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Memorandum

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Subject	**Town of Orleans, MA** **Water Quality and Wastewater Planning** **Task 10.1.B.1 - Lonnie's (Kescayo-Gansett) Pond Oyster Aquaculture Demonstration Project Year 1 Project Report - Final**
Project Number	60476644
From	Thomas Parece, P.E., AECOM Project Manager
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1. Technical Memorandum Organization

This Technical Memorandum for the Kescayo-Gansett (Lonnie's) Pond Oyster Aquaculture Demonstration (Tech Memo) documents the planning history, installation details and data collected during the 2016 Demonstration. Detailed information related to full-scale implementation of an oyster program for nitrogen-removal is also presented in this Tech Memo. Section contents are as follows:

- Section 1 describes the Technical Memorandum Organization;
- Section 2 describes the Technical Memorandum Overview;
- Section 3 describes the overall purpose and rationale for shellfish demonstration projects;
- Section 4 explains the specific purpose of the Lonnie's Pond demonstration project;
- Section 5 details the process that was used for deciding to pursue a demonstration project in Lonnie's Pond;
- Section 6 presents the system that was used for installing this demonstration project in the field;
- Section 7 presents the data collection methodology and results obtained for growth, mortality and nitrogen-content of the oysters that were grown;

- Section 8 describes the operation and maintenance of the project;
- Section 9 presents the overwintering system that was developed and installed;
- Section 10 is a review of relevant sections of the demonstration monitoring technical report (Howes et al. 2017) that was prepared by staff from the University of Massachusetts School for Marine Science and Technology (SMAST);
- Section 11 details several long-term implementation options for oysters as a nitrogen-removing strategy in Lonnie's Pond that are based on the results of 2016 Demonstration Project;
- Section 12 describes the second year of the demonstration that is aimed at validating several key parameters of the long-term plan, with a detailed budget;
- Section 13 describes the next steps for continuing this demonstration project; and
- Section 14 summarizes the references utilized as part of the demonstration project.

2. Technical Memorandum Overview

Results from the first year of the Lonnie's Pond demonstration project (2016 Demonstration) have confirmed that oysters can remove the annual target amount of nitrogen that is prescribed by the Massachusetts Estuaries Project (MEP) for Lonnie's Pond. This nitrogen-removal target is approximately 300 kg of nitrogen per year, and can be met within a full-scale implementation area of one acre. Nitrogen amounts in water are measurable, and agencies have targeted nitrogen measurements as one indicator of the overall health of a system. While nitrogen reduction is the measurable goal, the overarching goal by reducing nitrogen is for habitat restoration. Cultivating oysters for nitrogen removal and to meet TMDL requirements is anticipated to lead to improvements to murky water and mucky bottoms.

In addition to meeting the yearly target, oysters can achieve the daily nitrogen removal target for Lonnie's Pond during the critical impairment period of July and August (Howes et al. 2006, Howes et al. 2017). This target is approximately 0.8 kg of nitrogen per day, and is the amount that must be removed daily in order to achieve the Total Maximum Daily Load (TMDL) for this sub-embayment.

The first-year project quantitatively evaluated:

- Total nitrogen removal associated with uptake from oyster growth;
- Total nitrogen removal through denitrification from enhanced biochemical processes in the sediment caused by oyster biodeposits through the growing season. An increase in total nitrogen removed through denitrification is expected in the year two data due to the denitrification process continuing even after the oysters were removed from the area;
- Capacity within Lonnie's Pond for grow-out in high density gear at a small scale;
- Site suitability for overwintering; and
- Potential to scale to full implementation.

It is important to remember that the 2016 Demonstration began approximately six weeks after the start of the oyster growing season. The reported weights and lengths for the growth of oysters during the 2016 Demonstration were obtained between June 22nd (when they were deployed) and December 9th (prior to overwintering), and should not be used to calculate the outcome of a full scale, full season program without taking into consideration the fact that there would have been more growth if the project had started early enough to make use of a full growing season.

The projections for full-scale implementation are based on a full growing season (rather than the truncated 2016 Demonstration Project season) and estimate the nitrogen that would be removed by two pathways: uptake in new tissue and shell as the oysters grow, and enhancement of denitrification in the bottom sediments induced by biodeposits from the oysters. The methodology consistently used in this Tech Memo for the projections of nitrogen removal by both these pathways utilizes the dry weights of oysters. These dry weights are necessarily different from the dry weights of oysters monitored during the

2016 Demonstration at Lonnie's Pond, because those oysters did not grow over a full season. The wet harvest weights that were measured during the 2016 Demonstration were correlated to dry tissue weights, as shown in Figure 14, for the specific purpose of accurately projecting the outcome of a full season program. It is important to understand that dry tissue weight is the best quantity to use to calculate the nitrogen removal performance of oysters by the two pathways considered in this report, but wet harvest weight is the most feasible way to track actual performance in the field. As discussed in Section 7B, there is a basis in published literature for recognizing the ratio of harvest wet weight to dry tissue weight and this relationship will be further evaluated during Year 2 of the Demonstration Project.

The first-year demonstration was designed to install 200,000 second year oysters. Due to the oyster availability, approximately 65 percent of the oysters deployed were second-year, averaging 63 mm/18 g each. The balance of the oysters installed was early first-year seed and averaged 22 mm/1.2 g each at the time they were installed in Lonnie's Pond. This mass of oysters was adequate for preliminary assessment of oyster-enhanced sediment denitrification, while also allowing the growth rates and associated nitrogen uptake for both first and second year oysters to be measured. The monitoring results (Howes et al. 2017) show that the mass of nitrogen removed by denitrification is approximately 67 percent of the mass of nitrogen taken out of the water column by the growth of the oysters (Section 7).

Section 7B of the Technical Memorandum explains how the amount of direct nitrogen uptake by oysters in Lonnie's Pond during the 2016 Demonstration Year was estimated, using estimated dry weights of the oysters, and nitrogen uptake quantified by Boston University (BU) Laboratory measurements. Both dry weights and wet harvest weights of a sample of oysters were measured by the BU laboratory on two dates and these data were plotted in Figure 14 to establish a relationship between dry weight and wet harvest weight. Average dry weight was calculated from measured harvest weights using the relationship shown in Figure 14. Tables TMO-1 and TMO-2 below summarize some of the important values used to estimate nitrogen uptake by the oysters as well as projected full-scale implementation nitrogen uptake. Note that the estimated nitrogen uptake numbers in Tables TMO-1 and TMO-2 do not include denitrification removal, which was separately estimated for full-scale implementation projections as explained in Section 10E and Figure 36.

Table TMO-1: Estimated Oyster Weights and Nitrogen Uptake from 2016 Demonstration by Installation Size Class

	Estimated Dry Tissue Weight (g)						
Description	Initial	Final	Increase	Estimated N Uptake per Oyster (g)	Estimated Initial # of Oysters	Estimated Mortality	Total Estimated Uptake (kg)
Y1	0.055	1.04	0.985	0.103	60,000	6.6%	5.80
Falmouth Small	0.055	0.79	0.74	0.076	11,700	6.6%	0.83
Y2	0.562	2.12	1.56	0.160	126,690	6.6%	19.0
Totals					198,390	6.6%	25.6

There were three distinct populations of oysters deployed during the 2016 Demonstration (refer to Section 6B). As described in Section 7B, the total nitrogen uptake of the oysters in the 2016 Demonstration project was estimated as 25.9 kilograms (kg). This value was calculated using the data in Table 2 and Figure 14 in the Technical Memorandum. As an example of how to estimate total nitrogen removal, the relevant data are summarized in Table TMO-1.

Using the information in Table TMO-1, the Y1 oysters used for the 2016 Demonstration were deployed at a calculated initial average dry tissue weight of 0.055 g and ended the growing season with an estimated dry tissue weight of 1.04 g, for an increase of 0.985g per individual. The nitrogen uptake rate is 10.5 percent of this value, or 0.103 g per oyster (refer to Table 2 in Section 7B). To calculate the total nitrogen uptake in living oysters at the end of the season, the initial population of 60,000 is reduced by the measured mortality of 6.6 percent and the resulting 56,040 individuals are multiplied by the 0.103 g/individual to obtain a total uptake of 5.80 kg (dry weight) over the course of the 2016 Demonstration. Note that the total of 25.9 kg in Figure 12 includes the 25.6 kg shown in Table TMO-1 as well as 0.3 kg of nitrogen contained in the shells of removed dead oysters.

The ‘Falmouth Small’ population in Table TMO-1 does not represent a class of oysters that would be used in a full scale program, so in the interest of clarity the data were not displayed in the Figures in Section 7. However, the contribution of these oysters was included in the calculation of nitrogen removal for the 2016 Demonstration.

Table TMO-2: Projections a for Full Season, Full Scale Program

	Dry Tissue Weight (g)						
Description**	Initial	Final	Increase	N Uptake per Oyster (g)	Initial # of Oysters	Mortality	Total Annual Uptake (kg)
Y1 for Y1A,B,C,D	0.02	1.04	1.02	0.107	2,120,000	15%	193
Y1 for Y2E,F,G,H	0.02	0.49	0.47	0.049	*	*	*
Y2 for Y2E,F,G,H	0.49	2.05	1.56	0.164	*	*	*
Y2 for Y2G	0.49	2.05	1.56	0.164	1,330,000	15%	185

*values vary according to Scenario'

**refer to Table 3 in Section 11 for a description of the eight scenarios considered.

Two management approaches are presented in Section 11 for full-scale implementation: (1) grow oysters through the Town’s municipal propagation program, and (2) establish a private aquaculture license site (grant). Four scenarios for implementation under each management approach were considered. These are listed in Section 11 (see Table 3). The footprint over the growing season and economics associated with these different scenarios are also provided in Section 11.

Table TMO-2 displays the projected total annual update of nitrogen for eight different full scale scenarios that were considered. The assumptions for the projections shown in Table TMO-2 are based on dry tissue weights expected for a full growing season, which are shown in Figure 13 of Section 7B. Note that the assumed mortality was based on feedback from Orleans growers about their experience, and is much higher than the actual mortality experienced during the 2016 Demonstration. As noted above, the estimated total annual uptake in Table TMO- 2 does not include denitrification, which was separately calculated as explained in Section 10E and Figure 36; the total projected nitrogen removal is the sum of uptake into biomass (tissue and shell) and enhanced denitrification in the sediment. Data from the 2016 Demonstration indicate that Y1 oysters grown in Lonnie’s Pond will have a larger end weight than Y1 oysters grown at other sites. The long-term implementation projections for Y2-oyster scenarios are made assuming intermediate seed similar to what was used in the 2016 Demonstration, not oysters that would be as big as those from Lonnie’s Pond.

The weights and sizes of the oysters recommended for the 2017 Demonstration are also different from what would be used in a full-scale implementation, because the 2017 Demonstration is designed to provide data for validating all eight of the proposed long-term Scenarios, while a full scale implementation would use only one approach. It is important to understand the above considerations when evaluating the report. All the assumptions used for estimating nitrogen-removal for full-scale implementation are clearly enumerated in Sections 5B, 8E and 9, and are believed to be reasonable and conservative.

If the Town opens Lonnie's Pond to a commercial grower, then the expense associated with the removal of nitrogen would be limited to administrative oversight of the private grower and is estimated to be on the order of $11 per kilogram of nitrogen. In this scenario, the Town would not need to expand its workforce or facilities to accommodate the activities associated with growing and managing oysters. A threshold consideration for the commercial grower approach is whether an aquaculture license site (also known as a grant) could be permitted in Lonnie's Pond and whether a commercial propagation permit would be granted for this location. Key permitting considerations include granting of this site for aquaculture at the local level and the determination of bottom condition. If bottom conditions are found to be productive for shellfish in this section of Lonnie's Pond, a rotational system that moves the one-acre growing area within Lonnie's Pond each year might be employed (if allowed by MA Division of Marine Fisheries [DMF]) so that no one area is permanently removed from the public resource. Detailed costs for all four options are provided in Section 11.

Seven of the evaluated scenarios meet TMDL and MEP annual targets for Lonnie's Pond. The Town-managed option of maintaining an ongoing population of older oysters inside Lonnie's Pond does not meet the nitrogen-removal target when the available space for the floating bags is limited to one acre, due to the space needed to hold these larger oysters. Section 11 also discusses options for using these larger oysters outside Lonnie's Pond to help test the feasibility of establishing long-term oyster beds in areas with suitably hard sediment types.

The recommended plan for the second year of the demonstration program (2017 Demonstration) is also presented in this Technical Memorandum. The weights and sizes of the oysters to be used in the 2017 Demonstration are different from the weights and sizes of the oysters reported for the partial growing season of the 2016 Demonstration because the plan is to start at the beginning of the growing season in early May, not late June. The second year of this demonstration will further refine the long-term implementation plan and continue to collect data that are needed to obtain regulatory approvals for the use of shellfish aquaculture to achieve nitrogen goals. There are several key parameters related to full-scale implementation that will be evaluated during the second year of the demonstration, including:

- Nitrogen-removal rates through growth and denitrification as a function of time to ensure that TMDL requirements can be met during the critical impairment period of July and August; and
- Suitable growth rates across the full project area with the required stocking densities.

The details of the second-year demonstration program that were planned as of the original date of this memorandum in spring 2017 are presented in Section 12. It should be noted that since that time, some of the plans for the second year were curtailed due to budget constraints. The implemented year two demonstration project will be fully documented in the year 2 report. This demonstration for year two will provide evidence for achievable rates of nitrogen removal through uptake during oyster growth as well as nitrogen removal through enhanced denitrification. Several aspects of denitrification will be measured, including multi-season rates as well as differences between Y1 and Y2 oysters. Other impacts to sediment quality will also be measured.

3. Overall Purpose and Rationale for Shellfish Demonstrations

A. Overall Purpose

The overall purpose of the Town's shellfish demonstration projects is to monitor the nitrogen-removal impacts of both *Crassostrea virginica* (oysters) and *Mercenaria mercenaria* (hard clams) and utilize these measured results to develop full-scale plans for shellfish cultivation that help meet regulatory standards for nitrogen.

The Town must achieve specific, quantitative goals for removing nitrogen within each of its subwatersheds to meet the Total Maximum Daily Load (TMDL) standards. A TMDL establishes the maximum amount of a pollutant (nitrogen) allowed in a waterbody and serves as the starting point or planning tool for restoring water quality. Therefore, by meeting TMDL standards the ultimate goal is a healthy ecosystem with reductions of murky water and mucky bottoms. The design of a full-scale shellfish project for a particular waterbody to meet a prescribed nitrogen-removal target must be based on certain site-specific values, such as total annual growth in shellfish biomass and enhanced denitrification rates. The size and spatial configuration of a full-scale project are directly related to the values measured through these demonstrations.

Demonstrations are designed to:

- Evaluate the efficacy of oysters and quahogs in achieving reduced nitrogen concentrations within the Town's impaired waters;
- Determine the most advantageous approaches for growing the quantities of shellfish prescribed to meet nitrogen removal goals; and
- Develop realistic cost estimates for the preferred approaches to growing shellfish to meet nitrogen removal goals in specific waterbodies.

These demonstrations will help determine the role of shellfish in the overall strategy for reducing the nitrogen loads within the Town's impaired estuaries as was presented in the Town's ACWMP (2017).

B. Rationale for Use of Oysters

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.

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.

As oysters filter nitrogen-containing particles out of the water column, some of this nitrogen is reformulated into feces and pseudofeces (biodeposits). These feces and pseudofeces remove nitrogen from the water column and then become part of the sediment. The pathway by which additional nitrogen is removed that relates to biodeposition is called denitrification. Feces are the by-products of metabolized particles, while pseudofeces are particles that are not ingested or metabolized by an oyster. These particles are collected on the oyster's gills and coated in mucus. Biodeposits are heavier than water and rapidly sink to the sediment, where the organic nitrogen that was contained in the original particles is buried. Biodeposition is proportional to the weight of an oyster as well as the temperature of the system and the total suspended solids in the water column (Powell et al., 1992; Sisson et al, 2011). The long-term fate of the nitrogen in these biodeposits is difficult to assess, and will vary depending on the bottom chemistry and sediment type. At this time, it is not possible to state conclusively how much nitrogen is permanently removed from the water column by biodeposition. Therefore, it is assumed that there is no long-term removal of nitrogen from the estuary by biodeposition alone.

Biodeposits introduce materials to the bottom sediment that can stimulate additional, permanent removal of nitrogen from these sediments in the form of nitrogen gas, effectively removing it from the estuarine ecosystem permanently. Nitrogen removal within benthic sediment is a process driven by microbes that either produce nitrates (nitrifying bacteria) or metabolize nitrates (denitrifying bacteria). First, bacteria that prefer oxygen-rich environments produce NO_3 (nitrates) as one metabolic by-product. Then, bacteria that prefer oxygen-depleted environments metabolize nitrates, using the oxygen and releasing the nitrogen as a gas. Denitrification within the sediment can be increased by oyster biodeposits that feed a population of these bacteria. This biological process that takes place in the sediment is similar to the process used in advanced septic systems and Permeable Reactive Barriers to remove nitrogen. However, addition of too much organic matter (biodeposits) can lead to anoxic conditions under which denitrification will decrease and ultimately reach zero. Burial is a third, negligible pathway for nitrogen-removal. Because burial is assumed to be negligible, it was not measured in this Demonstration project.

Inorganic nitrogen (primarily ammonium) is excreted by the oysters to the water column, where it is typically incorporated in organic matter, some of which becomes food for organisms including oysters. Oysters have not imported this nitrogen into the water column; they have simply taken it from one organic material already in the water column to another organic material in the water column. When the nitrogen contained in ammonium excreted by oysters returns to another organic material in the water column, there has been no net change in the ecosystem from a nitrogen balance perspective.

The Cape Cod Cooperative Extension conducted an extensive study on the nitrogen content of both oysters and quahogs (Reitsma, Murphy and Franklin 2014). To accomplish this research, market sized oysters and quahogs were collected throughout Cape Cod and the mass of nitrogen sequestered in the shell and soft tissue was analyzed. The average size of the animals involved in the study was approximately 3.2-inch/2.3-ounce (84mm/66g). Key findings include:

- The nitrogen content is typically 0.21 percent of the shell dry weight and 7.95 percent of the tissue dry weight per adult oyster grown in off-bottom gear on Cape Cod. These values are consistent with those reported from the Chesapeake at 0.17 percent of the shell dry weight and 7.28 percent of the tissue dry weight per adult oyster (Higgins et al. 2011);
- The average nitrogen content of market size oysters grown in off-bottom gear on Cape Cod was approximately 0.42 percent of their total live weight. For a 3.2-inch (84mm) oyster weighing 2.3 ounce (66 g), this would equal 0.01 ounce (0.282g);
- The average nitrogen content in the soft tissue of market size oysters grown in off-bottom gear in Pleasant Bay was approximately 0.49 percent of their live weight. For a 3.1-inch (79mm) oyster weighing 2.1 ounce (60 g) wet, this would be 0.01 ounce (0.3g);

- The nitrogen densities of oysters and quahogs is similar, but because adult oysters are larger and heavier than littleneck sized quahogs (average shell length 2.2-inch [56.1mm], 31.2g shell weight and 2.22g dry tissue weight), removing a single oyster will extract more nitrogen than removing a single littleneck sized quahog;
- Both oysters and quahogs have a higher nitrogen density in the fall, partly because the mass of the soft tissue at this time of year is a larger fraction of the animal's total weight; and
- The nitrogen content of oysters is impacted by the type of growing system used, which primarily affects shell thickness. Wild oysters and oysters grown on the bottom had higher nitrogen content than cultured oysters grown off the bottom.

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). In some locations, denitrification is minimally increased by oysters (Higgins et al. 2013). In other studies, denitrification is significantly increased (Humphries et al. 2016; Kellogg et al. 2013). Some studies have shown that the nitrogen removed by the increase in denitrification caused by oysters is equal to the mass of nitrogen contained in the oyster (Newell et al. 2005; Kellogg et al 2013). Sediment chemistry and dissolved oxygen concentration at the sediment surface, as well as overall oyster biomass density are key factors that impact the likelihood that oysters will enhance denitrification (Burkholder et al. 2011).

There is a strong scientific basis for using oyster cultivation to decrease water column nitrogen concentration and improve water clarity. Demonstration projects focusing on oysters were seen as an important first step in order to validate the quantities of nitrogen removed through uptake in the body of the oyster and enhanced denitrification in local waters. These projects provide the field-verified basis for including oyster cultivation in the Town's wastewater plans.

C. Quahogs for Water Quality Improvements

Quahogs also uptake nitrogen (Reitsma, Murphy and Franklin 2014), and have been shown to modestly enhance denitrification rates (Nizzoli et al. 2006). A separate demonstration project is currently in process for Town Cove which is focused on enhancing municipal propagation of quahogs. A full discussion of the impacts of quahogs on water and sediment quality will be prepared as part of the Technical Memorandum describing enhanced quahog propagation demonstration in Town Cove. This Technical Memorandum is scheduled for completion after the quahog survey is completed by SMAST, where they will use local harvesters to accomplish the survey with oversight from SMAST staff.

There are several reasons why quahogs were not selected for the 2016 Demonstration in Lonnie's Pond:

- Oysters are an important species commercially and aquaculture leaseholders in Pleasant Bay are mainly growing oysters, so this species needs to be evaluated for its nitrogen-removal ability;
- Quahogs grow more slowly than oysters, so the water quality impacts would not be as easily measured in the first year of a demonstration project;
- Lonnie's Pond is a good experimental site for measuring the impact of oyster aquaculture on both water quality and sediment denitrification rates;
- The Massachusetts Department of Environmental Protection (MassDEP) must approve shellfish as part of the Town's Amended Comprehensive Wastewater Management Plan (ACWMP). The size, quantity and survival of oysters grown in gear can be directly measured. From a regulatory perspective, this enables consistent and repeatable implementation of oyster biomass to meet water quality goals. Quahogs are bottom-planted and buried, and are known to move, making a demonstration more difficult to monitor and evaluate;
- Quahogs have a smaller mass of sequestered nitrogen per animal than oysters at harvestable size;

- The nitrogen sequestered in shellfish is not removed from the system until the animal is removed from the system. Harvesting quahogs is very labor intensive, whereas oysters grown in gear are easily harvested. This is a consideration for full-scale, long-term implementation as well as during the demonstration phase.
- The effects of quahog propagation have not been studied to the extent of oysters in terms of their impact on water and sediment quality. The science of oyster interactions in eutrophic systems has been well-characterized; and
- Town Cove is a focus area for municipal propagation and commercial and recreational harvest of quahogs, therefore a quahog demonstration project is more appropriately being planned for this area.

Based on the findings of these projects, both oysters and quahogs could become a cost-effective part of the solution for reducing the nitrogen that is polluting the Town's estuaries.

4. Specific Purpose of the Lonnie's Pond Oyster Aquaculture Demonstration Project (Lonnie's Pond demonstration)

The first year of this three-year demonstration was designed to confirm the suitability of Lonnie's Pond for growing oysters, and provide preliminary estimates of the amount of nitrogen that can be removed by both uptake during oyster growth as well as enhanced denitrification.

The 2016 Demonstration in Lonnie's Pond was designed to:

- Quantify the total oyster biomass needed in Lonnie's Pond to fully achieve water quality goals. To make this determination, several parameters were monitored and measured:
  - Sediment denitrification enhancement attributable to oysters in Lonnie's Pond;
  - Growth of oysters in Lonnie's pond (grams/season);
  - Nitrogen content in the shell and soft tissue of oysters that reach harvestable size;
  - Water quality changes due to filter feeding by introduced oysters; and
  - Oyster impacts on other sediment and benthic processes.
- Develop and test a system for growing oysters that optimizes the use of space and addresses the known predation and soft sediment issues in Lonnie's Pond;
- Develop and test a system for overwintering oysters that addresses the known predation and soft sediment issues in Lonnie's Pond;
- Evaluate costs associated with oyster aquaculture at this site; and
- Assess use conflicts.

One of the most important reasons for the 2016 Demonstration was to determine whether oysters will increase ambient sediment denitrification rates. Because of the site-specific nature of denitrification, this parameter must be measured directly in Lonnie's Pond. Based on published studies (Humphries et al. 2016; Kellogg et al. 2013, Newell et al. 2005), it was believed that denitrification may reduce by half the actual quantity of oysters needed to meet the regulatory requirements for nitrogen-removal in Lonnie's Pond. If denitrification can reduce the overall biomass of oysters being grown annually, the size of the growing area, as well as the scale and costs of the long-term program are significantly reduced.

During the first year of the Lonnie's Pond demonstration, the size and density of oysters installed was specified to allow denitrification enhancement that is attributable to oysters to be measured. The data on denitrification are essential to accurately calculate the minimum biomass of oysters needed for full-scale implementation that will achieve the nitrogen reduction target for Lonnie's Pond. Denitrification and water quality monitoring results are discussed in the SMAST technical report (Howes et al. 2017). These monitoring results are summarized in Section 10.

A practical and cost-effective long-term program for Lonnie's Pond also needs to be based on actual growth and mortality rates observed at the site. Section 7 includes a detailed discussion of oyster growth. Nitrogen uptake as well as biodeposition rates that enhance denitrification are directly related to oyster dry tissue weight. For different starting size oysters, the amount of growth in terms of weight over a season required quantification. Like denitrification rates, the amount of biomass added over a growing season and nitrogen-content of oysters are site-specific, though increased biomass and nitrogen content is very similar throughout Cape Cod estuaries. It was also expected that localized changes in water quality parameters such as turbidity and particulate organic nitrogen concentrations would be measurable. These effects also impact the sizing of full-scale implementation.

Field-verification of implementation logistics and costs of oyster aquaculture *for the purposes of nitrogen-removal* was also evaluated during the 2016 Demonstration. The actual time needed for operations and maintenance as well as for overwintering were monitored. A specialized system needed to be developed if the oysters were to be overwintered in Lonnie's Pond. Equipment was designed and installed so that the feasibility of this design could be tested. Keeping oysters over several growing seasons is very different from the approach used in private aquaculture. Commercial growers usually keep first-year oysters for grow-out to select size in the second growing season or mobilize early to sell petite oysters after the first year of growth.

The first-year project in Lonnie's Pond also enabled neighbor reactions to be reviewed. This field-demonstration allowed people to become familiar with the details of oyster cultivation. Stakeholders experienced the visual and other impacts of growing oysters at this location firsthand, so their perspectives come from actual experiences.

In sum, the 2016 Demonstration was intended to provide site-specific data for key parameters of a long-term program, such as:

- Increase in oyster biomass and nitrogen uptake associated with this growth;
- Oyster-enhanced denitrification rates;
- Costs and feasibility of overwintering; and
- Stakeholder perspectives.

The projections for both the long-term implementation strategy as well as the 2017 Demonstration are based on the findings of the 2016 Demonstration. Eight different scenarios for full-scale implementation using two different management approaches are presented in Section 11. One management approach grows oysters through the Town's municipal propagation program, and the other establishes a private aquaculture license site (grant) and allows a grower to carry out the program. Verifying key components of these long-term implementation strategies guides the work proposed for the second year of the demonstration. The proposed plan for the second year of the Lonnie's Pond demonstration is presented in Section 12.

The purpose of the second year of the demonstration is to:

- Assess nitrogen removal rates through growth and denitrification as a function of time to ensure that TMDL requirements can be met; and
- Confirm that suitable growth rates are achieved across the full project area with the required stocking densities.

The purpose of the third year of the demonstration is to transition to an ongoing full scale program that is operated either by the Town or by a commercial grower.

It is important to remember that the MassDEP has stated that three years of data are needed to fully quantify the extent to which oysters can make a measurable difference in water quality parameters for regulatory purposes. MassDEP must approve shellfish cultivation as part of the Town's ACWMP. The three-year Lonnie's Pond demonstration was designed to provide the data needed to support the use of shellfish within the Town's ACWMP. During 2017 it is anticipated that discussions with regulatory agencies will allow for any necessary refinements for data collection in order to gain approval.

Although nitrogen removal rates from oyster cultivation is site-specific, the results from Lonnie's Pond are expected to be applicable to other impaired sections of Pleasant Bay because the conditions found there are similar to other areas such as Meetinghouse Pond, The River, Arey's Pond, Namequoit River and Paw Wah Pond.

5. Background: Planning the Lonnie's Pond Oyster Aquaculture Demonstration Project

A. Selection of Lonnie's Pond as the Demonstration Location

Lonnie's Pond was selected as the first demonstration site using a three-phase process. The first phase of site selection involved a detailed analysis of seven sites that were originally presented during the Town-sponsored June 2015 Shellfish Forum where scientists, engineers, growers and regulators met to review the shellfish component of the Orleans Consensus Agreement. Review of these seven sites was accomplished by the AECOM Technical Team that included representatives from AECOM, Science Wares, Biomimicry New England/UMass Boston, and Beach Point Oysters.

To facilitate a systematic and objective evaluation of each of the potential demonstration sites, a decision support tool, called a Site Selection Matrix was developed. This Site Selection Matrix includes a number of criteria for Site Suitability, Permitting, and Project Evaluation. These criteria were first presented as part of the process of developing the Orleans Consensus Plan. The Shellfish Technical Team refined the criteria after reviewing site-specific data to include the following:

1) Site Suitability
   - Available Growing Area/Adequacy of Acreage;
   - Water Quality Indicators;
   - Disease/Predation;
   - Ease of Access;
   - Aesthetic Impacts;
   - Representativeness of the Site (Transferability);
   - Use Conflicts; and
   - Ability to Co-locate with other Non-Traditional Technologies.
2) Permitting
   - Abutter Compatibility;
   - Wild Harvest Conflicts (DMF); and
   - Grow-Out to Harvest Size Allowed (DMF).
3) Permittability
   - Project Evaluation;
   - Expected Survival; and
   - Overall Likelihood of Monitoring Plan to Yield Quantified Results.

Site Suitability criteria address the environmental, land use and implementation characteristics of each proposed demonstration location. Permitting criteria assess the regulatory issues related to each proposed demonstration location. Project evaluation criteria estimate the likelihood of obtaining meaningful results from a proposed demonstration site. Other/Overriding Considerations refer to any threshold issue that precludes a demonstration at a given site. The results of the first phase analysis for demonstration site selection are documented in the Technical Memorandum on Final Site Characterization and Evaluation for Aquaculture/Shellfish Propagation (evaluation criteria and ranking) dated March 13, 2016 and is available on the Town's website.

The second phase of site selection began with a peer review of the Site Characterization Technical Memorandum, involving review and comments from several outside experts including:

- Diane Murphy, Barnstable County Cooperative Extension;
- Josh Reitsma, Barnstable County Cooperative Extension;
- Sandy Macfarlane, former Orleans Shellfish Constable;
- Henry Lind, former Eastham Shellfish Constable;
- Chatham Shellfish Propagation Department; and
- Dave Slack, Orleans oyster grower.

Written comments from these outside experts and responses from the Shellfish Technical Team are included in Appendix A of the Technical Memorandum. After this detailed peer review of the Site Selection Technical Memorandum, the third phase of site selection included discussion of potential sites with staff from SMAST and the Orleans Shellfish Working Group (SWG). The SWG consists of the Shellfish Constable/Harbormaster and representatives from the Shellfish and Waterways Advisory Committee, Orleans Marine and Freshwater Quality Task Force, Orleans Pond Coalition, Citizens Peer Review Committee, and Orleans Water Alliance. The SWG recommended that Lonnie's Pond be re-evaluated for the first shellfish demonstration location when floating constructed wetlands were no longer being considered as an option for 2016. Lonnie's Pond was then reassessed for a shellfish demonstration project. The technical review of Lonnie's Pond included:

- Studying the information on measured tidal exchange and benthic conditions that are presented in the Massachusetts Estuaries Project Report for Pleasant Bay (Howes et al. 2006) and reviewing annual water quality data sets provided by SMAST;
- Evaluating the report from Cape Cod Cooperative Extension (Clark 2007) documenting a project in Lonnie's Pond where remote set oysters were grown and bottom planted; and
- Discussing suitability and monitoring of Lonnie's Pond for an oyster demonstration with SMAST staff.

Lonnie's Pond was found to be the most suitable for purposes of a demonstration project because growing conditions are favorable and the site's configuration is the most likely to result in meaningful monitoring results for both water quality and sediment denitrification. In terms of tidal exchange, the local residence time is 1.1 days, meaning that the equivalent of the entire water volume moves out of Lonnie's in a little over one day. Freshwater inputs (groundwater and surface water) were also included in the salinity model of the Pleasant Bay estuary system by SMAST. The site-specific input for Lonnie's Pond was calculated as 1.32 ft^3/sec of flow (Howes et al 2006). Table 1 shows relevant water quality data provided annually to the Town by SMAST indicating suitability of Lonnie's Pond for oyster aquaculture. Averages computed from 2000-2014 samples analyzed for SMAST Sample ID PBA-15S.

Specific site characteristics that aid in monitoring success include the ease of access, the distinctive and measurable hydrological inputs and outputs, the overall shallow pond depths that are like other areas within Little Pleasant Bay, and the potential lack of user conflict.

Table 1 - Water Quality Data from Lonnie's Pond (Station PBA-15, Surface, Summer Sampling)

Parameter	Minimum Requirement	Lonnie's Pond Average Condition
Temperature (°C)	0	23.5 ± 2.4
Salinity (ppt)	20	28.9 ± 1.8
Dissolved Oxygen - DO (mg/L)	4	5.1 ±0.9
Particulate Organic Nitrogen (ug/L)	2	10.4 ± 3.8
Chlorophyl *a* (ug/L)	2	6.4 ± 4.5

Key reasons for selecting Lonnie's Pond as the first demonstration location were:

- Water quality is most degraded in terminal ponds, so a method of introducing non-traditional technologies in these areas is needed; Lonnie's Pond is one of the most degraded terminal ponds;
- Ample tidal flushing and exchange;
- Adequate food supply for growing oysters;
- Area is available for both demonstration and full scale implementation;
- Sediment type is expected to be conducive to denitrification;
- Ability to monitor inputs from the channel and herring run effectively;
- Ability to monitor impacts of oysters effectively; and
- Replicability to other sites (other terminal ponds are impaired and will also require floating gear due to soft bottom conditions).

Through this three-phase process, Lonnie's Pond was determined to be the best location to demonstrate the nitrogen-removing benefits of oyster propagation. Documentation for the Lonnie's Pond site evaluation is contained in both the Technical Memorandum on Shellfish Cultivation – Preliminary Engineering Design and Work Plan for Preferred Site(s) dated May 4, 2016 and the Technical Memorandum for Site Characterization and Evaluation for Floating Constructed Wetlands dated May 25, 2016, both of which are available on the Town's website.

B. Design of Lonnie's Pond Demonstration

The Lonnie's Pond demonstration was designed to meet two key project goals:

- Ability to monitor water and sediment impacts from oysters; and
- Ability to replicate growing system in other impaired waters.

Two possible field locations and a design for the Lonnie's Pond demonstration are detailed in the Technical Memorandum on Shellfish Cultivation - Preliminary Engineering Design and Work Plan for Preferred Site(s) dated May 4, 2016. This design was finalized by a technical team with expertise in engineering, oyster aquaculture and estuary science. This review occurred by:

- AECOM's Shellfish Technical Team;
- SMAST; and
- The SWG.

To select the final location within Lonnie's Pond and finalize the layout for the demonstration, the Technical Team, SWG and SMAST reviewed pond circulation data that was collected in June 2016 using an Acoustic Doppler Current Profiler (ADCP). These data showed that the incoming tide enters the pond and water fans out evenly across the pond area. Since the results of the SMAST analysis indicated that circulation patterns were not a limiting factor in locating the oyster field, the southern end of Lonnie's Pond was selected because this location was more advantageous in terms of access for operating and maintaining the field and visibility for patrolling. In addition, the northern end of Lonnie's Pond was identified as a preferred area for wild harvesting of quahogs. Siting the oyster field in the southern end of the pond ensured that quahog harvesting in the northern section of Lonnie's Pond would not be affected by this demonstration project.

1) Choice of Floating Gear

The process used to determine the choice of growing system and determine the layout of the demonstration included detailed review of current practices used for oyster farming on Cape Cod as well as consultation with: local growers, regional suppliers of shellfish gear, and the Cape Cod Cooperative Extension. After considering the specific attributes of the Lonnie's Pond location as well as the monitoring and replicability goals of the project, floating bags were determined to be the best choice for gear.

Growing oysters in gear-based systems has several advantages, including:

- Protection from predators;
- Ability to farm areas where the sediment is soft and deep; and
- Creation of a contained oyster population that can be readily maintained, measured and harvested.

Floating bags are commonly used by private aquaculture operations because they have been proven to allow oysters to grow quickly with high survival rates and uniform shape. Additional advantages of floating bags include the high density of oysters that can be grown in a given area relative to other systems, the low-profile aesthetics of floating bags and the ability to maintain a field in a way that bottom sediment is undisturbed (important during the demonstration phase when denitrification is being measured).

Floating gear also mitigates two significant factors prevalent in Pleasant Bay and Town Cove: predation and muck. Pleasant Bay has a significant population of oyster drills (*Urosalpinx cinerea*), a virulent predator. A significant population of oyster drills negatively impacted a 2006 through 2007 project in Lonnie's Pond where oyster remote set was grown in gear for one season and then was bottom-planted. The results of this project were documented in a report for the Cape Cod Cooperative Extension (Clark, 2007). The design of the current demonstration builds on the findings of this early project. In addition, growers in Little Pleasant Bay regularly encounter drills in their overwintered oysters. Floating bags are an effective system for protecting oysters from drills. Many areas within Pleasant Bay, including the terminal ponds (Lonnie's, Areys, Meetinghouse and PawWah) have fine-grained, soft sediment that experience periods of low dissolved oxygen, which precludes bottom-planting and installing trays located on the bottom. Floating bags provide a practical means of keeping oysters off this mucky bottom.

Oyster aquaculture in gear also creates a controlled and repeatable system for introducing, maintaining and measuring the amount of shellfish in a specific location. This consistency in implementation is important as the Town seeks to use shellfish in the context of regulatory compliance with the Total Maximum Daily Load (TMDL) requirements for nitrogen in its impaired waters. Regulatory approval is required if the Town includes shellfish as part of implementing its ACWMP. Gear-based oyster aquaculture creates a growing approach that is more likely to meet with regulatory approval because it can be duplicated, monitored and evaluated effectively.

The use of floating bags for growing oysters that is being demonstrated in Lonnie's Pond has wide applicability for other waterbodies in Orleans.

2) Choice of Second Year Oysters

The goal of the 2016 Demonstration was to monitor the impacts of oysters on sediment denitrification and water column nitrogen concentrations. The purpose of quantifying these parameters was to help in determining the biomass of oysters needed to fully remove the nitrogen reduction target for Lonnie's Pond. Therefore, a key consideration for the design of the first-year demonstration was to facilitate the assessment of two pathways for nitrogen removal.

The first pathway is through uptake in the shell and tissue of oysters. Once the oysters are removed, the nitrogen incorporated into oyster growth leaves the system. This pathway can be measured directly by tracking the change in biomass of oysters in the field over the growing season. The total increase in oyster biomass over a growing season is directly related to the total amount of nitrogen they sequester. The reported value for the nitrogen content of oysters cultured off-bottom in Pleasant Bay averages 10.9 percent of dry tissue weight (Reitsma, Murphy and Franklin 2014, Reitsma et al. 2017).

The second pathway involves removal of nitrogen by sediment denitrification. Because the existing sediment already cycles nitrogen, the challenge is to assess the extent to which oysters increase the denitrification pathway. For a given site, the effect of oysters on the rate of denitrification depends on several factors including sediment chemistry and organic matter content as well as dissolved oxygen concentrations. In order to measure enhanced denitrification caused by oysters, an appropriate amount of feces and pseudofeces must be deposited to the sediment. It was expected that a larger mass of biodeposition would have a more measurable effect on denitrification. The mass of biodeposits is proportional to the weight of the oysters as well as the temperature of the system and the concentration of total suspended solids in the water column (Powell et al., 1992; Sisson et al, 2011). The Virginia Institute of Marine Science developed a model for the Army Corps of Engineers (Sisson et al. 2011) that suggests the biodeposition rate of oysters (BD) can be modeled as follows:

BD = $\alpha * T * TSS^{\beta} * e^{-\delta * TSS}$ mg per gram dry tissue per hour, where

TSS is the total suspended solids in mg/L

T is temperature is in degrees C.

α = a parameter that determines peak biodeposition rate proportional to temperature

β = a parameter that determines both peak biodeposition rate and the position of the peak as a positive function of seston concentration (seston are the minute materials moving in water including both living and nonliving matter)

δ = a parameter that determines both peak biodeposition rate and the position of the peak as an inverse function of seston concentration.

The parameters α, β, δ as well as TSS and T are characteristic of the system being modeled and provide an estimate of the expected biodeposition rate as a function of the tissue mass of the oysters in the field.

This model accounts for three notable features of biodeposition rates observed from studies cited in Sisson's publication:

- A threshold effect of low seston concentration upon filtration;
- A clogging effect of high seston concentration upon filtration; and
- A positive correlation between filtration and temperature.

Biodeposition is proportional to the dry tissue weight. Therefore to maximize the amount of biodeposition that takes place for a given volume of gear deployed, there is a clear benefit to deploying oysters with a large initial weight. It is possible to obtain either first or second year seed at the beginning of the season. First year seed (Y1) can be purchased as small or medium, starting at sizes of approximately 3mm or 12mm respectively but have a dry tissue weight of only a few or tens of milligrams each. Second year seed (Y2) is considered an intermediate size of approximately 65mm and would typically have a dry tissue weight of 0.5g. Figure 1 shows projected dry tissue weights for typical Y1 and Y2 oysters leading up to and including the critical impairment period of July and August.

The values in Figure 1 can be combined with Sisson's biodeposition model (Sisson et al, 2011) to predict the mass of feces and pseudofeces deposited as the oysters grow as a function of oyster dry weight. As shown in Figure 2, the Y2 oysters are expected to produce a significantly greater accumulation of biodeposits, even at lower stocking densities per bag. At the beginning of the critical impairment period, approximately 5.8-times more biodeposits are deposited by Y2 oysters than what would have been accumulated if Y1 oysters were to have been deployed. Note that the exact amounts of biodeposition will depend on the values of α, β, δ used in the model, but for a wide range of values that have been used to model actual conditions, the trend and magnitude of the Y2/Y1 ratio remains very close to what is shown in Figure 2.

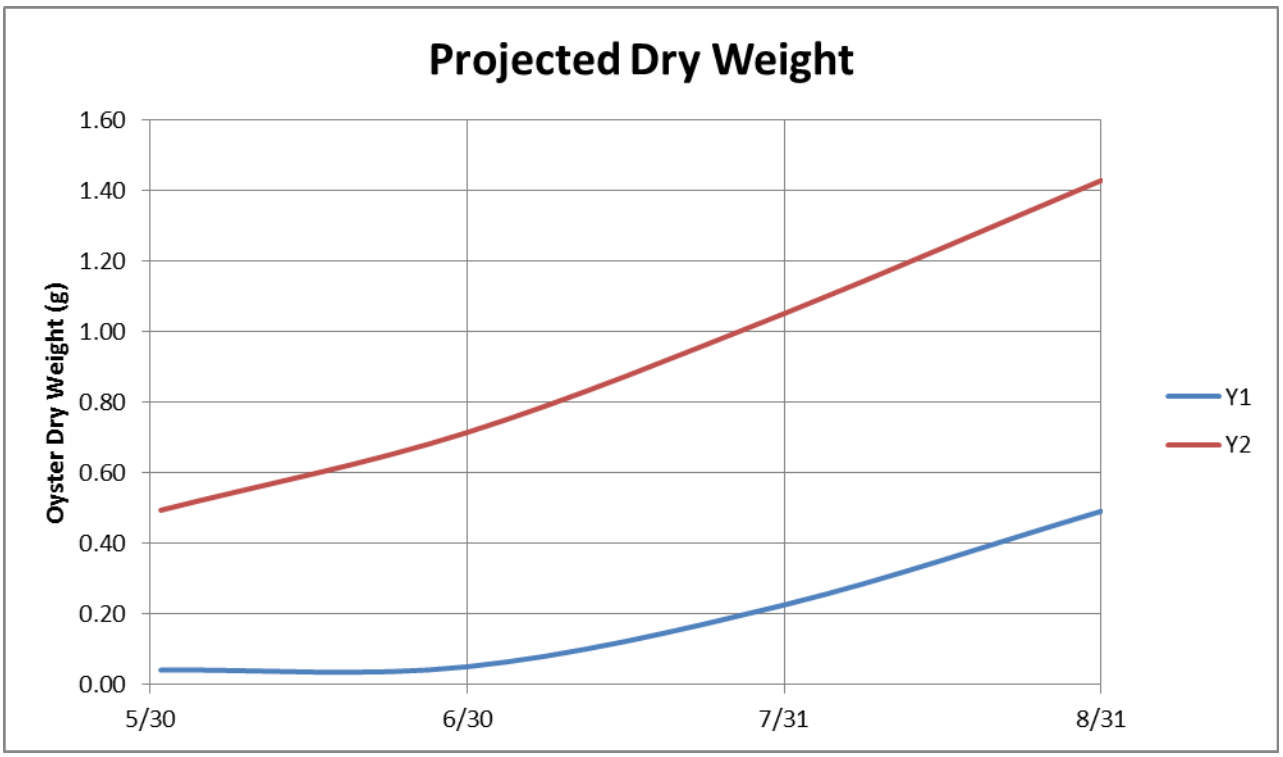

Figure 1 - Projected Dry Weight Starting from either Year 1 or Year 2 Oysters

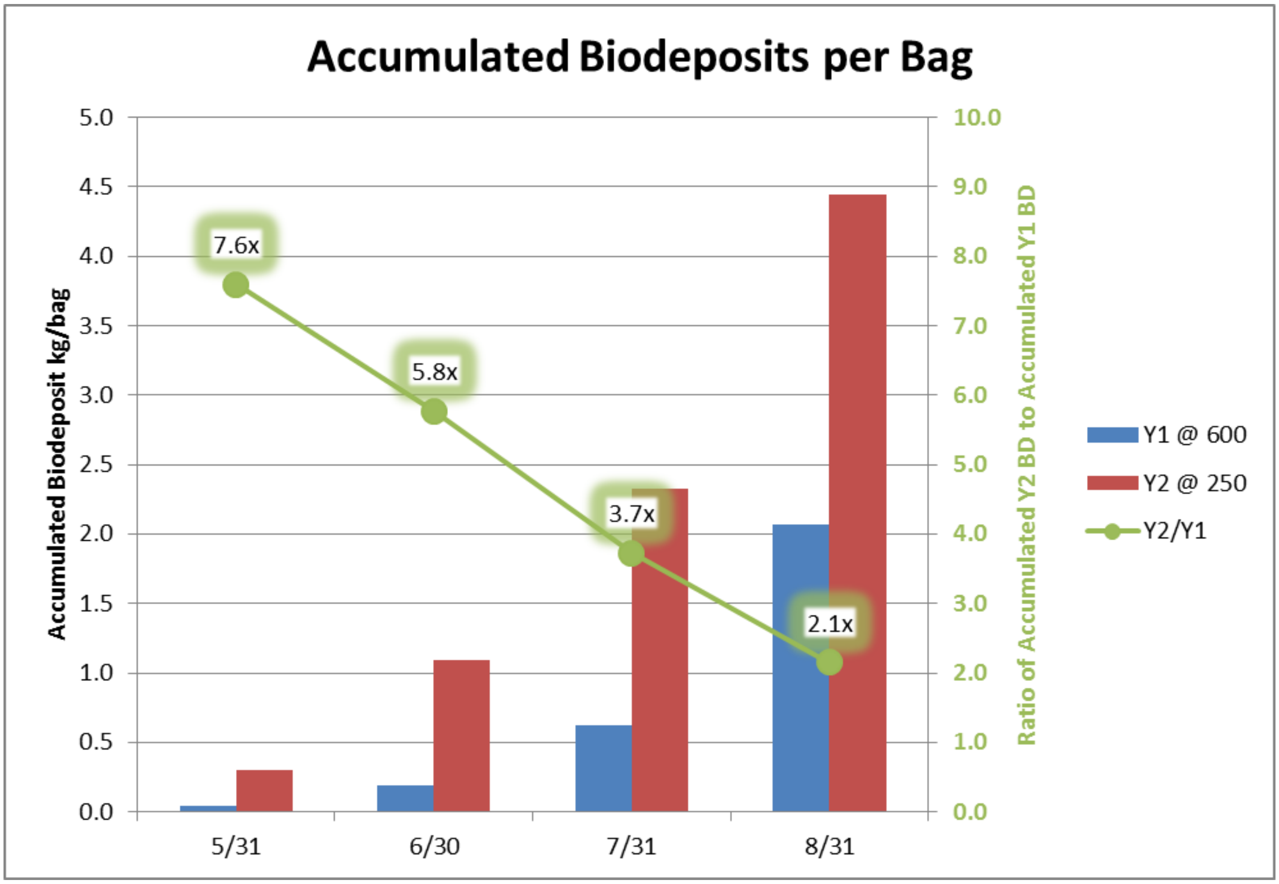

Figure 2 - Comparison of Accumulated Biodeposits for First Year (Y1) and Second Year (Y2) Oysters

3) Choice of Field Configuration

Proper design of the actual growing area is important for monitoring any enhancement to sediment denitrification caused by oyster aquaculture. The field was designed in consultation with SMAST, an organization whose scientists have decades of experience monitoring the benthic flux of nitrogen, and which has monitored oyster-enhanced sediment denitrification for several shellfish demonstration projects. The oyster field was deployed as a rectangle of densely-packed floating bags to maximize the ability to measure enhanced denitrification rates under the floating bags. The field was also designed to allow operation and maintenance to occur without disturbing the sediment or monitoring equipment. Consequently all field maintenance activities were performed from boats so the bottom was not disturbed.

6. Installation Details

A. Permitting and Ongoing Public Information for Lonnie's Pond Demonstration

The permitting process for the Lonnie's Pond demonstration included two steps: a modification to the Town's Municipal Propagation Permit through the DMF and a Conservation Commission Request for Determination of Applicability (RDA) with abutter notification. At the request of the Shellfish Constable, DMF included the Lonnie's Pond demonstration in the Town's propagation permit. A map showing the proposed demonstration location as well as a description of the shellfish being grown was submitted for this permit modification. Signs closing the project area to the harvest of oysters were also posted by the Orleans Shellfish Constable at the request of DMF. This species-specific closure allowed quahogs to continue to be harvested near this area while the demonstration occurred.

The standard RDA application form from MassDEP entitled WPA-1 was submitted to the Conservation Commission, and abutters were notified pursuant to local regulations. A public hearing was held on June 21, 2016 where several abutters commented on the project. Key concerns were the visual and navigation impacts of the project and the long-term plan for the installation. After a robust discussion, most abutters were supportive of the 2016 Demonstration because of the value of the research being done. The RDA was approved by granting a "negative determination", which means that the project could move forward without filing a Notice of Intent. The conditions of this approval included removing the floating bags from the surface by December 31, 2016 and providing a project update during a Conservation Commission meeting in January, 2017. Submerging the oysters in Lonnie's Pond as an overwintering strategy was also approved as part of the RDA approval. In addition to the abutter notification that was required as part of the RDA hearing process, an ongoing effort was made to keep the community informed about this demonstration.

As part of continued public outreach and education, the following occurred:

- Attendance at the Friends of Lonnie's Pond Annual Meeting in the spring of 2016 to present details of the demonstration project;
- Informational flyer posted below the parking lot located near the project;
- Project updates sent approximately every two weeks by email to a broad stakeholder group, including the Friends of Lonnie's Pond. Six updates were sent from July to early October with a final update sent after the overwintering was completed in mid-December;
- Newspaper articles describing key events such as volunteer bag building day, oyster installation in Lonnie's Pond, project operation and maintenance and overwintering;
- Monthly (at a minimum) Shellfish Working Group meetings; and
- Two presentations at Lonnie's Pond in September as part of the Orleans Pond Coalition event entitled "Celebrate our Waters".

Overall, there was positive stakeholder support for this demonstration. Residents of Orleans are eager to learn the extent to which shellfish can contribute to water quality improvements and regulatory compliance with nitrogen TMDLs in Orleans. This demonstration is seen as an effective approach for characterizing the effect of oysters on the nitrogen concentrations in the water column.

B. Details of Field Installation

The first step in deploying oysters in the field was building the floating bags and long line system that kept the bags in place. Clips were attached at regular intervals along the line and the floating bags were attached to these clips. The floating bags consisted of several component parts that were purchased separately and then assembled, including the bags themselves, floats, ties, a PVC slider closure, and rope. The bags were punched on a jig and hog rings were used to attach rope to the bags through the punched holes. Two volunteer days were held in Orleans (June 4, 2016 and June 17, 2016) so community members could get first-hand experience with the bag assembly process. Eight people came from the community on the first day, and four came on the second day. Paid staff and unpaid members of the shellfish technical team also worked on these volunteer days, and paid staff completed assembly of all 800 bags over several additional days of work. The long line system was assembled exclusively by paid staff.

On June 22, 2016, the installation of oysters in Lonnie's Pond began by setting a series of augers and attaching the long lines. Floating bags were then clipped to the long line at regular intervals. As shown in Figure 3, the demonstration layout included 800 bags in an 80' x 120' field. Floating bags were installed in 8 strings that were kept in place using 16 augers (one auger on each end of each set of long lines). The strings were spaced approximately 10 feet apart. Approximately 250 oysters were installed per floating bag. This was accomplished by counting 250 oysters into a measuring vessel and then using this volume to fill subsequent bags. Figure 4 shows the team filling the floating bags with oysters.

Figure 3 - Layout of Lonnie's Demonstration (Year One)

Figure 4 - Installing Oysters in Floating Bags (Year One)

Two sources of seed were used in Lonnie's Pond. Second-year seed that had been overwintered was purchased from the Town of Falmouth. Approximately 126,690 2.5-inch (Y2 oysters) and 11,700 1.5-inch animals (Y1 oysters) (for a total of 138,390) were available. The goal for the demonstration was 200,000 oysters and therefore an additional 60,000 oysters that were almost 1-inch (Y1 oysters) were purchased from Cape Cod Oyster. Both batches of seed were delivered to the site via refrigerated truck (Figure 5), and installed the same day as delivery. The 138,000 oysters from Falmouth were distributed among 560 bags and the 60,000 oysters from Cape Cod Oyster were distributed among the remaining bags and were installed in the field on June 22, 2016 and June 27, 2016, respectively.

7. Measuring Oyster Growth

A. Data Collection Methodologies

Over the project period, seven floating bags at the locations shown in Figure 6 were monitored regularly for growth rates. The entire field was evaluated for mortality during each session when the bags were maintained.

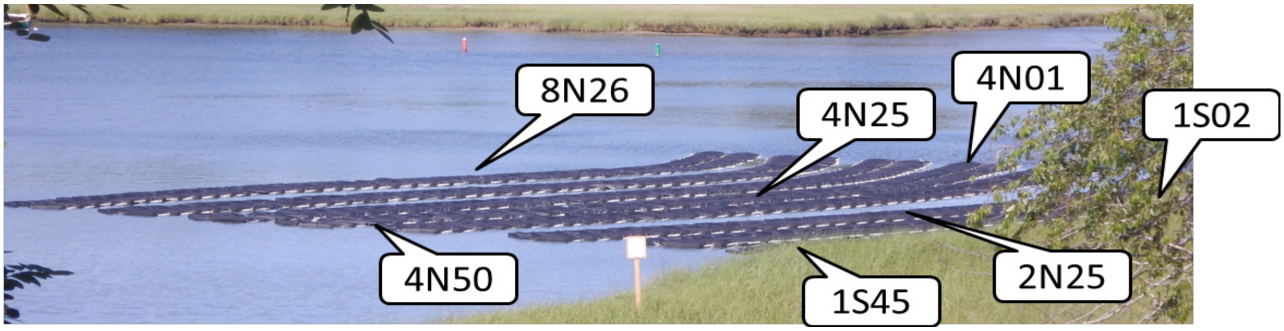

Figure 6 - Locations of the Floating Bags Monitored for Growth and Mortality

On each sampling day, 25 to 30 oysters were randomly selected from each tracking bag. The oysters were transported from the bags to shore in labeled sampling buckets. On shore, the oysters were lightly scrubbed to remove surface fouling and 25 were placed on a scaled mat and photographed. The images were loaded into a custom LabView application developed for this project that allowed each oyster to be measured individually. The user interface for this program is shown in Figure 7. The photographed oysters were then weighed using a SmartWeigh scale with a 2kg capacity and 0.1g accuracy, with a readability of 0.01g. Normally, the total weight of the 25 oysters was recorded so that an average could be calculated. Individual oysters were weighed to validate averages. An example of this data is shown in Figure 8. The volume occupied by oysters as they grew was determined by placing 30 oysters in a cylindrical container, agitating them briefly to allow them to settle, and lowering a gauge plate until it contacted the top of the oysters. The level of the plate was recorded so the volume occupied inside the cylindrical container could be calculated. Figure 9 shows staff performing the growth measurements.

Figure 7 - Custom LabView Program for Measuring Oyster Length

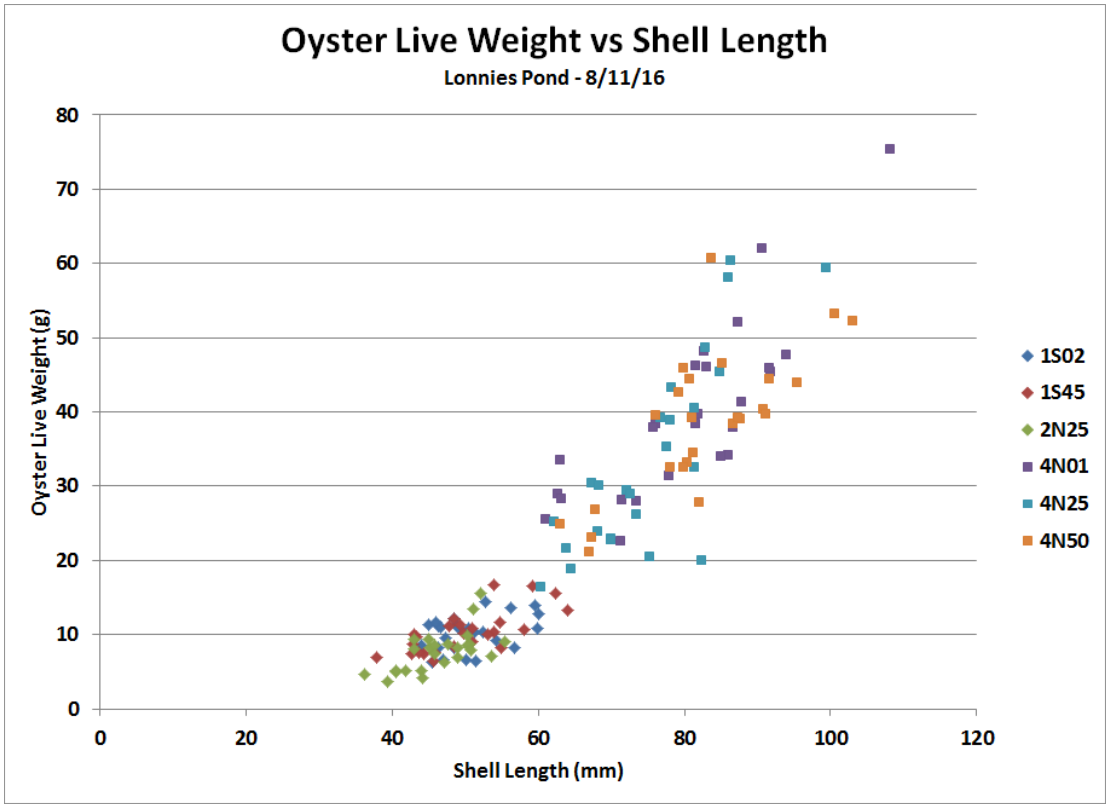

Figure 8 - Oyster Live Weight and Shell Length for One Sampling Event

Figure 9 - Photodocumentation of field measurements (weight and length) being taken

Mortality was monitored visually and audibly in each bag during maintenance operations. The shell of a dead oyster opens within a few days, and once the oysters get above approximately 50 mm, a single shell makes a distinctive rattle when the bag is flipped. The seven sampling bags were also inspected for mortality each time oysters were withdrawn for measurements, and dead shells were inspected, counted, and removed. No mortality was noticed by any of these methods until mid-September, when an occasional dead oyster was noticed in the tracking bags. From this point forward, mortality counts were 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 previously uncounted bags for each population class (four Y1 and four Y2), and the overall final mortality rate prior to overwintering was established at 6.6 percent.

B. Presentation of Data on Growth, Mortality and Nitrogen Content

Figure 10 shows the data for size and weight measurements taken over the course of the season, demonstrating that the oysters grew well and at an average rate approximating data from other Cape Cod locations. The first-year oysters finished at an average length of about 74mm (2.9-inches) and an average weight of 35 g (1.23 oz.). The second-year oysters finished at an average length of about 94 mm (3.7 inches) and an average weight of about 69 g (2.4 oz.). The distribution of sizes is shown in Figure 11, and demonstrates that approximately eighty percent of the first-year oysters reached either petite (64 mm/2.5-inches) or regular market size (76 mm/3-inches) in one shortened growing season. A typical season would begin in May, not late June. All of the second-year 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 next season.

It is important to understand the relationship between dry tissue weight and wet harvest weight, and the following outlines key points in regard to this relationship:

- Dry tissue weight is the best quantity to use to calculate the nitrogen removal performance of oysters by the two pathways considered in this report because
  - Nitrogen is incorporated into the dry solids of the oyster, so the total amount of nitrogen in any given oyster is a consistent percentage of the dry tissue weight;
  - The denitrification enhancement associated with an oyster is proportional to the amount of biodeposits produced by an oyster as long as saturation of organic matter into the sediments leading to anoxic conditions hasn't yet occurred. The amount of biodeposits produced is directly proportional to the dry tissue weight of the oyster;
- While dry tissue weight is the best way to forecast how a system will perform, wet harvest weight is the most feasible way to track actual performance in the field; and
- There is a basis in published literature for recognizing that the ratio of harvest weight to dry tissue weight may vary over the course of the season, and characterizing this variation will enable more accurate calculation of nitrogen removal based on harvest weight for programs that permanently remove oysters from the growing area at different times during the growing season.

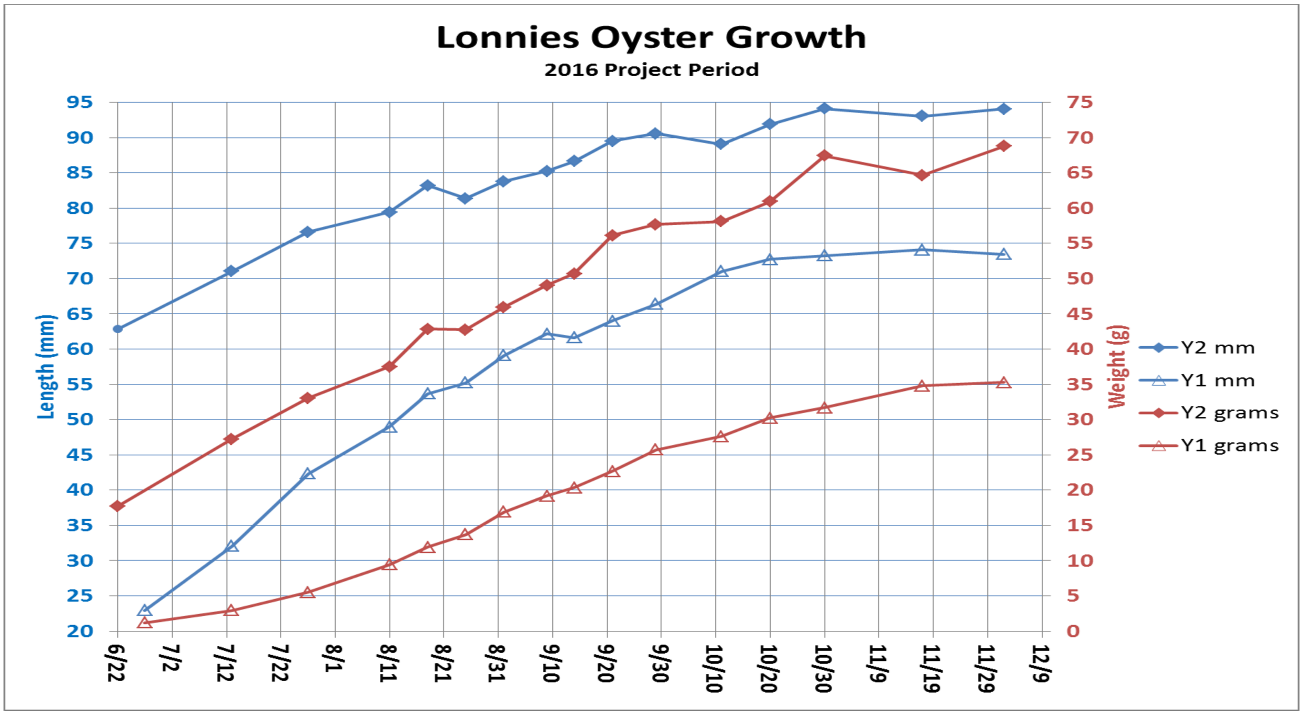

Figure 10 - Size and Weight Measurements for Lonnie's Pond Oysters

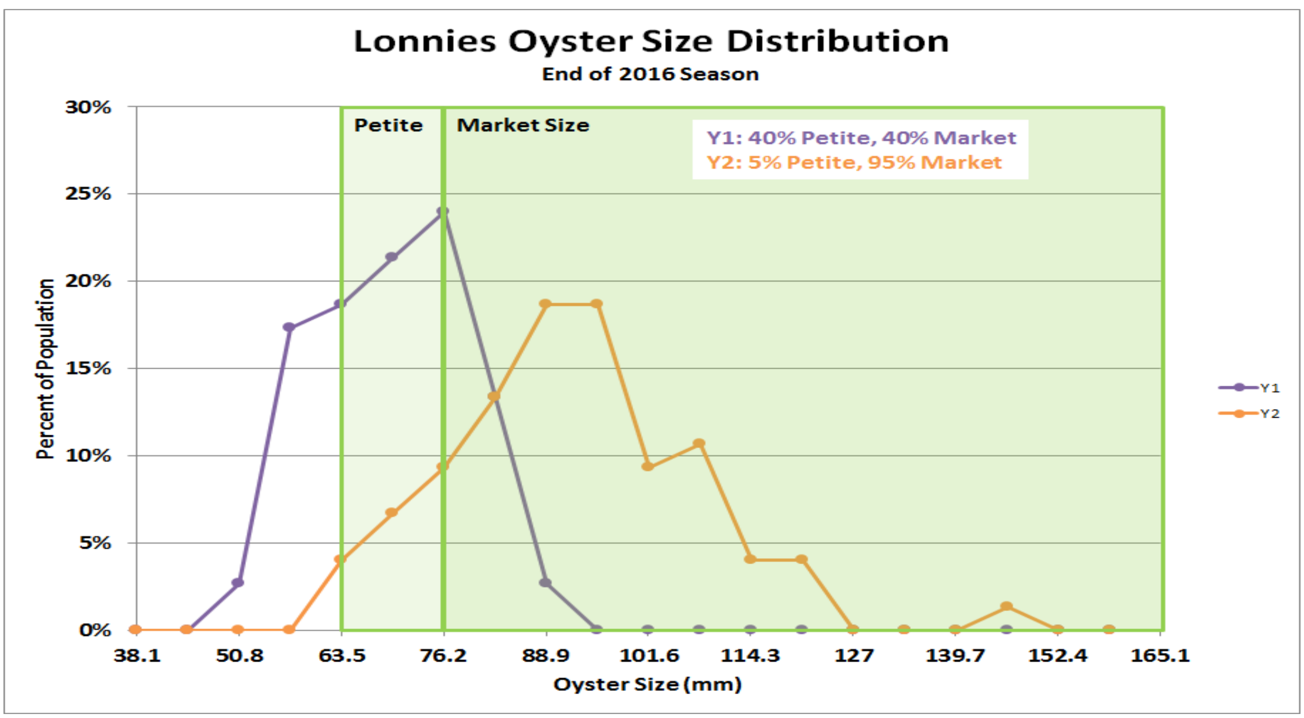

Figure 11 - Histogram of Size and Weight Distribution for Lonnie's Pond Oysters

Figure 12 shows both the increase in total oyster biomass over the growing season as well as the amount of nitrogen sequestered in the shells and tissues of these oysters. As shown in Figure 12, the live harvest (i.e., wet) weight of the oysters increased from approximately 2 metric tons at the beginning of the project on June 22, 2016 to approximately 10 metric tons at the end of the growing season. This value has been adjusted by the measured mortality of 6.6 percent. This biomass contains approximately 25.9 kg of nitrogen that was removed from the water column during the growing season. This nitrogen-uptake value of 25.9 kg was estimated using several measured values that are shown in Table 2 and the average dry weight, which was calculated from measured harvest weights using the relationship shown in Figure 14.

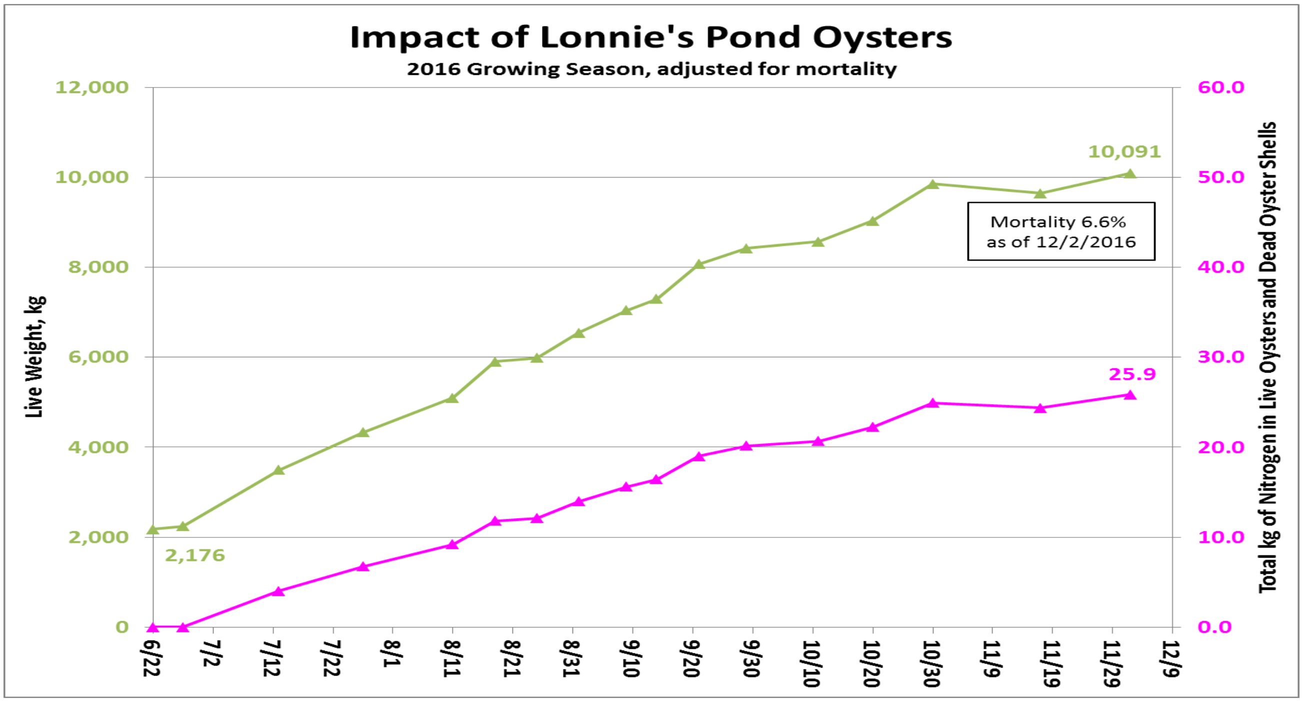

Figure 12 - Total Live Weight and Nitrogen Removed by Oyster during Year 1 of the Lonnie's Demonstration Project

As indicated above, harvest wet weights were measured in the field over the course of the 2016 Demonstration Project, but dry tissue weight is the best quantity to use to calculate the nitrogen removal. To establish dry weights of Lonnie's pond oysters, samples of Y2 oysters were sent to the Boston University (BU) Stable Isotope Laboratory at the beginning of the demonstration on June 22, 2016, and both size classes were sent after the end of the growing season on December 19, 2016. Both the wet harvest weight and dry weight of these oysters were recorded, and these data were plotted as shown on Figure 14, and used to establish a relationship between the dry and wet harvest weights of Lonnie's Pond Y1 and Y2 oysters. Figure 13 shows the calculated dry weight of oysters over the 2016 Demonstration Season. The oysters sent to the BU Laboratory were also analyzed for percent content of nitrogen in shell and soft tissue, as reported in Table 2. Analysis of these samples indicates that the total amount of nitrogen in oysters grown in Lonnie's Pond is 10.3 to 10.5 percent of the dry tissue weight of the oyster, comparable to measured results from other studies in Pleasant Bay (Reitsma, Murphy and Franklin 2014, and Reitsma et al. 2016).

To estimate the nitrogen- uptake value, the average dry weight calculated for Y1 and Y2 oysters was multiplied by the mortality-adjusted population and size-class appropriate nitrogen content (10.3 percent for Y2 and 10.5 percent for Y1, as shown in Table 2) to estimate the total nitrogen removed from the water by the growth of the living oysters. No nitrogen credit was taken for the tissue of dead oysters. This method provided an estimated total of 25.9 kg of nitrogen removed from the water column. The primary significance of this total nitrogen removal value is for comparison with the amount of nitrogen estimated to have been removed by increased denitrification, which was 17.3 kg, or about 67 percent of the amount removed through tissue and shell uptake (Howes et al. 2017).

Table 2 - Total Nitrogen Content of Lonnie's Pond Oysters as Compared to Other Pleasant Bay Data

Location	Sample Time	Length (mm)	Whole (wet) Weight (g)	Dry Tissue Weight (g)	Total N, Shell and Tissue (g)	N as a percent of Dry Tissue Weight (%)
Pleasant Bay (other studies)	Spring	77.7	64.9	2.31	0.29	12.6
Pleasant Bay (other studies)	Fall	80.9	54.3	2.99	0.30	10.0
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

Source of data for Lonnie's are actual measurements, source for other studies: Reitsma, Murphy and Franklin 2014, Reitsma et al. 2016

The Y2 and Y1 values for new growth in Lonnie's Pond shown in Table 2 were used to estimate total nitrogen content of the oysters at the end of the growing season. Figure 12 shows the progress of nitrogen uptake over the course of the season. This uptake was scaled according to the measured increase in the harvest (wet) weight of the oysters throughout the season. Based on measured results from the 2016 Demonstration, first year seed that is grown in Lonnie's Pond is likely to have a higher dry weight than seed that can be purchased elsewhere. The Y2-L line is an estimate for expected oyster dry weight for first year seed that is started in May in Lonnie's Pond and then used again in Lonnie's Pond as the second-year seed. The initial stocking density for these larger Y2-L oysters would be lower than for the smaller intermediate seed obtained from other suppliers, but the active biomass per unit volume of gear will not be effectively different. Net performance for a Y2 optimized system would be equivalent whether larger or smaller intermediate seed is used.

The nitrogen content of oyster tissue over the course of the growing season is slightly different depending on location (Reitsma, Murphy and Franklin 2014, Reitsma et al. 2016). As shown in Table 2, 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 for oysters that are removed from Lonnie's Pond at different points in the growing season. Note for example the variation in spring versus fall oysters from previous studies of Pleasant Bay oysters reported in Table 2. Nitrogen content for oysters that have been grown and overwintered in Lonnie's Pond will need to be measured to determine whether there is a significant seasonal variation in the nitrogen content of oysters grown to marketable size in Lonnie's Pond. Part of the purpose of the second year of the demonstration project is to obtain this information. It is not expected that such variations will have a substantial effect on the overall viability or costs of the program.

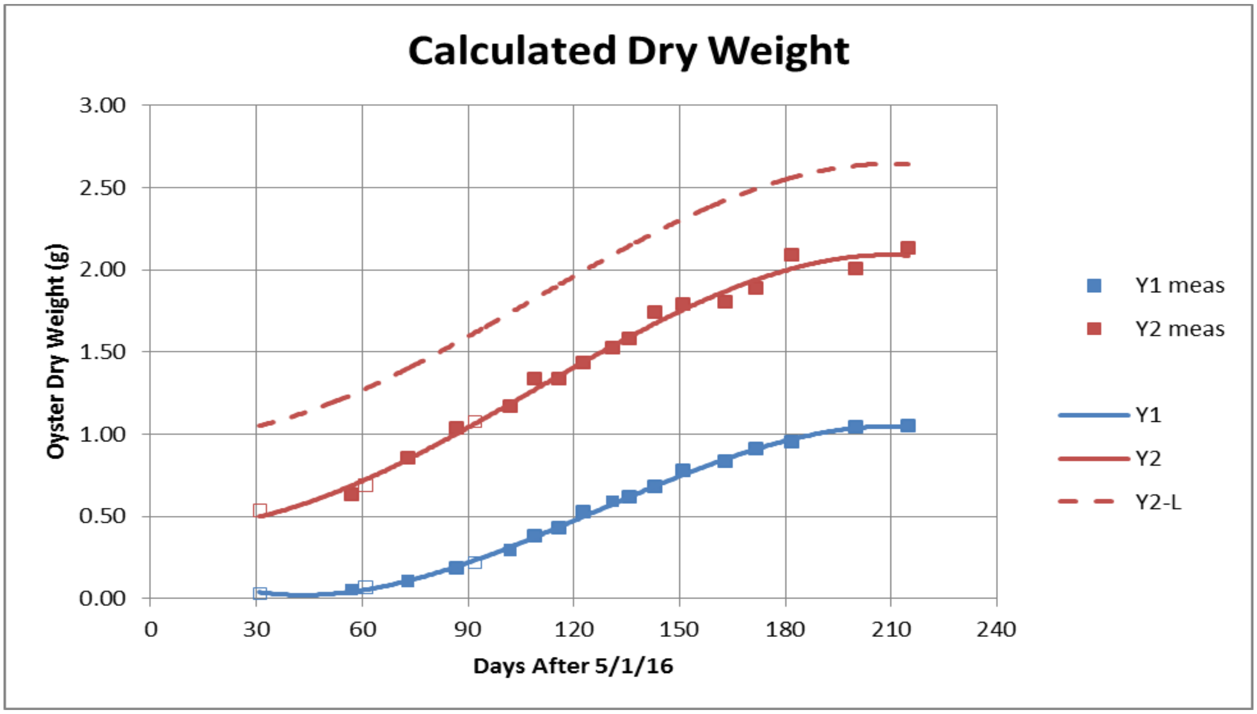

Figure 13 - Calculated Dry Tissue Weight of Oysters Over the Course of the Growing Season

It is not practical or cost effective to measure the size of each oyster or the actual dry weight of the tissue in oysters that are removed over the course of the season. Wet harvest weight is the parameter that is practical to track. To estimate the total nitrogen uptake as a function of wet harvest weight, dry tissue mass is the best predictor of nitrogen content. The relationship between wet harvest weight and dry tissue mass is not strong, as shown in Figure 14. As shown in Figure 15, there is typically a strong correlation between dry shell mass and harvest weight. Unlike shell mass, tissue mass may vary over the course of the growing season.

The relationship between dry tissue weight and wet harvest weight can be established by plotting the slope of the green line as a function of days after initial oyster installation. This will create a tool for calculating the total nitrogen content of an oyster as a function of its harvest weight at any given time during the growing season. A key objective of the sampling planned for the second year of this demonstration project is to establish this relationship over the growing season for both Y1 and Y2 oysters, so that the nitrogen-removal achieved when harvesting at times earlier than the end of the season can be calculated.

Measurements of the volume occupied by the oysters as they grew are shown in Figure 16. When the number of oysters per liter (inverse of volume) is plotted as a function of harvest weight, it can be seen that the volume occupied increases dramatically as the harvest weight increases. This is a quantitative illustration of the need to 'split' bags as oysters grow. While it is possible to start with a high stocking density of approximately 1,000 oysters per bag, they must be distributed among more bags as they grow. It also highlights an important feature of small oysters: they occupy less space per individual early in the season. Thus when a surface gear installation begins with the deployment of smaller oysters, it will have less of a visual impact in terms of the amount of gear needed on the water in the early stages. The change in the volume requirement of a fixed population of Y1 and Y2 oysters as grown in Lonnie's Pond during the 2016 Demonstration project is shown in Figure 17.

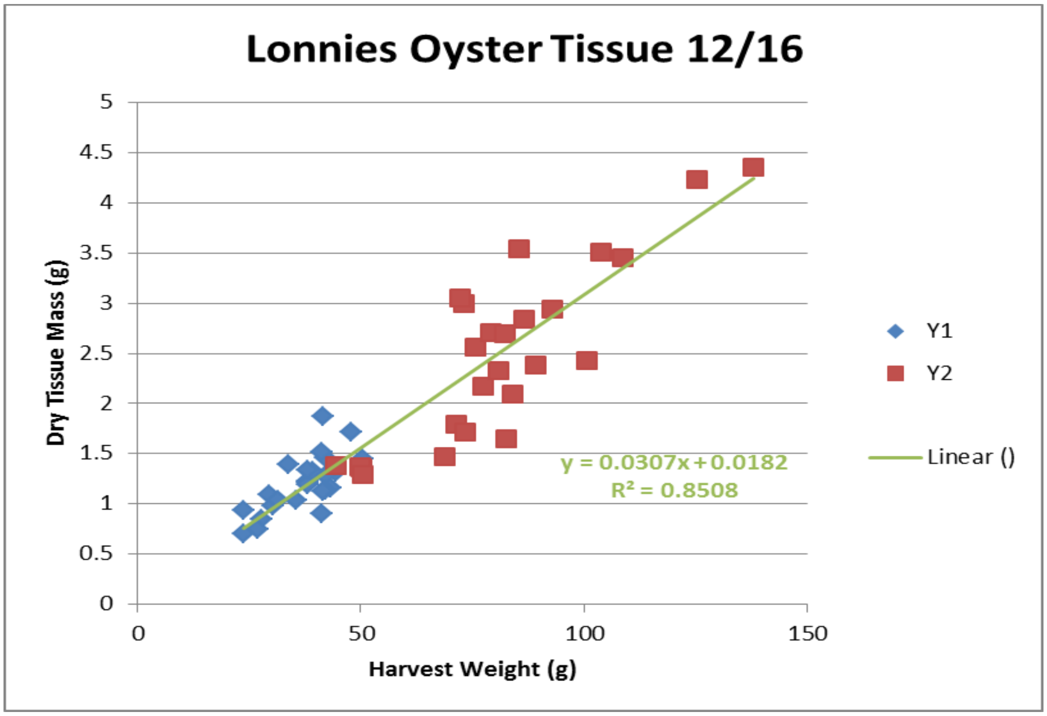

Figure 14 - Relationship Between Dry Tissue Mass to Harvest Weight

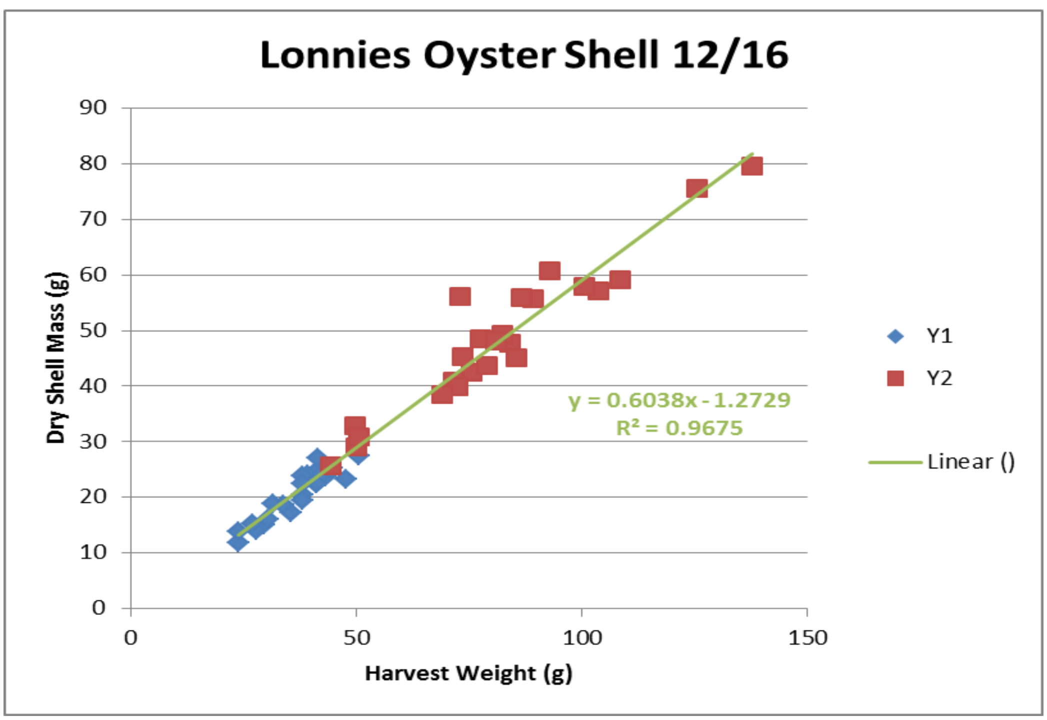

Figure 15 - Relationship Between Dry Shell Mass to Harvest Weight

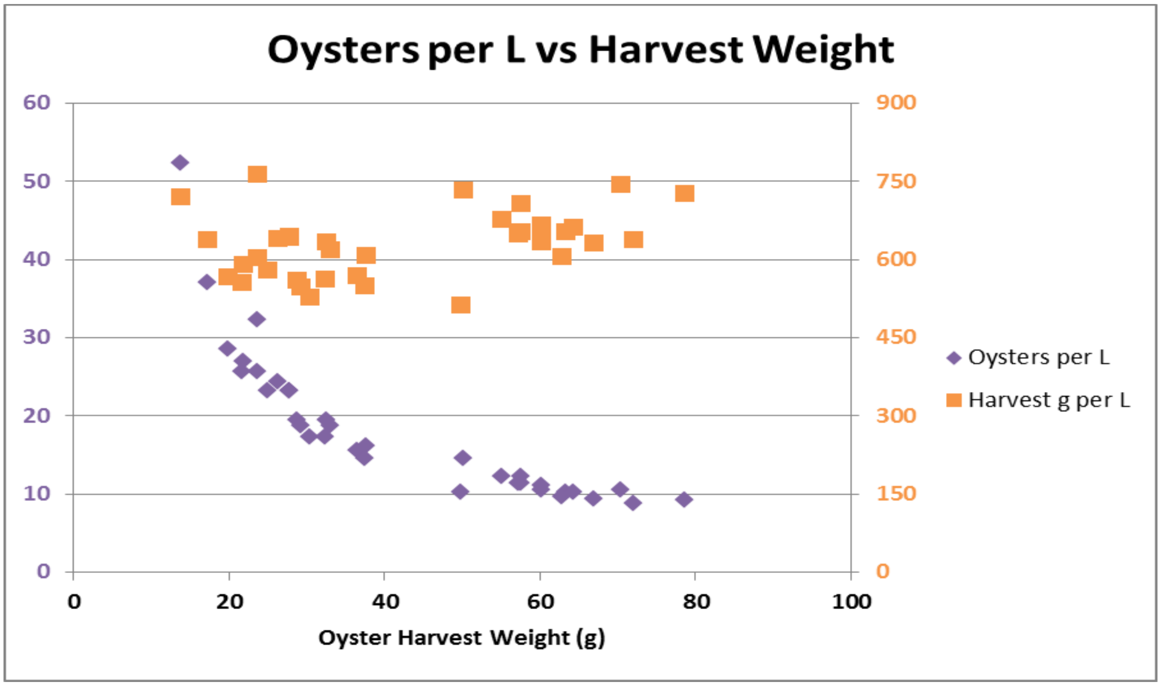

Figure 16 - Number of Oysters per Liter and Harvest Weight per Liter as a function of Harvest Weight for oysters grown in Lonnie's Pond

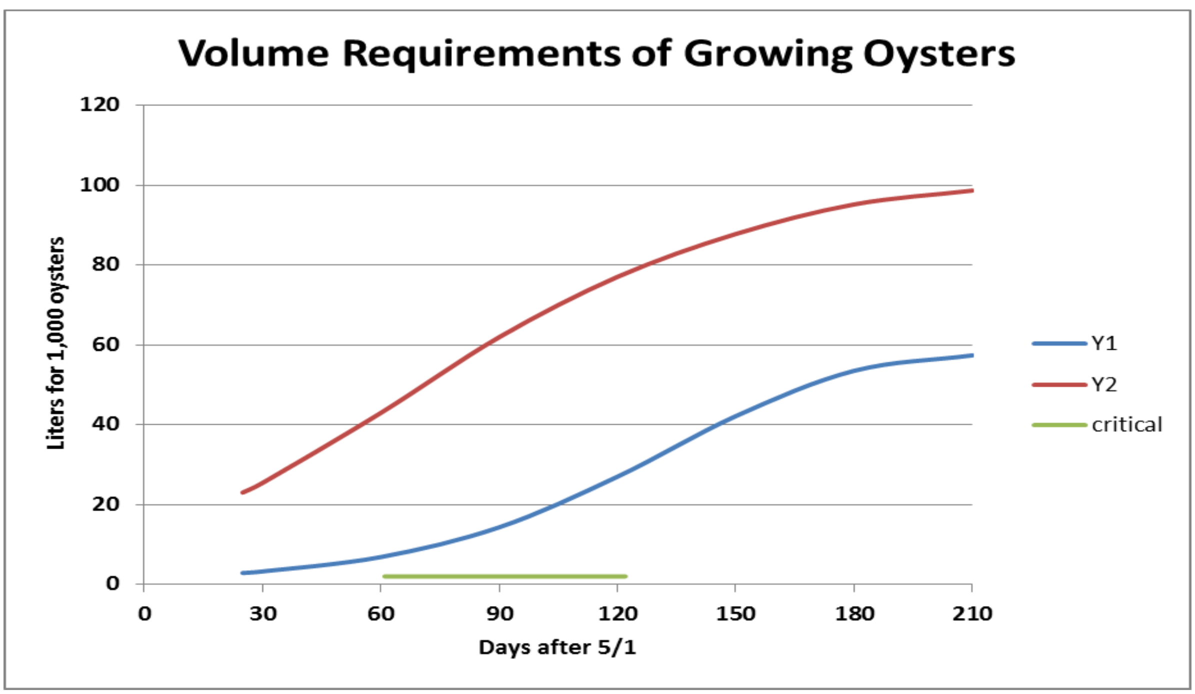

Figure 17 - Number of Liters occupied by Y1 and Y2 oysters over the course of the 2016 growing season

The slopes of the lines shown in Figure 17 represent the rate of increase in the required volume. Some notable features arising from an observation of the change in the slope of these lines over the course of the growing season include:

- Y1 oysters start with a low volume requirement that remains low through the initial part of the growing season, then accelerates quickly after the midpoint of the growing season, with a deceleration towards the end of the growing season; and
- Y2 oysters start with a significant volume requirement which increases through the season, but levels off during the last 30 days or so of the growing season.

The ramification of this difference in volume requirements between Y1 and Y2 oysters is that for a certain end population of oysters, a Y1 system requires fewer bags on the water early in the season and during the critical impairment period. This can result in a lower visual impact from late June to early September, and may be a factor in deciding whether to select a Y1 or Y2 pathway.

8. Operation and Maintenance

To prevent fouling all bags were flipped, which exposes the side previously in the water to air, killing biofouling organisms. Bags were flipped on a schedule that was approximately weekly during the peak impairment period and bi-weekly as the growing season concluded. A key constraint in the operation and maintenance routine was meeting the requirement of no disturbance to the bottom sediment in order for SMAST to accurately determine denitrification rates. This required that the bags be flipped by boat, at high tide. The preferred method of flipping involved two people working simultaneously in a 10-foot rigid inflatable boat or 17-foot whaler. In both cases, one person would work from each side of the boat when passing between lines, and both on the same side when working the outside edges. Another method used a sit-on-top kayak facing perpendicular to the long axis of the kayak, with legs hanging in the water but not touching the bottom. There were several incidents of accelerated biofouling involving tunicates (sea-squirts) and algae during July and August, but nothing that was not sufficiently controlled by flipping the bags once a week.

In addition to reducing biofouling, the activity of flipping the bags helps to keep the oysters from growing together, which is important for marketability. It also achieves some edge trimming that is similar to what is accomplished by running the oysters through a grader. This helps to shape the oyster in a way that makes it more marketable. Flipping the bags also helps extract feces and pseudofeces from the population inside the bag. Some of these materials drop directly to the bottom, but others collect on top of oysters in the bag when they are densely packed. While there was a conscious effort to avoid walking on the bottom to enable a study of the impact of the undisturbed biodeposits on denitrification rates, it is possible that allowing workers to walk on the bottom will not only make the operation and maintenance easier, but may also increase denitrification enhancement as the biodeposits get worked into the sediments via the activity of workers walking on the bottom.

Gear remained in-place all season. 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 added to the long lines to accommodate a possible storm surge increasing the water level. The storm never arrived, and the extra line length was removed.

Overall the gear performed well. The water level typically changed by several feet each 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.

9. Overwintering Details

A. General

By December 23, 2016, all of the Lonnie's Pond oysters had been submerged for overwintering. This process included customizing a design for the overwintering system, building the system and installation. The design process included detailed review of current practices used for overwintering as well as consultation with numerous experts including:

- Local growers;
- Regional suppliers of shellfish gear;
- East Coast Shellfish Growers Association;
- Maine Cooperative Extension, Barnstable County Cooperative Extension;
- Mook Sea Farms; and
- Canadian grower, Raspberry Point Oysters.

Several prototype systems were designed, built, and tested before the approach was finalized. Key considerations included:

- Keeping oysters from sinking into soft sediment;
- Designing with enough water clearance to avoid ice damage;
- Maintaining enough flow for the cold weather metabolic activity of oysters;
- Controlling mortality from predation; and
- Practicality of installation.

B. Overwintering System Design

As shown in Figure 18, the final system design included a three-part PVC tube frame attached to two plastic pallets. The overall assembly footprint is approximately 50-inches wide, 82-inches long, and 57-inches high. The assembly holds ten floating bags in a vertical orientation at two different levels for a total of twenty bags per assembly. By removing one float from each bag used to grow the oysters, the bag orients vertically in the water as shown in Figure 18, and becomes negatively buoyant. When attached to the frame, the negative buoyancy of the bags combines to pull the assembly to the bottom of the water column. Assuming ice will project a maximum of 1 foot below the surface, the two-level assembly requires a minimum of 6 feet of water at the lowest possible tide. Lonnie's Pond is at least six feet at low tide where these assemblies were submerged.

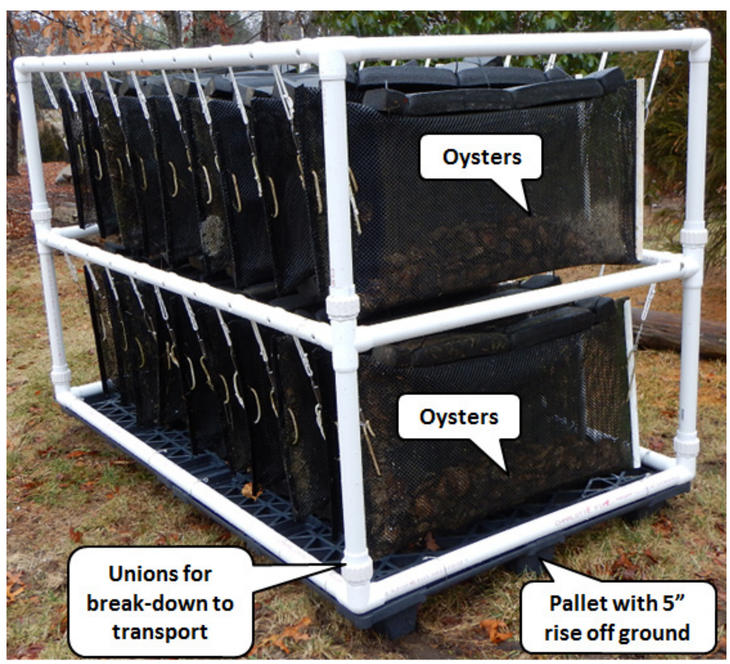

Figure 18 - Overwintering System

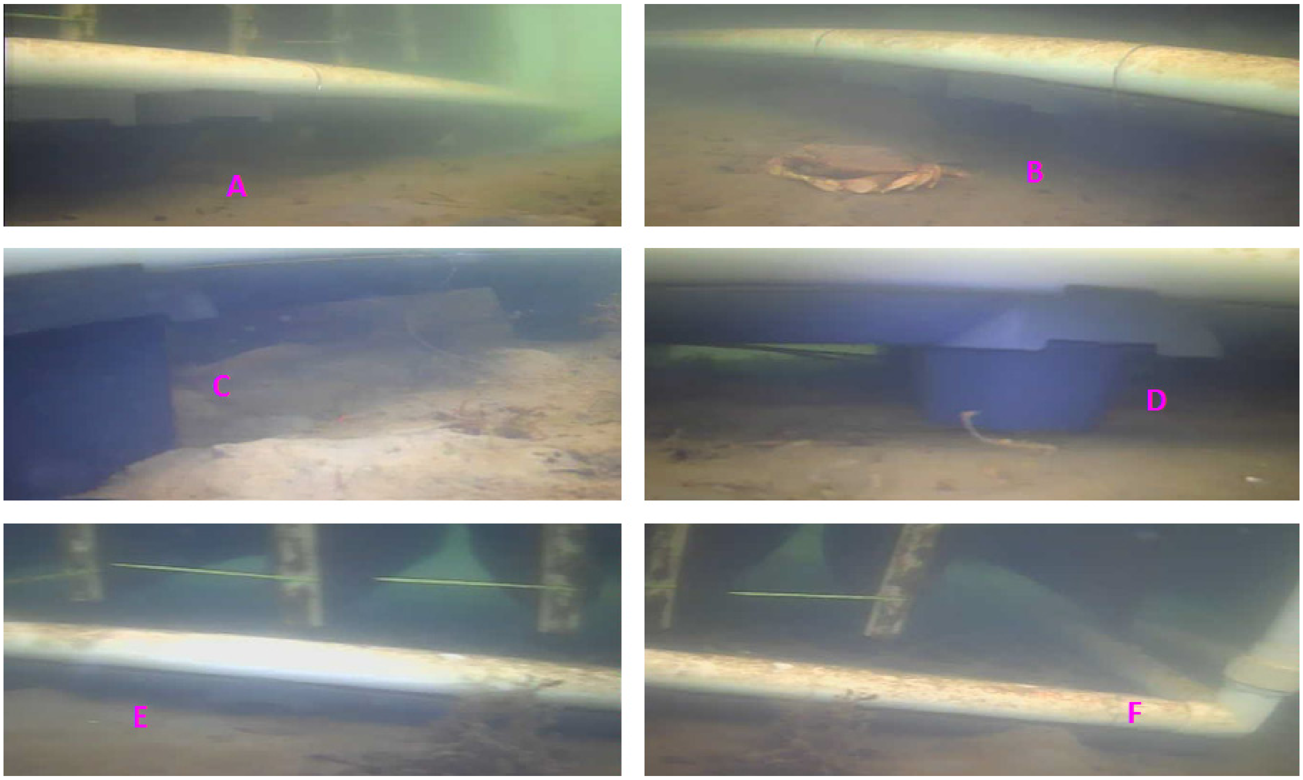

Figure 19 - Underwater image of the assembly showing both levels of bags and pallets resting on the sediment surface

Photo Notes: A and B show the bottom of the plastic pallet clearly above the 'hardest' bottom found in the overwintering area, with details of the feet shown in C and D. The system meets its design objective of keeping the rack above the softest bottom as shown in E and F, which show images taken of the northeastern most racks.

The pallets were specified with wedge shaped feet that prevent the loaded assembly from descending into soft bottom. The total net negative buoyancy of this overwintering system is approximately 100 pounds distributed over nine feet on each of the two pallets. The total initial contact surface area is approximately 100 square inches. This results in a pressure of approximately 1 psi, or approximately 40 percent of the pressure from the water on the bottom at a depth of 6 feet. As the assembly descends, the wedge-shaped feet increase the contact surface area laterally as well as vertically, so the effective pressure per unit area becomes lower as the assembly sinks, making it more difficult for the assembly to continue descending.

The decks of the pallets provide a contact area of over 1000 square inches, so if the assembly manages to sink lower than three to four inches below the sediment surface, the effective pressure per unit area is reduced to under 0.1 psi which is less than 4 percent of the pressure from the water on the bottom at a depth of 6 feet. As a result of this design, the assembly does not sink into even thick layers of soft sediment, as shown in Figure 19 E and F.

This system made it possible to leave the oysters in the grow-out bags for overwintering. Figure 20 shows a top view of the overwintered bags and a close-up of the oysters from under the water. There are several advantages of this approach. First, although oyster metabolism slows considerably as temperatures drop, they continue to filter. Maintaining some flow is important to survival, even in water that is 0°C. Packing oysters too tightly will also lead to toxicity as oysters continue to filter and purge over the winter. This system maintains water flow across the oysters. It also minimizes the handling of oysters that could damage the shells and lead to higher mortality over the winter.

Figure 20 - View of the top row of bags in a rack from under the water (left) and looking at the oysters in the bottom part of a bag (right)

Keeping the oysters in bags also provides a physical barrier against mature drills and other large predators, which cannot pass through the 6mm bag mesh. In addition, two different types of copper barriers were applied to some of the frames at the midpoint unions to discourage drills from traveling along the supports of the assembly to reach the bags. One type was an 8 AWG stranded copper grounding wire, the other was a 1-inch wide band of 0.025-inches thick copper flashing. These details are shown at A and B respectively in Figure 21. Figure 22 shows predators unable to reach the overwintered oysters.

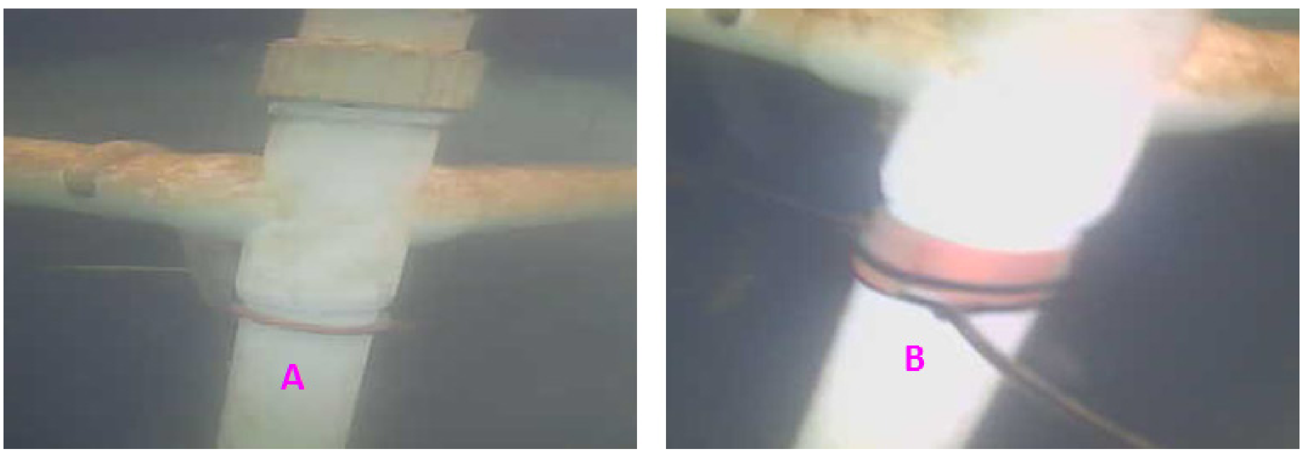

Figure 21 - Copper Barriers on Overwintering System

Figure 22 - Conch and starfish predators in foreground prevented from reaching overwintered oysters in the background

C. Overwintering System Installation

A custom raft was constructed for installation of this overwintering system. The raft held a frame in the water while workers attached bags to the lines on the frame. When loading of the frame with bags was complete, staff used lines to lower the frame to the bottom. The assembly was designed to be light enough in the water for staff to manipulate the frames for positioning on the bottom. Furthermore, the low net weight of the assembly made it feasible for staff to retrieve the loaded frame from the bottom in the spring time.

The process of installing bags on the racks and sinking them to the bottom took place over the course of three days. After the first overwintering deployment day (December 14, 2016), half of the field had been submerged. Then there was a cold weather event that lasted 2 days. The oysters that had not been submerged on racks remained in floating gear that kept them immersed in water. The oysters were inspected after this cold event and appeared to be unharmed.

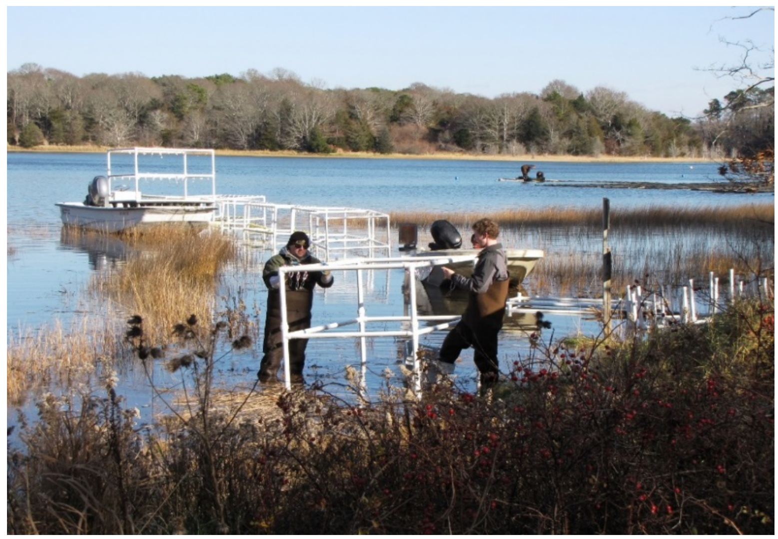

Figure 23 - Staging of Overwintering System for Installation

Figure 23 shows the staging of the overwintering racks during the installation period and Figure 24 shows a close-up of the overwintering system submerged for field installation.

The location of the overwintering system is shown in Figures 25 and 26, with a Sidescan Sonar image of the entire installation upon completion shown in Figure 27.

Figure 24 - Submerged Overwintering Assembly

Based on data shown in Figure 12, 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. Over the course of the growing season, the shells of dead oysters were removed when bags were evaluated to assess mortality. In the spring, these oysters will be graded and an overwintering mortality will be determined.

Figure 25 - Winter stick markers showing location of overwintering racks

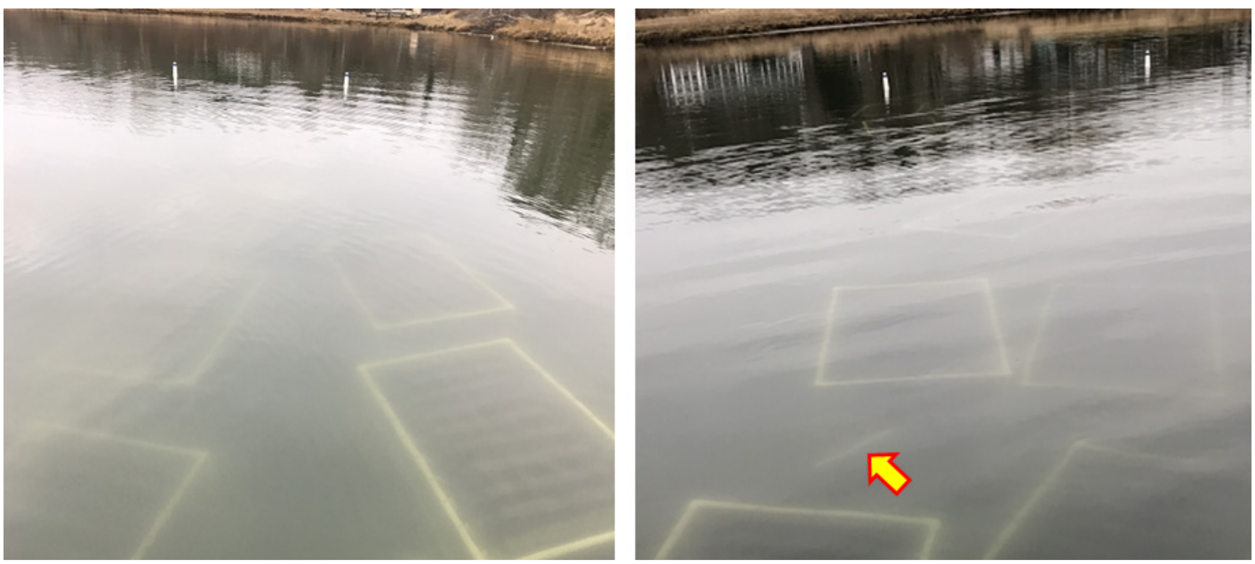

Figure 26 - View of winter racks at low tide on a calm day, looking east at the left and west at the right. Corner markers are visible at the top of both photos. The arrow shows the location of the monitoring sensors referenced in Figure 29.

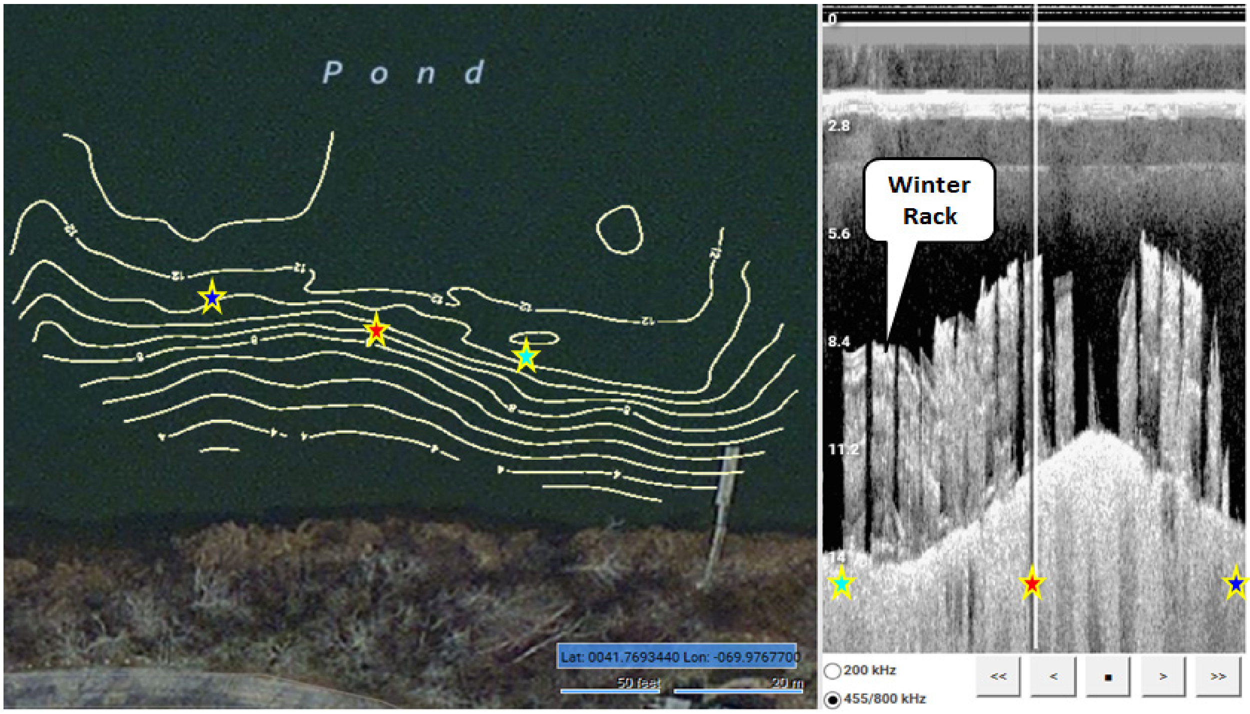

Figure 27 - Sonar scan of winter rack system after deployment on the bottom

D. Ongoing Monitoring

Prior to overwintering, temperature sensors were used to establish field conditions. Two temperature sensors were placed near the intended overwintering site to monitor water temperature at 10 minute intervals. One sensor was located a few inches below the surface, moving up and down with the water level, and the other sensor was fixed about a foot off the bottom near where the deepest overwintered oysters would be. The values from these two sensors are shown in Figure 28, along with a line showing the time period when overwintering was undertaken. Typically oysters are kept on the surface where environmental conditions including temperature and dissolved oxygen are frequently optimal for health as late into the winter season as possible. 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. During 2016, this threshold was preceded by a forecast of low temperatures for several days that put the water surface at risk of freezing. Consequently, overwintering was undertaken earlier than usual. The water surface never froze, but it can be seen that the water temperature did drop considerably during the cold weather period. Seawater typically freezes at -2°C, a temperature that clearly was not reached even at the surface. The close tracking of the surface and bottom temperatures highlights two important features of Lonnie's Pond:

- There is a high turnover rate of water coming in from Pleasant Bay; and
- There is not persistent stratification at the location where the oysters are being maintained.

For ongoing monitoring, 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. These sensors are shown in Figure 29. The data is downloaded from the sensors regularly and evaluated for events of concern. Figure 30 shows that the dissolved oxygen content does not fall below 12 mg/L, consistent with typical winter conditions. Figure 30 also shows that the normal cycle of tidally-influenced water levels can be affected by weather conditions. Figure 30 shows a decreasing water level trend from 1/4 to 1/6/17 which was likely the result of a northerly wind keeping water in the south end of Pleasant Bay.

It can also be seen that the water level range over the two-week span shown in Figure 30 was 1.65m (65”), and that the typical daily oscillation involves one change of about 0.9m (36”) and another change of about 1.2m (48”). These large tidal variations offer further evidence that there is a high rate of exchange of water with Pleasant Bay.

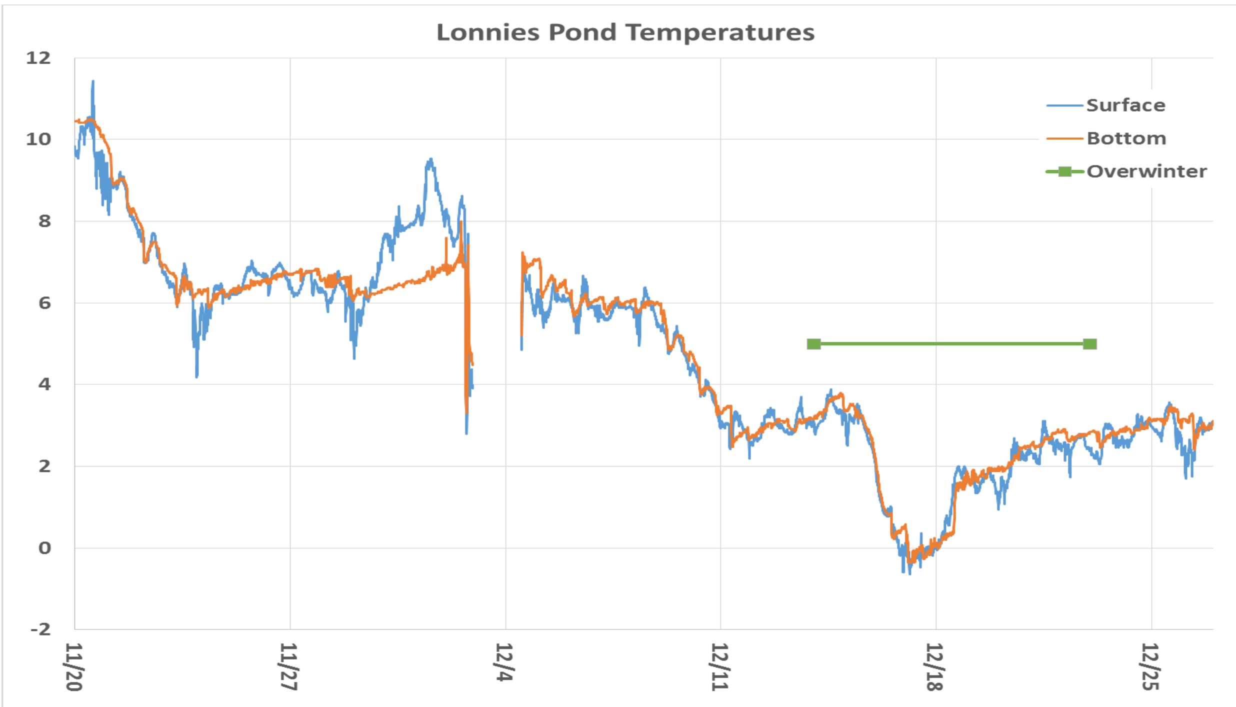

Figure 28 - Water temperature at the site where oysters were overwintered

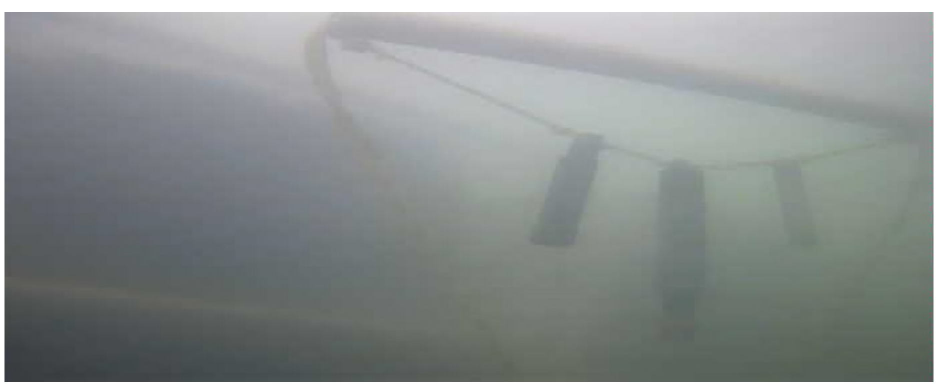

Figure 29 - Cluster of sensors for monitoring temperature, water level, salinity, and dissolved oxygen shown in the foreground at the right, with a rack in the background at the left

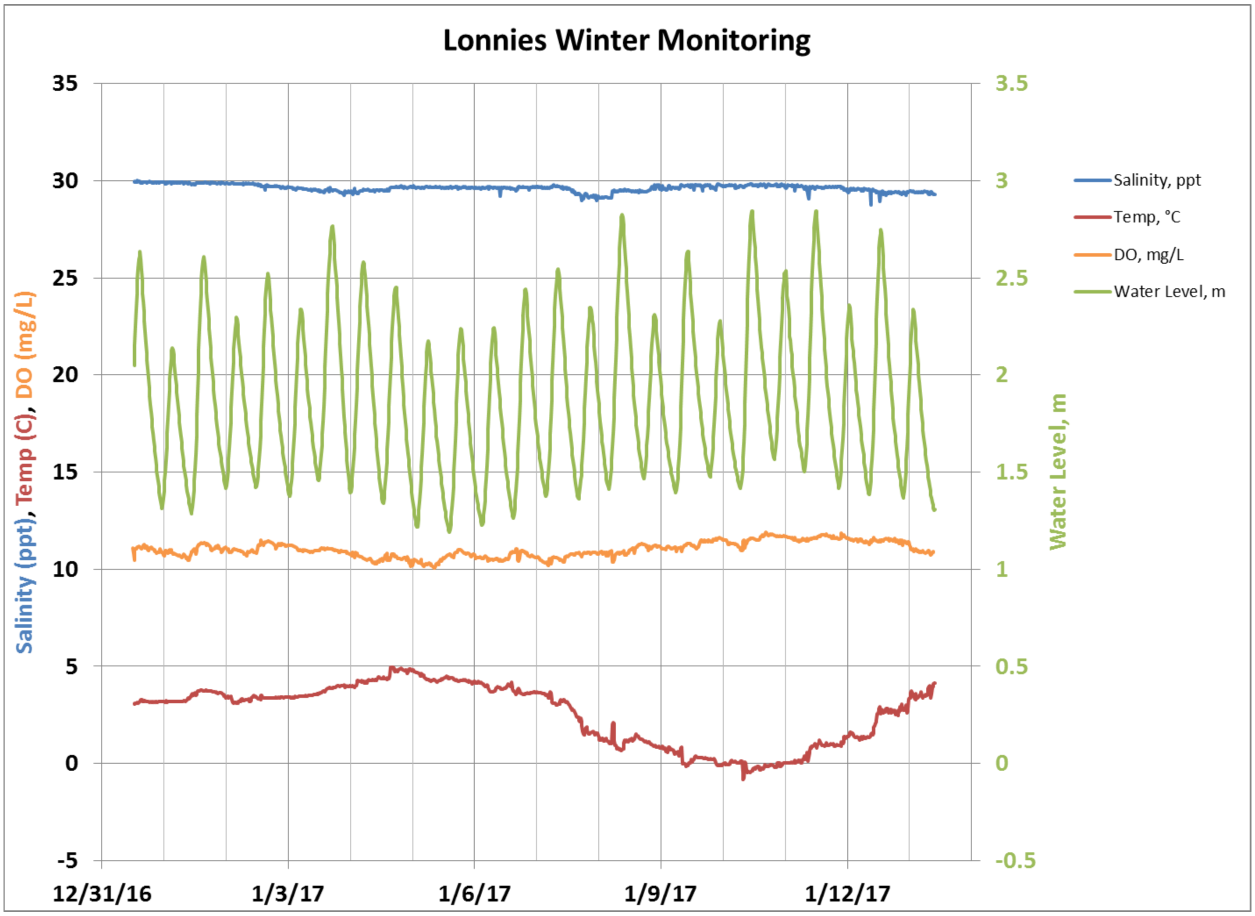

Figure 30 - Winter Sensor Data

10. Summary of Monitoring Results and Recommendations

A. Summary of Water Quality Monitoring Results for the 2016 Demonstration

The Lonnie's Pond demonstration area was monitored by SMAST staff over the 2016 Demonstration project. The technical report (Howes et al. 2017) presents the detailed methodology and findings of this data collection and analysis program. In general, a sampling program was implemented to establish both a 2016 water quality benchmark for Lonnie's 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. These stations are shown in Figure 31 and were selected 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. In parallel with the water column sampling, an Acoustic Doppler Current Profiler (ADCP) was deployed to measure current direction through the oyster area relative to the sampling points. The purpose of this sampling regime was to quantify changes in water column constituents through the oyster field. It was expected that particulate organic nitrogen (PON), total chlorophyll-*a* and bioactive N would be affected by the demonstration, although the complete suite of nitrogen components, orthophosphate and dissolved oxygen were also assessed.

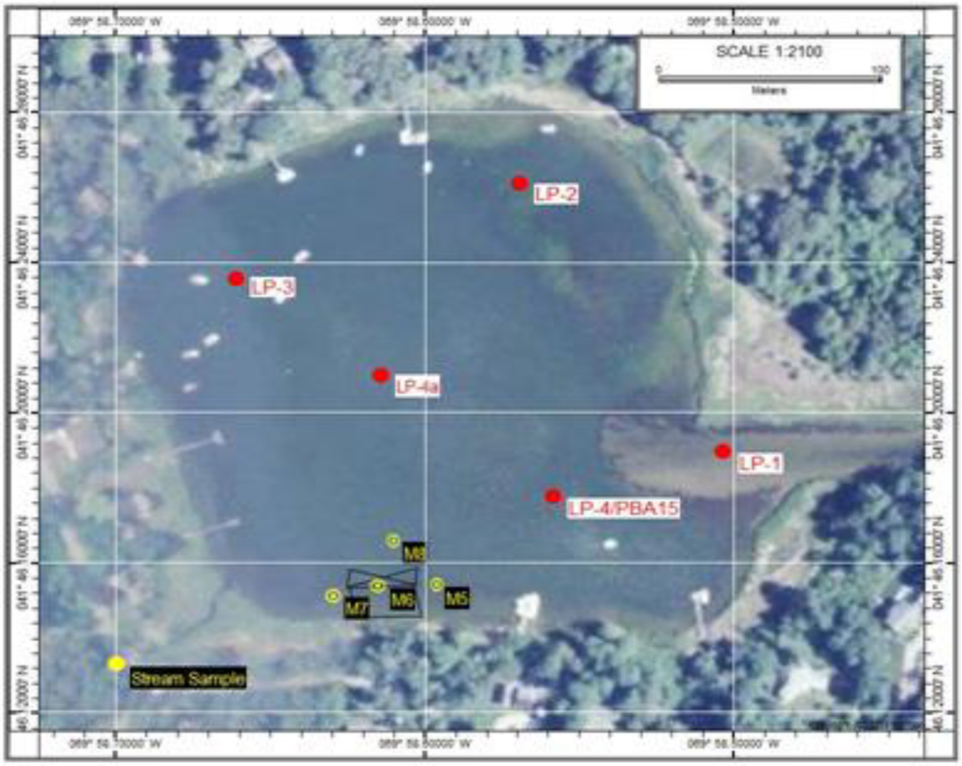

Figure 31 - SMAST Monitoring Stations (Howes et al. 2017)

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. The constituents of total nitrogen include (nitrate + nitrite), ammonia, dissolved organic nitrogen (DON) and PON. 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.

The major results of the 2016 Demonstration monitoring in Lonnie's Pond include:

- 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);

- Biodeposition of feces and pseudofeces from the oysters was observed in the oyster deployment, and these biodeposits stimulated overall sediment respiration rates and denitrification;
- Biodeposition of nitrogenous particles by the oysters was more than twice the amount of nitrogen the oysters incorporated into shell and tissue biomass;
- Denitrification (transformation of fixed nitrogen to nitrogen gas, N_2) was enhanced in sediments receiving oyster biodeposits; and
- 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.

B. Planning Calculations Based on Water Quality Monitoring Results

Several key future planning calculations can be made based on the data presented in Section 7 and information presented in the SMAST technical report (Howes et al. 2017), including:

- Oyster food availability (Chl-*a* PON and POC); and
- Denitrification contribution to nitrogen-removal over the course of the critical impairment period (July through mid-September).

These factors play a significant role in determining both the daily as well as total nitrogen-removal accomplished by a given quantity and size of oysters. The results of these calculations demonstrate that either Y1 or Y2 oysters can be used to meet both TMDL requirements and total nitrogen load reduction targets.

One key finding of the SMAST report is the food concentrations for summer and fall of 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). Thus the SMAST findings suggest that oyster growth will not be food-limited in Lonnie's Pond. When a high density of grow-out gear is being used, it is important to verify that there is sufficient water flow throughout the field to maintain adequate food concentration. An assessment of this will be undertaken during the second and third years of the demonstration program.

As described in Section 5, an important goal of the water quality monitoring during the 2016 Demonstration was to determine the level of enhanced denitrification achieved by the oysters. The preliminary assessment indicates that the demonstration system removed 17.3 kg of nitrogen by denitrification and 25.9 kg of nitrogen by uptake (increased biomass). 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 study indicated that denitrification should continue to be enhanced past the time of oyster harvest as the sediment incorporated biodeposits continue to contribute to enhanced denitrification. It is not possible at this point to determine from this partial year the full extent of nitrogen removal through denitrification, as the effect will likely continue into the next spring and summer increasing the estimated N removal.

C. Modeling Biodeposition in Lonnie's Pond

It is believed that the denitrification enhancement will be proportional to the amount of biodeposition. Measurements from the SMAST technical report have been used to predict what would happen as the number of oysters deployed in Lonnie's Pond is scaled up using a model developed by the Virginia Institute of Marine Science for the Army Corp of Engineers (Sisson et al, 2011). This model makes it possible to determine two critical parameters:

- Total biodeposition; and
- Timing of biodeposition.

The model suggests that the biodeposition rate of oysters (BD) can be calculated as follows:

BD = $\alpha * T * TSS^{\beta} * e^{-\delta * TSS}$ mg per gram tissue per hour, where:

TSS is the total suspended solids in mg/L; and

T is temperature in degrees C.

The parameters α, β, δ as well as TSS and T are characteristic of the system being modeled and provide an estimate of the expected biodeposition rate as a function of the tissue mass of the oysters in the field. This model accounts for three notable features of biodeposition rates:

- A threshold effect of low seston concentration upon filtration;
- A clogging effect of high seston concentration upon filtration; and
- A positive correlation between filtration and temperature.

In order to model the biodeposition in Lonnie's Pond, values for TSS and T as a function of time were required. Table V.1 of the SMAST technical report (Howes et al. 2017) indicates a declining relationship between TSS and POC. POC changes from 19.5 percent of TSS for 'Summer' to 9.0 percent of TSS for 'Fall'. It was assumed that there was a linear decline in the relationship between TSS and POC from June to October. The TSS values were calculated by applying this linear decline to the measured POC values and are shown by the green line in Figure 32. The plotted points for temperature and POC include values interpolated and extrapolated for the model.

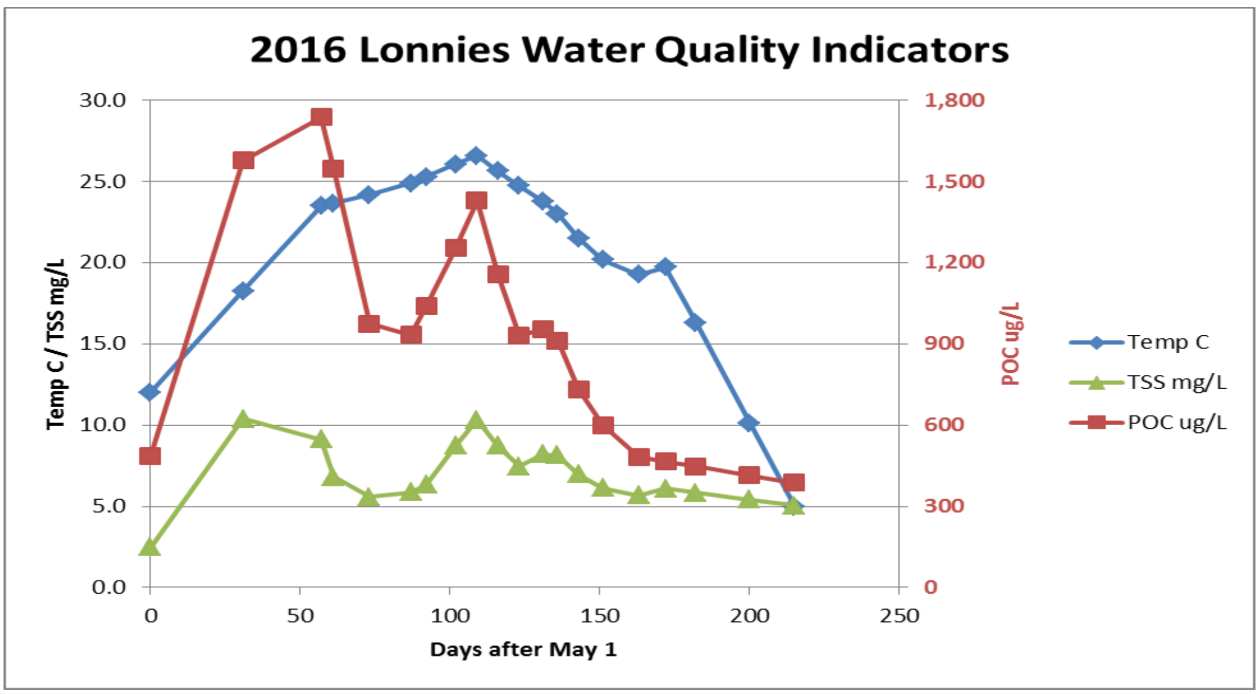

Figure 32 - Data for Temperature and Total Suspended Solids (TSS) Used for Biodeposition Modeling

The values for Temp and TSS shown in Figure 32 were used in equation 1 to determine α=0.0015, β=5.12 and δ=0.64. These values are suitable for modeling Lonnie's Pond, and the predicted biodeposition matches the rates calculated from SMAST Table V.2. The biodeposition data in SMAST Table V.2 was associated with August 16 (Day 107 after May 1) and October 5 (Day 157 after May 1). The biodeposition rates were calculated as:

- August 16, 2016: BD = 35.1 kg/day / 155.2 kg DW = 9.42 mg/g DW/hr; and
- October 5, 2016: BD = 31.7 kg/day / 222.2 kg DW = 5.94 mg/g DW/hr.

These observed points are shown in Figure 33, along with the predictions of equation 1 using the parameters listed.

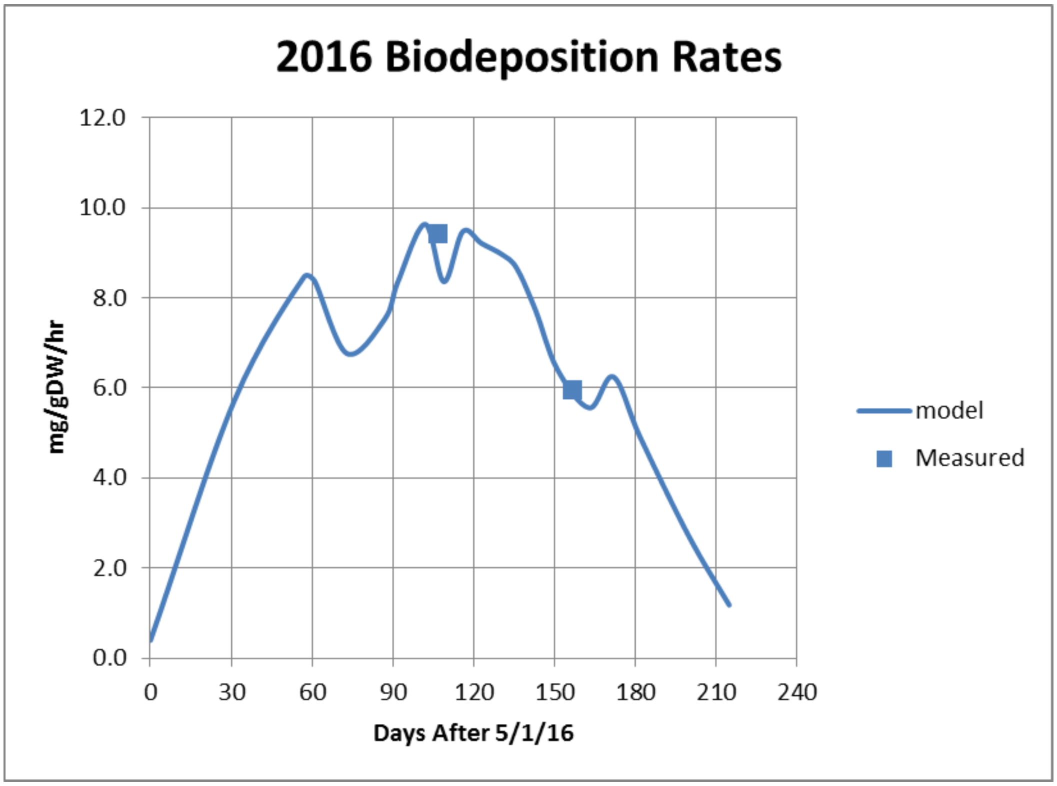

Figure 33 - Predicted Biodeposition Rates during the Growing Season

D. Using the Biodeposition Model to Predict Denitrification in Lonnie's Pond

Figure 13 in Section 7 shows the tissue dry weight as a function of time for first year (Y1) and second year (Y2) oysters. These values were determined based on measurements made during 2016, and extrapolated to cover a full growing season. Multiplying the tissue dry weight in Figure 13 by the biodeposition rate in Figure 33 provides an estimate of the biodeposition rate per oyster over the course of the growing season. The total amount of biodeposits generated by a given deployment of oysters can then be determined by multiplying this rate by the number of oysters per bag, and integrating as a function of time. The results of this calculation are shown in Figure 34. These values were then normalized to the total amount of biodeposits produced over the season to obtain a function expressing the percentage of the total biodeposits that had been produced at any given time during the season. This function is shown in Figure 35.

SMAST provided an estimate of total amount of nitrogen removed by enhanced denitrification that is 67 percent of total nitrogen uptake due to oyster growth over the 2016 demonstration period. The timing of this nitrogen removal by enhanced denitrification was not specified. To estimate when the nitrogen removal by enhanced denitrification would occur in a full-scale system, it was assumed that enhanced denitrification was proportional to the total amount of the biodeposition that had occurred and the amount of accumulated biodeposits is adequate to enhance denitrification by July 1. Using this estimate, the percent of the total nitrogen removed by enhanced denitrification would be equal to the percent of biodeposition that had occurred at each point in the growing season. The amount of projected denitrification for a full-scale system was estimated to be the product of the function in Figure 35, the total amount of nitrogen taken out of the water by growth of tissue and shell, and the enhanced denitrification factor of 67 percent. The results of this calculation are shown in Figure 36. In addition, the line labeled “critical” in Figure 36 represents the critical impairment period which is from July 1 to August 31 (Howes et al. 2006, Howes et al. 2017).

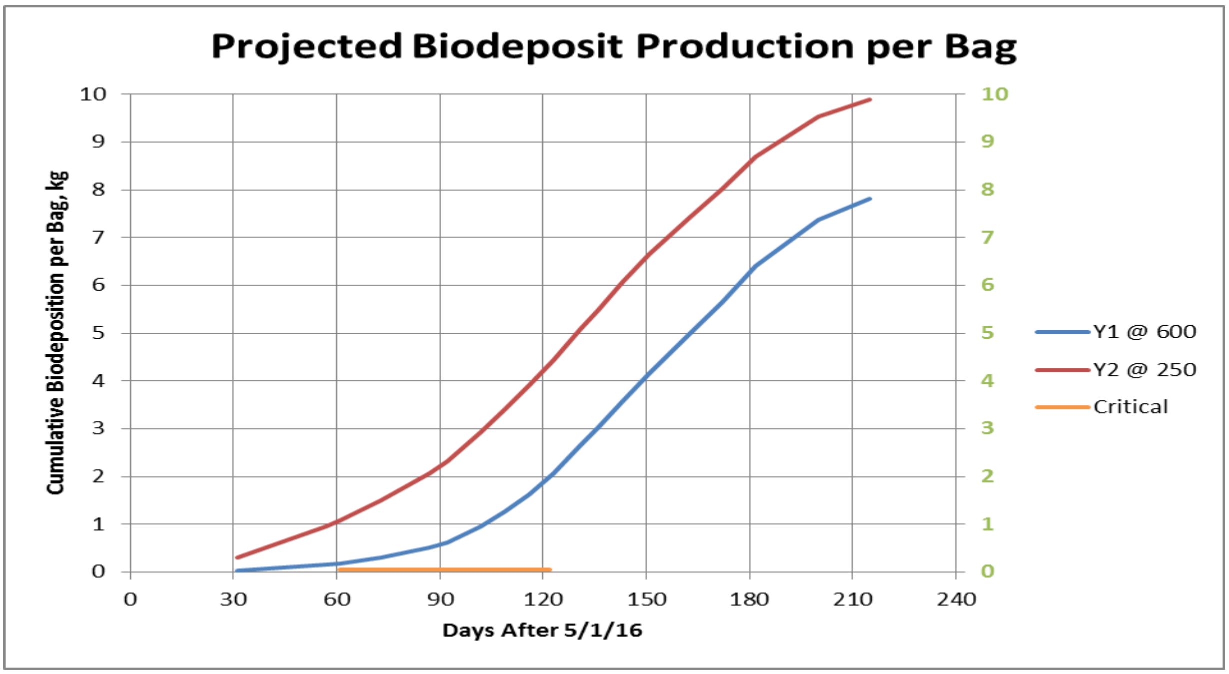

Figure 34 - Predicted accumulation of biodeposits over the course of the growing season from Y1 oysters at a stocking density of 600 per bag and Y2 oysters at a stocking density of 250 per bag

E. Full-Scale Grow-Out: Predictions of Total Nitrogen Removal by Oysters in Lonnie’s Pond

The TMDL for Lonnie’s Pond is 3 kg N/day, which includes nitrogen input from all sources, including controllable sources such as septic systems, fertilizer and stormwater runoff and non-controllable sources such as atmospheric deposition and benthic flux. The nitrogen removal target for Lonnie’s Pond has been calculated by the MEP (Howes et al. 2006) and is approximately 300 kg N/year. This mass, divided by 365 days per year is the daily nitrogen removal that is needed to achieve the overall Total Maximum Daily Load (TMDL) for Lonnie’s Pond. The daily reduction in nitrogen to achieve this TMDL is approximately 0.8 kg N/day.

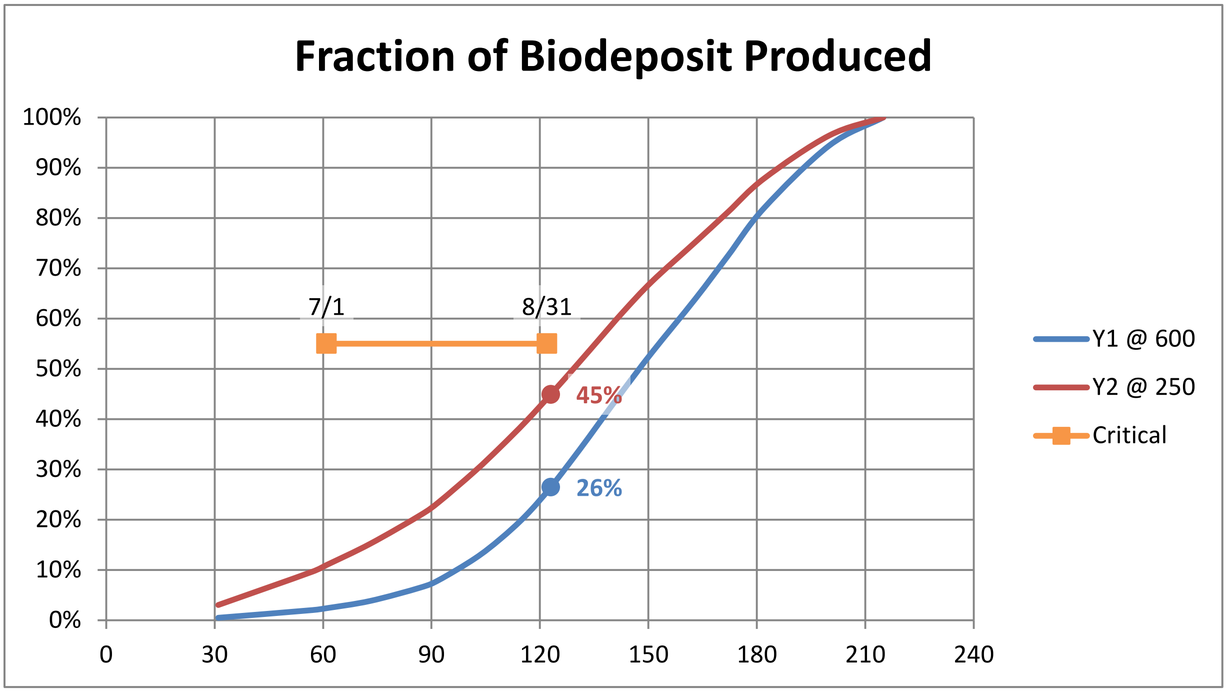

Figure 35 - Fraction of the Total Biodeposits that will be Produced over the Course of the Growing Season

The total nitrogen removed by oysters is the sum of uptake into biomass (tissue and shell) and enhanced denitrification in the sediment. To predict the amount of nitrogen removed from the water due to uptake in the growth of oyster tissue and shell, the tissue dry weight in Figure 13 was multiplied by the nitrogen content from Table 2, which is 10.3 percent for Y2 oysters and 10.5 percent for Y1 oysters. These values, multiplied by 3,000 bags for a Y1 deployment and 4,500 bags for a Y2 deployment, are shown by the double compound lines labeled ‘Y1 U’ and ‘Y2 U’ in Figure 36.

The nitrogen removed through enhanced denitrification added to the nitrogen removed through uptake is shown by the triple compound lines labeled ‘Y1 U+D’ and ‘Y2 U+D’ in Figure 36. The slope of these triple compound lines corresponds to the daily nitrogen removal rate which is associated with TMDL compliance. This calculation is shown by the single solid lines in Figure 36. It is helpful to note that projections for both the nitrogen removal by uptake and the nitrogen removal by enhanced denitrification are made using models driven by the dry tissue weight of the oysters. In order to provide a more efficient and cost effective method for monitoring the actual performance of a full-scale deployment, the second year of the demonstration project will examine whether there is significant seasonal variation in the relationship between tissue dry weight and live harvest weight for oysters grown in Lonnie’s Pond. This is an important factor because dry weight is not a practical parameter for routine monitoring for compliance during full-scale implementation. Harvest wet weight biomass is the measurement that will be routinely made.

The solid single lines are associated with the daily nitrogen removal rate on the left side vertical axis, and include a consideration of the total nitrogen removed by both uptake (U) through new growth as well as enhanced denitrification (D). The compound lines are associated with the annual removal amount on the right side vertical axis. Lines labeled U represent only the nitrogen removed by uptake through new growth. Lines labeled U+D represent the nitrogen removed by uptake through new growth as well as enhanced denitrification.

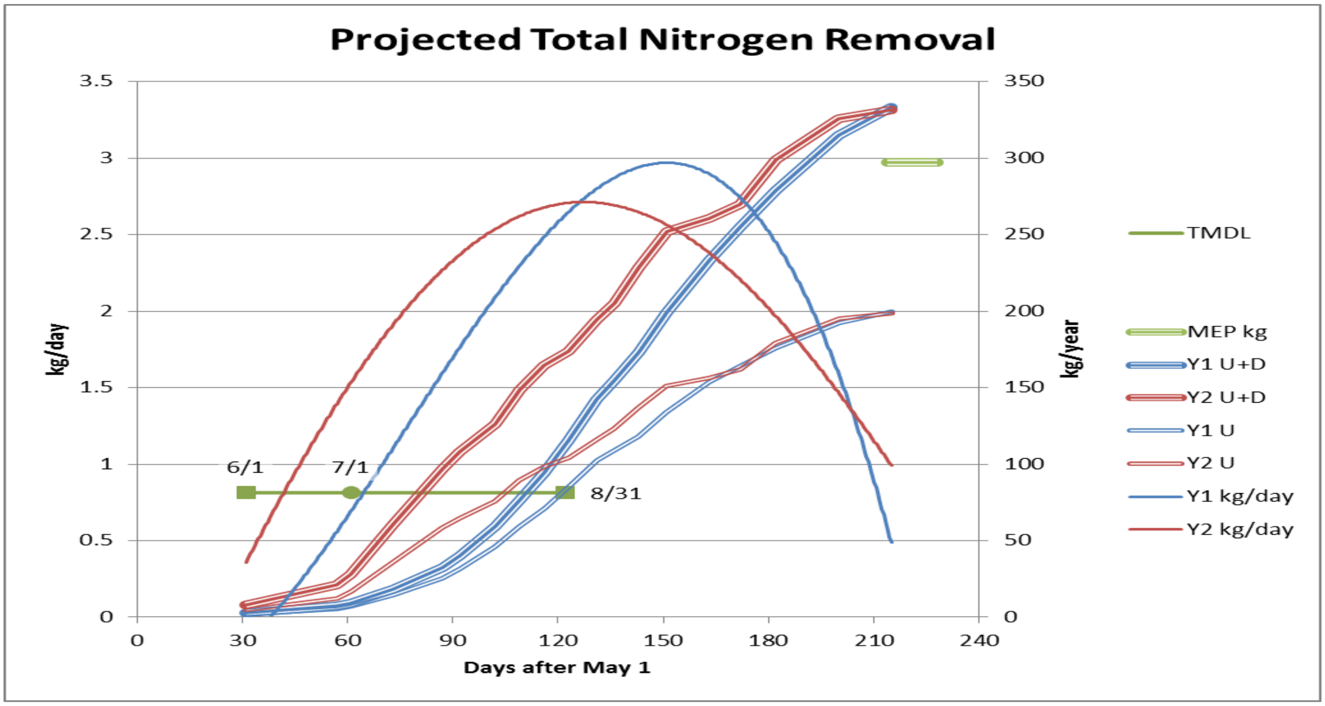

Figure 36 - Projected nitrogen removal for full scale Y1 (3,000 bags at 600 oysters/bag) and Y2 (4,500 bags at 250 oysters/bag) scenarios in Lonnie's Pond

Key observations for the Y1 system as shown in Figure 36 include:

- The Y1 system is expected to meet TMDL requirements in early July and exceed the MEP annual reduction target before the end of the growing season;
- The Y1 system could be scaled up by another 50 percent using a total of 4,500 bags within the one-acre footprint. This scenario would both meet the TMDL requirement approximately ten days earlier than the 3,000-bag deployment, as well as exceed the target total nitrogen removal goal. Approximately 500 kg N will be removed by uptake and denitrification enhancement, and uptake alone would account for the target removal rate of 300 kg N; and
- TMDL compliance and annual load reductions are not affected by the sale of oysters because the oysters are not removed during the growing season.

It may be possible to increase the ending Y1 stocking density to 1,000 oysters per bag. If growth and enhanced denitrification rates scale linearly with the population increase, then a system ending with 4,500 bags at 1,000 oysters per bag would be capable of removing 500 kg of nitrogen through uptake and 832 kg of nitrogen through combined uptake and denitrification enhancement. If these stocking and bag densities can be achieved but there is no interest in removing nitrogen beyond what is required to be removed by TMDL and MEP targets, then the system could be scaled back to end with 1,800 bags covering less than half an acre. While these grow-out densities may seem high to commercial growers, the municipal propagation program operated by the Town of Falmouth has demonstrated comparable growth rates with 900 oysters per bag deployed at 4,300 bags per acre (Figure 37). Part of the activity of the second-year demonstration program will include evaluation of the feasibility of Y1 grow-out of 1,000 oysters per bag with a 4,500 bag per acre density.

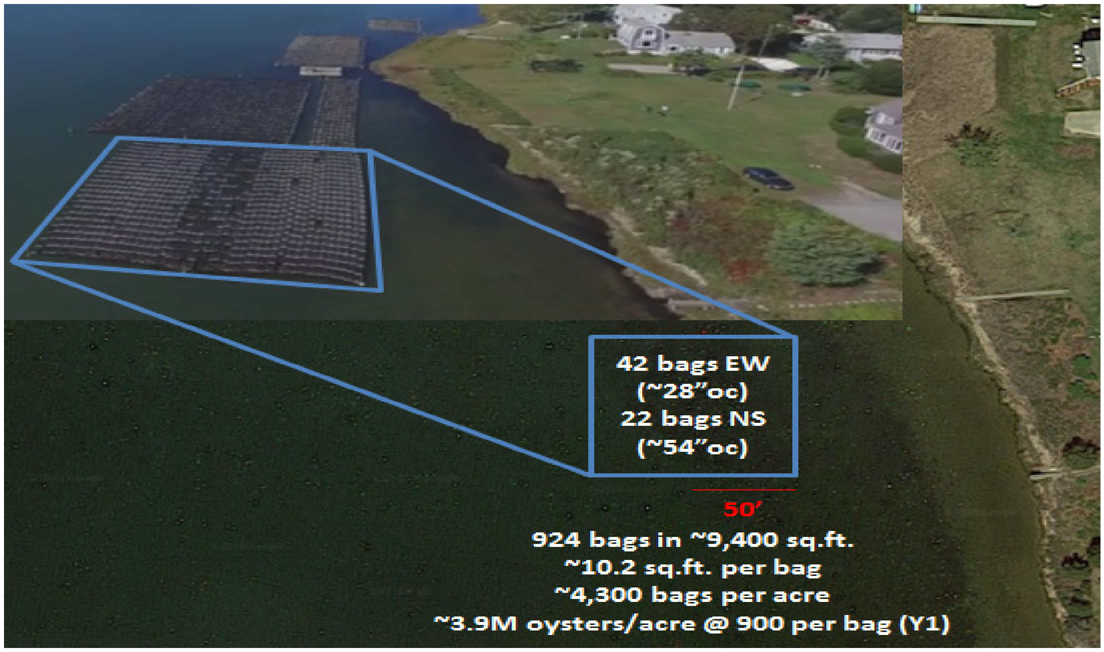

Figure 37 - Municipal Oyster Propagation in Floating Bags in an Estuary, Operated by the Town of Falmouth in 2016

Key observations for the Y2 system as shown in Figure 36 include:

- The Y2 system is expected to meet TMDL requirements in early June and exceed the MEP annual reduction target before the end of the growing season;
- The Y2 system involves nearly complete coverage of the 1 acre site by 4,500 bags through the end of the season. There is no appreciable space for additional bags;
- The Y2 projection was designed to exceed the MEP annual target due to the anticipated removal of some marketable oysters prior to the end of the growing season;
- The removal of a modest number of marketable oysters during the critical impairment period (July and August) is not expected to affect TMDL compliance because the daily nitrogen removal rate (kg per day) ranges from 1.5 to over 2.6 times the requirement during this timeframe when calculated with no removal; and
- There is not adequate space to significantly increase the number of Y2 oysters deployed within the assumed 1-acre area that will be available for use. In order to ensure sufficient uptake and denitrification for TMDL and total annual compliance, the majority of the Y2 oysters need to remain in the field for the majority of the growing season. As a result, a private grower may need to make use of a sizeable line of credit to support the cash flow requirements of a commercial operation. Forcing the grower to wait until late in the season to sell may also reduce the market price they can obtain.

Based on these results, the following recommendations are made for second-year monitoring:

- Assess indicators of food availability such as Chl-*a* and PON at regular intervals throughout the growing season both within and outside the growing area;
- Measure biodeposition and denitrification rates during and after the critical impairment period; and
- Install tide gauges to verify local residence time and tidal exchange from MEP Report (Howes et al. 2006).

11. Long Term Approach for Lonnie's Pond with Cost Analysis

A. Key Considerations

The results of the 2016 Demonstration provide empirical data and information on which to base future planning decisions. This demonstration established several key parameters for Lonnie's Pond, including:

- Over the course of the project, Y1 oysters grew by approximately 1 gram dry tissue per individual and Y2 oysters grew by approximately 1.6 grams dry tissue per individual. Note that due to the late funding of the project by the Town, the project period spanned only 80 to 85 percent of the typical growing season;
- The total nitrogen uptake by oyster growth, as a fraction of the dry tissue weight of the oysters, was approximately 10.5 percent for Y1 oysters and approximately 10.3 percent for Y2 oysters;
- The nitrogen removal by enhanced denitrification was approximately 67 percent of the nitrogen uptake by new growth;
- Throughout the growing season the food availability in Lonnie's Pond remains well above the threshold of 300µg C/L seawater (Tenore and Dunstan 1973) for maximum feeding rate;
- Y2 oysters grow well through the peak impairment period with stocking densities of 230 to 250 per bag;
- Y1 oysters also grow well through the peak impairment period and are expected to grow well at stocking densities of 600-1,000 per bag during this time;
- Floating bags were installed at a density of over 3,600 per acre. Density could be increased to 4,950 per acre with some adjustments to the layout;
- If the project were operated by a commercial grower, then 100 percent of Y2 oysters installed in June could have been sold as petite or standard market size by the end of the same growing season;
- Over 80 percent of Y1 oysters that have been started from 2 to 3 mm seed in early May will be market ready (2.5-inches/64 mm or larger) by early in the next growing season;
- The bottom sediment is believed to be too soft for planting oysters, and predators including oyster drills, conches, starfish, and blue crabs are present in Lonnie's Pond, so off-bottom overwintering and second year grow-out strategies must be used; and
- Lonnie's Pond is 15 acres, but the useable water surface area for aquaculture is not more than one acre so as not to encroach on other uses of this waterbody.

Related factors that impact long-term implementation as well as the second year of this demonstration include:

- 2 to 3 mm seed is available in early May but special arrangements including a cash deposit must be made with a qualified supplier at least six months before the seed is needed;
- Massachusetts now allows the sale of petite oysters which are between 2.5 to 3-inches (64 mm to 72 mm) in length, measured from hinge to shell margin;
- There is an existing pathway for a commercial grower to sell intermediate seed (the end product of growing Y1 oysters) and a probable pathway for the Town to sell intermediate seed. The revenues would be in the range of $125 to $190 per thousand oysters sold;
- Only commercial growers can harvest and sell Y2 oysters (the Town is not allowed to sell through the propagation permit), and the market value ranges from $0.35 to $0.65 per oyster depending on quality;

   - Growers report grow-out stocking densities of 500 to 1,000 for Y1 oysters and 150 to 250 for Y2 oysters. This project in year one has demonstrated the ability to support low mortality growth on the high end of stocking densities. The marketability of these oysters is believed to be high based on communication of photographs and size and weight data to wholesalers; and
   - Nitrogen reduction targets for the Lonnie's Pond watershed are 297 kilograms per year and 0.81 kg/day during the critical impairment period of July and August (Howes et al. 2006).
- These considerations can be used to inform the selection of a long-term implementation strategy for oyster cultivation in Lonnie's Pond. The strategy is limited by the need to maintain the oysters in gear, off the bottom. Since this project is constrained by other recreational uses in Lonnie's Pond, the maximum practical surface grow-out area is one acre, and the maximum anticipated bag density is 4,950 bags per acre. Project options are thus limited to final populations that will fit in 4,950 bags or less. The assessment of nitrogen removal by uptake as well as denitrification discussed in Section 10 and shown in Figure 36 reveals that a deployment with this many bags in a one-acre plot could achieve TMDL compliance and achieve MEP annual targets for nitrogen-removal using either Y1 or Y2 oysters.
- The use of either Y1 or Y2 oysters and different endpoint options for the oysters after the first season of growth leads to eight possible long-term implementation scenarios. Some endpoint options including sale of both intermediate seed and market-size oysters offset the costs associated with growing the oysters. There are four scenarios for each of the two oyster size classes. Scenarios are grouped into categories based on the starting size of seed because costing assumptions and nitrogen-removal are directly linked to these starting seed sizes. Note that some of the scenarios presented do not meet required TMDLs and MEP reduction targets. These options are presented for completeness because the question of retaining oysters for several years has been discussed at SWG meetings, and to show the maximum nitrogen-reduction possible for these scenarios given the constraints of the site.
- Key details of the four Y1 scenarios are summarized in Table 4. Key details of the four Y2 scenarios are summarized in Table 6. Information in these tables includes the total number of bags deployed, the starting and ending number of seed, the sale price of oysters needed for approximate break-even, costs and the kilograms of nitrogen removed. Costs and time requirements are based on estimates extrapolated from the 2016 Demonstration project and other field experience, and are subject to adjustment after the completion of the second year of the demonstration project.
- Assumptions for Table 4 and Table 6 are presented in Table 5 and Table 7 respectively. The Town-managed options assume that existing infrastructure can be used for gear fabrication and storage, and that a boat comparable to a 19-foot Carolina Skiff will be available when needed for occasional field activities. The Town's fully loaded labor rate is assumed to be $27.50 per hour for field work and $35.00 per hour for program oversight. The commercial options assume an investment in all new equipment to support the program, financed at 8 percent over 5 years. It also assumes the employment of insured, skilled labor at a rate of $25.00 per hour including benefits (paid vacation and sick time) and full health insurance.

B. Y1 Scenarios

For the Y1A scenario, the cost per kilogram of nitrogen removed for a Town propagation program that involves disposing of the oysters after one growing season is expected to be approximately $341. It may be possible to bottom plant the Y1 oysters at the end of the season, but this would not substantially change the economics of the program. Moreover, bottom-planting oysters that are only one season old could result in a reversal of the nitrogen benefit if the bottom planted oysters do not survive. The nitrogen contained in the oyster tissue typically represents more than two-thirds of the total nitrogen taken up by growth. If the oyster dies, the nitrogen in the tissue will remain in the estuary in some form.

Table 3 - Summary of Long Term Implementation Scenarios

Program	Description	TMDL / MEP %	$/kg N Cost to Town
Y1A	The Town purchases 2.1 million 2 to 3 mm early start seed every year, grows it in Lonnie's Pond for one season, removes the oysters at the end of the growing season, and disposes of them.	Yes / 110%	$341
Y1B	The Town purchases 2.1 million 2 to 3 mm early start seed every year, grows and overwinters it in Lonnie's Pond, and sells it as intermediate seed.	Yes / 110%	$(67)
Y1C	Commercial grower purchases 2.1 million 2 to 3 mm early start seed every year, grows and overwinters it in Lonnie's Pond, and sells it as intermediate seed.	Yes / 110%	$11
Y1D	Commercial grower purchases 2.1 million 2 to 3 mm early start seed every year, grows it in Lonnie's Pond for one season, overwinters it in Lonnie's Pond, and grows it out for a second year on a site outside Lonnie's Pond and using no more than 20,000 square feet of surface gear.	Yes / 110%	$11
Y2E	The Town purchases 1 million 2 to 3 mm early start seed every year, grows and overwinters it in Lonnie's Pond, uses survivors to stock a predominantly Y2 field in Lonnie's Pond, and bottom plants the Y2 survivors at the end of the second growing season outside of Lonnie's Pond.	No / 92%	$411
Y2F	The Town maintains oysters that are older than Y2 continuously inside Lonnie's Pond, overwintering them there and also purchasing ~190,000 2 to 3 mm early start seed every year to replenish the field as these older oysters die and are removed.	No / 78%	$600
Y2G	Commercial grower purchases all intermediate seed, grows it out in Lonnie's Pond over the course of one season, and by the end of the year has sold everything that survives.	Yes / 106%	$11
Y2H	Commercial grower purchases 1 million 2 to 3 mm early start seed every year, grows and overwinters it in Lonnie's Pond, uses survivors to stock a predominantly Y2 field in Lonnie's Pond, and sells all the Y2 survivors by the end of the growing season	No / 92%	$12

For the Y1B scenario, the Town program includes overwintering the oysters and establishing a pathway for selling intermediate seed at the start of the second growing season. For this scenario, as long as the sale price is $0.139 per oyster for this intermediate seed, the Town is expected to generate over $60 in revenues for each kilogram of nitrogen that is removed. This results in a revenue rather than a cost per kilogram of nitrogen removed. The critical task in this scenario is to establish a pathway for the sale of intermediate seed to private growers. This may be accomplished through the Town obtaining permission to sell directly to growers, or may involve partnering with a seed supplier such as Aquaculture Research Corporation (ARC).

For the Y1C scenario, a commercial grower cultivates and sells intermediate seed. The Town expense associated with the removal of nitrogen is limited to program oversight and is estimated to be on the order of $11 per kilogram of nitrogen. In the Y1C scenario, the Town would not be involved in the growing of oysters. It is expected that a commercial grower could cover their costs at a price for intermediate seed of $0.173 per oyster. The estimates used for a newly established private business result in costs that are higher than the costs that would be experienced by the Town, and the price point for privately-grown intermediate seed is consequently higher than for intermediate seed that is grown by the Town, but is still well within the range of anticipated market prices.

It is likely that a market for intermediate seed exists. Buyers for this intermediate seed would likely be existing growers interested making a profit by cultivating these large oysters for the short period of time required to have these intermediate seed reach marketable size. As shown in Figure 11, eighty percent of the Y1 oysters grown in Lonnie's Pond were market legal as petites or standard oysters by shell length standards at the end of the first-year growing season. If a grower maintained these oysters during the start of the next growing season, they are likely to be standard harvestable size and acceptable to the high quality restaurant market within a few weeks.

To illustrate the likelihood of a market for intermediate seed at the costs presented in this analysis, it is assumed that a grower purchases intermediate seed for $0.20 each, and these oysters are sold for $0.40 each one month later, during the peak season of July and August. At these prices, a grower using 500 bags with 200 oysters each would have a net revenue after paying for the seed of $20,000. The expenses beyond the seed would be minimal and would include a few weeks of work on the water in the summer. Less than 20 such growers across the Cape would be sufficient to create a demand for the oysters produced by the Y1B and Y1C options. The intermediate seed market is not widely developed, and should be verified before a program is selected that depends heavily on the existence of such a market. As part of the work plan for the second year of the demonstration project, issues relating to the sale of intermediate seed will continue to be discussed with DMF and others.

For the Y1D scenario, a grower is allowed to secure a location for second-year grow-out outside of Lonnie's Pond. This is a viable commercial business and all costs incurred are met with a market price of only $0.342 per oyster, including an eight percent profit. There are no restrictions on what time of year the oysters are sold, but in order to maintain typical supply/demand locally a restriction of selling into an off-Cape market is recommended. It is significant to note that the business represented in the Y1D scenario would involve full time employment of six people, with benefits including health insurance.

C. Y2 Scenarios

The Y2E scenario estimates the cost per kilogram of nitrogen removed for a Town propagation program that involves bottom planting Y2 oysters at the end of the growing season would be $411/kg N. This is considered less risky than bottom planting Y1 oysters because the shells will be tougher, therefore the oysters will be less likely to succumb to predators.

The Y2F scenario maintains an oyster population older than Y2, and is projected to cost over $600/kg N while also being unable to achieve MEP annual nitrogen removal targets due to the limited area available for placing floating gear. This option is presented to provide a complete breakdown of all options, it is not recommended for pursuing.

For the Y2G scenario, a commercial grower purchases intermediate seed and grows it out in Lonnie's Pond until it can be taken to market. The activity of removing oysters for market will have an impact on the system's ability to remove nitrogen. Until this effect is quantified using the findings from the second year of the demonstration program, it has been assumed that the program would require the grower to maintain all the oysters in the field through the majority of the growing season in order to meet the TMDL target goal. This increases the grower's costs over what would be experienced if the only consideration was taking oysters to market as soon as they are ready, and is part of what contributes to the relatively high break-even price of $0.398 per oyster.

The Y2H scenario allows the grower to use part of the available area inside Lonnie's Pond to raise Y1 oysters (similar to option Y2E) resulting in a slightly lower break-even price of $0.363 per oyster. The difference is that the profit that would otherwise go to the entity that provided the intermediate seed is retained in this business. If the oyster shape is good and the cup is appropriately deep, this is not an unreasonable price point to achieve. The timing of harvest/sale will also impact market price.

D. Other Considerations

Shellfish aquaculture is a technique for nitrogen removal that can generate a product with significant economic value. The current market for east coast oysters is over $10 million annually - evidence that it pays to grow oysters (Carter Newell, NACE 2017). As shown in Table 3, if the oysters grown for nitrogen removal are not sold, the cost per kilogram of nitrogen removed by shellfish aquaculture rises significantly. The Y1D, Y2G and Y2H scenarios involve selling a significant quantity of standard market size oysters. This could have an unfavorable impact on local market prices. Consequently, it has been assumed that the commercial grower would be required to sell Y2 oysters into an off-Cape market. This increases the grower's costs, as it is necessary to have a refrigerated truck and pay a driver to regularly transport market ready oysters to regional buyers. Key permitting considerations related to the scenarios involving a commercial grower include the granting of this site for private aquaculture at the local level and the determination of unproductive bottom conditions by DMF. If bottom conditions are found to be productive in this section of Lonnie's Pond, a rotational system that moves the one-acre growing area within Lonnie's Pond each year might be employed so that no one area is permanently removed from the public resource. Part of the work plan for the second year of this demonstration is to begin this permitting process in order to evaluate what is feasible to permit.

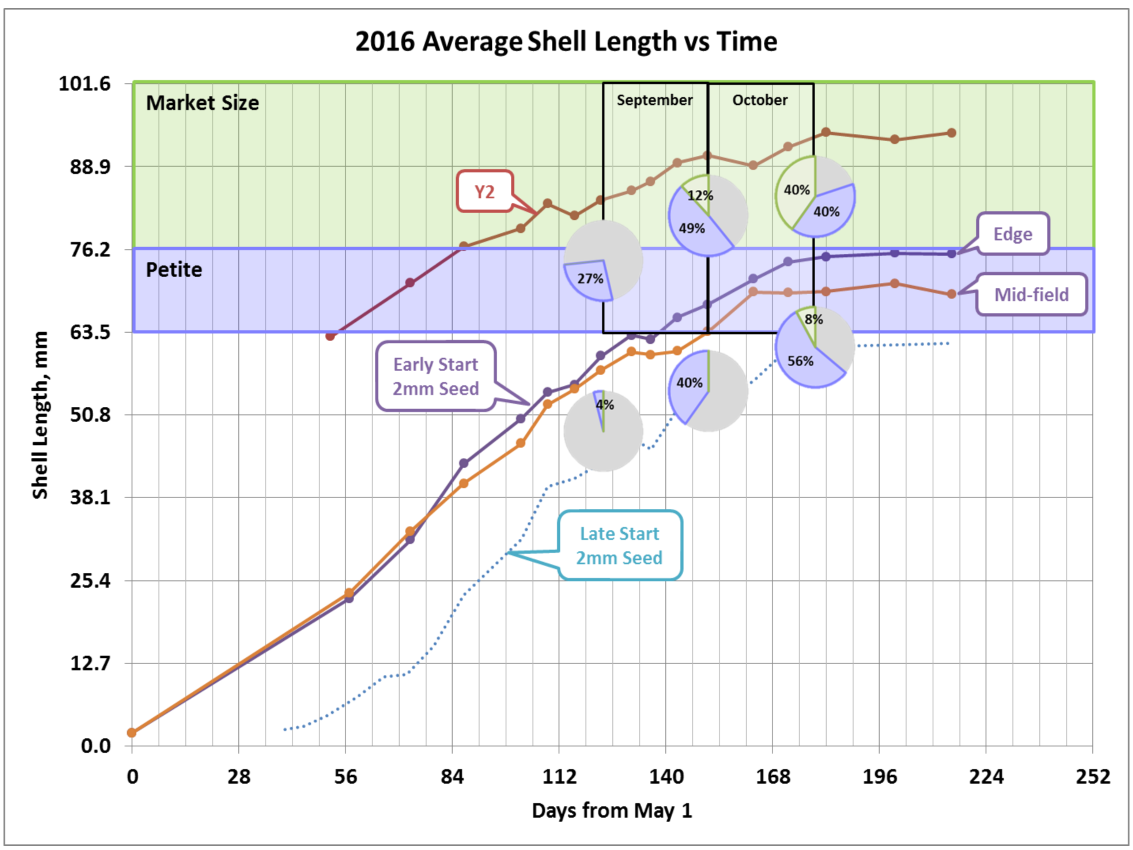

Figure 38 - Average Shell Length and Percent of Total Population to Reach Market Size Over One Growing Season in Lonnie's Pond

Table 4 - Y1 Seed Scenarios for Long Term Implementation

	Y1A	Y1B	Y1C	Y1D
	Town	Town	Grower	Grower
	No Sale	Intermed. Sale	Intermed. Sale	Standard Sale
Y1 Bags	3,000	3,000	3,000	3,000
Y2 Floating Bags				2,100
Y2 Bottom Bags				6,130
Oysters Started	2,120,000	2,120,000	2,120,000	2,120,000
Y1 End Oysters Removed from Lonnies	1,802,000	1,802,000	1,802,000	1,802,000
Y2 End Oysters Grown Outside Lonnies				1,531,700
Sale Price of Program Oysters	$ -	$ 0.139	$ 0.173	$ 0.342
Total Market Value of Program Oysters	$ -	$ 250,478	$ 311,746	$ 725,040
Total Capital for Floating Gear	$ 6,996	$ 6,996	$ 6,996	$ 34,926
Labor for Fabrication of Floating Gear	$ -	$ -	$ -	$ 26,250
Over-winter Gear (Fab Labor & Materials)	$ 30,910	$ 30,910	$ 34,375	$ 34,375
Capital for Other Equipment	$ 5,000	$ 9,300	$ 9,300	$ 241,725
Y1 Capital Requirement	$ 42,906	$ 47,206	$ 50,671	$ 337,276
Amortized Financed Gear Cost	$ 8,581	$ 9,441	$ 12,329	$ 82,065
Seed Cost	$ 33,920	$ 33,920	$ 33,920	$ 33,920
Field Labor Cost	$ 55,350	$ 144,450	$ 200,625	$ 396,844
Business Overhead			$ 39,703	$ 153,085
Total Annual Expenses	$ 97,851	$ 187,811	$ 286,577	$ 665,913
Program Oversight	$ 3,360	$ 42,735	$ 3,360	$ 3,360
Town Net Cost	$ 101,211	$ (19,932)	$ 3,360	$ 3,360
Grower Net Profit			$ 25,169	$ 59,127
Net Profit % of Gross Revenue		8.0%	8.1%	8.2%
N Removed by Uptake, kg	193	193	193	193
N Removed by Denitrification, kg	130	130	130	130
N Removed in Shells (Mortality), kg	3	3	3	3
Annual N Removal, All Pathways, kg	326	326	326	326
% of MEP Annual Removal Target	110%	110%	110%	110%
Ongoing Labor Requirement (hrs/yr)	2,050	5,850	5,850	12,583
Labor Full Time Equivalents	1.0	2.9	2.9	6.3
$/kg of N Removed, All Pathways	$ 341	$ (67.11)	$ 11.31	$ 11.31

Table 5 - Assumptions for Y1 Seed Scenarios for Long Term Implementation

	Y1A	Y1B	Y1C	Y1D
	Town	Town	Grower	Grower
	No Sale	Intermed. Sale	Intermed. Sale	Standard Sale
Number of spat bags	2,120	2,120	2,120	2,120
Spat bag cost	$ 6,996	$ 6,996	$ 6,996	$ 6,996
Floating bag material cost	$ -	$ -	$ -	$ 27,930
Floating bag fab labor cost	$ -	$ -	$ -	$ 26,250
Bottom bag material cost				$ 55,170
Bottom bag fab labor cost				$ 34,481
Winter racks	110	110	110	110
Winter rack materials cost	$ 22,000	$ 22,000	$ 22,000	$ 22,000
Winter rack labor	$ 8,910	$ 8,910	$ 12,375	$ 12,375
Initial Deployment Labor	$ 20,250	$ 20,250	$ 28,125	$ 105,281
Recondition winter bags labor		$ 8,100	$ 11,250	$ 11,250
Split Labor	$ 13,500	$ 13,500	$ 18,750	$ 18,750
Flip Labor	$ 8,100	$ 8,100	$ 11,250	$ 19,125
Remove Gear Labor	$ 13,500	$ 13,500	$ 18,750	$ 31,875
Y1 Grade Labor per cycle		$ 20,250	$ 28,125	$ 28,125
Y1 Grade cycles	0	1	1	1
Y1 Winter Labor	$ -	$ 20,250	$ 28,125	$ 28,125
Y1 Bag to Market		$ 40,500	$ 56,250	
Y2 Bag to Market	$ -	$ -	$ -	$ 154,313
Storage racks	67			183
Storage rack materials	$ 6,667			$ 18,289
Storage rack labor	$ 2,700			$ 7,407
Storage rack sq.ft.				4,572
Other space sq.ft.			500	1,000
Space rental			$ 6,000	$ 57,722
Administrative hours		500	500	2,000
Administrative labor		$ 39,375	$ 18,750	$ 75,000
Fuel cost			$ 390	$ 1,800
NAP Coverage			$ 6,563	$ 6,563
Business insurance			$ 4,000	$ 4,000
Equipment insurance			$ 4,000	$ 8,000
Trips to wholesale			4	20
Other miles, total			500	2,000
Oyster Grader, pkg 5				$ 38,100
Generator				$ 2,000
Salt water pump				$ 500
Grader Float System				$ 20,000
Winter deployment rafts		$ 2,800	$ 2,800	$ 2,800
Equipment delivery costs	$ 500	$ 2,000	$ 2,000	$ 4,000
Number of boats				3
Carolina Skiff 19DX				$ 9,620
Yamaha F90LA				$ 10,405
Boat Trailer				$ 2,650
Number of trucks				2
Chevy 4500 Reefer				$ 49,900
Utility trailer	$ 2,500	$ 2,500	$ 2,500	$ 2,500
Totes, tools	$ 2,000	$ 2,000	$ 2,000	$ 4,000

	Y1	Y2	
Mortality	15%	15%	
Target Seed/bag	1,000		
Target Oysters/bag	600	250	
Denitrification factor (% of uptake)	68%	68%	
Average Weight at Death (DW g/Oyster)	0.500	1.20	
Deployment Weight (DW g/Oyster)	0.02	1.04	
Ending Weight (DW g/Oyster)	1.04	2.50	
	Base	**Loaded**	**Load Factor**
Town Labor Rate	$ 18.00	$ 27.00	1.5
Program Oversight Labor	$ 35.00	$ 52.50	1.5
Grower Labor Rate	$ 25.00	$ 37.50	1.5
Shell N% of DW	2.00%		
Meat N% of live wt	10.50%		
MEP Target kg N	297		
Cost per 1,000 Oysters	$ 16.00		
Materials cost per floating bag	$ 13.30		
Materials cost per bottom bag	$ 9.00		
Cost of spat bag for 2-3mm seed	$ 3.30		
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		
Bags available after Y3	3000		
Storage rental	$ 10.00		
Office rental	$ 12.00		
Inspections per season	6		
Hours per inspection	8		
Report hrs	16		

Table 6 - Y2 Seed Scenarios for Long Term Implementation

	Y2E	Y2F	Y2G	Y2H
	Town	Town	Grower	Grower
	Bottom Plant	Reuse	Intermed. Start	2-3mm Start
Y1 Bags	1,500	187	0	1,500
Y2 Bags	3,000	4,500	4,500	3,000
Y1 Oysters Started	1,040,000	187,000	0	1,040,000
Y2 Oysters Started	890,000	1,058,824	1,330,000	890,000
Oysters Extracted (or retained by Town)	756,500	900,000	1,130,500	756,500
Sale Price of Program Oysters	$ -	$ -	$ 0.395	$ 0.366
Total Market Value of Program Oysters	$ -	$ -	$ 446,548	$ 276,879
Total Capital for Floating Gear	$ 23,382	$ 23,054	$ 19,950	$ 23,382
Labor for Fabrication of Floating Gear	$ 18,750	$ 21,088	$ 18,750	$ 18,750
Over-winter Gear (Fab Labor & Materials)	$ 9,835	$ 54,612	$ -	$ 10,938
Capital for Other Equipment	$ 7,800	$ 7,800	$ 76,575	$ 79,375
Y1 Capital Requirement	$ 59,767	$ 106,554	$ 115,275	$ 132,445
Amortized Financed Gear Cost	$ 11,953	$ 21,311	$ 28,048	$ 32,226
Seed Cost	$ 16,640	$ 2,992	$ 172,900	$ 16,640
Field Labor Cost	$ 80,595	$ 110,517	$ 143,438	$ 149,438
Business Overhead			$ 65,813	$ 56,479
Total Annual Expenses	$ 109,188	$ 134,820	$ 410,198	$ 254,783
Program Oversight	$ 3,360	$ 3,360	$ 3,360	$ 3,360
Town Net Cost	$ 112,548	$ 138,180	$ 3,360	$ 3,360
Grower Net Profit			$ 36,349	$ 22,096
Net Profit % of Gross Revenue			8.1%	8.0%
N Removed by Uptake, kg	161	132	185	161
N Removed by Denitrification, kg	109	89	125	109
N Removed in Shells (Mortality), kg	4	9	5	4
Annual N Removal, All Pathways, kg	274	230	314	274
% of MEP Annual Removal Target	92%	78%	106%	92%
Ongoing Labor Requirement (hrs/yr)	2,985	4,093	4,325	4,485
Labor Full Time Equivalents	1.5	2.0	2.2	2.2
$/kg of Target N Removed	$ 411	$ 600	$ 11.31	$ 12.26

Table 7 - Assumptions for Y2 Seed Scenarios for Long Term Implementation

	Y2E	Y2F	Y2G	Y2H
	Town	Town	Grower	Grower
	Bottom Plant	Reuse	Intermed. Start	2-3mm Start
Number of spat bags	1,040	187	0	1,040
Spat bag cost	$ 3,432	$ 617	$ -	$ 3,432
Floating bags material cost	$ 19,950	$ 22,437	$ 19,950	$ 19,950
Floating bags fab labor cost	$ 18,750	$ 21,088	$ 18,750	$ 18,750
Winter racks	35	194	0	35
Winter rack cost	$ 7,000	$ 38,870	$ -	$ 7,000
Winter rack labor	$ 2,835	$ 15,742	$ -	$ 3,938
Initial Deployment Labor	$ 27,270	$ 31,637	$ 42,188	$ 37,875
Recondition winter bags labor	$ 4,050	$ 12,655		$ 5,625
Split Labor	$ 6,750	$ 842	$ -	$ 9,375
Flip Labor	$ 12,150	$ 12,655	$ 16,875	$ 16,875
Remove Gear Labor	$ 20,250	$ 21,092	$ 28,125	$ 28,125
Y1 Winter Labor	$ 10,125	$ 31,637	$ -	$ 14,063
Y2 to Market Labor			$ 56,250	$ 37,500
Storage racks	67	0	100	67
Storage rack materials	$ 6,667	$ -	$ 10,000	$ 6,667
Storage rack labor	$ 2,700	$ -	$ 4,050	$ 2,700
Storage rack sq.ft.			2,500	1,667
Other space sq.ft.			500	500
Space rental			$ 31,000	$ 22,667
Administrative hours			500	500
Administrative labor			$ 18,750	$ 18,750
NAP Coverage			$ 6,563	$ 6,563
Business insurance			$ 2,500	$ 2,500
Business misc			$ 3,000	$ 2,000
Equipment insurance			$ 4,000	$ 4,000
Equipment delivery costs	$ 500	$ 500	$ 2,000	$ 2,000
Carolina Skiff 19DX			$ 9,620	$ 9,620
Yamaha F90LA			$ 10,405	$ 10,405
Boat Trailer			$ 2,650	$ 2,650
Winter rafts	$ 2,800	$ 2,800		$ 2,800
Chevy 4500 Reefer			$ 49,900	$ 49,900
Utility trailer	$ 2,500	$ 2,500		
Totes, tools	$ 2,000	$ 2,000	$ 2,000	$ 2,000

	Y1	Y2	Y3
Mortality	15%	15%	15%
Seed cost/1,000 oysters	$ 16	$ 130	
Target Seed/bag	1,000		
Target Oysters/bag	600	250	200
Denitrification factor (% of uptake)	68%	68%	68%
Average Weight at Death (DW g/Oyster)	0.250	1.20	2.50
Deployment Weight (DW g/Oyster)	0.020	0.49	2.10
Ending Weight (DW g/Oyster)	0.49	2.1	3.5
	Base	**Loaded**	**Load Factor**
Town Labor Rate	$ 18.00	$ 27.00	1.5
Program Oversight Labor	$ 35.00	$ 52.50	1.5
Grower Labor Rate	$ 25.00	$ 37.50	1.5
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	$ 3.30		
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		
Bags available after Y3	3000		
Storage rental	$ 10.00		
Office rental	$ 12.00		
Inspections per season	6		
Hours per inspection	8		
Report hrs	16		

E. Gear Layout for Long Term Implementation

The overall footprint of the required gear and the timing of gear installation are both important considerations. Figure 39 illustrates the gear footprint required for a full-scale deployment as well as the time during the growing season when different phases of installation occur. The estimates for the number of floating bags required are made by combining a consideration of the volume requirement for the oysters with the size and number of bags in the plots that would be used.

The Y1 and Y2 scenarios have important timing differences. The Y2 scenarios require more total bags to be deployed, and the bags would be installed earlier in the season. By June 15, two-thirds of the one-acre site is filled, and the entire acre is used starting August 15 in the Y2 scenarios. For Y1 oysters, two-thirds of the site would be filled by approximately August 1, and the entire site would not be used until October 1, well past peak season. The dates shown for when the field would be expanded are approximate, and would depend on actual growth of the oysters. It is possible they would shift earlier or later by as much as 7 to 10 days.

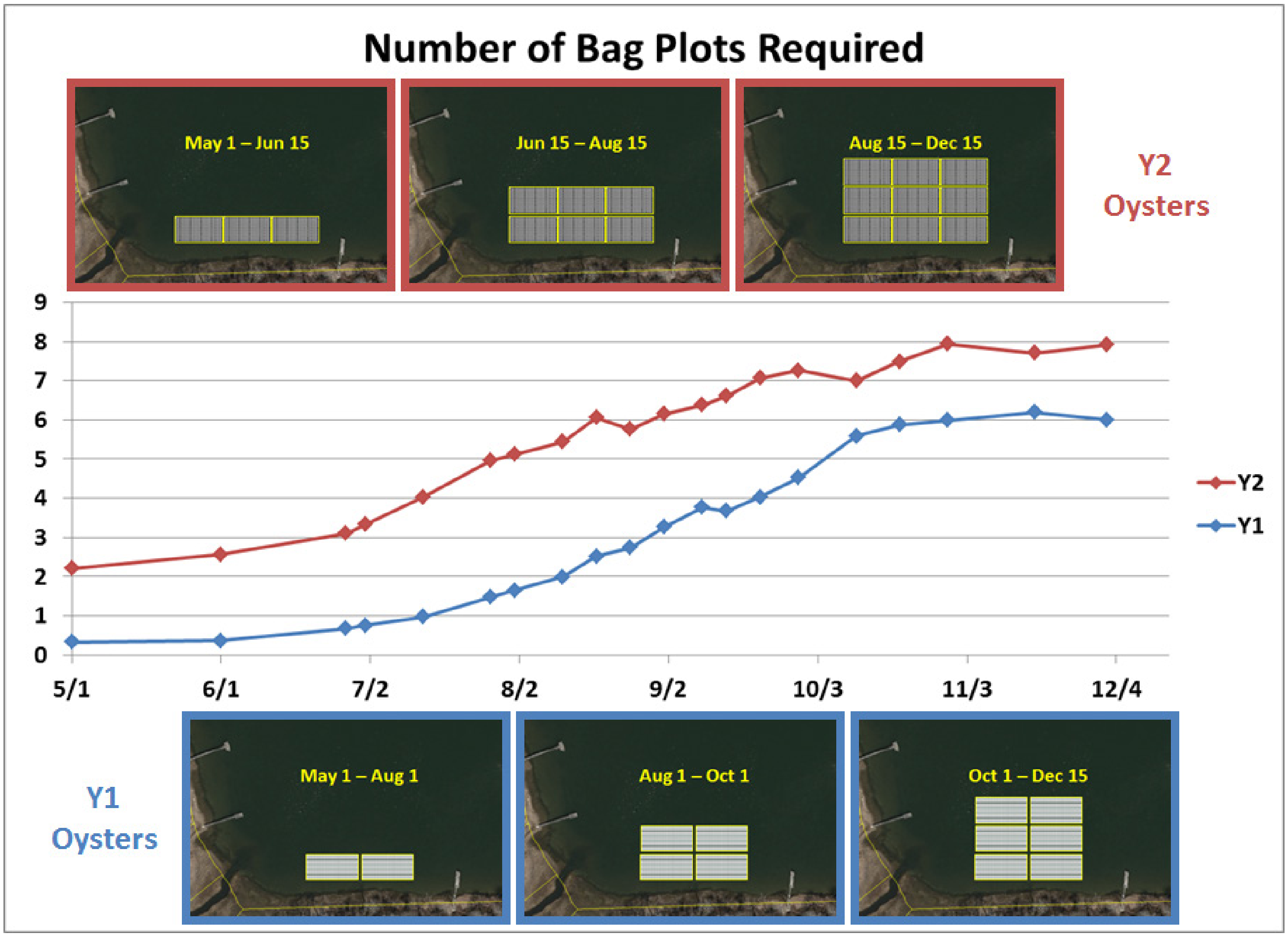

Figure 39 - Installation of Gear Throughout the Growing Season

F. Details of Plot Configurations

The first-year demonstration project footprint used a proven heavy-duty long line layout and achieved a density of approximately 3,600 bags per acre. Recognizing that Lonnie's Pond is well sheltered, it is possible to achieve a slightly higher-density layout of 4,800 bags per acre by simply locating the adjacent strings closer together. The layout of a 500 bag plot is shown below. Nine such plots could be placed inside a one acre field, achieving a layout density of 4,500 bags per acre. This bag configuration has been used for all Y1 scenarios.

An alternative configuration used in estuaries of other towns involves stringing bags in rows between perimeter lines. The bags are easily flipped by a worker traveling in between the rows. A typical configuration would involve placing 550 bags in a 50 foot x 92 foot plot as shown below. Nine such plots could be placed inside a one acre field, achieving a layout density of 4,950 bags per acre. This bag configuration is used for Y2 scenarios.

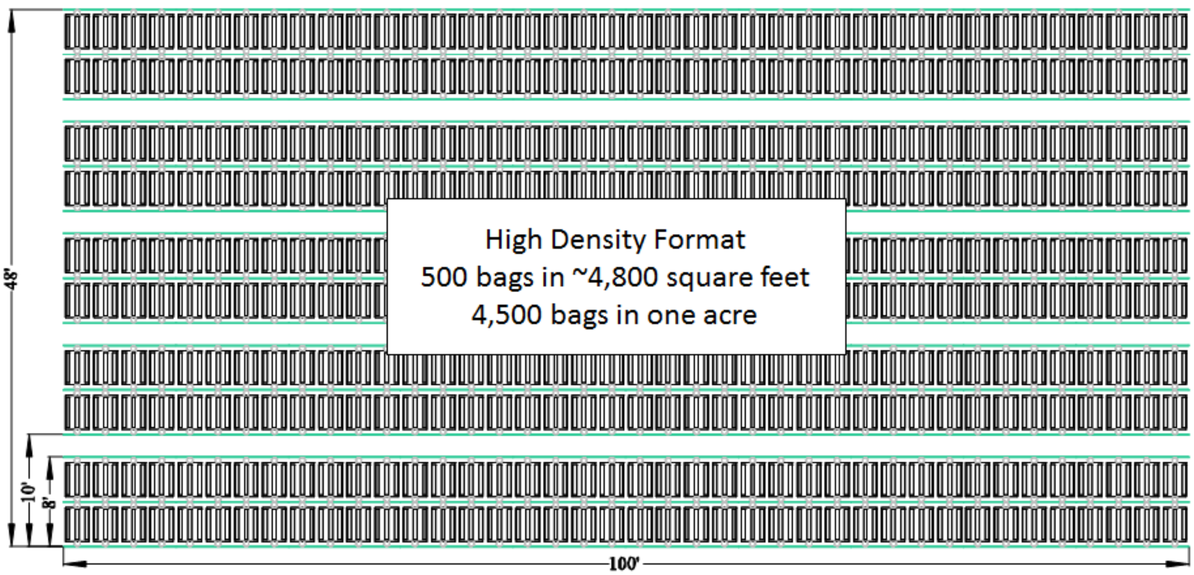

Figure 40 - Layout for Plots with 500 Floating Bags Each for Y1 Installations

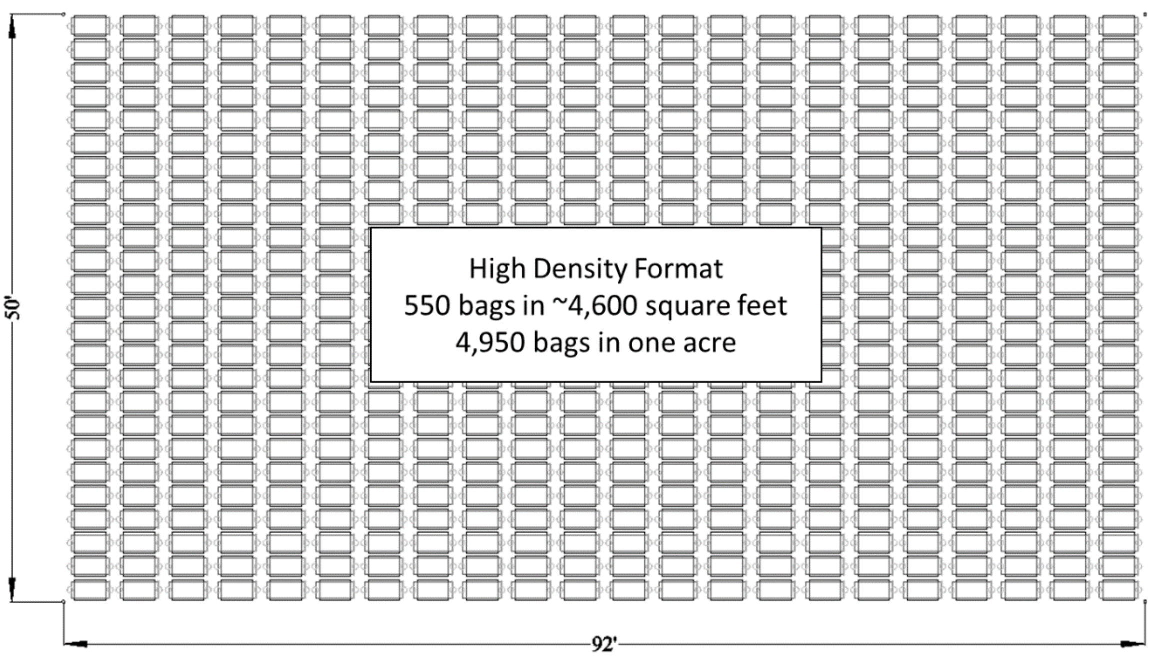

Figure 41 - Layout for Plots with 550 Floating Bags Each for Y2 Installation Scenarios

12. Second Year Program for Lonnie's Pond Demonstration

This section outlines the original recommendations for year 2 of the Lonnie's Pond Demonstration at of late winter 2017 when the draft version of this year 1 report was first completed. It should be noted that since that time, some of the plans for the second year were curtailed due to budget constraints as well as Town of Orleans decisions regarding program implementation; therefore, some of the original recommendations below were not implemented. The implemented year two demonstration project will be fully documented in the year 2 report.

A. General

The purpose of the second-year demonstration is to further refine a long-term implementation plan that uses shellfish to remove nitrogen in Lonnie's Pond, and continue to collect data needed to obtain regulatory approvals for the use of shellfish aquaculture to achieve nitrogen removal goals. Eight possible scenarios were presented in Section 11. These scenarios are based on the findings from the 2016 Demonstration, and include both Y1 and Y2 oysters.

The second-year demonstration for Lonnie's Pond involves deploying oysters within four separate plots, and initiating a scientific evaluation of quahogs for nitrogen removal. This configuration will provide additional information about the nitrogen uptake and denitrification effects of both Y1 and Y2 oysters as well as quahog growth rates, nitrogen-uptake and enhanced denitrification effects.

The following activities will be undertaken during the second year of the demonstration project:

- Assess the growth of Y1 oysters starting at 2 to 3 mm and targeting an average of 1 gram of dry tissue weight in a single growing season with a final grow-out stocking density of 600 oysters per bag;
- Assess the growth of Y1 oysters starting at 2 to 3 mm and targeting an average of 1 gram of dry tissue weight in a single growing season with a final grow-out stocking density of 1,000 oysters per bag;
- Assess the growth of Y2 oysters from intermediate seed with an average dry tissue weight of 0.5 grams and length of 2mm and an initial stocking density of 280 oysters per bag; and targeting market-ready oysters with a dry tissue weight averaging 2.5 grams with a final grow-out stocking density of 250 oysters per bag;
- Assess the growth of 2-inch, overwintered oysters installed in 510 bags;
- Assess the growth of Y1 and Y2 oysters using floating bag densities achieving up to 4,950 bags per acre;
- Assess the growth and survival rates and nitrogen content of quahogs in Lonnie's Pond;
- 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 amount of difference, if any, in the denitrification rate if maintenance is done on foot as opposed to by boat;
- Evaluate public and abutter acceptance;
- Evaluate acceptance and compatibility with other local growers and commercial shellfish harvesters;
- Review the options with DMF for sale by the Town of intermediate seed; and
- Identify any permitting issues for a commercial site license (grant) in Lonnie's Pond.

This second year of the three-year demonstration project involves deploying a system that is approximately half the size of the long-term implementation plan. This demonstration year will install approximately 168,000 Y2 oysters and 950,000 Y1 oysters distributed among four plots. Each plot will have 500 bags and be similar in size and shape to that envisioned for full-scale implementation. A fifth plot not influenced by the oyster plots will be bottom-planted with 100,000 seed quahogs made uniquely identifiable prior to harvesting. The purpose of each plot is to monitor the time dependence of nitrogen removal rates via uptake into biomass, biodeposition, and denitrification for the following:

- Plot A: 140,000 Y2 oysters at an initial stocking density of approximately 280 oysters per bag, adding biodeposits on top of the same area used by last year's site;

- Plot B: 60,000 Y2-L oysters (grown in Lonnie's in 2016) at an initial stocking density of approximately 150 oysters per bag and 28,000 Y2 oysters at an initial stocking density of approximately 280 oysters per bag, over deeper water and softer bottom than last year's site;
- Plot C: 590,000 Y1 oysters at a stocking density of approximately 1,000 oysters per bag, over bottom similar to that used by last year's site;
- Plot D: 360,000 Y1 oysters at a stocking density of 600 oysters per bag over deeper water and softer bottom than last year's site; and
- Plot Q: 100,000 15 to 20 mm quahogs at a suitable location to be determined.

This scale of demonstration will provide the second year of evidence for achievable rates of nitrogen removal through uptake during oyster growth as well as nitrogen removal through enhanced denitrification. Conducting a second year of this demonstration at half-scale allows details regarding monitoring requirements for regulatory approvals to be refined with MassDEP.

Another feature of installing the oyster fields as configured for the second-year demonstration is that it will give the community an opportunity to preview the visual and operational impacts that are comparable to a full-scale program. Demonstrating at this scale also reduces any uncertainty for the Town as well as potential growers. The results of this second year will verify key parameters that are important to the entity implementing this approach over the long-term.

The area that will be used for the second year of the project is shown in Figure 42.

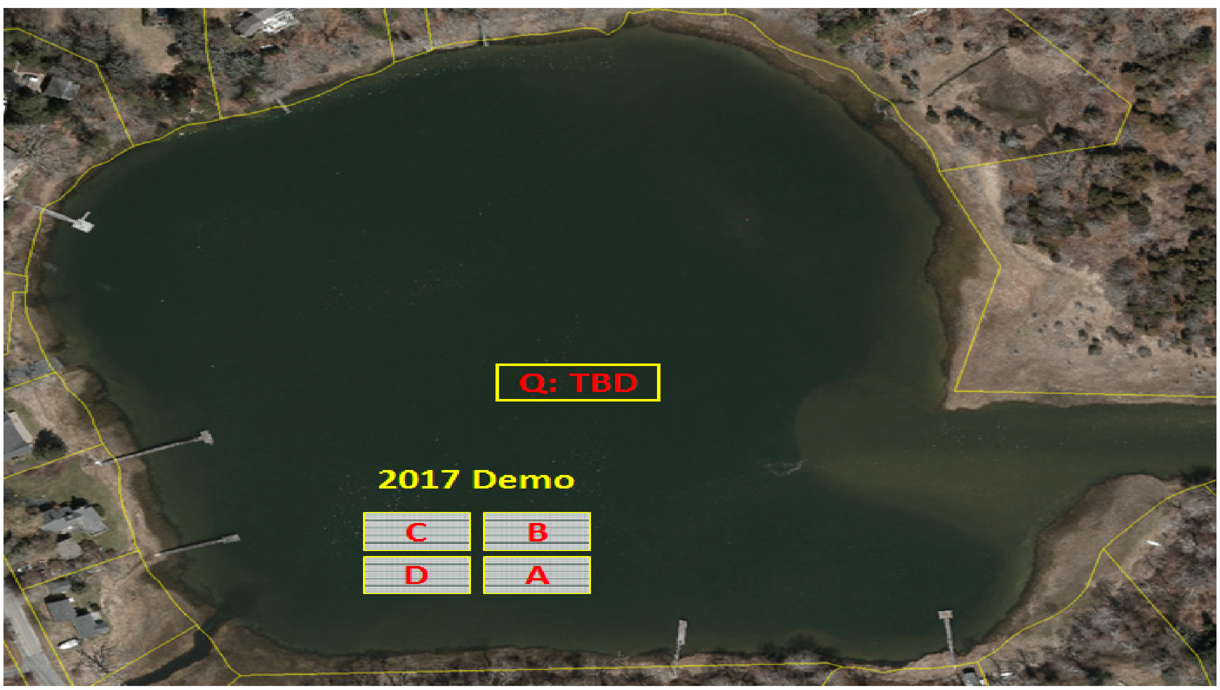

Figure 42 - Layout of floating gear for oyster plots anticipated during the second year of the demonstration project. The location of the quahog site is still to be determined.

B. Seed Procurement and Installation Steps

Both oyster and quahog seed will be procured as follows:

- A population of 100,000 15 to 20 mm quahogs will be ordered from ARC; and
- Arrangements will be made for an early May delivery of 950,000 oyster seed (2 to 3 mm) and 168,000 intermediate oyster seed.

An additional 1,020 floating bags will be needed to support the second year of the project, and the materials for these bags and reconditioning of the overwintered bags will be ordered. Standard procurement practices will be used to ensure competitive bids are received.

The more favorable Y2 scenarios described in Section 11 involve deploying intermediate seed with an average dry tissue weight of about 0.5 grams per oyster on May 1 at an initial stocking density that targets a final population of 250 per bag. To achieve this population density, the initial stocking density will be 280 oysters per bag to account for 15 percent mortality. The assumption of dry weight per individual is based on expected availability from suppliers for the 2017 growing season. As soon as they are available, a population of 140,000 intermediate seed oysters will be used to stock 500 bags at 280 individuals per bag to occupy Plot A. This will provide a system for monitoring the outcome of long term strategy Y2G, and will also support an analysis of increased denitrification rates due to multi-year accumulation of biodeposits from Y2 oysters.

As soon as the water temperature rises sufficiently, expected by mid-April, the oysters that were overwintered at the end of 2016 will be brought to the surface and removed from the bags that were submerged. Shells of dead oysters will be separated out for a mortality assessment. Total live weights will be measured, and representative samples of individuals will be weighed and measured to assess growth during the winter period. These oysters will be used as part of the second-year demonstration.

The population of approximately 60,000 oysters that was grown as Y1 in 2016 will be grown for a second year in 2017. These oysters are larger than the intermediate seed available from other suppliers. These oysters are identified as Y2-L because they were grown as Y1 in Lonnie's Pond and are consequently larger when they enter their second year of growth. 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. These 60,000 Y2-L oysters will be used to stock 400 bags at a density of 150 per bag. These bags will be deployed in Plot B, and the plot will be filled out with 100 bags of 280 intermediate seed oysters having an initial dry weight averaging 0.5g. This plot B, along with Plot A, will support monitoring related to long term strategies Y2E, Y2F, and Y2H.

The 950,000 early-start 2 to 3 mm seed will be distributed into spat bags at densities of about 1,000 per bag, resulting in 950 loaded spat bags. Reitsma (NACE 2017) has reported excellent success placing two spat bags in one floating bag, and this recommendation will be followed for the initial growth period, thus requiring a deployment of 475 floating bags, which will take place at Plot D.

When the oysters have grown to a point where they can be retained in a floating bag with 6 mm mesh, they will be removed from the spat bags, which will be cleaned and stored for reuse. This is expected to occur in the beginning of July. When the oysters have doubled in volume, expected to be by the end of July, they will be distributed into 500 floating bags at approximately 700 per bag (targeting 600 per bag at an 85 percent survival rate), which will be placed at Plot C, and 500 floating bags at about 1,200 per bag (targeting 1,000 per bag at an 85 percent survival rate) which will be placed at Plot D. These plots will support monitoring related to the long-term strategies Y1A, Y1B, Y1C, Y1D, Y2E, and Y2H. Plot D will also support an analysis of increased denitrification rates due to multi-year accumulation of biodeposits from Y1 oysters.

C. Options for Overwintered Third Year Oysters

As detailed in Section 11, there is no economically favorable long term scenario that involves retaining oysters that are older than Y2 inside Lonnie's Pond. At $600/kg, the Y2F strategy has the highest cost per kg of nitrogen removed of all the envisioned strategies. The main cost driver for the Y2F scenario that retains oysters over several years is the need to overwinter a large number of bags. In addition, the projected new growth per unit volume of gear occupied slows down as the oyster gets older. The space required to hold oysters in floating bags exceeds the area available in Lonnie's Pond. For this reason, oysters that are larger than Y2 cannot meet the TMDL or MEP nitrogen reduction targets within Lonnie's Pond.

Because the oysters that were Y2 for the 2016 project are entering their third year of growth, they should be more resistant to predators and disease. It is recommended that these now Y3 oysters be bottom planted in an area with suitable bottom outside Lonnie's Pond where they can be studied to monitor mortality and growth if desired. This would help the Town assess the feasibility of establishing bottom planted oyster beds as part of an overall strategy for improving water quality. For example, bottom planting at Kent's Point could provide valuable information for the viability study currently planned at this location, allowing predation to be monitored over the spring, summer, and fall of 2017, before the remote-set has grown to a suitable size for bottom-planting. This approach would first be discussed with the Shellfish Working Group and other stakeholders.

D. Budget for Second Year of Lonnie's Pond Demonstration

The budget for the second-year spans two Town fiscal years (2017 and 2018) for the Lonnie's Pond demonstration project. The details of the costs associated with operating the second year of the demonstration program through the end of the Town's 2017 fiscal year ending June 30, 2017 are shown in Table 8. For budgeting purposes in 2016, the placeholder planning number of oysters was estimated as 500,000. This estimate was for budgeting purposes before the 2016 Demonstration had begun. At that time, no monitoring data had been collected. Based on the monitoring results of the 2016 Demonstration, two sizes of oysters are recommended. The budget for these oysters does not exceed the initial planning budget for seed that was proposed.

After carrying forward this credit, an additional $192,000 is required to complete the second year of the demonstration and an additional $152,000 is needed for the portion of the third year of the demonstration that occurs during FY2018. The total FY2018 budget is $344,000, which includes both completing the second year and starting the anticipated third year of the demonstration. Table 9 details the costs included in the FY2018 budget.

Table 8 - Fiscal Year 2017 for Second Year of the Lonnie's Pond Demonstration Cost for Second Year through June 30, 2017

Description	Amount
Project Management	$21,000
2 to 3mm oyster seed	$15,200
Intermediate oyster seed	$31,920
15 to 20mm quahog seed	$5,000
Spat bags	3,140
Materials for 1,200 bags and lines	$15,960
Materials to recondition 800 bags	$2,400
Labor for 1,200 new bags and lines	$16,00
Labor to recondition 800 winter bags	$4,000
Field sampling and sample prep	$7,200
N analysis	$3,900
Materials and supplies for sampling	$3,180
Oyster Deploy Labor	$20,000
Quahog Deploy Labor	$2,000
Flip and Maintain Labor	$4,000
Engineering	$27,300
Contingency	$15,500
Total for Demo Year 2 – Part 1	**$197,700**

Table 9 - Fiscal Year 2018 Overlapping Second and Third Year of the Lonnie's Pond Demonstration Costs to Complete Second Year Demonstration Project by December 30, 2017

Description	Amount
Project Management	$24,600
Field sampling and sample prep	$24,000
Materials and supplies for sampling	$6,400
N analysis	$7,800
Additional overwinter gear	$3,200
First split Labor	$2,000
Second split Labor	$2,000
Flip and Maintain Labor	$10,000
Overwintering Labor	$10,000
Y2 Bottom Planting Labor	$4,000
Permitting	$25,000
Year 2 Final Report	$30,000
Public Engagement	$6,000
Engineering	$27,900
Contingency	$9,000
2017-2018 SMAST Monitoring	$47,000
Total for Demo Year 2 – Part 2	**$239,000**

Anticipated costs to begin third year demonstration project and operate through June 30, 2018

Description	Amount
Project Management	$23,400
2 to 3mm oyster seed	$33,920
15 to 20mm quahog seed	$10,000
Materials for 1,000 bags and lines	$13,300
Materials to recondition 1,000 winter bags	$980
Recondition 1,000 winter bags	$5,000
Full Scale Y1X Deployment Labor	$30,000
Engineering	$23,700
Contingency	$11,700
Total for Demo Year 3	**$152,000**

13. Next Steps

- Decision to proceed with the second year of the Lonnie's Pond demonstration (decision needed by early March if the demonstration is to continue, otherwise it may not be possible to obtain seed for an early start and materials for floating bags);
- Permitting for the second-year demonstration (Request for Determination of Applicability with Conservation Commission);
- Purchasing and assembly of gear and shellfish seed; and
- Work with SMAST to finalize the monitoring program.

Implementing the second year of the Lonnie's Pond demonstration requires a Request for Determination of Applicability decision from the Conservation Commission, purchasing seed and gear, assembling 1,200 floating bags, bringing up the 800 bags of shellfish overwintered in 2016, and working with SMAST to finalize the experimental program. Funding for these tasks has been authorized by both May 2016 Town Meeting and a town-wide ballot vote in October 2016.

A decision to proceed in early March by the Board of Selectmen is important to the successful implementation of this proposed program.

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