

Memorandum

To George Meservey, Director of Planning & Community Development
Michael Domenica, PE, Program Manager

CC Betsy Shreve, AICP, AECOM Project Director
Sia Karplus, Science Wares, Inc.

Subject **Town of Orleans, MA
Water Quality and Wastewater Planning
Task 8.2.2 - Adaptive Management Implementation
Technical Memorandum on the Review of SMAST Limited Monitoring Program
for Lonnie's Pond Demonstration Project**

Project Number 60476644

From Thomas Parece, P.E., AECOM Project Manager

Date August 12, 2016

1. Introduction

The Purpose of this Technical Memorandum is to document the review of the Scope of Work provided by the University of Massachusetts Dartmouth School for Marine Science and Technology (SMAST) entitled *Quantifying Oyster Aquaculture Impacts on Estuarine Nitrogen Related Water Quality: Lonnie's Pond, Upper Pleasant Bay*, dated June 28, 2016 (Appendix A). The goal of this Scope of Work is to conduct a monitoring program over the 2016 demonstration project period that will provide data to help guide the design of full-scale implementation. These data include:

- Extent and type of changes in water column nitrogen species and other key eutrophication metrics that are caused by the filter feeding activities of oysters;
- Response of nitrogen cycling in sediments associated with oyster depositional processes; and
- Confirmation of nitrogen-content of shell and soft tissue.

The number and size of shellfish specified for ongoing shellfish propagation in Lonnie's Pond will be based on the findings of this monitoring program. This Scope of Work details the methodology used to accomplish the following six tasks:

1. Water quality sampling and analysis via grab sampling;
2. Water quality sampling and analysis using time-series dissolved oxygen (DO) and chlorophyll-a (/Chl-a) continuous data loggers (SONDES);

3. Analysis of bio-deposition rates and mapping of impact areas;
4. Analysis of denitrification and oyster bio-deposits using Sediment Core Incubations; and
5. Water quality and tidal volume sampling and analysis of two surface water inflows (herring run and stormdrain).

A final report that contains data tables and analysis, as well as data sets in Excel format will be provided by SMAST. Tables and graphs will be presented comparing pre- and post-oyster aquaculture water quality results. Emphasis will be on changes in water column total nitrogen, pigment concentrations and water clarity and shifts between various nitrogen species within the total nitrogen budget.

In addition, a Sampling Analysis Plan (SAP) will be submitted that modifies the Quality Assurance Program Plan (QAPP) for the Massachusetts Estuaries Project for water quality, benthic and tidal volume monitoring. A map and GPS coordinates will be included in the SAP for all sampling locations. Methods for assessing bio-deposition rates and denitrification impacts will also be part of the SAP.

Confirmation of the nitrogen content in the shell and soft tissue of the oysters will be accomplished by sending 25 oysters to the Boston University Stable Isotope Laboratory (BUSIL) for analysis. The first sample set of oysters was sent on the day the demonstration was installed in Lonnie's Pond (June 29, 2016) to establish the starting nitrogen content of the shellfish. At the end of the growing season, the second sample set will be sent to BUSIL to determine the final nitrogen content. The endpoint of the growing season will be determined in one of two ways:

- Water temperature of 6 °C for seven consecutive days; or
- Oysters are moved out of Lonnie's Pond.

The starting nitrogen content will be subtracted from the final nitrogen content to establish the nitrogen content of the shellfish that can be attributed to the growing season in Lonnie's Pond.

2. Findings/Results

A review of the Scope of Work provided by SMAST included both internal review by the AECOM technical team as well as review by MassDEP. Internal review found that all of the items enumerated in the monitoring program outlined in the AECOM Technical Memo entitled *Final Technical Memorandum on Shellfish Cultivation - Preliminary Engineering Design and Work Plan for Preferred Site(s)* dated May 4, 2016 were adequately addressed. These items are summarized from the tasks described in the SMAST Scope of Work as follows:

Task 1 - Water quality sampling and analysis via grab sampling

The purpose of this task is to quantify the nitrogen removal within the water column that can be attributed to the oyster demonstration in Lonnie's Pond. The sampling and chemical analyses will include the full suite of nutrient-related water quality parameters established as part of the Massachusetts Estuaries Project (MEP), and currently monitored as part of ongoing SMAST water quality sampling in Orleans. Eight sampling locations will be established, including previously-established monitoring stations. Samples will be taken every two weeks throughout the demonstration period (June, 2016 – November, 2016).

Samples will be analyzed for: temperature, salinity, total nitrogen (nitrate + nitrite, ammonia, dissolved organic nitrogen (DON), particulate organic nitrogen (PON), particulate organic carbon (POC), total suspended solids (TSS), chlorophyll-a (Chl-a), pheophytin-a, orthophosphate, dissolved oxygen (DO), transparency (secchi depth), and alkalinity. Key parameters that will be evaluated include changes in the grab sample concentrations of Chl-a, PON, POC, ammonium, TSS, and DO as water moves through the oyster deployment area.

Task 2 - Water quality sampling and analysis using time-series data loggers for DO, light attenuation and Chl-a (SONDES)

Four YSI-6600 moorings will be deployed at three locations with accompanying light sensors. Two SONDES will be deployed within the footprint of the oyster deployment area, one will be located at the surface and the other at 30 cm from bottom sediment. The third SONDE will be located in the mixed layer at the margin of the growing area and the fourth SONDE will be located at a control site opposite the growing area. DO, light attenuation (as an indicator of turbidity), Chl-a (via fluorescence), salinity, and water temperature will be recorded at 15-minute intervals. The SONDES will be maintained from July 1, 2016 through September 30, 2016 when the oysters are most actively filtering pond water and the water quality is lowest.

Analysis of this data will address any temporal changes in measured parameters, as well as the frequency and duration of measured values above and below key benchmark levels reported in the MEP Report for Pleasant Bay. When presented in conjunction with grab sampling data of nutrients, it is expected that it will be possible to discern water quality parameters that have been changed as a result of oyster cultivation. Based on previous work, SMAST hypothesizes that increases in water clarity and a lowering of Chl-a and POC/PON will occur.

Task 3 - Analysis of bio-deposition rates and mapping of impact areas

Particulate capture and deposition from oysters within oyster aquaculture areas will be accomplished using particle traps attached to the oyster bags over a range of oyster sizes. For each bio-deposition measurement event, the traps will be deployed for approximately 24 hours, with a minimum of three events within the project timeframe. Surface water samples will be collected during the particle trap deployment for comparative purposes. Data collected from particle traps will be used to track PON deposited by oysters and determine individual oyster bio-deposits and 24-hour bio-deposition rates. This will enable quantification of total PON removed to sediments from the water column via oyster bio-deposition and water column clearance rates of phytoplankton facilitated by large scale oyster culture. The overall area of sediment surface affected by oyster culture will be determined by tracking fecal pellet settling and dispersion using acoustic methods for measuring horizontal and vertical velocities. Two Acoustic Doppler Current Profilers (ACDPs) will be deployed in the demonstration site to determine average horizontal and vertical velocities of sinking particles on ebb tide and tidal spreading of bio-deposits. Based upon these velocities and the average depth of the system, the approximate boundaries for fecal pellet deposition to the sediment will be calculated and the concomitant areal extent of impact beyond the culture area will be delineated.

Contour maps of tidally averaged fecal pellet deposition will be presented with overlays of oyster aquaculture boundaries. These maps will include deployment period references to water column particulate concentrations, TSS and Chl-a to further link in situ water column clearance rates and particle concentration dependent bio-deposition rates.

Task 4 - Analysis of denitrification and oyster bio-deposits using sediment core incubations

Changes in the sediment rates of regeneration and nitrogen removal via denitrification are important aspects of the impact of oysters on water quality. While harvest provides a partial measure of nitrogen removal by oysters, it appears that this is only a fraction (10 to 50 percent) of the total nitrogen removal mediated by oyster deployment. Accurate determination of denitrification and nitrogen regeneration response is critical to accurate sizing of projects that use oysters to meet target nitrogen reduction loads.

To establish a planning value for denitrification for Lonnie's Pond, intact sediment cores will be collected and incubated under in situ conditions during a period in the summer where water temperature is high. Cores will be analyzed for sediment carbon and nitrogen re--mineralization rates, denitrification and sediment nitrogen regeneration rates. These data will help to determine how oysters effect overall water column nitrogen levels in Lonnie's Pond during the critical summer management period, and will inform the design of future projects.

Task 5 - Water quality and tidal volume sampling and analysis of two surface water inflows (herring run and stormdrain)

There are two identified surface water discharges on the south side of Lonnie's Pond, a herring run and a stormdrain. These will be instrumented with ADCPs to determine when flow is occurring. Periodic nutrient samples will be collected, with point measurement of flow. The purpose of this sampling is to determine those periods where high flow rates may be interacting with the oyster effect within Lonnie's Pond. If the data indicates that these flows are confounding the in-pond measurements, then additional work will be conducted in subsequent project years.

3. MassDEP Coordination Meeting

A meeting with Massachusetts Department of Environmental Protection (MassDEP) was held on August 4, 2016. Sia Karplus from Science Wares, Brian Howes from SMAST, Brian Dudley from MassDEP, and Thomas Parece from AECOM attended. The following comments were provided by MassDEP prior to this meeting.

- There is no mention of a Quality Assurance Project Plan (QAPP) in the sampling and analytical protocols;
- Sampling locations are not shown in the scope;
- With respect to the sedimentation studies, there should be greater detail on the hypothesized mechanisms of changes in the sediment rates of regeneration and nitrogen removal via denitrification. There should also be a discussion on how the experimental protocol will demonstrate and support evidence of these changes; and
- Actual data should be provided as a deliverable rather than limiting data analysis to graphical representations or maps.

During this meeting, responses to these comments were provided to the satisfaction of MassDEP. These responses are as follows.

- A Sampling and Analysis Plan (SAP) will be provided as an update to the QAPP currently in place for Massachusetts Estuaries Project water quality and benthic sampling;
- The sampling locations will be shown on a map with GPS coordinates and included in the SAP;

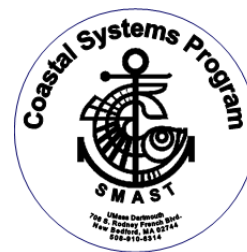
- The mechanism by which enhanced sediment denitrification is hypothesized to occur is the greater efficiency of Particulate Organic Nitrogen (PON) through oyster deposition as compared to gravity settling of PON; and
- The Town of Orleans will receive data tables of results in Excel format.

4. Action Items

The single remaining action item relative to review of the SMAST proposal for the limited monitoring program for the Lonnie's Pond demonstration project is to review the SAP once it is provided by SMAST to ensure that it includes all of the agreed-upon elements.

Appendix A

Scope of Work - University of Massachusetts Dartmouth School for Marine Science and Technology (SMAST) entitled Quantifying Oyster Aquaculture Impacts on Estuarine Nitrogen Related Water Quality: Lonnie's Pond, Upper Pleasant Bay, June 28, 2016



Scope of Work

**QUANTIFYING OYSTER AQUACULTURE IMPACTS ON ESTUARINE NITROGEN
RELATED WATER QUALITY: LONNIES POND, UPPER PLEASANT BAY**

Submitted to:

TOWN OF ORLEANS, MA.

By:

**Brian Howes Ph.D.
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**June 20, 2016
(rev) June 28, 2016**

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

An approach gaining momentum in many communities is the use of shellfish, particularly oysters to increase water clarity and remove nitrogen, while also supporting recreational fishing and the local economy. CSP has been at the fore front investigating oyster deployments/reefs as an in-estuary means to improve nitrogen related water quality. CSP scientists have begun quantifying the mass removal of water column nitrogen and quantifying the enhancement of associated enhancement of sediment denitrification ($\text{NO}_3^- \rightarrow \text{N}_2$) to gauge the nitrogen removal efficiencies of oysters. Oysters are being considered due to their high filtration rates, rapid growth, and ability to thrive in nutrient rich, warm, shallow waters over a range of estuarine salinities, as well as providing local economic benefits.

In this context, many Massachusetts communities have begun oyster propagation. However, almost none have quantified the integrated nitrogen removal through filtration, deposition and sediment denitrification and harvest. Similarly, water quality improvements associated with oyster deployments have not generally been accurately assessed. As a result, it is difficult to compare the cost/benefit of using shellfish compared to other nitrogen management approaches (Traditional WW treatment, PRBs, floating wetlands, enhanced natural attenuation) the accurately assessed to allow incorporation of shellfish culture into management or regulatory processes. The proposed scope of work focuses on quantifying the nitrogen processing and removal by the oyster/sediment complex within Lonnie's Pond, a terminal salt pond tributary to Upper Pleasant Bay, Orleans, MA. The pond has been selected due to its high level of nitrogen enrichment, its physical structure, its suitability for oyster culture and appropriateness for measuring nitrogen removal rates. The results are aimed at providing quantitative information to the Town of Orleans as it seeks to implement new nitrogen removal approaches and considers the use of shellfish for remediation of nitrogen related impairment of its coastal resources.

Project Purpose¹: In order to design and install a full-scale system for ongoing propagation that is based on an appropriate number and size of shellfish, additional information on necessary design parameters is needed. For oyster propagation, the AECOM engineers are seeking three factors from the Lonnie's Pond Oyster Demonstration Project:

- Extent and type of changes in water column nitrogen species and key eutrophication metrics. due to the filter feeding activities of oysters;
- Response of nitrogen cycling in sediments associated with oyster depositional processes;
- Confirmation of nitrogen-content of shell and soft tissue.

¹ From AECOM Project Description

Oyster Demonstration Deployments, Years 1-3. To provide the operable information needed for full-scale engineering design, the demonstration project designed for Lonnie's Pond is phased as follows:

- Year 1 (Summer, 2016): Cultivate approximately 200,000 single oysters in floating bags, starting at 2 inches (150,000) and 1 inch (50,000) starting in late June 2016;
- Year 2 (Fall, 2016 – Summer, 2017): Design and install a system for cultivating a sufficient quantity of oysters to achieve nitrogen related water quality goals and USEPA TMDL, based upon the monitoring results from Year 1. This goal may be expressed as bioavailable nitrogen or total nitrogen. The design will be refined, as necessary, for a permanent, ongoing installation in Year 3.
- Year 3: (Fall 2017 – Summer 2018): Continue refined program from Year 2.

The precise location of the oyster deployment in year 1 will be near the southern shoreline, see map below of both the southern as well as a northern locations. The plots are 1 acre, but only approximately one-third of the area will be used in Year 1, given the number of oysters to be deployed. A short-term ADCP deployment to assess circulation found no strong flows within the Pond, such that one site might be more appropriate than the other. The exact location of gear at the southern site needs to avoid direct discharge from the nearby surface water inflow. The year 1, deployment will employ floating surface bags to avoid potential hypoxic/anoxic conditions in bottom waters and to allow management of biofouling.

Lonnie's Pond South

 = Possible 1-Acre Demo Location



Monitoring Summary: Since the purpose of the Oyster Demonstration Project is to quantify changes in nitrogen levels and associated habitat quality parameters. Standard water quality sampling will occur biweekly with surface and bottom and possibly mid-depth samples depending on each sampling location's depth. At least 8 sites will be sampled in each full event, inclusive of one in the tidal river. In addition, autonomous sensors will be deployed at a minimum of 5 sites with surface and bottom at the oyster site, to record oxygen, chlorophyll,

salinity and water clarity. Sensors will be deployed to capture at least 2 months of record, one near the beginning of the deployment period and one near the end, when the oyster effect is predicted to be greatest.

DESCRIPTION OF PROJECT TASKS SUMMER 2016

Task 1 - Sampling for Lonnie's Pond Oyster Deployment (Water Quality). A sampling program will be implemented to quantify nitrogen removal by the oyster culture pilot deployment in Lonnie's Pond. Volunteers from the Town of Orleans will work with CSP scientists on the collection of freshwater inflow samples and as appropriate assist in logistical support. All chemical analyses and data synthesis will be performed through the Coastal Systems Analytical Facility at SMAST. The sampling and chemical analyses will include the full suite of nutrient related water quality parameters consistent with on-going and previous water quality monitoring in Lonnie's Pond and the overall Pleasant Bay system to be directly cross comparable to long term records. Eight (8) sampling locations will be monitored in Pond, building upon pre-existing monitoring stations. In addition, samples will be collected at the outflow from the cranberry bog and herring run, as possible. Additional stations will be added as appropriate and sampled bi-weekly throughout the oyster deployment period. Data collected under the proposed project will be compared to long term records and a pre-oyster deployment sampling of water quality for comparison to the multi-year post-oyster deployment conditions. Post-deployment analysis will also be structured on an upstream / downstream difference approach (based on flow direction by ADCP) where the total nitrogen transport/removal through the deployment area will be tracked with and without oysters (if time allows predeployment). Key components will be changes in chlorophyll, POC/PON, ammonium, TSS, D.O. concentrations in water as it moves through the oyster deployment areas, as well as data from autonomous sensors (described below). This latter approach is facilitated by the morphology and hydrodynamics of Lonnie's Pond that supports only one tidal inlet. Samples will be analyzed for: temperature, salinity, total nitrogen (nitrate + nitrite, ammonia, dissolved organic nitrogen, particulate organic nitrogen), chlorophyll-a (Chl-a), pheophytin-a, orthophosphate, dissolved oxygen, transparency (secchi depth), and alkalinity. Weather, tide-status, and results of water quality monitoring will be documented, with sampling performed so as to minimize both tide (ebb tide sampling) and weather-related effects on samples. Salinity measurements will be correlated to rainfall and other relevant parameters. Quality assurance samples (field duplicates) will be collected (5%-10% of total number of samples collected) with the goal of gaining acceptance of study results by MassDEP and USEPA.

Deliverable: Tables and graphs will be presented comparing pre- and post-oyster aquaculture water quality results for Lonnie's Pond. Emphasis will be on changes in water column nitrogen, pigment concentrations and water clarity and shifts between various constituent pools.

Task 2 - Time-series Dissolved Oxygen (DO)/Chl-a Moorings (Intensive Sampling). CSP-SMAST scientists will conduct continuous monitoring via a mooring program of key water quality parameters to assess the impact of oyster aquaculture on the ambient water column in concert with the field water sampling effort. This task will follow protocols and procedures established under the time-series mooring (DO, Chl-a) program for the MEP analysis of the Pleasant Bay Estuary System and for the Town of Falmouth's and Mashpee's Oyster

Demonstration Projects for cross comparability. Data collected will also be compared to time-series baseline DO/Chl-a data previously collected from Lonnie's Pond during the MEP assessment in the summer of 2003 under pre-oyster conditions. A total of four YSI-6600 moorings will be deployed at three locations with accompanying light sensors: within, at the margins and distant from the oyster deployment area. DO, light attenuation (as an indicator of turbidity), Chl-a (via fluorescence), salinity, and water temperature will be recorded at high frequency (15-minute intervals). Moorings will be deployed in the mixed layer, with a surface and bottom (30cm from sediment) mooring within the footprint of the oyster deployment. The moorings will be maintained from Jul. 1 – Sept. 30 when the oysters are most actively filtering pond water ("oyster effect" largest) and the pond water quality is lowest. Moorings will be calibrated bi-weekly. Calibration samples will be collected at the specific depth and location of each sonde. At the time of calibration, each sonde will be inspected, cleaned and the data will be downloaded. Sondes will then be returned to the moorings and secured. Calibration sampling will include triplicate Winkler samples for dissolved oxygen determination as well as collection of whole water for chlorophyll extraction, with light profiles being made for independent extinction determination.

Deliverable: Graphical representation of data, analysis relating temporal changes in parameters, frequency and duration above and below key benchmark levels, and correlations between inferred biological and physical processes producing observed changes. When presented with water column sampling data of nutrients we anticipate being able to determine transformations that have occurred as a result of oyster cultivation. Preliminary work indicates that water clarity increases and a lowering of chlorophyll a and POC/PON should be seen with this analysis.

Task 3 - Bio-deposition Rates and Bio-deposit Impact Areas. CSP scientists will quantify particulate capture and deposition from oysters within oyster aquaculture areas using particle traps attached to the oyster bags or separate cages over a range of particulate concentrations. A particle trap design was developed and successfully deployed in Green Pond, Falmouth, MA during summer 2015 to measure oyster bio-deposition (C,N) rates. While effective, it was determined based on 2015 results that modifications to the trap design maybe necessary to strengthen the signal of oyster bio-deposition in the treatment particle traps. As such and for the purposes of the Lonnie's Pond effort, a modified particle trap design maybe considered. The frame and funnel are secured below 0.5 m² floating oyster bags. Prior to deployment, traps will be filled with seawater that was previously collected from the system, filtered, and equilibrated to ambient temperature. The step of pre-filling of traps minimizes the background particulate concentration increasing the accuracy of the measurements. For each bio-deposition measurement event, the traps will be deployed for approximately 24 hours with a minimum of three (3) but as many as five (5) events per July-September. Surface pond water samples will be collected during the particle trap deployment for comparative purposes. The surface pond water samples, as well as the trap samples, will be processed by the CSP analytical laboratory for chlorophyll-a, total suspended solids, and particulate organic carbon and nitrogen. TSS filters will be analyzed for particulate organic carbon and nitrogen using a Perkin Elmer 2400 elemental analyzer. Data collected from particle traps will be used to track PON deposited by oysters and determine individual oyster bio-deposits and 24 hour bio-deposition rates. This will enable quantification of total PON removed to sediments from the water column via oyster bio-deposition and water column clearance rates of phytoplankton facilitated by large scale oyster culture. The overall area of sediment surface affected by oyster culture will be determined by

tracking fecal pellet settling and dispersion using acoustic methods for measuring horizontal and vertical velocities. Two acoustic doppler current profilers will be deployed to determine average horizontal and vertical velocities of sinking particles on ebb tide and tidal spreading of bio-deposits. The ADCPs will be deployed in the oyster areas. Based upon these velocities and the average depth of the system, the approximate boundaries for fecal pellet deposition to the sediment will be calculated and the concomitant areal extent of impact beyond the culture area will be delineated. This acoustic method was developed by the CSP under funding from Nortek, with method testing in Green Pond, Falmouth, MA., specifically for investigating bio-deposition in estuaries across the region.

Deliverables: Maps of tidally averaged fecal pellet deposition contours will be presented with overlays of oyster aquaculture boundaries. These maps will include deployment period references to water column particulate concentrations, TSS and Chl-a to further link in situ water column clearance rates and particle concentration dependent bio-deposition rates.

Task 4 - Sediment Core Incubations and Analysis of Denitrification and Oyster Bio-deposits. Intact sediment cores will be collected and incubated under in situ conditions during summer to determine the effect of oyster bio-deposits on sediment carbon and nitrogen remineralization rates, including denitrification. It will also provide sediment nitrogen regeneration rates to determine how oysters effect overall water column nitrogen levels in Lonnie's Pond during the critical summer management period, when impairment is greatest. Changes in the sediment rates of regeneration and nitrogen removal via denitrification are primary factors for inclusion of oysters in nitrogen remediation plans. While harvest provides a partial measure of nitrogen removal by oysters, it appears that this is only a fraction (10%-50%) of the total nitrogen removal mediated by oyster deployment. Accurate determination of the larger nitrogen removal and nitrogen regeneration response is critical to understanding the efficacy of oyster aquaculture as a water quality management strategy. The CSP will measure nitrogen regeneration as in the MEP estuaries and denitrification using a state-of-the art N_2/Ar method also part of routine CSP analysis and tailored to investigating affects from oyster bio-deposition. The methods have been developed for these shallow estuaries where oyster deployments are in place.

Core Collection and Incubation: The concept in determining sediment nutrient regeneration rates and how these maybe altered by bio-deposition from oyster propagation will be to collect undisturbed sediment samples and incubate them under in situ conditions to allow natural exchange of nutrients between the sediment and overlying water under controlled conditions. The approach and methods which will be employed in this effort are currently being used by the CSP Technical Team for numerous estuarine studies in southeastern Massachusetts including the work undertaken by the Massachusetts Estuaries Project. Rates of nitrogen release will be determined using undisturbed sediment cores incubated so as to obtain quantitative rates of oxygen uptake, nutrient flux and N_2 gas emission rate. Three time points will be collected at not less than 30 minute intervals. It is foreseen that incubations may last for up 8-12 hours. It is important to tailor the time of incubation to ensure an adequate "signal" for each of the flux constituents. 8 sediment cores (15 cm inside diameter) will be collected by SCUBA divers and cores will be transported to a shore side field lab. Water from Lonnie's Pond will be collected and filtered for each batch of 4-8 core sites to replace the headspace water of the flux cores prior to incubation. The collected water will be filtered and used to replace the headspace water of the flux cores prior to incubation. All of the sediment incubations will be performed immediately

upon return to shore side lab. Upon arrival at the shore-side field laboratory sediment cores are inspected for any surface disturbance or large fauna (for example crabs or fish) which cause rejection of these cores. The cores will be transferred to pre-equilibrated temperature baths, the headspace water overlying the sediment will be replaced with filtered water collected from the field collection site, the water overlying the sediment gently mixed with a magnetic stirrer and the headspace is sealed with a gas-tight closure fitted with sampling ports. The headspace will be set so as to maximize the signal and minimize the incubation time (ideally ~10 hours). Periodic 90 mL water samples will be withdrawn (volume replaced with filtered water), filtered into acid leached polyethylene bottles and held on ice for nutrient analysis. Ammonium and ortho-phosphate assays will be conducted within 48 hours and the remaining samples frozen (-20°C) for assay of nitrate + nitrite (Cd reduction: Lachat Autoanalysis), DON, silicate, dissolved organic phosphorus, total nitrogen, total phosphorus. Rates will be determined from linear regression of analyte concentrations through time. A “significant flux” is defined as one where the least squares regression of the headspace analyte concentration over time has a slope different from zero.

N₂ Analysis using High Precision Membrane Inlet Mass Spectrometry: N₂ excess will be measured using membrane-inlet mass spectrometry (MIMS). In the field, periodic 60 mL samples will be collected in glass serum bottles for denitrification assays. Bottles will be overflowed with three volumes of core water to ensure sample integrity. Samples will be held at air temperature and transported back to CSP-SMAST for analysis by mass spectrometry. All fluxes will be adjusted for water removals and measured activities within the headspace water. In the laboratory, sample water is pumped at ml/min rates through a gas permeable membrane in order to extract gas into the mass spectrometer inlet. Sample gas is analyzed by the mass spectrometer for masses 28 and 40 for determining the N₂ to Ar ratio. Calibration is made by comparison with a reference gas of known composition. A quadrupole mass spectrometer is used for its sensitivity and speed of analysis. Based on series of method related experiments, it was determined that the samples collected should be preserved before capping and once capped, samples should be kept at room temperature to prevent bubble formation. Sample preservative will be either mercuric chloride or hydrochloric acid such that the pH is lowered to a value of 2.

Deliverables: Tabular and graphical summaries of benthic nutrient regeneration and denitrification rates will be presented with sediment carbon concentrations that drive those rates. Rate measurements will be presented in the context of water column particulate nitrogen levels, rates and areal extent of oyster bio-deposition to further delineate the impact area and role of oyster culture in modifying overall water quality.

Task 5 - Project Management and Reporting. There are 2 identified surface water discharges to Lonnie's Pond. These will be instrumented with stage recorders to determine when flow is occurring. Periodic nutrient samples will be collected, with point measurement of flow. The purpose is to determine those periods where high flow rates may be interacting with the Oyster effect within the Pond. If the data indicates that these flows are confounding the in-pond measurements, then additional work will be conducted in Years 2 and 3.

Task 6 - Project Management and Reporting. This element includes the project management costs and the costs of data synthesis and interpretation. The synthesis includes graphical representation of the data, a Draft and Final Technical Report and presentation/meetings with the Town of Orleans Water Quality Management Committee.

Deliverables: Tabular and graphical summaries of benthic infaunal community characteristics will be presented in context of historical infauna data collected by the MEP in 2003.

SCHEDULE

Work will begin in late June and continue until November 1, 2016 or until the oysters are removed/submerged. The draft first year Technical Memorandum will be delivered by January 31, 2017, with the final Technical Memorandum completed by March 30, 2017 depending on the time required for Town comments.

MEETINGS

SMAST-CSP team contact will be available for regular project communication with the Town's designated committee. The CSP-SMAST Project Team will be available to attend three Orleans Water Quality Advisory Panel meetings, including a presentation at the beginning of the summer 2016 monitoring to describe the plan, at the completion of Tasks 1-4 to discuss findings and recommendations for summer 2017 growing and monitoring if this is programmed, and presentation of the final report.

The CSP-SMAST Project team will also be available to discuss project result with regulators (1 meeting) if required at appropriate stages in the process. Additional meetings (more than the 4 noted) will be supported by the Town (time and materials).

BUDGET

Cost Breakdown for Monitoring Oyster Deployment in Lonnie's Pond

	<u>Total Task Cost</u>
Task 1: Sampling for Lonnie's Pond Oyster Deployment (Water Quality)	\$11,000
Task 2: Time-series Dissolved Oxygen (DO)/Chl-a Moorings	\$6,000
Task 3: Bio-deposition Rates and Bio-deposit Impact Areas	\$2,000
Task 4: Sediment Core Incubations and Analysis of Denitrification and Oyster Bio-deposits	\$5,000
Task 5: Monitoring of 2 surface water inflows	\$2,000
Task 6: Reporting and Project Management	\$2,500
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Lonnie's Pond System	
Oyster Deployment Monitoring 2016	TOTAL = 28,500 ¹

¹ It is anticipated that additional funding will be made available, as possible, to enhance the final reporting and dissemination of results.