Lonnie's Pond Aquaculture Management Plan

Town of Orleans, MA | Water Quality and Wastewater Planning

August 4, 2017


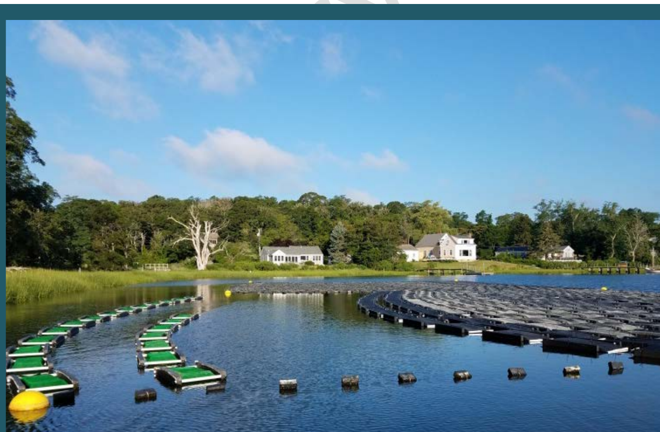

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Table of Contents

Executive Summary 1
1.0 Introduction 4
1.1 Background 4
1.1.1 Cape Cod Commission 208 Plan 4
1.1.2 Orleans Amended CWMP 4
1.1.3 Non-Traditional Technologies 4
1.1.4 Aquaculture/Shellfish Propagation 4
1.1.5 Lonnie's Pond 5
1.2 Goals and Organization of Document 5
1.3 Board of Selectmen Approval of Management Plan 5
1.3.1 Shellfish and Waterways Improvement Advisory Committee Review 5
1.3.2 Board of Selectmen Review 6
1.3.3 Consideration at the Fall Town Meeting 6
1.4 Town Staffing and Communication Channels 6
2.0 Lonnie's Pond Demonstration Project Planning 7
3.0 Lonnie's Pond Demonstration Project Year 1 (2016) 7
3.1 Field Installation 7
3.2 Operation and Maintenance 8
3.3 Growing Season Monitoring 8
3.4 Oyster Growth, Mortality, and Nitrogen Content 9
3.5 Quality Control 10
3.6 Overwintering System Design and Installation 11
3.7 Overwintering System Installation 11
3.8 Winter Monitoring 11
3.9 Resurfacing of Overwintered Oysters 12
3.10 Cost 13
4.0 Lonnie's Pond Demonstration Project Year 2 (2017) 13
4.1 Field Design and Equipment 13
4.2 Shellfish Acquisition and Installation 13
4.3 Maintenance and Monitoring 14
4.4 Shellfish Data Collection and Reporting 14
4.5 Additional Activities 14
4.6 Cost 15
5.0 Lonnie's Pond Demonstration Project Year 3 (2018) 15
5.1 Field Design and Equipment 15
5.2 Shellfish Acquisition and Installation 16
5.3 Management Measures 17
5.3.1 Equipment Monitoring and Maintenance Tasks 17

5.3.2 Additional Permitting 17
5.4 Shellfish Data Collection and Reporting 17
5.5 Shellfish Disposition 17
5.6 Cost 17
5.7 Preparation and Approval of Budget Items 18
5.8 Key Decisions and Associated Dates 18
6.0 Oyster Viability Considerations 19
6.1 Ocean Acidification 19
6.2 Predators 19
6.2.1 Crabs: Green, Blue, Calico (aka Lady), Mud, Spider, Rock, Asian Shore 19
6.2.2 Oyster Drills 19
6.2.3 Birds: Oyster Catchers and Seagulls 19
6.3 Disease 19
6.3.1 Juvenile Oyster Disease 20
6.3.2 Dermo, Perkinsus marinus 20
6.3.3 MSX or Multinucleated Sphere Unknown, *Haplosporidian nelson* 20
6.4 Algal Blooms 20
6.4.1 Macroalgae/Seaweed 20
6.5 Biofouling/ Pests 20
6.5.1 Sea Squirts/ tunicates/ hydroids 20
6.5.2 Mud Blisters 20
6.5.3 Boring Sponge 20
6.6 Storm Damage 21
6.7 Theft 21
7.0 Water Quality Monitoring and Reporting 21
7.1 Summary of Water Quality Monitoring Results for the 2016 Demonstration 21
7.2 Sampling Details 21
7.3 Major Results of 2016 Demonstration Monitoring 22
7.4 Planning Calculations Based on Water Quality Monitoring Results 22
7.5 Modeling Biodeposition in Lonnie's Pond 23
7.6 Using the Biodeposition Model to Predict Denitrification in Lonnie's Pond 23
7.7 Full-Scale Grow-Out: Predictions of Total Nitrogen Removal by Oysters in Lonnie's Pond 25
7.8 Key observations for the Y1 System 26
7.9 Key observations for the Y2 System 26
7.10 Recommendations for Second-Year Monitoring 26
8.0 Considerations for Full-Scale Aquaculture at Lonnie's Pond 27
8.1 Design 27
8.2 Costs 27
8.3 Transition to Commercial Growers 27
8.4 State Revolving Fund (SRF) or other financing options 28

8.5 Permitting ... 28
8.6 Public Engagement ... 28
8.7 Town Staffing ... 28
9.0 Next Steps ... 28
9.1 Coordination with MassDEP ... 28
9.2 Other Gear-Based Oyster Aquaculture Sites ... 29
9.3 Other Aquaculture and CHR Options for Future Consideration ... 30
9.4 Regulatory Considerations ... 30
Appendix A: Boston University Stable Isotope Laboratory QAQC ... 31
Appendix B: QAPP Outline ... 34

List of Tables

Table 3-1 Oyster Seeds for Year 1 ... 8
Table 3-2 Total Nitrogen Content of Lonnie's Pond Oysters ... 9
Table 3-3 Oyster Weights and Nitrogen Uptake from 2016 Demonstration by Installation Size Class ... 10
Table 3-4 Resurfaced oysters from 2016 ... 12
Table 3-5 Demonstration Project cost by year ... 13
Table 4-1 Demonstration Project cost in Year 2 ... 15
Table 5-1 Demonstration Project cost in Year 3 ... 18
Table 5-2 Key decisions for Year 3 ... 18
Table 9-1 Potential sites for gear-based oyster aquaculture and planning values for full scale implementation ... 29
Table 9-2 Potential sites for other aquaculture and CHR and planning values for full scale implementation ... 30

List of Figures

Figure 1-1 Organizational chart for Lonnie's Pond Demonstration ... 6
Figure 4-1 Year 2 (2017) demonstration field ... 14
Figure 5-1 Year 3 (2018) demonstration field ... 16
Figure 7-1 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* ... 24
Figure 7-2 Fraction of the total biodeposits that will be produced over the growing season* ... 24
Figure 7-3 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 ... 25

Executive Summary

The goals of this management plan are to summarize activity to date at Lonnie's Pond to date and identify remaining actions between now and the end of the three-year Demonstration Project, which include acquisition and disposition of shellfish, budgeting for the demonstration, long-term considerations for an aquaculture program in Lonnie's Pond. It is the role of the Shellfish and Waterways Improvement Advisory Committee to review this document and provide the Board of Selectmen with a recommendation about whether to continue with the demonstration and the funds that should be appropriated for the project. The Board of Selectmen will consider approval of this management plan prior to the fall 2017 Town Meeting, where, if placed on the ballot, the Town will vote to approve the budget for the spring 2018 Demonstration Project.

In 2016, the Town of Orleans initiated a three year oyster Demonstration Project in Lonnie's Pond. The overall purpose of the Demonstration Project is to assess the effectiveness of using aquaculture to remove nitrogen from the water as a component of the Town's strategy to meet TMDL requirements and total nitrogen load reduction targets.

The installation project is an outcome of planning efforts, including the Cape Cod Commission's 208 Plan Update, a Consensus Plan, and subsequent amendments to the Town's 2010 Comprehensive Wastewater Management Plan, which identified the need to improve water quality in coastal waters surrounding Cape Cod. Both traditional sewered technologies and non-traditional technologies, including shellfish aquaculture/coastal habitat restoration, were identified as strategies to reduce the nitrogen load within Pleasant Bay.

Lonnie's Pond was identified as the preferred location for the town's first shellfish Demonstration Project. This selection was made based on two key factors: the town's strong desire to improve the environmental conditions in the town's terminal ponds, and the expected ability to monitor water quality and other impacts caused by shellfish in this semi-closed sub-embayment.

Nearly 200,000 Year 1 and Year 2 oysters were deployed Lonnie's Pond on June 22, 2016. The oysters were placed in floating bags containing 250 oysters each that were installed in an 80 foot x 120 foot system. Each bag contained 250 oysters. The bags were maintained on a weekly basis during the peak impairment season of July and August and then bi-weekly for the remainder of the growing season. Monitoring occurred every two weeks between June and December 2016. Monitoring consisted of assessing growth rate of 25-30 oysters that were randomly selected from each of seven tracking bags. The weight, length, and volume of the oysters were recorded. In addition, the oysters were monitored for mortality.

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

To measure the nitrogen content of the shell and dry weight tissue of the oysters, the samples were sent to the Boston University Stable Isotope Laboratory. The nitrogen content for Y1 and Y2 was 10.5 percent and 10.3 percent, respectively. The preliminary assessment indicates that the demonstration system removed 25.9 kg of nitrogen by uptake (increased biomass). The demonstration monitoring project in Lonnie's Pond showed favorable results achieving nitrogen reduction. The results from year one of the project indicate that the use of shellfish to meet TMDL requirement and total nitrogen reduction targets is promising.

The oysters were overwintered in Lonnie's Pond in a system designed to keep oysters from sinking into soft sediment, avoid ice damage, maintain enough flow for the cold weather metabolic activity of oysters, and control mortality from predation. The oysters were resurfaced in mid-April 2017 and removed from the bags and assessed for mortality. Overall, the mortality rate for Y1 oysters from June 2016 through the resurfacing in 2017 was less (1.78%) than the Y2 oysters (10.8%). The total live weight of the oysters was measured and a representative sample of individuals was weight and measured to assess growth during the winter period. These oysters were then used as a part of the second year demonstration. Year 2 oysters from the 2016 season, now three years old, were relayed to Falmouth.

The Demonstration Project was continued for a second year in late spring of 2017. Both Y1 and Y2 oysters were deployed in bags in a similar system as 2016. The population of oysters for the second demonstration year consisted of oysters from the first year of the Demonstration Project (former 2016 Y1 oysters), 480,000 2 mm oyster seed from Mook Sea Farms, and 58,000 2 inch oysters from Falmouth. The total number of oysters deployed in the second year was approximately 607,000 oysters. Year 2 oysters were installed in 510 bags with a targeted final grown-out stocking density of 250 oysters per bag. Over the course of the 2017 growing season, the density of Y1 oyster bags will be adjusted as their size increases for a final grown-out density similar to the Y2 oysters. Monitoring in Year 2 will further refine the relationship between live wet weight and nitrogen content to provide a tool for quantifying estimated nitrogen removal by oysters without analyzing the nitrogen content of dry shell and tissue.

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

The disposition of oysters at the end of the 2018 is dependent on the status of the MassDEP approval of aquaculture as part of a Watershed Permit and associated Watershed Management Plan. Potential options include using shellfish for an ongoing demonstration of full-scale project or putting oysters out for harvest. Decisions regarding disposition will be made after discussions with MassDEP.

The preliminary cost estimate for Year 3 of the Demonstration Project is approximately $250,000. Funding for Year will require approval at the fall 2017 Special Town Meeting.

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

Biodeposition has not been approved by MassDEP as an approach to meet nitrogen targets. However, monitoring results should that it supplement nitrogen removal by update. Further use of the biodeposition model to predict denitrification in Lonnie's Pond is warranted.

Considerations for full-scale aquaculture at Lonnie's Pond include design, permitting, costs, transition to commercial growers, financing options, staffing, and public engagement. It is estimated that the MEP nitrogen reducing goal for Lonnie's Pond (660 lbs/yr or 300 kg/yr) could be met by harvesting 1,200,000 Y1 oysters annually. The design of a full scale TMDL compliance program is anticipated to be similar to but larger than the Year 3 Demonstration Project and would focus solely on Y1 oysters in order to maximize nitrogen removal in a given space.

After the conclusion of the Demonstration Projects, the next steps include coordination with MassDEP to gain consensus about the implications of the Demonstration Project for full-scale aquaculture and to determine the regulatory treatment of non-traditional technologies like shellfish aquaculture as a component of meeting TMDL requirements. In addition, both other gear-based oyster aquaculture sites and other types of aquaculture and coastal habitat restoration (CHR), such as oyster beds and quahog propagation, have been identified as sites where a Demonstration Project similar to Lonnie's Pond may be replicated. These sites should be further considered for aquaculture that can aid in meeting nitrogen goals. Finally, a cursory review of the Town's existing shellfish and shellfish grant regulations revealed several potential amendments the Town may wish to consider in order to facilitate aquaculture in Orleans.

1.0 Introduction

1.1 Background

1.1.1 Cape Cod Commission 208 Plan

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

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

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

1.1.2 Orleans Amended CWMP

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

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

1.1.3 Non-Traditional Technologies

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

1.1.4 Aquaculture/Shellfish Propagation

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

1.1.5 Lonnie's Pond

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

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

1.2 Goals and Organization of Document

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

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

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

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

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

1.3 Board of Selectmen Approval of Management Plan

1.3.1 Shellfish and Waterways Improvement Advisory Committee Review

The Shellfish and Waterways Improvement Advisory Committee (the committee) serves as an advisor to the Town Administrator, Board of Selectmen, Harbormaster/Shellfish Constable, and other town boards and committees. The Town calls upon this committee for issues related to preserving, protecting, managing, and enhancing natural resources, including shellfish and waterways. It is the committee's role to provide a recommendation to the Board of Selectmen whether or not to continue with Year 2 of the oyster Demonstration Project at Lonnie's Pond. The committee should also provide a recommendation of the amount of money that should be appropriated for the project. The committee must provide Nathan Sears, Orleans Natural Resources Manager with a recommendation by October 16, 2017.

1.3.2 Board of Selectmen Review

The Board of Selectmen will need to consider and vote on the approval of the Lonnie's Pond Management Plan prior to the fall 2017 Town Meeting on October 16, 2017. Pending the Selectmen's approval of the Plan, the budget for work in the spring of 2018 would be determined. Prior to consideration of the budget at Town meeting, it is the role of the Finance Committee to review the proposed budget and inform citizens of Orleans of its findings and recommendations. This is discussed in further detail in Section 5.7.

1.3.3 Consideration at the Fall Town Meeting

To be considered on the warrant for the October 16, 2017 Town Meeting, a Warrant Article must be submitted to the Town by September 16, 2017. Citizens will vote on the ballot question to approve the budget for spring 2018 Demonstration Project work on October 24, 2017.

1.4 Town Staffing and Communication Channels

Figure 1-1 displays the organization and staffing structure for the Lonnie's Pond Demonstration Project.

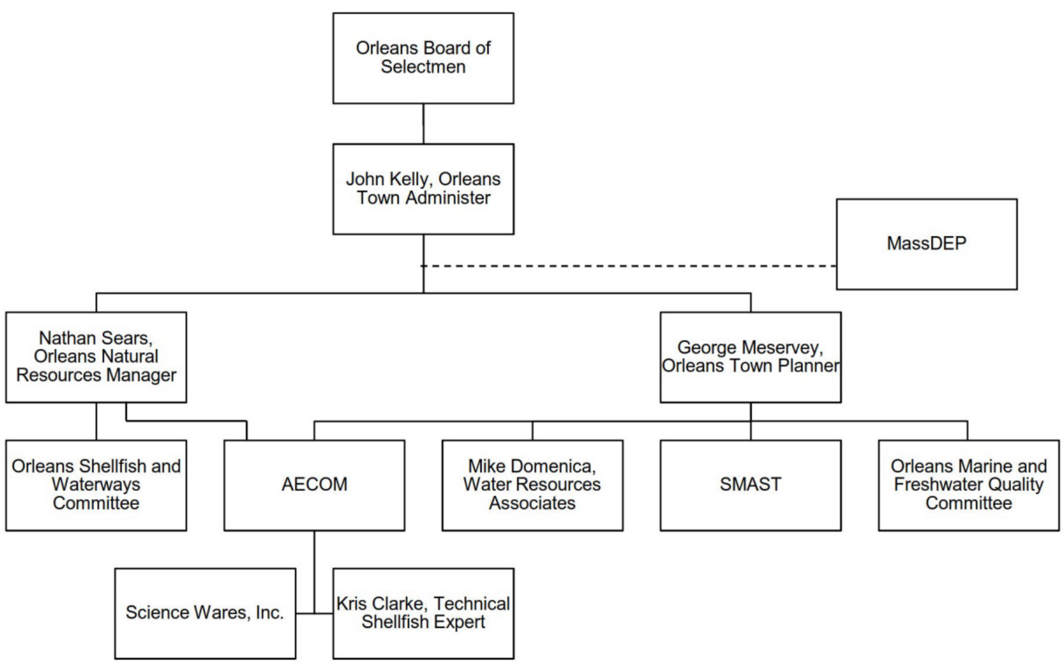


Figure 1-1 Organizational chart for Lonnie's Pond Demonstration

2.0 Lonnie's Pond Demonstration Project Planning

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

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

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

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

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

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

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

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

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

3.1 Field Installation

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

Table 3-1 Oyster Seeds for Year 1

Year 2 (2- inch)	126,690	Town of Falmouth	560	6/22/17
Year 1 (1-inch)	11,700	Town of Falmouth		
Year 1 (< 1-inch)	60,000	Cape Cod Oyster	240	6/27/16
TOTAL	198,390		800	

3.2 Operation and Maintenance

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

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

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

3.3 Growing Season Monitoring

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

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

The seven tracking bags were further inspected for mortality each time oysters were withdrawn for measurements, at which point any dead shells were inspected, counted, and removed. Mortality was first observed during these inspections of the tracking bags in mid-September. From mid-September through December, mortality counts were also made from additional representative bags during every other bag flip cycle. The final mortality assessment was made by counting live and dead oysters in four (4) bags of Year 1 (Y1) oysters and four bags of (Y2) Year 2 oysters that had not been previously counted. The overall final mortality rate prior to overwintering (12/2/16) was 6.6 percent.

3.4 Oyster Growth, Mortality, and Nitrogen Content

The oysters grew well and at rate typical of other Cape Cod locations. The Y1 oysters finished at an average length of about 74mm (2.9in) and an average weight of 35g (1.23oz). The Y2 oysters finished at an average length of about 94mm and an average weight of about 69g (2.4oz).

Approximately 80 percent of the Y1 reached either petite (64mm/2.5in) or regular market size (76mm/3in) in one shortened growing season (the typical season begins in May). All of the Y2 oysters had reached petite or full market size by the end of their second growing season. From discussions with wholesalers, it is believed that the Y1 oysters will have a significantly higher market value if they are overwintered, and it is expected that many of them would be market-ready within the first few weeks of the next season.

The live weight of the oysters increased from approximately 2,176 metric tons at the beginning of the project on June 22, 2016 to approximately 10,091 metric tons at the end of the growing season (adjusted for 6.6 percent measured mortality).

During the growing season, the volume requirement of the oysters increases, resulting in the need to split bags. The rate of change of volume requirement for Y1 and Y2 oysters is different, resulting in fewer bags on the water early in the season and during the critical impairment period for Y1 oysters as well as a lower visual impact from late June to early September.

Samples were sent to the Boston University Stable Isotope Laboratory to determine the nitrogen content of the shell and dry weight tissue. The nitrogen content for Y1 and Y2 was 10.5 percent and 10.3 percent, respectively Table 3-2). The preliminary assessment indicates that the demonstration system removed 17.3 kg of nitrogen by denitrification (about 67 percent of the amount removed through tissue and shell uptake) and 25.9 kg of nitrogen by uptake (increased biomass). A summary of oyster weights and nitrogen update from Year 1 is displayed in Table 3-3.

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

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

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

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

Oyster	Dry Tissue Weight (g)			N Uptake per Oyster (g)	Initial # of Oysters	Mortality	Total Uptake (kg)
	Initial	Final	Increase				
Y1	0.055	1.04	0.985	0.103	60,000	6.6%	5.81
Falmouth Small	0.055	0.79	0.735	0.077	11,700	6.6%	0.84
Y2	0.562	2.12	1.558	0.164	126,690	6.6%	19.41
Totals					198,390	6.6%	26.06

3.5 Quality Control

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

A sampling and analysis plan (SAP) addressing the Demonstration Project sampling and analytical procedures and the associated quality control and quality assurance measures provides transparency to the process so that the stakeholders are assured of the capabilities and the process is reproducible. The term SAP is used to describe a document that includes a field sampling plan (FSP) and quality assurance project plan (QAPP). The document may also be referred to as a QAPP. Field procedures are a critical part of this project. BU has protocols that are followed for N analysis; however, the initial years of the Demonstration Project have been conducted without written protocols. These protocols can be developed now to improve transparency and quality control for data collected in the field.

QAPPs range in complexity and detail depending on the complexity of the project, the data quality needs, the severity of the outcome if the data are not of sufficient quality and the wrong decisions are made, and regulatory oversight. Although the detail and complexity vary, all QAPPs should address the requirements of ANSI/ASQ E4-2004, "Quality Systems for Environmental Data and Technology Programs- Requirements with Guidance for Use," American National Standard, 2004.

EPA QA/R5, "EPA Requirements for Quality Assurance Project Plans" USEPA, 2001 (https://www.epa.gov/sites/production/files/2016-06/documents/r5-final_0.pdf), is based on the ANSI standard. QA/R-5 and the sister guidance document, EPA QA/G-5, "Guidance for Quality Assurance Project Plans" USEPA, 2002 (https://www.epa.gov/sites/production/files/2015-06/documents/g5-final.pdf) provide guidance on writing QAPPs based on the ANSI standard.

This project does not require the greatest level of detail or complexity for an environmental project, which would merit a Unified-Federal Programs (UFP-QAPP) or similar approach. The QAPP should however, address all the categories from the QA/R-5 and QA/G-5 as presented on the attached outline in Appendix B.

For this project, the following information, in particular, will provide transparency:

- The specific objectives of the project for short-term and long-term (the questions that need to be answered);
- Explanation of how the program designed and the data gathered will answer the questions that are posed;
- Field sampling procedures that are used in enough detail that they could be reproduced
- Data that will be used and calculations performed to generate the basis for conclusions (i.e., total volume nitrogen removed);

- Specifics on the laboratory sample preparation and analyses, including laboratory SOPs;
- The measurement performance indicators and criteria for the laboratory procedures;
- The data review process for external review of laboratory data;
- The quality control associated with the field testing (equipment testing, calibration, quality control samples);
- Internal quality control and assessment steps and procedures for the project (review of all data generation activities, data manipulation, report generation, etc.).

3.6 Overwintering System Design and Installation

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

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

3.7 Overwintering System Installation

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

3.8 Winter Monitoring

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

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

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

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

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

3.9 Resurfacing of Overwintered Oysters

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

Table 3-4 summarizes the number of Y1 and Y2 oysters from 2016 that were processed and the mortality rate from June of 2016 through May of 2017. Year 1 oysters from the first year of the Demonstration Project (now Y2 oysters) were moved to the south side of the Demonstration Projects year two field. The Y2 oysters from 2016 (now Y3 oysters) were relayed to Falmouth.

Table 3-4 Resurfaced oysters from 2016

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

3.10 Cost

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

Table 3-5 Demonstration Project cost by year- Costs reported are preliminary draft numbers that are currently being reviewed to confirm accuracy

Year 1 Costs*	
Site Characterization	
Memo on Site Characterization for Aquaculture/Shellfish Propagation (criteria and ranking).	$ 20,997
Engineering	
Preliminary Engineering Work Plan for Aquaculture/Shellfish Propagation (drawings, preliminary specifications, cost estimates, funding sources, and monitoring plan).	$ 62,991
Planning and Implementation (July 2016 through October 2016)	
Lonnie's Pond Demo Project Maintenance	$ 59,680
Quanset Pond Viability Study	$ 11,900
Quahog Population Survey Planning	$ 11,900
Existing Growers	$ 11,900
FY17 Planning and Implementation (November 2016 through June 2017)	
Build and Install Overwintering System (2016 Oysters)	$ 80,000
2016 Project Report	$ 17,000
Assemble bags	$ 90,000
Install oysters (June 2017)	$ 110,000
Monthly Operation and Maintenance	$ 15,000
FY 18 Planning and Implementation	
Monthly Operation and Maintenance	$ 101,500
Project Report and BOS Meeting	$ 18,000
TOTAL	**$ 610,868**

* costs reported are preliminary draft numbers that are currently being reviewed to confirm accuracy

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

4.1 Field Design and Equipment

The Demonstration Project at Lonnie's Pond was continued for a second year to further refine a long-term implementation plan that that uses shellfish to remove nitrogen and to continue to collect data needed to obtain regulatory approvals for the use of shellfish aquaculture to achieve nitrogen goals. The design of Year 3 Demonstration Project is similar to the Year 1 project, with two plots containing Y1 and Y2 oysters (Figure 4-1).

4.2 Shellfish Acquisition and Installation

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

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

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

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

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

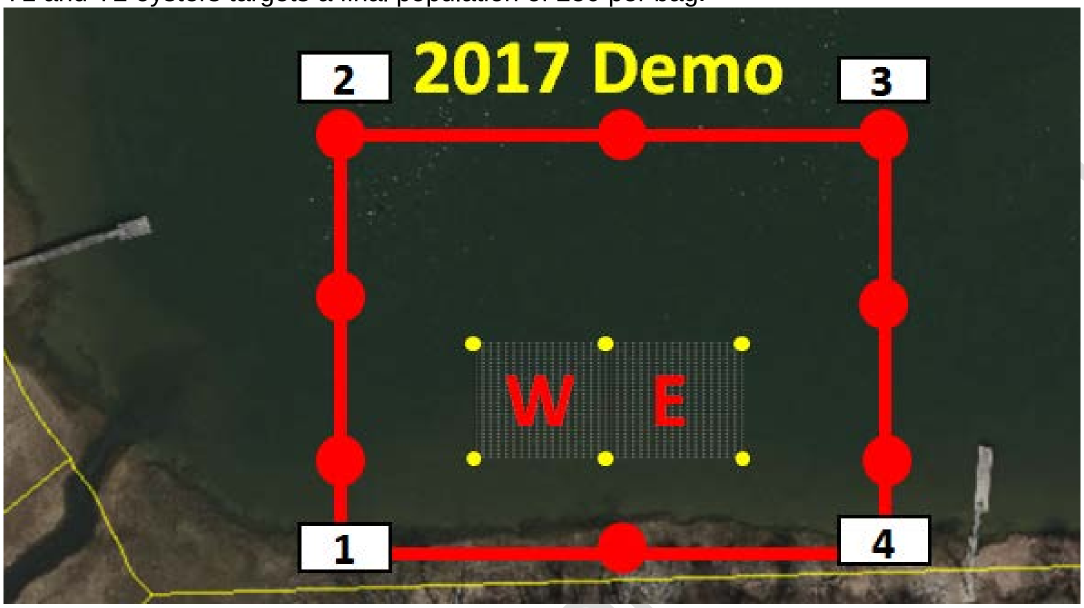


Figure 4-1 Year 2 (2017) demonstration field

4.3 Maintenance and Monitoring

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

4.4 Shellfish Data Collection and Reporting

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

At the end of the sampling period 25 oysters will be randomly selected and analyzed for nitrogen content at the Boston University laboratory. Based on the data collected in Year 1 and Year 2, a tool will be developed to allow the nitrogen content of live wet oysters to be estimated, which will be necessary for future quantification of nitrogen removal at full scale implementation.

4.5 Additional Activities

Additional activities in Year 2 of the Demonstration Project include:

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

- Evaluate public and abutter acceptance;
- 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.

4.6 Cost

Table 4-1 displays a cost estimate for the aquaculture Demonstration Project in Lonnie's Pond for the second year of the demonstration. Year 2 costs include labor for deploying, maintaining, monitoring, overwintering, and reconditioning the bags. Project management, engineering, and a final report are included. Oyster seeds were also purchased to supplement the existing supply of overwintered oysters (2016 Y1) oysters. Finally, lab costs for the nitrogen analysis are included.

Table 4-1 Demonstration Project cost in Year 2 - Costs reported are preliminary draft numbers that are currently being reviewed to confirm accuracy

	Year 2 Costs*	
	April - June 30, 2017	**July 1 - Dec 31, 2017**
Project Management	$ 18,000	$ 21,000
Engineering	$ 22,500	
Labor		
Overwintered Oyster Processing Labor	$ 14,400	
Labor for bags and fixed field alignment gear	$ 11,000	
2017 Oyster Deployment Labor in Lonnie's	$ 10,000	
Flip & Maintain Labor	$ 2,000	
First split Labor		$ 1,000
Second split Labor		$ 1,000
Flip & Maintain Labor		$ 5,000
Overwintering Labor		$ 5,000
Y2 Bottom Planting Labor		$ 2,000
Labor to recondition 500 winter bags		
Half Scale Y1X Deployment Labor		
Field sampling & sample prep	$ 7,680	$ -
Analysis of Monitoring Data		$ 7,800
Permit Options Analysis		$ 1,000
Lab		
N analysis	$ 2,600	$ 7,800
Oyster Seed		
2-3mm oyster seed	$ 5,760	
Intermediate oyster seed	$ 12,760	
Materials		
Spat bags	$ 1,920	
Materials for two 500 bag fixed fields	$ 8,320	
Materials & supplies for sampling	$ 2,180	$ 6,600
Materials for 1,000 bags & lines		
Materials to recondition 500 winter bags		
Year 2 Final Report		$ 30,000
Contingency	$ 10,700	$ 6,200
TOTAL	**$ 129,820**	**$ 94,400**
		$224,220

***** Costs reported are preliminary draft numbers that are currently being reviewed to confirm accuracy

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

5.1 Field Design and Equipment

The design of the Year 3 Demonstration Project is recommended to be similar to the Year 2 project with the exception that the number of oysters in the Y1 field is recommended to be increased to be half the size of a full scale operation in Lonnie's Pond. Thus, there would be two Y1 fields in 2018, each comprising 750 bags by the end of the season for growing out 2-3mm seed from an initial population of 1,060,000. These Y1 oysters are intended to validate the ability of Y1 focused scenarios to meet TMDL and MEP requirements. Increasing the size of the Demonstration Project in Year 3 for Y1 oysters will increase confidence in the accuracy of the data due to the larger sample size.

The Y2 field is proposed to remain the same as in 2017. This field would have approximately 130,000 Y2 oysters in 510 bags and is focused on denitrification studies being conducted by SMAST, although weight, length, and nitrogen content will also be measured.

Figure 5-1 illustrates the anticipated layout for the three oyster fields, pending approval by the Shellfish and Waterways Committee and the Board of Selectmen.

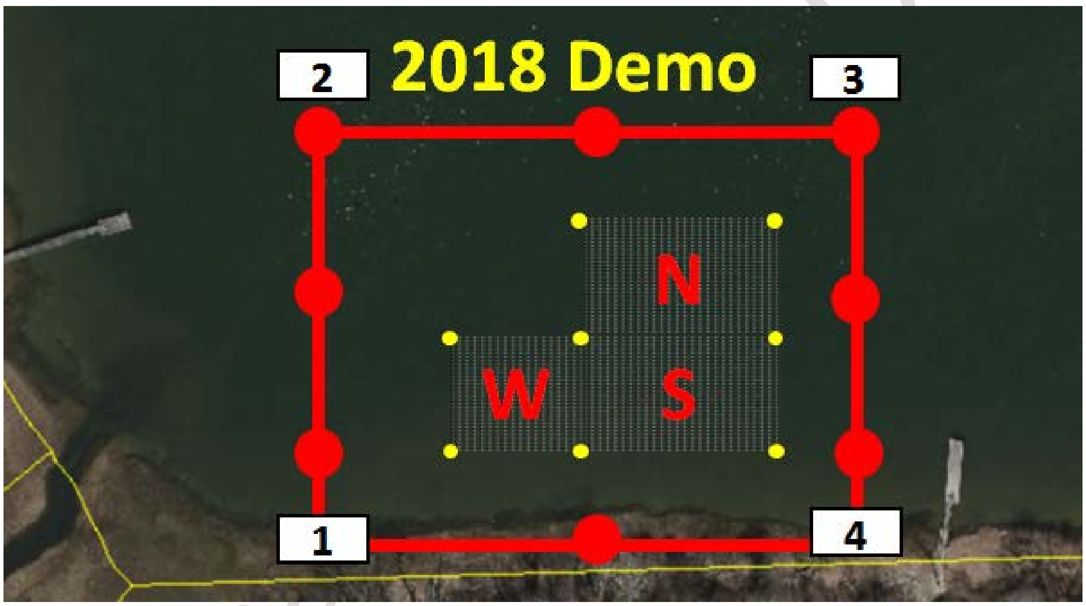


Figure 5-1 Year 3 (2018) demonstration field

The bags needed for the 2018 Y2 oysters would include a total of 2,010 6mm diamond mesh bags, which will consist of the following:

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

In addition, 180 spat bags will be needed for grow-out of the 1.06 million 2-3mm seed until they can be kept in 6mm diamond mesh bags:

- 80 bags from demo Year 2 (the 2017 growing season) can be reused but will need new internal frames;
- 100 new bags will need to be constructed.

5.2 Shellfish Acquisition and Installation

The Y1 and Y2 oysters for the third year of the Demonstration Project will be obtained from a combination of 2017 Y1 oysters that will be Y2 in 2018 after overwintering, as well as additional procurement of Y1 oysters in 2018 from an outside source. For the 130,000 Y2 oysters needed in Lonnie's Pond in 2018, all of these are anticipated to be provided by overwintered Y1 oysters. For the 1,060,000Y1 2018 oysters, all of these would need to be procured from a nursery in spring 2018, which necessitates submitting a request for procurement in December 2017 and submitting a deposit to secure delivery in the spring.

5.3 Management Measures

5.3.1 Equipment Monitoring and Maintenance Tasks

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

5.3.2 Additional Permitting

Permitting requirements for 2018 are anticipated to be similar to those that were required in 2017. The Negative Determination of Applicability obtained from the Orleans Conservation Commission for the 2017 work was only valid for the 2017 year of the Demonstration Project. Therefore, another Request for Determination of Applicability (RDA) will need to be submitted this fall to authorize overwintering of oysters in the 2017/2018 winter and deployment of the proposed oyster fields in spring 2018. No additional permitting is anticipated to be needed for Year 3. Based on last year's Conservation Commission review, it is anticipated that a Negative Determination would be issued again, and that a full Notice of Intent submittal and Order of Conditions would not be required.

5.4 Shellfish Data Collection and Reporting

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

5.5 Shellfish Disposition

Disposition of oysters at the end of the 2018 season will be dependent upon the status of MassDEP approval of aquaculture as part of a Watershed Permit and associated Watershed Management Plan, as discussed in greater detail in Section 9.1. Potentially, the shellfish remaining at the end of 2018 could be used in an ongoing demonstration or full-scale project, or put out for harvest. Final decisions regarding this issue cannot be determined until after additional discussions with MassDEP. Critical dates for these discussions are identified below in Section 5.8.

5.6 Cost

Costs for the 2018 Year 3 of the Demonstration Program will include the following:

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

Based on the 2017 invoice the cost would be $13 per 1,000, for a total of $13,780. There needs to be a 50% deposit by 12/31/17 to get early seed for 2018, so there would be a prepayment of

$6,890 and a payment on delivery of $6,890 in May 2018. The complete summary of costs for Year 3 are shown in Table 5-1 below.

Table 5-1 Demonstration Project cost in Year 3 - Costs reported are preliminary draft numbers that are currently being reviewed to confirm accuracy

	Year 3 Costs	
	Jan 1 - June 30, 2018	**July 1 - Dec 31, 2018**
Project Management	$ 19,200	$ 22,400
Process Engineering & Optimization	$ 12,000	$ 6,000
Labor		
Overwintered Oyster Processing Labor	$ 2,860	
Labor for bags and fixed field alignment gear	$ 16,060	
2017 Oyster Deployment Labor in Lonnie's	$ 5,610	
Seed Flip & Maintain Labor	$ 1,320	$ 1,980
Seed Splitting Labor	$ 8,360	$ 7,920
Bag Flip & Maintain Labor	$ 1,130	$ 10,960
Field sampling & sample prep	$ 20,020	$ 34,320
Y1 Bottom Planting Labor		$ 8,800
Y1 Bottom Cultivation Labor		$ 4,400
Y2 Bottom Planting Labor		$ 2,250
Lab		
N analysis	$ 3,110	$ 9,310
Materials		
Materials & Supplies for Sampling	$ 1,000	$ 3,060
New floating spat bag materials	$ 3,940	
Materials for one 510 and two 750 bag fixed fields	$ 8,930	
Permitting	$ 5,000	
Year 3 Final Report		$ 30,000
TOTAL	**$ 108,540**	**$ 141,400**
		$ 249,940

***** Costs reported are preliminary draft numbers that are currently being reviewed to confirm accuracy

5.7 Preparation and Approval of Budget Items

Funding for Year 3 of the Demonstration Project will require finalization of an Article for the Warrant for the special fall town meeting. The deadline for the Warrant is September 16, 2017 and the Town meeting is October 16, 2017, followed by the vote on October 24, 2017. Approval of funding for at least costs that will be incurred from fall 2017 through June 30 2018 will need to be included on the Warrant article. If desired by the Town, costs for ongoing operation and maintenance activities between July 1, 2018 and June 30, 2019 could be omitted from the fall 2017 warrant and instead included on a separate warrant for the spring 2018 Town meeting.

5.8 Key Decisions and Associated Dates

Key decisions related to Year 3 of the Demonstration Project are summarized in Table 5-2, along with the date by which each decision is needed.

Table 5-2 Key decisions for Year 3

Key Decision Point	Critical Date
Finalize Warrant Article for Town Meeting for Funding for spring 2018 portion of Year 3 Demonstration Project	September 16, 2017
Prepare Request for Determination of Applicability for Conservation Commission for Overwintering Preparation and implementation of Year 3	October 2017
Submit Request to Selectmen for Temporary Increase in Commercial Harvest Shellfish Limit for Harvest of Excess Year 2 Oysters	November 1, 2017
Meet with MassDEP to discuss Watershed Permit and Compliance Requirements	September 2017
Submit RDA and Attend Conservation Commission Meeting	November 2017
Receive 113,000 oysters from Falmouth for Harvest	November 2017

Put excess 2017 Oysters out to Harvest	December, 2017
Place Deposit on Order for 2018 Seed Shellfish for Year 3 Demonstration Project	December 31, 2017
Prepare Y1 oysters for overwintering	December 2017/January 2018
Develop Watershed Management Plan and submit application to MassDEP for Non-traditional program approval	Winter 2017/2018
Place Final Order for 2018 Year 3 Shellfish	April 1, 2018
Receive early start seed and establish bags	May 1, 2018
Deploy Y2 field	May 1, 2018
Evaluate status of DEP discussions and whether to continue with Demonstration Project, expand to full scale, or curtail program	Summer 2018

6.0 Oyster Viability Considerations

6.1 Ocean Acidification

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

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

One response could be that younger, more vulnerable oysters with thin shells could be cultivated elsewhere and the older and more rugged oysters (Year 2) oysters grown in Lonnie's Pond, doing the heavy lifting of the Nitrogen uptake. The overall uptake of Nitrogen in this water body would be less than is currently proposed, though, if the younger oysters weren't grown in Lonnie's Pond.

6.2 Predators

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

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

6.2.2 Oyster Drills

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

6.2.3 Birds: Oyster Catchers and Seagulls

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

6.3 Disease

6.3.1 Juvenile Oyster Disease

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

6.3.2 Dermo, Perkinsus marinus

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

6.3.3 MSX or Multinucleated Sphere Unknown, *Haplosporidian nelson*

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

6.4 Algal Blooms

6.4.1 Macroalgae/Seaweed

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

6.5 Biofouling/ Pests

6.5.1 Sea Squirts/ tunicates/ hydroids

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

6.5.2 Mud Blisters

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

6.5.3 Boring Sponge

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

6.6 Storm Damage

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

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

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

6.7 Theft

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

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

7.0 Water Quality Monitoring and Reporting

7.1 Summary of Water Quality Monitoring Results for the 2016 Demonstration

SMAST staff monitored the Lonnie's Pond demonstration over the 2016 growing season. A sampling program was implemented to establish both a 2016 water quality benchmark for Lonnie's Pond, as well as to initially quantify nitrogen removal due to denitrification enhancement attributable to the oyster installation. From June 29, 2016 to October 19, 2016, eight sampling stations were monitored to further refine the long term water quality sampling database that was initiated for Lonnie's Pond as part of the MEP (Howes et al. 2006).

7.2 Sampling Details

Sampling occurred every other week during mid-ebb tide at the surface, bottom, and at mid-water column, if possible. During the demonstration period, intensive water column sampling also occurred over complete tidal cycles on August 10, 2016, August 24, 2016, September 13, 2016, and October 12, 2016. Samples were collected at nominal hourly intervals over consecutive flooding and ebbing tides.

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

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

7.3 Major Results of 2016 Demonstration Monitoring

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

- **Phytoplankton biomass was removed by the oysters as water flowed through the oyster deployment.** This is evidenced by the reduction in Chl-*a* concentrations and PON within the oyster field, relative to samples taken adjacent to the installation. These reductions in Chl-*a* and PON are statistically significant ($p<0.5$) and were seen during the tidal studies designed to capture water ebbing through the demonstration area;
- **Bioactive N levels declined by 12 to 20 percent during passage through the oyster field.** The decrease in bioactive nitrogen concentrations is likely due to the lowering of PON concentrations;
- **Observed nitrogen removals are conservative estimates** due to the oblique patterns of flow through the oyster area in the surveys, which underestimates uptake;
- **There was a clear temporal trend with higher levels of PON, Chl-a and bioactive N in mid-summer**, which is consistent with increased eutrophic conditions in estuaries in warmer summer months (poorest water quality July through mid-September);
- 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.

7.4 Planning Calculations Based on Water Quality Monitoring Results

Either Y1 or Y2 oysters can be used to meet both TMDL requirements and total nitrogen load reduction targets. Planning calculations including oyster food availability and denitrification contribution to nitrogen-removal over the course of the critical impairment period (July through mid-September) can be made based on the data collected on growth, mortality, and nitrogen-content of the oysters and the information presented in the SMAST technical report (Howes et al. 2017).

The SMAST findings suggest that oyster growth will not be food-limited in Lonnie's Pond. Food concentrations for summer and fall were 1,740 (±213) and 633 (±57.8) µg C/L seawater, respectively. Observations by others suggest that there is no increase in oyster feeding rates at food concentrations above 300 µg C/L (Tenore and Dunstan 1973). During the second and third years of the demonstration program, water flow to maintain adequate food concentration will be assessed throughout the field.

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

7.5 Modeling Biodeposition in Lonnie's Pond

MassDEP is not validating results of denitrification enhancement at this time; however, is anticipated that the denitrification enhancement will be proportional to the amount of biodeposition. Measurements from the SMAST technical report have been used to predict the effect of scaling up the demonstration to determine total biodeposition and the timing of biodeposition. This was modeled[1] based on parameters including total suspended solids (TSS), temperature. 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.

The values for TSS and temperature as a function of time were derived from the SMAST technical report (Howes et al. 2017), which indicates a declining relationship between TSS and particulate organic carbon (POC), and were used to determine suitable model variables for modeling Lonnie's Pond. The predicted deposition matches the rates calculated from SMAST.[2] The biodeposition rates calculated for August 16 (Day 107 after May 1) and October 5 (Day 157 after May 1) were 9.42 mg/g DW/hr and 5.94 mg/g DW/hr, respectively. These observed points aligned with the predicted biodeposition rate of the model, which generally increased from < 1 mg/g DW/hr at the beginning of May to a peak of around 9.5 mg/g DW/hr in mid-August, then declined through early December.

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

The biodeposition rate per oyster over the course of the growing season was estimated by multiplying the tissue dry weight as a function of time for Y1 and Y2 oysters by the deposition rate obtained from the model described above. The total amount of biodeposits generated by a given deployment of oysters was then determined by multiplying this rate by the number of oysters per bag, and integrating as a function of time (see Figure 7-1). These values were then

[1] The model developed by Virginia Institute of Marine Science for the Army Corp of Engineers (Sisson et al, 2011) 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 total suspended solids in mg/L, T is temperature in degrees Celsius, and the parameters α, β, δ and TSS and T are characteristic of the system being modeled.
[2] SMAST Table V.2.

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 7-2.

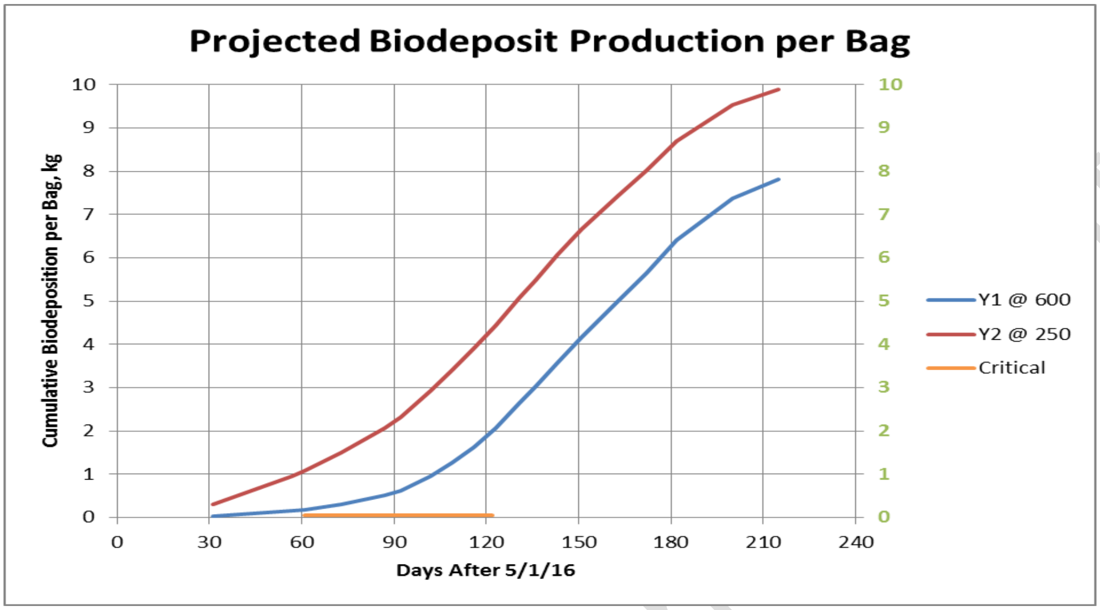


Figure 7-1 Predicted accumulation of biodeposits over the course of the growing season from Y1 oysters at a stocking density of 600 per bag and Y2 oysters at a stocking density of 250 per bag*

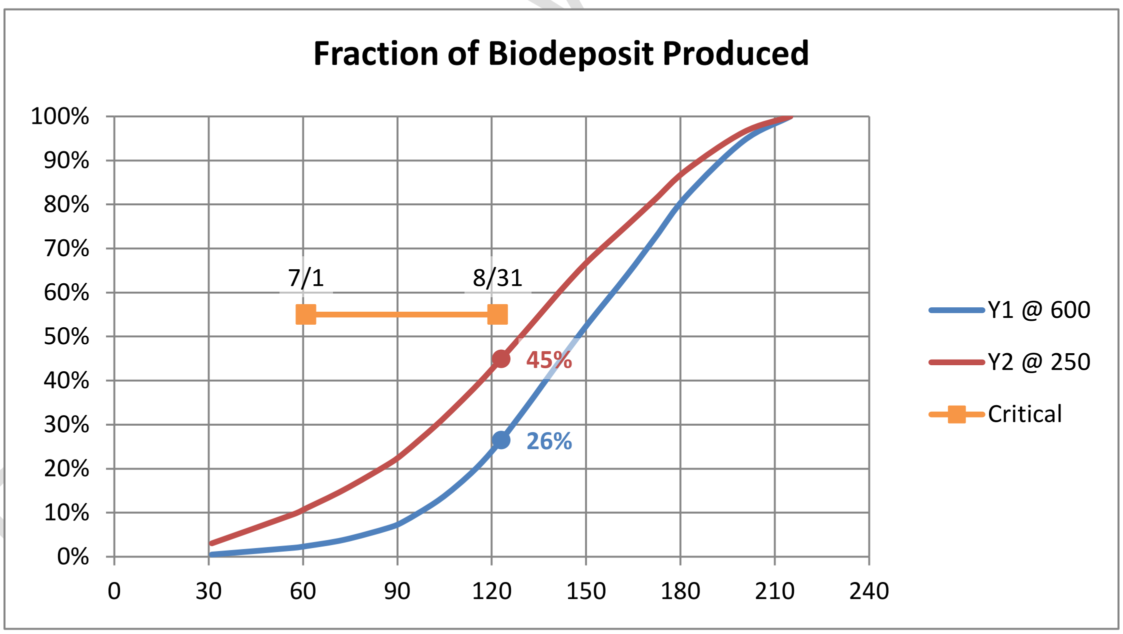


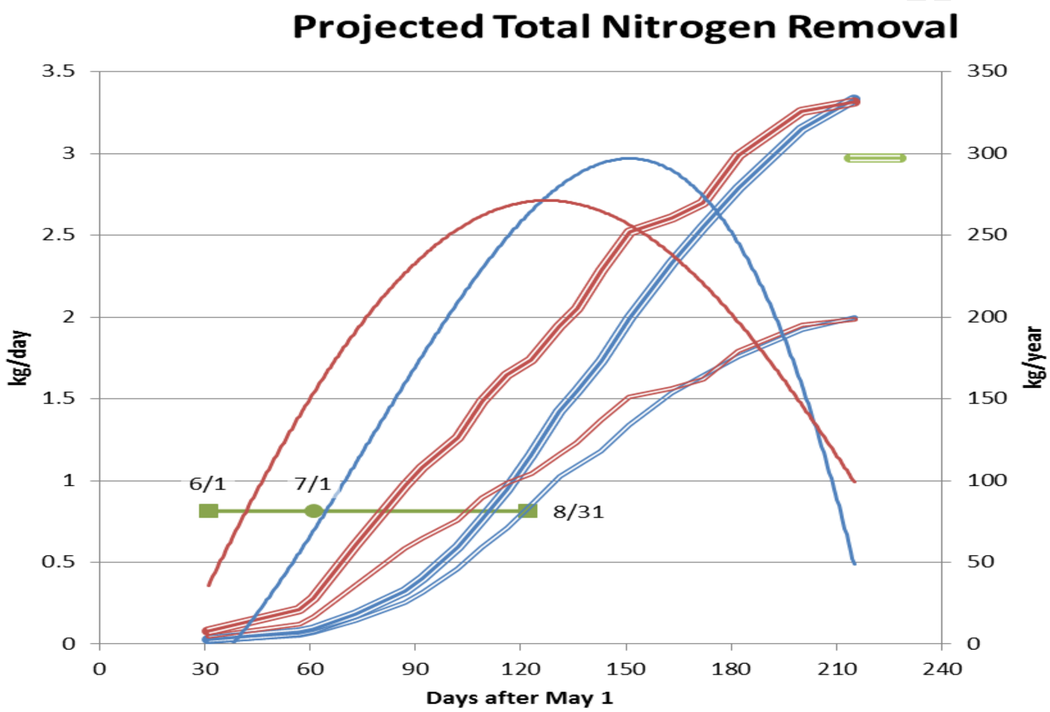
Figure 7-2 Fraction of the total biodeposits that will be produced over the growing season*

*Note: the line labeled “critical” represents the critical impairment period, which is from July 1 to August 31 (Howes et al. 2006, Howes et al. 2017).

7.7 Full-Scale Grow-Out: Predictions of Total Nitrogen Removal by Oysters in Lonnie's Pond

The daily nitrogen removal needed to achieve the overall TMDL for Lonnie's Pond (3 kg N/day) is 0.8 kg N/day based on the nitrogen removal target for Lonnie's Pond, 300 kg N/year (calculated by MEP). The TMDL for Lonnie's Pond 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 total nitrogen removed by oysters is the sum of uptake into biomass (new tissue and shell)("U") and enhanced denitrification in the sediment ("D"). The projections in Figure 7-3 are based on the dry tissue weight of the oysters. The second year of the Demonstration Project will examine whether there is significant seasonal variation in the relationship between dry weight and wet harvest weight for Lonnie's Pond Oysters in order to provide a more efficient and cost effective method for monitoring the actual performance of a full-scale deployment in which wet harvest weight will be measured.



Key		
	TMDL	0.8 kg N/day removal by oysters to achieve overall TMDL
	MEP	300 kg N/year nitrogen removal target for Lonnie's Pond (calculated by MEP)
	Y1 U+D	Kg N/year uptake into biomass + enhanced denitrification in sediment of Y1 oysters
	Y2 U+D	Kg N/year uptake into biomass + enhanced denitrification in sediment of Y2 oysters
	Y1 U	Kg N/year uptake into biomass of Y1 oysters
	Y2 U	Kg N/year uptake into biomass of Y2 oysters
	Y1	Predicted kg N/day removal by Y1 oysters (tissue dry weight over the course of the growing season x total nitrogen content of Lonnie's Pond Oysters x 3,000 bags)
	Y2	Predicted kg N/day removal by Y2 oysters (tissue dry weight over the course of the growing season x total nitrogen content of Lonnie's Pond Oysters x 4,500 bags)

Figure 7-3 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

7.8 Key observations for the Y1 System

- 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.
- 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 Y1 stocking density of Y1 oysters to 1,000 oysters per bag and 4,500 bag, which would result in an estimated 500 kg of nitrogen removal through uptake and 332 kg of nitrogen removal through enhanced denitrification for a combined nitrogen removal of 832 kg of nitrogen (assuming growth and enhanced denitrification rates scale linearly with the population increase).
- It may be possible to scale the system to 1,000 oysters per bag and 1,800 bags, which would cover less than half an acre, while still removing the amount of nitrogen required to meet TMDL and MEP targets.

7.9 Key observations for the Y2 System

- 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.
- 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.

7.10 Recommendations for Second-Year Monitoring

- Evaluate the feasibility of Y1 grow-out of 1,000 oysters per bag with a 4,500 bag per acre density;
- Determine the preferred orientation of plots for second-year monitoring based on measured flow patterns;
- Determine the feasibility of walking within one experimental plot to compare enhanced denitrification rates with an undisturbed plot;
- 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 before, during, and after the critical impairment period, and coordinate this sampling with nitrogen content analysis to enable assessment of what is required for both Y1 and Y2 systems to achieve TMDL target levels during the critical impairment period;
- Install tide gages to verify local residence time and tidal exchange from MEP Report (Howes et al. 2006); and
- Assess the utility of running the MEP model to determine the impacts to Lonnie's Pond if the target nitrogen load is removed but no other nitrogen-removal occurs in the Pleasant Bay system, including average inflows from both the herring run from Pilgrim Lake and the nearby cranberry bog.

8.0 Considerations for Full-Scale Aquaculture at Lonnie's Pond

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

The MEP nitrogen reduction goal for Lonnie's Pond is approximately 660 pounds per year (300 kg/yr). The potential annual nitrogen per acre per year in Lonnie's was calculated based on the nitrogen content of oysters, densities, and weights measured in the Lonnie's Pond during Year 1 of the Demonstration Project. Based on these values, the number of oysters needed is estimated to be approximately 1,200,000 Year 1 oysters harvested annually, which was calculated by dividing the annual goal by the mass uptake of nitrogen over a season by a Y1 oyster and rounding up to the nearest 100,000. The area required to grow the necessary numbers of shellfish in Lonnie's Pond was estimated to be 0.9 acres which was calculated by dividing the annual goal by the projected annual uptake/acre/year.

8.1 Design

The design of a full-scale TMDL compliance program at Lonnie's Pond is anticipated to be similar but larger than the Year 3 Demonstration Project and would focus only on Y1 oysters in order to maximize nitrogen removal in a given space. The Year 3 design is approximately half of the full scale implementation scenario. Thus, the full scale scenario would include four Y1 fields in 2018, each comprising 750 bags by the end of the season for growing out 2-3mm seed from an initial population of 2,120,000.

Approximately 360 spat bags will be needed for grow-out of the 2.12 million 2-3mm seed until they can be kept in 6mm diamond mesh bags. At full grow-out there would be approximately 3,000 6mm diamond mesh bags.

Under this full-scale implementation scenario, each year there would be a significant number of non-harvestable size oysters that would require disposition. These oysters could be grown to harvestable size in floating gear elsewhere in Pleasant Bay, or could be bottom-planted as part of an Oyster Reef Demonstration Project.

8.2 Costs

The cost of full scale implementation cannot yet be definitively determined due to uncertain compliance documentation that will be required by MassDEP. However, the cost would be approximately double the cost of the Year 3 Demonstration Project, minus currently projected Year 3 costs associated with ongoing engineering, management, and data collection, which would all be presumed to be less under a full-scale implementation program since these issues would have been substantially vetted and resolved during the demonstration period. Thus, annual full-scale implementation costs would be anticipated to in the order of approximately $200,000.

8.3 Transition to Commercial Growers

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

- It may be beneficial to modify the current aquaculture regulations to allow growers to transition their grants to others, as allowed by Commonwealth law; current Orleans regulations do not allow such a transition, which is a financial disincentive for a grower to take on a grant since there is no pathway for them to recover financial investments at the conclusion of their interest in operating the grant
- The aquaculture regulations may need to be modified to allow a new grant category for this operation, which specific reporting and compliance requirements as well as requirements for growing the oysters in floating gear at specified densities
- Options for alternative types of floating gear that may be desired by commercial growers would need to be investigated with MassDEP and would need to be specified in the grant license
- The requirement to avoid sediment disturbance may be unrealistic, but also unnecessary if the nitrogen removed via the denitfrification pathway is discounted
- Additional grants in Pleasant Bay may be needed for growers to either sell the Y1 oysters and have others grow them out, or for a single grower to transfer the Y1 oysters to new locations in Pleasant Bay.

8.4 State Revolving Fund (SRF) or other financing options

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

8.5 Permitting

Permitting requirements are anticipated to be similar to those that were required in the Demonstration Project phase, although the applicability of a Negative Determination of Applicability versus a full Notice of Intent submittal and Order of Conditions would need to be discussed with the Conservation Commission.

8.6 Public Engagement

For a full scale implementation at Lonnie's Pond, additional public outreach and engagement is recommended. This could take the form of either a public meeting at the Town Hall where the plan is presented and comments are solicited; posting the draft Lonnie's Pond management Plan on line and solicitation of written comments; or hosting a public visitation day at Lonnie's Pond; or a combination of any of the above. It is recommended that the public engagement occur during the latter part of 2017 or early 2018, after further confirmation from MassDEP is received regarding the details of the Watershed Permit review and approval process as well as requirements for compliance documentation.

8.7 Town Staffing

9.0 Next Steps

The next steps in the aquaculture/coastal habitat restoration (CHR) program include coordination with MassDEP and identification of potential other sites and programs that could be implemented in Orleans.

9.1 Coordination with MassDEP

Coordination with MassDEP is necessary to gain consensus regarding the implications of the Demonstration Project for full-scale implementation of aquaculture/CHR in Orleans and to determine the regulatory treatment of non-traditional technologies as a major component of the Town's efforts to meet TMDL requirements and its strategy to manage wastewater.

MassDEP is in the process of developing guidance for a Watershed Permit that would include non-traditional technologies. This new wastewater management and impact mitigation permitting program would provide for a watershed-based approach to restore embayment water quality on Cape Cod. It is anticipated that enrollment in the program will demonstrate that the Town is taking action to address wastewater.

The Town and its consultants plan to meet with the Cape Cod Commission and MassDEP soon to discuss the guidance to ensure that critical regulatory issues for Lonnie's Pond, with would fall under a watershed permit for the Pleasant Bay watershed, are addressed.

It is anticipated that the Watershed Permit process will likely require that the Town submit a Watershed Permit Plan that:

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

9.2 Other Gear-Based Oyster Aquaculture Sites

The gear-based oyster aquaculture program implemented at Lonnie's Pond could be replicated at other locations in Pleasant Bay and Town cove. The Consensus Plan of 2015 identified several potential sites for full-scale gear-based oyster aquaculture. A refined list of these sites based on the Consensus Plan from the Technical Memorandum Draft Aquaculture Full-Scale Implementation Program (7/7/17) is included in Table 9-1. The total annual goal for nitrogen removal for gear-based oyster aquaculture sites, including Lonnie's Pond, is 1,206 kg/year. In advance of initiating any work at these other locations, a DMF survey of productive bottom should first be initiated well in advance of a projected start date, so evaluation of these areas for existing shellfish could begin in the near future

Table 9-1 Potential sites for gear-based oyster aquaculture and planning values for full scale implementation

Location	Method	Annual Goal for Kg N Removed	Waterbody Surface Area and Notes[1]	Annual Number of Shellfish Harvested/Removed
Arey's	Y1 Oyster Aquaculture in Floating Gear	136	0.4 acres with high stocking density	1,100,000
Meetinghouse	Y1 Oyster Aquaculture in Floating Gear	150	0.4 acres with high stocking density	1,200,000
Pleasant Bay: Existing Aquaculture Grant Sites[2]	Oyster Aquaculture in Floating Gear	620	5 acres within existing grant areas, includes equal numbers of Y1 and Y2 size classes growing	2,200,000
Lonnie's Pond	Y1 Oyster Aquaculture in Floating Gear	300	0.9 acres with high stocking density	2,400,000

1. High stocking density is considered to 45 oysters per square foot
2. Based on the results of the Enhanced Aquaculture demonstration, it is believed that there is space on each of the 15 currently-approved shellfish to grow 2.2 million more oysters per year.

9.3 Other Aquaculture and CHR Options for Future Consideration

In addition to gear-based oyster aquaculture, there are opportunities to implement other types of aquaculture and coastal habitat restoration (CHR) within Pleasant Bay and Town Cove. One location in Pochet was identified for a 2 acre oyster bed, which would provide and estimated 480 kg of nitrogen removal per year as well as coastal habitat restoration. Two larger areas were targeted for quahog propagation: the River, Namequoit and associated areas, and Town Cove/Mill Pond. The total annual goal for nitrogen removal for these sites is 2,370 kg/year (Table 9-2).

Table 9-2 Potential sites for other aquaculture and CHR and planning values for full scale implementation

Location	Method	Annual Goal for Kg N Removed	Waterbody Surface Area and Notes[1]	Annual Number of Shellfish Harvested/Removed
Pochet	Oyster Bed[2]	480	2 acres with high planting density	3,800,000
The River, Namequoit and Associated Areas	Quahog Propagation	530	13 acres (includes area under netting and final broadcast)	2,300,000
Town Cove/Mill Pond	Quahog Propagation	1,360	31 acres (includes area under netting and final broadcast)	5,700,000

1. High stocking density is considered to 45 oysters per square foot
2. Coastal habitat restoration

9.4 Regulatory Considerations

As Orleans endeavors to reduce nitrogen in Pleasant, the Town may wish to consider amending its Shellfish Regulations (Town Code Chapter 176) and Shellfish Grant Regulations/Aquaculture Regulations to lessen potential barriers to entry into the shellfish harvesting market. Suggested amendments to the Shellfish Grant Regulations include:

- Consolidating the permitting process for holding both a shellfish grant license and a commercial license. Consider eliminating the requirement of Section B.
- Consider allowing more than one individual per household to a license for a shellfish grant (Section B).
- Add language to Section E to allow the Board of Selectmen to consider licensing grants for the purposes of environmental restoration and increased nitrogen removal capacity, regardless of whether the specified total grant area limit has been reached.
- Consider lowering the minimum threshold for annual expenditures on shellfish to be planted on a shellfish grant lease site, such as to $1,000 or $1,500 per acre per year, imposing a harvest production threshold after the first three years of a license, as opposed to a planting and purchasing threshold for the duration of the license.
- Allowing a shellfish grant license to be transferred from one individual to another, with Board of Selectmen or with Town Manager approval.

Appendix A: Boston University Stable Isotope Laboratory QAQC

BOSTON UNIVERSITY
STABLE ISOTOPE LABORATORY

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Boston, MA 02215
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Quality Assurance and Quality Control

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

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

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

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

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

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

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

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

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

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

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

Percent organics and nitrogen protocol

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

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

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

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

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

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

Appendix B: QAPP Outline

QAPP Outline (Based on *EPA Guidance for Quality Assurance Project Plans,* EPA QA/G-5)

A. Project Management

- A1 Title and Approval Sheet
- A2 Table of Contents and Document Control Format
- A3 Distribution List
- A4 Project/Task Organization and Schedule
- A5 Problem Definition/Background
- A6 Project/Task Description
- A7 Quality Objectives and Criteria for Measurement Data
- A8 Special Training Requirements/Certification
- A9 Documentation and Records

B. Measurement/Data Acquisition

- B1 Sampling Process Design
- B2 Sampling Methods Requirements
- B3 Sample Handling and Custody Requirements
- B4 Analytical Methods Requirements
- B5 Quality Control Requirements
- B6 Instrument/Equipment Testing, Inspection, and Maintenance Requirements
- B7 Instrument Calibration and Frequency
- B8 Inspection/Acceptance Requirements for Supplies and Consumables
- B9 Data Acquisition Requirements (Non-Direct Measurements)
- B10 Data Management

C. Assessment/Oversight

- C1 Assessments and Response Actions
- C2 Reports to Management

D. Data Validation and Usability

- D1 Data Review, Validation and Verification Requirements
- D2 Validation and Verification Methods
- D3 Reconciliation with Data Quality Objectives