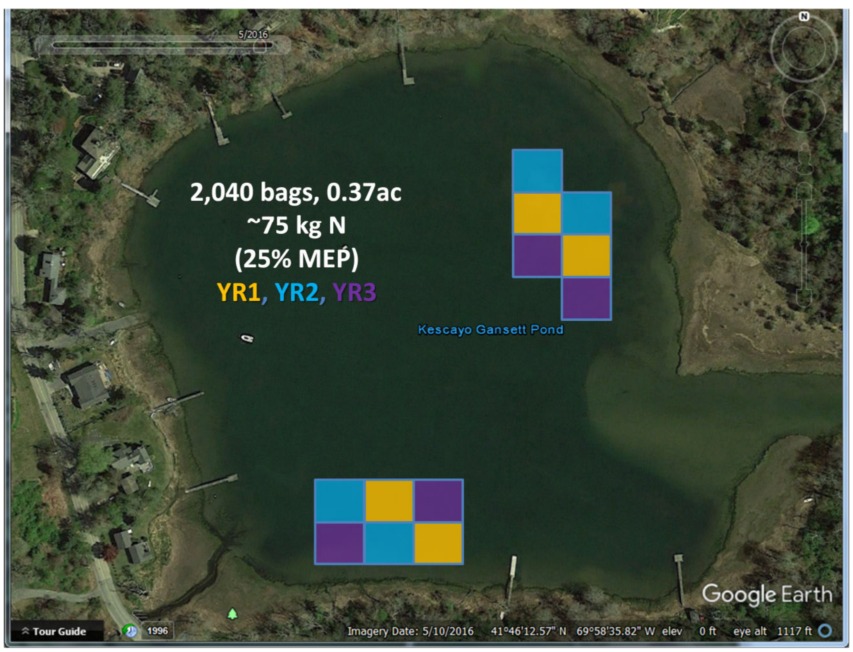
Lonnie's Pond Aquaculture and Nitrogen Management Plan

FINAL

October 2018

Town of Orleans



Prepared by:

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Prepared for

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Prepared By

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Lonnie's Pond Aquaculture and Nitrogen Management Plan

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I. Introduction

The Town of Orleans is working on options to reduce nitrogen loads to its estuaries through a variety of strategies, including improved wastewater treatment, but also through lower-cost, non-traditional approaches. These efforts reflect an understanding of the community and economic links to healthy ecosystems and clean water, but also strive to attain reductions required by the Massachusetts Department of Environmental Protection (MassDEP) through their adoption of Total Maximum Daily Loads (TMDLs) for impaired waters under the federal Clean Waters Act.

In 2007, MassDEP finalized TMDLs for Pleasant Bay that identified portions of the estuary, including Lonnie's Pond, as having excessive nitrogen.[1] The Massachusetts Estuaries Project report for Pleasant Bay[2], which is the technical basis for the TMDLs, suggested that 300 kg/yr of nitrogen would need to be removed from Lonnie's Pond to mitigate the impairments.

In 2016, the Town began a non-traditional oyster aquaculture demonstration project in Lonnie's Pond as part of examining innovative non-sewering approaches to nitrogen management. The aquaculture project was planned as a three-year effort to evaluate the water quality impacts and implementation issues associated with enhanced aquaculture for nitrogen reduction and potential achievement of the TMDL without sewering within the Pond watershed.

Monitoring during the Lonnie's Pond Demonstration Project has found significant removal of nitrogen due to shellfish growth and bio-deposition and some water quality improvements. However, as the amount of nitrogen removal by oyster aquaculture is becoming clear, focus has shifted to the logistical, regulatory, monitoring, and public coordination components that are needed for its long-term use in the Town's nitrogen management program for its estuaries.

This Lonnie's Pond Aquaculture and Nitrogen Management Plan provides a detailed framework for the long-term use of oyster aquaculture in this basin. Its goal is ensuring the reliability of regular oyster aquaculture for nitrogen removal. The overall concept of the plan is that the Town will partner with private contractors to oversee and implement the Plan. An Aquaculture Contractor will be responsible for purchasing, installing, maintaining, and removing oysters deployed in floating bags started as seed and then grown from spring to removal in early winter. A separate, Monitoring Contractor will coordinate with the Aquaculture Contractor to complete nitrogen monitoring of both the shellfish and water quality. The Monitoring Contractor will also work with the Town Natural Resource Manager (or designee) to provide an annual aquaculture report detailing the number and amount of shellfish grown, the amount of nitrogen removed and resulting water quality relative to compliance with the TMDL. This Plan will be coordinated initially through the Shellfish and Waterways Improvement Advisory Committee to ensure that start-up information is publicly shared and available. After initial start-up, the Town Natural Resource Manager (or designee) will be ensure regular public sharing of project performance.

[1] MassDEP. 2007. FINAL Pleasant Bay System Total Maximum Daily Loads For Total Nitrogen (Report # 96-TMDL-12, Control #244.0). 53 pp.

[2] Howes B., S. W. Kelley, J. S. Ramsey, R. Samimy, D. Schlezinger, E. Eichner (2006). Linked Watershed-Embayment Model to Determine Critical Nitrogen Loading Thresholds for Pleasant Bay, Chatham, Massachusetts. Massachusetts Estuaries Project, Massachusetts Department of Environmental Protection. Boston, MA.

The Plan includes shellfish and water quality management decision points and their likely schedule within each year, a communications plan to ensure that all Town decision makers and citizens understand the in-pond activities, results, and responsibilities for costs and associated contingencies, such as dealing with catastrophic loss/death of oysters. This Plan also includes details such as the ideal number of oysters, their sizes, their deployment areas, and their ultimate use/sale.

II. Lonnie's Pond Aquaculture Deployment

To initiate the Aquaculture portion of the Lonnie's Pond Aquaculture and Nitrogen Management Plan, an Aquaculture Contractor will be selected by the Town through a public procurement process in November for a 3 year renewable contract. The Aquaculture Contractor will be responsible for purchasing, installing, maintaining, transporting, and harvesting the shellfish. The Aquaculture Contractor will be provided access to the available aquaculture gear previously developed and used during the demonstration project, including 680 floating spat bags and 2,040 6 mm diamond mesh floating bags. A floating bag setup similar to that used in the Demonstration Project is to be used, although the oyster density may be varied based on growth results.

II.A. Aquaculture Contractor: Selection, Responsibilities

II.A.1. Aquaculture Contractor: Selection

The selection of an Aquaculture Contractor for Lonnie's Pond will be completed through a public procurement process, including the development of a Request for Proposals (RFP). The Town will publicly advertise a RFP in November of each year a new contractor is needed and select the Aquaculture Contractor by December 1. A pre-bid conference will be held by the Town to allow inspection of the gear previously developed and used during the demonstration project; details for the pre-bid conference will be included in the RFP. The RFP will follow standard Town procurement requirements and include the following criteria for review:

1) Experience in oyster aquaculture, both on Cape Cod and within Orleans.
2) Experience deploying more than 2 million oyster seed prior to June 1.
3) Certification of access to approved upweller space and experience in growing seed in an upweller or ability to provide the required amount of oyster seed.
4) Experience with use and maintenance of existing Town gear or equivalent.
5) Description of planned oyster deployment, including specific shellfish deployment areas, shellfish care between installation and harvesting, maintenance, and any additional equipment that will be deployed (see II.B for details of deployment); alternative strategies for oyster counts and management to attain the required N removal will be considered,
6) Description of planned use of public facilities, such as the boat ramp, including anticipated time of day and frequency of access
7) Description of how the Contractor will minimize visual, noise and traffic relative to abutters
8) Documentation of liability insurance
9) Presentation of any necessary regulatory documents

II.A.2. Aquaculture Contractor: Contractor Responsibilities

The Aquaculture Contractor will be responsible for purchasing, transporting, installing, maintaining and removing the shellfish. Associated details of Aquaculture Contractor responsibilities include:

1) Creation of 23,400 kg of net weight of oysters by the time the oysters are removed (based on Demonstration Project results, this should remove 75 kg of total nitrogen). This growth will be achieved by selecting either of the following scenarios:

   Scenario A:
   - acquisition and deployment of a minimum of 5.5 million Year 1 seed oysters (2 to 3 mm) by June 1 in floating spat bags;
   - then selecting and transferring the best growing oysters to 2,040 6 mm mesh bags, such that there will be 1,000 oysters per bag;
   - then allowing these oysters to grow and removing them between November 15 and December 15

   OR

   Scenario B:
   - acquisition and deployment of a minimum of 2.1 million Year 1 seed oysters (10 to 25 mm) by July 1 in 2,040 6 mm mesh bags, such that there will be 1,000 oysters per bag;
   - then allowing these oysters to grow and removing them between November 15 and December 15

   If monitoring results show that this amount of oysters needs to be reduced or increased in subsequent years, it will be addressed in annual modifications and/or a future contract.
2) providing documentation to the Town by December 31 that adequate supply of Year 1 seed oysters has been ordered and planned delivery is prior to June 1. The Aquaculture Contractor will ensure that oysters are certified disease-free and obtained from a certified facility.
3) providing (or fabricating) any additional gear necessary for oyster installation and necessary trucks or alternatives for transport of shellfish. All bags shall be black to minimize visual aesthetics, anchoring of gear shall use of telescoping auger system (helix anchors) or equivalent, and other gear equivalent to the current Demonstration Project will be required. Note that if screw anchors (augers) are used to anchor the deployment, no Army Corps of Engineers permit is needed, but the Contractor should confirm with Massachusetts Division of Marine Fisheries (MassDMF).
4) boats and other equipment for installation, movement, maintenance, and collection of the bags and racks,
5) an acceptable plan for restoring shellfish if oysters die or are lost before August 1 in any given year, including the time needed for re-deployment. The Aquaculture Contractor should identify potential mid-season suppliers of Year 1 oysters as part of the replacement plan. If shellfish are lost the Aquaculture Contractor must submit a brief memorandum documenting the reason for the loss, the timing of loss and reinstallation (including costs to contractor) and how it will be avoided in the future,
6) installation and removal of gear in spring and fall with gear removed by 2 weeks after harvest each year, but no later than December 15, except with permission of the Natural Resource Manager (or designee),

7) ensuring that oysters are certified disease-free prior to their removal from Lonnie's Pond and obtaining permission from MassDMF and the Natural Resource Manager (or designee) to move the oysters to a new site.
8) storage of all gear used and emergency backup gear over the course of the contract,
9) notifying the Town of any survival or growth concerns, including signs of disease,
10) coordinating any water quality or shellfish sampling with Monitoring Contractor,
11) providing all necessary information to the Monitoring Contractor for the completion of the Lonnie's Pond Aquaculture/TMDL Annual Report,
12) coordinating response to any catastrophic events (*e.g.*, significant shellfish die-off, significant gear destruction) with Town and Monitoring Contractor, and
13) providing a plan for the oyster sale or the transfer to other sites independent of town funding or liability.
14) obtaining a renewable annual permit from MassDMF for the oyster deployment.
15) providing a commitment to work to address abutters concerns as much as possible, including visual impacts, minimizing noise, restricting work hours, and minimizing vehicle parking at the boat ramp.

<u>II.A.3. Aquaculture Contractor: Town Responsibilities</u>

II.A.3.a. Regulatory Approvals

Following the selection of the Aquaculture Contractor, the Town will work with the Aquaculture Contractor to secure: 1) a Board of Selectmen license from the Town, 2) a Division of Marine Fisheries certification of the Town license, 3) a Notice of Intent (NOI) or Request for Determination of Applicability (RDA) from the Town Conservation Commission. These permitting steps will be completed prior to Spring deployment. The Town will also ensure that the Aquaculture Contractor has obtained a renewable annual permit from MassDMF for the oyster deployment. If any other regulatory approvals are required, the Town will work with the Aquaculture Contractor to obtain necessary approvals.

II.B. Shellfish: Deployment Details

<u>II.B.1. Shellfish: Type, Quantity</u>

Shellfish will be deployed with the purpose of removing a Net Live Biomass (shell + tissue) of 23,400 kg (removal weight minus deployment weight) to yield a Net Nitrogen Removal of 75 kg N per year following one of the two acceptable scenarios below:

<u>Scenario A</u>:

- acquisition and deployment of a minimum of 5.5 million Year 1 seed oysters (2 to 3 mm) by June 1 in floating spat bags;
- then between July 1 and July 20, transferring seed to 2,040 6 mm mesh bags, such that there will be 1,000 oysters per bag;
- then allowing these oysters to grow and removing them between November 15 and December 15.

OR

<u>Scenario B</u>:

- acquisition and deployment of a minimum of 2.1 million Year 1 seed oysters (10 to 25 mm) by July 1 in 2,040 6 mm mesh bags, such that there will be 1,000 oysters per bag;
- then allowing these oysters to grow and removing them between November 15 and December 15.

If monitoring results show that this amount of oysters needs to be reduced or increased in subsequent years, it will be addressed in annual modifications and/or a future contract. The target population will be sustained with documentation of any replacements until at least November 15. All oysters and accompanying gear must be removed from Lonnie's Pond no later than December 15 with certification that the shellfish are disease-free prior to their removal from Lonnie's Pond and with permission from MassDMF and Natural Resource Manager to move the Year 1 oysters to a new site. The Aquaculture Contractor shall complete both the initial shellfish deployment and end of season removal within seven days.

II.B.2. Shellfish: Deployment Locations

The maximum extent of the deployment locations will be approximately 0.37 acres, similar to the area used during the Demonstration Project in 2017 and 2018 (Figure 1). It is anticipated that a rotational deployment strategy will be implemented where the same site is not used for more than two consecutive years. Locations will be finalized with the Town Natural Resource Manager (or designee) assisted by the Monitoring Coordinator who will provide GPS coordinates, before submission for Conservation Commission review.

II.B.3. Shellfish: Density and Care

Based on estimates developed through the Demonstration Project, after the shellfish reach the size where they can be deployed in the 6 mm mesh bags, the density will be approximately 1,000 oysters per bag from July to removal. Installation will be similar to the Demonstration Project setup with 800 floating bags along lines approximately 10 ft apart within each of the 80 ft X 120 ft areas (as shown in Figure 1). As the oysters grow, it is anticipated that actual growth conditions may require adjustments by the Contractor. The final number of bags is anticipated to be approximately 2,040 bags based on information developed during the Demonstration Project (see Appendix A). The number of spat bags in Scenario A is flexible, but must fit in the deployment footprint.

Bags will be flipped on a weekly basis at the beginning of the growing season and bi-weekly later in the season; this practice was deemed to be sufficient to prevent tunicate (sea-squirt) and algal growth and help extract feces and pseudofeces. Dead oysters should be removed, at a minimum, when bags are split. Gear will be inspected and repaired if necessary during these visits. Aquaculture activities on the pond shall be performed between the hours of 7 AM and 6 PM Monday through Friday, except in preparation for storms.

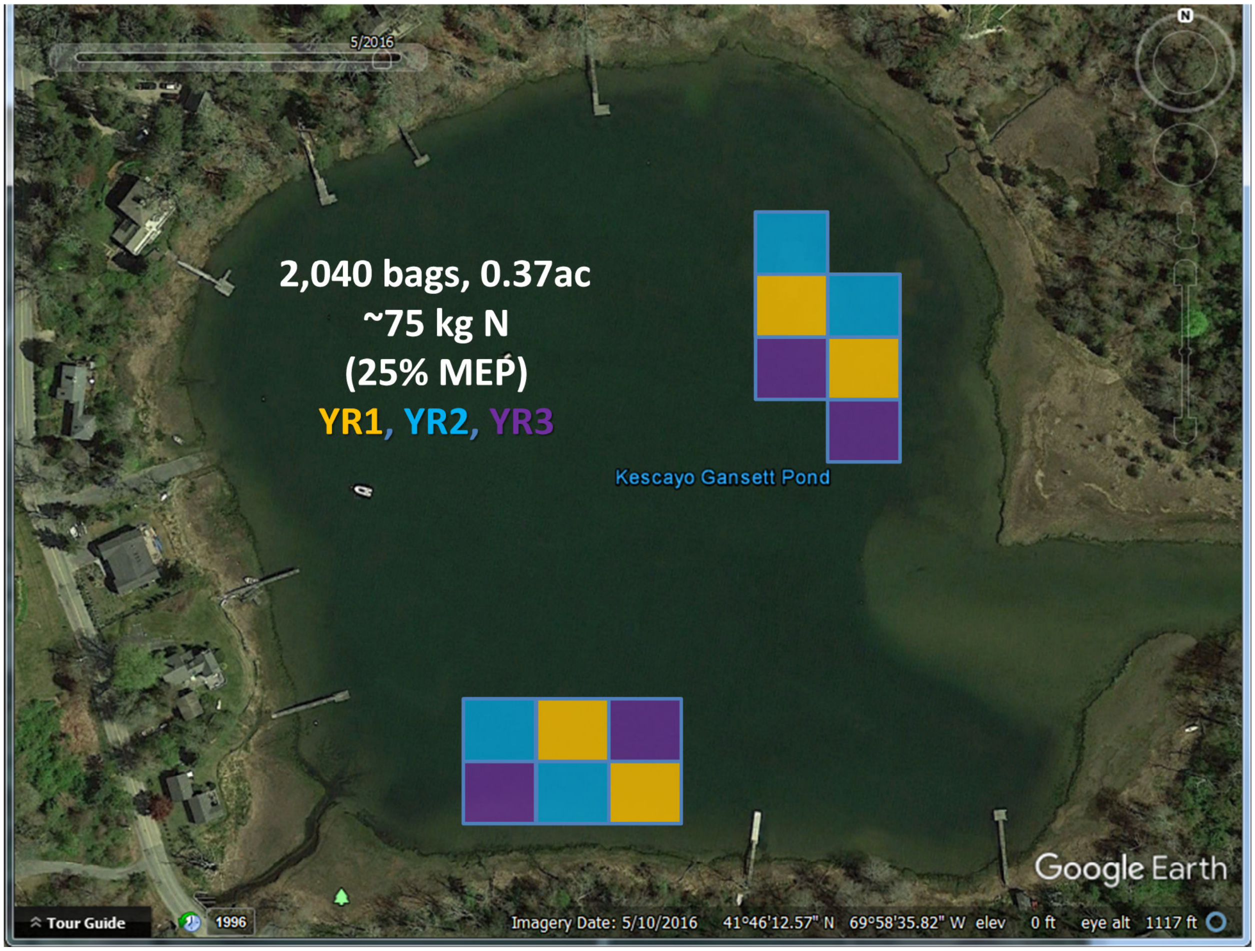


Figure 1. Lonnie's Pond Oyster Aquaculture Deployment Area. During Year 1, the deployment will be within the four boxes shaded yellow shown above, which have an area of 0.37 acres (or 2% of the pond area). This area is the same surface coverage as utilized during the Demonstration Project. During Years 2 and 3, the deployment area will remain 0.37 acres, but will shift to other boxes within the overall deployment area as shown. Rotation of deployment areas will occur to encourage additional nitrogen reductions and will try to avoid use of the same areas for more than two consecutive years. Deployment areas will be adjusted and reviewed annually based on the oyster count needed to attain N removal.

II.B.4. Shellfish: Catastrophic Loss (Death) or Equipment Loss

The timing of replacement noted herein is based on the maximum deployment being spring (no later than June 1 in Scenario A and July 20 in Scenario B) to December 15 each year, with no overwintering of oysters in Lonnie's Pond. Once oysters are removed from Lonnie's Pond by the Aquaculture Contractor, they are solely the responsibility of the Aquaculture Contractor.

In the event of a catastrophic loss of the shellfish population (defined as more than 50% of the initial annual population), the Aquaculture Contractor shall strive to replace the population as soon as replacements are available if the loss occurs in April, May, June, or July. If the loss occurs in August, September, October, November, or December, the Aquaculture Contractor will not be required to replace the population.

In the event of a significant gear loss due to a storm or theft during April, May, June, or July, the Aquaculture Contractor will be responsible to replace the gear at their cost and strive to install the replacement gear within the same month. If significant gear loss due to a storm or theft occurs during August, September, October, November, or December, the Aquaculture Contractor will be responsible to replace the gear at their cost, but re-installation of the replacement gear will not be required until normal spring deployment the following year.

III. Lonnie's Pond Nitrogen and Ecosystem Monitoring

Monitoring in Lonnie's Pond will be completed in order to provide the Town with TMDL compliance documentation. Monitoring will include sampling of water quality, shellfish nitrogen content, and sediment N content. Monitoring will occur at different frequencies depending on the source being sampled. It is anticipated that the Town will select a Monitoring Contractor to complete the ecosystem monitoring tasks.

III.A. Monitoring Contractor: Selection, Responsibilities

<u>III.A.1. Monitoring Contractor: Selection</u>

The selection of a Monitoring Contractor for Lonnie's Pond will be completed through a public procurement process, including the development of a Request for Proposals (RFP). The Town will publicly advertise a RFP in January prior to the first year of deployment for a 3 year duration (same as Aquaculture Contractor, also renewable) and select the Monitoring Contractor by February 15. The RFP will follow standard Town procurement requirements and include the following criteria for review:

1) Experience in water quality sampling, both on Cape Cod and within Orleans
2) Experience with types of sampling required for Lonnie's Pond, including potentially use and coordination of volunteers, shellfish sampling, and sediment sampling
3) Description of planned sampling strategy, including anticipated laboratories for assays, detection limits, and how procedures may differ from those used during the Demonstration Project and the MEP.
4) Documentation of applicable insurance
5) The Monitoring Contract must also demonstrate acceptability of data produced for regulatory agencies (*i.e.*, compliance)

<u>III.A.2. Monitoring Contractor: Contractor Responsibilities</u>

The Monitoring Contractor will be responsible for collecting, handling, and transporting all samples to matrix-specific laboratories within timeframes required by assay methods (see Sections III.B and III.C). The Monitoring Contractor will select the laboratories and ensure that assay methods and detection limits are comparable or superior to those used during the Demonstration Project. The Monitoring Contractor will submit a Lonnie's Pond Nitrogen Management QAPP to the Town and, following Town review, to MassDEP for approval to ensure MassDEP acceptability of results for TMDL compliance. The Monitoring Contractor will also be responsible for preparing both a Semi-Annual Technical Memorandum and a Lonnie's Pond Aquaculture/TMDL Annual Report with the assistance of the Aquaculture Contractor (see Section IV). Each of these reporting documents will be submitted to the Town for review and final approval before being submitted to MassDEP. Monitoring activities on the pond shall be performed between the hours of 6 AM and 6 PM Monday through Friday, except in preparation for storms.

III.B. Water Quality Sampling

Water quality sampling will include both grab samples and continuous monitoring and will focus on summer months, which are the primary focus for TMDL compliance. Sampling methods and chemical assays will generally be the same as previous MEP and Town samplings and comparability to historic data is required.

III.B.1. Water Quality Grab Sampling

Water quality grab sampling will be conducted every two weeks between June 15 and September 15 in each of the three years. A reduction in sampling to only once a month may be allowed in future years if system variability within the monitoring results becomes sufficient documented and less frequent sampling is deemed sufficient for compliance by MassDEP. The Monitoring Contractor will participate in any discussion about sampling with the Town and MassDEP. Water quality samples will be collected during the morning (6 AM to 9 AM) on the outgoing (ebb) tide. Sampling dates will be selected so that sampling occurs at approximately mid-ebb tide (1 hr before to 1 hr after). Since these are the same procedures utilized in the Demonstration Project and the volunteer sampling coordinated through the Marine and Fresh Water Quality Committee, it is envisioned that volunteers will continue to collect this portion of the Lonnie's Pond sampling.

Samples will be collected at two depths:

- one sample at 0.25 meters below the surface (the "surface" sample).
- one sample from 0.5 meters above the bottom (the "bottom" sample)

Field measurements for total depth, temperature, dissolved oxygen, and clarity/Secchi will also be recorded at the same location (Figure 2). All sampling methods and sample handling will follow previously approved Town and MEP QAPP procedures and the project-specific Lonnie's Pond Nitrogen Management QAPP.

Collected grab samples will be assayed for the following parameters: nitrate+nitrite, ammonium, total dissolved nitrogen, particulate nitrogen, ortho-phosphorus, chlorophyll a, pheophytin a, and particulate carbon. All assay methods will follow those described in previously approved Town and MEP QAPP procedures and the project-specific Lonnie's Pond Nitrogen Management QAPP.

III.B.2. Water Quality Continuous Monitoring

Two continuous monitoring devices will be installed at two depths (0.5 m and 3.5 m). Both devices will have the following probes: dissolved oxygen, depth, salinity, temperature, and chlorophyll-a. The devices will be installed in late May/early June and removed in September to coincide with the critical compliance period. The device will be programmed to record readings every 15 minutes.

III.B.3. Contingency Monitoring

Funding will be reserved to address "emergency" monitoring associated with extreme events that are anticipated to occur rarely during the growing season, including harmful algal blooms, signs of shellfish disease, and hurricanes.

Figure 2. Water Quality Monitoring Sampling Location. Location indicated with green bullseye.

III.C. Shellfish Sampling

Shellfish sampling will be conducted by the Monitoring Contractor to measure the amount of nitrogen retained and removed through removal of oysters at the end of each growing season. This sampling will be coordinated with the Aquaculture Contractor. The amount of nitrogen removed will be part of the TMDL compliance reporting, as will the size and number of oysters harvested.

At the beginning of the growing season, the Aquaculture Contractor will coordinate with the Monitoring Contractor to measure the total weight and estimate the total population of oysters deployed in Lonnie's Pond. At the end of the growing season, the Aquaculture Contractor will coordinate with the Monitoring Contractor to weigh all the oysters as they are removed from Lonnie's Pond.

A representative sample of one hundred (100) shellfish will be collected, weighed, and analyzed for nitrogen twice during the shellfish season in batches of no more than 10 oysters, once at the beginning of the growing season prior to deployment and once at the end of the growing season. If seed are being assayed, screening for size can be used, and groups of seed can be weighed and analyzed as long as at least 5 groups are assayed. Analysis will be completed on whole shellfish. End of season oysters will be measured individually. Comparison of the nitrogen content in the pre- and post-growing season samples combined with the weight and post-growing count will determine the amount of nitrogen removed by shellfish in Lonnie's Pond. All nitrogen content assay methods and sampling procedures will follow those described in the Demonstration Project (see Appendices A and B).

III.D. Sediment Sampling

Sediment sampling will be conducted to measure the forms and amount of nitrogen deposited to the sediments by the shellfish during a given growing season. The amount removed will be part of the TMDL compliance reporting.

Eight (8) cores will be collected and incubated twice to determine the amount and forms of nitrogen under a selected portion of the shellfish area. Cores will be collected in August and early October to determine N_2 release as an additional removal of nitrogen associated with the aquaculture deployment. Four (4) cores under the floating bags and 4 cores at a "control" area adjacent (>15 m outside) the floating bags will be collected during each core collection run. Core collections will follow procedures used during the demonstration project (Appendix B). Core data will be reviewed each year to assess the need for additional sampling in the following year (agreement between the Town, Monitoring Contractor and MassDEP). Water column sampling at each meter will also be conducted at the time of core collection to determine the water column nitrogen forms. Comparison of the forms of nitrogen in the pre- and post-growing season samples and in the various redox conditions of the cores will be considered with the water quality data to estimate the amount of nitrogen that has been removed via denitrification. All nitrogen content assay methods will follow those described in the Demonstration Project (see Appendix B).

IV. Lonnie's Pond Reporting and Communication

The Lonnie's Pond Aquaculture Management Project will include two primary communication pathways: a) reporting to state agencies and b) reporting to Orleans decisionmakers, citizens, and staff. Two documents will be prepared by the Monitoring Contractor with cooperation from the Aquaculture Contractor and analytical facilities and will be the main focal points for providing updates on Project status: 1) Lonnie's Pond Aquaculture/TMDL Annual Report and 2) Lonnie's Pond Aquaculture/TMDL Semi-Annual Status Update Technical Memorandum. However, these formal reports do not take the place of on-going communication between the Aquaculture Contractor, Monitoring Contractor, the Natural Resource Manager (or designee) and the Shellfish and Waterways Improvement Advisory Committee.

IV.A. Lonnie's Pond Reports: Annual Report and Semi-Annual Status Update

The Lonnie's Pond Aquaculture/TMDL Annual Report will, at a minimum, include summaries of the following:

1) Aquaculture summary, including shellfish counts, size, and weight at initial deployment and at removal, and notes on maintenance and care,
2) Shellfish nitrogen mass removal based on comparison of average weight and nitrogen content at the time of delivery and upon removal at the end of the growing season,
3) Progress toward meeting TMDL and MassDEP surface water standards based on water quality results (both continuous records and grab samples), shellfish nitrogen removal, and sediment core results, and
4) Recommendations for refinements in any Project procedures and likely changes in costs for refinements.

The Lonnie's Pond Aquaculture/TMDL Semi-Annual Status Update will be a technical memorandum prepared by the Monitoring Contractor by the end of the July and will summarize initial insights from shellfish deployment and ecosystem monitoring (including dates of installation, maintenance, and monitoring), but will not significantly review any monitoring data. The Semi-Annual Status Update will incorporate information and data collected by the Aquaculture Contractor. The Update will be submitted to Natural Resource Manager (or designee) and the Shellfish and Waterways Improvement Advisory Committee and presented by the Monitoring Contractor at a Committee meeting.

IV.B. Lonnie's Pond Reports: Delivery Dates and Public Presentations/Outreach

The Lonnie's Pond Aquaculture/TMDL Annual Report will be prepared by the Monitoring Contractor with cooperation from the Aquaculture Contractor and analytical facilities. The Annual Report will be publicly presented in draft form at a forum acceptable to both the Town and the Monitoring Contractor in December and a final form will be delivered to the Town in January following the receipt of any comments. The Lonnie's Pond Aquaculture/TMDL Semi-Annual Status Update Technical Memorandum will be delivered to the Town in July and will be publicly presented at a forum acceptable to both the Town and the Monitoring Contractor. The Town will mail agendas and any meeting materials to Lonnie's Pond abutters and other concerned citizens prior to the presentations on both the Semi-Annual Status Update and the Annual Report. Both the Technical Memorandum and the Annual Report will be available on the Town website, as will any presentation materials.

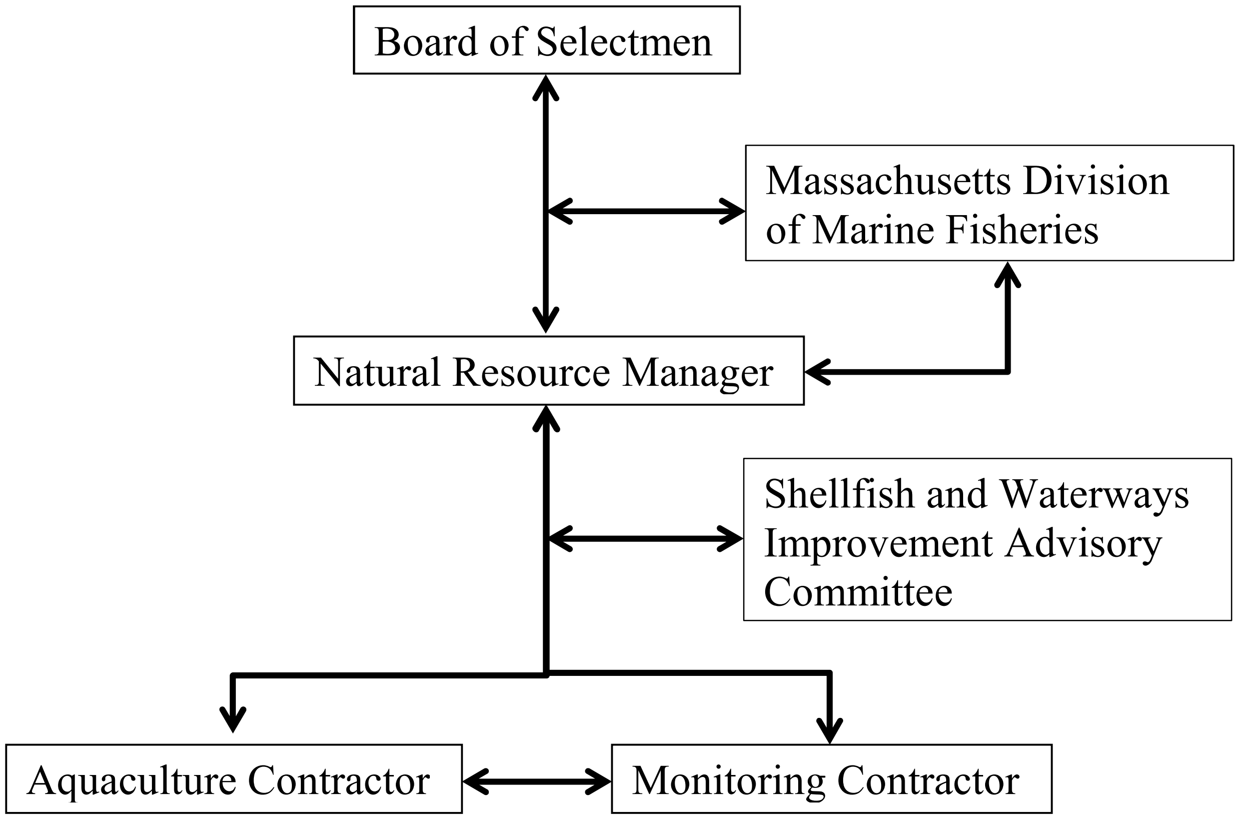
IV.C. Lonnie's Pond Reports: Regulatory Reporting

The Town, with support from the Monitoring Contractor, will submit the Lonnie's Pond Aquaculture/TMDL Annual Report to MassDEP in support of TMDL and Surface Water Quality Standards compliance. A copy of the Annual Report will also be submitted to MassDMF and the Town.

V. Lonnie's Pond Aquaculture and Nitrogen Management Plan Schedule

Action	Date	Frequency
Town Actions		
Town publicly advertises Aquaculture Contractor RFP	November	YR1 and as needed
Town holds Aquaculture Contractor pre-bid conference to review available, used Lonnie's Pond Aquaculture gear	November	YR1 and as needed
Town selects Aquaculture Contractor	December 1	YR1 and as needed
Town publicly advertises Monitoring Contractor RFP	January	YR1 and as needed
Town grants license to Aquaculture Contractor following MassDMF certification of the Town license	January/February	YR1 and as needed
Town and Aquaculture Contractor obtain RDA or NOI from Conservation Commission	January/February	YR1 and as needed
Town selects Monitoring Contractor	February 15	YR1 and as needed
Aquaculture Contractor Actions		
Aquaculture Contractor confirms to Town that sufficient Year 1 seed oysters will be delivered	By December 31	Annually
Aquaculture Contractor deploys oysters	no later than June 1 (Scenario A) or July 1 (Scenario B)	Annually
Aquaculture Contractor removes oysters and gear	Between November 15 and December 15	Annually
Monitoring Contractor Actions		
Monitoring Contractor completes and submits Nitrogen Management QAPP to Town and MassDEP	February/March	Every 3 years
Monitoring Contractor measures initial shellfish N content through coordination with Aquaculture Contractor	Collection at time of deployment	Annually
Monitoring Contractor completes sampling every two weeks	June 15 to September 15	Annually
Monitoring Contractor installs and maintains continuous monitoring devices	Late May/early June to September	Annually
Monitoring Contractor submits and publicly presents Lonnie's Pond Aquaculture/Semi-Annual Status Update	By July 30	Annually
Monitoring Contractor measures sediment N removal	August	Annually
Monitoring Contractor measures harvest shellfish N content	Late Fall	Annually
Monitoring Contractor measures sediment N removal	October	Annually
Monitoring Contractor submits and publicly presents draft Lonnie's Pond Aquaculture/TMDL Annual Report	By December 31	Annually
Monitoring Contractor submits final Lonnie's Pond Aquaculture/TMDL Annual Report	By January 31	Annually

VI. Lonnie's Pond Aquaculture and Nitrogen Management Plan Organizational Chart



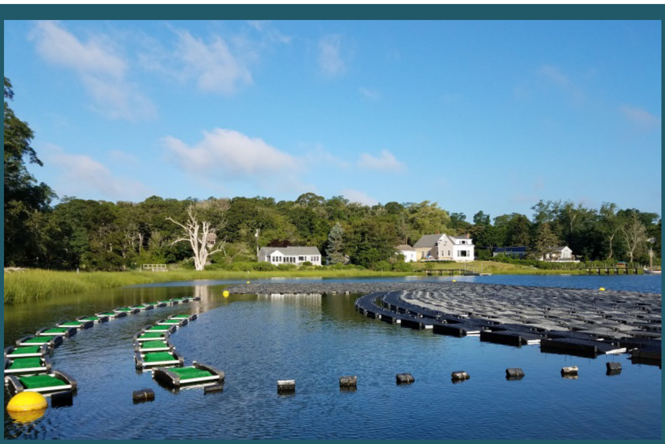
Appendix A. AECOM Lonnie's Pond Aquaculture Management Plan. Final. January 2018.

Appendix B. Coastal Systems Program, School of Marine Science and Technology (CSP/SMAST), University of Massachusetts-Dartmouth, Lonnie's Pond Shellfish Demonstration Project: Year 2 Monitoring Summer/Fall 2017 Oyster Deployment. September 2018.

Lonnie's Pond Aquaculture Management Plan

Town of Orleans, MA | Water Quality and Wastewater Planning

August 4, 2017
Revised December, 2017
Final January 2018



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Executive Summary

In 2016, the Town of Orleans initiated a three year oyster Demonstration Project in Lonnie's Pond. The Demonstration Project is an outcome of planning efforts, including the Cape Cod Commission's 208 Plan Update, a Consensus Plan, and subsequent amendments to the Town's 2010 Comprehensive Wastewater Management Plan, which identified the need to improve water quality in coastal waters surrounding Cape Cod. The overall purpose of the Demonstration Project is to assess the effectiveness of using aquaculture to remove nitrogen from the water as a component of the Town's strategy to meet TMDL requirements and total nitrogen load reduction targets. Lonnie's Pond was identified as the preferred location for the town's first shellfish Demonstration Project based on a few key factors, including: the town's strong desire to improve the environmental conditions in the town's terminal ponds, many of which include anoxic, muddy sediments; and the expected ability to monitor water quality and other impacts caused by shellfish in this semi-closed sub-embayment.

The goals of this management plan are to summarize activity to date at Lonnie's Pond and identify management tasks between now and the end of the three-year Demonstration Project, including acquisition and disposition of shellfish, installation and monitoring of shellfish and water quality in Lonnie's Pond, budgeting, and long-term considerations for an aquaculture program in Lonnie's Pond.

Nearly 200,000 First Year (Y1) and Second Year 2 (Y2) oysters were deployed in Lonnie's Pond on June 22, 2016. Y1 are those oysters beginning the year as seed oysters approximately 2 to 3 mm in size and growing throughout one season. Y2 are those oysters that are in their second year of growth. The oysters were placed in floating bags containing 250 oysters each that were installed in an 80 foot by 120 foot system. Each bag contained 250 oysters. The bags were maintained on a weekly basis during the peak impairment season of July and August and then bi-weekly for the remainder of the growing season. Monitoring occurred every two weeks between June 2016 and December 2016. Monitoring consisted of assessing growth rate of 25 to 30 oysters that were randomly selected from each of seven tracking bags. The weight, length, and volume of the oysters were recorded. In addition, the oysters were monitored for mortality.

The oysters in the Lonnie's Pond demonstration grew well and at a rate that is typical for Cape Cod oysters during the first year of the demonstration. The Y1 oysters finished the season at an average length of about 74 mm (2.9 in) and an average weight of 35 g (1.23 oz.). The Y2 oysters finished at an average length of about 94 mm and an average weight of about 69 g (2.4 oz.). All of the Y2 oysters and approximately 80 percent of the Y1 oysters reached either petite or regular market size by the end of the Demonstration Project season, which started later than the normal growing season would. The live weight of the oysters increased from approximately 2,176 metric tons at the beginning of the project in June to approximately 10,091 metric tons at the end of the growing season (adjusted for 6.6 percent measured mortality prior to overwintering).

To measure the nitrogen content of the shell and dry weight tissue of the oysters, the samples were sent to the Boston University Stable Isotope Laboratory. The nitrogen content for Y1 and Y2 was 10.5 percent and 10.3 percent, respectively. The preliminary assessment indicates that the demonstration system removed 25.9 kg of nitrogen by uptake (increased biomass). The demonstration monitoring project in Lonnie's Pond provided data indicating that nitrogen reduction was achieved, which is a goal of the TMDL established by MassDEP for Lonnie's Pond.

Between 2016 and 2017, the oysters were overwintered in Lonnie's Pond in a system designed to keep oysters from sinking into soft sediment, avoid ice damage, maintain enough flow for the cold weather metabolic activity of oysters, and control mortality from predation. The oysters were resurfaced in mid-April 2017 and removed from the bags and assessed for mortality. Overall, the mortality rate for Y1 oysters from June 2016 through the resurfacing in 2017 was less (1.1 percent) than the Y2 oysters (10.9 percent). The total live weight of the oysters was measured and a representative sample of individuals was weighed and measured to assess growth during the winter period. These oysters were then used as a part of the second year demonstration. Year 2 oysters from the 2016 season, now three years old, were relayed to Falmouth, MA; approximately 113,000 oysters were relayed to Falmouth, MA with the intention that Falmouth, MA would return the same number of harvestable size oysters in fall 2017. However, due to oyster losses in Falmouth, MA, Orleans, MA and Falmouth, MA mutually agreed that 113,000 harvestable size quahogs would be returned in lieu of oysters. These quahogs were made available for residential family harvest.

The Demonstration Project was continued for a second year in late spring of 2017. Both Y1 and Y2 oysters were deployed in bags in a similar system as 2016. The population of oysters for the second demonstration year consisted of oysters from the first year of the Demonstration Project (former 2016 Y1 oysters), 480,000 two mm oyster seed from Mook Sea Farms, and 58,000 two inch oysters from Falmouth, MA. The total number of oysters deployed in the second year was approximately 607,000 oysters. Year 2 oysters were installed in 510 bags with a targeted final grown-out stocking density of 250 oysters per bag. Over the course of the 2017 growing season, the density of Y1 oyster bags was adjusted as their size increased, for a final grown-out density similar to the Y2 oysters.

Science Wares' monitoring in Year 2 included data collection to further refine the relationship between live wet weight and nitrogen content to provide a tool for quantifying estimated nitrogen removal by oysters without analyzing the nitrogen content of dry shell and tissue. These results will be included in the Year 2 report, due to be released in draft form in late January 2018.

The demonstration monitoring project in Lonnie's Pond data showed that oysters are achieving nitrogen reduction. The results from year one of the project indicate that oysters are removing nitrogen and could be used to meet part or all of the TMDL requirement in regard to nitrogen reduction. The oysters removed sufficient nitrogen through uptake alone. Mortality was low and the bag and line system and overwintering installation provided sufficient protection from predation and supported strong oyster growth. Year 2 and Year 3 of the Demonstration Project in Lonnie's Pond will provide additional data to evaluate the effectiveness of this non-traditional technology.

Year 3 of the demonstration project is proposed to be designed similarly as Year 1 and Year 2 but oysters in the Y1 field will be deployed in a system that is half the size of a full scale operation. These oysters are intended to validate the ability of Y1 focused scenarios to meet TMDL and MEP requirements. The field of Y2 oysters is proposed to remain the same as in 2017 with approximately 130,000 oysters. Maintenance and monitoring are proposed to be the same as in previous years, with bi-weekly measurements taken during the growing season. Samples will be sent to the BU laboratory for nitrogen analysis at the beginning and end of the demonstration year.

In fall of 2018, oysters that have been used for the Demonstration Project and are of harvestable size will be put out for harvest by residents, while smaller oysters are anticipated to be traded to Falmouth, MA for quahogs that will also be put out for harvest. It is necessary to remove these oysters from Lonnie's Pond because the oysters have incorporated nitrogen from the water column, and this nitrogen is only removed from the estuarine system when the oysters are removed from the pond.

The viability of oysters is impacted by a number of factors, including ocean acidification, predators, disease, algal blooms, biofouling and pests, storm damage, and theft. Environmental conditions should be monitored over the long term to ensure the health and survival of the oysters. Regular maintenance and the design of the system proved effective in controlling for predators and bio-monitoring to date. The overwintering system design protected from storm damage to date.

Biodeposition has not been approved by MassDEP as an approach to meet nitrogen targets. However, monitoring results indicated that it should supplement nitrogen removal by uptake. Further use of the biodeposition model to predict denitrification in Lonnie's Pond is warranted, however this management plan focuses on removal by direct uptake.

A number of scenarios are under consideration for full-scale implementation subsequent to 2018. It is estimated that the MEP nitrogen reducing goal for Lonnie's Pond (660 lbs/yr or 300 kg/yr) could be met by growing and harvesting Y1 oysters annually or by growing a combination of Y1 and Y2 oysters in a larger area of the pond, with oysters removed when they are of harvestable size. The selected scenario will be determined based on review of the last two years of data as well as the status of the MassDEP approval of aquaculture as part of a Watershed Permit and associated Watershed Management Plan.

1.0 Introduction

1.1 Background

1.1.1 Cape Cod Commission 208 Plan

Massachusetts Department of Environmental Protection (MassDEP) directed the Cape Cod Commission to update the 1978 Water Quality Management Plan in accordance with Section 208 of the federal Clean Water Act. The updated was necessary due to the impairment of water quality in coastal waters resulting from excess nitrogen. The plan was prepared by the Commission and approved by MassDEP and US EPA in 2015.

The 208 Plan Update identified a number of recommendations to improve water quality in coastal waters surrounding Cape Cod. Among these were a number of alternative technologies that should be considered to reduce nitrogen loadings from wastewater on the Cape, in addition to the consideration of traditional sewering, treatment, and effluent discharge approaches.

Following the update to the 208 Plan, a Consensus Agreement was developed under the guidance of the Orleans Water Quality Advisory Panel (OWQAP), which convened in 2014 to achieve consensus and build widespread community support for a customized, affordable water quality management plan for Orleans. The Consensus Agreement led to the preparation of an Amended Comprehensive Wastewater Management Plan,

1.1.2 Orleans Amended CWMP

In 2010, a Comprehensive Wastewater Management Plan (CWMP) was prepared that proposed to meet state and federal mandates through an expansion of the municipal sewer system under an Adaptive Management Plan.

The Amended CWMP was developed to provide the Town of Orleans with an alternative, more cost effective strategy for managing wastewater and reducing nitrogen in the Rock Harbor, Nauset Marsh, Pleasant Bay, Namskaket, and Little Namskaket Watersheds. This strategy included a hybrid approach to managing wastewater through a combination of traditional (sewered) technologies and several non-traditional technologies.

1.1.3 Non-Traditional Technologies

Non-Traditional nitrogen control strategies can reduce the volume of wastewater that requires treatment at wastewater treatment facilities and result in lower treatment costs for the Town. The Consensus Agreement recommended three Non-Traditional Technologies for use in key locations in Orleans' sub-watersheds in order to reduce nitrogen loading in the Town's coastal estuaries: Floating Constructed Wetlands (FCW), aquaculture/shellfish propagation, and permeable reactive barriers (PRB). An additional innovative Non-Traditional Technology, a Nitrogen Reducing Biofilter (NRB), was also considered for implementation in areas of Orleans.

1.1.4 Aquaculture/Shellfish Propagation

Orleans chose to include shellfish propagation as a means to reduce the amount of nitrogen entering watersheds where sewering was not currently planned. Four different Demonstration Projects were discussed and planned in order to obtain site specific information within Orleans' waterbodies and the viability of pursuing full-scale implementation. The Demonstration Projects were scaled to allow meaningful monitoring and quantifiable results, while expending only the minimal amount of necessary funds during this experimental phase. The purpose of the Orleans shellfish demonstrations is to both locally measure the nitrogen-removal benefits of shellfish cultivation as well as to demonstrate the practical applications of shellfish propagation and aquaculture expansion within the Town of Orleans.

1.1.5 Lonnie's Pond

Potential demonstration sites for non-traditional technologies were systematically evaluated and ranked using a site selection matrix that included criteria for site suitability, permitting, and project evaluation. Initially, Lonnie's Pond was not evaluated by the Shellfish Team during the site selection process because it was selected by as the best alternative for Floating Constructed Wetland implementation. Once the Town put implementation of the Floating Constructed Wetland technology on-hold until further refinement of estuarine nitrogen removal and costs were evaluated, Lonnie's Pond was identified as the preferred location for the town's first shellfish Demonstration Project. This selection was made based on two key factors: the town's strong desire to improve the environmental conditions in the town's terminal ponds, and the expected ability to monitor water quality and other impacts caused by shellfish in this semi-closed sub-embayment.

At the time of the preparation of this plan, Year 1 (Y1) of the oyster Demonstration Project at Lonnie's pond had been completed and the growing season of Year 2 (Y2) was underway. Work completed to date, including the installation, monitoring, overwintering, and analysis of 200,000 oysters, is summarized in the following sections of this management plan. The remaining work of the Demonstration Project includes monitoring and data collection for Y2, installation, monitoring, and data collection for Year 3 (Y3), and an assessment of considerations for full-scale aquaculture at Lonnie's Pond. A discussion of this work is included in the following sections of this management plan.

1.2 Goals and Organization of Document

The goals of the document are to summarize activity to date at Lonnie's Pond to date and identify remaining actions between now and the end of the three-year Demonstration Project. Remaining actions include:

- Important decision points such as acquisition of oysters for additional years of investigation;
- Disposition of shellfish at the conclusion of each season;
- Budgeting for the remaining years of the Demonstration Project; and
- Long-term considerations and next steps for maintaining an aquaculture program in Lonnie's Pond, and potentially other terminal ponds in Orleans.

The introduction of this document, Section 1, describes past plans and activities that led up to the planning and installation of a Demonstration Project at Lonnie's Pond. Section 2, Section 3, Section 4, and Section 5 provide an overview of the planning and implementation of the three year Demonstration Project. A description of the planning, installation, monitoring program and results, and overwintering for Y1 is included in this document. At the time this document was prepared (August 2017), Y2 of the demonstration at Lonnie's Pond was underway and sampling results were not yet available. Additional details about the demonstration field, installation, and monitoring can be found in the Technical Memorandums titled *Demonstration Project Year 1 Project Report* (2/17/17) and *Draft Aquaculture Full-Scale Implementation Program* (7/7/17).

A discussion of oyster viability considerations, including disease, pests, and environmental threats, is included in section 6 of this document. Section 7 provides an overview of the results of water quality monitoring from Year 1, which is described in more detail in the memorandum *Demonstration Project Year 1 Project Report.*

The final sections of this document review considerations for full scale aquaculture at Lonnie's Pond as well as other opportunities for shellfish aquaculture and coastal habitat restoration.

1.3 Town Organization

1.3.1 General

The Lonnie's Pond Demonstration Project and Full Scale Implementation is an outcome of planning efforts, including the Cape Cod Commission's 208 Plan Update, a Consensus Plan, and subsequent amendments to the Town's 2010 Comprehensive Wastewater Management Plan, which identified the need to improve water quality in coastal waters surrounding Cape Cod. The organization for the Project is shown on Figure 1-1.

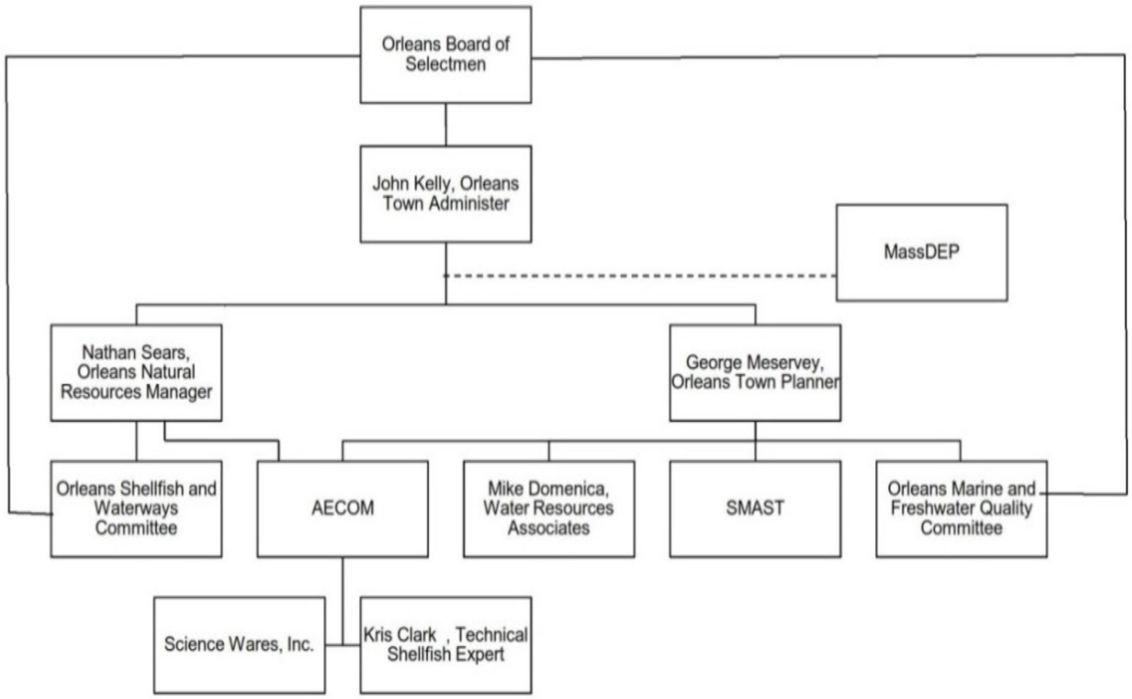

Figure 1-1 - Organizational Chart for Lonnie's Pond Demonstration

1.3.2 Board of Selectmen

The Board of Selectmen has a delegate who coordinates with the Committee and apprises the Board of status of the Lonnie's Pond aquaculture project, as well as any issues requiring decisions and resolution. The Board of Selectmen considered the Lonnie's Pond Management Plan in fall 2017 and voted to place an item on the Warrant for the fall 2017 Town Meeting for approval of funding for the spring 2018 portion of Y3 of the Demonstration Project. Prior to consideration of the budget at Town meeting, the Finance Committee reviewed the proposed budget and informed citizens of Orleans of its findings and recommendations. This is discussed in further detail in Section 5.7.

1.3.3 Shellfish and Waterways Improvement Advisory Committee Review

The Shellfish and Waterways Improvement Advisory Committee (the committee) serves as an advisor to the Town Administrator, Board of Selectmen, Harbormaster/Shellfish Constable, and other Town boards and committees. The Town calls upon this committee for issues related to preserving, protecting, managing, and enhancing natural resources, including shellfish and waterways. It is the committee's role to review the progress of, and provide recommendations as needed, in regard to the oyster Demonstration Project and Full Scale Implementation at Lonnie's Pond. The Committee provides recommendations to Nate Sears, Orleans Natural Resources Manager and the Board of Selectmen. In fall 2017, the Committee reviewed a draft version of the Management Plan and made a recommendation to Nate Sears and the Board of Selectmen to continue with Year 3 of the oyster Demonstration Project at Lonnie's Pond Board of Selectmen Review,

1.3.4 Town Meeting Budget Approvals

The Board of Selectmen submitted a Warrant Article for the fall Town Meeting, and citizens voted to approve the budget for spring 2018 Demonstration Project work on October 24, 2017. Ongoing budgets for the Lonnie's Pond aquaculture will be subject to approval at future Town Meetings as appropriate.

2.0 Lonnie's Pond Demonstration Project Planning

Orleans is pursuing oyster cultivation as the first demonstration because many scientific papers published in peer-reviewed journals demonstrate the nitrogen uptake and water quality improvements caused by oyster cultivation (Bricker 2015; Carmichael et al. 2004; Higgins et al. 2011; Kellogg et al. 2013, 2014; Nelson et al. 2004; Porter et al. 2004).

Oysters feed by filtering algae and other particles that contain nitrogen out of the water column. Through this filter-feeding process, oysters both improve water clarity and impact nitrogen concentrations (Newell et al. 2002, 2004, 2005; Officer 1982). Oysters remove nitrogen from the water column by filtering phytoplankton and other organic particles from the water. These inorganic materials are incorporated into the shell and soft tissue and are removed when the oysters are harvested. In the sediment, nitrogen compounds in the feces and pseudofeces are mineralized into inorganic nitrogen through oxidation to nitrates. Denitrification of nitrates releases nitrogen gas which leaves the system. A small fraction of the nitrogen is buried in the sediment and does not re-enter the water column.

Thus, the main pathways by which oysters remove the mass of nitrogen in an estuary are:

- Uptake into shell and soft tissue (which harvesting removes);
- Enhancement of sediment denitrification (nitrogen removed as a gas); and
- Packaging of particles into feces and pseudofeces (biodeposits), which sink into the estuary bottom and are not denitrified (burial).

It is important to remember that all of the nitrogen that is sequestered in the body of an oyster, as well as the nitrogen contained in biodeposits and excretions, comes originally from the water column. Therefore, following the principle of the conservation of mass, oysters do not contribute new nitrogen, but instead both sequester and reformulate the nitrogen already contained in an ecosystem. Biodeposition and excretion of inorganic nitrogen does not add any new nitrogen to the water column or estuary bottom. The nitrogen was already in the system.

Removing oysters that have grown in the water column directly removes a mass of nitrogen that was previously in the water. This nitrogen-removal value can be measured directly by weighing the shell and soft tissue and applying a measured value for the percent nitrogen contained therein. While the amount of nitrogen sequestered in the shell and tissue of adult oysters is reasonably consistent, rates of enhanced sediment denitrification vary widely and are highly site-specific (Kellogg et al. 2013). Therefore this management plan focuses on nitrogen removed via direct uptake.

There is a strong scientific basis for using oyster cultivation to decrease water column nitrogen concentration and improve water clarity. A Demonstration Projects focusing on oysters was seen as an important first step in order to validate the quantities of nitrogen removed through uptake in the body of the oyster. The Lonnie's Pond project provides the field-verified basis for including oyster cultivation in the Town's wastewater plans.

3.0 Lonnie's Pond Oysters Demonstration Project Year 1 (2016)

Two-hundred thousand (200,000) oysters between 1 and 2 inches in size were installed in floating bags in Lonnie's Pond in the summer of 2016. The oysters were maintained and monitored through the winter of 2016 and 2017. In April of 2017, the oysters were removed from the bags and evaluated for survival and growth. The work accomplished and results of Project Y1 are summarized below.

3.1 Field Installation

Oysters were deployed in Lonnie's Pond in an 80 foot by 120 foot system comprised of 800 floating bags that were installed along long lines spaced approximately 10 feet apart. Each floating bag contained 250 oysters. Volunteers, staff, and unpaid members of the shellfish technical team assisted with assembling and deploying the oysters, which were purchased from the Town of Falmouth, MA and Cape Cod Oyster (Table 3-1).

Table 3-1 - Oysters for Demonstration Year 1 (2016)

Size Classes	Number of Oysters	Source of Oysters	Number of Bags
Year 2 (2-inches)	127,000	Town of Falmouth, MA	560
Year 1 (1-inch)	11,700		
Year 1 (< 1-inch)	60,000	Cape Cod Oyster	240
TOTALS	**198,700**		**800**

3.2 Operation and Maintenance

In order to prevent fouling, bags were flipped on a weekly basis during the peak impairment period and bi-weekly as the growing season concluded. Tunicates (sea-squirts) and algae were found on several occasions but were controlled by the weekly flipping. Bags were flipped by boat at high tide to prevent disturbance to the bottom sediment and allow for SMAST to accurately determine denitrification rates. Flipping the bags also helped to prevent oysters from growing together and to trim edges, both of which are important for marketability. In addition, flipping the bags helps extract feces and pseudofeces.

Gear remained in-place all season and performed well overall. Minor repairs were performed as needed on the water, and primarily involved replacing broken zip-ties that held the side floats to some of the bags. On one occasion when there was a risk of a hurricane in the forecast, an additional 10-foot of length was temporarily added to the long lines to accommodate a possible storm surge increasing the water level.

The water level typically changed by several feet each the day. Scope between the end of the middle long line and the auger on each end was sufficient to allow the strings to withstand this range of water level change. On extreme tides, two to four bags located on the ends of the field would occasionally stand up on end, but the oysters would redistribute as the tide went out and the bags laid back down flat. Extended periods of wind and moon cycles could increase or decrease the average water height by about a foot. Regardless of the wind, the surface conditions were calm, and the gear was never at risk of damage due to wind or wave action.

3.3 Growing Season Monitoring

Seven floating bags were monitored for growth rates and mortality every two weeks between June and December, 2016 (total of 18 times). To assess growth rate, 25 to 30 oysters were randomly selected from each of the seven tracking bags and lightly scrubbed to remove surface fouling. Twenty-five oysters were placed on a scaled mat and photographed for measurement. The oysters were weighed as a group to determine an average weight. Individual oysters were also weighed to validate the averages. In addition, the total volume occupied by the sample was measured inside a cylindrical container with a gauge plate.

The entire oyster field was monitored for mortality during each session when bags were maintained and flipped. Two methods were used to assess oyster mortality: (1) visual inspection to look for open shells, and (2) audible inspection to listen for the distinctive rattle of a single oyster shell that could be heard when the bag was flipped. Mortality was noted during maintenance sessions, but dead oysters were not removed from the bags at that time.

The seven tracking bags were further inspected for mortality each time oysters were withdrawn for measurements, at which point any dead shells were inspected, counted, and removed. Mortality was first observed during these inspections of the tracking bags in mid-September. From mid-September through December, mortality counts were also made from additional representative bags during every other bag flip cycle. The final mortality assessment was made by counting live and dead oysters in four (4) bags of Y1 oysters and four bags of Y2 oysters that had not been previously counted. The overall final mortality rate prior to overwintering (December 2, 2016) was 6.6 percent.

3.4 Oyster Growth, Mortality, and Nitrogen Content

The oysters grew well and at rate typical of other Cape Cod locations. The Y1 oysters finished at an average length of about 74 mm (2.9 in) and an average weight of 35 g (1.23 oz.). The Y2 oysters finished at an average length of about 94 mm and an average weight of about 69 g (2.4 oz.). Approximately 80 percent of the Y1 reached harvestable size (76 mm / 3 in) in one shortened growing season (the typical season begins in May). The live weight of the oysters increased from approximately 2,176 metric tons at the beginning of the project on June 22, 2016 to approximately 10,091 metric tons at the end of the growing season (adjusted for 6.6 percent measured mortality). During the growing season, the volume requirement of the oysters increases, resulting in the need to split bags. The rate of change of volume requirement for Y1 and Y2 oysters is different, resulting in fewer bags on the water early in the season and during the critical impairment period of July and August for Y1 oysters as well as a lower visual impact from late June to early September.

Samples were sent to the Boston University Stable Isotope Laboratory to determine the nitrogen content of the shell and dry weight tissue. The nitrogen content for Y1 and Y2 was 10.5 percent and 10.3 percent, respectively Table 3-2). The preliminary assessment indicates that the demonstration system removed 17.3 kg of nitrogen by denitrification (about 67 percent of the amount removed through tissue and shell uptake) and 25.9 kg of nitrogen by uptake (increased biomass). A summary of oyster weights and nitrogen update from Y1 is displayed in

Location	Sample Time	Length (mm)	Whole Weight (g)	Dry Tissue Weight (g)	Total N, Shell and Tissue (g)	N as a percent of Dry Tissue Weight (%)
Y2 Lonnie's Incoming	Spring	62.9	17.73	0.66	0.0683	10.3
Y2 Lonnie's New Growth	Fall	100	80.4	2.49	0.257	10.3
Y1 Lonnie's New Growth	Fall	74.3	37.4	1.20	0.126	10.5

Table 3-3.

Table 3-2 - Total Nitrogen Content of Lonnie's Pond Oysters

Location	Sample Time	Length (mm)	Whole Weight (g)	Dry Tissue Weight (g)	Total N, Shell and Tissue (g)	N as a percent of Dry Tissue Weight (%)
Y2 Lonnie's Incoming	Spring	62.9	17.73	0.66	0.0683	10.3
Y2 Lonnie's New Growth	Fall	100	80.4	2.49	0.257	10.3
Y1 Lonnie's New Growth	Fall	74.3	37.4	1.20	0.126	10.5

Table 3-3 - Oyster Weights and Nitrogen Uptake from 2016 Demonstration by Installation Size Class

Oyster	Dry Tissue Weight (g)			N Uptake per Oyster (g)	Initial # of Oysters	Mortality	Total Uptake(kg)
	Initial	Final	Increase				
Y1	0.055	1.04	0.98	0.103	60,000	6.6%	5.81
Falmouth, MA Small	0.055	0.79	0.74	0.076	11,700	6.6%	0.84
Y2	0.562	2.12	1.56	0.160	126,690	6.6%	19.41
Totals					**198,390**	**6.6%**	**26.06**

The percent of total nitrogen contained in Lonnie's Pond oysters are typical for cultured off-bottom oysters; however, the actual value may be different depending on at which point in the growing season oysters are removed from Lonnie's Pond. Additional information about seasonal variation in the nitrogen content of oysters grown to marketable size will be obtained during the second year of the Demonstration Project. It is not expected that such variations will have a substantial effect on the overall viability or costs of the program. The relationship between dry tissue weight and harvest weight will also be established in the second year in order to develop a tool for quantifying nitrogen removal over the course of the growing season and at different harvest times.

3.5 Quality Control

As stated above, samples were sent to the Boston University Stable Isotope Laboratory for nitrogen analysis. The Laboratory weighs out samples to 0.001 mg into tin capsules on a microbalance, then combusts them in an elemental analyzer and measures them using software. Check standards are inserted into the run to ensure precision and quality control. Any anomalous samples are reweighed and rerun. The precision for replicate samples is 0.5 percent for nitrogen. Boston University (BU) protocols were approved by MassDEP. Refer to the QAQC document in Appendix A for a description of the protocol.

In addition to laboratory analysis, other procedures included sampling and analyzing oysters for harvest weight and dry weight. Science Wares' Standard Operating Protocols (SOPs) for these procedures are also included in Appendix A.

3.6 Overwintering System Design and Installation

Oysters were submerged for overwintering by December 23, 2016. The overwintering system was designed to keep oysters from sinking into soft sediment, avoid ice damage, maintain enough flow for the cold weather metabolic activity of oysters, and control mortality from predation, in addition to being practical to install. The system includes a three-part PVC tube frame attached to two plastic pallets, and is illustrated in Figure 3-1. The assembly footprint is 50 in wide by 82 in long by 57 in high and requires a minimum water depth of six feet. The negative buoyancy and nine wedge-shaped feet of each assemble prevent the loaded assembly from descending into soft bottom. Copper barriers were applied to some of the frames to discourage drills from traveling along the supports to reach the bags. This system made it possible to leave the oysters in the grow-out bags for overwintering, and is advantageous because it:

- Allows oysters to continue to filter water;
- Maintains water flow across the oysters to enable survival and prevent toxicity as oysters continue to filter and purge over the winter;
- Minimizes the handling of oysters that could damage the shells and lead to higher mortality over the winter; and
- Provides a physical barrier (6 mm bag mesh, copper wire) against mature drills and other predators.

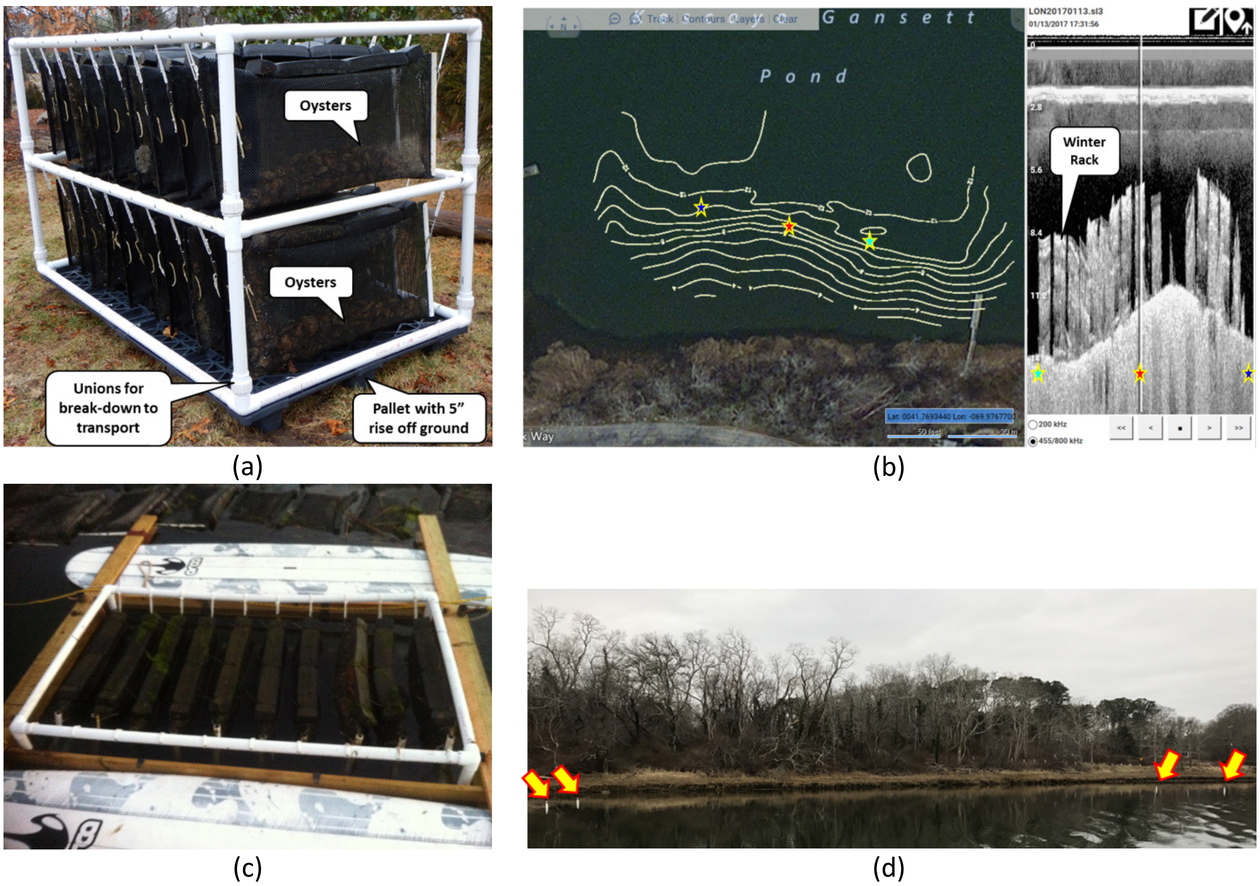

Figure 3-1. Science Wares overwintering system for 2016 Demonstration Year at Lonnie's Pond: (a) rack on dry ground showing two levels of bags oriented with one float up and oysters down (b) depth profile and sonar scan showing orientation of racks on the bottom (c) loaded rack about to be submerged (d) a view of the surface of Lonnie's Pond after all bags had been submerged, with corner markers showing the location of the overwintering racks.

A custom raft was constructed for installation of this overwintering system. Bags were installed on the racks and sunk to the bottom over the course of three days. The total biomass of oysters that were submerged was over 10,000 kg. This total weight was determined by direct measurement of oyster weights and survival. In the spring, the oysters were graded and an overwintering mortality was determined.

3.7 Winter Monitoring

Two temperature sensors were placed in different locations near the overwintering site to establish field conditions prior to overwintering. The sensors monitored water temperature at 10 minute intervals a few inches below the surface (moving up and down with the water level), and at a fixed location about a foot off the bottom near where the deepest overwintered oysters would be.

Typically oysters are kept on the surface as late into the winter season as possible, depending on environmental conditions including temperature and dissolved oxygen. Although common practice is to submerge the oysters below the surface as soon as the water temperature drops below 6°C for six days in a row, the demonstration oysters were overwintered earlier than usual due to forecasted low temperatures and risk of the water freezing at the surface. Ultimately, the water temperature at the surface did not reach -2°C, the temperature at which seawater typically freezes. The results of temperature monitoring show close tracking of the surface and bottom temperatures, highlighting two important features of Lonnie's Pond:

- A high turnover rate of water coming in from Pleasant Bay; and
- An absence of persistent stratification at the location where the oysters are being maintained.

In addition to monitoring temperature, a cluster of sensors was placed in the field of winter racks to measure temperature, water level, salinity, and dissolved oxygen at 15 minute intervals throughout the winter season. Data revealed that:

- Dissolved oxygen content does not fall below 12 mg/L, which is consistent with typical winter conditions;
- Normal cycle of tidally-influenced water levels can be affected by weather conditions, such as a northerly wind; and
- Large tidal variations (including changes of 1.65 m over a two-week period in early 2016 and typical daily oscillations of about 0.9 m and 1.2 m) indicate that there is a high rate of exchange of water with Pleasant Bay.

3.8 Resurfacing of Overwintered Oysters

The 2016 oysters were resurfaced between April 17 and April 24, 2017 after the water temperature had risen sufficiently. The oysters were brought to the surface, removed from the submerged bags, and assessed for mortality. The shells of dead oysters were separated out. The total live weight of the oysters was measured and a representative sample of individuals was weight and measured to assess growth during the winter period. These oysters were used as a part of the second year demonstration.

Table 3-4 summarizes the number of Y1 and Y2 oysters from 2016 that were processed and the mortality rate from June of 2016 through May of 2017. Year 1 oysters from the first year of the Demonstration Project (now Y2 oysters) were moved to the south side of the Demonstration Projects year two field. The Y2 oysters from 2016 (now Y3 oysters) were relayed to Falmouth, MA because they were of harvestable size but there was no location in late spring, 2017 within Orleans where they could be put out for harvest due to the time of year and lack of appropriate regulations in place at the time.

Table 3-4 - Resurfaced Oysters From 2016

Oyster Age and ID		Oysters Processed After Overwintering	Mortality Since 6/22/16	Location of Oysters in 2017
2016	2017			
Y1	Y2-L	70,769	1,320 (1.78%)	Oysters moved to south side of the Y2 field on May 4, 2017
Y2	Y3	127,346	13,772 (10.8%)	113,574 live oysters relayed to Falmouth, MA

3.9 Cost

Table 3-5 displays a cost estimate for the Year 1 of the aquaculture Demonstration Project in Lonnie's Pond in 2016.

Table 3-5 - Demonstration Project Year 1

Description		Cost
Planning and Implementation (July 2016 through October 2016)		$59,680
FY17 Planning and Implementation (November 2016 through June 2017)		
Build and Install Overwintering System		$80,000
2016 Project Report		$17,000
Assemble bags		$115,000
Install oysters (June 2017)		$135,000
Monthly Operation and Maintenance		$15,000
SMAST		$28,500
	TOTAL	**$450,180**

4.0 Lonnie's Pond Oysters Demonstration Project Year 2 (2017)

4.1 Field Design and Equipment

The Demonstration Project at Lonnie's Pond was continued for a second year to further refine a long-term implementation plan that that uses shellfish to remove nitrogen and to continue to collect data needed to obtain regulatory approvals for the use of shellfish aquaculture to achieve nitrogen goals. The design of Y2 Demonstration Project is similar to the Y1 project, with two plots containing Y1 and Y2 oysters (Figure 4-1).

4.2 Shellfish Acquisition and Installation

The population of oysters that was grown as Y1 in 2016 was grown for a second year in 2017. These oysters are larger than the intermediate seed available from other suppliers. These oysters have an average dry tissue weight of about 1 gram, so the equivalent initial stocking density would be 150 oysters per bag to achieve the projected Y2 performance comparable to placing intermediate seed with a 0.5g dry tissue weight at an initial stocking density of 280 per bag.

Additional seed was ordered to continue the Demonstration Project, including:

- 480,000 2 mm oyster seed from Mook Sea Farms; and
- 58,000 2 inch (i.e. overwintered Y2 oysters) was ordered from Falmouth, MA.

Approximately 607,000 oysters, including 127,000 Y2 oysters and 480,000 Y1 oysters were deployed in two plots in Lonnie's Pond. The Y2 oysters were deployed in 510 bags with a targeted final grown-out stocking density of 250 oysters per bag in the west (W) plot (Figure 4-1).

Y1 oysters were grown from 2 mm seed in spat bags in the east (E) plot shown in Figure 4-1. The number of bags and Y1 oysters occupying each bag was adjusted over the growing season to accommodate oyster growth. It is anticipated that there will be 510 bags of Y1 oysters at the end of the growing season. The number of bags and initial stocking density for Y1 and Y2 oysters targets a final population of 250 per bag.

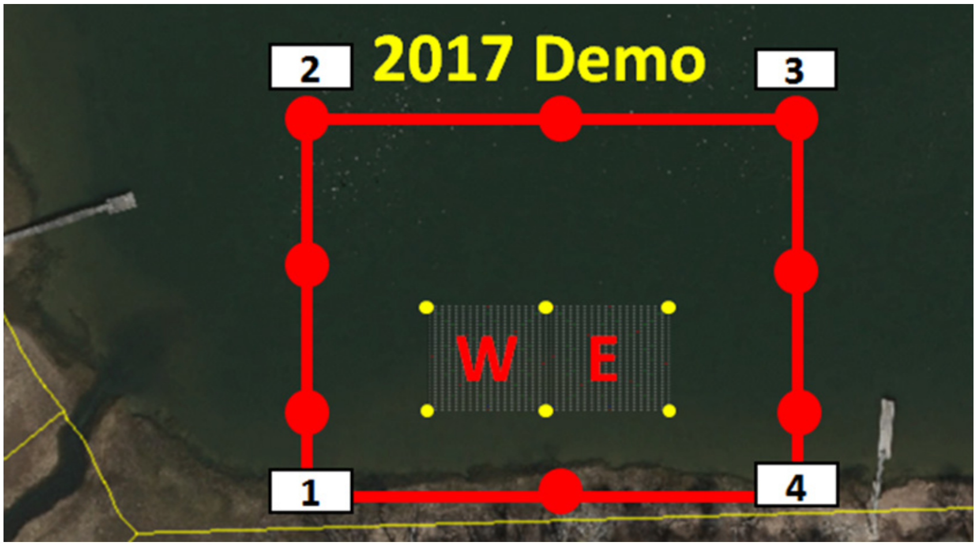

Figure 4-1 - Year 2 (2017) Demonstration Field

4.3 Maintenance and Monitoring

As in 2016, the 2017 management measures consist of flipping the bags on a weekly basis at beginning of the growing season and bi-weekly later in the season as growth begins to curtail. Gear will continue to be maintained and repaired as needed.

4.4 Disposition of Excess Oysters

All of the Y1 oysters from the 2017 Demonstration Year will be overwintered and re-deployed in spring 2018. Approximately 100,000 harvestable size oysters were removed from Lonnie's Pond in November 2017 and transported to the Town Landing on Route 28 for harvest by residents with a family harvest license. In addition, arrangements are underway to receive approximately 113,000 harvestable size quahogs from Falmouth, MA for residential harvest, in exchange for the 113,000 harvestable size oysters that were provided to Falmouth, MA in spring, 2017.

4.5 Shellfish Data Collection and Reporting

Similar to 2016, oysters were monitored for growth and mortality. Fourteen bags of Y2 oysters and 2 to 3 percent of the Y1 bags were monitored for weight and mortality on a weekly basis. During each monitoring session, 15 oysters were extracted from a different bag each time at approximately two-week intervals for size, harvest weight, and dry tissue weight analysis. Similar measurements will be made of the oysters in the Y1 field once they have a harvest weight of approximately 5 grams each. The weights and lengths were measured in the same manner as in the first year of the Demonstration Project.

At the end of the sampling period 25 oysters will be randomly selected and analyzed for nitrogen content at the Boston University laboratory. Based on the data collected in Y1 and Y2, a tool will be developed to allow the nitrogen content of live wet oysters to be estimated, which will be necessary for future quantification of nitrogen removal at full scale implementation.

4.6 Additional Activities

Additional activities in Year 2 of the Demonstration Project include:

- Work with SMAST to obtain food availability, biodeposition, and denitrification enhancement measurements from suitable locations before, during, and after the critical impairment period of July and August; and determine the feasibility of measuring the difference, if any, in the denitrification rate if maintenance is done on foot as opposed to by boat;

- Evaluate public and abutter acceptance by person interviews and/or surveys;
- Evaluate acceptance and compatibility with other local growers and commercial shellfish harvesters by personal interviews and/or surveys;
- Review the options with DMF for sale by the Town of intermediate seed;
- Identify any permitting issues for a commercial site license (grant) in Lonnie's Pond; and
- Investigate permitting for intermediate seed sale by growers

4.7 Cost

Table 4-1 displays a cost estimate for the aquaculture Demonstration Project in Lonnie's Pond for the second year of the demonstration. Y2 costs include labor for deploying, maintaining, monitoring, overwintering, and reconditioning the bags. Project management, engineering, and a final report are included. Oyster seeds were also purchased to supplement the existing supply of overwintered oysters (2016 Y1) oysters. Finally, lab costs for the nitrogen analysis are included.

Table 4-1 - Demonstration Project Cost in Year 2

Description	April - June 30, 2017	July 1 - Dec 31, 2017
Project Management	$18,000	$21,000
Engineering	$22,500	
Labor		
Overwintered Oyster Processing Labor	$14,400	
Labor for bags and fixed field alignment gear	$11,000	
2017 Oyster Deployment Labor in Lonnie's	$10,000	
Flip & Maintain Labor	$2,000	
First split Labor		$1,000
Second split Labor		$1,000
Flip & Maintain Labor		$5,000
Overwintering Labor		$5,000
Y2 Bottom Planting Labor		$2,000
Labor to recondition 500 winter bags		
Half Scale Y1X Deployment Labor		
Field sampling & sample prep	$7,680	$0
Analysis of Monitoring Data		$7,800
Permit Options Analysis		$1,000
Lab		
N analysis	$2,600	$7,800
Oyster Seed		
2 to 3mm oyster seed	$5,760	
Intermediate oyster seed	$12,760	
Materials		
Spat bags	$1,920	
Materials for two 500 bag fixed fields	$8,320	
Materials & supplies for sampling	$2,180	$6,600
Materials for 1,000 bags & lines		
Materials to recondition 500 winter bags		
Year 2 Final Report		$30,000
Contingency	$10,700	$6,200
SMAST Water Quality Sampling		$67,000
TOTALS	**$129,820**	**$161,400**
		$291,220

5.0 Lonnie's Pond Oysters Demonstration Project Year 3 (2018)

5.1 Field Design and Equipment

The layout of the 2018 Y3 of the Demonstration Project is illustrated in Figure 5-1. The northwest (NW) and southwest (SW) fields are Y2 oysters, the southeast (SE) field is comprised of Y1 oysters, and the northeast (NE) field would be comprised of a combination of Y1 and Y2 oysters.

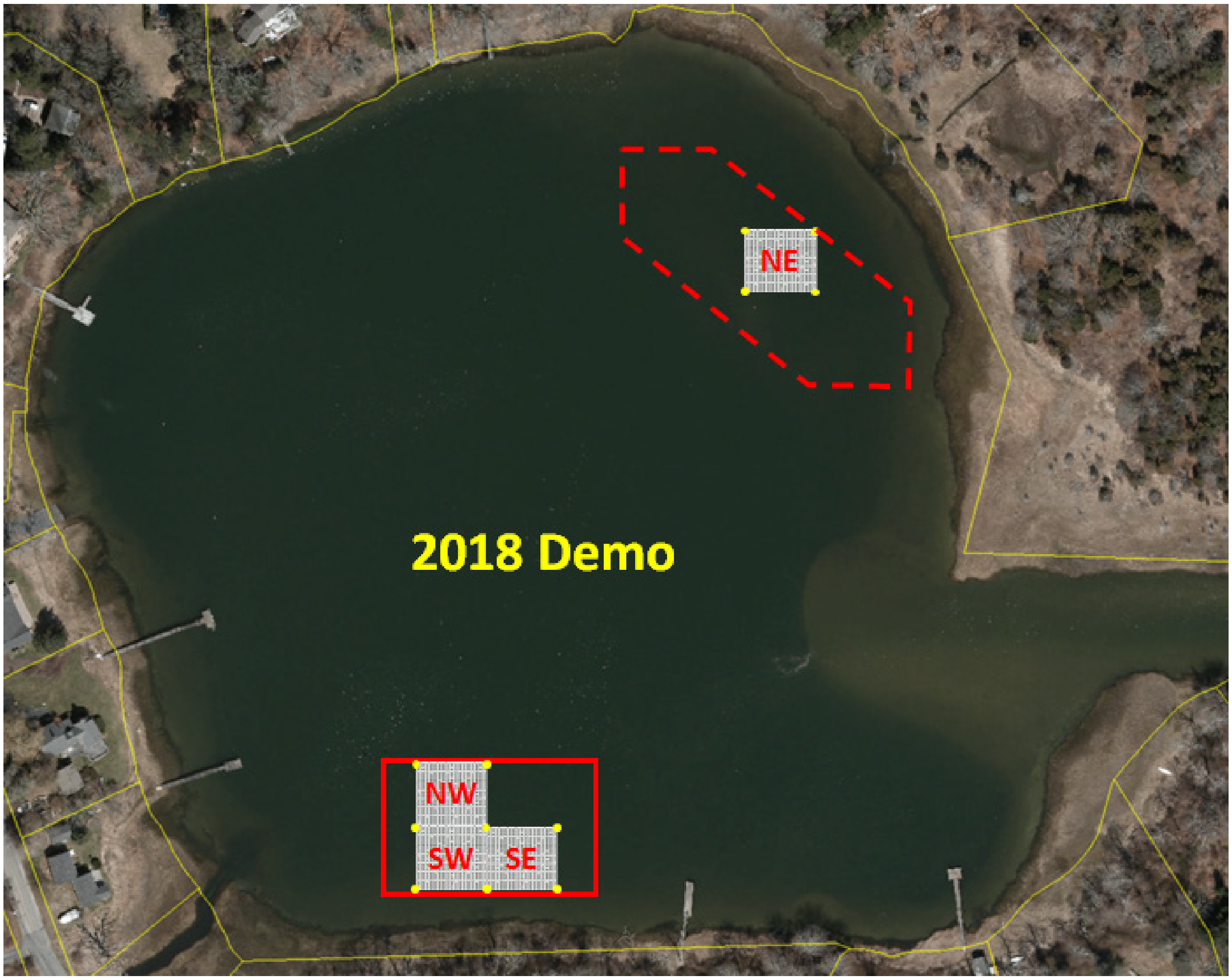

Figure 5-1 - Year 3 (2018) Demonstration Field

The design of the Y3 Demonstration Project is recommended to be approximately twice as big as the 2017 Y2, and approximately half of a full scale implementation scenario that would achieve 100% compliance with the MEP Nitrogen removal goals. The larger design is planned because it will facilitate evaluation of a deployment in which Y2 oysters occupy the majority of the deployment space to verify their nitrogen uptake in a high density layout. In addition, the expanded layout involves installing an oyster field in the northeast part of the pond, where oyster fields would be required if full scale implementation were to meet 100 percent of the MEP nitrogen removal goals. The 2018 layout will provide additional data to validate assumptions made for all scenarios currently under consideration for scale implementation depending on scenario ultimately selected (see Section 9.0).

The bags needed for the 2018 Y2 oysters would include a total of 2,040 6 mm diamond mesh bags, which will consist of the following:

- 1,020 from demo Year 2 (the 2017 growing season);
- 800 from the demo Year 1 (the 2016 growing season) which need to be reconstructed; and
- 220 new bags.

In addition, 360 spat bags will be needed for grow-out of the 2.04 million 2 to 3 mm seed until they can be kept in 6 mm diamond mesh bags:

- 80 bags from demo Y2 (the 2017 growing season) can be reused but will need new internal frames; and
- 100 new bags will need to be constructed.

5.2 Shellfish Acquisition and Installation

The Y1 and Y2 oysters for the third year of the Demonstration Project will be obtained from a combination of 2017 Y1 oysters that will be Y2 in 2018 after overwintering, as well as additional procurement of Y1 oysters in 2018 from an outside source. For the Y2 oysters needed in Lonnie's Pond in 2018, all of these are anticipated to be provided by overwintered Y1 oysters. For the 1,060,000 Y1 2018 oysters, all of these would need to be procured from a nursery in spring 2018, which necessitates submitting a request for procurement in December 2017 and submitting a deposit to secure delivery in the spring.

5.3 Management Measures

5.3.1 Equipment Monitoring and Maintenance Tasks

As in 2017, the 2018 management measures will consist of flipping the bags on a weekly basis at beginning of the growing season and bi-weekly later in the season as growth begins to curtail. As needed, gear will be repaired. Only minor repairs are anticipated to be needed, such as replacing broken zip-ties that hold the side floats to the bags, although bi-weekly inspections will occur to monitor the oyster fields and make any repairs needed. As in past years, work will be performed from kayak/skiff in order to avoid disturbance of the bottom sediments to facilitate the SMAST sampling that is ongoing.

5.3.2 Additional Permitting

Permitting requirements for 2018 are anticipated to be similar to those that were required in 2017. The Negative Determination of Applicability obtained from the Orleans Conservation Commission for the 2017 work was only valid for the 2017 year of the Demonstration Project, including the overwintering between 2017/2018. Therefore, another Request for Determination of Applicability (RDA) or a Notice of Intent (NOI) will need to be submitted to authorize deployment of the proposed oyster fields in spring 2018.

5.3.3 Shellfish Data Collection and Reporting

Shellfish data collected will be similar to 2017. Approximately bi-weekly between May 1 and December 1, 25 oysters from the 7 selected sampling bags will be removed for weight and length measurements in the field. Weights and lengths will be measured in the same manner as in past years. In addition, 25 oysters will be collected at the beginning and end of the sampling period and analyzed for nitrogen content at the Boston University laboratory.

5.3.4 Shellfish Disposition

Disposition of oysters at the end of the 2018 season will be dependent upon the status of MassDEP approval of aquaculture as part of a Watershed Permit and associated Watershed Management Plan, as discussed in greater detail in Section 9.1. Potentially, the shellfish remaining at the end of 2018 could be used in an ongoing demonstration or full-scale project, or put out for harvest if they are of harvestable size. In addition, arrangements are underway to arrange for an exchange of excess Y1 oysters with Falmouth, MA, in return for harvestable size quahogs from Falmouth, MA.

5.4 Cost

Costs for the 2018 Y3 of the Demonstration Program are envisioned to include the following:

- Labor and materials to overwinter the required number of Y1 oysters;
- Acquisition of required number of 2 to 3 mm seed;
- Labor and materials to deploy the Y1 and Y2 oysters;
- Labor to operate and maintain the four fields of oysters between May and November 2018;
- Labor to collect length, weight, and nitrogen measurements; and
- Labor to prepare the year-end report (Table 5-1).

Based on the 2017 invoice the cost for 2 to 3 mm seed would be $13 per 1,000, for a total of $26,500. A 50 percent deposit of $13,250 is required by December 31, 2017 to get early seed for 2018; a second payment on delivery of $13,250 in May 2018 will be required. The complete summary of costs for Y3 is shown in Table 5-1 below.

Table 5-1 - Demonstration Project Cost in Year 3

Description	Jan 1 - June 30, 2018	July 1 - Dec 31, 2018
Project Management	$ 44,200	$47,400
Process Engineering & Optimization	$ 12,000	$6,000
Labor		
Overwintered Oyster Processing Labor	$ 11,220	$11,220
Labor for bags and fixed field alignment gear	$ 16,900	
2017 Oyster Deployment Labor in Lonnie's	$ 11,220	
Seed Flip & Maintain Labor	$ 1,320	$1,980
Seed Splitting Labor	$ 8,360	$7,920
Seed Deployment Labor	$ 3,960	
Bag Flip & Maintain Labor	$2,250	$11,130
Field sampling & sample prep	$ 20,020	$34,320
Y2 Bottom Planting Labor		$4,490
Lab		
N analysis	$ 3,110	$9,310
Materials		
Materials & Supplies for Sampling	$ 1,000	$3,060
New floating spat bag materials	$ 3,940	
Materials for four 510 bag fixed fields	$ 9,650	
Purchase of 2 to 3 mm seed	$26,500	
Permitting	$ 5,000	
Year 3 Final Report		$30,000
TOTALS	**$180,650**	**$166,830**
		$347,480

5.5 Key Actions and Associated Dates

Key decisions for Y3 of the Demonstration Project in 2018 are summarized in Table 5-2, along with the date by which each decision is needed.

Table 5-2 - Key decisions for Year 3

Key Decision Point	Critical Date
Prepare Notice of Intent for Conservation Commission for Implementation of Year 3	January 2017
Put excess 2017 Oysters out to Harvest	November, 2017
Place Deposit on Order for 2018 Seed Shellfish for Year 3 Demonstration Project	December 31, 2017
Prepare Y1 oysters for overwintering	December 2017
Place Final Order for 2018 Year 3 Shellfish	April 1, 2018
Confirm FY 2018 Budget and Include for Town Meeting Approval	February, 2018
Receive early start seed and establish bags	May 1, 2018
Deploy Y2 field	May 1, 2018
Contact Falmouth, MA to arrange for exchange of oysters and shellfish	January 1, 2018
Evaluate status of DEP discussions and whether to continue with Demonstration Project, expand to full scale, or curtail program	Summer 2018
Identify number and location for harvestable oysters	October 2018
Inventory Overwintering and Deployment Equipment and Identify Storage/Disposition	October 2018

6.0 Oyster Viability Considerations

6.1 Ocean Acidification

Tracking the acidity (pH) of the growing area will enable a grower to be aware if concerns for ocean acidification are warranted. Primarily a cause for concern at shellfish hatcheries where young oyster larvae and juvenile seed are reared, acidity of estuarine waters is not expected to interfere with growing oysters. But tracking the pH, not only through the year, but over the past few years would give information about the pH trend so that a response can be developed.

It is likely that responses are severely limited should pH start trending down significantly. Any large-scale buffering attempts done in Lonnie's Pond would have to go through the Orleans Conservation Commission and would likely be challenged at that level. But if acidity begins to be a problem, it would not likely be in the near future and a lot may change in Lonnie's Pond and in regulations by then.

One response could be that younger, more vulnerable oysters with thin shells could be cultivated elsewhere and the older and more rugged oysters (Y2) oysters grown in Lonnie's Pond, doing the heavy lifting of the Nitrogen uptake. The overall uptake of Nitrogen in this water body would be less than is currently proposed, though, if the younger oysters weren't grown in Lonnie's Pond.

6.2 Predators

6.2.1 Crabs: Green, Blue, Calico (aka Lady), Mud, Spider, Rock, Asian Shore

Crabs can devastate an oyster crop, especially when the oysters are young. Having the oysters in floating bags off the bottom is helpful in keeping the vulnerable oysters away from the crabs' primary habitat on the bottom of the Pond. Regular maintenance of the crop to remove the crabs from the bagged oysters will serve to preserve the crop.

6.2.2 Oyster Drills

Oyster Drills may be overlooked due to being tiny and being approximately the same color as oysters in the field. Regular maintenance of an oyster crop with vigilant observations for oyster drills, oyster drill eggs and oyster drill damage can keep any loss to a minimum. Use of copper rings as a barrier on bottom structures (like the overwintering cages) can repel oyster drills from moving towards the oysters.

6.2.3 Birds: Oyster Catchers and Seagulls

Oysters can be protected from predation by these birds by being covered in cages, oyster bags and similar predation exclusion devices.

6.3 Disease

6.3.1 Juvenile Oyster Disease

This disease strikes first year oysters and is infectious. It can be minimized by making sure that the hatchery from where the seed comes utilizes good animal husbandry practices. It is expressed in the early stages of growth, from around July to September and can cause collapse of a crop in its early stage of development. Affected oyster seed exhibits cupped shells with a brown ring or deposit on the inner shell. Removing the diseased animals from the rest of the crop is advised. Moving crop to lower salinities (fresher water) can help. Obtaining seed from certified hatcheries is advised.

6.3.2 Dermo, Perkinsus marinus

Watery oyster meat is an effect of Dermo, whereby the oyster slows its growth (because of poor health) and eventually dies. Mortality is exhibited in the fall and typically affects second year oysters. Growing oysters in lower salinities helps. It's better to grow the oysters fast and sell them before diseases mature. Disease resistant oyster seed has been developed by hatcheries and has increased the survival of oysters with some prevalence of Dermo in the growing areas. Obtaining seed from certified hatcheries is advised.

6.3.3 MSX or Multinucleated Sphere Unknown, Haplosporidian Nelson

Watery oyster meat is also a hallmark of MSX. Disease resistant seed has been developed by hatcheries and has been helpful in the grow-out of oysters in the Northeast. Obtaining seed from certified hatcheries is advised.

6.4 Algal Blooms

Algal and/or seaweed mats can clog oyster growing gear, limiting the amount of oxygenated water and phytoplankton (microalgae) from feeding the oysters. If algal mats form, harvest the seaweed and dispose of it (or compost it) away from the grow-out site. Bag flipping serves to expose fouling organisms to the sun which effectively bakes them off. Regular bag flipping keeps fouling minimized on both sides of the bag.

6.5 Biofouling/ Pests

6.5.1 Sea Squirts/ tunicates/ hydroids

Fouling organisms found with oyster growing gear which may contribute to clogging the oyster growing devices and interfering with the ability of the oysters to maximize feeding. Brush off growing gear of fouling like sea squirts, tunicates and hydroids. Regular bag flipping keeps fouling at bay.

6.5.2 Mud Blisters

Mud Blisters are caused by worms in the mud that get incorporated into the oyster shell and make the oyster less marketable. Not only is it unsightly, but mud blisters may make the meat of the oyster muddy should a shucker pierce the mud blister on the inside of the oyster shell. Mud blisters are avoided by not growing oysters in the mud.

6.5.3 Boring Sponge

Affecting the integrity of the shell, not the oyster meat itself, the boring sponge penetrates an oyster shell and makes the shell too brittle to open without shattering the shell. With a brittle shell, it is not likely to open an oyster without getting shell fragments in the meat and makes it undesirable for shucking. It is evidenced by tiny holes in the shell once the sponge is rubbed or brushed off. It can be treated with air drying or brine dipping (with air drying), to kill the sponge without killing the oyster. Treating juvenile oysters this way can put the oyster at risk.

6.6 Storm Damage

Threats to a farm by storm can be devastating. With news of an impending hurricane or strong winds, extra anchors on the support structure may help "weather the storm". If a very serious hurricane is expected to hit, a decision could be made to retrieve all floating gear with the shellfish in it and store out of water for one-two day duration (a very long low tide) to preserve the crop from storm damage, keeping the oysters cool and hydrated. This, of course, would have to be done with the Shellfish Constable's knowledge and support. After the storm passes, the crop would have to be re-deployed.

Another strategy for protecting the crop from storm damage would be to employ the deep-water storage system to protect the floating gear from being thrashed around. There is concern for dissolved oxygen levels, though, especially in the summer months, when dissolved oxygen levels are typically at their lowest. Dissolved oxygen levels in the deep-water site could be tested periodically in the summer to have a course of action planned out in case of a hurricane.

The winter sinking of the crop in the deeper part of Lonnie's Pond appeared to be a successful strategy for overwintering the crop and protecting it from ice damage. It is a safe strategy that can be utilized if the deep-water space is available. From the experience last winter, the crops appeared to come through the icy part of the year in good shape and should be a good course of action should it successfully overwinter the crop again in the winter of 2017 and 2018.

6.7 Theft

Threats by theft can be very discouraging. Surveillance cameras can be set up and monitored by several outlets, especially with the Town if the aquaculture project has a municipal component to it. The Town of Barnstable has cameras set up to monitor some of the municipal shellfish growing areas. It would be advised that more than one camera be deployed to not only get the thieves in action, but also capture the numbers on license plates of the trailers and/or trucks used.

Another theft "alarm" is vigilance by neighbors living around the aquaculture site. If the neighbors are coached in what to do should they see trouble with the site, they might feel engaged to see that the project is successful. Phone numbers with 24-hour response (Police Department) could be shared as long as there are willing participants. Neighbors who serve on the watch might be proud to participate and may be able to be "paid" in oysters for their service if it was legally and procedurally found to be compatible with the project.

7.0 Water Quality Monitoring and Results

7.1 Water Quality Monitoring Methodology

SMAST staff monitored the Lonnie's Pond demonstration over the 2016 growing season. A sampling program was implemented to establish both a 2016 water quality benchmark for Lonnie's Pond, as well as to initially quantify nitrogen removal due to denitrification enhancement attributable to the oyster installation. From June 29, 2016 to October 19, 2016, eight sampling stations were monitored to further refine the long term water quality sampling database that was initiated for Lonnie's Pond as part of the MEP (Howes et al. 2006).Sampling occurred every other week during mid-ebb tide at the surface, bottom, and at mid-water column, if possible. During the demonstration period, intensive water column sampling also occurred over complete tidal cycles on August 10, 2016, August 24, 2016, September 13, 2016, and October 12, 2016. Samples were collected at nominal hourly intervals over consecutive flooding and ebbing tides.

An Acoustic Doppler Current Profiler (ADCP) was deployed to measure current direction through the oyster area relative to the sampling points in order to quantify changes in water column constituents through the oyster field. Particulate organic nitrogen (PON), total chlorophyll-*a*, bioactive N, orthophosphate, dissolved oxygen, and the complete suite of nitrogen components were assessed. The constituents of total nitrogen include (nitrate + nitrite), ammonia, dissolved organic nitrogen (DON) and PON. Samples were analyzed for: temperature, salinity, total nitrogen (TN), chlorophyll-*a* (Chl-*a*), pheophytin-*a*, orthophosphate, dissolved oxygen (DO), transparency (secchi depth), and alkalinity according to protocols outlined for the MEP.

Quality assurance samples (field duplicates) were also collected, as is protocol according to the Quality Assurance Project Plan (QAPP) under which SMAST collects MEP samples. DO and temperature profiles were performed at multiple depths and Winkler samples were collected in triplicate at water quality stations that had in-situ sensors. Continuous water quality monitoring of DO and Chl-*a* was conducted using five YSI-6600 sondes and HOBO® light sensors anchored at stations M5, M6, M7 and M8. Samples were also collected at the outflow from the cranberry bog and herring run when sufficient flow was available.

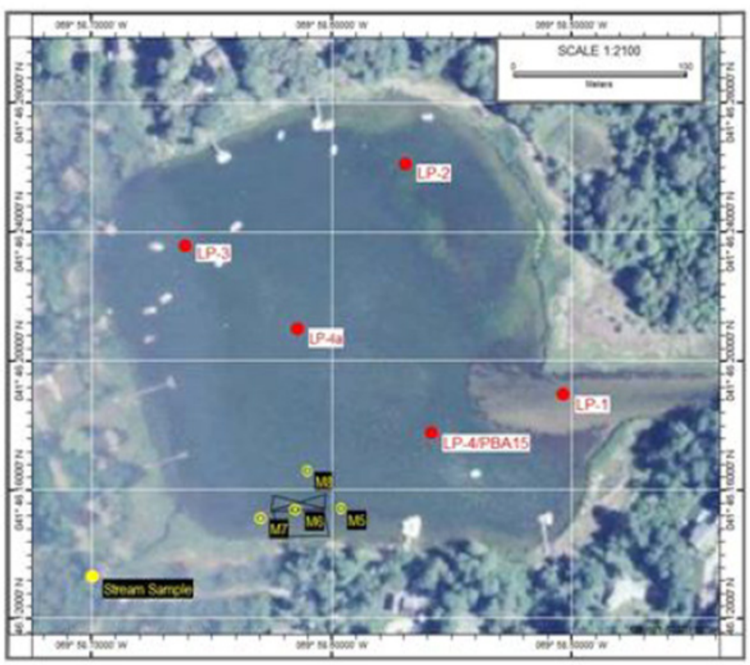

Figure 7-1 - SMAST Water Quality Monitoring Stations

7.2 Major Results of 2016 Water Quality Monitoring

A summary of key results from the SMAST Water Quality monitoring in year one follows.

- **Phytoplankton biomass was removed by the oysters as water flowed through the oyster deployment.** This is evidenced by the reduction in Chl-*a* concentrations and PON within the oyster field, relative to samples taken adjacent to the installation. These reductions in Chl-*a* and PON are statistically significant ($p<0.5$) and were seen during the tidal studies designed to capture water ebbing through the demonstration area;
- **Bioactive N levels declined by 12 to 20 percent during passage through the oyster field.** The decrease in bioactive nitrogen concentrations is likely due to the lowering of PON concentrations;
- **Observed nitrogen removals are conservative estimates** due to the oblique patterns of flow through the oyster area in the surveys, which underestimates uptake;
- **There was a clear temporal trend with higher levels of PON, Chl-a and bioactive N in mid-summer**, which is consistent with increased eutrophic conditions in estuaries in warmer summer months (poorest water quality July through mid-September);
- **Because of drought conditions, the oyster study was not influenced by surface water flows in 2016.** The nitrogen loading to Lonnie's Pond from Pilgrim Lake calculated during the low flow conditions of 2016 was significantly lower than was calculated for 2003 flows; and
- **The SMAST findings suggest that oyster growth will not be food-limited in Lonnie's Pond.** Food concentrations for summer and fall were 1,740 (±213) and 633 (±57.8) μg C/L seawater, respectively. Observations by others suggest that there is no increase in oyster feeding rates at food concentrations above 300 μg C/L (Tenore and Dunstan 1973). During the second and third years of the demonstration program, water flow to maintain adequate food concentration will be assessed throughout the field.

8.0 TMDL Compliance

Y1 of the demonstration monitoring project in Lonnie's Pond showed favorable results achieving nitrogen reduction. The results from year one of the project indicate that it is possible to use oysters to meet TMDL requirement and total nitrogen reduction targets. The oysters removed sufficient nitrogen through uptake alone. Mortality was low and the bag and line system and overwintering installation provided sufficient protection from predation and supported strong oyster growth. Y2 and Y3 of the Demonstration Project in Lonnie's Pond will provide additional data to evaluate the effectiveness of this non-traditional technology.

Overall, it appears that denitrification conservatively removes approximately 0.67 kg N for each 1 kg N removed in oyster harvest. In September/October when oysters had reached their mid-season biomass increase, an amount equivalent to almost one-third of the biodeposition rate was denitrified each day. The sediment incorporated biodeposits continue to continue to enhance denitrification after oyster harvest and will likely continue into the next spring and summer increasing the estimated N removal.

As further discussed in Section 9.0, either Y1 or Y2 oysters can be used to meet both TMDL requirements and total nitrogen load reduction targets. MassDEP is not validating results of denitrification enhancement at this time. Therefore, Full-Scale Implementation scenarios outlined in Section 9.0 focus on direct removal of nitrogen by uptake. However, is anticipated that the denitrification enhancement will be proportional to the amount of biodeposition, based on the 2016 SMAST Technical Report (Howes et. al, January 2017). If additional removal of nitrogen by denitrification is eventually demonstrated to the satisfaction of MassDEP, this removal would provide an additional margin of safety for regulatory compliance, beyond the removal predictions outlined in Section 9.0. Additional information regarding denitrification monitoring and modeling predictions is included in both the 2016 SMAST report and the Year 1 Lonnie's Pond Technical Memorandum dated October, 2017.

9.0 Full-Scale Aquaculture Scenarios

This section discusses what full-scale implementation at Lonnie's Pond would entail, based on data collected to date, TMDL scenario goal identified by MassDEP for Lonnie's Pond, estimated cost based on costs to date, and considerations regarding the feasibility of engaging commercial growers to assume responsibility for the Lonnie's Pond operation once MassDEP approves the removal data. This section also discusses anticipated permitting requirements for full-scale implementation at Lonnie's Pond as well as considerations regarding additional public engagement and future Town Staffing needs.

9.1 Scenarios and Cost

The MEP nitrogen reduction goal for Lonnie's Pond is approximately 660 pounds per year (300 kg/yr). The potential annual nitrogen per acre per year in Lonnie's was calculated based on the nitrogen content of oysters, densities, and weights measured in the Lonnie's Pond during Year 1 of the Demonstration Project. Based on these values, six different scenarios were developed identifying the number of Y1 and Y2 oysters and pond area needed to meet the full MEP removal goal as well as portions of the goal. It should be noted that the scenarios presented in Table 9-1 should be considered preliminary at this time, as they are based on one year of data collection, and will be further confirmed and refined based on data collected in Y2 and Y3 of the Demonstration project. The scenarios are summarized in illustrated in Figure 9-1 and described in Table 9-1, which summarizes oyster numbers and sizes, densities, pond area, number of bags, and other parameters associated with each potential scenario. Approximate costs for each of the scenarios are also detailed in Table 9-1. Table 9-2 provides assumptions used when developing these costs. The assumptions in Table 9-2 are subject to review and revision; therefore the costs in Table 9-1 are not necessarily final budgetary numbers, but do provide a comparative cost for each scenario.

9.2 Transition to Commercial Growers

The comparative cost numbers in Table 9-1 illustrate that a Full-Scale Implementation program at Lonnie's Pond could potentially be profitable for a commercial grower, and is likely more cost efficient for the Town than municipal operation of the aquaculture program on a long-term basis. Furthermore, results from the first two years of the Demonstration Program showed that the techniques used in Lonnie's Pond to date have resulted in high quality oysters with good market value. In 2016, approximately 80 percent of the Y1 reached harvestable size (76 mm / 3 in) in one shortened growing season (the typical season begins in May), and all of the Y2 oysters had reached petite or full market size by the end of their second growing season.

From discussions with wholesalers, it is believed that the Y1 oysters will have a significantly higher market value if they are overwintered, and it is expected that many of them would be market-ready within the first few weeks of the following season.

Operation of aquaculture at Lonnie's Pond could occur under a special grant category that would include reporting and compliance measures required by MassDEP, which are yet to be determined. A benefit of transitioning the program to a commercial grower would be that once the oysters reach harvestable size, the grower could sell the oysters for a profit, whereas the Town cannot sell shellfish under its municipal propagation permit, except to another town. Some considerations regarding the potential for transitioning the program to a commercial grower are outlined below:

- The aquaculture regulations may need to be modified to allow a new grant category for this operation, which specific reporting and compliance requirements as well as requirements for growing the oysters in floating gear at specified densities;
- The Town may want to consider issuing a Request for Proposals for the project, to allow multiple private growers as well as potentially licensed hatcheries to express their interest and approach to the project;

- It is expected that MassDEP approval of aquaculture in Lonnie's Pond as a means of nitrogen removal and TMDL compliance would be contingent upon using the same floating bag methodologies and oyster densities as have been implemented to date. If any options for alternative types of floating gear would need to be investigated with MassDEP and would need to be specified in the grant license; and
- The requirement to avoid sediment disturbance may be unrealistic, but also unnecessary if the nitrogen removed via the denitrification pathway is discounted.

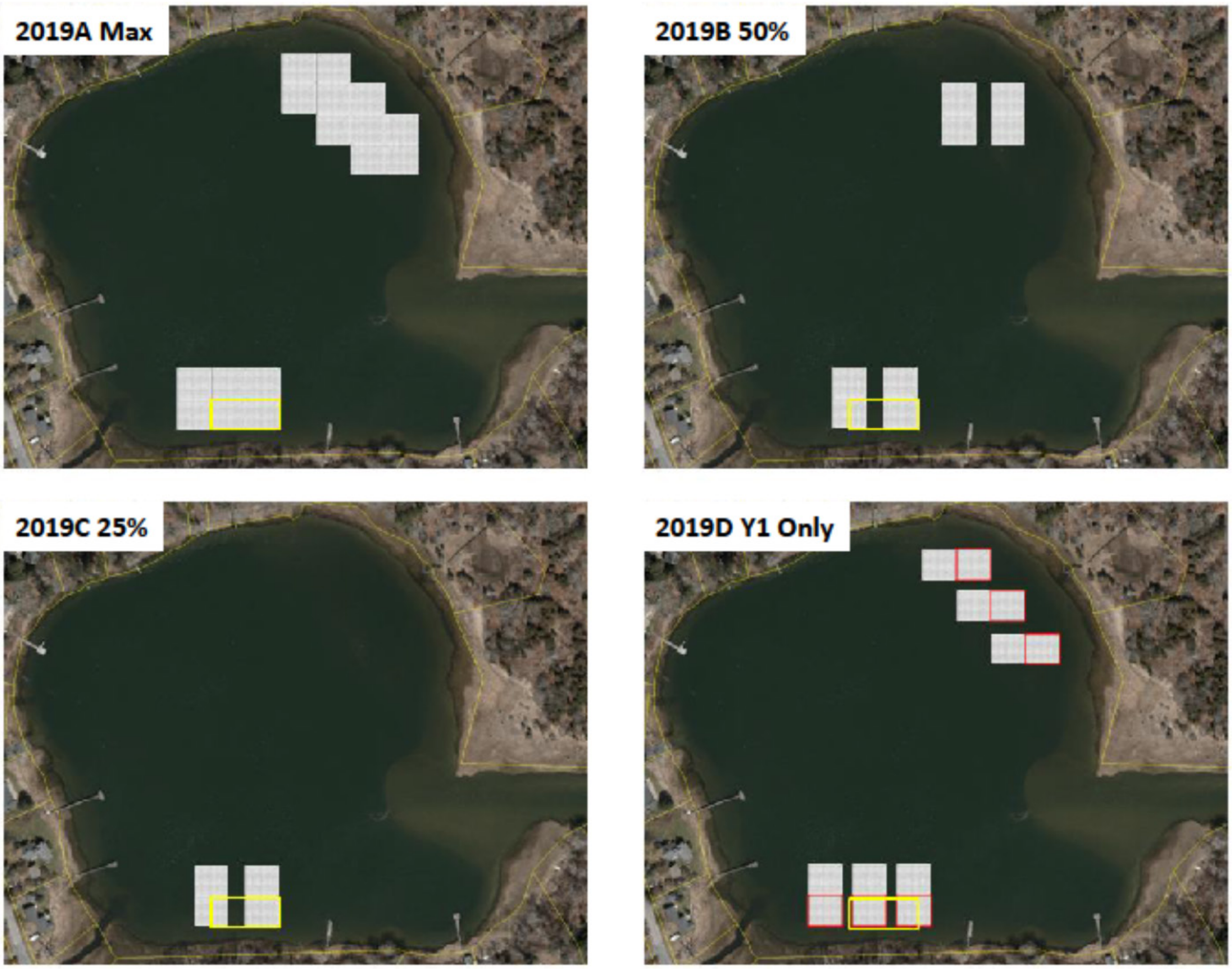

Figure 9-1 - Full Scale Implementation Scenarios

Table 9-1 - Full Scale Implementation Costs

	2019A	2019B	2019C	2019D	Town 2019B	Town 2019C
	Max Coverage	50% MEP	25% MEP	Y1 Only	50% MEP	25% MEP
Y1 Bags	1,650	843	422	6,280	843	422
Y2 Bags	6,600	3,372	1,688		3,372	1,688
Total Bags	8,250	4,215	2,110	6,280	4,215	2,110
Area Utilized	1.5	0.78	0.38	1.2	0.78	0.38
Y1 Oysters Started	1,740,000	888,000	445,000	6,610,000	888,000	445,000
Y2 Oysters Started	1,650,000	843,000	422,000	-	843,000	422,000
Live Oysters Extracted	1,530,000	784,000	392,000	6,280,000	784,000	392,000
Sale Price of Program Oysters	$ 0.35	$ 0.35	$ 0.350	$ 0.120	$ -	$ -
Total Market Value of Program Oysters	$ 535,500	$ 274,400	$ 137,200	$ 715,920	$ -	$ -
Total Capital for Floating Gear	$ 86,209	$ 25,444	$ -	$ 100,591	$ 25,444	$ -
Labor for Fabrication of Floating Gear	$ 71,625	$ 21,188	$ -	$ 47,000	$ 15,255	$ -
Over-winter Gear (Fab Labor & Materials)	$ 13,281	$ 672	$ -	$ 85,625	$ 604	$ -
Capital for Other Equipment	$ 95,375	$ 80,475	$ 62,175	$ 46,975	$ 7,800	$ 7,800
Y1 Capital Requirement	$ 266,490	$ 127,778	$ 62,175	$ 280,191	$ 49,103	$ 7,800
Amortized Financed Gear Cost	$ 64,841	$ 31,090	$ 15,128	$ 68,175	$ 9,821	$ 1,560
Seed Cost	$ 55,680	$ 28,416	$ 14,240	$ 211,520	$ 28,416	$ 14,240
Field Labor Cost	$ 278,063	$ 153,881	$ 76,488	$ 262,319	$ 110,795	$ 55,071
Business Overhead / Administration	$ 76,854	$ 49,546	$ 40,813	$ 100,863	$ 9,300	$ 9,300
Total Annual Expenses	$ 475,438	$ 262,933	$ 146,668	$ 642,876	$ 158,331	$ 80,171
Program Oversight	$ 3,360	$ 3,360	$ 3,360	$ 3,360	$ 3,360	$ 3,360
Town Net Cost	$ 3,360	$ 3,360	$ 3,360	$ 3,360	$ 158,331	$ 80,171
Grower Net Profit	$ 60,062	$ 11,467	$ (9,468)	$ 73,044		
Net Profit % of Gross Revenue	11%	4%	-6.9%	10.2%		
N Removed by Uptake, kg	290	148	74	297	148	74
N Removed in Shells (Mortality), kg	3	2	1	2	2	1
Annual N Removal, All Pathways, kg	293	150	75	298	150	75
% of MEP Annual Removal Target	99%	51%	25%	100%	51%	25%
Ongoing Labor Requirement (hrs/yr)	8,165	4,604	2,540	8,995	4,404	2,340
Labor Full Time Equivalents	4.1	2.3	1.3	4.5	2.2	1.2
Town $/kg of Target N Removed	$ 11	$ 22	$ 45	$ 11	$ 1,055	$ 1,069

Table 9-2 - Full Scale Implementation Assumptions

	2019A	2019B	2019C	2019D	Town 2019B	Town 2019C
	Max Coverage	50% MEP	25% MEP	Y1 Only	50% MEP	25% MEP
Number of new spat bags	400	116	0	2,023	116	0
Spat bag cost	$ 10,000	$ 2,900	$ -	$ 50,583	$ 2,900	$ -
Floating bags material cost	$ 76,209	$ 22,544	$ -	$ 50,008	$ 22,544	$ -
Floating bags fab labor cost	$ 71,625	$ 21,188	$ -	$ 47,000	$ 15,255	$ -
New Winter racks	43	2	0	274	2	0
Winter rack cost	$ 8,500	$ 430	$ -	$ 54,800	$ 430	$ -
Winter rack labor	$ 4,781	$ 242	$ -	$ 30,825	$ 174	$ -
Initial Deployment Labor	$ 81,094	$ 40,603	$ 19,781	$ 77,844	$ 29,234	$ 14,243
Recondition winter bags labor	$ 6,188	$ 15,806	$ 7,913	$ 23,550	$ 11,381	$ 5,697
Split Labor	$ 10,313	$ 5,269	$ 2,638	$ 39,250	$ 3,794	$ 1,899
Flip Labor	$ 30,938	$ 15,806	$ 7,913	$ 23,550	$ 11,381	$ 5,697
Remove Gear Labor	$ 51,563	$ 26,344	$ 13,188	$ 39,250	$ 18,968	$ 9,495
Y1 Winter Labor	$ 15,469	$ 7,903	$ 3,956	$ 58,875	$ 5,690	$ 2,849
Y2 to Market Labor	$ 82,500	$ 42,150	$ 21,100	$ -	$ 30,348	$ 15,192
Storage racks	107	35	0	0	35	0
Storage rack materials	$ 10,667	$ 3,493	$ -	$ -	$ 3,493	$ -
Storage rack labor	$ 6,000	$ 1,965	$ -	$ -	$ 1,415	$ -
Storage rack sq.ft.	2,667	873	0	0	873	0
Other space sq.ft.	500	500	500	500	500	500
Space rental	$ 32,667	$ 14,733	$ 6,000	$ 6,000		
Administrative hours	750	500	500	2,000	300	300
Administrative labor	$ 28,125	$ 18,750	$ 18,750	$ 75,000	$ 8,100	$ 8,100
NAP Coverage	$ 6,563	$ 6,563	$ 6,563	$ 6,563		
Disease testing				$ 4,800	$ 1,200	$ 1,200
Business insurance	$ 2,500	$ 2,500	$ 2,500	$ 2,500		
Business misc	$ 3,000	$ 3,000	$ 3,000	$ 2,000		
Equipment insurance	$ 4,000	$ 4,000	$ 4,000	$ 4,000		
Equipment delivery costs	$ 500	$ 500	$ 2,000	$ 2,000		
Carolina Skiff 19DX	$ 9,620	$ 9,620	$ 9,620	$ 9,620		
Yamaha F90LA	$ 10,405	$ 10,405	$ 10,405	$ 10,405		
Boat Trailer	$ 2,650	$ 2,650	$ 2,650	$ 2,650		
Winter raft	$ 2,800	$ 2,800		$ 2,800	$ 2,800	$ 2,800
Field maintenance craft	$ 3,000	$ 3,000	$ 3,000	$ 3,000	$ 3,000	$ 3,000
Field float	$ 12,000	$ 12,000		$ 12,000		
Chevy 4500 Reefer	$ 49,900					
Truck with coolers		$ 35,000	$ 30,000			
Utility trailer	$ 2,500	$ 2,500	$ 2,500	$ 2,500		
Totes, tools	$ 2,000	$ 2,000	$ 2,000	$ 2,000	$ 2,000	$ 2,000

Shell N% of DW	2.00%
Meat N% of live wt	10.50%
MEP Target kg N	297
Materials cost per bag	$ 13.30
Cost of spat bag for 2-3mm seed	$ 25.00
Years of service for Gear & Equipment	5
Commercial cost of capital, 5 yr @ 8%	21.7%
Winter bags/rack	20
Materials/rack	$ 200
Storage materials/rack	$ 100
Storage bags/rack	45
sq.ft. per storage rack	25
Winter racks available	40
Floating spat bags available after Y3	180
Bags available after Y3	2,520
Storage rental	$ 10.00
Office rental	$ 12.00
Inspections per season	6
Hours per inspection	8
Report hrs	16

	Y1	Y2
Mortality	5%	7%
Seed cost/1,000 oysters	$ 32	
Target Seed/bag	3,000	
Target Oysters/bag	1000	250
Denitrification factor (% of uptake)	68%	68%
Average Weight at Death (DW g/Oyster)	0.250	1.20
Deployment Weight (DW g/Oyster)	0.010	0.46
Ending Weight (DW g/Oyster)	0.46	1.78

	Base	Loaded
Town Labor Rate	$ 18.00	$ 27.00
Program Oversight Labor	$ 35.00	$ 52.50
Grower Labor Rate	$ 25.00	$ 37.50

9.3 Financing

Additional discussion and investigation is needed in regard to funding mechanisms for the overall wastewater management project, including the Aquaculture Non-Traditional Technology. Potential funding/financing options include: State Revolving Fund (SRF) loans, MassDEP 604b/319 grants, USDA rural development grants, 5 Star Wetland and Urban Waters Restoration grants, MA CZM Coastal Pollutant Remediation (CPR) Grant Program, North American Wetlands Conservation Act grants, and Massachusetts Environmental Trust (MET) General grants. Availability of these funding sources would be dependent on a successful application process, and can be further investigated if the Town elects to pursue these funding sources. Permitting.

9.4 Permitting

Depending on the scenario selected, permitting requirements are anticipated to include a Notice of Intent (NOI) or Request for Determination of Applicability as well as a Section 404 Permit approval from the US Army Corps of Engineers. If more than 10 percent of the pond area is utilized for aquaculture, it is likely that an NOI rather than an RDA would be required by the Conservation Commission, and that a Pre-Construction Notification (PCN) application would be required by the US Army Corps of Engineers rather than the project qualifying under the Self-Verification Process.

9.5 Communication Plan

For a full scale implementation at Lonnie's Pond, additional public outreach and engagement is recommended. This could take the form of either a public meeting at the Town Hall where the plan is presented and comments are solicited; posting the draft Lonnie's Pond management Plan on line and solicitation of written comments; or hosting a public visitation day at Lonnie's Pond; or a combination of any of the above.

9.6 Town Staffing

If the Town were to operate the Lonnie's pond full-scale aquaculture themselves, it is anticipated that additional season staff would be needed to for the installation and breakdown of the floating gear fields each year, as well as the ongoing splitting of the Y1 seed and redistribution into larger bags, as well as potentially relocating Y1 oysters into floating year or a bottom setting each winter. In addition, harvesting and associated reporting would be an additional task for town staff. It is likely that this additional field and reporting workload would require hiring one or two additional seasonal, or potentially permanent, year round staff.

10.0 Full Scale Implementation Management Plan

The next steps in the aquaculture include coordination with MassDEP to gain consensus regarding the implications of the Demonstration Project for full-scale aquaculture implementation and to determine the regulatory treatment of non-traditional technologies as a major component of the Town's efforts to meet TMDL requirements and its strategy to manage wastewater. At the conclusion of Y3 of the Demonstration Project in December, 2018, data from the three required years of deployment should be compiled and submitted to MassDEP for review, and a meeting should be held to discuss next steps required for MassDEP approval of aquaculture as a part of a long-term TMDL compliance program. Similarly, at this time, the town can review the final results of the three-year Demonstration Project to consider, select, and propose to MassDEP their selected scenario for Full-Scale implementation at Lonnie's Pond.

10.1 Watershed Permitting

MassDEP is in the process of developing guidance for a Watershed Permit that would include non-traditional technologies. This new wastewater management and impact mitigation permitting program would provide for a watershed-based approach to restore embayment water quality on Cape Cod. It is anticipated that enrollment in the program will demonstrate that the Town is taking action to address wastewater. The Pleasant Bay Alliance (PBA) has been meeting with the Cape Cod Commission and MassDEP to discuss the guidance to identify regulatory issues that would fall under a watershed permit for the Pleasant Bay watershed, potentially including Lonnie's Pond. It is anticipated that the Watershed Permit process will likely require that the Town submit a Watershed Permit Plan that:

- Identifies proposed technologies and approaches in the proposed watershed or subwatershed;
- Describes the adaptive management strategy or process for making implementation decisions;
- Specifies a monitoring plan and describe the contingency plan;
- Identifies all permits and approvals that are required by local, regional, state, and federal entities.

Further discussion with MassDEP and the PBA is necessary once it is determined that MassDEP will accept aquaculture as part of a TMDL compliance program and the Town selects their preferred scenario for implementation.

10.2 Oyster Configuration

Oyster configuration for Full-Scale Implementation will be determined once a scenario is selected. Section 9 describes the potential scenarios under consideration. This section can be updated to identify the number of oysters, size, bag layouts, multi-year planning once a scenario is selected for implementation. For planning purposes, a safety factor of approximately 15 to 20 percent is proposed in regard to number of oysters to be installed, in case an adverse event results in oyster loss. In addition, pending the results of the denitrification studies, denitrification may provide an additional margin of safety in terms of nitrogen removal for TMDL compliance.

10.3 Required Tasks and Schedule of Activities

The schedule will be determined once a scenario is selected. Section 9 describes the potential scenarios under consideration. An initial outline of required tasks and associated schedule dates is provided below:

- Compile and provide Demonstration Project results to MassDEP – spring, 2019;
- Select preferred Full Scale Implementation Schedule – spring, 2018;
- Meet with MassDEP to discuss – spring, 2019;
- Determine number and size classes of oysters needed, as well as equipment needs – TBD; and
- Develop RFP if commercial route is chosen – TBD.

10.4 Overwintering Protocols

These are described in Section 3.1 and briefly repeated here. Typically oysters are kept on the surface as late into the winter season as possible, depending on environmental conditions including temperature and dissolve oxygen. As a guide, oysters should be submerges below the surface as soon as the water temperature drops below 6°C for six days in a row, although if water temperature and observations suggest that the water will freeze imminently, it is prudent to submerge oysters in advance of this trigger. In the spring, oysters should be raised once water temperatures reach 6°C for six days in a row.

10.5 Long-Term Costs

Long-Term cost can be determined once a scenario is selected for implementation. This section can be updated at that time.

10.6 Excess Oyster Disposal

This will be determined once a scenario is selected. Options for disposal include harvesting full size oysters or trading to Falmouth, MA for quahogs (if Town run), or sale of intermediate seed, market size oysters or both, if grower run.

10.7 Catastrophic Loss and/or Project Abandonment

If there is a catastrophic loss for one year, then either the safety factor from previous years will account for this, or nitrogen removal will need to be made up in subsequent years. Any issues will be reported to MassDEP, and it is expected that discussion with them would be needed to reach resolution. If multiple years of the project showed less than expected nitrogen removal, it would be necessary to consider replacement of the aquaculture with alternative types of treatment, such as PRBs or conventional sewering.

Appendix A

Boston University Stable Isotope Laboratory QAQC

and

Science Wares SOPs

BOSTON UNIVERSITY
STABLE ISOTOPE LABORATORY

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Quality Assurance and Quality Control

Instrumentation for stable isotope analysis has expanded to include both the original Finnigan Delta-S and now two GV Instruments IsoPrime isotope ratio mass spectrometer. With the addition of the GVI instruments and associated peripherals, we have automated stable isotope analysis of most organic (and some inorganic) samples for carbon-13 and nitrogen-15. The workhorse of continuous flow measurements of solid samples for carbon and nitrogen isotopes is done by the GVI IsoPrime and a Eurovector elemental analyzer, combined with a diluter and reference gas box.

Samples for automated isotope analysis are first weighed out into tin boats to the nearest 0.01 mg on a Mettler AE240 or a Sartorious micro electronic balance. During a sequence run by the mass spectrometer, each sample is flash combusted at 1800ºC in the Eurovector CN analyzer; the combustion products (CO_2, N_2 and H_2O) are separated chromatographically and introduced into the mass spectrometer, with water removed in a chemical trap. The gases of interest are then introduced into the mass spectrometer for isotope analysis and the rest pumped away. The sample isotope ratio is compared to a secondary gas standard, whose isotope ratio has been calibrated to international standards. For $^{13}C_{V\text{-}PDB}$ the gas was calibrated against NBS 20 (Solenhofen Limestone), NBS 21 (Spectrographic Graphite), and NBS 22 (Hydrocarbon Oil); for $^{15}N_{air}$ the gas was calibrated against atmospheric N_2 and IAEA standards N-1, N-2, and N-3 (all are ammonium sulfate standards). All international standards were obtained from the National Bureau of Standards in Gaithersburg, MD.

International standards used for water samples include V-SMOW, GISP and SLAP, which were utilized to calibrate the secondary gas standard. In the past, water samples were prepared using the guanidine hydrochloride technique for oxygen-18 and the zinc reduction technique for deuterium (zinc obtained from John Hayes, Indiana University). With the addition of the GVI MultiFlow and ChromeHD systems, we are now able to automate both procedures. Oxygen-18 analysis is done via CO_2 equilibration and deuterium analysis is done via pyrolysis in the ChromeHD system.

When running gas samples on the Finnigan Delta-S, as a daily check on instrument performance we run a second gas (lecture N_2 or CO_2) that is isotopically distinct from our standard gas. If the isotope values are within 0.05 per mil of its long-term record, analysis proceeds; otherwise, further analysis stops until any problems are resolved. Internal precision for the instrument is $\pm$0.014 per mil. For solid continuous flow samples, a suite of in-house standards are first analyzed. If they fall within laboratory specifications, client sample analysis then proceeds.

Required external precision of a sample (i.e. replicate analysis) for either ^{15}N or ^{13}C is 0.2 per mil. Typically, our precision is better than 0.1 per mil for well-ground organic tissue samples using the trapping box.

Samples run in continuous flow mode are currently within 0.2 per mil for both nitrogen and carbon. In addition to carbon and nitrogen isotopes from the same sample, continuous flow will also report %C and %N data.

The lab runs one replicate per 10 samples, and any anomalous results are rerun. As a check on the combustion and cryogenic distillation steps, a laboratory standard is run every 15 samples. This standard is either peptone, a hydrolyzed animal protein from Sigma Chemical Company, glycine, or citrus leaves, SRM 1572. Both have been well documented by several stable isotope laboratories and their isotopic values are well known. Its value must be within 0.15 per mil of its documented value. If it does not, the samples preceding the standard are considered suspect and rerun.

The addition of the GVI Instrumentation precision of water samples (oxygen-18 and deuterium) has improved significantly and is extremely good. The lab generally runs duplicates of all samples for oxygen-18 if there is enough water. The precision is usually 0.1 permil or better. An internal lab standard is run after every 4 client samples as a check on the instrumentation. Deuterium samples are run with the ChromeHD pyrolysis system. Three injections are done with each sample, with the first injection discarded, due to memory effects in the system. The standard is Boston University deionized water, collected in batch fashion and stored. The water standard is within 0.2 per mil of its long-term value for deuterium and 0.2 per mil for oxygen-18. In addition, the metabolic samples are inputted to a spreadsheet that calculates FMR (Field Metabolic Rates). If calculated values are not within acceptable ranges, the suspect samples are rerun. The calculations are based on the equations of Lifson and McClintock (1966), as modified by Kenneth Nagy, UCLA.

For carbonate samples, NBS-20 and Carera-Z are used as two point calibration standards. Precision is currently 0.05 permil for carbon-18 and 0.06 permil for oxygen-18. CO_2 air and breath samples are calibrated using atmospheric air and a 1% CO_2/helium mix gas. The mix gas was checked against calibration gases obtained from Oztech Corporation, Texas.

Data is presented in a tabular form and can be sent by fax, mail or email. The sheet includes sample ID, mass/volume used, isotopic value and % organics (if applicable). All isotopic data are rounded to 2 decimal places.

We request that a sample list be included with all samples and that all samples be clearly identified. This allows the Laboratory Manager to look over the data and compare the isotope values against generally accepted values for that type of sample. Any samples that appear anomalous are rerun if possible to check their values; if preloaded, they are flagged as anomalous for the client.

Percent organics and nitrogen protocol

Samples are weighed out to 0.001 mg into tin capsules on a Sartorius XM1000P microbalance. They are combusted in a Fisons NA1500 elemental analyzer and measured using Eager200 software. Check standards are inserted into the run to ensure precision and quality control. Any anomalous samples are reweighed and rerun. Precision for replicate samples is 0.2 percent for carbon, and 0.5 percent for nitrogen, but will vary depending on the heterogeneity of the material.

The elemental analyzer is recalibrated each day using a size series of 5 acetanilide standards ranging from 2 to 0.2 mg. A sixth acetanilide is then measured to check for accuracy.

Samples can be either dried and ground by the client, or shipped to the laboratory in dried or wet form and ground by the lab. A mortar and pestle and liquid nitrogen are used to ensure a well-ground, homogenous mix. Samples can be stored in any container, but preferably in scintillation vials or Eppendorf tubes.

If samples are to be prepared by the client, they should be placed in 96 well trays, leaving slots 6 and 12 open for BUSIL internal standards. The amount of material will vary, but should be around 1, 2, and 5 mg for animal tissue, plant tissue, and soils, respectively. The spreadsheet found on the BUSIL website (http://www.bu.edu/sil/PDF%20files/BUSIL%20EA%20Sample%20Submission.xls) should be filled out and sent electronically to the laboratory manager prior to shipping.

The results are put into an Excel spreadsheet and sent to the client, reporting sample ID's, masses, and percent carbon and nitrogen.

Maintenance on the elemental analyzer is performed after a run of 120 samples. Excess tin and ash are removed from the combustion column. The reduction column is changed after 300 to 500 sample analyses. The combustion column is replaced after 1,500 samples.

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:

Brian Howes, Ph.D., David Schlezinger, Ph.D., Roland Samimy, Ph.D. , Micheline Labrie, Jennifer Benson, M.S., Sara Sampieri, M.S.,Amber Unruh, and Paul Mancuso

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September 2018

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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 (NO3-àN2) 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 Lonnies 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 Lonnies 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 Lonnies 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 Lonnies 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 Lonnies 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 Lonnies Pond

Sampling Program:

A sampling program was implemented in Lonnies 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 Lonnies 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 Lonnies 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.

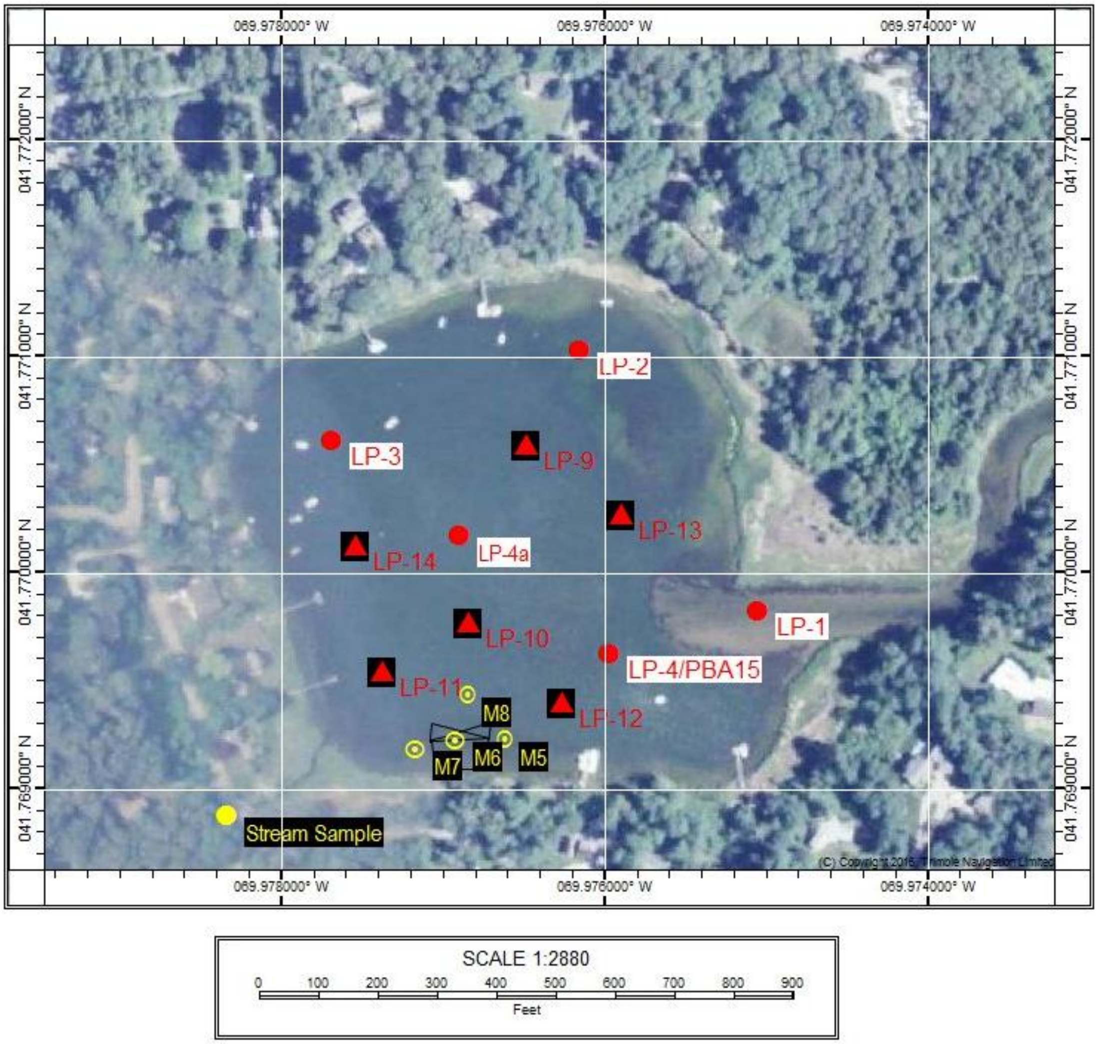


Figure II.1. Locations of water quality stations. Red dots denote stations established in 2016. Red triangles denote water quality stations added in 2017. LP4a was occupied during June 2016 until a permanent buoy was deployed by the harbormaster; hereafter LP4 was used to compliment previous water quality 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 N[2]). 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.

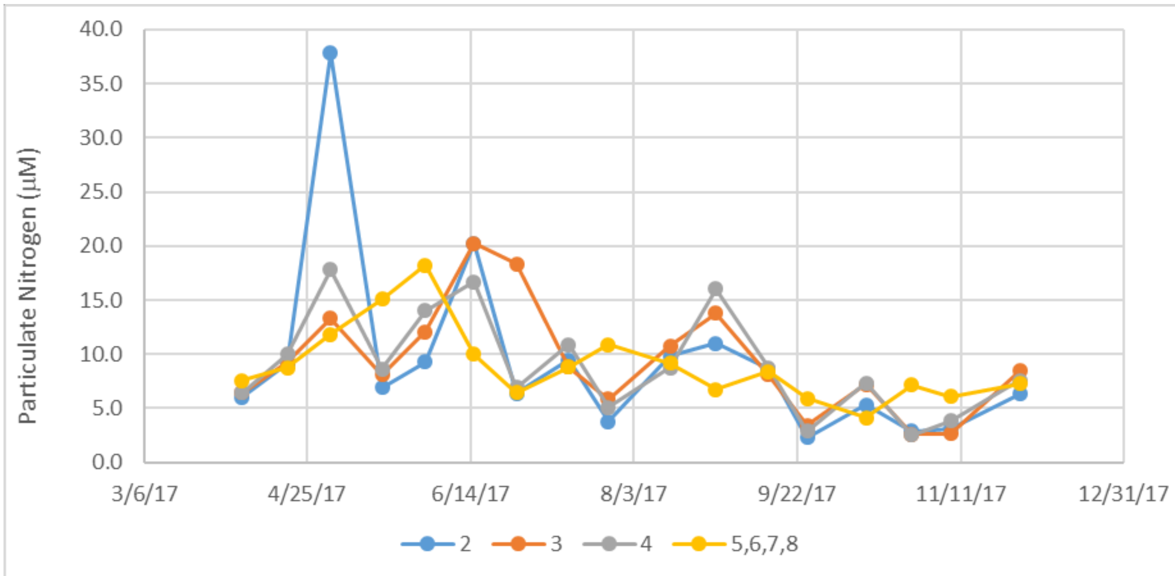
Within the generally homogeneous distribution of the major constituents across Lonnies Pond, there appeared to be lower levels of each constituent within the region of the oyster deployment. Although the magnitude of the constituents varied over time, each of the oyster influenced stations (M5-M8, Figure 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 (NH_4 and NO_3). 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 NH_4 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 Lonnies 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 Lonnies 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 NOx 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.



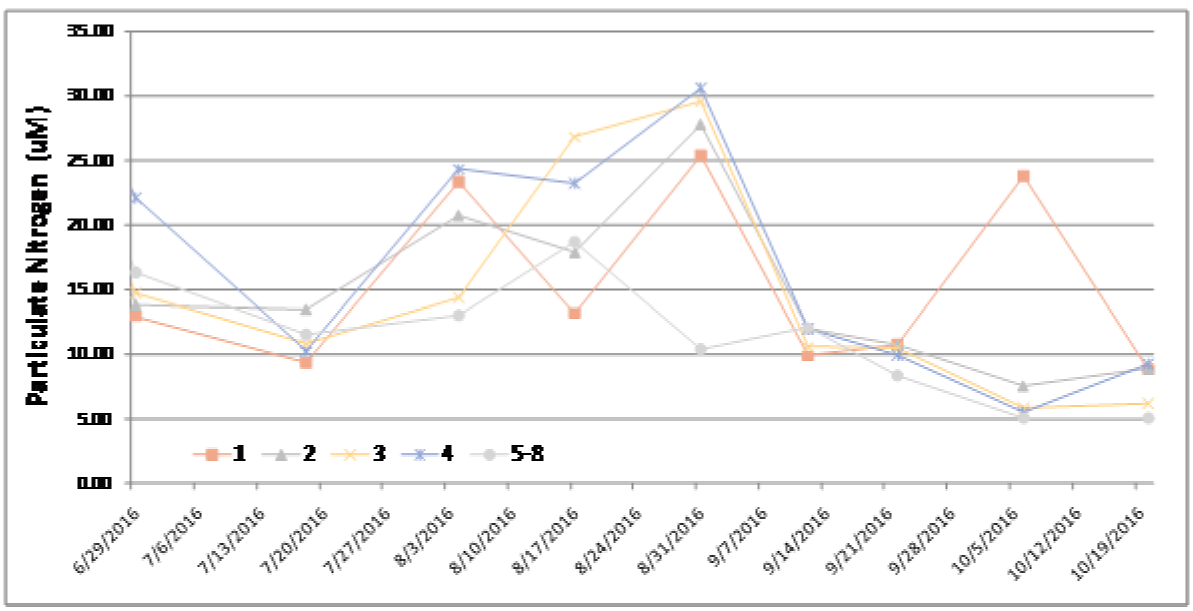
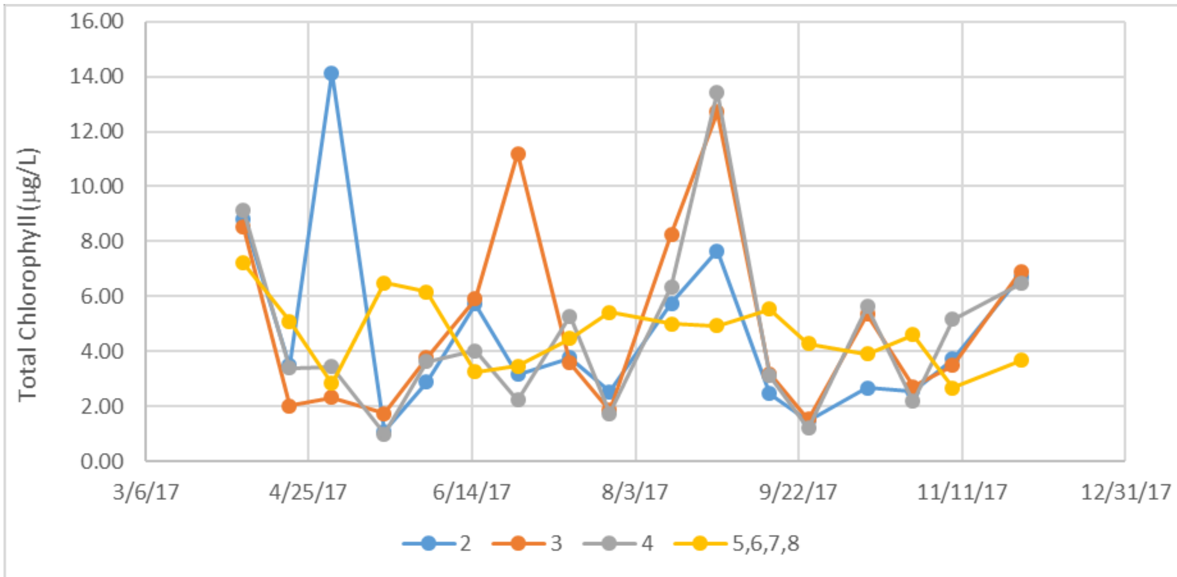


Figure II.2. Top Panel: 2017 time-series of mixed layer average particulate nitrogen at each individual sampling station and the average of the stations (5-8) associated with the oyster deployment. Bottom Panel: 2016 data shown for comparison. Station numbers refer to locations in station map above.



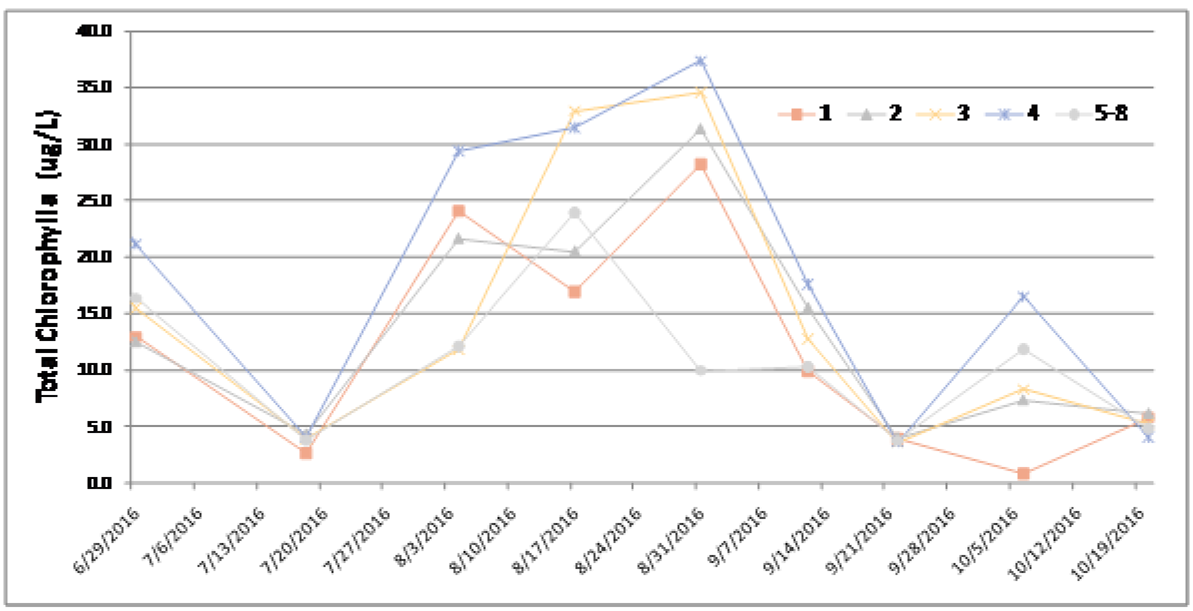
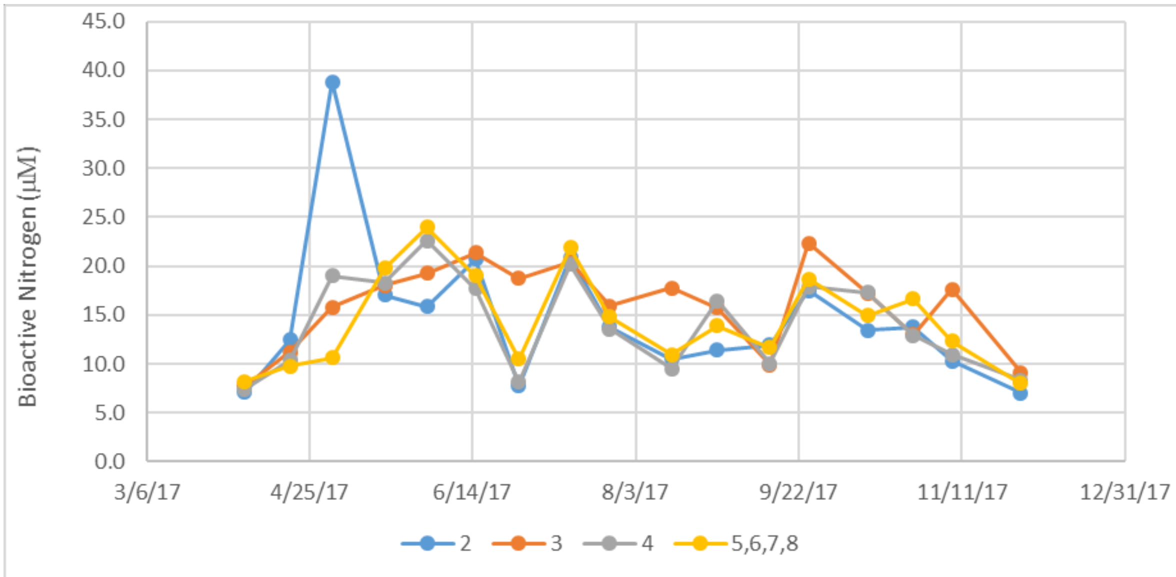


Figure II.3. Top Panel: 2017 time-series of mixed layer average total chlorophyll a at each individual sampling station and the average of the stations (5-8) associated with the oyster deployment. Bottom Panel: 2016 data shown for comparison. Station numbers refer to locations in station map above.



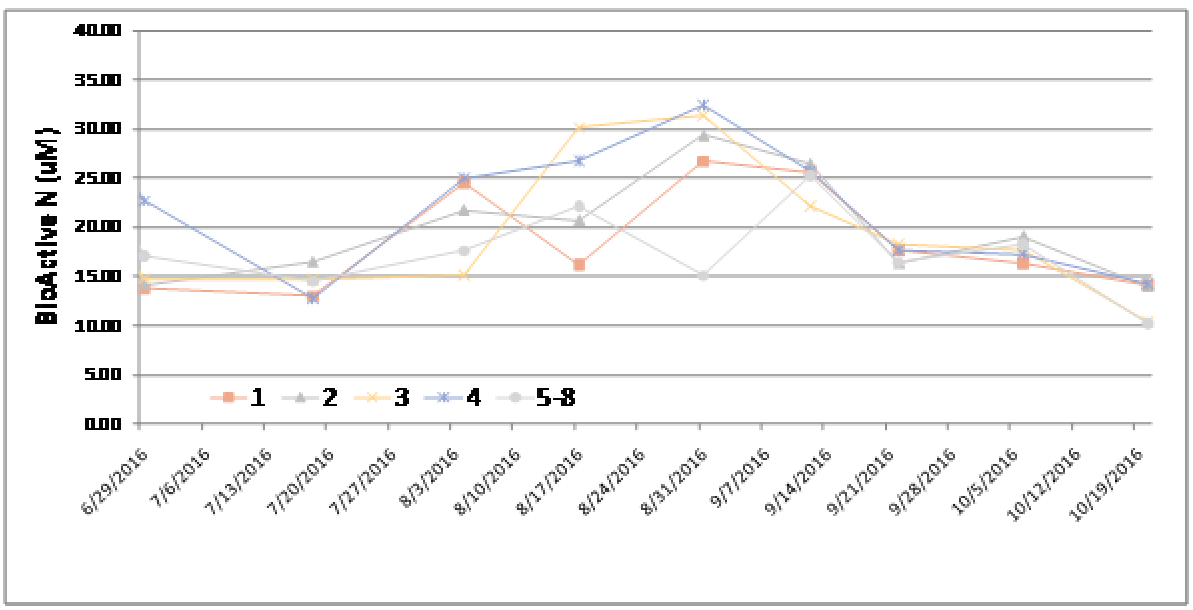


Figure II.4. Top Panel: 2017 time-series of mixed layer average bioactive nitrogen concentration at each individual sampling station and the average of the stations (5-8) associated with the oyster deployment. Bottom Panel: 2016 data shown for comparison. Station numbers refer to locations in station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

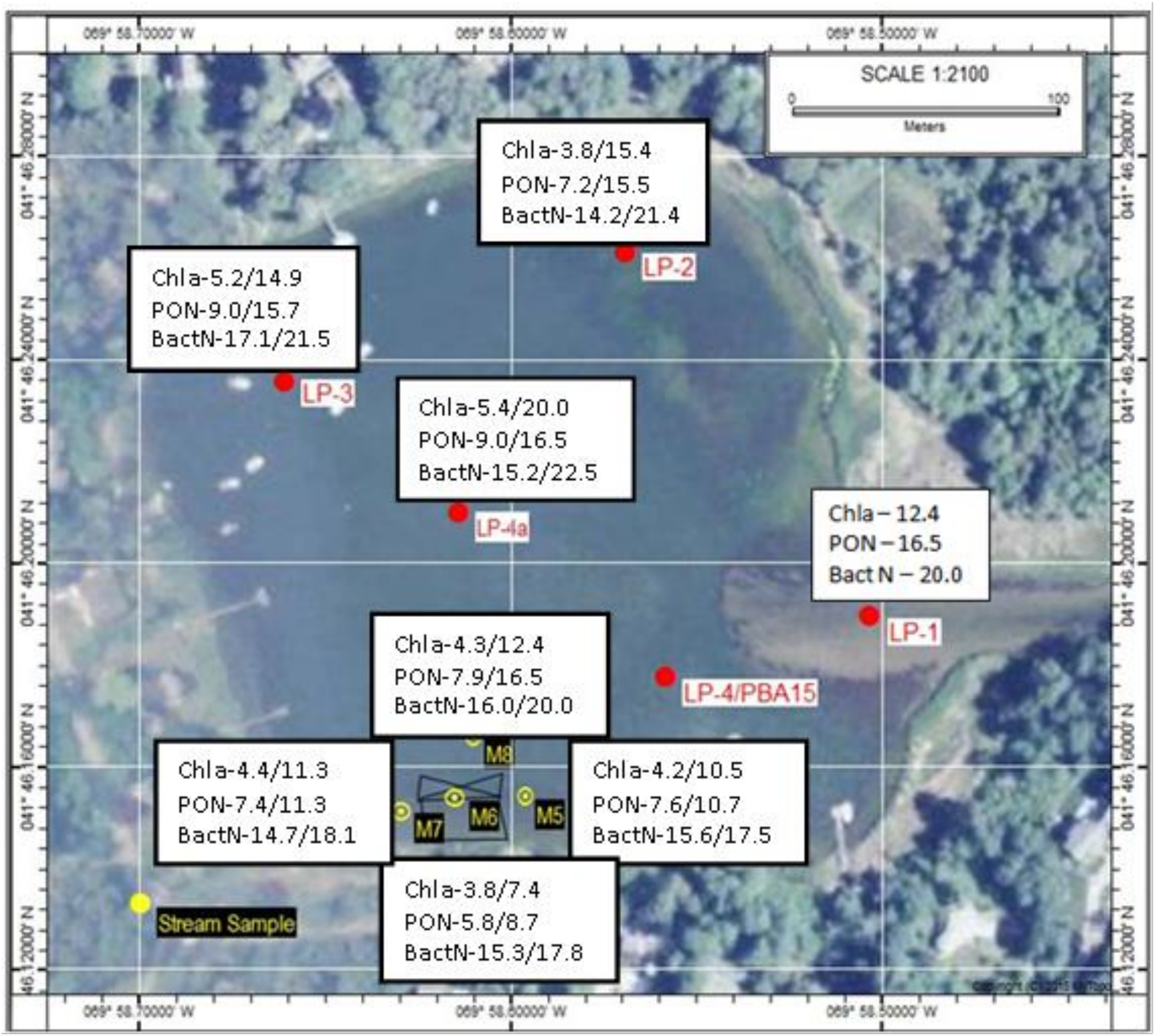


Figure II.5. Mixed layer average concentrations of total chlorophyll a ($\mu g\ L^{-1}$), particulate organic nitrogen (μM), and bioactive nitrogen (μM). Values represent average of samples collected from July 18 through October 6, 2016. Note the lower concentrations of each constituent in the region 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.

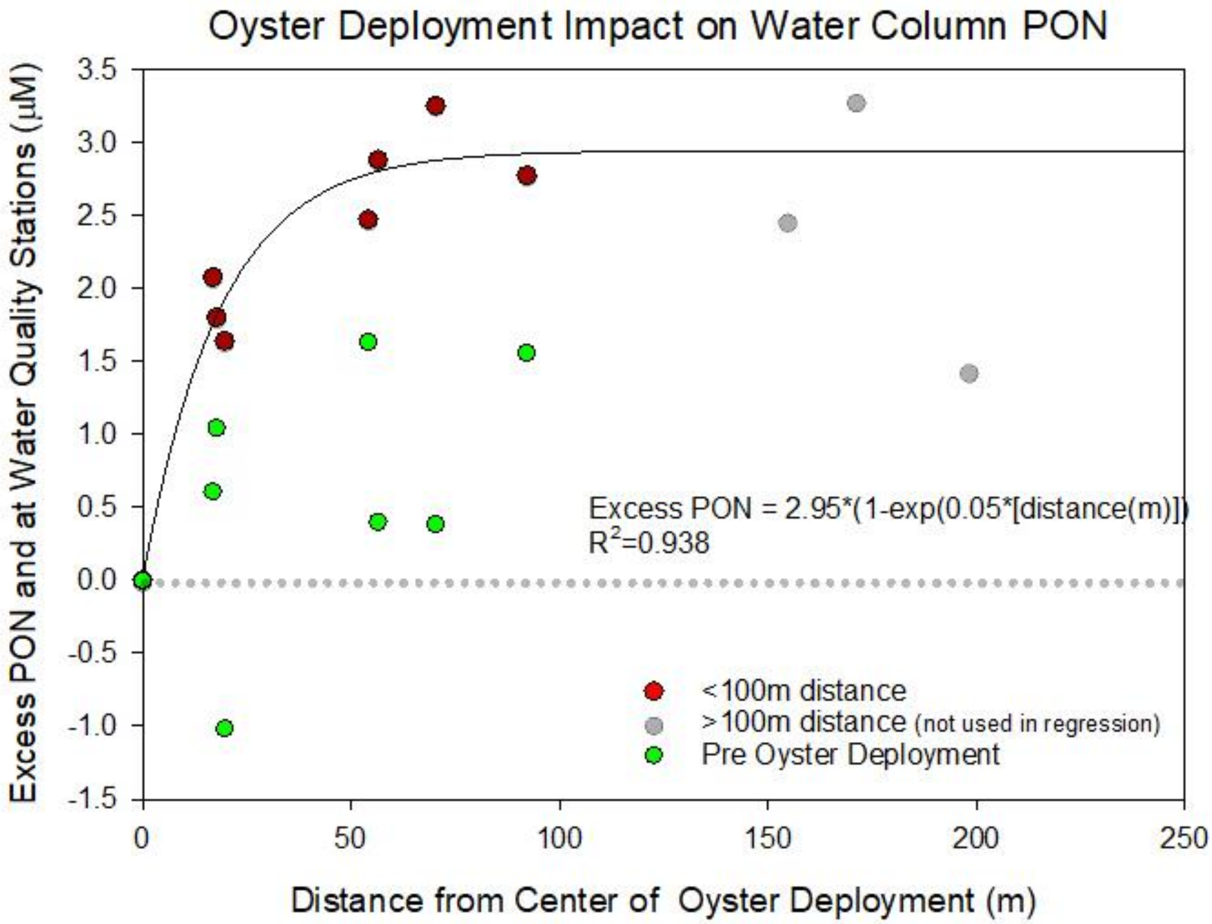


Figure II.6. Mixed layer average concentrations of particulate organic nitrogen (μM) in excess of that observed at M6 prior to oyster deployment (April –June 2017) and following oyster deployment (July-October). Note the concentrations of excess PON within 100m of oyster deployment increase exponentially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

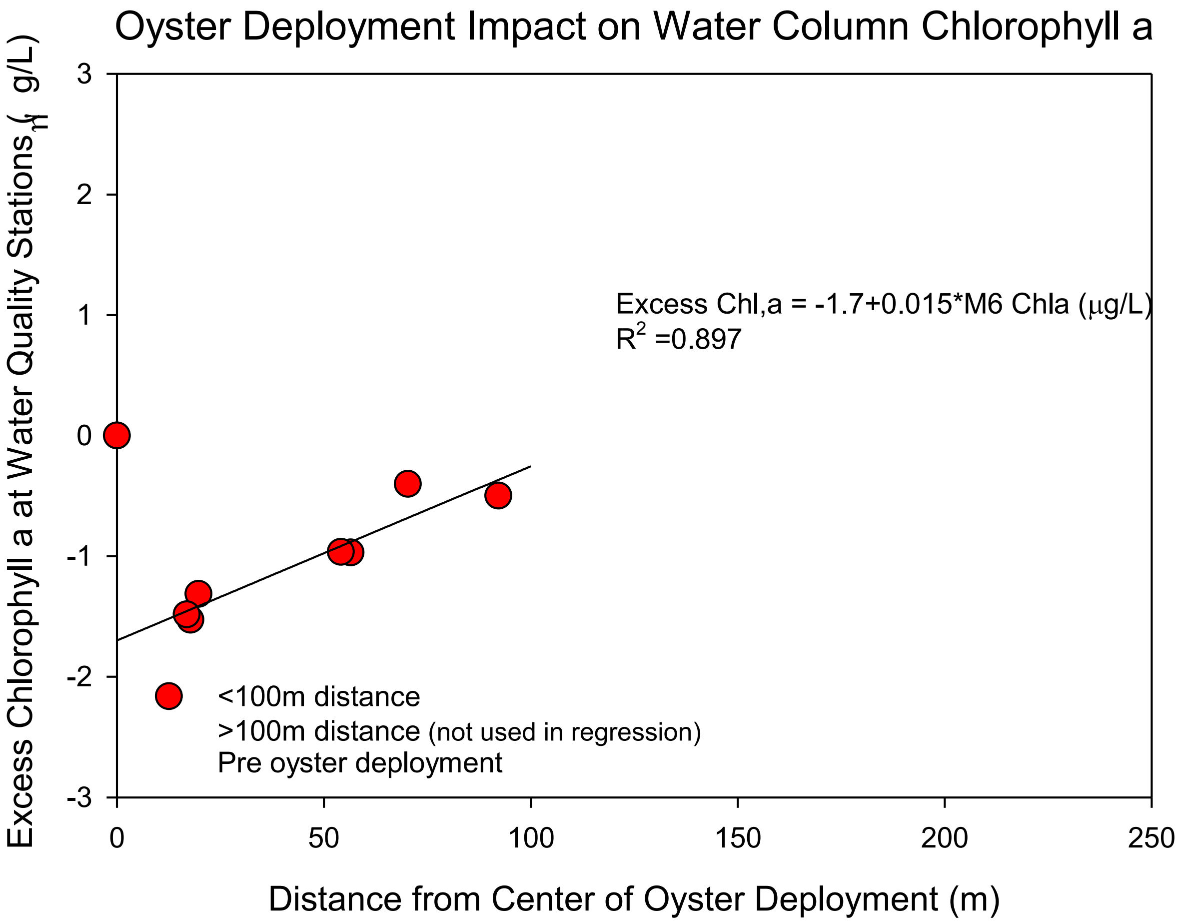


Figure II.7. Mixed layer average concentrations of chlorophyll a (µg/L) in excess of that observed at M6 prior to oyster deployment (April –June 2017) and following oyster deployment (July-October). Note the concentrations of excess Chla within 100m of oyster deployment increase linearly with distance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

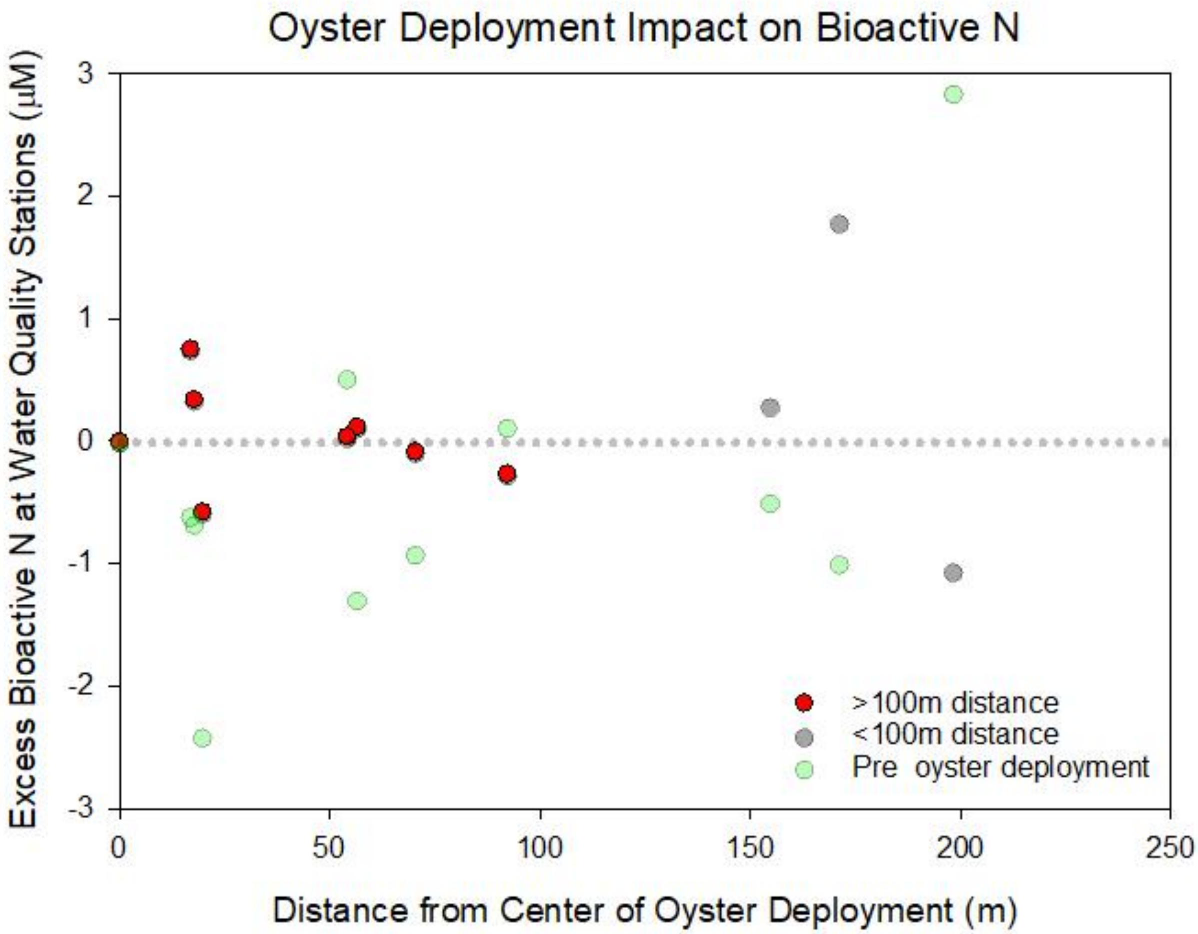


Figure II.8. Mixed layer average concentrations of bioactive N (μM) in excess of that observed at M6 prior to oyster deployment (April –June 2017) and following oyster deployment (July-October). Note that unlike PON and chlorophyll there is no obvious trend with increasing distance from the oyster deployment 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 Lonnies 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 Lonnies 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 Lonnies 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 partical 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 Lonnies 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 Lonnies Pond, Summer 2016. Highlighted pairs of means have significantly different levels after passing through the oyster deployment area.

Tide	Flow Direction	PON (uM) East	PON (uM) West	PON (uM) PB-15	T-pig (ug/L) East	T-pig (ug/L) West	T-pig (ug/L) PB-15	TN (uM) East	TN (uM) West	TN (uM) PB-15	BioActN (uM) East	BioActN (uM) West	BioActN (uM) 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	8.03	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	8.97	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:

SMAST 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 Lonnies 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 Lonnies 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.

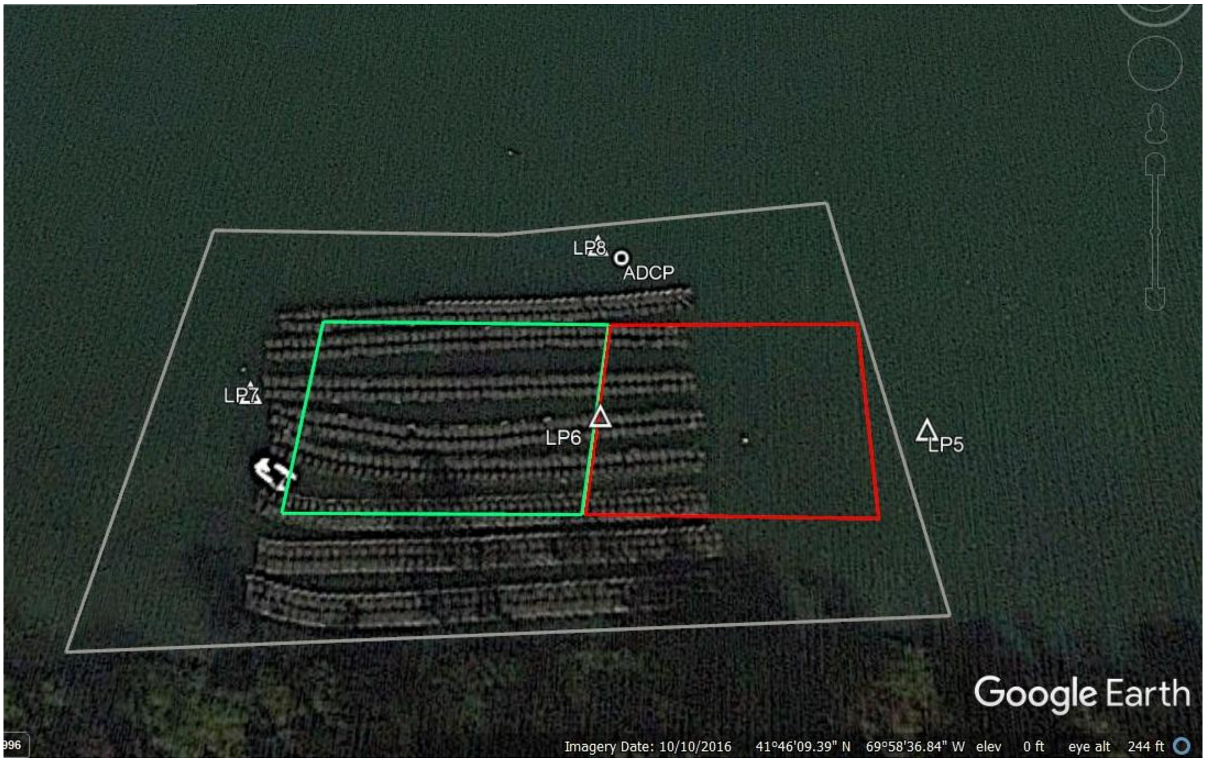
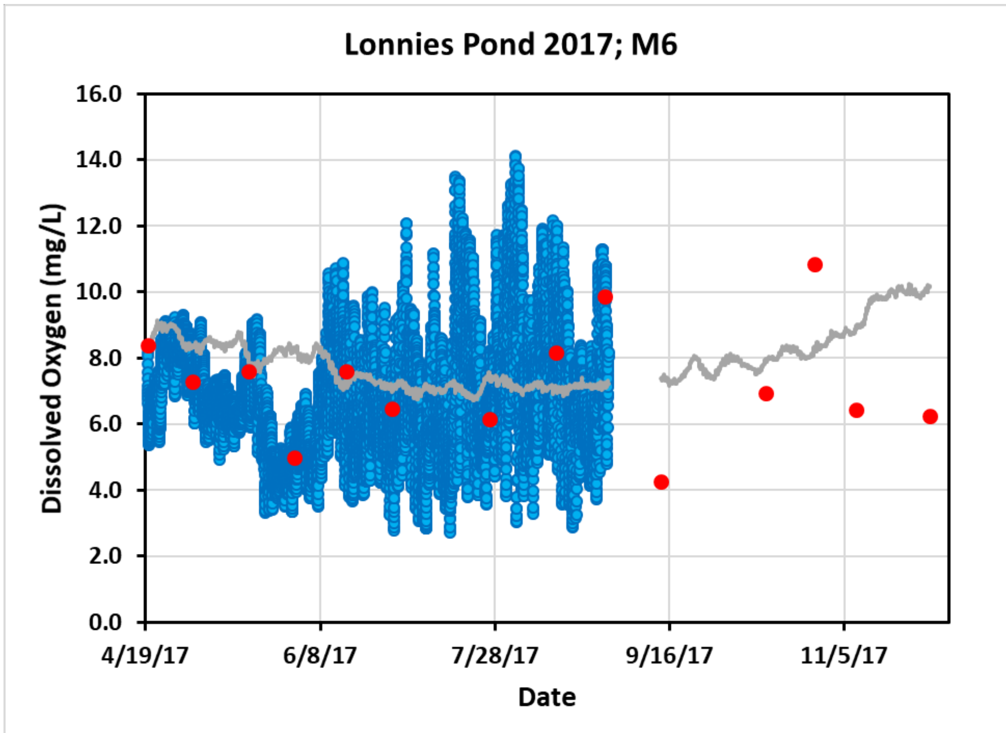


Figure IV.1. Aerial photograph showing the initial 2016 oyster deployment (floating bags) relative to mooring locations at the edges of the oyster deployment footprint (LP5 {bottom}, LP7 {bottom}, LP8 {surface+bottom}). The area defined by the green line is where the first year class oysters (2016) were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



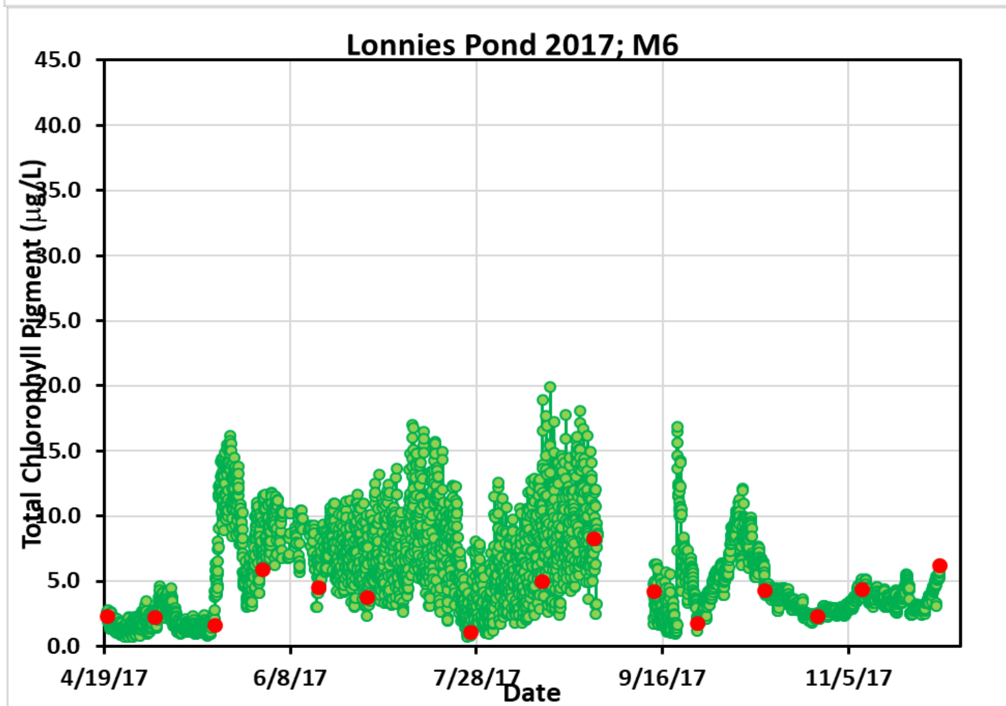
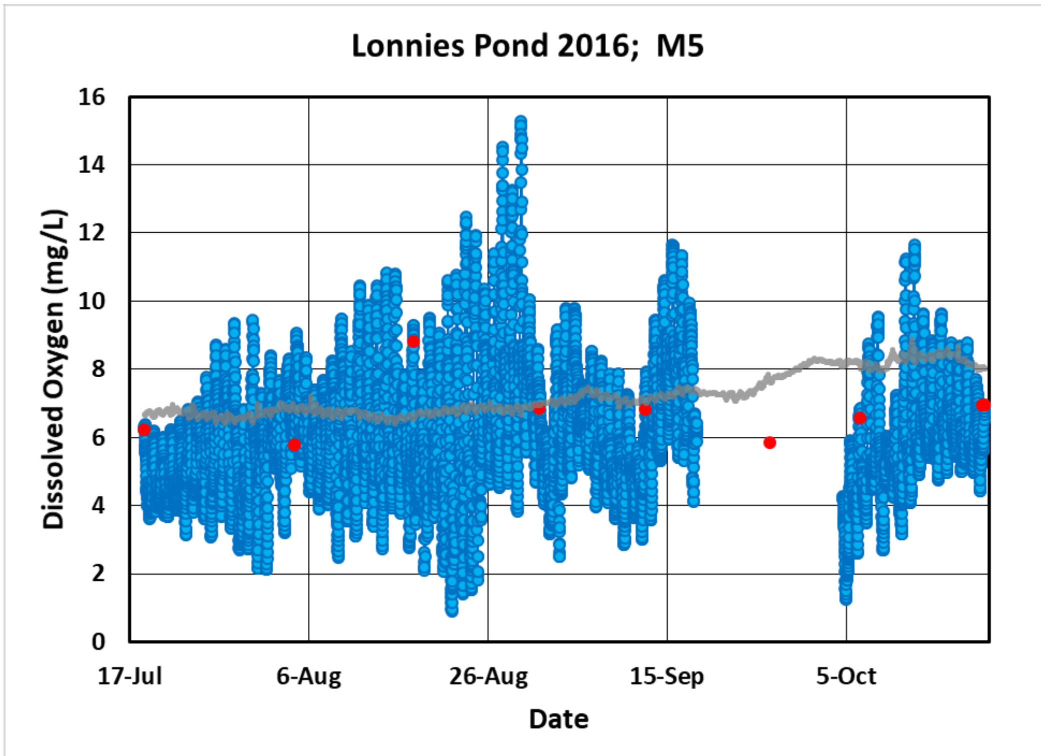


Figure **IV.1a** Time series dissolved oxygen at Lonnies Pond Mooring M6 located in the center of the oyster deployment footprint. Red markers indicate Winkler titration calibration points. The gray line represents the air saturation value (top). Time series chlorophyll measurements at Lonnies Pond Mooring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



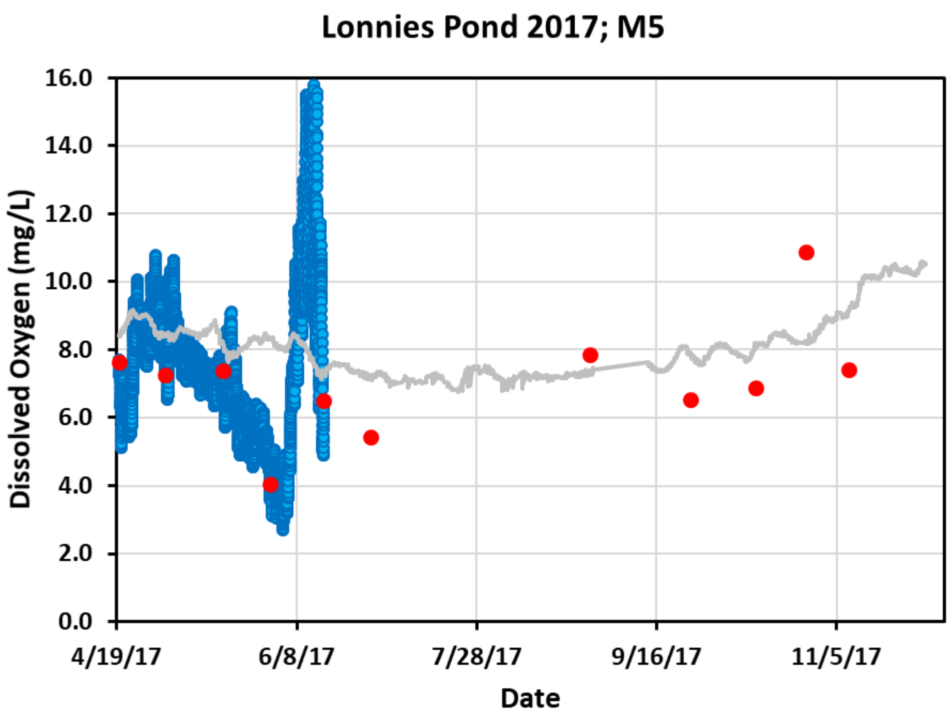


Figure IV.2. Time series dissolved oxygen at Lonnies Pond Mooring M5 located due east of the oyster deployment footprint. Red markers indicate Winkler titration calibration points. The gray line represents the air saturation value (top panel 2016 for comparison).

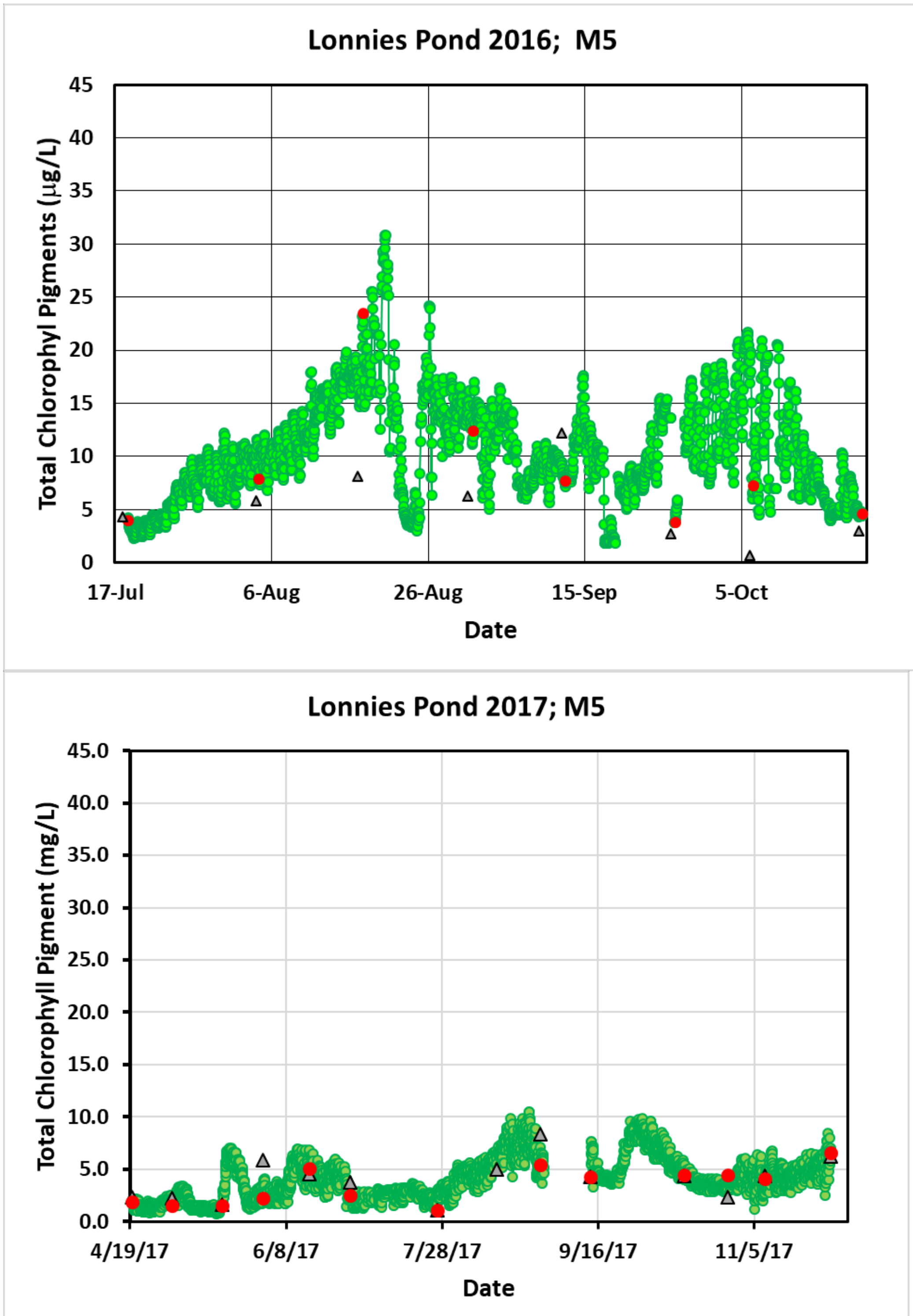


Figure IV.3. Time series chlorophyll measurements at Lonnies Pond Mooring M5 located due east of the oyster deployment footprint. Red markers indicate chlorophyll extraction calibration points. Green triangles indicate chlorophyll extractions conducted at Mooring M6 located in the middle of the deployment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

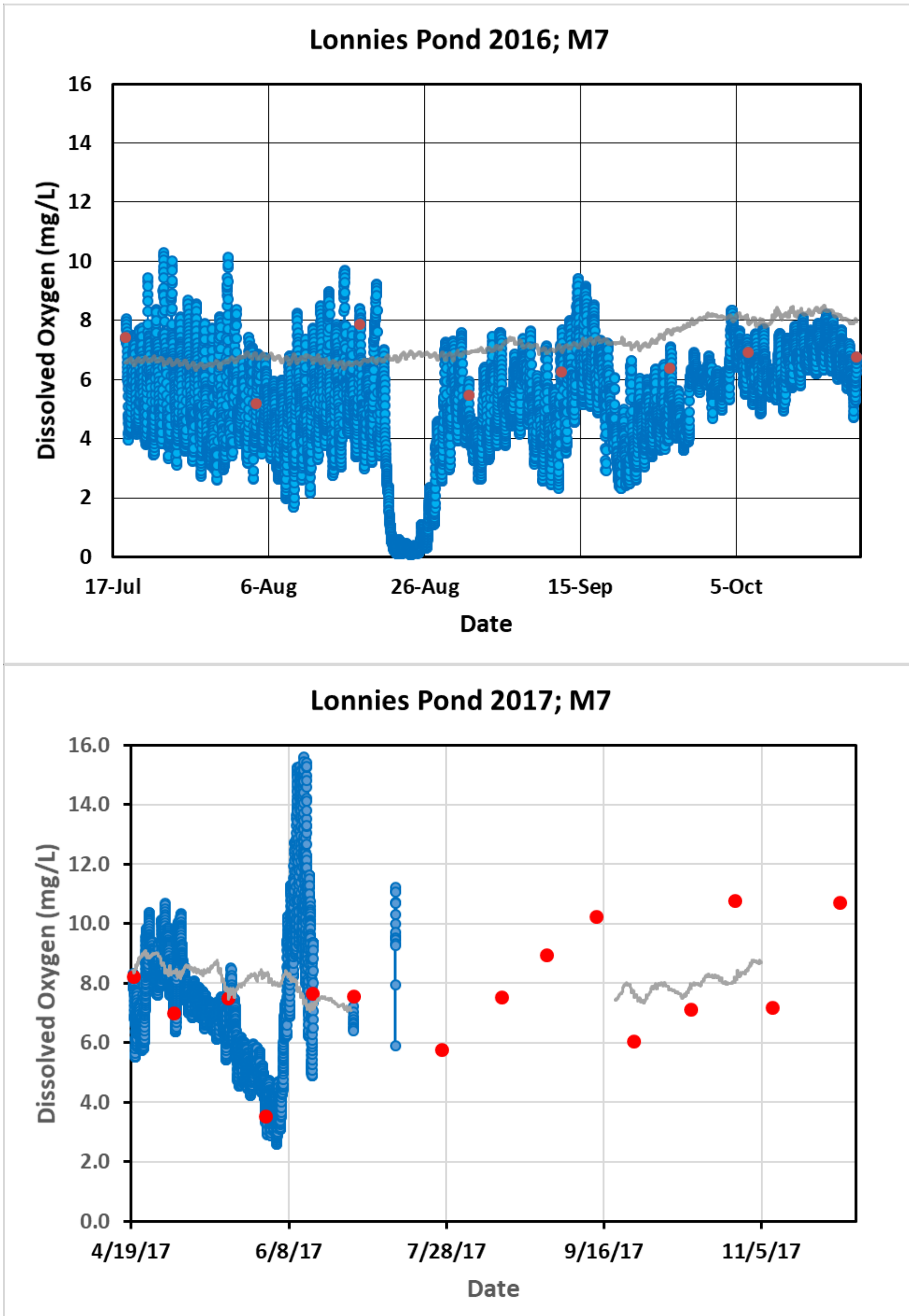


Figure IV.4. Time series dissolved oxygen at Lonnies Pond Mooring M7 located due west of the oyster deployment footprint. Red markers indicate Winkler titration calibration points. The gray line represents the air saturation value (top panel 2016 for comparison).

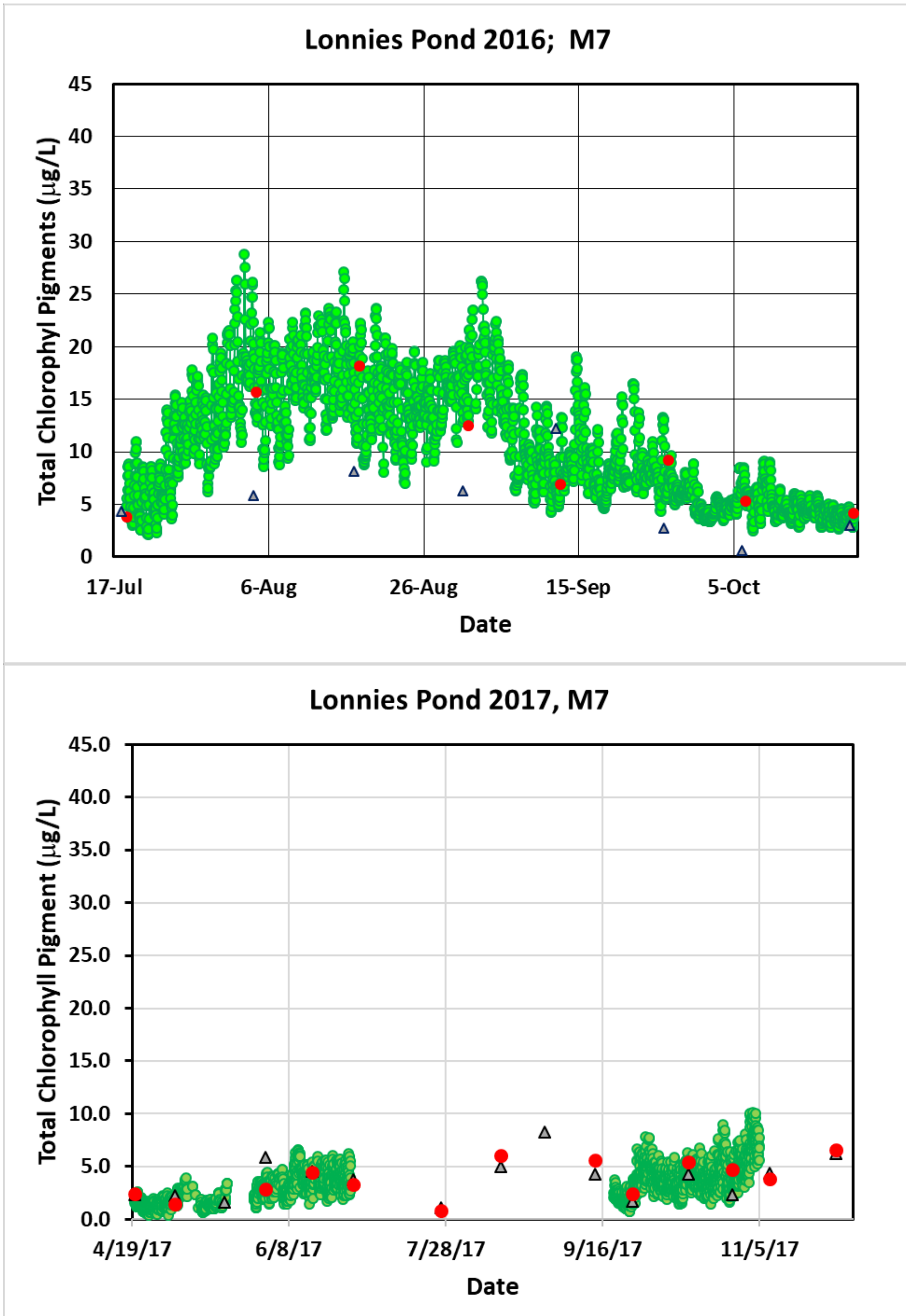


Figure IV.5. Time series chlorophyll measurements at Lonnies Pond Mooring M7 located due west of the oyster deployment footprint. Red markers indicate chlorophyll extraction calibration points. Green triangles indicate chlorophyll extractions conducted at Mooring M6 located in the middle of the deployment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

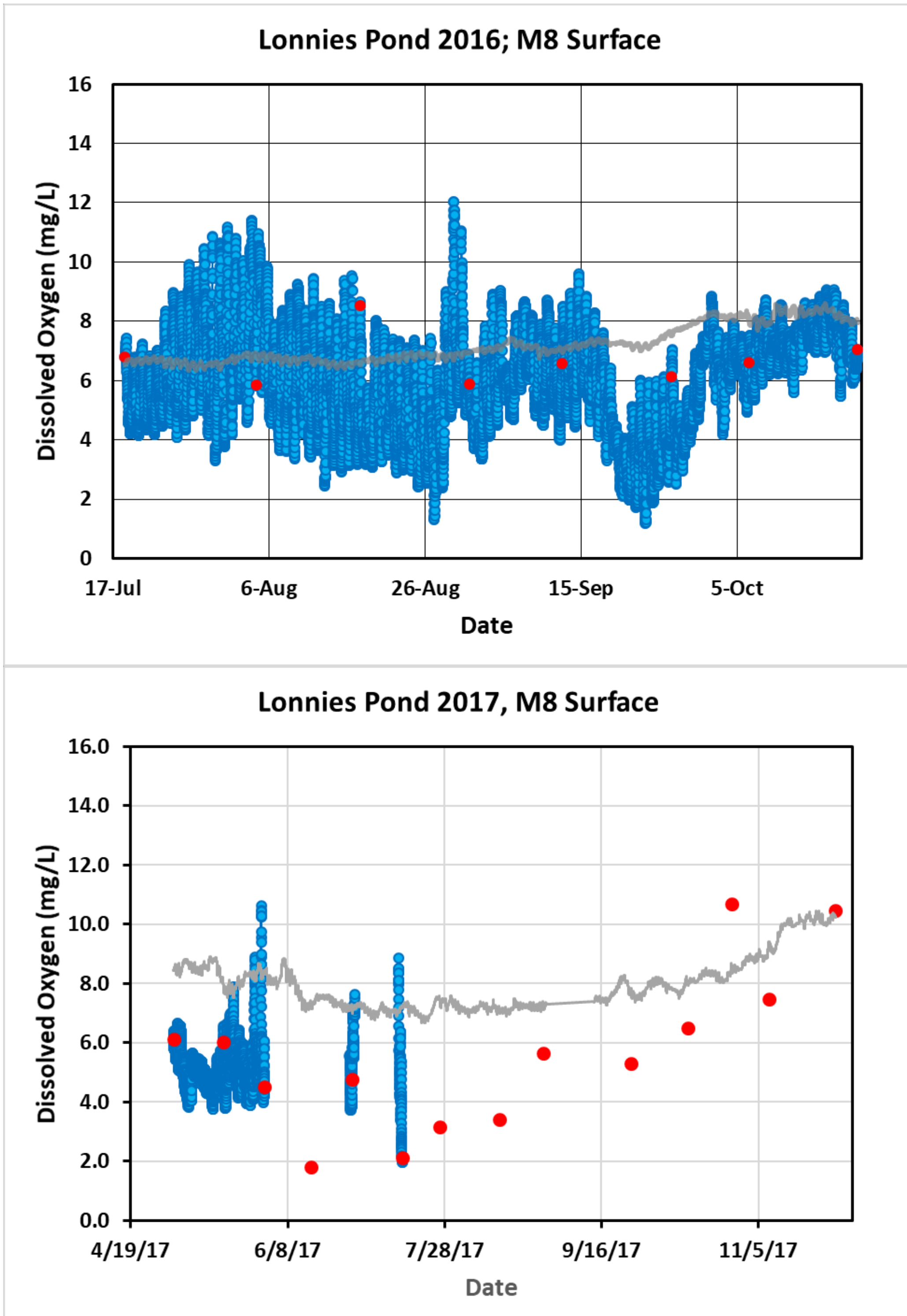
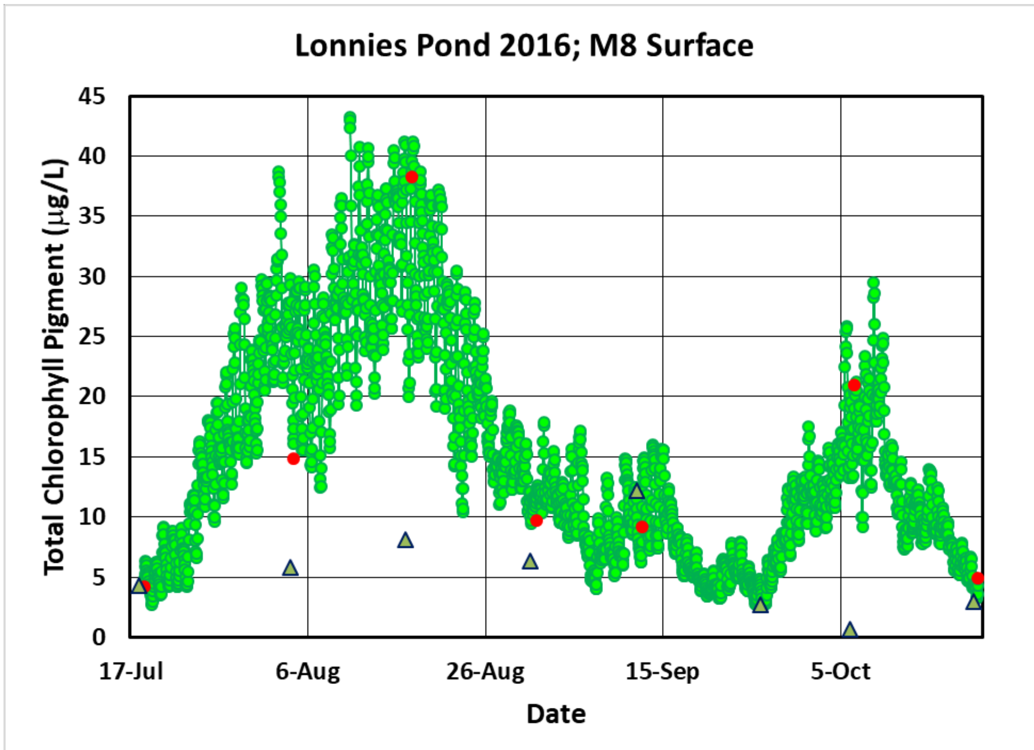


Figure IV.6. Time series dissolved oxygen at Lonnies Pond Mooring M8 (Surface) located due north of the oyster deployment footprint. Red markers indicate Winkler titration calibration points. The gray line represents the air saturation value (top panel 2016 for comparison).



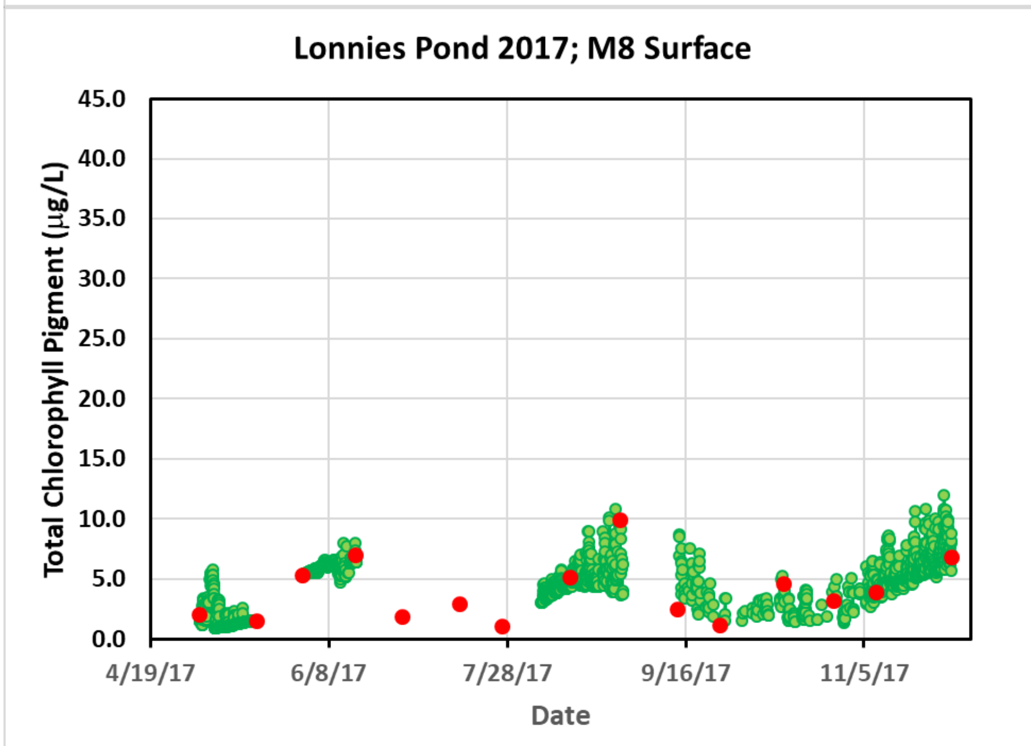
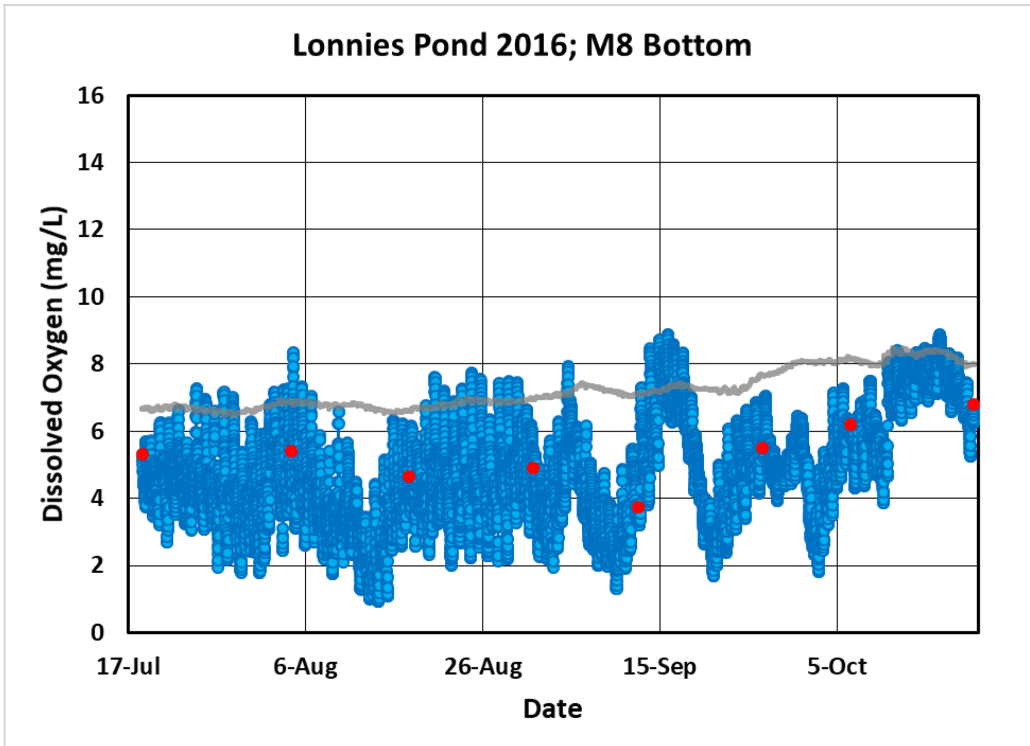


Figure IV.7. Time series chlorophyll measurements at Lonnies Pond Mooring M8 (Surface) located due north of the oyster deployment footprint. Red markers indicate chlorophyll extraction calibration points. Green triangles indicate chlorophyll extractions conducted at Mooring M6 located in the middle of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



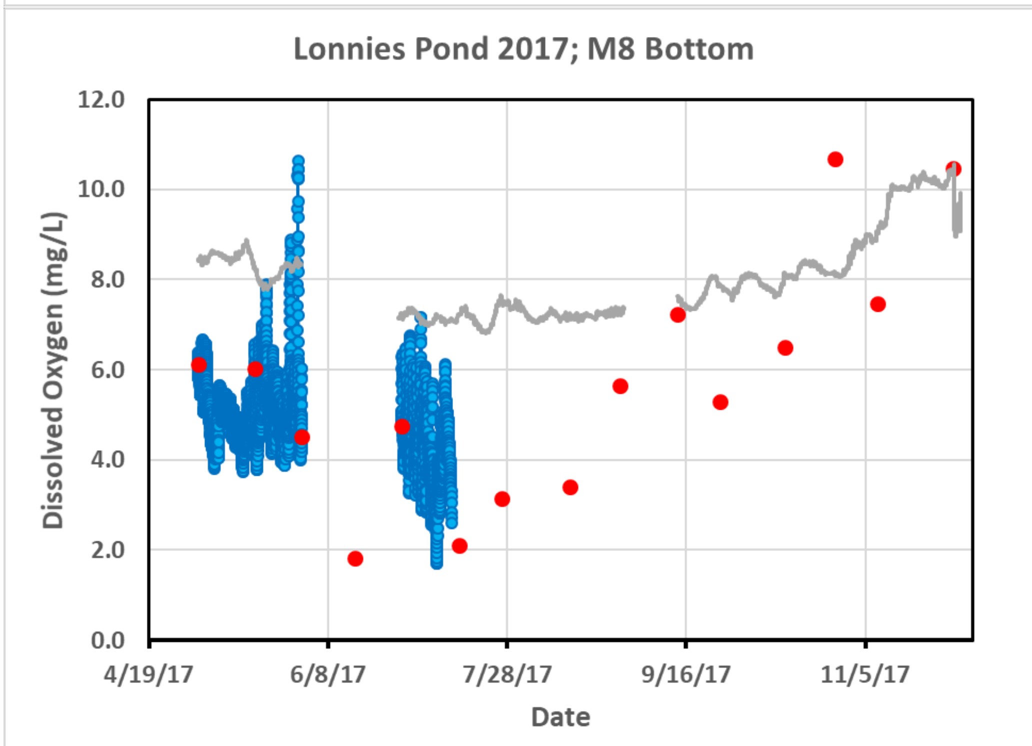
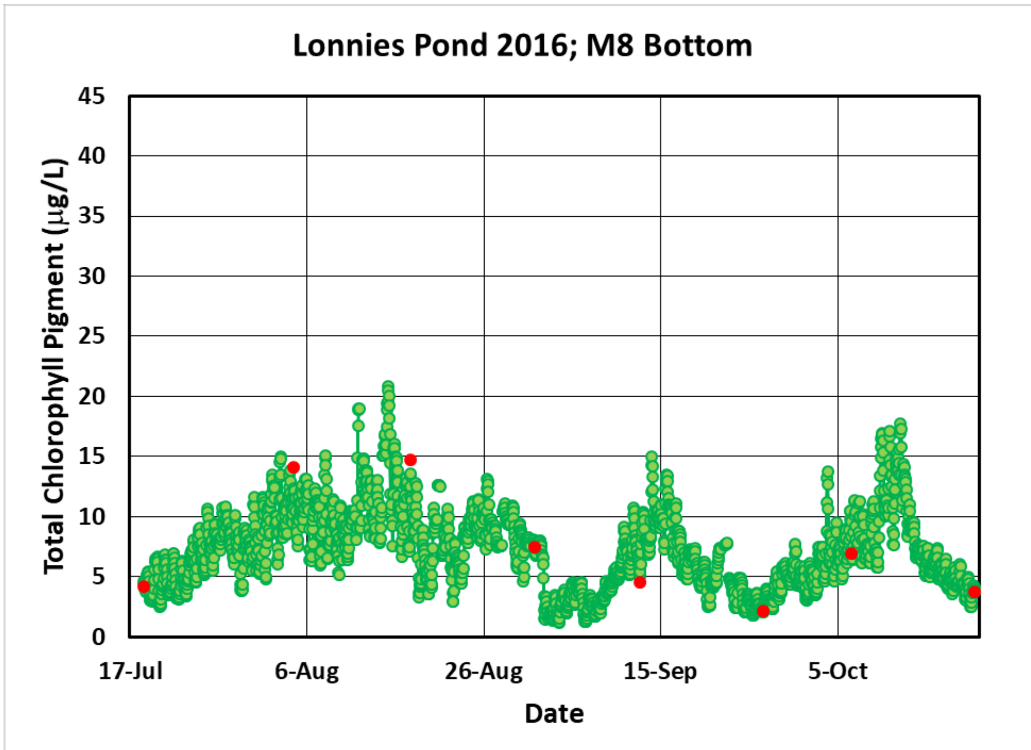


Figure IV.8. Time series dissolved oxygen at Lonnies Pond Mooring M8 (Bottom) located due north of the oyster deployment footprint. Red markers indicate Winkler titration calibration points. The gray line represents the air saturation value (top panel 2016 for comparison).



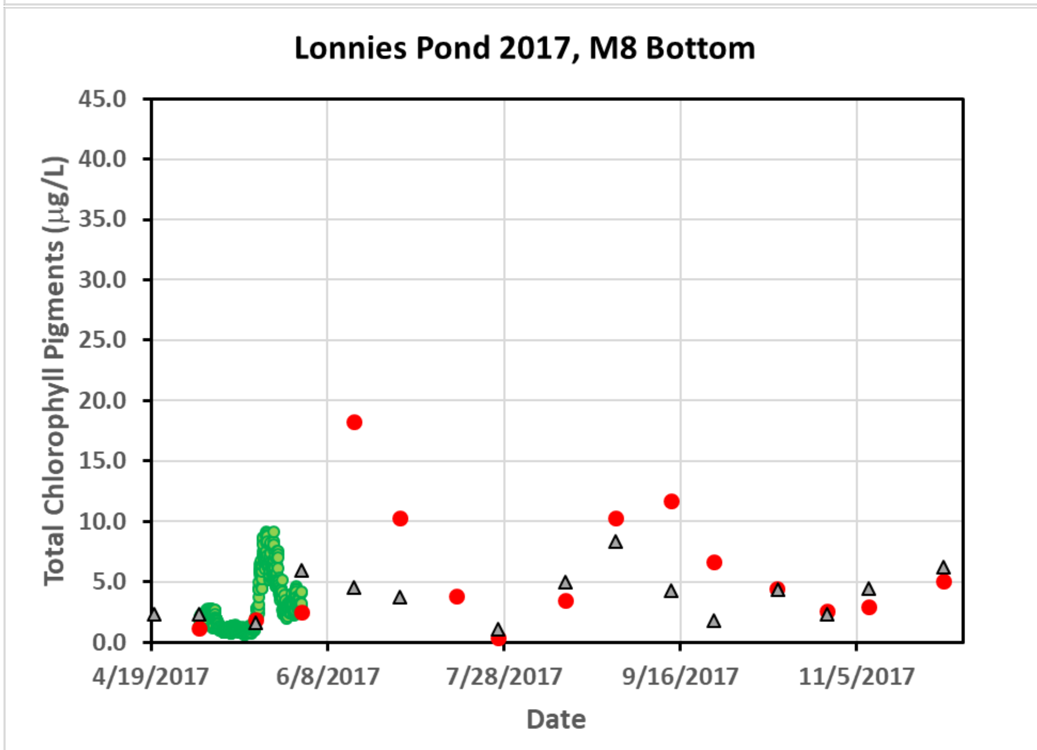


Figure IV.9. Time series chlorophyll measurements at Lonnies Pond Mooring M8 (bottom) located due east of the oyster deployment footprint. Red markers indicate chlorophyll extraction calibration points. Green triangles indicate chlorophyll extractions conducted at Mooring M6 located in the middle of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

Light pendants were used to measure light intensity in units of $\mu E/m^2/s$ 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, Lonnies 8, while also having the lowest chlorophyll concentration (Table IV.1). Note that station Lonnies 8 had additional surface samples collected. In contrast, when looking at only the data from stations in the oyster area, Lonnies 5, 6, 7, the lowest chlorophyll and lowest TSS (10.05 μg/L, 9.33 mg/L respectively) were found directly in the middle of the oysters, Lonnies 6. The chemical data represented specially as chlorophyll concentration and total suspeneded 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 Lonnies 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 (μE/m2/s)	Mean Penetration	Light Extinction Coefficient, k	TSS (mg/L)	Total Chlorophyll Pigments (μg/L)
Lonnies5 (east)	Surface	249.74				
Lonnies5 (east)	Bottom	88.00	35.21%	1.66	10.99	11.13
Lonnies6 (oysters)	Surface	216.94				
Lonnies6 (oysters)	Bottom	44.42	20.74%	2.41	9.33	10.05
Lonnies7 (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.

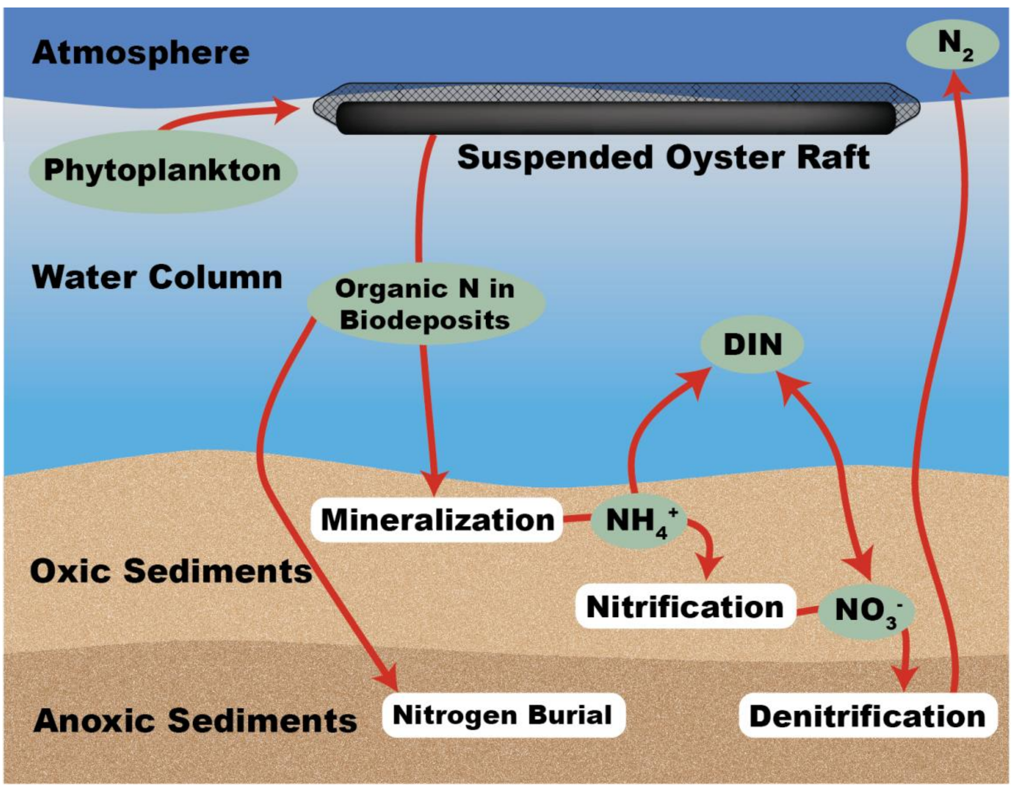


Figure V.1– Diagram of oyster's role in the marine nitrogen cycle in a shallow water estuary with oxygenated (oxic) and non-oxygenated (anoxic) sediments. White rectangles indicate microbial processes; green ovals indicate nitrogen species (Diagram adapted from Kellogg et al 2013).

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 Lonnies Pond aquaculture oyster population.

Figure V.2. Biodeposit trap deployed in Lonnies 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 Lonnies Pond oyster deployment to expand the biodeposition rate to include the total oyster biomass deployed in Lonnies 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-*a* (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 (±SE) total oyster deployment biodeposition rates and particulate organic nitrogen (PON) deposition. Biodeposit Characteristics: mean (±SE) particulate organic carbon (POC), particulate organic nitrogen (PON), and carbon/nitrogen molar ratio (C:N) of collected biodeposits. Surface Water Column Conditions: mean (±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-*a* (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 Lonnies Pond and Little Pond in August 2016.

Discussion:

Overall, the filtration of particulates from Lonnies 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 increseased 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 Lonnies 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 Aquapro 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±5.01mm/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 mg N/m^2/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 Lonnies Pond sediments from the deployed oyster arrays and to target areas to assess potential enhancements of sediment denitrification.

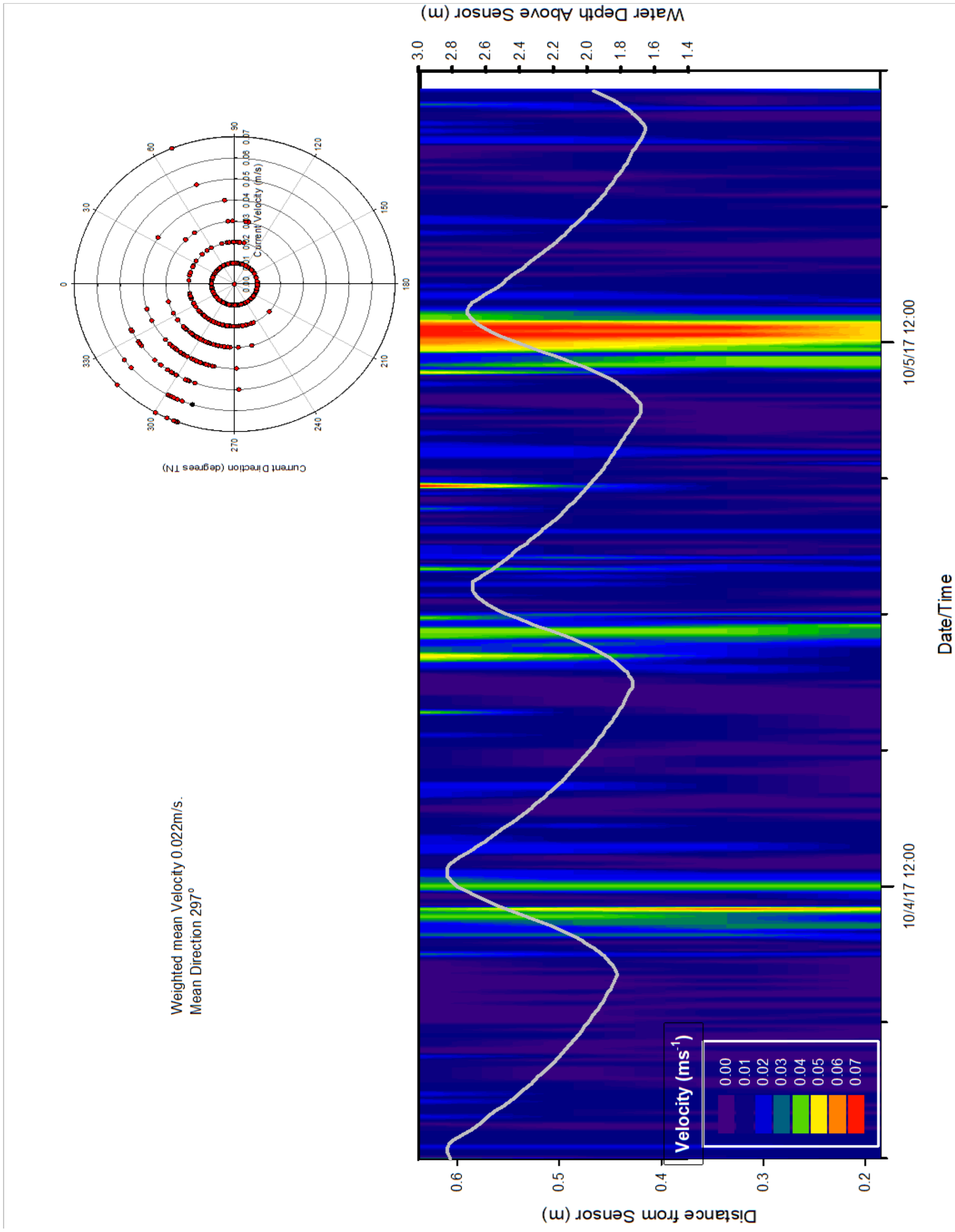


Figure VI.1. Top left: Compass rose plot of particle direction and velocity data for 23-24 August. Current direction was predominantly to the north north-west ~300 from True North . Velocities increase with distance from the center of the compass rose. Highest velocity was 0.07m/s (7cm/s). Right: Time 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.

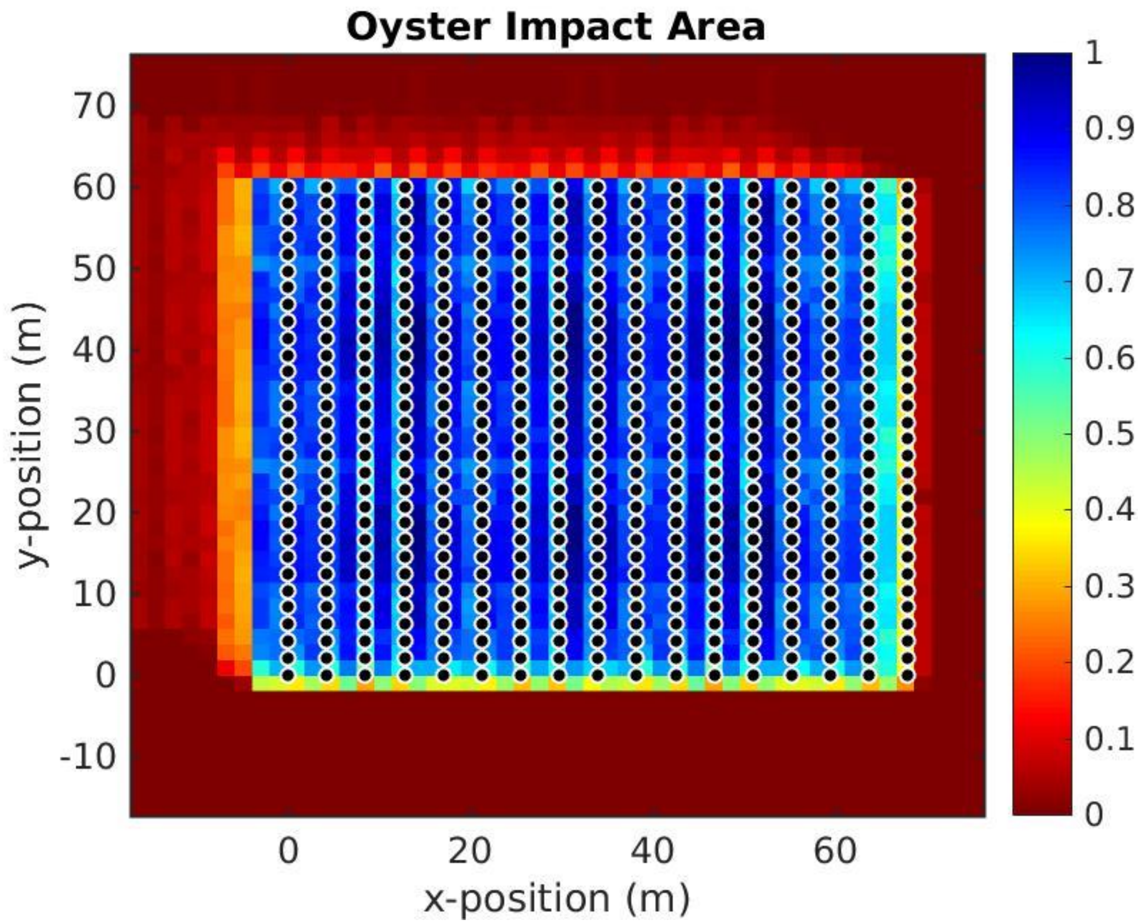


Figure VI.2. Expected deposition of fecal pellets to surficial sediments for pellets originating at the edge of the oyster array. Fecal pellet production was given as 1 and results are shown in the contours as a fraction of the pellet production. Thus, deposition areas colored dark blue received 100% 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.

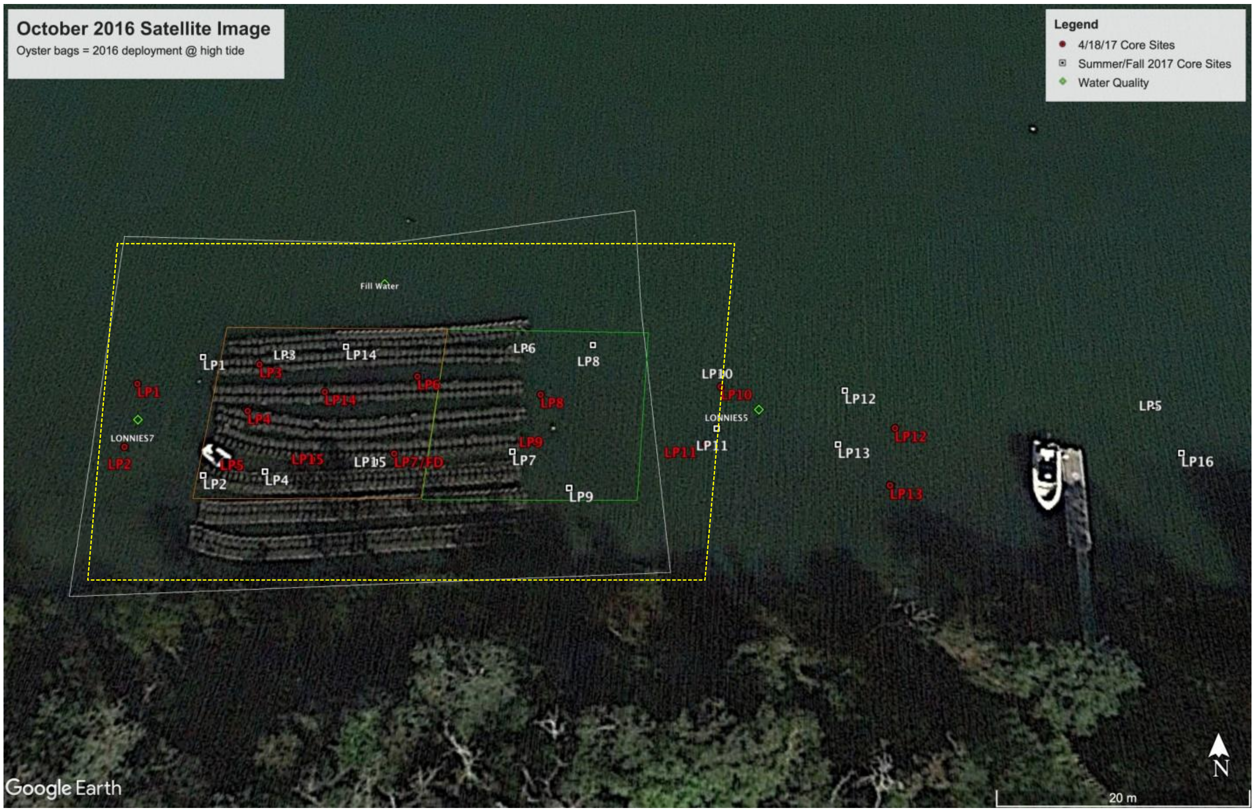


Figure VI.3. 2016 Aerial photograph showing the location of the oyster deployment area overlain with 2017 oyster deployment area (red and green polygons). Mooring and water column sampling locations (green diamonds), April (pre-oyster deployment red diamonds) and Summer/Fall (white square) flux core 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 Lonnies 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 Lonnies 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 Lonnies 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 Lonnies 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 Lonnies 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 Lonnies 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.

N_2 excess was measured using membrane-inlet mass spectrometry (MIMS). N_2 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 CO_2 gas. Sample gas was analyzed by the mass spectrometer for masses 28 and 40 for determining the N_2 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 Lonnies 5 and Lonnies 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 Lonnies 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)

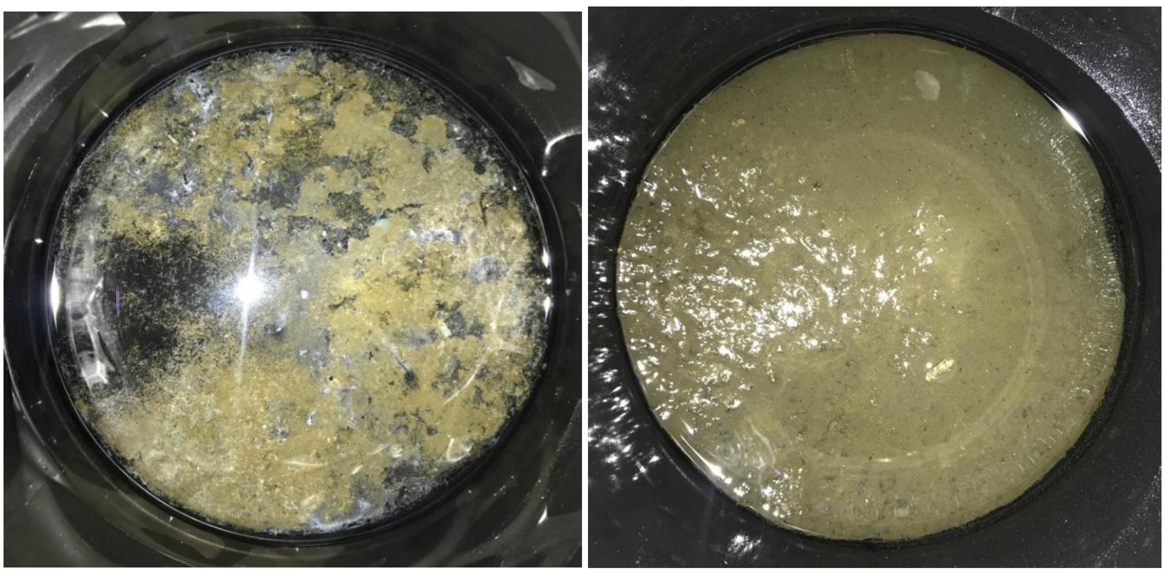


Figure VII.1 Images of sediment core surfaces from September 2017. Left: Core 3 within treatment area showed dark areas where reduced sediment was exposed to the water surface, thus preventing efficiently coupled nitrification and denitrification. Right: Core 13 outside of the treatment area showed a 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						
	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	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						
	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	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						
	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	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							
	SOD	**NH4**	**NO3**	**DIN**	**N2-N**	**Total N Cycled**	**Denitrified**
Site ID	**(mMol/m2/d)**	**(mMol/m2/d)**	**(mMol/m2/d)**	**(mMol/m2/d)**	**(mMol/m2/d)**	**(mMol/m2/d)**	**% 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							
	SOD	**NH4**	**NO3**	**DIN**	**N2-N**	**Total N Cycled**	**Denitrified**
Site ID	**(mMol/m2/d)**	**(mMol/m2/d)**	**(mMol/m2/d)**	**(mMol/m2/d)**	**(mMol/m2/d)**	**(mMol/m2/d)**	**% 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						
	SOD	**NH4**	**NO3**	**DIN**	**N2-N**	**Total N Cycled**
Site ID	**(mMol/m2/d)**	**(mMol/m2/d)**	**(mMol/m2/d)**	**(mMol/m2/d)**	**(mMol/m2/d)**	**(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 Temperature	4/18/17 12.7 C	6/27/17 22.1 C	8/1/17 23.6 C	9/19/17 23.5 C	9/19/17* 23.5 C	10/3/17 17 C	8/16/16 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 Temperature	4/18/17 12.7 C	6/27/17 22.1 C	8/1/17 23.6 C	9/19/17 23.5 C	9/19/17* 23.5 C	10/3/17 17 C	8/16/16 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)				
	Treated		Background		Oyster
Date	Mean	Std. Dev.	Mean	Std. Dev.	Effect
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/m2/d)				
	Treated		Background		Oyster
Date	Mean	Std. Dev.	Mean	Std. Dev.	Effect
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/m^2 N)	298.0	253.3
Enhanced Annual DeN2 (gm/m^2 N)	4.17	3.55
Impact area (m^2)	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 Lonnies 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 Lonnies 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 Lonnies 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 Lonnies 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 Lonnies 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 Lonnies 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 Lonnies Pond, the measured surface water load is the attenuated load.

Given the importance of quantifying the balance of nitrogen into and out of Lonnies 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 Lonnies 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:

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

where by:

Q = Stream discharge (m^3/s)
A = Stream subsection cross sectional area (m^2)
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 Lonnies 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 Lonnies 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 where 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 Lonnies 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 Lonnies 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 Lonnies 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 Lonnies Pond as appropriate. The "measured load" was representative of attenuated nitrogen entering Lonnies Pond directly from surfacewater as opposed to groundwater (recharge over area delineated by watershed).

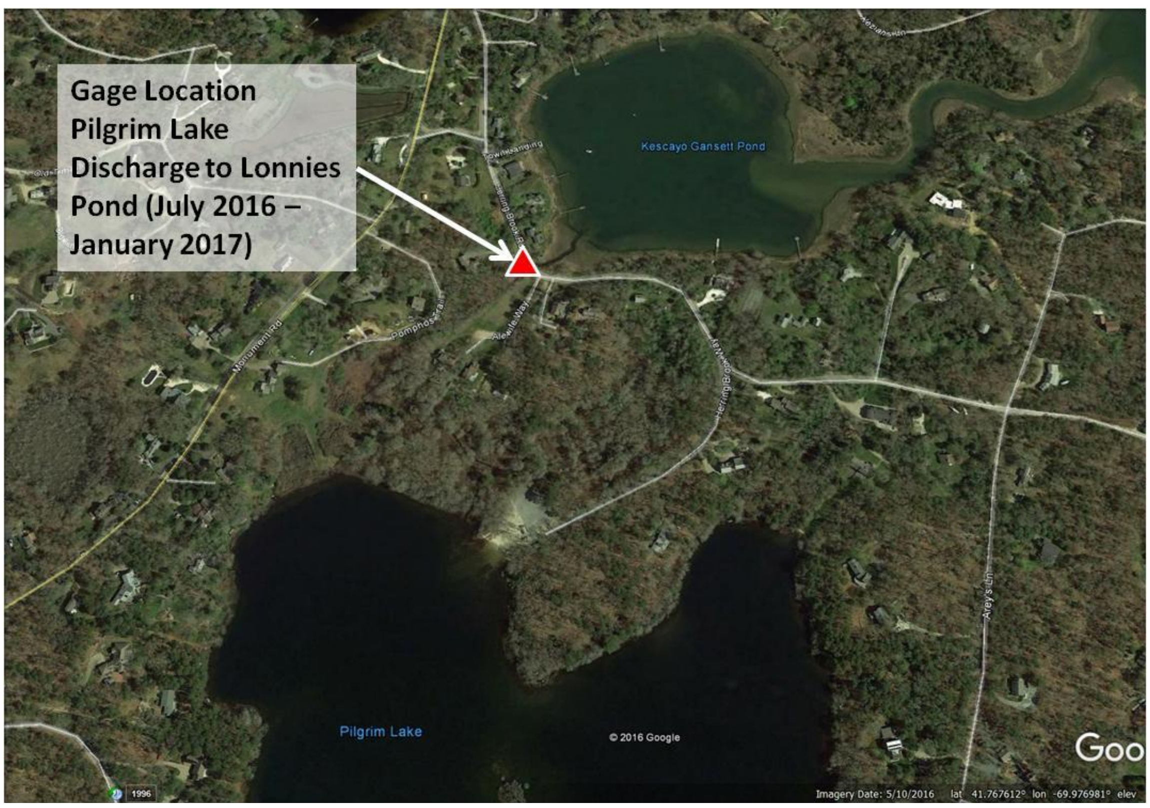


Figure VIII.1a. Location of stream gauge (red triangle) deployed at the base of the herring ladder between Pilgrim Lake and Lonnies Pond. An updated MEP rating curve initially developed in 2003 but refined with 2016-2017 flow measurements was utilized to determine daily flows and nutrient load to Lonnies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

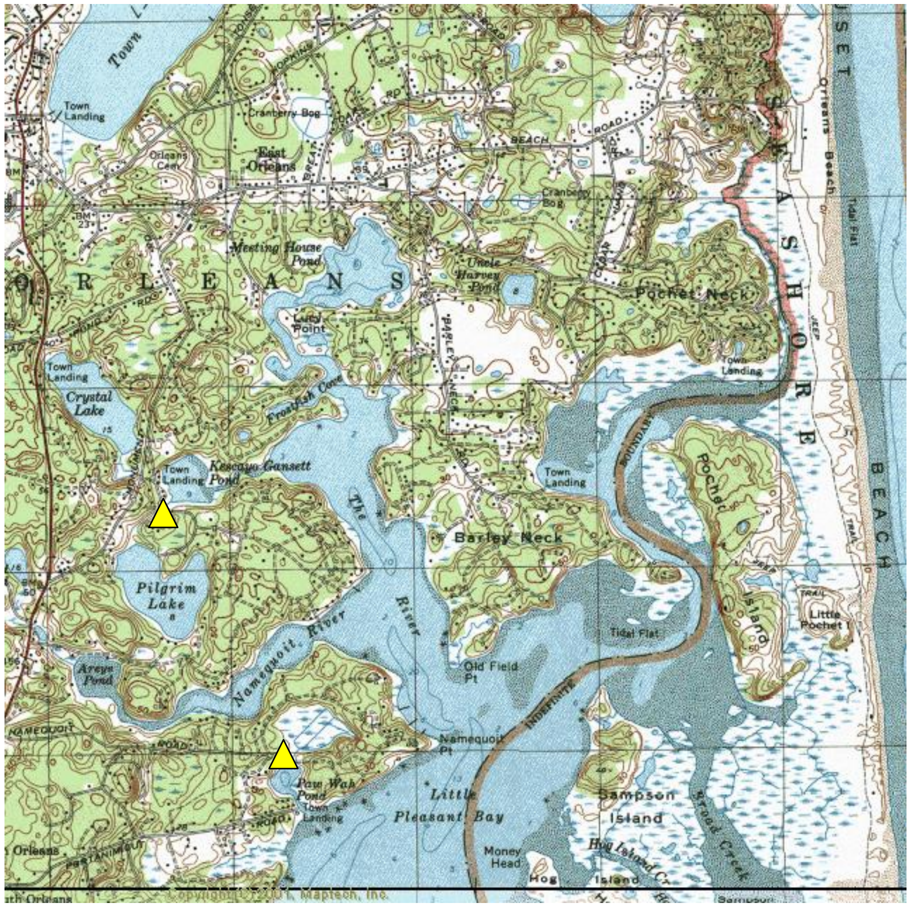


Figure VIII.1b. Location of MEP Stream gauge (yellow triangle) in the upper portions of the Pleasant Bay system embayment system. Rating curve, stage, flow, nutrient load and percent attenuation was determined for the 2002-2003 hydrologic year

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 Lonnies 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 m^3 (average daily flow = 514 m^3). By comparison, total surface water inflow to Lonnies Pond from Pilgrim Lake during the MEP study period (October 2002-September 2003) was 355,279 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.

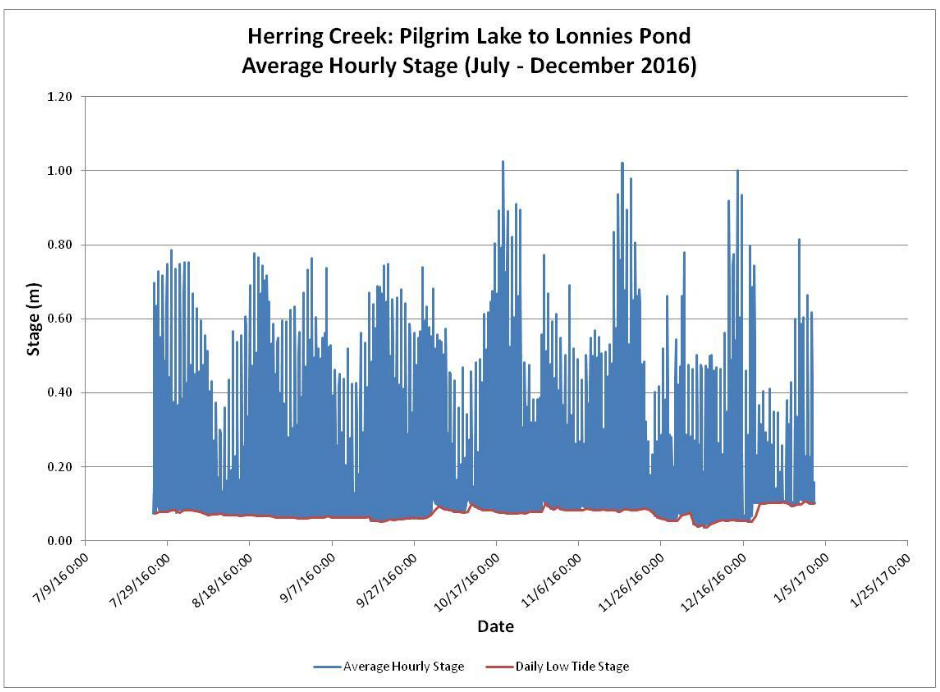


Figure VIII.2a. Average hourly stage record from Herring Creek discharge to Lonnies Pond from Pilgrim Lake, July 25, 2016 to December 31, 2016 and associated daily low tide stage used to calculate freshwater flow and nitrogen load to Lonnies Pond.

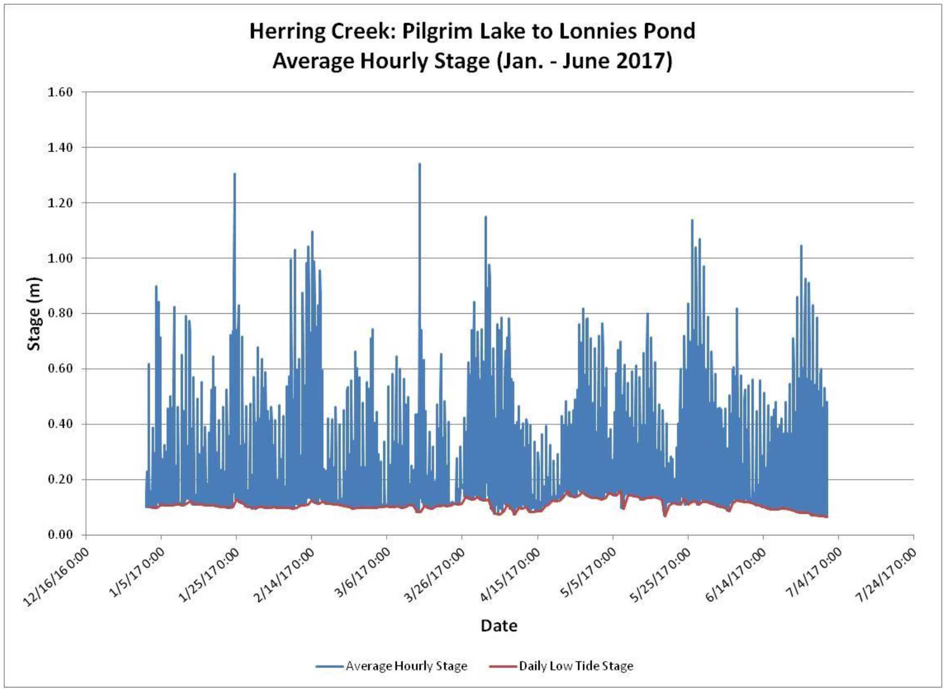


Figure VIII.2b. Average hourly stage record from Herring Creek discharge to Lonnies Pond from Pilgrim Lake, January 1, 2017 to June 30, 2017 and associated daily low tide stage used to calculate freshwater flow and nitrogen load to Lonnies Pond.

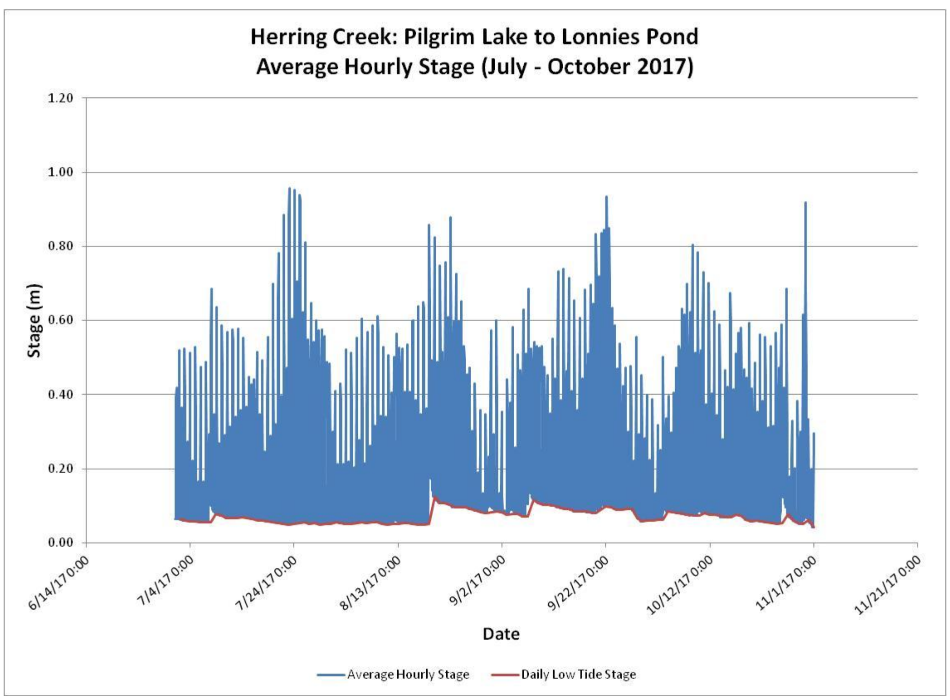


Figure VIII.2c. Average hourly stage record from Herring Creek discharge to Lonnies Pond from Pilgrim Lake, July 1, 2017 to October 31, 2017 and associated daily low tide stage used to calculate freshwater flow and nitrogen load to Lonnies Pond.

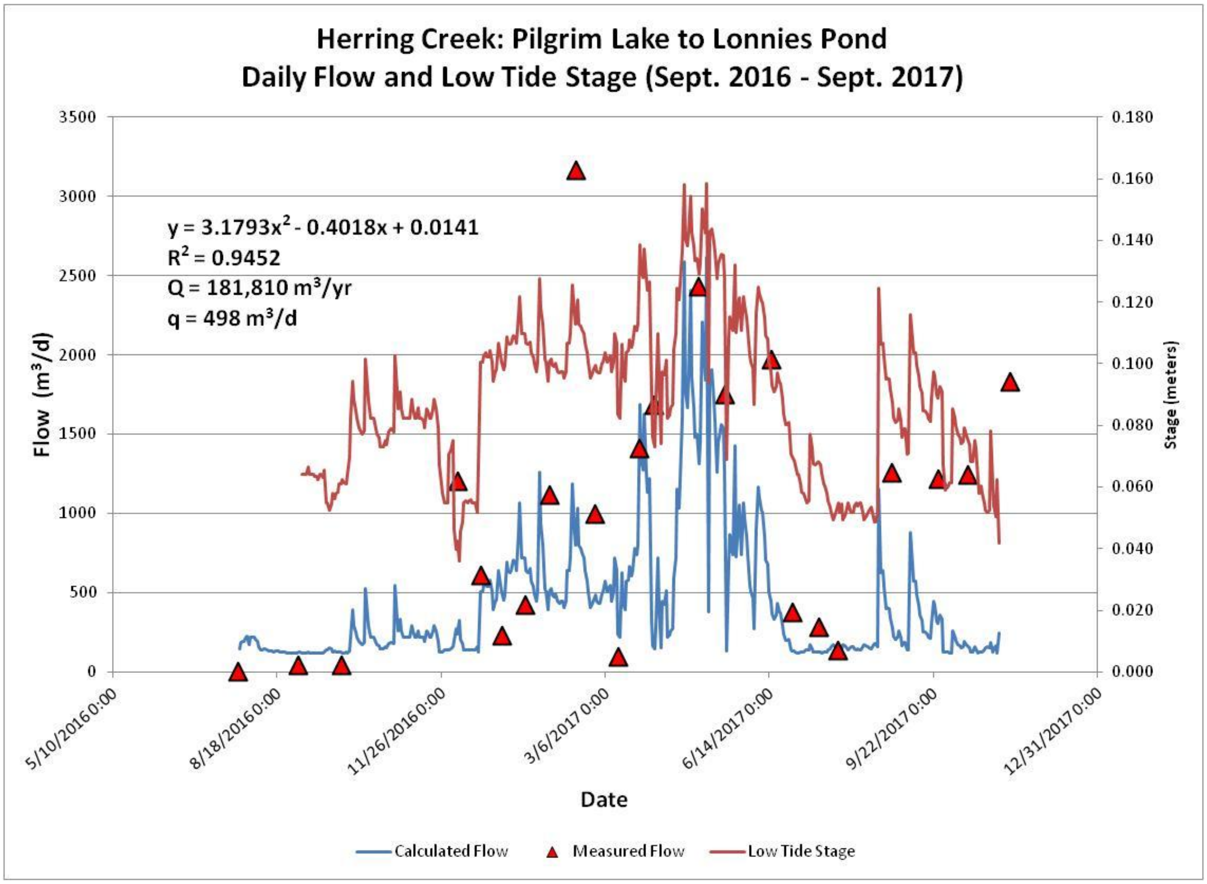


Figure VIII.3. Daily flow record from Herring Creek discharge to Lonnies Pond from Pilgrim Lake, September 1, 2016 to October 31, 2017, associated daily low tide stage used to calculate freshwater flow and nitrogen load to Lonnies Pond and comparison to measured point flow measurements.

Monthly Flow Pilgrim Lake to Lonnies Pond (August - December)		
MONTH	**2016-2017 FLOW (m^3/month)**	**2002-2003 FLOW (m^3/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 Lonnies 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 Lonnies Pond, sample concentrations were interpolated and then merged with the daily flows to obtain an estimate of the nitrogen load into Lonnies 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 (Table2). 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.

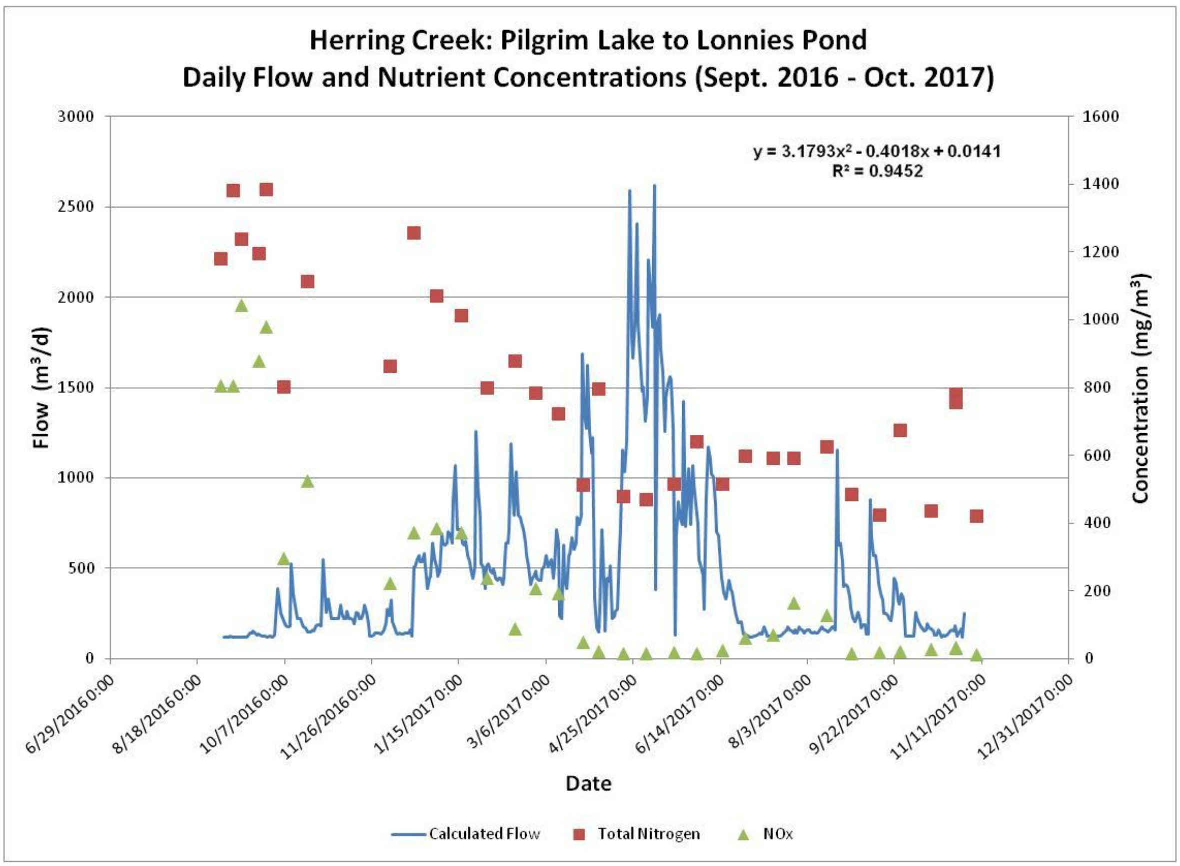


Figure VIII.4. Daily flow and stream nutrient concentration record from Herring Creek discharge to Lonnies Pond from Pilgrim Lake, September 1, 2016 to October 31, 2017 used to calculate freshwater flow and nitrogen load to Lonnies Pond.

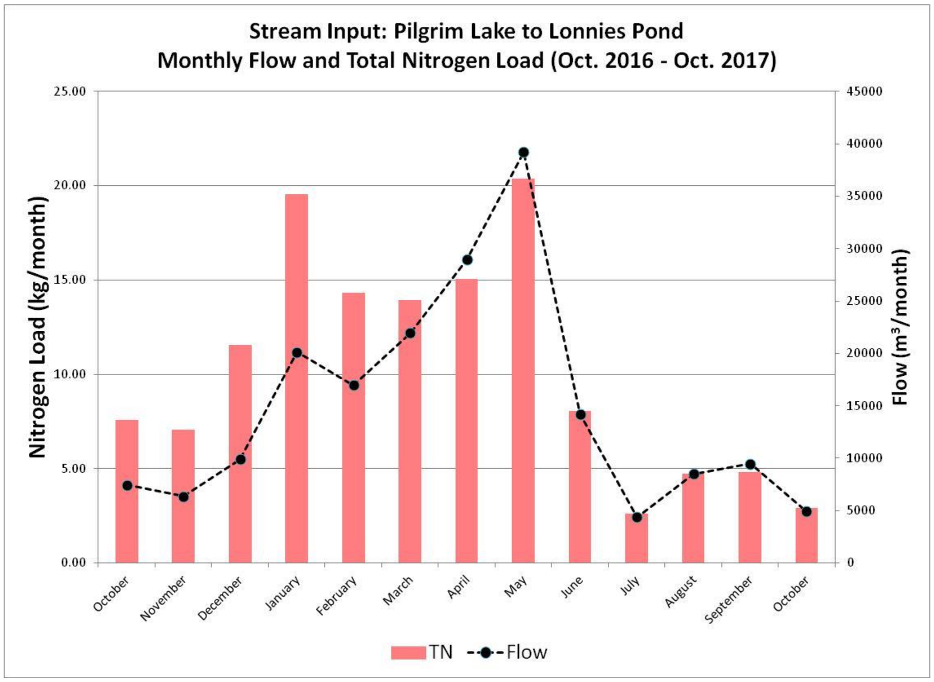


Figure VIII.5. Monthly flow and monthly stream total nitrogen load from Pilgrim Lake to Lonnies Pond.

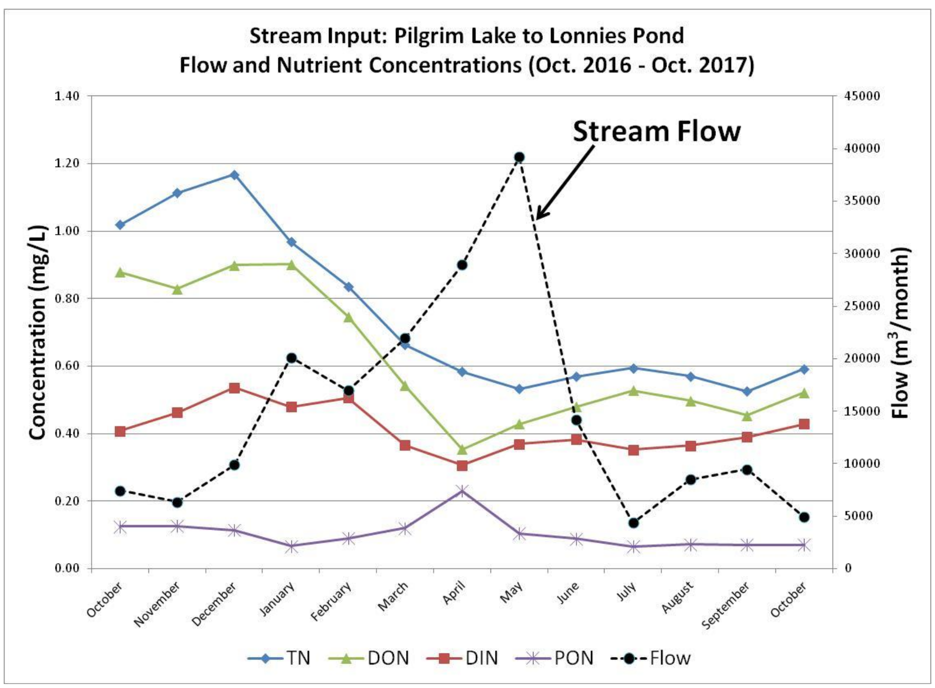


Figure VIII.6. Monthly flow and average monthly stream nitrogen constituent concentration record from Herring Creek discharge to Lonnies Pond from Pilgrim Lake, October 1, 2016 to October 31, 2017 used to calculate freshwater flow and nitrogen load to Lonnies Pond and determine seasonal differences in 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 Lonnies 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 Avg. Monthly (mg/L)	NOX Avg. Monthly (mg/L)	DIN Avg. Monthly (mg/L)	DON Avg. Monthly (mg/L)	PON Avg. Monthly (mg/L)	TN 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 Lonnies Pond in December 2016.						

Table VIII.2. Nutrient concentrations by month (August - December) 2016 vs. 2003 entering Lonnies 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 Lonnies Pond in December.						

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

Section IX. Lonnies Pond Oyster Study 2016 - 2017: Conclusions

The major results of the 2 year pilot deployment of oysters in Lonnies 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* of >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 kg N per 1 kg N in harvest, but when continued into the second year the increased removal was significantly higher ~0.37 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 Lonnies 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 m^3) with 2017 flows being higher (Aug.-Oct., 22,891 m^3) 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 m^3) 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 Lonnies 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 where lower than for the same period in 2017, average monthly TN load to Lonnies 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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