Lonnie's Pond Aquaculture Management Plan

Town of Orleans, MA | Water Quality and Wastewater Planning

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AECOM

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Executive Summary

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

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

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

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

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

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

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

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

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

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

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

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

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

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

1.0 Introduction

1.1 Background

1.1.1 Cape Cod Commission 208 Plan

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

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

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

1.1.2 Orleans Amended CWMP

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

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

1.1.3 Non-Traditional Technologies

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

1.1.4 Aquaculture/Shellfish Propagation

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

1.1.5 Lonnie's Pond

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

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

1.2 Goals and Organization of Document

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

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

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

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

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

1.3 Town Organization

1.3.1 General

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

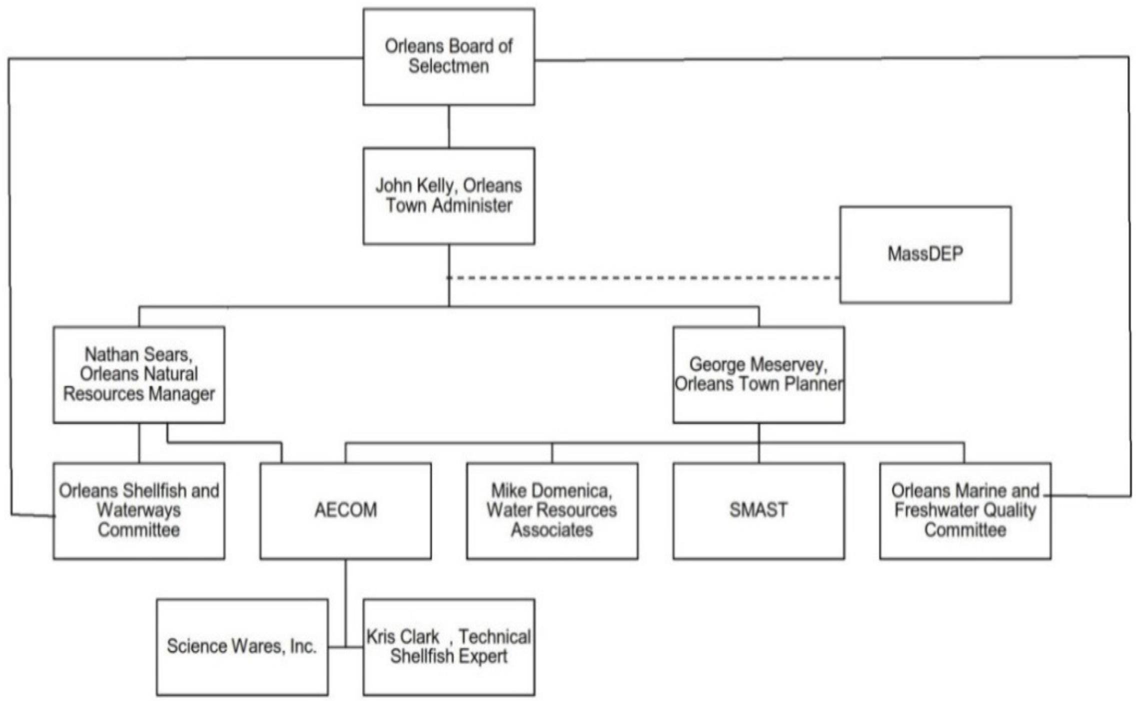

Figure 1-1 - Organizational Chart for Lonnie's Pond Demonstration

1.3.2 Board of Selectmen

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

1.3.3 Shellfish and Waterways Improvement Advisory Committee Review

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

1.3.4 Town Meeting Budget Approvals

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

2.0 Lonnie's Pond Demonstration Project Planning

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

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

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

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

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

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

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

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

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

3.1 Field Installation

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

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

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

3.2 Operation and Maintenance

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

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

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

3.3 Growing Season Monitoring

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

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

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

3.4 Oyster Growth, Mortality, and Nitrogen Content

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

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

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

Table 3-3.

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

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

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

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

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

3.5 Quality Control

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

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

3.6 Overwintering System Design and Installation

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

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

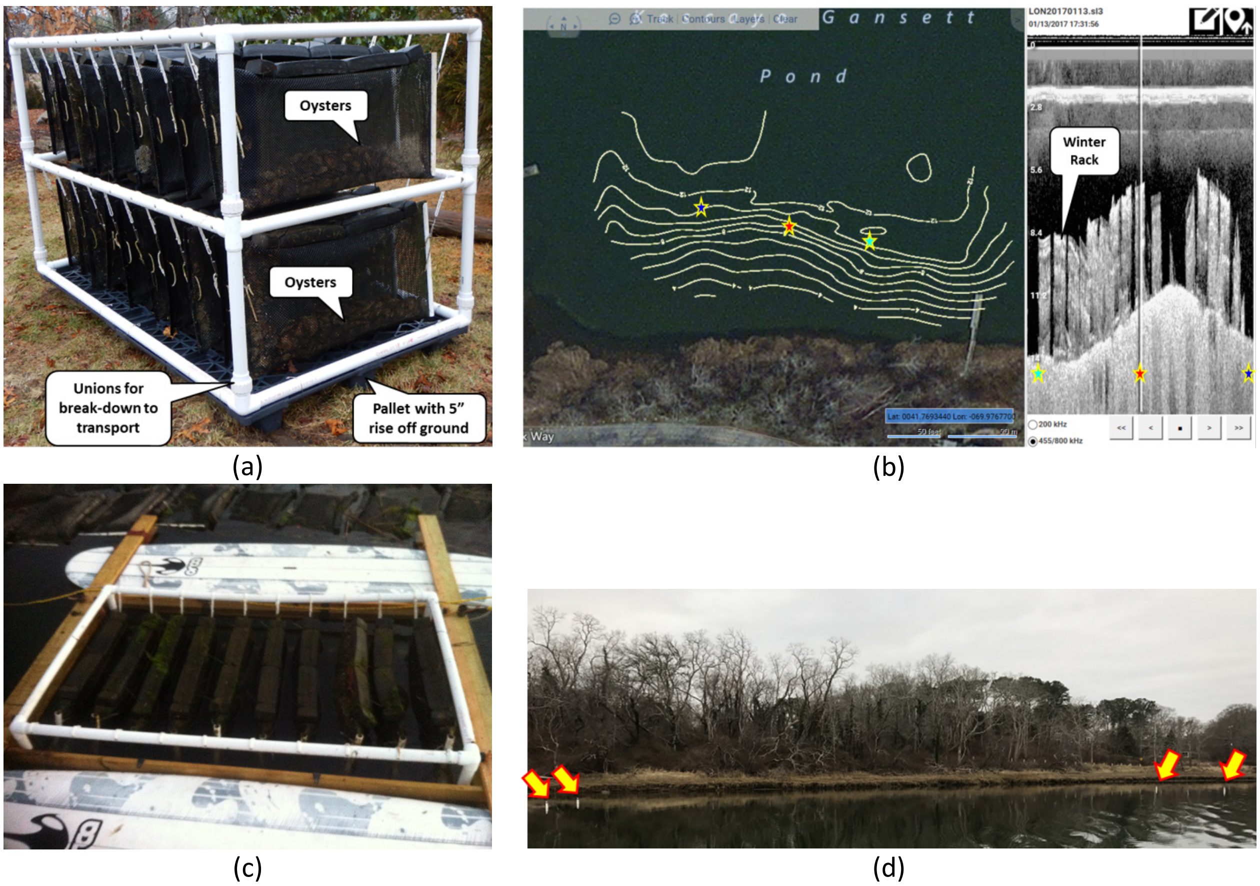

Figure 3-1. Science Wares overwintering system for 2016 Demonstration Year at Lonnie's Pond: (a) rack on dry ground showing two levels of bags oriented with one float up and oysters down (b) depth profile and sonar scan showing orientation of racks on the bottom (c) loaded rack about to be submerged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

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

3.7 Winter Monitoring

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

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

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

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

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

3.8 Resurfacing of Overwintered Oysters

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

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

Table 3-4 - Resurfaced Oysters From 2016

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

3.9 Cost

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

Table 3-5 - Demonstration Project Year 1

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

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

4.1 Field Design and Equipment

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

4.2 Shellfish Acquisition and Installation

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

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

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

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

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

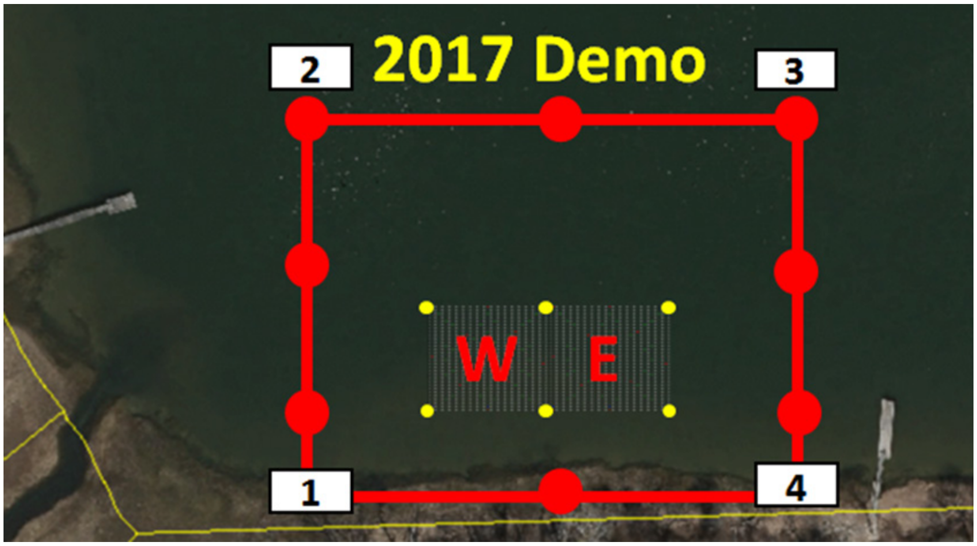

Figure 4-1 - Year 2 (2017) Demonstration Field

4.3 Maintenance and Monitoring

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

4.4 Disposition of Excess Oysters

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

4.5 Shellfish Data Collection and Reporting

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

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

4.6 Additional Activities

Additional activities in Year 2 of the Demonstration Project include:

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

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

4.7 Cost

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

Table 4-1 - Demonstration Project Cost in Year 2

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

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

5.1 Field Design and Equipment

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

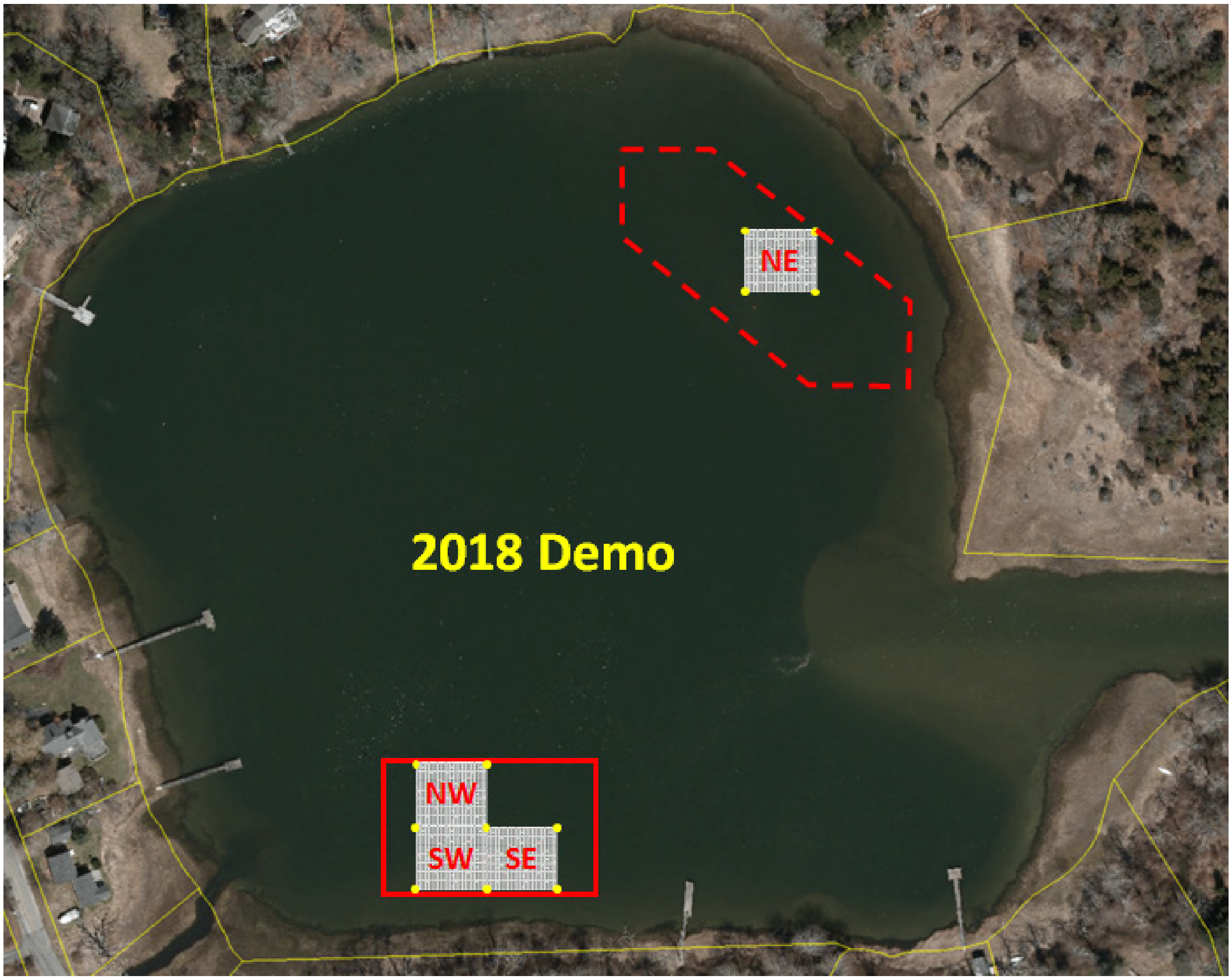

Figure 5-1 - Year 3 (2018) Demonstration Field

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

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

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

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

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

5.2 Shellfish Acquisition and Installation

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

5.3 Management Measures

5.3.1 Equipment Monitoring and Maintenance Tasks

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

5.3.2 Additional Permitting

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

5.3.3 Shellfish Data Collection and Reporting

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

5.3.4 Shellfish Disposition

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

5.4 Cost

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

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

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

Table 5-1 - Demonstration Project Cost in Year 3

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

5.5 Key Actions and Associated Dates

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

Table 5-2 - Key decisions for Year 3

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

6.0 Oyster Viability Considerations

6.1 Ocean Acidification

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

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

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

6.2 Predators

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

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

6.2.2 Oyster Drills

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

6.2.3 Birds: Oyster Catchers and Seagulls

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

6.3 Disease

6.3.1 Juvenile Oyster Disease

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

6.3.2 Dermo, Perkinsus marinus

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

6.3.3 MSX or Multinucleated Sphere Unknown, Haplosporidian Nelson

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

6.4 Algal Blooms

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

6.5 Biofouling/ Pests

6.5.1 Sea Squirts/ tunicates/ hydroids

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

6.5.2 Mud Blisters

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

6.5.3 Boring Sponge

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

6.6 Storm Damage

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

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

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

6.7 Theft

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

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

7.0 Water Quality Monitoring and Results

7.1 Water Quality Monitoring Methodology

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

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

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

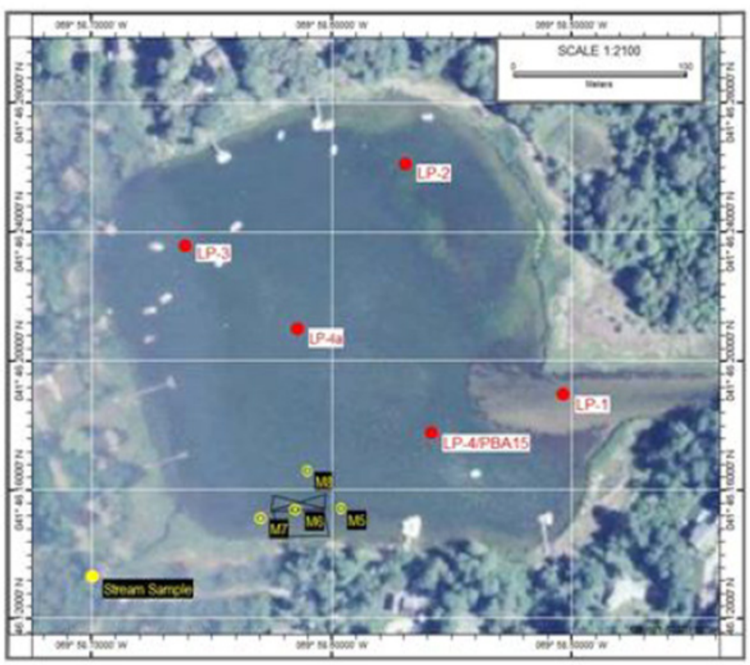

Figure 7-1 - SMAST Water Quality Monitoring Stations

7.2 Major Results of 2016 Water Quality Monitoring

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

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

8.0 TMDL Compliance

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

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

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

9.0 Full-Scale Aquaculture Scenarios

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

9.1 Scenarios and Cost

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

9.2 Transition to Commercial Growers

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

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

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

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

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

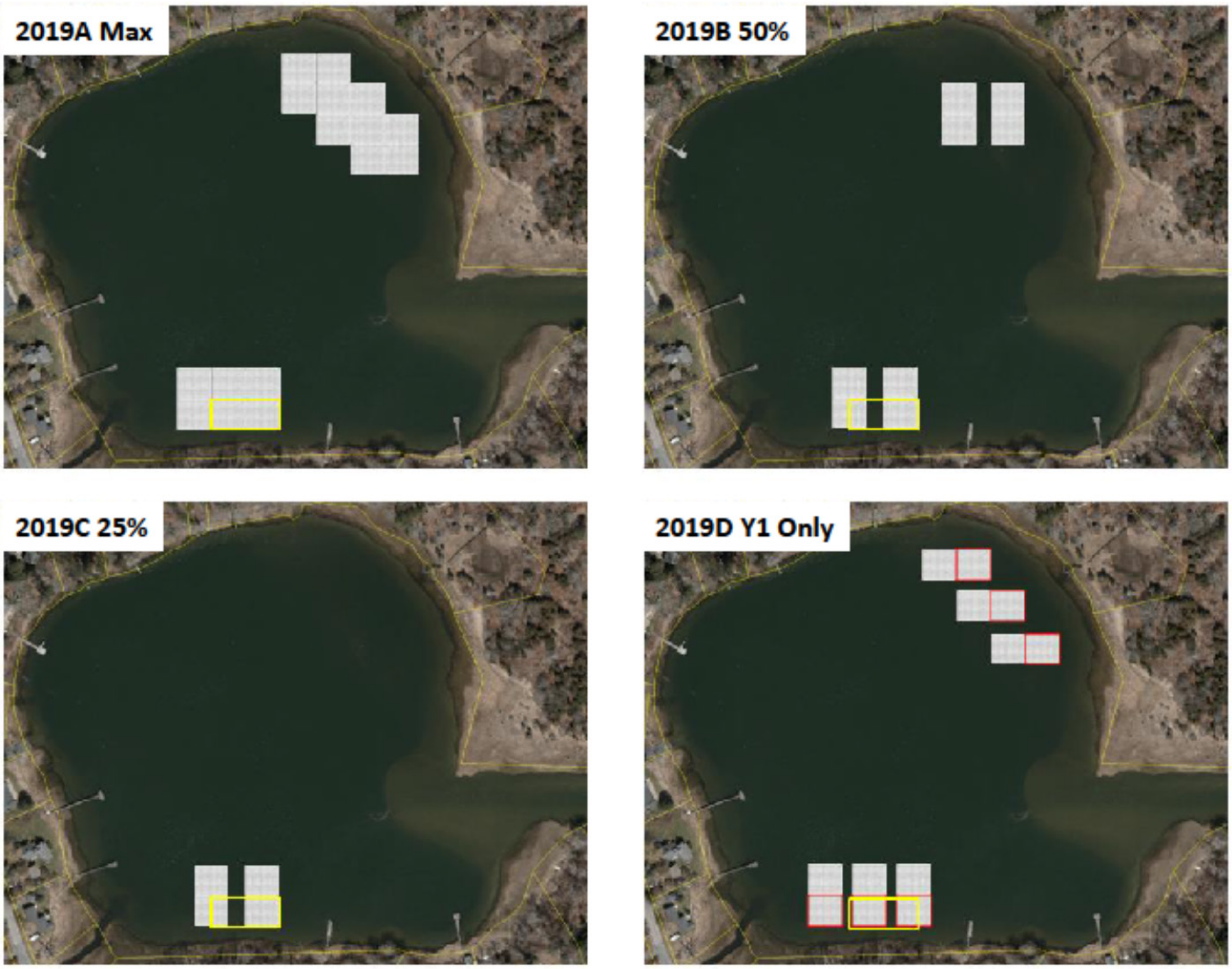

Figure 9-1 - Full Scale Implementation Scenarios

Table 9-1 - Full Scale Implementation Costs

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

Table 9-2 - Full Scale Implementation Assumptions

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

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

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

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

9.3 Financing

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

9.4 Permitting

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

9.5 Communication Plan

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

9.6 Town Staffing

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

10.0 Full Scale Implementation Management Plan

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

10.1 Watershed Permitting

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

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

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

10.2 Oyster Configuration

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

10.3 Required Tasks and Schedule of Activities

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

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

10.4 Overwintering Protocols

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

10.5 Long-Term Costs

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

10.6 Excess Oyster Disposal

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

10.7 Catastrophic Loss and/or Project Abandonment

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

Appendix A

Boston University Stable Isotope Laboratory QAQC

and

Science Wares SOPs

BOSTON UNIVERSITY
STABLE ISOTOPE LABORATORY

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Quality Assurance and Quality Control

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

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

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

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

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

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

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

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

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

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

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

Percent organics and nitrogen protocol

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

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

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

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

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

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

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