Town of Orleans
Lonnie's Pond Aquaculture and Nitrogen Management Plan

A Partnership with
Coastal Systems Program
School for Marine Science and Technology
University of Massachusetts Dartmouth

Lonnie's Pond Aquaculture/TMDL Annual Report (*Parts 1 & 2*)
January 26, 2020

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Prepared for:

Town of Orleans

1.0 Background

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

In 2007, MassDEP finalized TMDLs for Pleasant Bay that identified portions of the estuary, including Lonnie's Pond, as having excessive nitrogen with resulting impaired water and habitat quality.[1] The Massachusetts Estuaries Project report for Pleasant Bay,[2] which is the technical basis for the TMDLs, suggested that the nitrogen load to Lonnie's Pond would need to be lowered by 300 kg N/yr to mitigate the impairments.

In 2016, the Town began a demonstration project in Lonnie's Pond to evaluate a non-traditional, nitrogen reduction approach using oyster aquaculture. The Lonnie's Pond Demonstration Project was planned as a three-year effort to evaluate likely water quality improvements and determine any implementation issues associated with enhanced aquaculture for nitrogen reduction as part of achievement of the TMDL without sewering within the Pond watershed. Monitoring during the demonstration project found significant removal of nitrogen and some water quality improvements due to shellfish growth and bio-deposition.[3]

In 2018, the Town approved the Lonnie's Pond Aquaculture and Nitrogen Management Plan[4] to transition from an oyster aquaculture demonstration project to a sustainable long-term oyster aquaculture system for nitrogen removal toward achieving compliance with the TMDL. The Management Plan detailed the logistical, regulatory, monitoring, and public coordination components needed for long-term use of aquaculture as part of the Town's nitrogen management program for its estuaries, including two regular reports on Plan implementation to be prepared by the Monitoring Contractor [Coastal Systems Program, School for Marine Science and Technology, University of Massachusetts Dartmouth (CSP-SMAST)]: a Semi-Annual Status Update and Annual Report. A Quality Assurance Project Plan (QAPP) was submitted and approved by MassDEP in May 2019 to ensure regulatory acceptance of collected data for TMDL compliance. As specified in the Management Plan, the Annual Report summarizes aquaculture

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

[2] Howes B., S. W. Kelley, J. S. Ramsey, R. Samimy, D. Schlezinger, E. Eichner (2006). Linked Watershed-Embayment Model to Determine Critical Nitrogen Loading Thresholds for Pleasant Bay, Chatham, Massachusetts. Massachusetts Estuaries Project, Massachusetts Department of Environmental Protection. Boston, MA. 245 pp.

[3] Coastal Systems Program, School of Marine Science and Technology (CSP/SMAST), University of Massachusetts-Dartmouth, Lonnie's Pond Shellfish Demonstration Project: Year 2 Monitoring Summer/Fall 2017 Oyster Deployment. September 2018. 75 pp.

[4] Howes, B. and E. Eichner. 2018. Town of Orleans Lonnie's Pond Aquaculture and Nitrogen Management Plan. Coastal Systems Program, School of Marine Science and Technology (CSP/SMAST), University of Massachusetts-Dartmouth. New Bedford, MA. 128 pp.

activities, shellfish N removal, progress toward meeting the TMDL, and considerations for refinement of Plan activities and follows the 1st Semi-Annual Status Update, submitted July 31, 2019. This current document is part one of the 1st Annual Report relating directly to TMDL compliance monitoring, focusing primarily on the aquaculture N removal relative to the TMDL N target. Part two will focus on water quality impacts and ecosystem monitoring and their relationship to TMDL goals.

In 2019, Ward AquaFarms deployed year one (seed) and year two oysters in mid-July. Approximately, 1.5 million oysters, weighing 1,359 kg live wet weight, with 4.2 kg of N contained within their tissue and shell. Oysters remained in Lonnie's Pond until mid-December for an average deployment of 144 days. Upon harvest in December 2019 there were 718,596 live year 1 and 69,427 live year 2 oysters containing a total N mass of 63.9 kg N in their tissue and shell. Accounting for the N content in the YR1 and YR2 oysters at the times of installation and harvest resulted in a net removal of 59.7 kg of nitrogen (harvest N minus deployment N) by the implementation of the aquaculture plan. This removal was 79% of the plan goal (75 kg N) and 20% of the overall Lonnie's Pond TMDL nitrogen removal target. An additional 1.9 kg N was removed by YR3 and YR4 oysters that remained from the prior demonstration project, which makes the total N removed by oysters in 2019 ~61.9 kg N or 82.5%.

These harvest results were comparable to those achieved in 2018, the final year of the demonstration project, but were achieved in 62 fewer days due to the later initial deployment. Therefore, it is possible that additional nitrogen could be removed with longer oyster deployment (e.g. earlier deployment).

2.0 Water Quality Monitoring

Using procedures employed in the prior Oyster Demonstration Project and detailed in the approved Lonnie's Pond QAPP, CSP/SMAST staff began coordinating and conducting the 2019 biweekly water quality monitoring in Lonnie's Pond starting on April 24th, 2019. As per the 2019 monitoring plan, there were eleven sampling events from 4-24-19 through 10-23-2019 (Table 1). Samples were collected at nine locations in Lonnie's Pond (Figure 1). The deployment of continuous monitoring devices (dissolved oxygen, Chlorophyll-*a*, salinity, temperature) was delayed to coincide the later oyster deployment. Deployment of two moorings occurred on 08-06-2019 at LP6 and LP10 and recorded until retrieval on 12-04-2019.

All water quality samples were transported back to the Coastal Systems Analytical Facility to be processed for dissolved nutrients, particulate organic C and N, total nitrogen, total chlorophyll-*a* pigments, and salinity. In addition to the pond sampling, water samples and stream flow measurements were made biweekly at the two streams discharging into Lonnie's Pond: 1) the herring run from Pilgrim Lake and 2) the cranberry bog outlet downstream of Crystal Lake. A continuous stage meter is deployed in the stream from Pilgrim Lake to provide daily freshwater discharge volume. Flow from the cranberry bogs is minimal and intermittent and is only monitored at the time of the biweekly stream sampling. Flows in 2019 from the cranberry bog were very low and there were no flows from the bog, September through October.

Table 1. Sampling dates for water quality and laboratory assays performed on samples. Note that TDN is Total Dissolved Nitrogen, POCN is particulate organic carbon and nitrogen (mainly phytoplankton), TSS is total suspended solids, CHLA is total chlorophyll-*a* pigments.

		Assays							
Sample Date	**# of samples**	**NH4**	**PO4**	**NO3/NO2**	**TDN**	**POCN**	**TSS**	**CHLA**	**Salinity**
4/25/2019	20	X	X	X	X	X	X	X	X
5/13/2019	21	X	X	X	X	X	X	X	X
5/28/2019	21	X	X	X	X	X	X	X	X
6/12/2019	21	X	X	X	X	X	X	X	X
6/26/2019	21	X	X	X	X	X	X	X	X
7/11/2019	21	X	X	X	X	X	X	X	X
7/25/2019	21	X	X	X	X	X	X	X	X
8/8/2019	22	X	X	X	X	X	X	X	X
9/5/2019	21	X	X	X	X	X	X	X	X
9/23/2019	23	X	X	X	X	X	X	X	X
10/23/2019	23	X	X	X	X	X	X	X	X
Total	**235**								

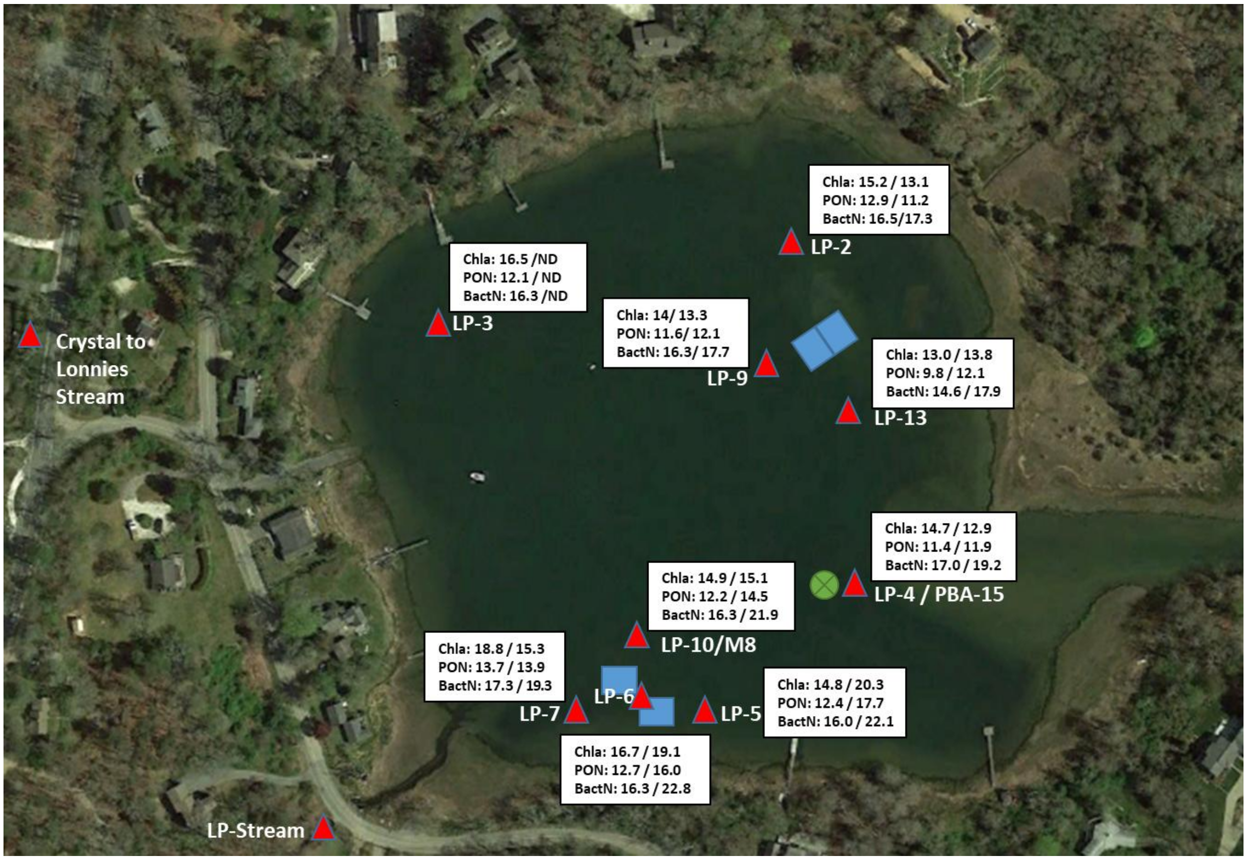

Figure 1. Station map of Lonnie's Pond 2019 water quality sampling locations. The red triangles were sampled by CSP staff April 24th – November 23rd, green circle is the PBA water quality station and was sampled biweekly by volunteers from July 15th – September 15th, and the blue squares represent the oyster deployment areas. White boxes show mixed layer average concentrations of total chlorophyll-*a* (µg L^{-1}), particulate organic nitrogen (µM), and bioactive nitrogen[5] (µM). Values represent averages of samples collected July – October for 2019 and 2018 (2019/2018), the active oyster growth period in those years.

Water quality monitoring indicated that Lonnies Pond in 2019 was generally horizontally mixed throughout. Using paired t-tests, there was no consistency in the differences around Lonnies Pond during pre-oyster WQ conditions (May 28th - July 11th) or post-oyster conditions (July 25th – September 5th). Generally, there was little variability in nutrient concentrations across the pond on any given sampling day. However, there were differences in nutrient concentrations over the season, with the highest concentrations in June and lowest in April and September/October (Figure 2). These variations can be attributed, in part, to meteorological conditions. An increase in freshwater inputs to Lonnies Pond from Pilgram Lake and Crystal Lake likely result in the increase of nutrient and total chlorophyll-*a* concentrations from April to June. This can be seen in the low surface salinity on 5-13-2019 at stations LP3 (18.1 ppt) and LP7 (14.9 ppt), compared to an average surface salinity of 25.1 ppt in the rest of the pond.

[5] Bioactive nitrogen consists of dissolved inorganic nitrogen and particulate organic nitrogen, representing the most biologically active nitrogen within the total nitrogen pool.

Oysters were deployed starting 7-09-2019 to 7-25-2019. Average water quality conditions from July 11th to October 23rd throughout the pond appear similar (Figure 1). However, samples from the control station, LP3, showed no significant difference in surface water PON, TN, total chlorophyll-*a*, bioactive N during pre-oyster deployment (May 28th – July 11th) compared to post-oyster deployment (July 25th – September 5th). Whereas, all other stations, except LP2, there was a significant reduction in PON and TN concentrations after the oysters were deployed. Similarly, all stations, except LP2, 5, and 13, showed significant reductions in bioactive N concentrations from samples pre-oyster versus post-oyster deployment. Only LP4 had significantly higher total chlorophyll-*a* concentrations pre-oyster versus post-oyster. And only LP2 had significantly lower total chlorophyll-*a* concentrations pre-oyster versus post-oyster. However, the bulk of the evidence indicates that the oysters were lowering the PON, TN and phytoplankton biomass levels in the regions associated with oysters while far field samples showed no change in these constituents when oysters were deployed. Note: significance for all tests was $p=0.1$.

We attempted to refine the water quality effect of the oysters by looking for changes in surface water quality as water moves through the oyster areas (e.g. water from LP7 to LP 5 or LP2 to LP13 on ebbing tide). However, no statistically significant differences were found. This is most likely the result of the highly variable flow field due to tidal and wind driven circulation. Tidal and wind driven flow velocities in the region of the oysters are very low and variable, such that flow through the bags can follow significantly different tracks. Therefore predicting the specific upgradient and downgradient sampling points on any given day has large uncertainties, even though over the long term there is a general particle track for the flood and ebb tides. This is confirmed by the sediment analysis which shows that fecal material from the oysters is deposited around the margins of the deployment area, consistent with a diffuse low velocity flow field. It should be noted that water quality data from samples in defined flow fields (e.g. flow path is defined) typically show significant declines from up to down gradient samples of 30% (Bournes Pond, Falmouth).

Additional water quality sampling was conducted by Chatham/Orleans volunteers from July 8th to September 4th at sentinel station PBA-15, which has a long-term water quality record of 20+ years provides a quality assurance check. Water quality results at this station (PBA-15) directly coincide with station LP-4. Comparison of water quality sampling by volunteers and SMAST staff between July and September show very similar results even though they are not taken on the same day, indicating that the volunteer WQ monitoring is performing well (Table 2).

Table 2. Comparison of average particulate organic nitrogen, bioactive N, and total N from July to September for similar sampling dates by CSP-SMAST (LP-4) and volunteer (PBA-15) WQ sampling efforts. The relative percent difference (RPD) between the monitoring programs (PBA-15, volunteer program and LP4, SMAST staff) indicates a high degree of congruence, which increases the confidence in the data.

	PBA15	LP4	RPD
PON (uM)	14.9	13.8	8%
Bioactive N (uM)	18.4	19.2	4%
TN (uM)	45.0	39.6	13%

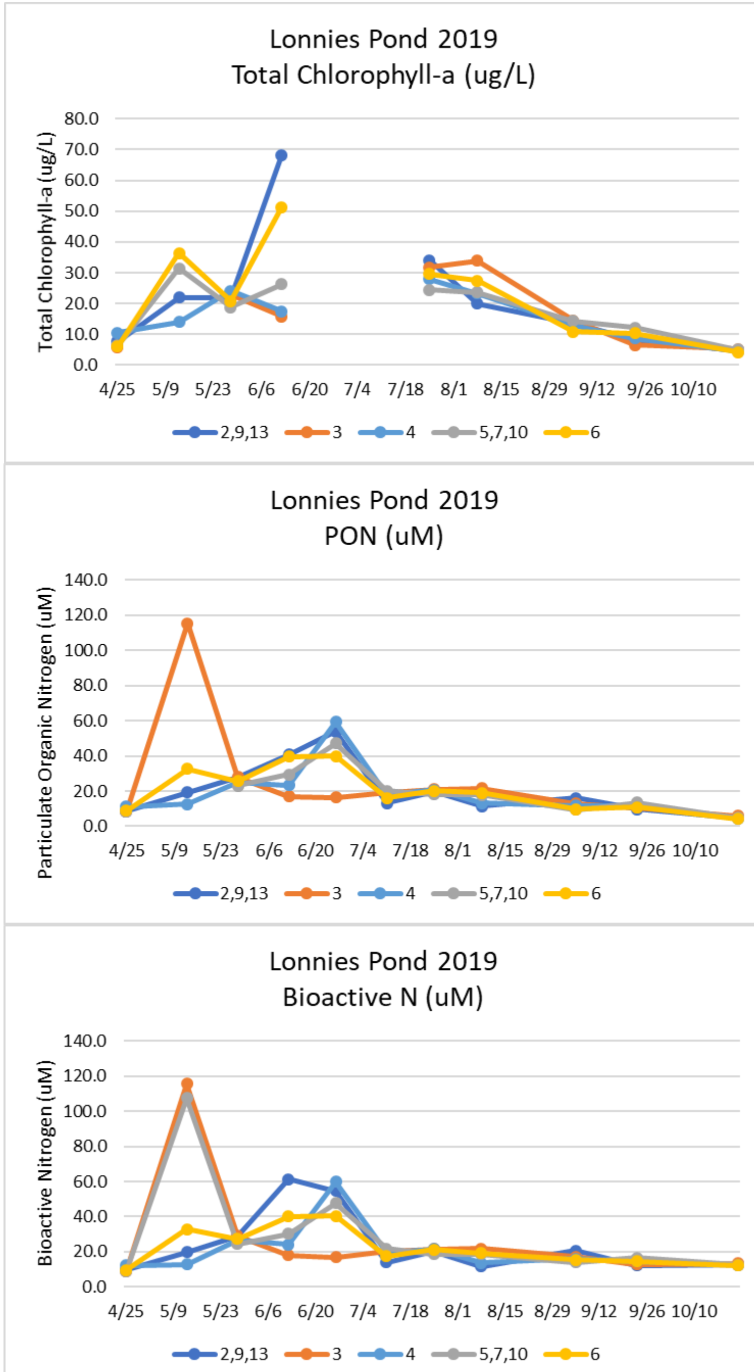

Figure 2. Time-series of mixed layer average total chlorophyll-*a*, particulate organic nitrogen, and bioactive nitrogen at stations LP-3, LP-4, LP-6 and averages of the stations (LP 5, 7, 10) and (LP 2, 9, 13) associated with the oyster deployment. Station numbers refer to locations in station map in Figure 1.

During the 2019 field season there appeared to be spring and summer phytoplankton blooms as seen in the chlorophyll a and PON records particularly at stations LP-3,5,7,10 (Figure 2). It also appears that phytoplankton and PON levels declined by September and remained low through November. This follows the season light and temperature cycle in Lonnies Pond and indicates that oyster growth should not be limited by food supply in an earlier deployment (e.g. April). The measured water quality parameters are also consistent with a nitrogen enriched eutrophic basin, where chlorophyll-*a* levels are above 20 ug/L for much of the spring and summer.

Steam Inflows: Lonnies Pond receives freshwater discharge from two streams, Pilgram Lake to Lonnies Pond and Crystal Lake Bog to Lonnies Pond. These additional nitrogen inputs to Lonnies Pond are important to measure as they play a role in setting the nutrient field in Lonnies Pond. Pilgrim Lake stream discharges freshwater to Lonnies Pond bringing nitrogen with it. The stream flows throughout the year with highest flows and nitrogen load regularly occurring in the spring, with lesser flow and load occurring in the summer months (Table 3). Water quality results from 2017, 2018 and 2019 also indicate that Lonnies Pond nitrogen concentrations decrease throughout the summer (Figure 2). In 2019 the Pilgram Lake stream input ~45.7 kg of nitrogen into Lonnies Pond from April – June (pre-oyster), compared to only ~14.7 kg N July – September (with oysters).

Overall, nitrogen inputs to the pond via stream discharges was lower than in 2018, but very similar to that in 2017. This results from the lower stream flow volumes in 2019 rather than lower concentrations in water originating in Pilgrim Lake.

Table 3. Total nitrogen load (kg) entering Lonnies Pond via Pilgram Lake Bog stream in 2019. April – June 2019 was without oysters, where as 2017 and 2018 had oysters during spring time freshwater flow conditions. July – September for all three years had oysters during these summer time freshwater flow conditions.

	FLOW (m^3)	Year	NH4 Load (kg/3-month)	NOX Load (kg/3-month)	DIN Load (kg/3-month)	DON Load (kg/3-month)	PON Load (kg/3-month)	TN Load (kg/3-month)
Apr-Jun 2017	93257	**Total Load (April-June 2017)**	3.86	1.92	5.79	32.59	12.08	50.46
Jul-Sept 2017	39420	**Total Load (July-Sept. 2017)**	1.83	2.12	3.95	14.64	2.85	21.45
Apr-Jun 2018	137888	**Total Load (April-June 2018)**	4.63	6.18	10.81	57.40	14.45	82.67
Jul-Sept 2018	100995	**Total Load (July-Sept. 2018)**	5.08	9.91	15.00	39.52	8.11	62.62
Apr-Jun 2019	51956	**Total Load (April-June 2019)**	6.09	2.66	8.74	25.81	11.19	45.74
Jul-Sept 2019	23178	**Total Load (July-Sept. 2019)**	1.20	2.06	3.26	8.58	2.89	14.73

Crystal Lake Bog to Lonnies Pond stream discharges a significant amount of total nitrogen load with higher flows during winter and spring time conditions. As summer continues, inputs from the Crystal Lake Bog stream dampen, eventually drying up for a portion of late summer. Thus, the total nitrogen load from Crystal Lake Bog into Lonnies Pond is minimal throughout the critical summer months (July and August), with ~ 7.5 kg N and ~3.2 kg N inputs in July and August, respectively (Figure 3).

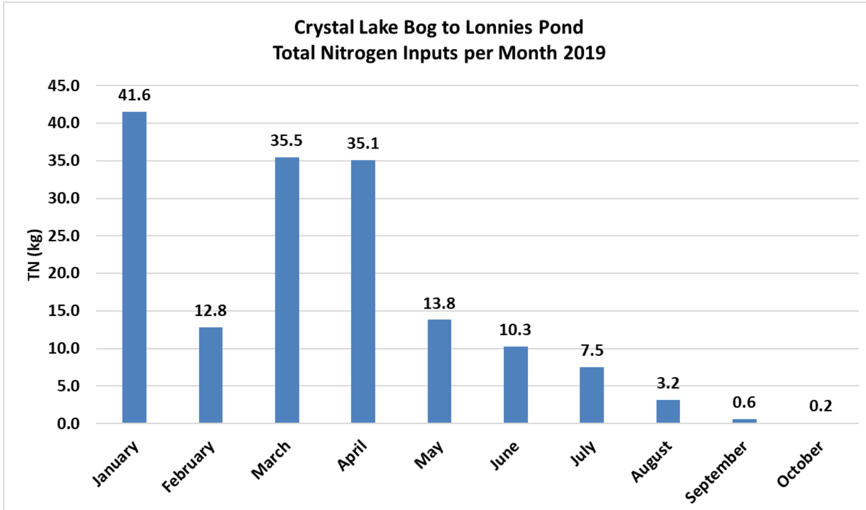

Figure 3. Total nitrogen load (kg) entering Lonnies Pond via Crystal Lake Bog stream in 2019.

3.0 Dissolved Oxygen and Chlorophyll-*a* Continuous Monitoring

Two autonomous recording multiparameter sondes were deployed from August 8 through December 4, 2019. One sonde was deployed 30 cm from the bottom in approximately 1 meter of water along the southwest corner of the aquaculture area (Lonnies Pond South, see Figure 4 and 6). The second sonde was deployed 30 cm from the bottom in approximately 3.5 meters of water slightly north of the northeast corner of the oyster deployment area. Oysters filter large quantities of water. Phytoplankton removed from the water are deposited as fecal pellets on the underlying sediment where they undergo remineralization releasing nutrients and consuming oxygen. Autonomous instrumentation was deployed to measure dissolved oxygen, chlorophyll, temperature, salinity and depth at 15 minute intervals. Then high frequency measurements provide a means to determine whether concentrated benthic organic matter beneath the oyster bags causes significant local oxygen depletion. The measurements also provide the information necessary to determine whether water column concentrations of chlorophyll-*a* differ between locations within the aquaculture and adjacent locations. Lastly, continuous measurements of physical parameters (temperature, salinity, depth) provide the data necessary to assess environmental factors that may affect growth and mortality, such as elevated temperatures or low salinity.

Oxygen levels were periodically low but the data was spotty and unreliable and is not shown here. The water column sampling program showed signs of depleted oxygen, however, the water column profile data did not indicate hypoxia within the oyster propagation area itself. Continuous monitoring may have revealed periodic short term oxygen excursions below 3 mg/L, however, water column profiles suggest that on the days when water column sampling was performed hypoxia was unlikely within the shallow waters underlying the oyster propagation area. Significantly, bottom water dissolved oxygen at Station 3, 4, and 10 was hypoxic on several dates prior to the installation of the sondes, but at depths of 4m. The deep basins of

Lonnies Pond have historically had depressed oxygen concentrations not related to oyster culture and this appears to be the case in 2019 as well.

Continuous chlorophyll data indicated only slight differences between the two sonde locations (Figure 4 and 5) except in late November when there was an apparent bloom isolated to the southern edge of the propagation area. Otherwise the records were similar though skewed with higher chlorophyll concentrations observed at Lonnies Pond South, where Pilgrim Lake Steam discharges. Comparison of chlorophyll data from the two sondes from August 8 – Nov. 20 (Nov. 20 – Dec. 4 data was excluded) gave a linear relationship:

LPNorth Chl (ug/L) = LPSouth Chl (ug/L)* 0.72 + 1.56 Chl (ug/L) [R2=0.75],

showing that chlorophyll concentrations along the northern edge of propagation area were generally 30 % lower than at the southern edge. This decline in chlorophyll as water flows through the propagation area is consistent with lower levels in Lonnies Pond when oysters are deployed compared to pre-deployment (see above). The clear pattern in this data compared to the water column water quality sampling results (above) stems from the high frequency sampling which allowed the pattern to emerge even in the temporally variable flow field.

Like the water column sampling result, the time series data indicated periodic blooms within the pond. Storm activity with pulses of surface runoff of rainwater and mixing of deep high nutrient water into the upper mixed layer likely accounts for the observed late season bloom (November/December). Whereas the Spring Bloom is typical of high nitrogen inflows increased sediment nutrient release as the system warms. The fall bloom was not seen in the water column sampling as it ended on Nov 23rd. Lonnies Pond South Salinity records (Figure 6, see Figure 7 for comparison) during the same time period indicate salinity excursions down to 0ppt. Examination of weather records from the Chatham Airport indicated rainfall during these periods of greater than an inch for each excursion.

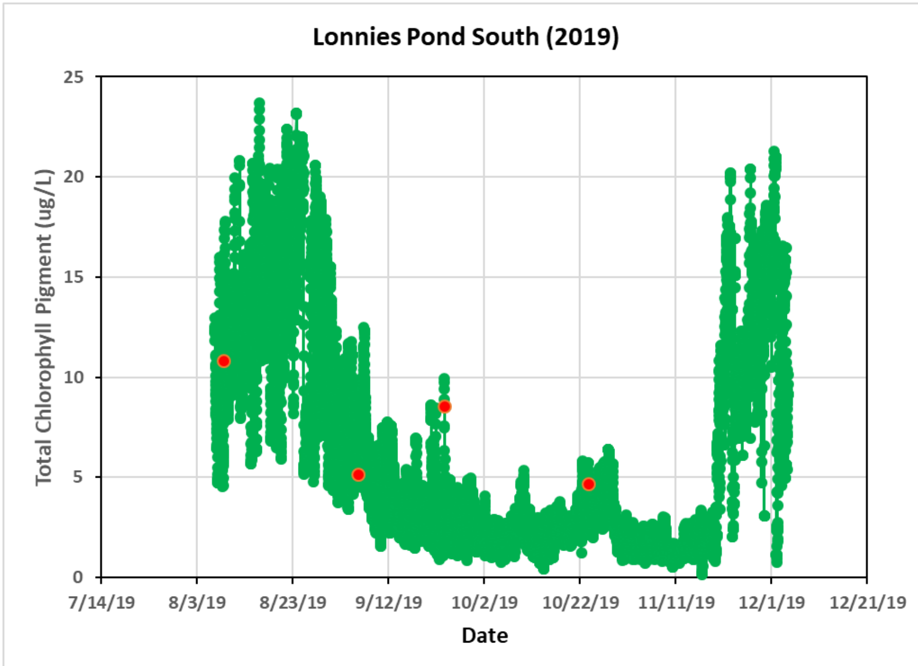

Figure 4. Lonnies Pond South mooring continuous record of chlorophyll pigments. Red dots indicate laboratory chlorophyll pigment extractions that served to calibrate the autonomous record.

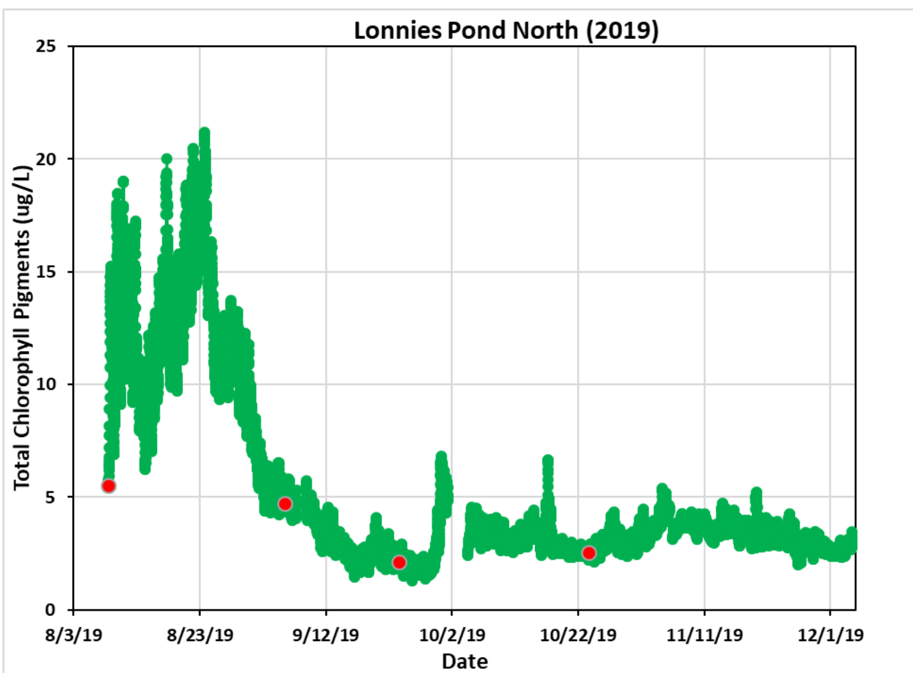

Figure 5. Lonnies Pond North mooring continuous record of chlorophyll pigments. Red dots indicate laboratory chlorophyll pigment extractions that served to calibrate the autonomous record.

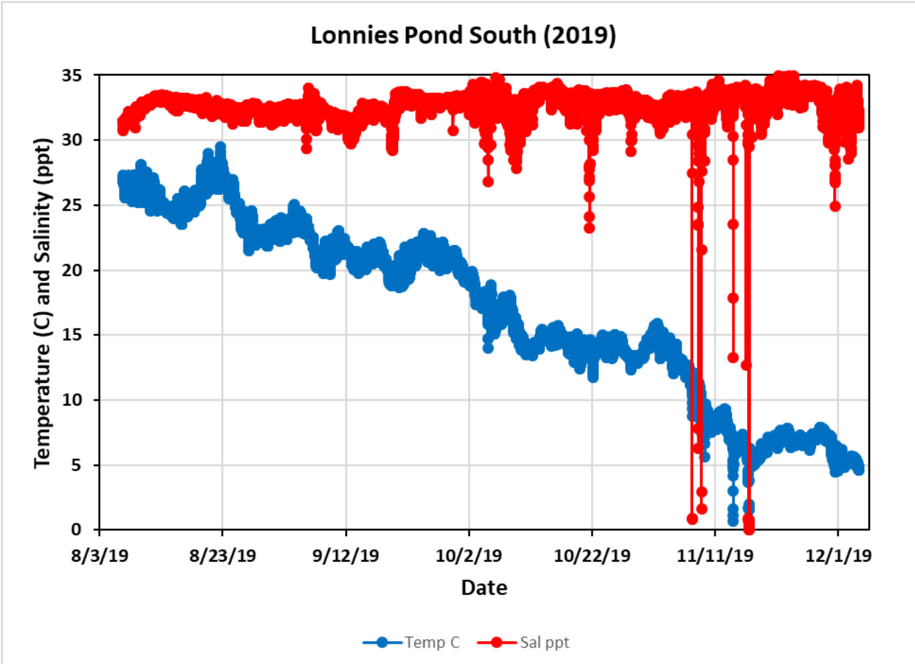

Figure 6. Lonnies Pond South mooring continuous record of Temperature and Salinity.

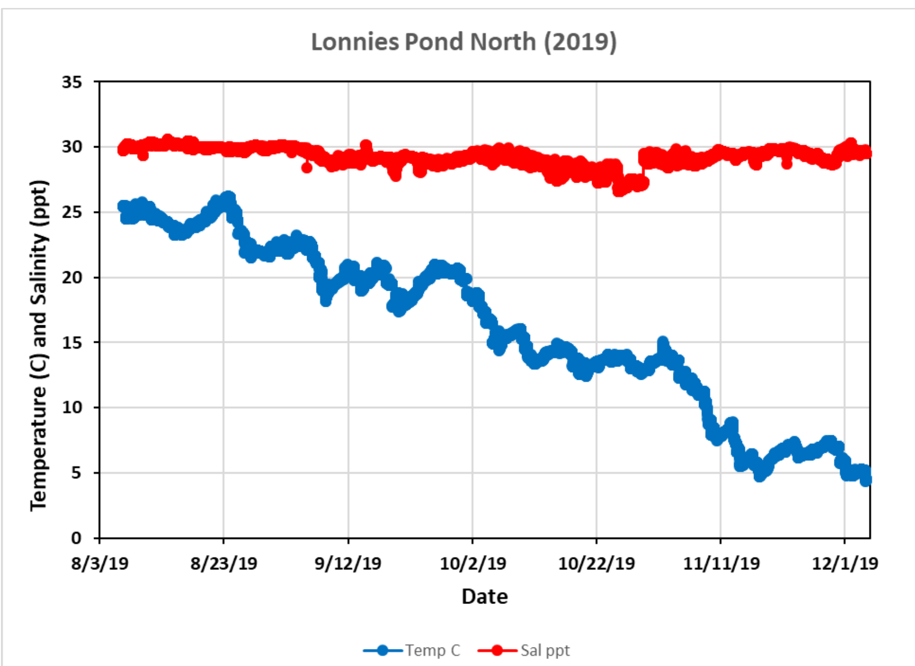

Figure 7. Lonnies Pond North Mooring continuous record of Temperature and Salinity.

4.0 Effects of Oysters on Nutrient Regeneration and Denitrification in Sediments

In estuarine systems such as Lonnies Pond, nitrogen (N) is transformed and recycled within the sediments and water column. This recycled nitrogen adds directly to the eutrophication of the estuarine waters in the same fashion as watershed inputs. In some systems under Massachusetts Estuaries Project investigation, recycled nitrogen can account for about half of the nitrogen supply to phytoplankton blooms during the warmer summer months. It is during these warmer months that estuarine waters are most sensitive to nitrogen loadings. Failure to account for this recycled nitrogen generally results in significant errors in determination of the effects of watershed nitrogen loadings, the overall nitrogen balance of the system and how oyster propagation may affect nitrogen dynamics at the sediment water column interface.

The organic rich nature and relatively shallow waters of coastal systems like the Lonnies Pond sub-basin of the Pleasant Bay Estuary and others on Cape Cod result in sediments playing a significant role in system biogeochemical cycles. Organic matter deposition to sediments, hence benthic respiration, tends to decrease with increasing depth of overlying waters due to interception by water column heterotrophic processes resulting in lower deposition of labile (decomposable) organic matter. The result is that embayment respiration rates are typically many-fold higher than in the adjacent deeper offshore waters. With potential stratification of embayment waters, sediment metabolism plays a major role in bottom water oxygen declines (an ecosystem structuring parameter). This applies particularly to Lonnies Pond which periodically goes hypoxic (i.e. < 4 mg/L dissolved oxygen) during the summer months. It should be noted that while water depth is important in altering the deposition of labile organic matter to sediments, filter feeders and especially large filter feeders like oysters can overwhelm the "depth effect" due to the large amount and size of packaged feces that they emit. In these situations, oysters are projected to increase deposition which subsequently increases sediment respiration rates.

Measurements of Benthic Nutrient Regeneration, Denitrification and Sediment Oxygen Uptake:

In order to determine the contribution of sediment regeneration to nutrient levels within the oyster aquaculture portion of the Lonnies Pond system and the effect the oysters may have on nitrogen recycling rates and oxygen levels, sediment samples were collected and incubated under *in situ* conditions on two dates, 8/6 and 10/8 in 2019. The August 6, 2019 sampling date was during the period of maximum oyster activity in the summer interval (July- September) and the October 8, 2019 sampling was during the period of maximum oyster biomass (October-December), but at a significantly lower temperature. Since the April 18, 2017 flux revealed that there was significant over winter carryover of nitrogen rich oyster biodeposits associated with the smaller oyster biomass in 2016 compared to 2017, early spring denitrification was measured April 22, 2019 for the associated 2018 carry over (Table 5).

Time series measurements of total dissolved nitrogen, nitrate+nitrite, ammonium and ortho-phosphate were made on each incubated core sample. The rate of oxygen uptake was also determined in order to: (1) evaluate sensitivity to oxygen depletion of the oyster aquaculture area of Lonnies Pond, (2) rank sediments as to organic matter deposition rates (not possible using organic content) and (3) develop a nitrogen model for how the oysters may be affecting the nitrogen cycle in the sediments associated with oysters. Measurements of sediment regeneration were limited to oysters deployed in the southern portion of the pond (referred to as the southwest deployment area). Assays were performed on eight cores from sites distributed throughout the southwest oyster deployment area and outside the area on each date. Cores were collected directly under the oyster aquaculture rafts and at distances east and west of the aquaculture area.

The distance of cores collected outside of the deployment area to the edge of the deployment area determined whether the core was within the biodeposit impact area. For comparison to previous years (2016-2018), an acoustic doppler current profiler (ADCP) was deployed to determine horizontal velocities proximal to the southwest deployment area. The ADCP was deployed at water quality station Lonnies 5 on 10/8/19 for 5 tidal cycles; 2019 ADCP data was consistent and generally confirmed velocity measurements made 2016-2018. Using the previously determined mean sinking velocity of fecal pellets (8.14±5.01mm/s), mean depth around the border of the oyster deployment area, and tidal range, fecal pellet settling was modeled step-wise assuming fecal pellet production was similar among all bags. The model results largely confirm the results from 2016-2018 concerning the extent of the impact area of surficial sediments. The 2019 impact area for the southwest deployment area was determined to be 2890 m^2; this is a decrease compared to the 2018 impact area (3250m^2). The 2019 impact area decreased because the southwest deployment area was reduced by approximately 1/3rd from 2018 to 2019.

The results allowed determination of the spatial pattern and rate of nutrient exchanges from the sediments to the water column and how these rates may be affected by the cultivation of oysters in Lonnies Pond. From our experience, sediment regeneration during the summer is a large and important source of nutrients supporting both phytoplankton and macroalgal blooms in embayments throughout SE Massachusetts. The degree to which intensive oyster aquaculture can change those rates through enhancement of denitrification needs to be determined if this approach is to be used for N management of these systems.

Denitrification was determined from time series N_2 excess measurements using isotope ratio mass spectrometry (IRMS). N_2 produced by denitrification is precisely detected by analysis of its ratio with the naturally occurring inert gas Argon. Water samples were collected and stored to prevent gas exchange or bubble formation. In the laboratory, sample water was pumped at ml/min rates through a gas permeable membrane in order to extract gas into the mass spectrometer inlet. The inlet was fitted with cryogenic traps to remove water vapor and CO_2 gas. Sample gas was analyzed by the mass spectrometer for masses 28 and 40 for determining the N_2 to Argon ratio. Calibration was made by comparison with a reference gas of known composition. Water column respiration measurements were collected east and west of the southwest deployment area at the Lonnies 5 and Lonnies 7 water quality monitoring sites.

Sediment Nutrient Cycling Results (2019):

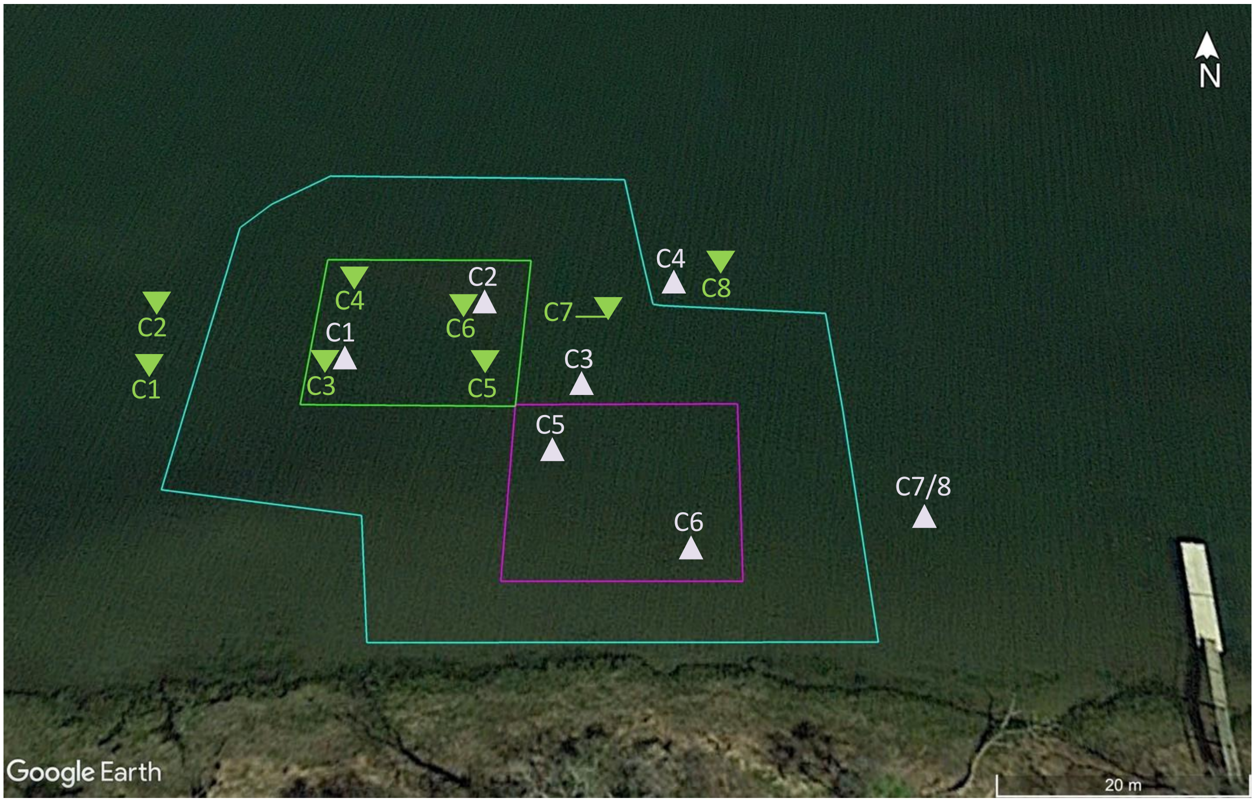

Figure 8. Aerial photograph showing the location of the oyster deployment area overlain with 2019 oyster deployment area (green and purple polygons) and summer (white markers) and fall (green markers) sediment flux locations. Total area impacted by the oyster deployment as determined by fecal pellet distribution in 2019 is shown as the outer bounded area (blue line).

Benthic flux results are summarized in Table 4 below. An April 2019 benthic flux was conducted to capture enhanced denitrification stimulated by the carryover of biodeposits accumulated during the 2018 oyster deployment. Sediment cores were collected in April before the start of the 2019 oyster growing season as water temperatures were starting to increase. April 2019 cores were collected relative to the 2018 oyster deployment and collection sites which approximated the 2019 summer and fall sites.

August cores were collected approximately 2-3 weeks following oyster deployment in the southwest deployment area. The benthic flux results suggest that the 2-3 weeks between oyster deployment and core collection was sufficient time for oyster biodeposits to influence the oxygenated surficial sediments as flux rates, particularly the SOD and NH^{4+} flux rates are comparable to rates from cores collected within the oyster impact area in previous years. However, this does not necessarily mean that there was sufficient time for the accumulation of biodeposit organic matter required for denitrification in the deeper low oxygen and anoxic sediment layers, as the organic material needs to be mixed into the surficial sediments. The effect of the biodeposits is seen in the SOD rates in the background cores (outside of the impact area) being only 65% of the SOD rates in cores from the area receiving biodeposits (within the impact area), which is attributable to the increased deposition of labile organic matter. In August, ammonium flux out of the sediment was the dominant pathway of regenerated nitrogen. This is to be expected as higher temperatures increase bacterial respiration which reduces oxygen availability for nitrification; the incubation temperature (set to match the in pond temperature) during the August flux was 24.8°C.

On October 8, 2019, a second set of eight cores were collected and incubated at 16.5°C. Core locations varied slightly between August and October; however, C1, C2, and C4 collected in August can be directly compared to C3, C6, and C8 collected in October, respectively (see Figure 8). October's average (C3, C6, and C8) SOD and NH^{4+} flux rates decreased by approximately 60% compared to August's average (C1, C2, and C4) rates. Nitrate uptake (typically due to direct denitrification) occurred in all cores except C5 during the October flux, which is related to the higher water column nitrate concentrations during the fall months. Nitrate efflux measured in October's C5 may be the result of its sandy sediment composition and presence of a burrow. Burrows have been found to have increased nitrification rates compared to surface sediments by increasing ammonium availability and oxygenated sites where nitrification can occur.[6] As in previous years, sediment heterogeneity exists among the treated cores, which leads to differences in sediment biogeochemistry; however, obvious patterns of nitrogen cycling are clear throughout the impact area.

Table 4 - Summary of benthic flux rates from core incubations conducted 4/22/19, 8/6/19 and 10/8/19. Rows shaded in gray indicate that the core location is outside of the area impacted by biodeposition (background). N_2-N detection limits were determined for each analysis date based on the consistency of seawater standards measured after every eighth sample. Below detection limit (BDL) indicates that the N_2-N flux rate was less than the detection limit determined for the day on which the samples were analyzed.

Date Cores Collected: 4/22/19; Incubation Temperature 11.8							
Site ID	**SOD**	**NH_4^+**	**NO_3^-**	**DIN**	**N_2-N**	**Total N Cycled**	**Denitrified**
	mMol/m²/d	**mMol/m²/d**	**mMol/m²/d**	**mMol/m²/d**	**mMol/m²/d**	**mMol/m²/d**	**% Total Cycled N**
C1	**58.56**	**2.17**	**-0.01**	**2.16**	**1.38**	**4.46**	**31%**
C2	**19.81**	**0.66**	**-0.03**	**0.63**	**0.61**	**2.25**	**27%**
C3	**36.17**	**0.49**	**-0.01**	**0.48**	**0.93**	**3.52**	**26%**
C4	**32.93**	**0.57**	**0.00**	**0.57**	**1.29**	**3.25**	**40%**
C5	**37.66**	**0.93**	**-0.01**	**0.92**	**0.63**	**2.54**	**25%**
C6	**62.63**	**1.47**	**-0.02**	**1.45**	**3.97**	**5.01**	**79%**
C7	**72.01**	**1.50**	**-0.02**	**1.49**	**2.80**	**4.09**	**68%**
C8	**41.36**	**-0.13**	**-0.02**	**-0.15**	**0.83**	**1.67**	**51%**

[6] Mayer, M. S., Schaffner, L., & Kemp, M. W. (1995). Nitrification potentials of benthic macrofaunal tubes and burrow walls: effects of sediment NH4+ and animal irrigation behavior. *Marine Ecology Progress Series*, 157-169.

C9	75.98	2.05	-0.01	2.04	2.40	4.16	58%
C10	72.63	1.44	-0.03	1.42	0.90	1.96	46%
C11	69.70	-0.19	-0.03	-0.23	BDL	0.44	BDL
C12	78.47	2.68	0.01	2.68	BDL	5.04	BDL
C13	ND	1.65	-0.01	1.65	0.41	1.96	21%
C14	106.99	0.11	-0.09	0.03	1.09	2.74	42%
C15	29.01	-0.09	-0.02	-0.11	BDL	0.46	BDL
C16	66.11	-0.04	-0.04	-0.08	0.86	1.54	54%

Date Cores Collected: 8/6/19; Incubation Temperature: 24.8 ℃							
Site ID	SOD	NH_4^+	NO_3^-	DIN	N_2-N	Total N Cycled	Denitrified
	$mMol/m^2/d$	$mMol/m^2/d$	$mMol/m^2/d$	$mMol/m^2/d$	$mMol/m^2/d$	$mMol/m^2/d$	% Total Cycled N
C1	147.07	9.69	1.05	10.75	2.14	12.89	17%
C2	107.32	7.18	0.08	7.26	1.30	8.56	15%
C3	171.74	11.27	0.70	11.97	2.67	14.64	18%
C4	95.06	8.29	1.04	9.33	1.04	10.37	10%
C5	109.92	11.83	0.06	11.89	1.36	13.25	10%
C6	114.08	12.12	0.03	12.14	1.44	13.59	11%
C7	65.97	13.21	0.06	13.27	0.43	13.70	3%
C8	92.00	6.63	0.14	6.76	0.98	7.74	13%

Date Cores Collected: 10/8/19; Incubation Temperature: 16.5 ℃							
Site ID	SOD	NH_4^+	NO_3^-	DIN	N_2-N	Total N Cycled	Denitrified
	$mMol/m^2/d$	$mMol/m^2/d$	$mMol/m^2/d$	$mMol/m^2/d$	$mMol/m^2/d$	$mMol/m^2/d$	% Total Cycled N
C1	58.82	-1.35	-0.32	-1.67	0.51	2.18	23%
C2	49.17	-0.30	-0.44	-0.74	0.32	1.06	30%
C3	43.04	4.89	-0.51	4.39	0.40	5.80	7%
C4	35.72	1.76	-0.26	1.50	0.36	2.37	15%
C5	35.76	1.93	0.44	2.37	0.68	3.05	22%
C6	40.58	2.28	-0.34	1.94	0.19	2.81	7%
C7	34.42	2.48	-0.28	2.20	0.68	3.44	20%
C8	41.37	1.43	-0.59	0.84	0.15	2.17	7%

A conservative estimate of the total nitrogen removed by oyster enhanced denitrification can be made using our measurements of background denitrification rates (outside biodeposit area) with the rates within the biodeposit impact area. The background rates may be a slight overestimate as spreading of the biodeposits by water currents was the only process examined and the particles may have spread over a wider area due to storm resuspension. The 2019 background rates and rates measured under the oyster treatment are shown in Table 5, as well as, the 2016-2018 rates for comparison. Average denitrification rates measured in control cores outside the impact area were considered to represent background rates. The difference between the average rate observed within the impact area and the average background rate was used to determine the level of enhanced denitrification produced by oyster biodeposits. Denitrification rates from the April 2019 flux were appended to the "Year 3 Mean Denitrification Rates" section in Table 5. The 2019 spring flux represents the end of the Year 3 oyster enhanced denitrification, as the enhanced denitrification rates were the result of biodeposits from the 2018 oyster deployment. The April 2019 "Oyster Effect" (1.5 mMoles/m^2/d; Table 5) is comparable to the Oyster Effect measured in April 2017 (1.9 mMoles/m^2/d); overall, denitrification rates were lower in April 2019 compared to April 2017. These data indicate the necessity of following denitrification into the following spring in order to capture the full level of denitrification enhancement. The October 2019 oyster enhanced denitrification was less than that measured in 2016 and 2017, but was consistent with the oyster effect measured in October 2018.

Table 5. Mean denitrification rates for cores collected in the biodeposit impact area associated with the oyster deployment area (Treated) and outside the impact area (Background). The difference in these two values should represent the contribution made by the ongoing oyster culture (Oyster Effect).

	Year 1 Mean Denitrification Rates (mMoles/m^2/d)				
	Treated		Background		Oyster
Date	Mean	Std. Dev.	Mean	Std. Dev.	Effect
8/16/16	2.1	0.6	1	0.4	1.1
10/5/16	4.1	2.5	1.2	1	2.9
4/18/17	2.9	1.8	0.9	0.4	1.9

	Year 2 Mean Denitrification Rates (mMoles/m^2/d)				
	Treated		Background		Oyster
Date	Mean	Std. Dev.	Mean	Std. Dev.	Effect
6/27/17	1.0	0.5	0.3	0.4	0.7
8/1/17	2.4	1.3	0.8	0.4	1.6
9/19/17	0.7	0.9	0.2	0.1	0.5
10/3/17	1.5	0.9	0.8	0.5	0.8

	Year 3 Mean Denitrification Rates (mMoles/m^2/d)				
	Treated		Background		Oyster
Date	Mean	Std. Dev.	Mean	Std. Dev.	Effect
7/26/18	2.5	2.5	1.3	0.4	1.2
10/2/18	0.5	0.3	0.4	0.2	0.2
4/22/19	1.8	1.2	0.3	0.5	1.5

	Year 4 Mean Denitrification Rates (mMoles/m^2/d)				
	Treated		Background		Oyster
Date	Mean	Std. Dev.	Mean	Std. Dev.	Effect
8/6/19	1.8	0.6	0.8	0.3	1.0
10/8/19	0.6	0.2	0.3	0.2	0.3
4/XX/2020	TBD	TBD	TBD	TBD	TBD

A summary of the measured N removal from Lonnies Pond via oyster harvest and enhanced denitrification during the four years of study is found in Table 6. The mass of nitrogen removed from the system through enhanced denitrification was calculated by multiplying this enhanced rate by the impact area. Weighting of rates obtained during different parts of the season allowed the determination of annual nitrogen removal by denitrification. Enhanced Annual DeN_2 calculated for Year 4 (2019) in Table 6 is less than that reported for 2016-2018; however, this difference is attributable to the timing and duration of the 2019 oyster deployment compared to previous years. The 2019 southwest deployment area was not fully stocked until July 25th and primarily consisted of first year oysters with shell lengths ranging from 10 – 15 mm. The result being that there wasn't sufficient time for the accumulation of biodeposit organic matter in the sediments to fully support the enhancement of denitrification beyond what was observed in August and October 2019. In addition, the spring carry over related denitrification has not yet been measured which will raise the nitrogen removal by denitrification once it is available.

Table 6. Annual Nitrogen Removal Budget for the oyster impact area showing contributions from enhanced denitrification and oyster harvest. Note that Spring carryover denitrification has not been added to Year 3 (2018) or Year 4 (2019) data.

Year	Year 1 (2016)	Year 2 (2017)	Year 3 (2018)	Year 4 (2019)
Deployment Duration (days)	146	195	240	155
Enhanced Annual DeN_2 ($mmol/m^2$ N)	298	253.3	179.8	77.6***
Enhanced Annual DeN_2 (g/m^2 N)	4.17	3.55	2.52	1.1***
Impact area (m^2)	2287	2735	3250	2890
Total Annual Enhanced DeN_2 (g N)	9541	9699	8190	3140
Total Annual Enhanced DeN_2 (kg N)	9.54	9.70	8.19	3.14***
Net Annual N removed by oysters[7] (kg N)	39.1	27.2	36.2 [57.3]*	30.8 [61.6]*
Enhanced DeN_2 as percent of N removed by oysters	24.4%	35.7%	22.6%**	10.2%**

* Includes the northeast deployment area that did not have denitrification measurements.
** Based on denitrification and harvest data from southwest deployment area only.
*** Due to the fewer sampling events and timing of those events relative to the later oyster deployment in 2019, the overall rate is an underestimate of the annual enhanced denitrification rate; in addition, April 2020 rates are not yet included, which also lowers the annual rates at this point. They will be added once they become available.

[7] For interannual comparison purposes, the NE oyster deployment area was omitted from the calculation of Net Annual N removed by oysters. The NE area was initially deployed in 2018 in the NE section of Lonnies Pond and is not included in the assessment of sediment nutrient cycling. The 2016-2018 oyster survival and growth analysis and nitrogen removed by harvest was conducted by Science Wares Inc.

5.0 Oyster Aquaculture Deployment and Removal

The Aquaculture Contractor (Ward Aquafarms, LLC) began the installation of oysters in Lonnie's Pond on July 9th. This installation was later than the July 1st date specified in the QAPP and Management Plan, but was required to ensure that the oyster seed was large enough (10 mm) to remain in the floating bags used for grow-out. Ward Aquafarms speculated that the 2019 seed grew slower than usual in the pre-deployment upwellers due to the cooler weather conditions in the spring and early summer. Whatever the precise cause, an additional week of seed growth was required before deployment. Oysters were deployed on Tuesdays and Thursdays beginning July 9th over 3-weeks, totaling six deployment days; full deployment was completed on July 25th.

Ward Aquafarms and CSP/SMAST staff coordinated weighing and counting of oysters on each deployment day. Staffs worked with each other to cross-check count estimates by bag and subsample weights and volumes, as well as, documenting values for each of the various oyster strains. Comparison of methods generally showed differences in individual counts of less than 5%, typically <2.5%. Over the six deployment days, a total of 1.55 million oysters were deployed. The total deployed oysters were comprised of approximately 1.47 million year 1 (YR1) oyster seed comprised of Maine, Connecticut, Mixed, and Triploid strains and 77,000 year 2 (YR2) oysters relayed from Falmouth (Table 7).

For all oyster strains deployed in 2019, subsamples of oysters at the time of deployment were collected for weighing and determination of N content. As specified in the QAPP, % N content was determined for tissues and shells. These were used to determine the total N mass in the initial deployment and the same procedures were utilized at the harvest.

The nitrogen content of the oysters deployed in July totaled 4.2 kg N (see Table 7). While oysters were in the pond, Ward Aquafarms staff maintained the bags by flipping them regularly and rotating them around Lonnie's Pond for optimal growth based on insights developed during the Lonnie's Pond Demonstration Project 2016-2018. CSP staff noted very little algae on floating bags with the overall result being a relatively low mortality, below 9% for all strains.

Table 7. Total weight and counts of the initial oyster deployment. Oyster subsamples collected for each strain for percent nitrogen determination (Revised from Semi-Annual Report 2019).

Oyster Deployment Data							
ID	**Date**	**Oyster Strain**	**Total Wet Weight (kg)**	**Total # of live oysters**	**Dry Weight (kg)**	**%N Content (DW)**	**Total N inputs (kg)**
Day1	7/9/2019	YR1 CT	31.1	166,619	20.69	0.86	0.178
Day1	7/9/2019	YR1 MK	34.6	140,665	22.70	0.83	0.188
Day2	7/11/2019	YR1 CT	45.3	349,250	28.07	0.83	0.234
Day2	7/11/2019	YR1 MK	33.9	183,375	21.79	0.97	0.212
Day3	7/16/2019	YR1 Mixed	42.7	321,561	27.66	0.74	0.204
Day4	7/18/2019	YR1 Mixed 4mm	7.9	110,880	4.86	0.63	0.031
Day4	7/18/2019	YR1 Mixed 2mm	2.7	90,818	2.40	0.66	0.016
Day4	7/18/2019	YR1 Trips 2mm	1.7	57,551	1.15	0.83	0.010
Day5	7/23/2019	YR2 Fal	438.9	38,581	310.22	0.40	1.233
Day5	7/23/2019	YR2 Fal (Shells)	31.7	-	28.55	0.15	0.043
Day6	7/25/2019	YR2 Fal	641.8	38,477	447.37	0.40	1.775
Day6	7/25/2019	YR2 Fal (Shells)	43.5	-	39.13	0.14	0.056
Day6	7/25/2019	YR1 Trips 8mm	3.4	48,171	2.24	0.83	0.019
		Total YR1	**203.2**	**1,468,889**	**131.6**	**-**	**1.09**
		Total YR2	**1155.9**	**77,058**	**786.1**	**-**	**3.11**
		Total	**1359.2**	**1,545,947**	**956.8**	**-**	**4.20**

In December, oysters were relayed from the pond by Ward Aquafarm on five days over a period of 16 days. CSP staff worked with the Town of Orleans and Ward Aquafarm staff to collect oyster weights, counts, and subsamples. Large trucks were used to transport oysters from the Pond. At the start of each relay day, empty trucks were weighed at the Orleans Transfer Station and then weighed again after loading the oysters to determine the total mass of oysters removed from Lonnie’s Pond. CSP staff collected whole bag weights on a minimum of 25 bags for each of the variety of oyster strains: YR2, YR1 CT, YR1 MK, YR1 Triploid, YR1, Mixed. In addition, 5 to 10 bags (per strain) were further assessed to determine: empty bag weight, live oyster count and weight, and dead oyster count and weight. Data was collected on the Lonnie’s Pond boat ramp and occasionally the next day at the Ward Aquafarms storage facility when necessary. CSP weight data and Orleans Transfer Station Scale weights were within 3% of each other each day and within 0.5% for all days totaled (Table 8), indicating that the truck scale is an accurate way to determine total mass of oysters removed, as long as pre-loading truck weight is determined each day and there is an appropriate correction for the empty grow-out bags.

Table 8. Oyster weights determined by CSP staff compared to Orleans Transfer Station truck scale weights. This exercise was conducted as an independent verification of the harvested oyster weights by strain and showed excellent agreement between the methods.

Date	# of Bags Relayed	Tag Color	Strain	Average Oyster Wet Weight with Bag (kg)	Total Wet Weight with Bag By Strain (kg)	Net Wet Weight from Truck Scale (kg)	Percent Difference
12/2/2019	50	Purple	YR2 Fal Megansett	7.50	375		
12/2/2019	44	Barcode	Eric's Studs	12.30	541		
12/2/2019	44	No tag	Dan YR2 - x2 stock	9.82	432		
12/2/2019					**1348**	**1313**	**2.7%**
12/4/2019	179	Purple	YR2 Fal. Megansett	8.55	1531		
12/4/2019	25	No tag	Dan YR2 - x2 stock	9.53	238		
12/4/2019					**1769**	**1752**	**1.00%**
12/9/2019	272	Purple	YR2 Fal. Megansett	8.21	2234		
12/9/2019					**2234**	**2232**	**0.11%**
12/16/2019	427	Blue	YR1 Mixed	6.21	2650		
12/16/2019	168	Red	YR1 Triploid	7.36	1237		
12/16/2019	102	Green	YR1 Maine	11.22	1145		
12/16/2019					**5032**	**5126**	**1.84%**
12/18/2019	241	Green	YR1 Maine	13.58	3272		
12/18/2019	463	Yellow	YR1 Conneticutt	9.67	4477		
12/18/2019	8	Blue	YR1 Mixed	4.17	33		
12/18/2019					**7782**	**7829**	**0.60%**
Total -All Relay Days					**18166**	**18250**	**0.46%**

Over the five days of harvesting, over 20 tons of oysters and bags were relayed from Lonnie's Pond comprised of approximately 719,000 YR1 oysters at ~50 mm shell length and 70,000 YR2 oysters at ~72.5 mm shell length (Table 9). Using deployment counts, relay counts, and mortality of YR1 oysters, it is estimated that approximately 653,000 oysters were lost by falling through the mesh in the grow-out bags when deployed into 8 mm mesh bags before reaching a minimum size of 10 mm (see Table 9). YR1 Maine (MK) oysters were deployed at ~11.5 mm size, resulting in a loss of only 54,000 oysters. Whereas, the strain, YR1 Mixed, were deployed at a size of ~7.9 mm size, resulting in a loss of 340,000 oysters.

Due to poor growing conditions the oyster seed was still small in July when the oysters were deployed to Lonnies Pond. The "lost" oysters represent a significant amount of nitrogen removal potential that could have been realized if the oysters had survived and grown throughout the season. Based on the average weight and % N content of the harvested oysters, it is estimated that the "lost" oysters represent up to 38 kg of potential nitrogen removal, given that oyster seed was all a minimum 10 mm size.

Table 9. Total counts, sizes, and mortality of each oyster strain. "Lost*" oysters are oysters that fell through the 8mm mesh bags upon deployment due to their small size. "% mortality**" is based on # of dead oysters (open shells) compared to total individuals in a bag, which does not include "lost" oysters.

Strain	# of Oysters Deployed	# of Live Oysters Relayed	# of Oysters "lost"*	avg. size at deployment (mm)	avg. size at relay (mm)	% Mortality **
YR1 Conneticut (CT)	515,869	243,992	239,847	10.09	51.8	6%
YR1 Maine (MK)	324,040	242,937	54,136	11.58	51.1	8%
YR1 Mixed	523,258	150,556	340,330	9.46	47.5	6%
YR1 Triploids	105,722	81,110	19,183	7.90	49.3	5.1%
YR2 Fal	77,058	69,427	NA	40.6	72.5	9%
Total	**1,545,947**	**788,022**	**653,496**	**-**	**-**	**-**

Weights and count data documented that a total of 791,564 live oysters totaling 17,560 kg were harvested from Lonnie's Pond in December 2019 (Table 10). Oysters from all five strains, plus the YR3 and YR4 oysters from the 2016-2018 demonstration project were subsampled for dry tissue weight, dry shell weight, and %N content of each; these were combined to determine the total nitrogen in the oysters harvested from Lonnie's Pond. Generally, %N content (dry weight) of oysters was not significantly different for YR1 (0.66 to 0.75 %N) and YR2 (0.46 to 0.83 %N) oysters (see Table 10) and were also similar to the YR3 & YR4 oysters (0.68 %N). Collectively, the oysters (YR1, YR2, YR3, YR4) in Lonnie's Pond incorporated a total of 61.6 kg of nitrogen in their tissues and shells during growth from their initial deployment.

If only the YR1 and YR2 oysters are considered, they incorporated a total of 59.7 kg N during the 2019 growing season in Lonnie's Pond. It should also be noted that significant additional removal of nitrogen through sediment processes (8 to 10 kg N in 2016-2018) is not included in these removal numbers, which reflect only the actual net removal in harvested tissue and shell.

Table 10. Total weight and counts of the oyster relay. Oyster subsamples collected for each strain for percent nitrogen determination.

Oyster Relay Data							
ID	Date	Oyster Strain	Total Wet Weight (kg)	Total # of live oysters	Dry Weight (kg)	%N Content (DW)	Total N removed (kg)
Day1	12/2/2019	YR2 Fal	2649	5,500	1,689	0.46	7.820
Day1	12/2/2019	YR2 Fal (Shells)	53	-	48	0.19	0.092
Day1	12/2/2019	YR2 Fal x2stocked	342	6,516	211	0.51	1.070
Day1	12/2/2019	YR2 Fal x2stocked (Shells)	63	-	52	0.14	0.074
Day1	12/2/2019	YR3 & YR4 Studs	461	3,542	284	0.68	1.931
Day1	12/2/2019	YR3 & YR4 Studs (Shells)	61	-	50	0.42	0.214
Day2	12/4/2019	YR2 Fal	1124	19,213	702	0.60	4.236
Day2	12/4/2019	YR2 Fal (Shells)	171	-	189	0.16	0.307
Day2	12/4/2019	YR2 Fal x2stocked	186	3,400	114	0.60	0.690
Day2	12/4/2019	YR2 Fal x2stocked (Shells)	25	-	28	0.16	0.045
Day3	12/9/2019	YR2 Fal	1711	34,798	1,047	0.83	8.653
Day3	12/9/2019	YR2 Fal (Shells)	249	-	216	0.32	0.682
Day4	12/16/2019	YR1 Triploids	1038	81,110	472	0.75	3.533
Day4	12/16/2019	YR1 Triploids (Shells)	105	-	84	0.28	0.233
Day4	12/16/2019	YR1 Mixed	2019	147,884	1,015	0.75	7.583
Day4	12/16/2019	YR1 Mixed (Shells)	40	-	32	0.22	0.070
Day4	12/16/2019	YR1 MK	994	71,369	528	0.66	3.472
Day4	12/16/2019	YR1 MK (Shells)	59	-	51	0.20	0.102
Day5	12/18/2019	YR1 MK	2485	171,568	1,320	0.66	8.681
Day5	12/18/2019	YR1 MK (Shells)	210	-	182	0.20	0.362
Day5	12/18/2019	YR1 CT	3615	243,992	1,999	0.76	15.285
Day5	12/18/2019	YR1 CT (Shells)	248	-	202	0.27	0.543
Day5	12/18/2019	YR1 Mixed	25	2,672	14	0.78	0.112
Day5	12/18/2019	YR1 Mixed (Shells)	1	-	1	0.26	0.001
		Total YR1	**10838**	**718,596**	**5900**	**-**	**40.0**
		Total YR2	**6261**	**69,427**	**4027**	**-**	**22.9**
		Total YR3&YR4	**461**	**3,542**	**284**		**1.9**
		Total	**17560**	**791,564**	**10211**	**-**	**65.79**
			Total Harvested N from Lonnies Pond: 65.79 kg - 4.20 kg =				**61.59**

4.0 Future Considerations

Overall, the 2019 oyster deployment achieved the general goals outlined in the Lonnie's Pond Aquaculture and Nitrogen Management Plan. CSP staff working with Ward Aquafarm and Town staff were able to document ~62 kg N net removal in the oyster harvest, 82.5% of the project goal. When enhanced denitrification is added in it appears that the total N removal approximated the 75 kg N net removal target[8]. In addition, the oysters continue to lower particulate constituents to improve water quality, but a pond-wide improvement is difficult to quantify as (a) only a portion of the TMDL is being removed (25%) and no reductions have been made in the downgradient basins that supply nutrient enriched flood tidal waters to Lonnies Pond. None-the-less nitrogen removal was clear and some localized water quality impairments documented.

As 2019 was the first year of oyster aquaculture under the 2018 Management Plan, we also documented ways to make the future deployment and monitoring more efficient. Considerations for future deployments include:

1) Try to identify the best 2 or 3 oyster strains. Multiple strains of oysters increase the time and effort for documenting nitrogen removals, since each strain must have a complete tracking, evaluation, subsampling, analysis, etc.

2) Use of the Orleans Transfer Station Scale "truck scale" for harvest weight determinations appears to be adequate on its own for determining oyster mass harvested, although individual strains must be accommodated.

3) A) The Town should explore the possibility of installing a spring upweller in Lonnie's to increase the nitrogen removal in May/June. Oysters in upwellers are efficient at taking up nitrogen as the seed grow to the size needed for deployment in floating bags. The siting and logistics would need to be worked out, but portable upwellers are in regular use on Cape Cod. This type of installation would address the uncertainties of attaining sufficiently sized seed in years like 2019, including the loss of seed.

   B) Alternatively, the Town can refine the Aquaculture Contractor conditions to allow small year 2 oysters to be placed in the pond in March/April and be removed and replaced with sufficiently sized seed in July. This would increase N removal during the critical period.

4) Continue examination of new approaches to streamline deployment and harvest and to optimize monitoring.

5) Continue to strengthen coordination between the Town, aquaculture contractor and monitoring contractor. The procedures developed during 2019 worked very well and need to be codified and followed in future years. A meeting in February among the parties should be held each year to formalize refinements and target dates.

[8] Approximation based upon prior years and 2019 measurements, awaiting the April 2020 measurements to capture the "carry over effect".