use the total dry tissue biomass determined for the Lonnie's Pond oyster deployment to expand the biodeposition rate to include the total oyster biomass deployed in Lonnie's Pond.

The biodeposit measurements (total mass and particulate organic carbon and nitrogen collected per day) and surface water parameters (total suspended solids and particulate organic carbon and nitrogen) were used to determine the mass of particulate organic nitrogen settling from the suspended oyster bags.

Results:

Table V.1. 2017 biodeposition rates, biodeposit characteristics, and surface water column conditions associated with particle trap deployment. Deposition Rates: mean (\pm SE) total oyster deployment biodeposition rates and particulate organic nitrogen (PON) deposition. Biodeposit Characteristics: mean (\pm SE) particulate organic carbon (POC) and particulate organic nitrogen (PON) content and carbon/nitrogen mass ratio (C:N) of collected biodeposits. Surface Water Column Conditions: mean (\pm SE) surface water total suspended solids (TSS), particulate organic carbon (POC), particulate organic nitrogen (PON), carbon/nitrogen mass ratio (C:N), total chlorophyll- a , temperature, and salinity at particle trap locations.

	8/1/17	8/17/17	9/14/17	10/5/17	10/30/17
Deposition Rates					
Biodeposition (kg dry wt. day ⁻¹)	18.1 (2.1)	56.0 (9.8)	21.9 (3.2)	24.1 (4.6)	30.6 (1.6)
Biodeposit N deposition (kg N day ⁻¹)	0.7 (0.2)	2.1 (0.4)	0.8 (0.1)	0.4 (0.1)	0.5 (0.0)

Biodeposit Characteristics					
C content (%C)	21.1 (0.2)	22.8 (0.7)	21.9 (0.1)	11.8 (0.2)	9.8 (0.4)
N content (%N)	4.0 (0.1)	3.9 (< 0.1)	3.9 (0.1)	1.9 (< 0.1)	1.6 (0.3)
C:N	6.2 (< 0.1)	6.9 (< 0.1)	6.6 (< 0.1)	7.3 (< 0.1)	7.1 (0.2)
Surface Water Column Conditions					
TSS (mg dry wt./L seawater)	19.2 (4.7)	6.8 (0.9)	5.7 (0.2)	5.0 (0.5)	5.4 (0.1)
POC (uM C)	138.3 (42.2)	101.3 (9.4)	93.8 (2.1)	47.8 (1.4)	68.9 (10.5)
PON (uM N)	18.3 (5.1)	13.2 (2.1)	13.2 (1.2)	6.8 (0.2)	7.9 (0.9)
C:N	7.3 (0.3)	7.9 (0.6)	7.3 (0.8)	7.1 (0.1)	8.6 (0.4)
Total Chlorophyll- <i>a</i> (ug/L seawater)	16.7 (8.6)	15.9 (3.5)	12.9 (0.6)	7.3 (0.1)	8.9 (3.4)
Temperature (Celsius)	23.6 (0.8)	22.5 (0.8)	20.6 (0.6)	15.8 (0.3)	12.8 (0.6)
Salinity	29.5 (0.0)	29.5 (0.2)	28.2 (0.2)	29.0 (0.1)	25.7 (1.3)

Greater seston concentrations and seston organic content were observed in the surface waters during the summer biodeposit trap deployments relative to the fall trap deployments (Table V.1). Greater organic content is associated with higher oyster food quality, but oyster feeding rates appear to stabilize once food concentrations reach ca. 300 µg C/L seawater (Tenore and Dunstan 1973). Observed food concentrations were greater than 300 µg C/L seawater in 2017 with a surface water maximum of 2559 µg C/L during the 8/1/17 biodeposit trap deployment and a minimum of 538 µg C/L seawater during the 10/5/17 biodeposit trap deployment. During periods when particulate organic carbon concentrations are above 300 µg C/L in surface waters, the oysters will reject more and more captured particulate matter as pseudofeces, which is less densely “packaged” than fecal matter/pellets (Tenore and Dunstan 1973). Because the pseudofeces is less dense than fecal pellets it is more likely to be affected by turbulence and lost from the biodeposit trap. Determining *in situ* biodeposition rates means compromising between eliminating environmental factors that interrupt biodeposit collection and maintaining natural environmental conditions. For this reason, biodeposition rates are a conservative estimate of the mass of organic nitrogen transferred from the water column to the sediment through oyster filtration and deposition of feces and pseudofeces.

Table V.2. Full oyster deployment biodeposition summary for the 2017 field season. Full oyster deployment deposition rates and biodeposit carbon and nitrogen content were averaged from May 2017 to December 2017 to determine the season total dry mass of biodeposits impacting the sediments, as well as, the total dry mass of POC and PON contained within the biodeposits.

	Whole Field Season: May 2017 – December 2017
Biodeposit dry mass (kg dry wt.)	4984.8 (163.7)
Biodeposit POC (kg dry wt.)	870.6 (0.5)
Biodeposit PON (kg dry wt.)	151.2 (0.1)

In Table V.2. (above), the whole field season deposition was calculated as follows: 1) determined a dry tissue weight corrected biodeposition rate (mg dry wt. biodeposits/day) for each oyster cohort and month that the oysters were deployed, 2) multiplied the dry tissue weight corrected biodeposition rate by the number of days per month, and 3) summed the mass of biodeposits calculated for each month.

Table V.3. 2016 biodeposition rates, biodeposit characteristics, and surface water column conditions associated with particle trap deployment. Deposition Rates: mean (\pm SE) total oyster deployment biodeposition rates and particulate organic nitrogen (PON) deposition. Biodeposit Characteristics: mean (\pm SE) particulate organic carbon (POC), particulate organic nitrogen (PON), and carbon/nitrogen molar ratio (C:N) of collected biodeposits. Surface Water Column Conditions: mean (\pm SE) surface water total suspended solids (TSS), particulate organic carbon (POC), particulate organic nitrogen (PON), carbon/nitrogen mass ratio (C:N), total chlorophyll-*a*, temperature, and salinity at particle trap locations.

	Summer 2016*	Fall 2016
Deposition Rates		
Biodeposition (kg dry wt. day ⁻¹)	35.1 (5.7)	31.7 (6.0)
Biodeposit N deposition (kg N day ⁻¹)	0.6 (0.1)	0.3 (0.1)
Biodeposit characteristics		
C content (%C)	12.0 (0.3)	7.4 (0.7)
N content (%N)	1.6 (0.0)	0.9 (0.2)
C:N	7.3 (0.1)	9.8 (1.3)
Surface Water Column Conditions		
TSS (mg dry wt./L seawater)	8.9 (0.8)	7.2 (0.7)
POC (uM C)	144.6 (17.7)	52.7 (4.8)
PON (uM N)	22.9 (3.3)	6.7 (0.6)
C:N (molar ratio)	6.33 (0.12)	7.92 (0.07)
Total Chlorophyll- <i>a</i> (ug/L seawater)	23.2 (2.8)	8.0 (1.3)
Temperature (Celsius)	30.5 (0.3)	17.3 (0.7)
Salinity	24.2 (1.7)	28.7 (0.0)

* Summer 2016 data is from a 8/13/16 biodeposit trap deployment conducted in Little Pond, Falmouth, MA. Ambient water column conditions were comparable between Lonnie's Pond and Little Pond in August 2016.

Discussion:

Overall, the filtration of particulates from Lonnie's Pond waters and its packaging into feces and pseudofeces appears to support a large amount of biodeposition to bottom sediments. This

provides the mechanism for the observed increase in water clarity within the oyster grow-out area, the reduction in particulate concentrations in water flowing through the oyster bags, and the increases in sediment respiration associated with the oyster deployment area. While all of these features are supportive of potential enhanced nitrogen removal, none result in significant negative impacts to the associated pond water column and sediments. As seen in the water quality results the chlorophyll concentrations were elevated within the oyster deployment area, but the effects disappeared by the border of the oyster rafts. This suggests that increases in local production stimulated by the increased biodeposition of organic material to the sediments and excretion of inorganic nitrogen were more than offset by oyster removal of plankton with the net effect of increasing the overall water quality which extended a significant distance outside of the deployment area. In addition it is notable that biodeposition rates were similar in summer and fall, even with nearly 3 fold higher chlorophyll levels in summer. This is consistent with there being more than sufficient phytoplankton in Lonnie's Pond for oysters (as indicated above, ca. 300 µg C/L). However, the increased food quality in summer is seen in the much higher nitrogen levels in the biodeposits in summer compared to fall.

Section VI. Biodeposit Impact Area

Although fecal pellets fall rapidly through the water column, variables such as pellet density and current velocity can affect the dispersion of pellets before they settle to the sediment surface. During the 2016 and 2017 sediment flux measurements an Acoustic Doppler Current Profiler (ADCP, Nortek Aquaprobe HR) was deployed near the sediment surface facing upward at the northern edge of the oyster propagation area. Later measurements included deployments near the eastern and western edges of the deployment area. Velocity measurements were made at 10 minute intervals and conducted at the highest possible sensitivity. Velocities measured acoustically are determined by sound reflected off particles within the water column with the assumption that the particles movement is also indicative of water movement. The particles in the vicinity of the oyster rafts are comprised of a mixture of fecal pellets and phytoplankton, with the latter being numerically dominant. Therefore, measured velocities are probably biased towards under estimation since the larger sized fecal pellets would tend to settle faster and move less laterally. The underestimation would be seen in vertical velocities as well since the velocity of falling fecal pellets would be confounded by phytoplankton with a lower settling velocity. Taking these caveats into consideration, the measured velocities should be regarded as conservative.

Velocity measurements completed in 2017 were consistent and generally confirmed measurements made in 2016 with the exception that the Nortek device is more sensitive than the ADCP used in 2016 so that more accurate directionality was available in 2017 (Figure VI.1). As seen in the time series velocity plot, the high velocities were recorded during flooding tides. During other parts of the tidal cycle velocities were minimal. Over the tidal period average water column current direction roughly correlated with prevailing wind direction, North-Northwest. However, longer deployments at the start of the 2018 season at the margins of the

eastern and western borders of the array indicated that average water column current direction, which were in shallower water, were predominately oriented east to west through the oyster deployment array.

Using the previously determined mean sinking velocity of fecal pellets (8.14 ± 5.01 mm/s) and the median depth around the border of the oyster deployment area, fecal pellet settling was modeled step-wise assuming fecal pellet production was similar among all bags. (Figure VI.2). The model results largely confirm the results from 2016 concerning the extent of the impact area of surficial sediments. At 5 meters from the edge of the deployment area deposition remains at 30 % of that directly under the deployment area and declines to 10%-15% at 10 meters. With the additional data captured in 2018 the impact area would extend around the entire deployment area with the same distances and gradients.

From the above discussion of biodeposition, a deposition value of $710 \text{ mg N/m}^2/\text{d}$ was derived. Applying this calculated distribution of nitrogen derived from oyster fecal pellets to the actual footprint of the oyster deployment area, the area affected (impact area) increases from 760 m^2 , actually occupied by the oyster bags, out 11.75 m from the edge to where there was no longer direct contribution of fecal pellets to the sediment surface. Including the 11.75 m extension beyond the edges of the deployment area, the total impact area was calculated to be 2735 m^2 , an increase of 448 m^2 over the estimated 2016 area of 2287 m^2 , which was determined using less sensitive instruments (Figure VI.3). These spatial estimates for biodeposition represent direct settling to sediments, but do not include bordering areas that may be effected by particle transport along the sediment surface post-deposition. These direct deposition areas and extended areas of deposition were used to determine the enhanced particulate nitrogen loading to Lonnie's Pond sediments from the deployed oyster arrays and to target areas to assess potential enhancements of sediment denitrification.

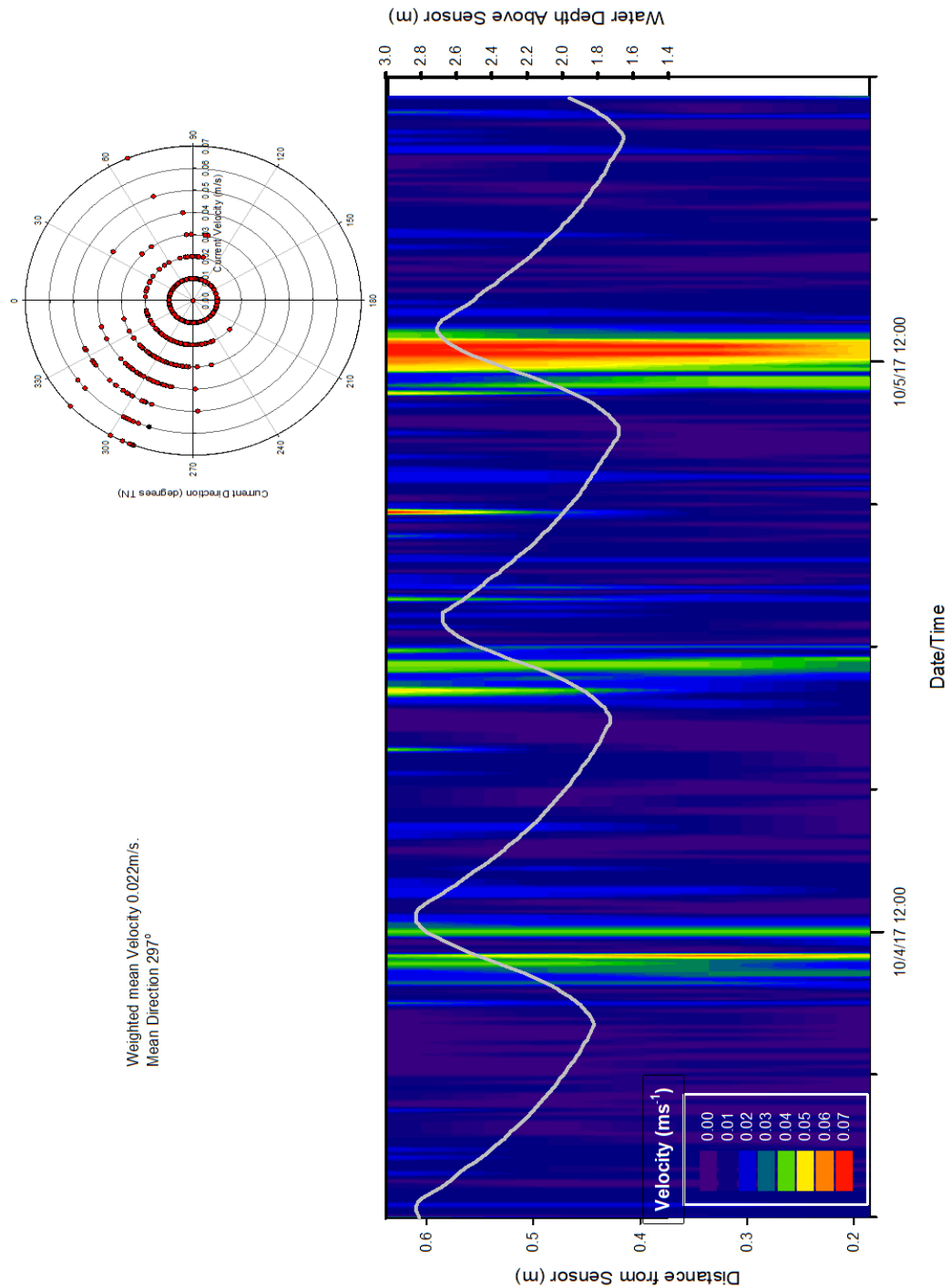


Figure VI.1. Top left: Compass rose plot of particle direction and velocity data for 23-24 August. Current direction was predominantly to the north north-west ~300 from True North . Velocities increase with distance from the center of the compass rose. Highest velocity was 0.07m/s (7cm/s). Right: Time series velocity data from the ADCP are shown at 10cm intervals above the ADCP which was moored to the bottom. Velocities at specific times and heights above the ADCP are displayed as different colors with purple indicating no water movement and red indicating the maximum observed velocity of 0.07 m/s. For reference the tidal height change in water depth is shown as a continuous white line indicating the depth of water above the ADCP.

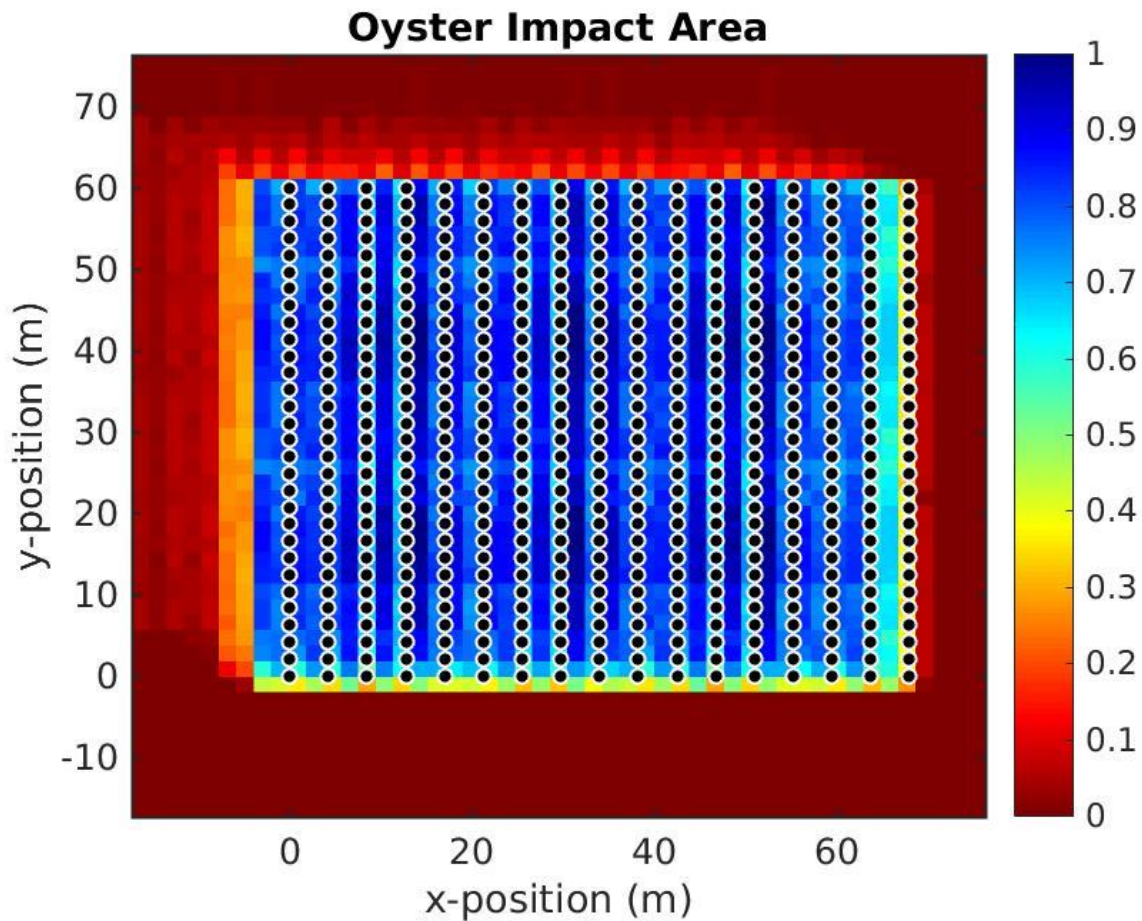


Figure VI.2. Expected deposition of fecal pellets to surficial sediments for pellets originating at the edge of the oyster array. Fecal pellet production was given as 1 and results are shown in the contours as a fraction of the pellet production. Thus, deposition areas colored dark blue received 100% of the average areal fecal pellet production and deposition; areas colored dark red received 0% of the average areal fecal pellet production and were not directly impacted by the oyster deployment. Note that the dimensions of the model grid are equal in both directions, but this has no impact on the deposition gradients at the edges of the model deployment area.

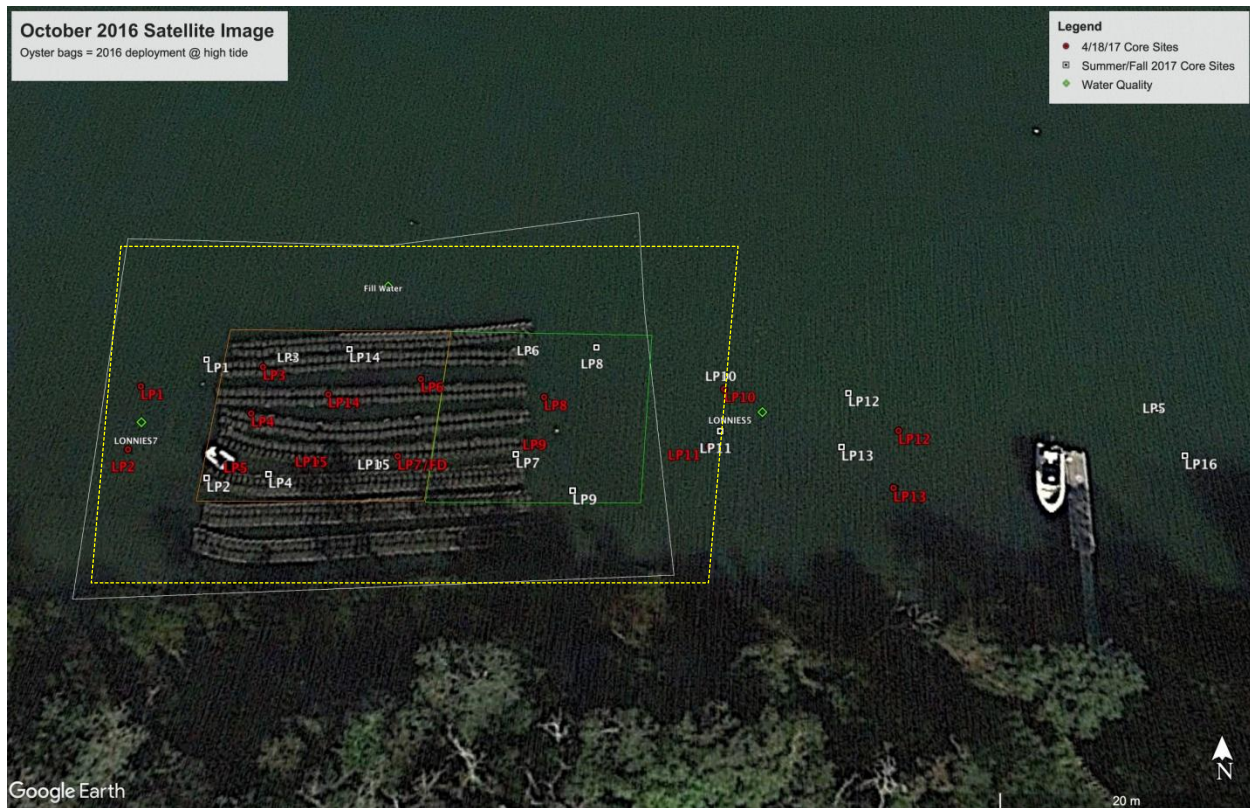


Figure VI.3. 2016 Aerial photograph showing the location of the oyster deployment area overlain with 2017 oyster deployment area (red and green polygons). Mooring and water column sampling locations (green diamonds), April (pre-oyster deployment red diamonds) and Summer/Fall (white square) flux core locations. Total area impacted by the oyster deployment as determined by fecal pellet distribution in 2016 is shown as the white bounded area while the 2017 total impact area is bounded by the yellow dashed area.).

Section VII. 2016 Nitrogen Cycling and Oyster Culture: Regeneration and Denitrification

In estuarine systems such as Lonnie's Pond, nitrogen is transformed and recycled within the sediments and water column. This recycled nitrogen adds directly to the eutrophication of the estuarine waters in the same fashion as watershed inputs. In some systems under MEP investigation, recycled nitrogen can account for about half of the nitrogen supply to phytoplankton blooms during the warmer summer months. It is during these warmer months that estuarine waters are most sensitive to nitrogen loadings. Failure to account for this recycled nitrogen generally results in significant errors in determination of the effects of nitrogen loadings, the overall nitrogen balance of the system and how oyster propagation may affect nitrogen dynamics at the sediment water column interface.

The organic rich nature and relatively shallow waters of coastal systems like the Lonnie's Pond

sub-basin of the Pleasant Bay Estuary and others on Cape Cod result in sediments playing a significant role in system biogeochemical cycles. Organic matter deposition to sediments, hence benthic respiration, tends to decrease with increasing depth of overlying waters due to interception by water column heterotrophic processes resulting in lower deposition of labile (decomposable) organic matter. The result is that embayment respiration rates are typically many-fold higher than in the adjacent deeper offshore waters. With potential stratification of embayment waters, sediment metabolism plays a major role in bottom water oxygen declines (an ecosystem structuring parameter). This applies particularly to Lonnie's Pond which periodically goes hypoxic (i.e. low D.O) during the summer months. It should be noted that while water depth is important in altering the deposition of labile organic matter to sediments, filter feeders and especially large filter feeders like oysters can overwhelm the "depth effect" due to the large amount and size of packaged feces that they emit. In these situations, oysters are projected to increase deposition which subsequently increases sediment respiration rates.

Measurements of Benthic Nutrient Regeneration, Denitrification and Sediment Oxygen Uptake:

In order to determine the contribution of sediment regeneration to nutrient levels within the oyster aquaculture portion of the Lonnie's Pond system and the effect the oysters may have on nitrogen recycling rates and oxygen levels, sediment samples were collected and incubated under *in situ* conditions on 5 dates, 4/18, 6/27, 8/11, 9/19 and 10/3. The April 18, 2017 sampling occurred prior to the oyster deployment which began on April 5 and concluded on June 22. Thus the June 27 sampling represents the first sediment sampling of 2017 with a full complement of oysters and their associated biodeposition. The August 11 and September 19, 2017 sampling dates were during the period of maximum oyster activity in the summer interval (July- September) and the October 3, 2017 sampling was during the period of maximum oyster biomass (October-December). The April 18, 2017 sampling did not include deposition from 2017, but did include the residual effect from the 2016 deployment. The 2016 oysters were held over winter and replaced in 2017, but since their late 2016 season depositions were not fully degraded in the sediments, due to the cold temperatures (December – March), they were available to stimulate nitrogen cycling in the early spring 2017. Therefore the early spring denitrification enhancement needed to be measured to determine: 1) its magnitude and 2) if there were sufficient carry over to require addition to the 2016 deployment impacts and rates.

Time series measurements of total dissolved nitrogen, nitrate+nitrite, ammonium and ortho-phosphate were made on each incubated core sample. The rate of oxygen uptake was also determined in order to: (1) evaluate sensitivity to oxygen depletion of the oyster aquaculture area of Lonnie's Pond, (2) rank sediments as to organic matter deposition rates (not possible using organic content) and (3) develop a nitrogen model for how the oysters may be affecting the nitrogen cycle in the sediments associated with oysters. Assays were performed on 16 cores from sites distributed throughout the oyster aquaculture area on each date. Cores were collected directly under the oyster aquaculture rafts and at distances east and west of the aquaculture area.

The results allowed determination of the spatial pattern and rate of nutrient exchanges from the sediments to the water column and how these rates may be affected by the cultivation of oysters in Lonnie's Pond. From our experience, sediment regeneration during the summer is a large and important source of nutrients supporting both phytoplankton and macroalgal blooms in embayments throughout S.E. Massachusetts and the degree to which intensive oyster aquaculture can change those rates through enhancement of denitrification needs to be determined to support innovative management of these systems.

N₂ excess was measured using membrane-inlet mass spectrometry (MIMS). N₂ produced by denitrification is precisely detected by analysis of its ratio with the inert gas Argon. Water samples were collected and stored to prevent gas exchange or bubble formation. In the laboratory, sample water was pumped at ml/min rates through a gas permeable membrane in order to extract gas into the mass spectrometer inlet. The inlet was fitted with cryogenic traps to remove water vapor and CO₂ gas. Sample gas was analyzed by the mass spectrometer for masses 28 and 40 for determining the N₂ to Ar ratio. Calibration was made by comparison with a reference gas of known composition. A quadrupole mass spectrometer (e.g. Pfeiffer 422) was used for its sensitivity and speed of analysis and the analysis of the samples conforms to the same methods as was utilized during a comprehensive survey undertaken by the CSP in 2008. Water column respiration measurements were collected east and west of the oyster area at the Lonnie's 5 and Lonnie's 7 water quality monitoring sites.

Results:

Benthic results are summarized in Table VII.1 below. April cores were collected approximately six weeks prior to the deployment of the oyster rafts. The second set of cores was collected in late June just after the oyster rafts were fully deployed. Both the August and September cores were collected to coincide with peak growing season and the time during which the most stressed water quality conditions were expected. Finally, October cores were collected near the end of the growing season when the full effects of oyster culture could be determined. For comparison, the 2016 values are shown appended to the 2017 data in Table VII.1. To more easily view the data through the season the averages of the cores impacted by the oysters are shown in Table VII.2. The table also includes corresponding values for oyster impact area in 2016, during August 2016, when water temperatures were greatest. For comparison the 2016 data is also presented with values adjusted conservatively by a factor of 1.9 to correct for temperature differences to allow cross comparison throughout the season and for comparison to peak 2016 values. Higher temperatures increase bacterial respiration while lower temperatures decrease respiration. The actual factor for Lonnie's Pond, found empirically varies from ~2.3-1.6.

Small scale sediment heterogeneity was common in the oyster deployment area in 2016. Sediment heterogeneity was observed by divers collecting the cores in 2017, however in 2017 significantly smaller differences were observed in the sediment biogeochemistry evaluated in

duplicate cores (LP7/7Dup). Nonetheless specific patterns were immediately obvious. Average sediment oxygen uptake rates were correlated positively with changing temperatures as was NH_4 flux. Nitrate uptake, however, was predominant only during April and October, which were the coolest months and had the lowest sediment oxygen uptake. During all other sampling dates the average nitrate flux was out of the sediment. This suggests that increased ammonium production did result in increased nitrate production but that the nitrate was not fully denitrified, possibly due to a very thin surface oxidized layer, which allowed a larger proportion of the nitrate to escape to the overlying waters, thus the efficiency of nitrate uptake into denitrification may be affected by redox status of the sediment during the most stressful parts of the summer (Figure VII.1)



Figure VII.1 Images of sediment core surfaces from September 2017. Left: Core 3 within treatment area showed dark areas where reduced sediment was exposed to the water surface, thus preventing efficiently coupled nitrification and denitrification. Right: Core 13 outside of the treatment area showed a uniform oxidized surface.

Most importantly, the fraction of regenerated nitrogen used in denitrification was greatest in the shoulder months of summer, 74% and 29%, for April and October, respectively. While oysters had not yet been deployed in April, the residual organic matter from the previous Fall continued to be regenerated as water temperatures began warming accounting for the high rates observed. Low rates in June may represent a temporary deficit in sediment carbon between exhaustion of the previous year deposition, prior to a buildup of the current year's deposition.

Twice as many stations were sampled for benthic processes in 2017 than in 2016 to better quantify both background rates as well as treatment rates of sediment flux including denitrification. A conservative estimate of the total nitrogen removed by oyster enhanced denitrification can be made using our measurements of background denitrification with the

rates in the oyster deployment area. The background rates may be slightly high as spreading of the biodeposits by water currents was the only process examined. The 2017 background rates and rates measured under the oyster treatment are shown in Table VII.3 as well as the 2016 rates for comparison. By summing the product of the measured rates of denitrification by the intervals between the denitrification measurements and extending measurements forwards to Nov 15 when temperatures began to become too cold for significant oyster activity in both years it is possible to obtain an annual mass of nitrogen that was denitrified and compare that value to the total mass of nitrogen removed from the system by incorporation into oyster tissue. The annual enhanced denitrification resulted in a net loss of 9.7 kg N in 2017 compared to 9.54 kg N in 2016 (Table VII.4). Although the 2017 value was slightly larger than seen in 2016 both the impact area and the deployment duration were greater in 2017 than in 2016. The differences in the number of oysters, the size of the oysters, and the quantity of food (phytoplankton) available are all potential factors that can affect these results. The effect of all these factors is integrated environmentally and reflected in two primary variables: the incorporation of nitrogen into oyster tissue and the enhanced biodeposition of organic matter within the impact area. Examining these two variables (Table VII.4) we found that while enhanced denitrification was similar, the amount of nitrogen removal through assimilation in oyster tissue declined slightly from 2016 to 2017. However, denitrification, as a percent of nitrogen removal increased from 24.7% to nearly 36%. This result suggests that after a year of oyster culture the sediments were more enriched in organic matter and thus the ability to denitrify was enhanced. This result in 2017 can be partly attributed to carry over of deposition from the previous season oyster culture as seen in the high rates of denitrification in April 2017.

Table VII.1. Summary of benthic flux rates from core incubations conducted in August and October. The bottom panel shows October rates adjusted using a Q10 factor of 1.9 for direct comparison to August cores which were incubated at a temperature 10C warmer.

4/18/2017, Temperature 12.7 C							
Site ID	SOD (mMoles/m2/d)	NH4 (mMoles/m2/d)	NO3 (uMoles/m2/d)	DIN (uMoles/m2/d)	N2-N (uMoles/m2/d)	Total N Cycled (uMoles/m2/d)	Denitrified % Total Cycled N
LP1	33.05	0.03	-0.06	-0.03	2.44	2.53	97%
LP2	44.43	0.52	0.08	0.60	1.03	1.63	63%
LP3	66.73	1.23	-0.08	1.15	1.41	2.73	52%
LP4	113.37	1.38	-0.18	1.20	6.10	7.67	80%
LP5	62.83	1.75	-0.03	1.72	2.09	3.87	54%
LP6	71.46	0.20	-0.16	0.04	0.75	1.11	68%
LP7	ND	0.06	-0.03	0.03	ND	ND	ND
LP7 FD	71.13	-0.05	-0.22	-0.27	0.72	0.99	73%
LP8	37.68	0.00	-0.12	-0.12	0.89	1.01	88%
LP9	61.09	0.61	0.20	0.81	1.72	2.53	68%
LP10	22.01	-0.31	-0.09	-0.40	1.20	1.59	75%
LP11	29.66	-0.09	-0.07	-0.16	1.10	1.26	87%
LP12	36.84	-0.14	-0.11	-0.24	1.15	1.39	83%
LP13	44.92	-0.18	-0.10	-0.27	0.65	0.92	70%
LP14	55.52	-0.16	-0.09	-0.26	2.40	2.66	90%
LP15	42.90	-0.31	-0.08	-0.39	0.23	0.62	37%
6/27/2017, Temperature 22.1 C							
Site ID	SOD (mMoles/m2/d)	NH4 (mMoles/m2/d)	NO3 (uMoles/m2/d)	DIN (uMoles/m2/d)	N2-N (uMoles/m2/d)	Total N Cycled (uMoles/m2/d)	Denitrified % Total Cycled N
LP1	118.47	4.58	0.03	4.61	0.85	5.46	16%
LP2	105.29	5.02	0.14	5.16	1.93	7.09	27%
LP3	130.52	14.17	0.01	14.18	0.23	14.41	2%
LP4	134.04	12.33	0.04	12.37	1.08	13.45	8%
LP5	51.73	6.59	0.15	6.74	-2.53	9.27	-27%
LP6	239.20	5.43	0.44	5.87	1.12	6.99	16%
LP7	64.53	3.45	0.07	3.53	ND	ND	ND
LP8	60.98	3.90	0.25	4.15	-4.67	8.82	-53%
LP9	81.37	1.08	0.04	1.12	-4.05	5.17	-78%
LP10	68.06	0.26	0.05	0.30	-3.77	4.08	-93%
LP11	69.29	2.73	0.06	2.79	-2.74	5.53	-50%
LP12	169.23	7.84	0.11	7.95	-2.62	10.57	-25%
LP13	69.87	5.66	0.21	5.87	-2.71	8.59	-32%
LP14	138.06	11.18	0.40	11.58	1.08	12.66	9%
LP15	152.34	10.98	0.03	11.01	1.00	12.01	8%
LP16	101.77	6.17	0.40	6.56	0.69	7.26	10%
8/1/2017, Temperature 23.6 C							
Site ID	SOD (mMoles/m2/d)	NH4 (mMoles/m2/d)	NO3 (uMoles/m2/d)	DIN (uMoles/m2/d)	N2-N (uMoles/m2/d)	Total N Cycled (uMoles/m2/d)	Denitrified % Total Cycled N
LP1	58.14	8.69	-0.04	8.65	2.16	10.89	20%
LP2	72.81	6.41	-0.03	6.37	0.83	7.27	11%
LP3	176.21	26.93	-0.10	26.83	2.91	29.94	10%
LP4	124.63	11.47	0.15	11.63	2.58	14.20	18%
LP5	63.99	4.88	0.71	5.59	2.78	8.37	33%
LP6	101.91	7.76	2.70	10.47	4.75	15.21	31%
LP7	60.31	2.67	-0.04	2.63	ND	ND	ND
LP8	64.18	3.96	0.17	4.13	0.44	4.58	10%
LP9	112.24	7.38	0.44	7.83	1.06	8.89	12%
LP10	39.20	4.52	0.07	4.59	1.06	5.64	19%
LP11	222.07	9.72	0.26	9.98	3.40	13.38	25%
LP12	48.26	5.96	0.14	6.10	2.37	8.47	28%
LP13	92.45	0.98	0.06	1.04	3.04	4.09	74%
LP14	120.25	11.99	-0.08	11.90	0.58	12.65	5%
LP15	121.55	11.70	-0.10	11.60	2.33	14.13	17%
LP16	84.49	0.92	-0.05	0.88	2.55	3.52	72%

9/19/2017 Dark, Temperature 23.5 C							
	SOD	NH4	NO3	DIN	N2-N	Total N Cycled	Denitrified
Site ID	(mMoles/m2/d)	(mMoles/m2/d)	(uMoles/m2/d)	(uMoles/m2/d)	(uMoles/m2/d)	(uMoles/m2/d)	% Total Cycled N
LP1	83.42	6.89	0.07	6.97	-0.32	7.28	-4%
LP2	232.99	17.03	0.03	17.05	2.32	19.37	12%
LP3	116.82	4.91	0.05	4.96	0.37	5.33	7%
LP4	123.30	11.67	0.09	11.76	0.20	11.96	2%
LP5	53.60	5.78	0.16	5.94	0.30	6.24	5%
LP6	74.33	4.86	0.05	4.91	0.27	5.18	5%
LP7	61.67	1.81	0.05	1.86	ND	ND	ND
LP8	60.67	9.53	0.65	10.18	0.84	11.03	8%
LP9	49.81	2.52	0.13	2.65	-0.30	2.95	-10%
LP10	67.00	6.00	0.12	6.13	0.08	6.21	1%
LP11	79.18	4.76	0.31	5.06	0.39	5.45	7%
LP12	69.65	4.39	0.48	4.87	0.16	5.03	3%
LP13	82.69	5.31	0.12	5.43	4.21	9.64	44%
LP14	92.17	5.28	0.04	5.31	0.32	5.63	6%
LP15	59.95	3.55	0.06	3.61	-0.88	4.50	-20%
LP16	57.99	3.84	0.40	4.24	0.11	4.35	3%
9/19/2017 Light, Temperature 23.5 C							
	SOD	NH4	NO3	DIN	N2-N	Total N Cycled	Denitrified
Site ID	(mMoles/m2/d)	(mMoles/m2/d)	(uMoles/m2/d)	(uMoles/m2/d)	(uMoles/m2/d)	(uMoles/m2/d)	% Total Cycled N
LP1	78.09	0.22	-0.14	0.08	1.76	2.12	83%
LP2	154.22	7.65	-0.06	7.58	8.18	15.90	51%
LP3	109.51	15.71	-0.06	15.65	18.89	34.66	55%
LP4	102.15	9.79	0.02	9.81	12.22	22.03	55%
LP5	42.16	4.50	-0.06	4.44	5.17	9.73	53%
LP6	68.07	5.71	-0.13	5.58	5.96	11.80	51%
LP7	62.32	2.45	-0.04	2.41	ND	ND	ND
LP8	69.14	13.04	0.44	13.48	15.68	29.16	54%
LP9	62.60	0.64	-0.12	0.52	2.84	3.61	79%
LP10	78.93	4.99	0.02	5.01	5.49	10.50	52%
LP11	71.75	3.32	0.06	3.38	4.11	7.49	55%
LP12	49.93	5.33	0.09	5.42	5.97	11.38	52%
LP13	81.32	2.27	-0.11	2.16	3.25	5.63	58%
LP14	101.79	10.57	-0.21	10.36	13.86	24.65	56%
LP15	82.53	5.17	-0.07	5.11	7.06	12.30	57%
LP16	45.86	2.35	0.19	2.54	2.77	5.31	52%
10/3/2017, Temperature 17 C							
	SOD	NH4	NO3	DIN	N2-N	Total N Cycled	Denitrified
Site ID	(mMoles/m2/d)	(mMoles/m2/d)	(uMoles/m2/d)	(uMoles/m2/d)	(uMoles/m2/d)	(uMoles/m2/d)	% Total Cycled N
LP1	73.18	3.92	-0.12	3.79	2.02	6.07	33%
LP2	52.99	1.80	-0.27	1.53	2.95	5.01	59%
LP3	117.51	6.13	-0.37	5.75	1.71	8.21	21%
LP4	21.49	0.99	0.12	1.11	0.56	1.67	33%
LP5	39.66	0.52	-0.15	0.38	1.06	1.73	61%
LP6	42.03	2.97	-0.12	2.85	0.57	3.66	16%
LP7	36.52	3.57	0.05	3.62	ND	ND	ND
LP8	27.04	3.20	0.00	3.20	0.38	3.58	11%
LP9	37.00	0.59	-0.04	0.54	0.83	1.45	57%
LP10	19.21	0.89	0.00	0.89	0.30	1.20	25%
LP11	53.73	0.90	-0.12	0.79	1.02	2.04	50%
LP12	23.81	0.38	-0.03	0.35	0.20	0.62	33%
LP13	63.86	0.51	-0.15	0.35	1.46	2.12	69%
LP14	101.31	6.63	-0.29	6.34	2.55	9.47	27%
LP15	17.86	4.38	-0.17	4.21	0.86	5.41	16%
LP16	21.82	0.45	-0.07	0.39	0.85	1.37	62%

August 16, 2016; 28 C							
Site ID	SOD (mMol/m2/d)	NH4 (mMol/m2/d)	NO3 (mMol/m2/d)	DIN (mMol/m2/d)	N2-N (mMol/m2/d)	Total N Cycled (mMol/m2/d)	Denitrified % Total Cycled N
LP1	115.3	1.9	-0.03	1.9	5.5	7.4	74%
LP2	139.4	14.0	0.31	14.3	3.8	18.1	21%
LP2 DUP	379.3	36.4	0.18	36.5	1.8	38.3	5%
LP3	68.4	9.5	0.01	9.5	1.3	10.8	12%
LP4	97.9	14.1	0.05	14.2	1.4	15.6	9%
LP4 DUP	150.7	18.8	0.56	19.3	4.0	23.3	17%
LP5	108.7	7.2	0.03	7.2	1.4	8.6	16%
LP6	73.8	7.1	0.08	7.2	1.0	8.2	12%
LP8	55.4	4.6	0.21	4.8	1.2	6.0	20%
October 5, 2016; 18 C							
Site ID	SOD (mMol/m2/d)	NH4 (mMol/m2/d)	NO3 (mMol/m2/d)	DIN (mMol/m2/d)	N2-N (mMol/m2/d)	Total N Cycled (mMol/m2/d)	Denitrified % Total Cycled N
LP1	70.5	0.1	-0.38	-0.3	8.7	9.0	97%
LP2	17.1	5.9	-0.56	5.3	2.3	7.6	30%
LP2 DUP	72.3	0.3	-0.13	0.2	2.7	2.9	94%
LP3	101.8	7.1	0.22	7.3	3.9	11.1	35%
LP4	128.2	2.5	-0.16	2.4	0.7	3.0	23%
LP5	83.3	3.5	0.86	4.4	0.1	4.5	3%
LP6	59.0	-2.5	-0.31	-2.8	4.8	7.6	63%
LP7	31.9	1.3	0.05	1.4	1.5	2.8	52%
LP7 DUP	25.7	-1.1	-0.23	-1.3	2.0	3.3	60%
LP8	45.3	-2.1	-0.54	-2.7	5.1	7.8	66%
October 5, 2016; Adjusted to 28C using Q10=1.9							
Site ID	SOD (mMol/m2/d)	NH4 (mMol/m2/d)	NO3 (mMol/m2/d)	DIN (mMol/m2/d)	N2-N (mMol/m2/d)	Total N Cycled (mMol/m2/d)	
LP1	134.0	0.1	-0.7	-0.6	16.6	17.1	
LP2	32.6	11.2	-1.1	10.2	4.3	14.4	
LP2 DUP	137.3	0.6	-0.3	0.3	5.1	5.4	
LP3	193.4	13.4	0.4	13.8	7.3	21.1	
LP4	243.7	4.8	-0.3	4.5	1.3	5.8	
LP5	158.3	6.7	1.6	8.3	0.2	8.6	
LP6	112.2	-4.7	-0.6	-5.3	9.0	14.4	
LP7	60.6	2.5	0.1	2.6	2.8	5.4	
LP7 DUP	48.9	-2.0	-0.4	-2.5	3.7	6.2	
LP8	86.0	-4.0	-1.0	-5.0	9.7	14.8	

Table VII.2. Summary of benthic flux rates from core incubations conducted in 2017. The bottom panel shows 2017 rates adjusted using a Q10 factor of 1.9 only to allow direct comparison to August 2016 cores which were incubated at a temperature 28C.

2017 Mean Treatment Area Sediment Flux Rates							
Date	4/18/17	6/27/17	8/1/17	9/19/17	9/19/17*	10/3/17	8/16/16
Temperature	12.7 C	22.1 C	23.6 C	23.5 C	23.5 C	17 C	28 C
Rate (mMol/m ² /d)							
SOD	62.42	93.4	82.3	92.4	82.7	46.7	100.6
NH4	0.57	6.4	6.7	7.1	6.5	2.5	9.0
NO3	-0.06	0.1	0.2	0.1	0.0	-0.1	0.1
DIN	0.51	6.5	9.2	7.2	6.5	2.4	9.0
N2-N	1.91	1.0	1.8	0.4	NA	1.2	2.4
Total N Cycled	2.67	7.5	12.0	8.4	NA	3.6	11.4
Denitrified % Total N	71%	14%	15%	5%	NA	32%	21%
*Light Treatment							

2017 Mean Treatment Area Sediment Flux Rates Q ₁₀ =1.9 Reference temperature 28 C							
Date	4/18/17	6/27/17	8/1/17	9/19/17	9/19/17*	10/3/17	8/16/16
Temperature	12.7 C	22.1 C	23.6 C	23.5 C	23.5 C	17 C	28 C
Rate (mMol/m ² /d)							
SOD	166.7	136.3	109.1	123.3	114.0	94.5	100.6
NH4	1.5	9.3	8.8	9.5	8.9	5.0	9.0
NO3	-0.2	0.1	0.2	0.2	0.0	-0.2	0.1
DIN	1.4	9.5	12.2	9.7	8.9	4.8	9.0
N2-N	5.1	1.5	2.4	0.6	NA	2.3	2.4
Total N Cycled	7.1	10.9	15.9	11.2	NA	7.3	11.4
Denitrified % Total N	71%	14%	15%	5%	NA	32%	21%
*Light Treatment							

Table VII.3 Mean denitrification rates for cores collected in the biodeposit impact area associated with the oyster arrays (Treated) and outside the impact area (Background). The difference in these two values should represent the contribution made by the ongoing oyster culture (Oyster Effect).

	2017 Mean Denitrification Rates (mMoles/m ² /d)				Oyster Effect
	Treated		Background		
Date	Mean	Std. Dev.	Mean	Std. Dev.	
4/18/2017	2.9	1.8	0.9	0.4	1.9
6/27/2017	1	0.5	0.3	0.4	0.7
8/1/2017	2.4	1.3	0.8	0.4	1.6
9/19/2017	0.7	0.9	0.2	0.1	0.5
10/3/2017	1.5	0.9	0.8	0.5	0.8

	2016 Mean Denitrification Rates (mMoles/m ² /d)				Oyster Effect
	Treated		Background		
Date	Mean	Std. Dev.	Mean	Std. Dev.	
8/16/2016	2.1	0.6	1	0.4	1.1
10/5/2016	4.1	2.5	1.2	1	2.9
4/18/2017	2.9	1.8	0.9	0.4	1.9

Table VII.4 Annual Nitrogen Removal Budget for the oyster impact area showing including contributions from enhanced denitrification and oyster harvest.

Year	2016	2017
Deployment Duration (days)	146	195
Enhanced Annual DeN2 (mmol/m² N)	298.0	253.3
Enhanced Annual DeN2 (gm/m² N)	4.17	3.55
Impact area (m²)	2287	2735
Total Annual Enhanced DeN2 (gm N)	9541	9699
Total Annual Enhanced DeN2 (kg N)	9.54	9.70
Net Annual N removed by oysters (kg N)	39.1	27.2
Enhanced DeN2 as percent of N removed by oysters	24.4%	35.7%

Recommendations:

It is clear that denitrification adds significantly to the nitrogen removed by oyster harvest. But the previously reported 2016 data allowed only a very conservative estimate due to the lack of data from the following spring. At that time, it was recommended that better estimates can be supported if:

(1) additional time points were added to the denitrification time-series, particularly early and late in the season and in the following spring and early summer. The biodeposits take time to accumulate and will persist for months to 1+ years. Therefore, the period of enhanced denitrification is almost certainly longer than a single season that the oysters are in place. This was implemented in 2017 with a multi-year deployment with measured denitrification rates as accumulated biodeposits are remineralized and some denitrified. Also, more sampling of background denitrification rates were undertaken now that the footprint of biodeposition is more accurately mapped. In the 2016 surveys, area affected by biodeposits was only generally assessed (compared to 2017). Based upon these improvements the total nitrogen removed for 2016 and 2017 has been determined below (Section IX). All future denitrification surveys should include additional background sites, some farther away than in 2016.

Section VIII. Stream flow and Nutrient Load Measurement Method: Stream Discharge from Pilgrim Lake to Lonnie's Pond (2016 - 2017)

Predicting changes in coastal embayment nitrogen related water quality from nutrient load reduction strategies, enhanced flushing with low nutrient water or oyster propagation is based, in part, on determination of the inputs of nitrogen from the surrounding contributing land (watershed). Transport of nutrients (specifically nitrogen) to Lonnie's Pond from the watershed is effected namely via direct groundwater discharge or surface water inflow (and to a lesser extent direct atmospheric depositions). Rates of nitrogen loading to Lonnie's Pond via groundwater were based upon the delineated watersheds and land-use coverages developed by the Massachusetts Estuaries Project (MEP). Additionally, rates of surface water inflow and associated nitrogen load to Lonnie's Pond were determined using standard hydrologic techniques employed both for this study as well as the MEP nutrient threshold assessment of Pleasant Bay, which also included Lonnie's Pond.

Surface water flow and N load in each study was determined at the exact same gauging location situated at the base of the herring ladder connecting Pilgrim Lake to Lonnie's Pond (Figure 1a, 1b). The only difference between the stream gauging effort in 2016-2017 vs. 2002-2003 when the MEP assessment was completed is that the MEP extended the surface water N loading analysis to quantify percent nitrogen attenuation. By example, if all of the nitrogen applied or discharged within a watershed reaches an embayment the watershed land-use loading rate represents the nitrogen load to the receiving waters (0% attenuation). This condition exists in watersheds where nitrogen transport from source to estuarine waters is uniquely through groundwater flow in sandy outwash aquifers. The lack of nitrogen attenuation in these aquifer systems results from the lack of biogeochemical conditions needed for supporting nitrogen sorption and denitrification. However, in most watersheds in southeastern Massachusetts, nitrogen passes through a surface water ecosystem (pond, wetland, stream) on its way to the adjoining embayment. Surface water systems, unlike sandy aquifers, do support the needed conditions for nitrogen retention and denitrification. The result is that the mass of nitrogen passing through lakes, ponds, streams and marshes (fresh and salt) is diminished (attenuated) by natural biological processes that represent removal (not just temporary storage). For the current (2016-2017) investigation of the oyster filtering effect in Lonnie's Pond, the measured surface water load is the attenuated load.

Given the importance of quantifying the balance of nitrogen into and out of Lonnie's Pond to determine the effect of the oyster deployment, directly measured flow and load that integrates all contributors to upper watershed attenuation was undertaken by CSP scientists in a similar manner as was undertaken under the MEP (thereby allowing for direct comparison of data sets).

Surface water flow paired with weekly to bi-weekly sampling for nitrogen concentration of the discharge from Pilgrim Lake were combined to yield a nutrient load associated with the surface water flow into Lonnie's Pond that integrated all of the processes presently attenuating nitrogen in the sub-watersheds up-gradient from the gauging sites, which are the watershed to Pilgrim

Lake and a small stream-only watershed between the Lake and Lonnie's Pond. Flow, concentration and nitrogen load were determined at the stream gauging site for twelve (12) months comprising a complete hydrologic year (low flow to low flow) from October 1, 2016 to September 30, 2017. During the study period, periodic velocity profiles were completed at the stream gauge location to compare to calculated flows determined by passing the measured stage data from the gauge through the updated stage - discharge relation (rating curve) that was originally developed by the MEP. The updated rating curve reflects additional flow measurements made in 2016 and 2017. The summation of the products of stream subsection areas of the stream cross-section and the respective measured velocities represent the computation of instantaneous stream flow (Q).

Determination of stream flow was calculated and based on the measured values obtained for stream cross sectional area and velocity. Stream discharge was represented by the summation of individual discharge calculations for each stream subsection for which a cross sectional area and velocity measurement were obtained. Velocity measurements across the entire stream cross section were not averaged and then applied to the total stream cross sectional area.

The formula that was used for calculation of stream flow (discharge) is as follows:

$$Q = \sum (A * V)$$

where by:

Q = Stream discharge (m³/s)

A = Stream subsection cross sectional area (m²)

V = Stream subsection velocity (m/s)

Thus, each stream subsection will have a calculated stream discharge value and the summation of all the sub-sectional stream discharge values will be the total calculated discharge for the stream.

Under the MEP analysis for Pleasant Bay, periodic measurements of flows over a complete hydrologic year allowed for the development of a stage-discharge relationship (rating curve) that was used to obtain daily flow volumes from the detailed record of stage measured by the continuously recording stream gauge. In order to measure stage for the present Lonnie's Pond oyster study, a stream gauge was redeployed in 2016 and was maintained through 2017 in order to determine the annual flow and nutrient load to Lonnie's Pond during the study period. Water level data obtained every 10-minutes from the herring creek up-gradient of the culvert passing under Herring Brook Road was averaged to obtain hourly stages. These hourly stages values were then entered into the stage-discharge relation to compute hourly flow. Hourly flows were summed over a period of 24 hours to obtain daily flow and further, daily flows summed to obtain monthly flow. In the case of tidal influence on stream stage (as is the case with the herring creek), the diurnal low tide stage value was extracted on a day by day basis in order to obtain the stage value indicative of strictly freshwater flow. The lowest low tide stage

value for a given day was then entered into the updated MEP stage – discharge relation in order to compute daily flow for the Lonnie's Pond oyster study undertaken in the 2016-2017 period.

The flow record generated by passing measured stage data through the rating curve for the surface water flow into Lonnie's Pond via the herring ladder was merged with the nutrient concentration data obtained through the water quality sampling program to determine nitrogen loading rates to the head of Lonnie's Pond. Nitrogen discharge from the stream was calculated using the paired daily discharge and daily nitrogen concentration data to determine the mass flux of nitrogen through a gauging site. For the gauging location in this study, weekly water samples were collected (at low tide to account for tidal influence) in order to determine nutrient concentrations from which nutrient load was calculated. In order to pair daily flows with daily nutrient concentrations, interpolation between weekly nutrient data points was necessary. These data are expressed as nitrogen mass per unit time (kg/d) and can be summed in order to obtain weekly, monthly, or annual nutrient load to Lonnie's Pond as appropriate. The "measured load" was representative of attenuated nitrogen entering Lonnie's Pond directly from surfacewater as opposed to groundwater (recharge over area delineated by watershed).

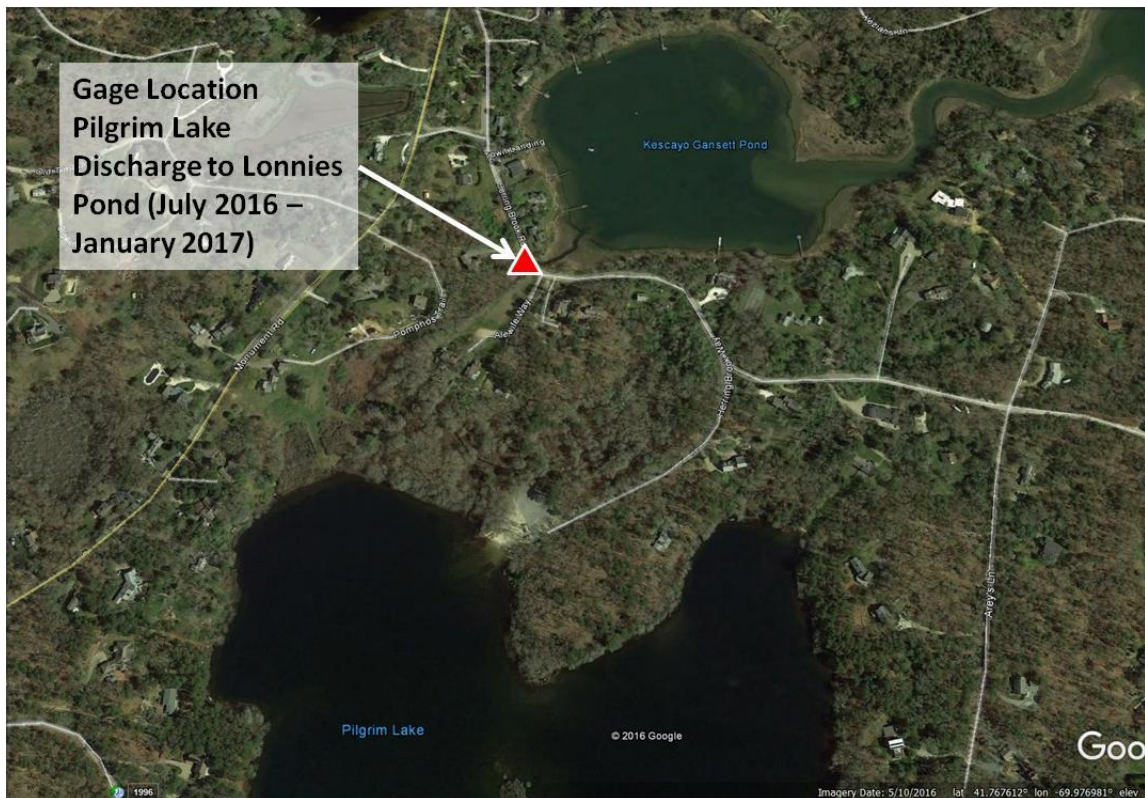


Figure VIII.1a. Location of stream gauge (red triangle) deployed at the base of the herring ladder between Pilgrim Lake and Lonnie's Pond. An updated MEP rating curve initially developed in 2003 but refined with 2016-2017 flow measurements was utilized to determine daily flows and nutrient load to Lonnie's Pond for the oyster study.

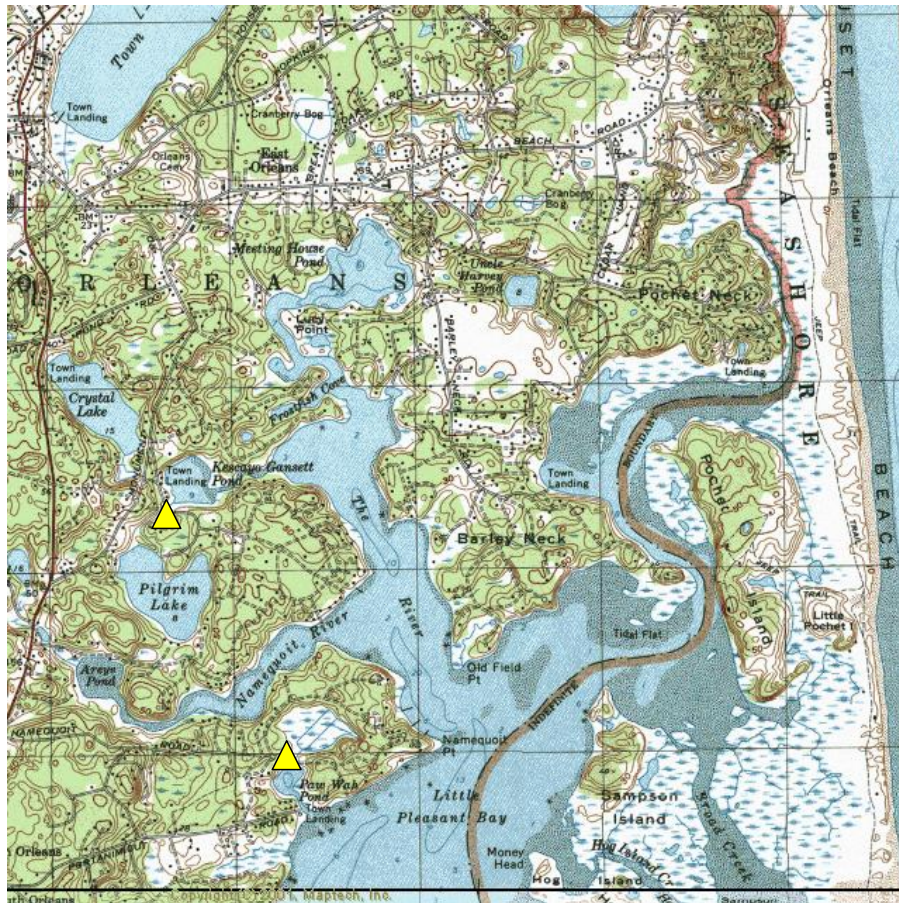


Figure VIII.1b. Location of MEP Stream gauge (yellow triangle) in the upper portions of the Pleasant Bay system embayment system. Rating curve, stage, flow, nutrient load and percent attenuation was determined for the 2002-2003 hydrologic year

Pilgrim Lake discharges freshwater and nitrogen load to the brackish waters of Lonnies Pond via a herring ladder that flows into a 1-meter diameter concrete culvert passing under Herring Brook Road. In order to quantify the flow of freshwater and the associated nitrogen load into Lonnies Pond, a stream gage was deployed immediately up-gradient of the culvert in the concrete box the herring ladder flows into on its way to Lonnies Pond.

The stream gage was deployed in late July 2016 and measured the stage of water in the box at a 10 minute frequency until October 31, 2017. As described above, the 10 minute stage data was averaged to obtain hourly stage which was then filtered to extract the lowest tide stage in a given day which would be representative of the freshwater portion of the flow (Figure 2a, b, c). The daily low tide stage was passed through the updated stage-discharge relationship originally developed by the MEP in 2002-2003 but modified to include flow measurements that were collected during the 2016-2017 Lonnies Pond study. Stage in 2016-2017 was measured at the same location as during the MEP stream analysis completed in 2002-2003. The 2016-2017

stage record was passed through the modified stage-discharge relation in order to calculate a volumetric flow in cubic meters per second (m^3/s) that was then converted to cubic meters per day (m^3/d) as depicted in Figure 3.

Total surface water inflow to Lonnie's Pond (Table VIII.1) from Pilgrim Lake for the period October 1, 2016 to September 30, 2017 (a complete hydrologic year, low flow to low flow) and based on measured stage for that period and the modified MEP developed rating curve was $187,507 \text{ m}^3$ (average daily flow = 514 m^3). By comparison, total surface water inflow to Lonnie's Pond from Pilgrim Lake during the MEP study period (October 2002-September 2003) was $355,279 \text{ m}^3$ (average daily flow = 973 m^3). The lower observed flows in 2016-2017 is primarily attributable to lower groundwater levels during the measurement period. During the 2002/2003 water year, January through May groundwater levels at the local long-term water level monitoring well were generally in the 75th and 90th percentile of all data and near average during the rest of the year, while groundwater levels were generally closer to the long-term averages during the 2016/2017 water year (Eichner, et al., 2018). As might be expected since precipitation and groundwater levels are related, lower precipitation rates were also noted during 2016/2017, but precipitation variations explained only 8% of the variation in streamflows, while groundwater variations explained 36% of flow variations.

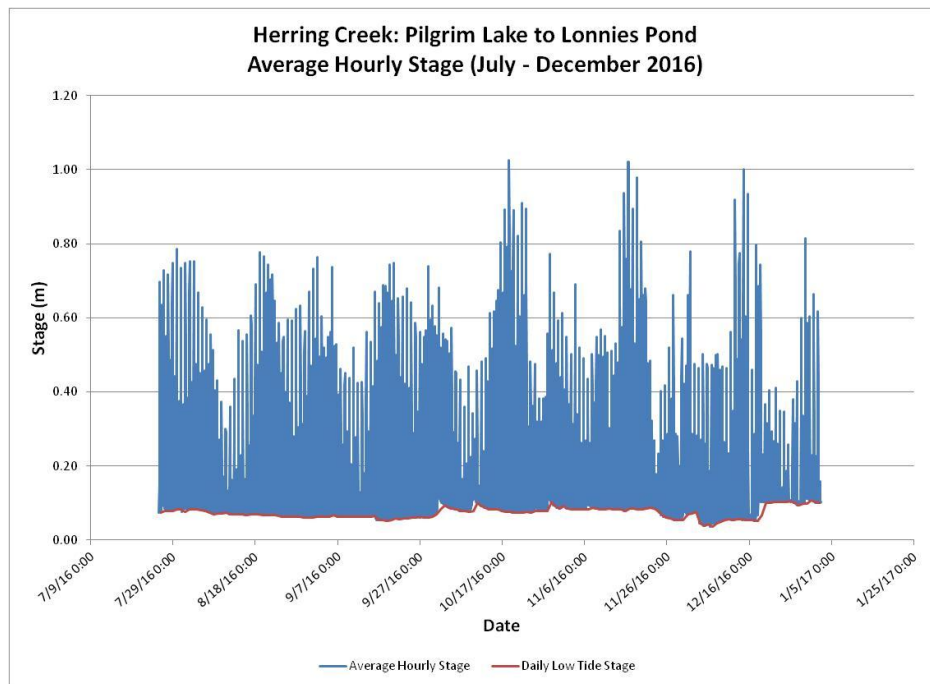


Figure VIII.2a. Average hourly stage record from Herring Creek discharge to Lonnie's Pond from Pilgrim Lake, July 25, 2016 to December 31, 2016 and associated daily low tide stage used to calculate freshwater flow and nitrogen load to Lonnie's Pond.

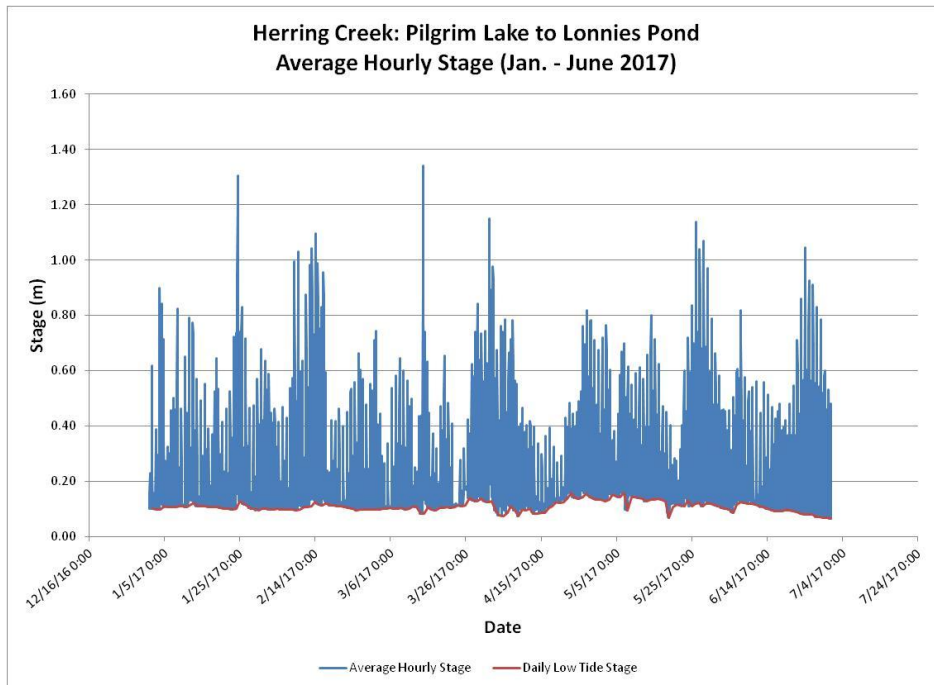


Figure VIII.2b. Average hourly stage record from Herring Creek discharge to Lonnie's Pond from Pilgrim Lake, January 1, 2017 to June 30, 2017 and associated daily low tide stage used to calculate freshwater flow and nitrogen load to Lonnie's Pond.

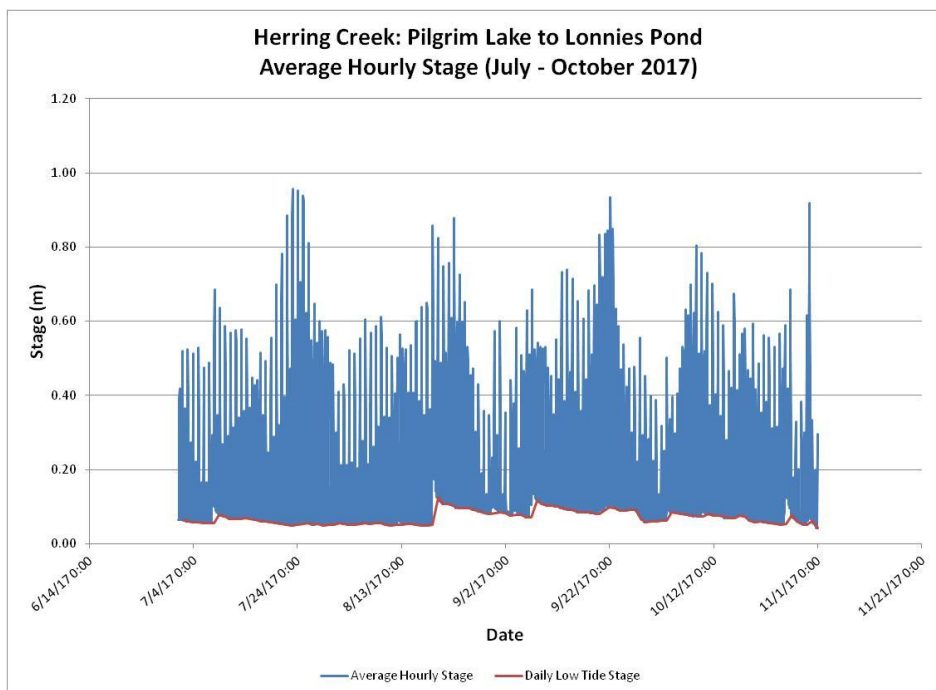


Figure VIII.2c. Average hourly stage record from Herring Creek discharge to Lonnie's Pond from Pilgrim Lake, July 1, 2017 to October 31, 2017 and associated daily low tide stage used to calculate freshwater flow and nitrogen load to Lonnie's Pond.

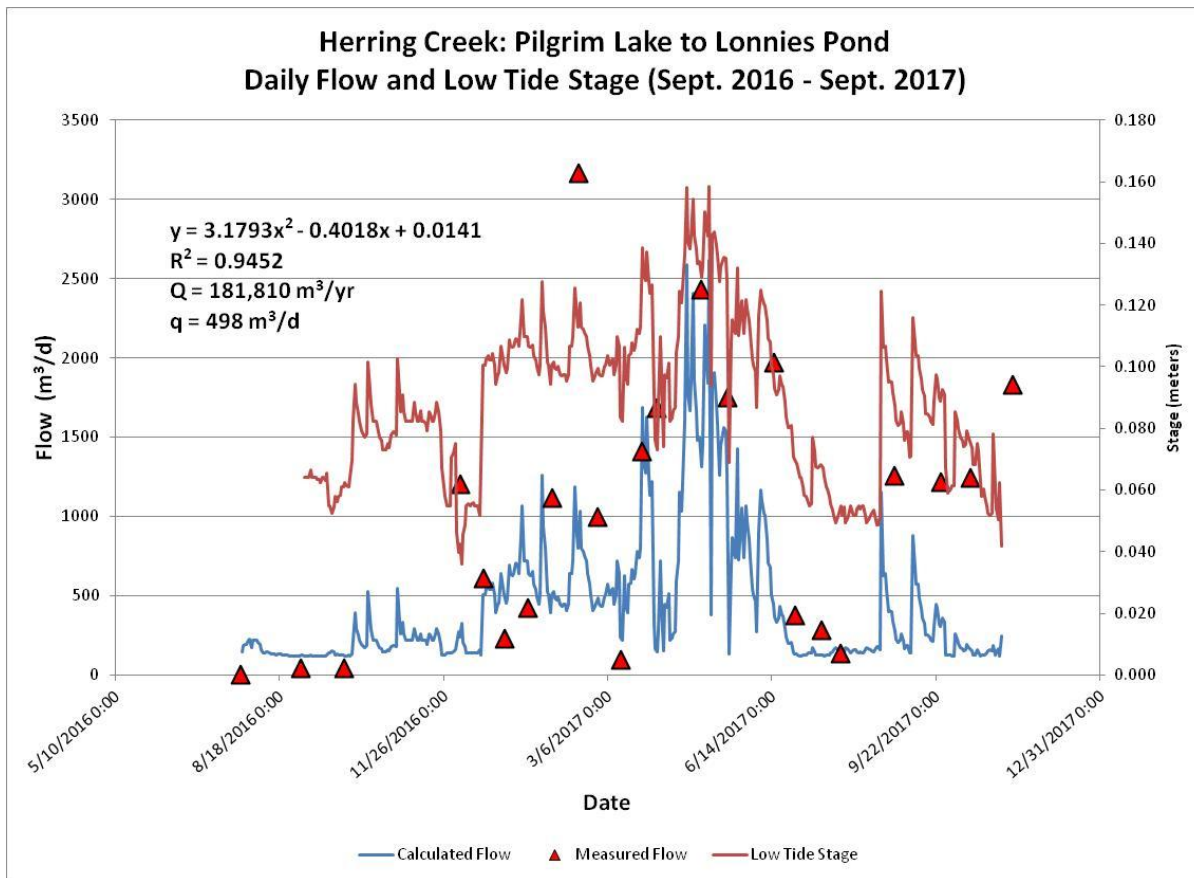


Figure VIII.3. Daily flow record from Herring Creek discharge to Lonnie's Pond from Pilgrim Lake, September 1, 2016 to October 31, 2017, associated daily low tide stage used to calculate freshwater flow and nitrogen load to Lonnie's Pond and comparison to measured point flow measurements.

Monthly Flow Pilgrim Lake to Lonnie's Pond (August - December)		
MONTH	2016-2017 FLOW (m³/month)	2002-2003 FLOW (m³/month)
August	4457	--
September	3791	--
October	7450	8342
November	6360	33240
December	9893	36363
January	20111	30,062
February	16981	46,759
March	21964	59,961
April	28947	41,072
May	39226	51,896
June	14210	14,367
July	4394	11,522
August	8504	9,280
September	9467	12,415
October	4920	10,754
November	--	16,887
December	--	17,520
Total (Oct.-Sept.)	187,507	355,279
NOTE:		
Annual Precip 2002-2003 = 54.39 inches		
Annual Precip 2016-2017 = 40.79 inches		
Avg. Annual Precip 1993-2015 = 46.11 inches		

Table VIII.1. Summary of monthly flow volumes discharged to Lonnie's Pond. 2016-2017 significantly lower total volume for the period October to September (hydrologic year) due primarily to lower groundwater levels compared to 2002-2003.

Based on the daily flows generated during the gauge deployment period and the approximately bi-weekly sampling of the flow into Lonnie's Pond, sample concentrations were interpolated and then merged with the daily flows to obtain an estimate of the nitrogen load into Lonnie's Pond during the critical period of the oyster deployment. On average for the period August through October 2016, total nitrogen concentration averaged 1.11 mg/L and ranged between 0.96 and 1.30 mg/L (Figure 4, Table 2). When combined with flow during the same period, TN load averaged 5.67 kg/month TN and ranged between a high monthly average load of 7.57 kg/month and a low of 4.67 kg/month (Figure 5, Table 3). By comparison, on average for the period August through October 2017, total nitrogen concentration averaged 0.59 mg/L and ranged between 0.55 and 0.66 mg/L (Table 2). When combined with flow during the same period, TN load averaged 4.15 kg/month TN and ranged between a high monthly average load of 4.83 kg/month and a low of 2.90 kg/month.

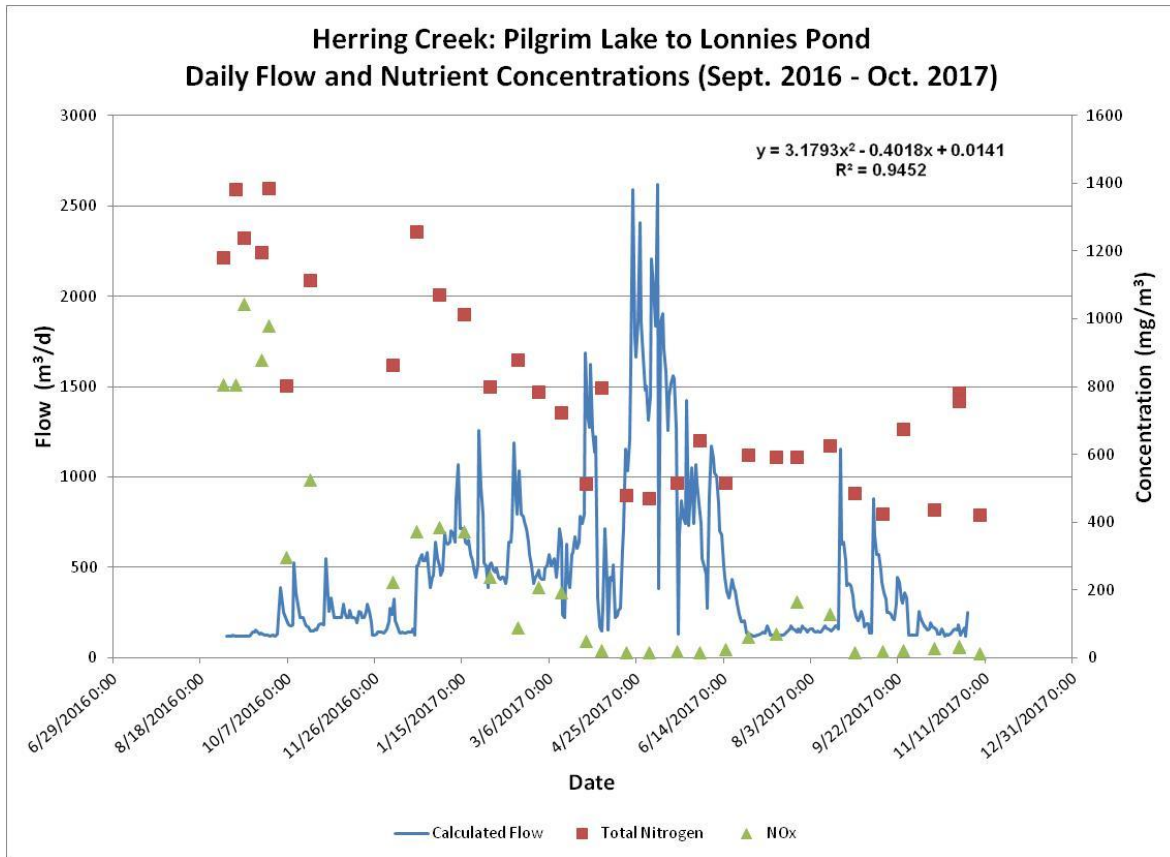


Figure VIII.4. Daily flow and stream nutrient concentration record from Herring Creek discharge to Lonnie's Pond from Pilgrim Lake, September 1, 2016 to October 31, 2017 used to calculate freshwater flow and nitrogen load to Lonnie's Pond.

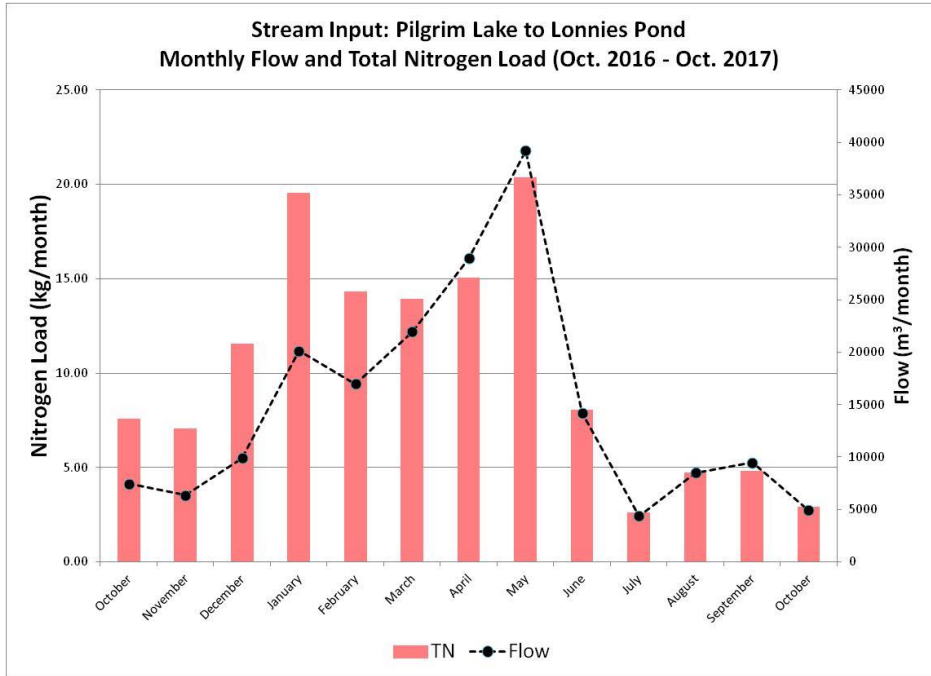


Figure VIII.5. Monthly flow and monthly stream total nitrogen load from Pilgrim Lake to Lonnie's Pond.

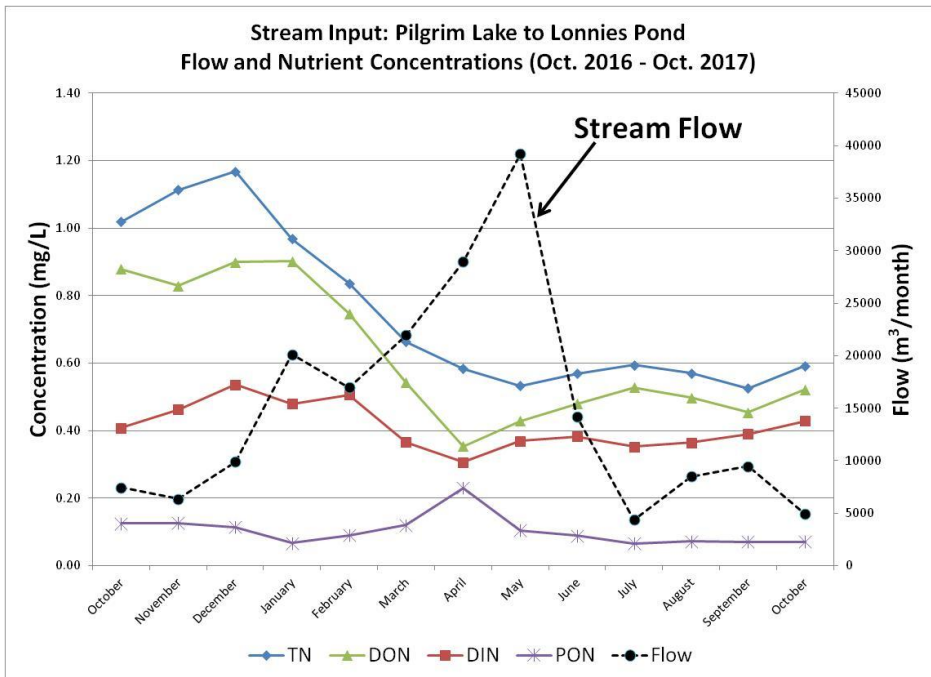


Figure VIII.6. Monthly flow and average monthly stream nitrogen constituent concentration record from Herring Creek discharge to Lonnie's Pond from Pilgrim Lake, October 1, 2016 to October 31, 2017 used to calculate freshwater flow and nitrogen load to Lonnie's Pond and determine seasonal differences in nutrient loading.

Considering the component nitrogen species, it appears that the total nitrogen concentration is

primarily comprised of dissolved inorganic nitrogen (DIN, 50%) and dissolved organic nitrogen (DON, 43%) with the remaining portion being comprised of particulate organic nitrogen (PON 7%). The 2003 stream nutrient concentrations determined during the MEP study were generally similar in that DIN and DON were the predominant fraction of the TN pool (30% and 58% respectively) with PON constituting the remainder of the total nitrogen (11%). DIN concentration in 2016 did appear higher than DIN levels in 2003 (50% vs. 30%), however, that may be the result of differences in annual hydrologic conditions and annually variable biogeochemical transformations in Pilgrim Lake. Lower volumetric discharge in the herring creek between Pilgrim Lake and Lonnie's Pond could result in the slightly higher constituent concentrations for nitrogen (e.g. average TN 2016 {Aug.-Oct.} 1.11 mg/L, average TN 2003 {Aug.-Oct.} 0.791 mg/L). By comparison, total precipitation in the period August to October 2017 was 13.69 inches with an average TN concentration for that period of 0.59 mg/L.

NUTRIENT CONCENTRATIONS (based on samples w/o interpolation)						
Year	NH4	NOX	DIN	DON	PON	TN
	Avg. Monthly (mg/L)	Avg. Monthly (mg/L)	Avg. Monthly (mg/L)	Avg. Monthly (mg/L)	Avg. Monthly (mg/L)	Avg. Monthly (mg/L)
2016						
August	0.07	0.50	0.57	0.40	0.10	1.07
September	0.07	0.93	0.99	0.21	0.10	1.30
October	0.02	0.41	0.43	0.41	0.11	0.96
November *	0.01	0.35	0.46	0.83	0.13	1.11
December	0.04	0.30	0.34	0.54	0.11	1.06
* November concentrations are based on interpolation						
2017						
January	0.079	0.331	0.410	0.479	0.072	0.961
February	0.070	0.149	0.219	0.505	0.109	0.833
March	0.036	0.122	0.158	0.352	0.110	0.620
April	0.026	0.019	0.044	0.306	0.287	0.637
May	0.045	0.017	0.062	0.371	0.109	0.542
June	0.062	0.044	0.106	0.372	0.079	0.557
July	0.072	0.117	0.189	0.345	0.058	0.592
August	0.033	0.072	0.105	0.373	0.078	0.556
September	0.052	0.020	0.072	0.411	0.067	0.550
October	0.079	0.031	0.110	0.478	0.071	0.659
2003						
August	0.14	0.22	0.36	0.36	0.08	0.80
September	0.06	0.14	0.20	0.43	0.07	0.70
October	0.06	0.20	0.26	0.51	0.11	0.88
November	0.03	0.08	0.11	0.59	0.09	0.79
December	0.08	0.21	0.28	0.50	0.12	0.91
Avg. Conc. (Aug.-Oct. 2016)	0.05	0.61	0.67	0.34	0.10	1.11
Avg. Conc. (Aug.-Oct. 2017)	0.05	0.04	0.10	0.42	0.07	0.59
Avg. Conc. (Aug.-Oct. 2003)	0.09	0.19	0.28	0.43	0.08	0.79

NOTE: Oysters Removed from Lonnie's Pond in December 2016.

Table VIII.2. Nutrient concentrations by month (August - December) 2016 vs. 2003 entering Lonnie's Pond from Herring Creek.

NUTRIENT LOADS						
Year	NH4 Monthly Load (kg/month)	NOX Monthly Load (kg/month)	DIN Monthly Load (kg/month)	DON Monthly Load (kg/month)	PON Monthly Load (kg/month)	TN Monthly Load (kg/month)
2016						
August	0.30	2.07	2.37	1.87	0.44	4.67
September	0.25	3.39	3.65	0.81	0.32	4.78
October	0.16	3.33	3.49	3.03	0.92	7.57
November	0.09	2.28	2.37	2.93	0.81	7.08
December	0.47	3.40	3.87	5.36	1.17	11.56
2017						
January	1.71	6.84	8.55	9.66	1.34	19.54
February	1.42	2.51	3.93	8.82	1.56	14.31
March	0.70	2.47	3.17	7.69	3.07	13.94
April	0.89	0.50	1.39	8.84	4.81	15.04
May	1.58	0.68	2.27	14.12	3.99	20.38
June	0.83	0.39	1.22	5.54	1.29	8.05
July	0.31	0.46	0.78	1.54	0.29	2.60
August	0.30	0.66	0.96	3.12	0.65	4.73
September	0.41	0.19	0.60	3.57	0.67	4.83
October	0.31	0.14	0.45	2.10	0.35	2.90
2003						
August	1.35	2.18	3.53	3.40	0.71	7.64
September	0.64	2.17	2.81	4.59	0.85	8.26
October	0.61	1.92	2.53	5.92	1.23	9.68
November	0.41	1.26	1.67	9.75	1.53	12.96
December	ND	ND	ND	ND	ND	ND
Total Load (Aug.-Oct. 2016)	0.71	8.80	9.51	5.71	1.69	17.02
Avg. Load (Aug.-Oct. 2016)	0.24	2.93	3.17	1.90	0.56	5.67
Total Load (Aug.-Oct. 2017)	1.02	0.98	2.01	8.79	1.66	12.46
Avg. Load (Aug.-Oct. 2017)	0.34	0.33	0.67	2.93	0.55	4.15
Total Load (Aug.-Oct. 2003)	2.60	6.27	8.87	13.91	2.79	25.57
Avg. Load (Aug.-Oct. 2003)	0.87	2.09	2.96	4.64	0.93	8.52
NOTE: Oysters Removed from Lonnie's Pond in December.						

Table VIII.3. Nutrient loads by month (August - December) 2016 and 2017 vs. 2003 entering Lonnie's Pond from Herring Creek.

Section IX. Lonnie's Pond Oyster Study 2016 - 2017: Conclusions

The major results of the 2 year pilot deployment of oysters in Lonnie's Pond are summarized below. While there are areas of the analysis that need refinement and additional quantification, a few things are clear from this extended deployment:

- 1) In both years there was a clear reduction in phytoplankton biomass by oyster filtration as water flowed through the oyster culture area. Significant reductions in total chlorophyll-*a* >50% were commonly observed in samples adjacent versus within the deployment area.

2) Significant ($p < 0.5$) reductions in total chlorophyll-*a* and particulate organic nitrogen (PON) were seen in tidal studies designed to capture water as it flowed through the culture area. Reduction in bioactive nitrogen was also seen, mainly due to the reduction in its main component, PON. Given the short time that any packet of water is in contact with the oysters, the large quantifiable reductions in all particulate groups is clear evidence of the ability of these types of oyster deployments to improve water quality even in nitrogen enriched waters

3) Biodeposition of feces and pseudofeces from the oysters was clearly evident in the region of the oyster deployment and the sediment region receiving biodeposits. The boundary of impact was refined in 2017 using a more sensitive acoustic device than in 2016. The oysters process more particulates than they incorporate by 2 fold. The deposition of organically labile particulate matter in biodeposits stimulated overall sediment respiration rates. This stimulation occurred in summer and fall with temperature moderating the absolute rate. Comparing October rates adjusted for temperature effects (Q_{10}) with August rates, overall respiration rates (carbon turnover) were similar, but with a shift towards greater uptake of NH_4 and NO_3 and greater denitrification. Rates of N biodeposition were directly measured yielding rates of 0.58 kg-N d^{-1} and 0.30 kg-N d^{-1} in August and October, respectively. The water quality surveys documented a peak in both particulate nitrogen and chlorophyll-*a* (phytoplankton biomass) in August compared to October due to a summer phytoplankton bloom (see Section II).

4) Denitrification (transformation of fixed nitrogen to nitrogen gas, N_2) was enhanced in sediments receiving oyster biodeposition. In September/October when oysters had reached their mid-season biomass, an amount equivalent to almost 1/3 of the biodeposition rate was denitrified each day. The study indicated that denitrification continues to be enhanced past the time of oyster harvest as the sediment incorporated biodeposits continue to contribute nitrogen to denitrification into the following spring when waters re-warm.

5) The second year of oyster deployment in the same area resulted in an increase in nitrogen removed through denitrification by 50% (2017[year 2] versus 2016 [year 1]) over the single season. It appears that the initial 1 year new deployment did not deliver sufficient biodeposits to the sediments to maximize the amount of nitrogen removal through denitrification. The second year also was initiated earlier than year 1 and with more oyster biomass early on, such that denitrification was higher for a longer period.

6) Should a rotational deployment be initiated, it will be important to gauge the potential reduction in denitrification if only 1 year deployments are made. At this point the optimal rotation time is unclear. In addition, it appears that the need to maximize biodeposits as early as April/May should be considered in planning where possible.

7) Re-examining the full denitrification and refined impact area and biodeposition over the 2 year deployment indicates that the initial deployment of seed only from June to December resulted in an increased nitrogen removal of $0.25 \text{ kg N per 1 kg N}$ in harvest, but when continued into the second year the increased removal was significantly higher $\sim 0.37 \text{ kg N per 1}$

kg N removed in harvest. These rates are lower than for Little Pond Falmouth, but are in the range of 0.5 kg N per 1 kg N removed in harvest found elsewhere.

8) As part of the overall effort, stream discharges to Lonnie's Pond were determined in summer 2016 and summer 2017 during the oyster deployment. The flow data are consistent with 2016 being part of the drought (Aug.-Oct., 15,698 m³) with 2017 flows being higher (Aug.-Oct., 22,891 m³) due to higher precipitation. Precipitation in 2016 was reduced over long-term averages and more significantly flow through the main surface water discharge (Herring Run) was far below historic levels (e.g. 2003), with 2016 rates being 52% of 2003 surface water flow volumes (32,449 m³) in the August through October period. Flow for the same period in 2017 compared to 2003 was 29% lower. At these flow levels, it did not appear that the oyster study was influenced by surface water flows in 2016 or 2017. It should be noted that at the low surface flows of 2016, average monthly nitrogen loading (Aug.-October.) to Lonnie's Pond waters from Pilgrim Lake was significantly lower than 2003 loads (5.67 vs. 8.52 kg/month). It should be noted that while flows in 2016 were lower than for the same period in 2017, average monthly TN load to Lonnie's Pond in 2016 (5.67) was slightly higher than for the same 2017 period (4.15) due to the very high measured TN concentrations in the stream water (1.11 mg/L and 0.59 mg/L respectively). The interannual variation in TN concentration is likely related to the turnover time of Pilgrim Lake, which is controlled by the freshwater inflow volume (lower in 2016 than 2017).

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