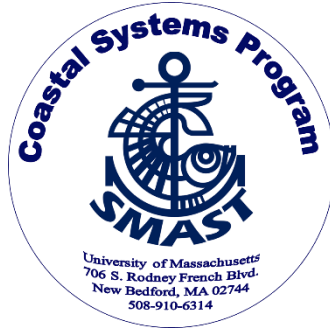


Town of Orleans Lonnie's Pond Aquaculture and Nitrogen Management Plan



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

Lonnie's Pond Aquaculture/TMDL 2020 Annual N Removal/Harvest Report Final Parts 1 and 2 February 1, 2021

Prepared by:

**Coastal Systems Program
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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 (e.g., IA septic systems, permeable reactive barriers, aquaculture, etc.) These efforts not only 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 (N) with resulting impaired water and habitat quality.¹ The Massachusetts Estuaries Project report for Pleasant Bay², which is the technical basis for the TMDLs, suggested that the N 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, N reduction approach using oyster aquaculture. The Lonnie's Pond Demonstration Project was planned as a three-year effort to evaluate the water quality impacts and determine any implementation issues associated with enhanced aquaculture for N reduction as part of achievement of the TMDL without sewerage within the Pond watershed. Monitoring during the demonstration project found significant removal of N 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 Plan⁴ and to continue operations with a commercial oyster aquaculture grower as a phase II demonstration project to test the sustainability for potential use for TMDL compliance. 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 N management program for its estuaries, including two regular reports each year on Plan implementation: Semi-Annual Status Update and an Annual Report. The reporting was to be completed by a Monitoring Contractor, who would conduct all water quality monitoring and, in coordination with the Aquaculture Contractor, would ensure sufficient monitoring to quantify N removal by the shellfish. 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.

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.

The Coastal Systems Program, School for Marine Science and Technology, University of Massachusetts Dartmouth (CSP/SMAST) was selected by the Town as the Monitoring Contractor and has prepared both the Annual Reports and Semi-Annual Updates as required under the QAPP since 2019. Each Semi-Annual Status Update summarizes insights from the initial shellfish deployment and ecosystem monitoring (including dates of installation, maintenance, and monitoring in coordination with the Aquaculture Contractor), but does not significantly review any monitoring data. Each Annual Report summarizes all water quality monitoring data and determines the N removed from the system through tracking of shellfish growth and N incorporation. During the initial implementation of the Management Plan, the Town selected a goal of 75 kg/yr N removal from Lonnie's Pond.

Based on 2019 monitoring and synthesis, CSP/SMAST determined that oyster harvest removed 60 kg of N from Lonnie's Pond between July 15th and December 9th, 2019. The report also concluded that "it is possible that additional nitrogen could be removed with longer oyster deployment (e.g., earlier deployment)". Subsequent discussions among the Town, CSP/SMAST, and the Aquaculture Contractor (Ward Aquafarms) led to a program change to allow deployment of oysters earlier in the year to achieve additional N removal and improve the N removal during the critical summer period (June – September), while continuing to utilize the same area within the Pond for the floating bag arrays. Since oyster seed is not generally available (at 10 mm) early in the season, it was agreed that first year (YR1) oysters pulled from Lonnie's Pond in December 2019, would be redeployed into the Pond as second year (YR2) oysters in March 2020. These YR2 oysters were slated for a July/August removal, at which time new YR1 seed would be deployed into Lonnie's Pond as the YR2 oysters were removed.

Part 1 of the 2020 Annual Report includes the N removal attained during the 2020 season and includes initial N mass at deployment and at removal of both YR2 oysters and subsequent replacement on harvest by new YR1 seed oysters throughout the July and September period and the N removed after these YR1 seed had grown and were removed in December 2020. YR2 oysters were deployed in March and a fraction (64 %) were removed and replaced over an extended period from July 9 through September 27 by the Aquaculture Contractor. As oysters were removed, oyster seed was put into the emptied bags and redeployed over the same period. All oysters were removed in December 2020. The CSP/SMAST team tracked all the oyster additions and removals, as well as water quality and sediment nutrient regeneration/denitrification throughout this period. The results of the 2020 water quality monitoring and sediment nutrient regeneration/denitrification efforts are the focus of Part 2 of the 2020 Annual Report. Parts 1 and 2 are synthesized in this Annual Report following the approach developed in 2019.

2.0 Water Quality Monitoring: Overview.

Using procedures detailed in the Lonnie's Pond QAPP, CSP/SMAST staff began conducting biweekly water quality monitoring in Lonnie's Pond starting on April 29, 2020. There were twelve (12) sampling events over the 2020 season: 4-29-20, 5-14-20, 6-11-20, 6-29-20, 7-14-20, 7-29-20, 8-11-20, 8-26-20, 9-9-20, 9-24-20, 10-8-20, and 11-9-20 (Table 1). During each sampling event, water quality samples were collected at nine locations in Lonnie's Pond (Figure 1). In addition, two continuous monitoring devices were deployed 4-14-20 at 0.30 m above the bottom

directly east and west of southernmost oyster deployment area, to record chlorophyll-*a*, dissolved oxygen, salinity, temperature and depth. On each sampling date, water clarity (Secchi depth), temperature and dissolved oxygen profiles were collected with water samples. Water samples were transported to the CSP Analytical Facility and have been processed for N constituents (nitrate+nitrite, ammonium, dissolved and particulate organic nitrogen), ortho-phosphate (PO₄), particulate organic carbon (POC), total chlorophyll-*a* pigment, and salinity. In addition to the pond sampling, stream flow and water quality measurements were made biweekly at the two streams discharging into Lonnie's Pond: 1) the herring run from Pilgrim Lake and 2) the small cranberry bog outlet downstream of Crystal Lake. A continuous stage meter is deployed in the stream from Pilgrim Lake to provide daily volumetric freshwater outflow to Lonnie's Pond. Flow from the cranberry bogs was sporadic and only instantaneous readings were measured at the time of the biweekly stream sampling.

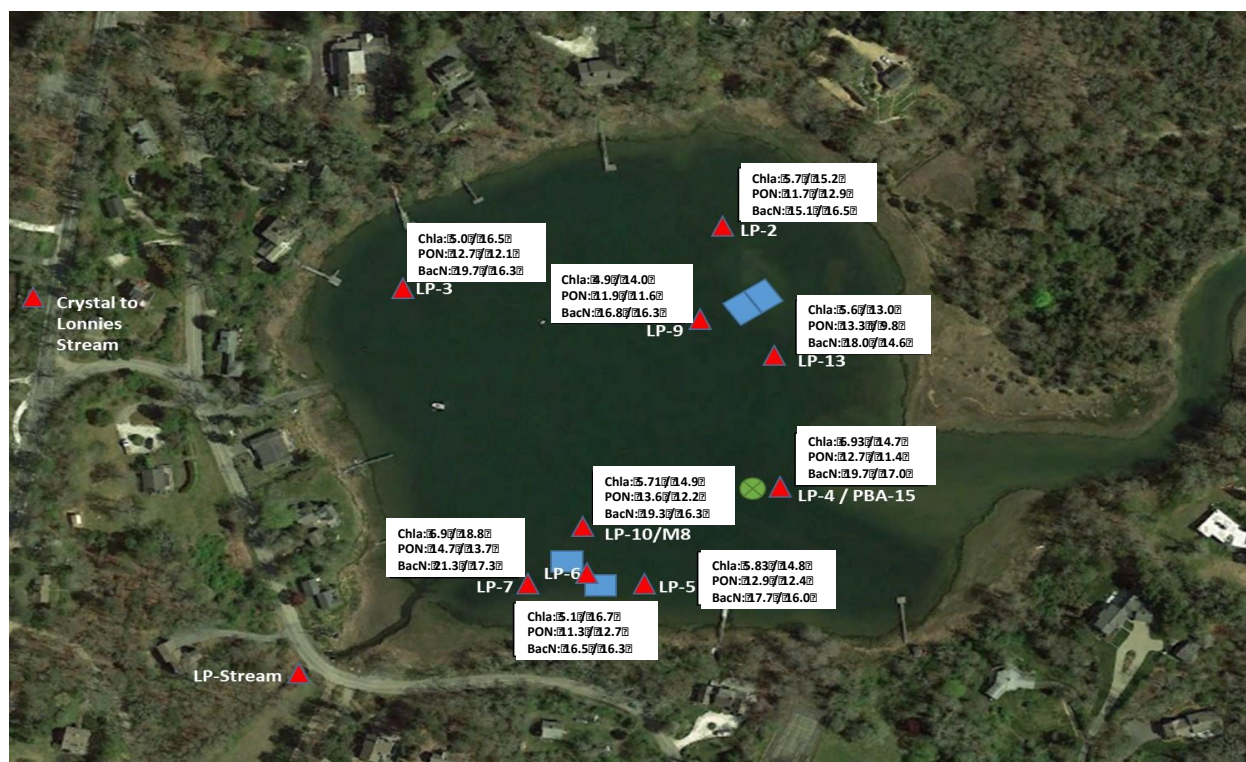


Figure 1. Map of Lonnie's Pond 2020 water quality sampling locations. The red triangles are sampled biweekly by CSP staff April 29 to November 9, 2020, green circle is sampled biweekly by Orleans citizen volunteers as part of the Pleasant Bay Alliance (PBA) from July 15-September 15, 2020, and blue squares represent the oyster deployment areas. White boxes show mixed layer average concentrations of total-chlorophyll-*a* ($\mu\text{g L}^{-1}$), particulate organic N (μM), and bioactive nitrogen⁶ (μM). Values represent averages of samples collected July-October for 2020 and 2019 (shown 2020/2019), the active oyster growth period in those years.

⁶ Bioactive nitrogen consists of dissolved inorganic nitrogen and particulate organic nitrogen, representing the most biologically active nitrogen within the total nitrogen pool.

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.

Sample Date	# of samples	Assays							
		NH4	PO4	NO3/NO2	TDN	POC/C	TSS	CHLA	Salinity
4/29/20	20	X	X	X	X	X	X	X	X
5/14/20	20	X	X	X	X	X	X	X	X
6/11/20	20	X	X	X	X	X	X	X	X
6/29/20	20	X	X	X	X	X	X	X	X
7/14/20	22	X	X	X	X	X	X	X	X
7/29/20	22	X	X	X	X	X	X	X	X
8/11/20	22	X	X	X	X	X	X	X	X
8/26/20	22	X	X	X	X	X	X	X	X
9/9/20	22	X	X	X	X	X	X	X	X
9/24/20	22	X	X	X	X	X	X	X	X
10/8/20	22	X	X	X	X	X	X	X	X
11/9/20	22	X	X	X	X	X	X	X	X
Total	256								

Water quality monitoring indicated that Lonnie’s Pond in 2020 was generally horizontally mixed throughout although there were spikes in chlorophyll-*a* concentration at LP2S on 6-11, LP4 on 7-14, and LP7 on 8-11 (Figure 2). These spikes in chlorophyll-*a* concentration were mirrored in the POCN data (Figure 2). 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 May, June, and July and lowest in September (Figure 2). These variations cannot be attributed to meteorological conditions since a comparison of the surface water salinity from LP3 (29.9 ppt) and LP7 (29.8 ppt) compared to the rest of the pond (29.8 ppt) was not significantly different. An increase in freshwater inputs to Lonnie’s Pond from Pilgrim Lake and Crystal Lake has in past years resulted in the increase of nutrient and total chlorophyll-*a* concentrations. Chlorophyll-*a* concentrations were highest in the spring and typically higher in (LP6) and around (LP2, 9, 13 and LP5, 7, 10) the oyster deployment areas throughout the year (Figure 2). Overall, the chlorophyll-*a*, PON, and bioactive N concentrations were lower in 2020 compared to 2019 with significant decreases in all nutrient constituents (Figure 1 & 4) consistent with the longer oyster deployment and greater oyster biomass in 2020 than previous years.

During the 2020 field season there appeared to be spring and summer phytoplankton blooms as seen in the chlorophyll-*a* and PON records particularly at station LP2, (Figure 2). It also appears that phytoplankton and PON levels declined by September and remained low through November. This follows the seasonal light and temperature cycle in Lonnie’s Pond and indicates that oyster growth was likely not limited by food supply in the earlier deployment in March. The measured water quality parameters from 2019 were consistent with a N enriched eutrophic basin, where chlorophyll-*a* levels were above 20 µg/L for much of the spring and summer. Chlorophyll-*a* levels in 2020 were much reduced with only two measured blooms in June and July exceeding 20 µg/L (Figure 2).

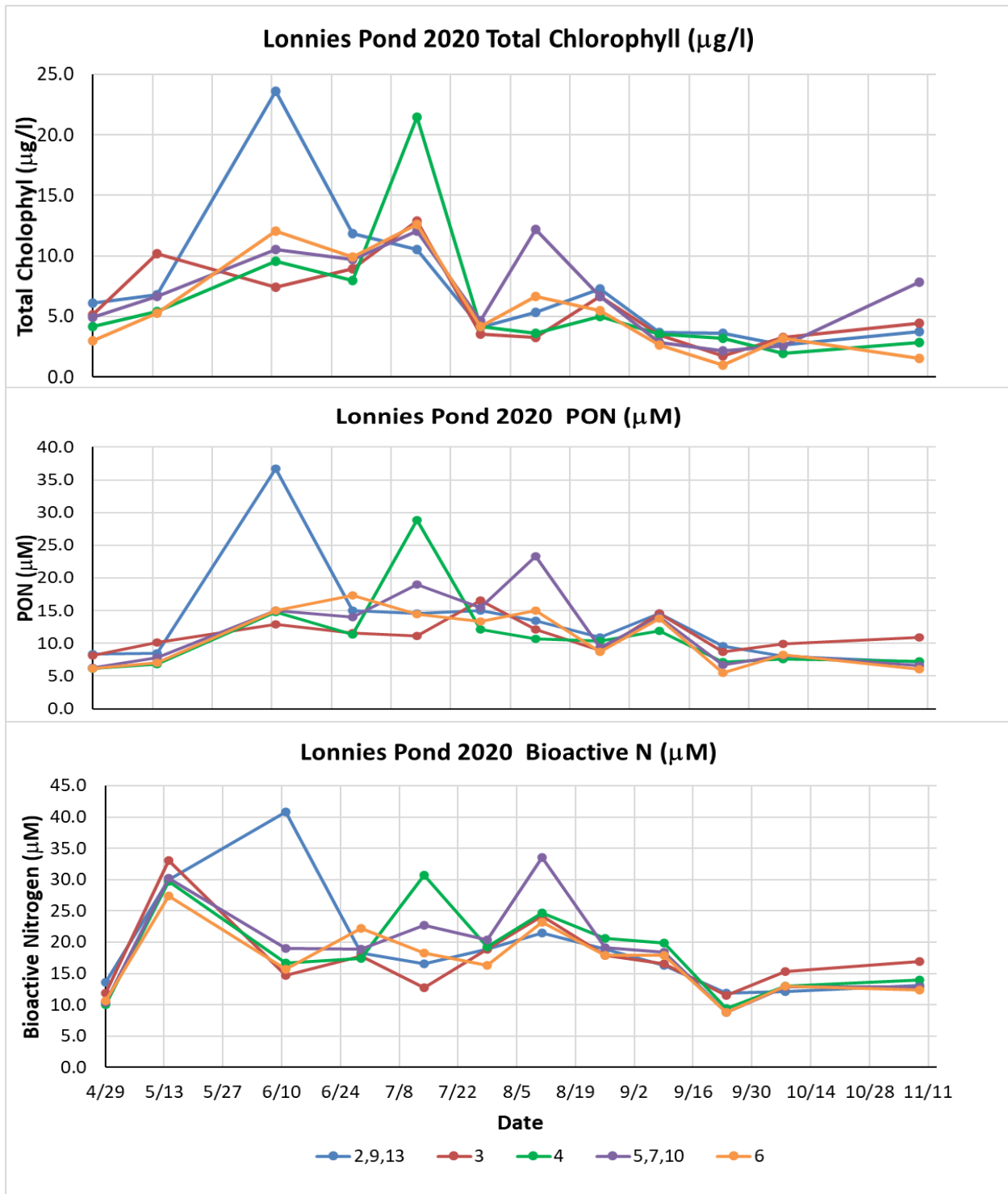


Figure 2. Time-series of mixed layer average total chlorophyll-*a*, particulate organic nitrogen (PON), and bioactive N at stations LP3, LP4, LP6 and averages of the stations (LP2, 9, 13) and (LP5, 7, 10) associated with the North and South oyster deployment. Station numbers refer to locations in station map in Figure 1.

Oysters were deployed March 12-20th. Oyster deployment occurred prior to the earliest planned water quality sampling on April 29th removing the potential for comparing water quality pre and post oyster aquaculture. Using paired t-test with 2019 water quality monitoring results showed significant increases in PON and significant decreases in dissolved inorganic nitrogen (DIN) post-oyster deployment (July 2019) ($p < 0.01$). Although a pre versus post oyster deployment comparison of water quality was not an option in 2020, significant increases in PON and TN were measured from June-October compared to April-May, while DIN was significantly reduced (Table 3 & 4). The generally high PON and chlorophyll in June-October versus April-May most likely results from higher light and warmer waters supporting greater phytoplankton growth. However, the lower bioactive nitrogen in June-October is a positive finding as the TMDL is based upon this parameter as the most active pool of nitrogen. Some of this seasonal shift (lower in summer) may also result from the similar reduction in bioactive N entering the pond during these 2 periods from Pilgrim Lake (see below). However, given the relative low discharge from Pilgrim Lake it is likely that the lower DIN in summer is due to internal processes related to uptake by phytoplankton since as the DIN was lower the PON was similarly higher. This coupled with the higher oyster filtration in summer versus April May results in the lower bioactive nitrogen levels in summer. However, these results were localized (Table 3) and were not as clear across the entire watershed of Lonnie's Pond except for PON and DIN (Table 4).

Average water quality conditions from July 14th to October 8th throughout the pond appear similar (Figure 1). Also, samples from the reference station, LP3, showed no significant difference in surface water PON, TN, total chlorophyll-*a*, or bioactive N compared to the rest of the stations, however chlorophyll-*a*, bioactive N and DIN were reduced at LP3 from the spring (Table 3.). Similarly, 6 of 9 stations showed reductions in bioactive N concentrations from samples in the spring versus the remainder of the growing season. The lack of clear horizontal gradients in 2020 versus previous years is at least partially, the result of the longer deployment of the oysters and the more even distribution across the pond. The distribution within the flow field should be allowing the oysters to filter phytoplankton more evenly across the watershed resulting in the observed distributions. Note: significance for all tests was $p=0.01$.

Table 3. Lonnie’s Pond mixed-layer water quality station averages from spring (April-May) and June-October 2020 for critical nutrient concentrations of chlorophyll-*a* (Total pigments), particulate organic nitrogen (PON), bioactive N, dissolved inorganic nitrogen (DIN), and total nitrogen (TN). Results in red indicate a reduction from spring averages.

April-May 2020						June-October 2020					
	Total						Total				
	Pigments	PON	Bioactive	DIN	TN		Pigments	PON	Bioactive	DIN	TN
Sta #	µg/L	µM N	µM N	µM N	µM N	Sta #	µg/L	µM N	µM N	µM N	µM N
2	6.55	9.25	24.77	15.52	44.51	2	10.57	16.77	20.57	3.79	51.08
9	6.19	7.56	21.49	13.93	36.90	9	6.55	12.98	17.25	4.27	40.58
13	6.53	8.46	19.04	10.58	34.21	13	8.27	16.23	20.58	4.36	54.43
3	7.66	9.14	22.44	13.31	40.80	3	5.69	11.83	16.62	4.79	45.95
4	4.78	6.48	19.84	13.36	38.26	4	7.35	12.75	19.08	6.33	46.34
6	4.14	6.62	18.99	12.37	35.77	6	6.40	12.41	17.02	4.61	43.72
5	4.22	5.94	18.90	12.96	40.27	5	7.53	13.81	18.98	5.17	44.02
7	5.59	6.29	19.93	13.64	36.80	7	7.27	14.60	20.64	6.04	46.94
10	7.49	8.80	22.14	13.35	38.97	10	6.30	13.26	18.32	5.06	43.43

Table 4. Seasonal results from paired t-tests with $p < 0.01$ used mixed-layer station averages sampled from Lonnie’s spring (April-May) and June-October 2020 for critical N constituents. Significant differences were seen in the PON (particulate organic nitrogen), DIN (dissolved inorganic nitrogen), and TN (total nitrogen) concentrations.

	Total Pigments µg/L		PON µM N		Bioactive µM N		DIN µM N		TN µM N	
	Apr-May	June-Oct	Apr-May	June-Oct	Apr-May	June-Oct	Apr-May	June-Oct	Apr-May	June-Oct
mean	5.91	7.33	7.62	13.85	20.84	18.78	13.22	4.94	38.50	46.28
variance	1.53	1.85	1.55	2.58	3.60	2.26	1.53	0.61	8.46	15.81
stdev	1.24	1.36	1.25	1.61	1.90	1.50	1.24	0.78	2.91	3.98
n	9	9	9	9	9	9	9	9	9	9
t	-2.26		-10.68		2.53		16.42		-4.80	
d.o.f	8		8		8		8		8	
critical value	3.36		3.36		3.36		3.36		3.36	
[t] > critical value	no significant diff.		significantly different		no significant diff.		significantly different		significantly different	

*Boldened [t] > critical value of significance indicates positive (black) or negative (red) changes in nutrient concentrations over seasonal change.

We attempted to refine the oysters’ effect on water quality by looking for changes in surface water quality as water moves through the oyster areas (e.g., water from LP7 to LP5 or LP2 / LP9 to LP13 on ebbing tide). The water transparency was significantly increased ($p < 0.01$) as the ebbing waters passed through the oyster bags, LP9 to LP13 (Figure 3). The water passing over the oysters on an ebb tide appears to be clarified by the oysters in the northern aquaculture area as was found in prior years. However, no statistically significant differences were found in the water transparency

in southern oyster aquaculture area (LP7 to LP5) consistent with the lack of reduction of PON, total chlorophyll-*a* pigments, or total suspended solids (TSS). This results from the difficulty in predicting the flow field around the southern deployment area. 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 up-gradient and down-gradient 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).

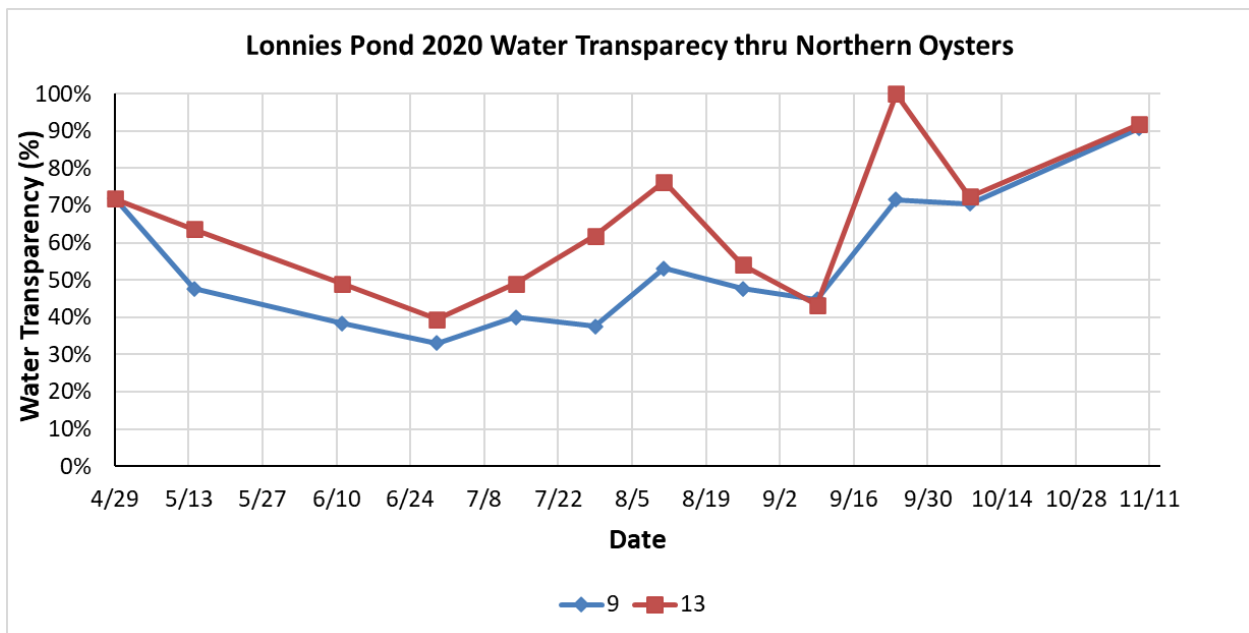
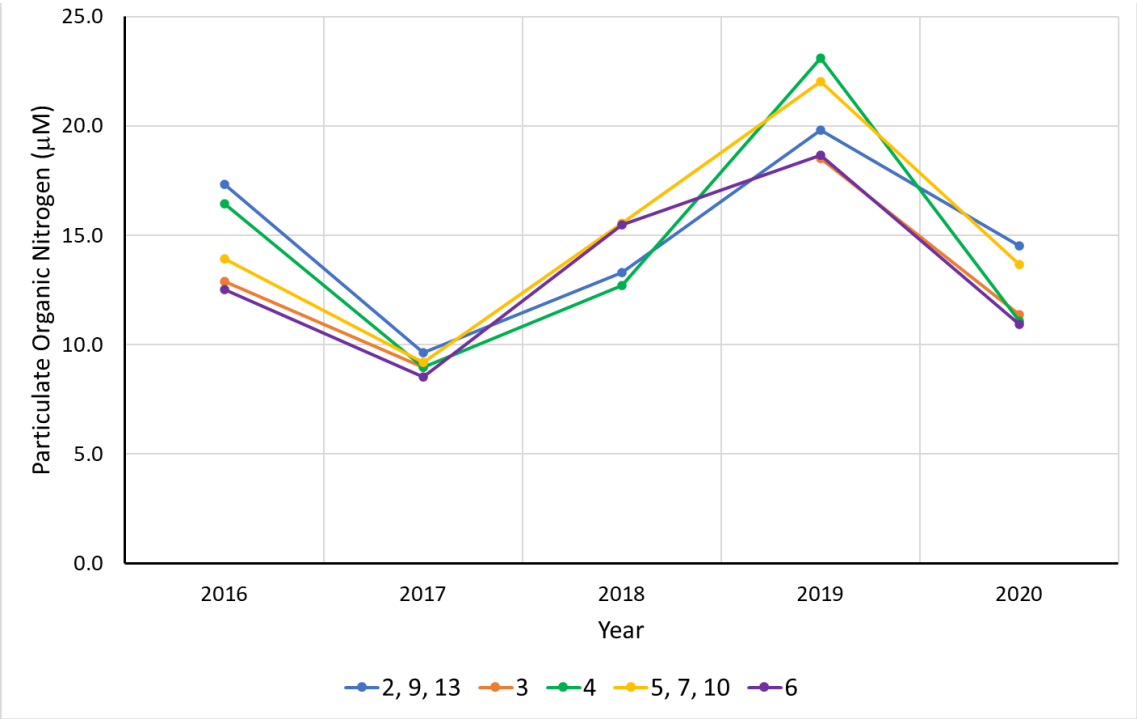
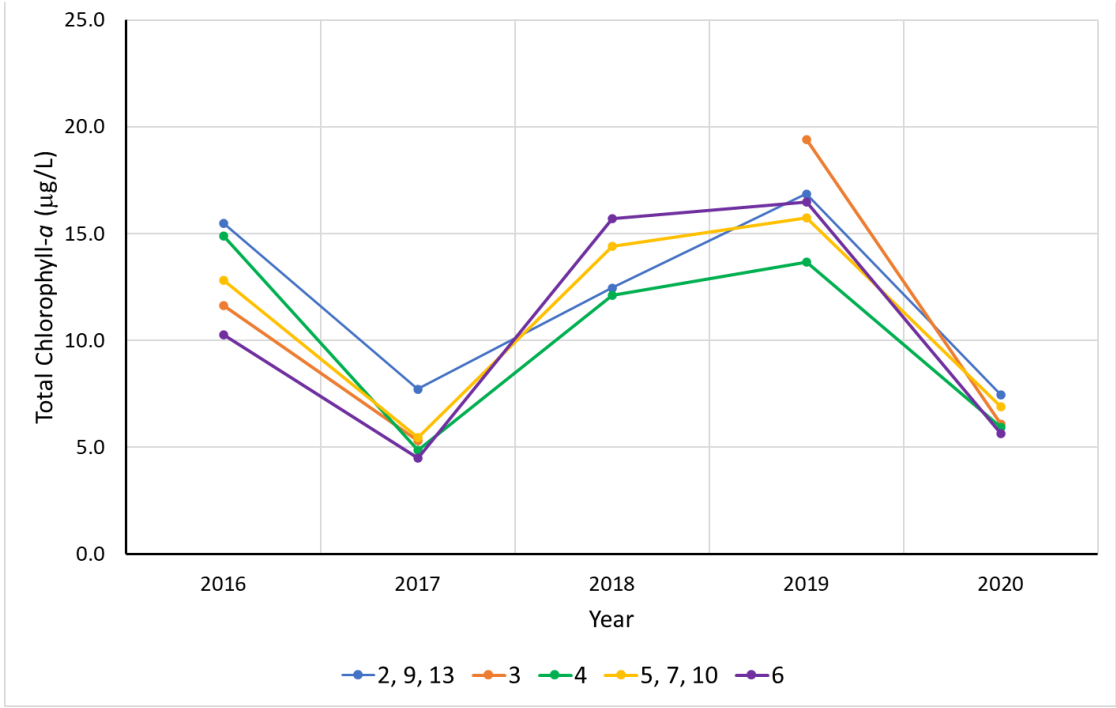


Figure 3. Time-series of water transparency during the 2020 water quality monitoring using the station secchi depth divided by the total station depth in the North oyster aquaculture area.

The study the efficacy of using shellfish to reduce N load began in 2016 and we investigated the water quality data for any trends over the five years. Based on the compiled results pond-wide chlorophyll-*a* concentrations have ranged from 4.5 – 19.4 µg/L with the highest levels for each respective station occurring in 2019 (Figure 4). All stations have lower chlorophyll-*a* concentrations in 2020 averaging 7.8 ug/L pond-wide for the year compared to the start of the study in 2016 which averaged 12.9 ug/L in total pigments. PON and bioactive N also reached its peak in 2019 and has lowered back down to the PON concentrations recorded in 2016. The sharp decrease in nutrients concentration from 2019 to 2020 may be explained by the earlier oyster deployment of March 2020 and the greater period of high oyster biomass allowing for a greater filtration of phytoplankton and N assimilation by the oysters.



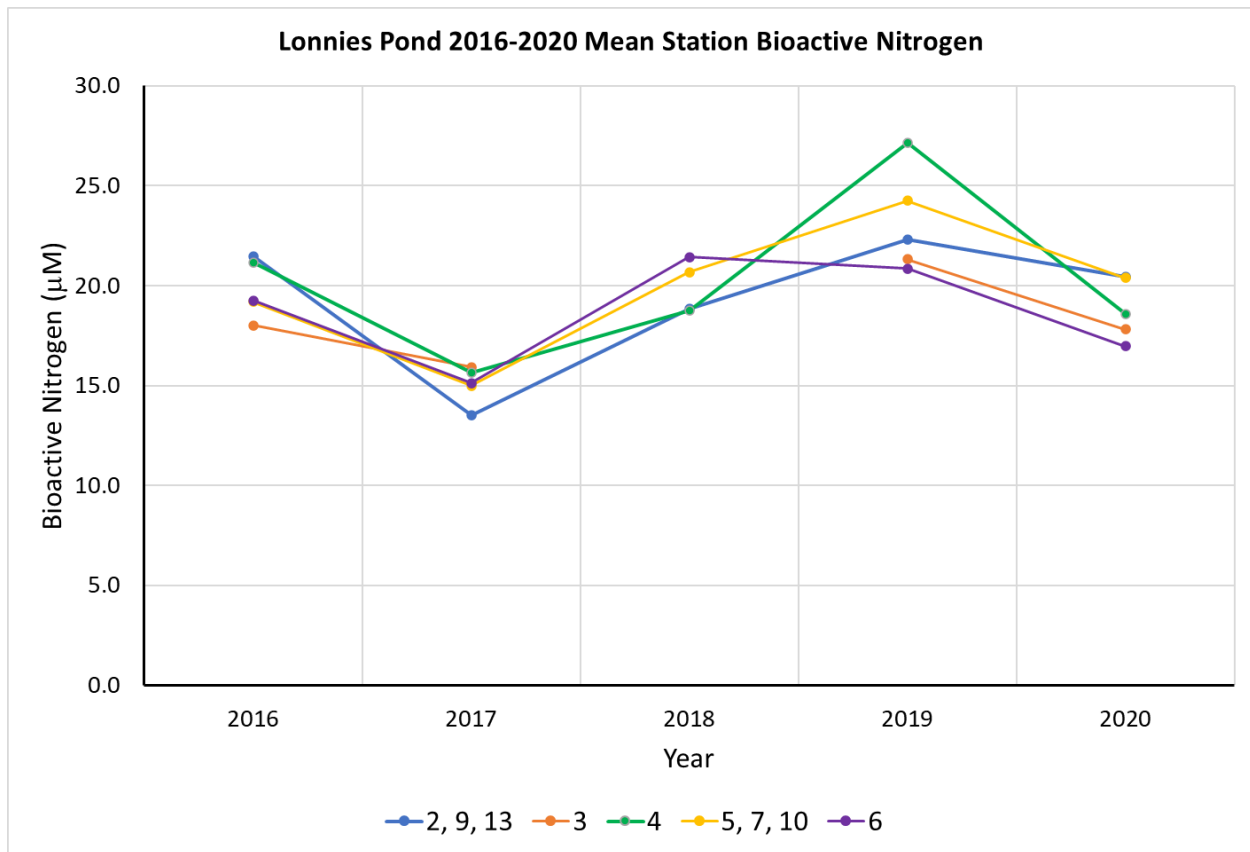


Figure 4. 2016-2020 Interannual variation in mixed layer average total chlorophyll-*a* (*top*), particulate organic nitrogen (*middle*), and bioactive N (*btm*) at stations LP-3, LP-4, LP-6 and averages of the stations (LP 5, 7, 10) and (LP 2, 9, 13) surround the oyster deployments. Station numbers refer to locations in station map in Figure 1.

TSS were measured in and around the South oyster aquaculture area consistently from 2016-2020. TSS represents the dry weight (mg) of suspended solids, which are not dissolved in the water column. Due to oysters filtering capacity, it is hypothesized that the TSS will be reduced as the water travels out of Lonnie's Pond into Little Pleasant Bay on an ebb tide. Station LP7 is up-gradient of the aquaculture area, LP6 is within the aquaculture area, and LP5 is down-gradient of the aquaculture area, (refer to station map in Figure 1). All samples were collected on the ebb tide and the highest TSS was indeed found up-gradient of the oysters (LP7) and reduced down-gradient of the oysters every year from 2016-2020 (Figure 5). Interestingly, TSS was lowest within the aquaculture area (LP6) every year except 2020 and was significantly reduced compared to LP7 ($p = 0.01$) showing a clear drawing down of suspended solids by the oysters. Consistent with generally higher water clarity.

One source of TSS is the herring run (Lonnie's Stream) that inputs just west of LP7. Station LP7 can be used as a measure of the TSS input from the stream and shows TSS has been increasing from 2016-2019. Despite the increased measured TSS up-gradient of the oysters, it appears the

aquaculture area has handled those increases and has not exceeded capacity for filtering out these suspended solids.

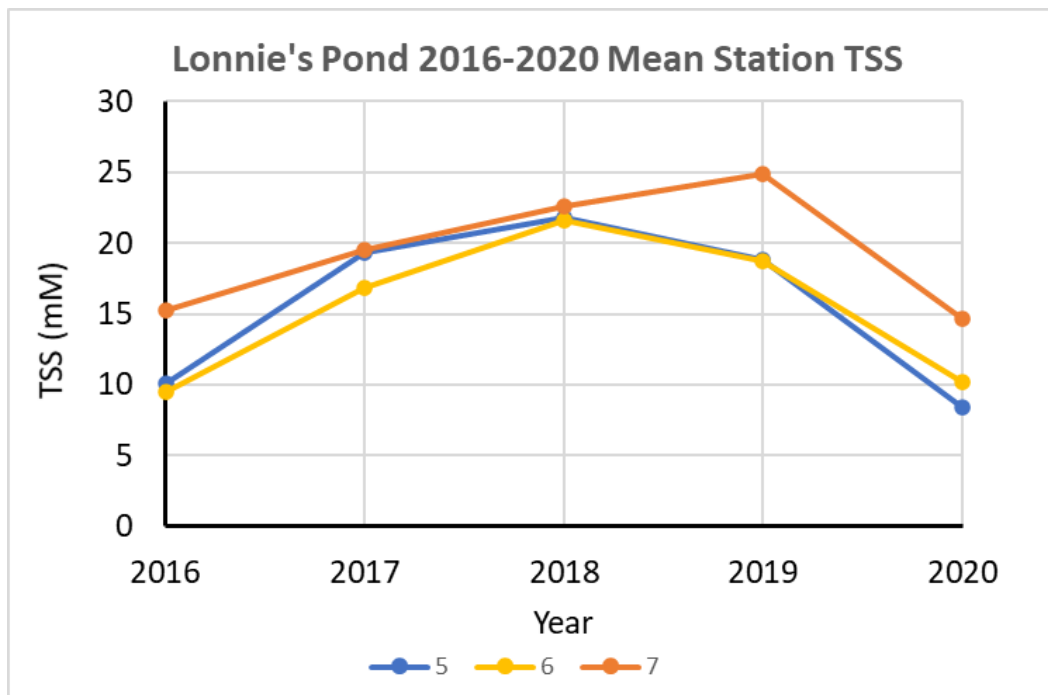


Figure 5. Compiled 2016-2020 averages for total suspended solids (TSS) at stations up-gradient (LP7), within (LP6), and down-gradient (LP5) of the Lonnie’s Pond South oyster aquaculture area. Station numbers refer to locations in station map in Figure 1.

Oysters have been shown to reduce water column particulates, PON, TSS, and chlorophyll-*a* in Lonnie’s Pond as well multiple other areas locally (e.g., Bournes Pond⁵) as well as regionally (Great Bay, New Hampshire⁶ and Long Island Sound⁷).

Additional water quality sampling was conducted by Chatham/Orleans volunteers from July 9 - September 9, 2020 at sentinel station PBA-15, which has a long-term water quality record of 20+ years and 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 and used different sampler gear and personnel, indicating that the volunteer

⁵ Howes, B. Unruh, A., Schlezinger, D., Labrie, M., Benson, J., 2018. Preliminary Assessment of Bournes Pond Oyster Aquaculture Effects on Water Quality and Nutrient Cycling. Coastal Systems Program, School for Marine Science and Technology (CSP/SMAST), University of Massachusetts-Dartmouth. New Bedford, MA. 28 pp.

⁶ Bricker, S.B., Grizzle, R.E., Trowbridge, P. et al. 2020. Bioextractive Removal of Nitrogen by Oysters in Great Bay Piscataqua River Estuary, New Hampshire, USA. *Estuaries and Coasts* 43, 23–38).

⁷ Bricker, S.B., Ferreira, J.G., Zhu, C., Rose, J.M., et al. 2018. Role of Shellfish Aquaculture in the Reduction of Eutrophication in an Urban Estuary. *Environmental Science & Technology* 52 (1), 173-183.

WQ monitoring is performing very well and can be relied upon by the Town for management purposes (Table 5).

Table 5. 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 LP 4, SMAST staff) indicates a high degree of congruence, which increases the confidence in the data. An RPD of less than 10% shows excellent agreement.

	PBA15	LP4	RPD
PON (µM)	11.77	12.72	8%
Bioactive N (µM)	22.49	23.04	2%
TN (µM)	53.47	52.49	2%

Stream Inflows and Nutrient Loads: Lonnie's Pond receives freshwater discharge from two streams, Pilgrim Lake to Lonnie's Pond and a small discharge from Crystal Lake Bog to Lonnie's Pond. These nitrogen inputs to Lonnie's Pond are important to measure as they play a role in setting the nutrient field in Lonnie's Pond. Pilgrim Lake stream discharges freshwater to Lonnie's Pond with its associated nitrogen load. The stream flow varies seasonally with highest flows and nitrogen load regularly occurring in the spring and lower flow and load occurring in the summer months (Table 6; Figure 6). Water quality results from 2020 show that total nitrogen and flow inputs are significantly lower throughout the summer than in the preceding spring (Figure 6). In 2020, as in previous years, summer stream flow declined to less than 1/3 and N load to less than 1/2 of the preceding spring. In 2019, the Pilgrim Lake stream input of ~45.7 kg of nitrogen into Lonnie's Pond from April – June (pre-oyster), only ~14.7 kg N July – September. Similarly, the Pilgrim Lake input ~31.7 kg N in the spring and 14.6 kg N during the summer was observed in 2020 (Table 6).

Overall, nitrogen inputs to the pond via stream discharges was lowest in 2020 of the 4 years of measurement (Table 6). This results 2020 having the lowest stream flow volumes of the 4 years, rather than lower concentrations in water originating in Pilgrim Lake.

Table 6. Total nitrogen load (kg) entering Lonnie's Pond via Pilgrim Lake stream in 2020. April – June 2019 was without oysters, whereas 2017, 2018, and 2020 had oysters during spring time freshwater flow conditions. July – September for all four 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)
93,257	Total Load (April-June 2017)	3.86	1.92	5.79	32.59	12.08	50.46
39,420	Total Load (July-Sept. 2017)	1.83	2.12	3.95	14.64	2.85	21.45
137,888	Total Load (April-June 2018)	4.63	6.18	10.81	57.40	14.45	82.67
100,995	Total Load (July-Sept. 2018)	5.08	9.91	15.00	39.52	8.11	62.62
51,956	Total Load (April-June 2019)	6.09	2.66	8.74	25.81	11.19	45.74
23,178	Total Load (July-Sept. 2019)	1.20	2.06	3.26	8.58	2.89	14.73
46,939	Total Load (April-June 2020)	3.71	4.38	8.09	17.44	6.18	31.71
12,238	Total Load (July-Sept. 2020)	1.93	4.64	6.56	4.99	2.74	14.62

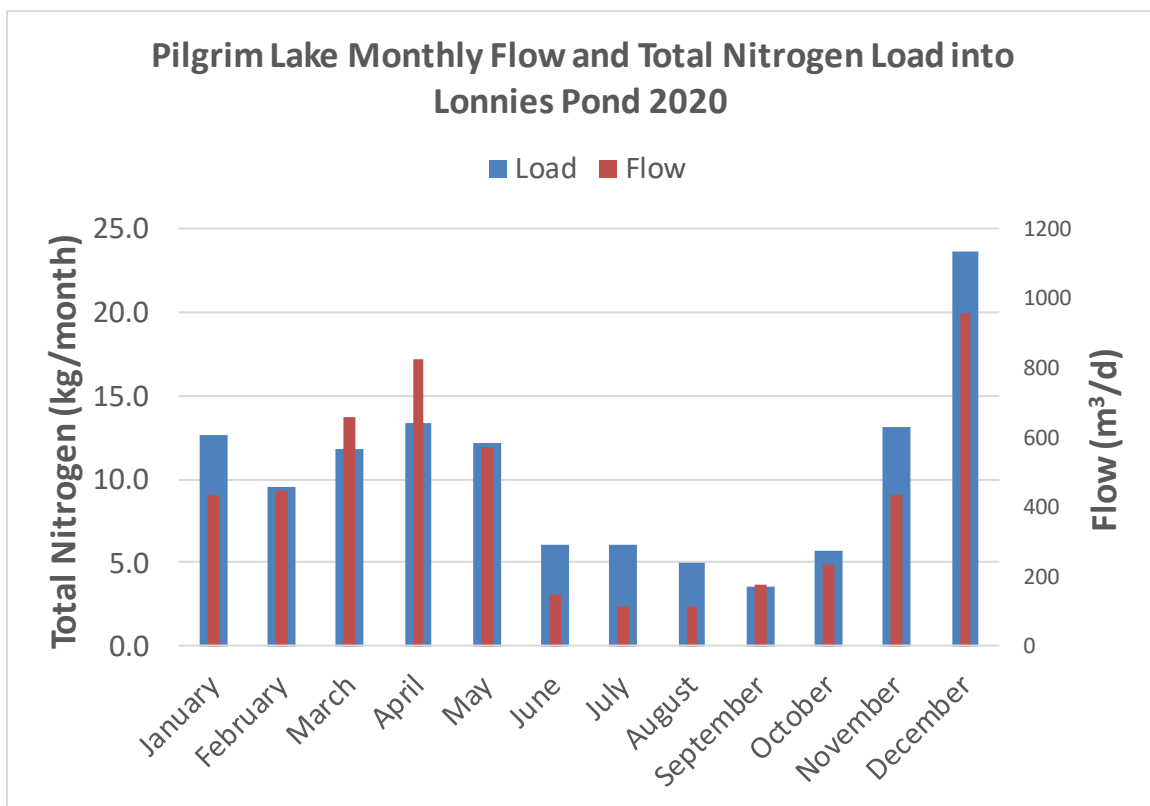


Figure 6. Total nitrogen load (kg) and freshwater volume (m³) entering Lonnie's Pond via Pilgrim Lake in 2020.

3.0 Dissolved Oxygen and Chlorophyll-*a* Continuous Monitoring

Two autonomous recording multiparameter sondes were deployed from April 14 through November 9, 2020. One sonde was deployed 30 cm from the bottom in approximately 1 meter of water along the eastern side of the aquaculture area (Lonnie's Pond East, see Figures 7, 8 and 9; top panel). The second sonde (Lonnie's Pond West, see Figures 7, 8 and 9; bottom panel) was deployed 30 cm from the bottom in approximately 1.6 meters of water slightly south of the where the two oyster deployment areas meet (4m south of LP6). Oysters filter large quantities of water. Phytoplankton removed from the water are deposited as feces and pseudofeces 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. These 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 concentrations at the two moorings were not significantly different from those at the other more distant monitoring stations within the pond, nor were the average daily concentrations, which generally approximated air saturation (Figure 7). However, significant differences between the magnitude of daily excursions were observed between the two moorings. The East mooring displayed daily excursions of up to 18 mg/L, primarily as a result of greater light availability; no anoxia was observed. The West mooring, which was more shaded by the oyster deployment bags, showed smaller daily excursions of up to 10 mg/L and six instances of anoxia lasting no more than 15-30 minutes. At both moorings high daily oxygen excursions and oxygen minima were coincident with high chlorophyll values associated with algal blooms. The similarity of oxygen data at the moorings and other sites indicates that the periodic hypoxia within the deployment area is part of a Lonnie's Pond basin-wide phenomenon, rather than a localized oyster effect. This is being examined further as part of an assessment of stocking density effects and will be reported upon in the future.

Algal blooms, as determined from high chlorophyll concentrations, were generally associated with rain events that were infrequent over the summer. Infrequent rain results in higher concentrations of nutrients entering the pond from overland flow as they have a longer time to accumulate on the soil and impermeable areas. Although the temporal record for 2020 is much longer than in 2019, peak chlorophyll concentrations were not larger than in 2019, nor was the adjusted frequency of algal blooms (i.e., ~2 events over the same time span, as also seen in the grab sampling program).

Continuous chlorophyll data indicated small differences between the two mooring locations (Figure 8) with concentrations at the East mooring higher than at the West mooring; and the peak chlorophyll concentrations occurring almost simultaneously. Otherwise, the records were similar though skewed with higher chlorophyll concentrations observed at Lonnie's Pond East. This is

opposite the pattern observed in 2019 when higher chlorophyll concentrations were observed to the west where Pilgrim Lake Steam discharges. The reason for this difference is not clear, but may be related to low rain fall and lower steam flow. The general direction of water flow through the oyster propagation area depends, in part, upon fresh water flow from Lonnie's Stream. With greatly reduced flow from Lonnie's Stream, a nutrient point source, gradients produced by fresh water inflow may have been minimized. Another possibility stems from the deeper depth of the Western mooring (1.6m vs 0.6m for the East mooring). Salinity records (Figure 9) from the shallower East mooring indicate that fresh water was mixing down to bottom of the water column following rain events, while the West mooring displayed mixing down to 1.6m. The greater availability of nutrients born by fresh water inputs and the shallower depth of the East mooring (more light) may also contribute to the reversed pattern observed in 2020.

Overall, the mooring records were extremely similar between 2019 and 2020 for both the magnitude and frequency of phytoplankton blooms. While the time series record of oxygen from 2019 was spotty, comparison of the values obtained during the monitoring activities indicated no significant differences between 2019 and 2020. It appears that Lonnie's Pond currently supports periodic hypoxia during July and August due to its overall nitrogen loading.

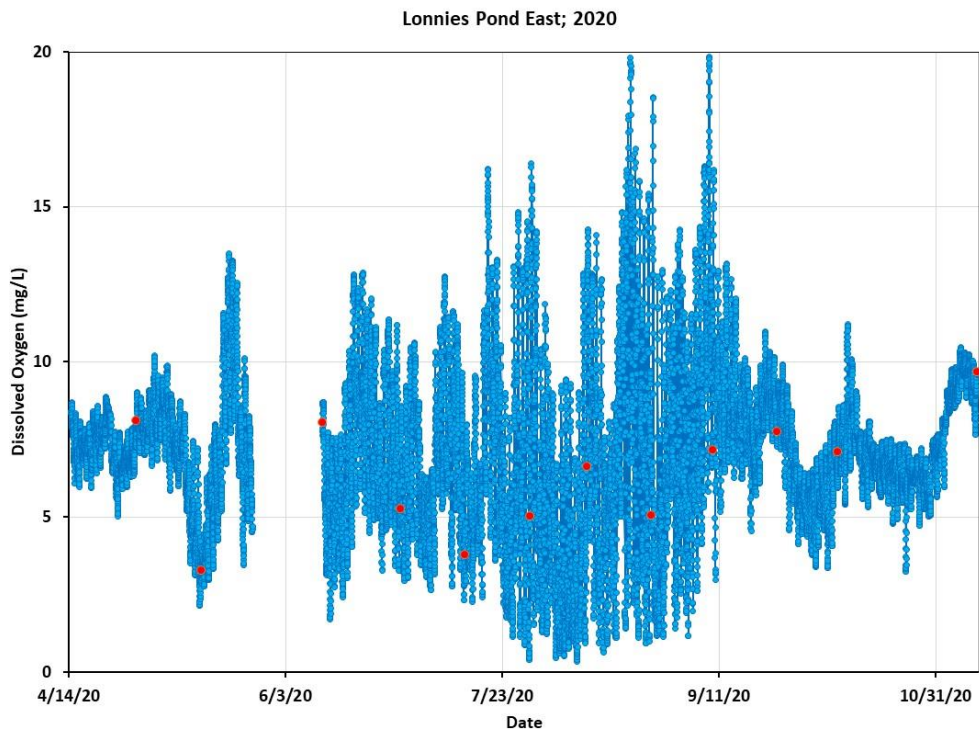
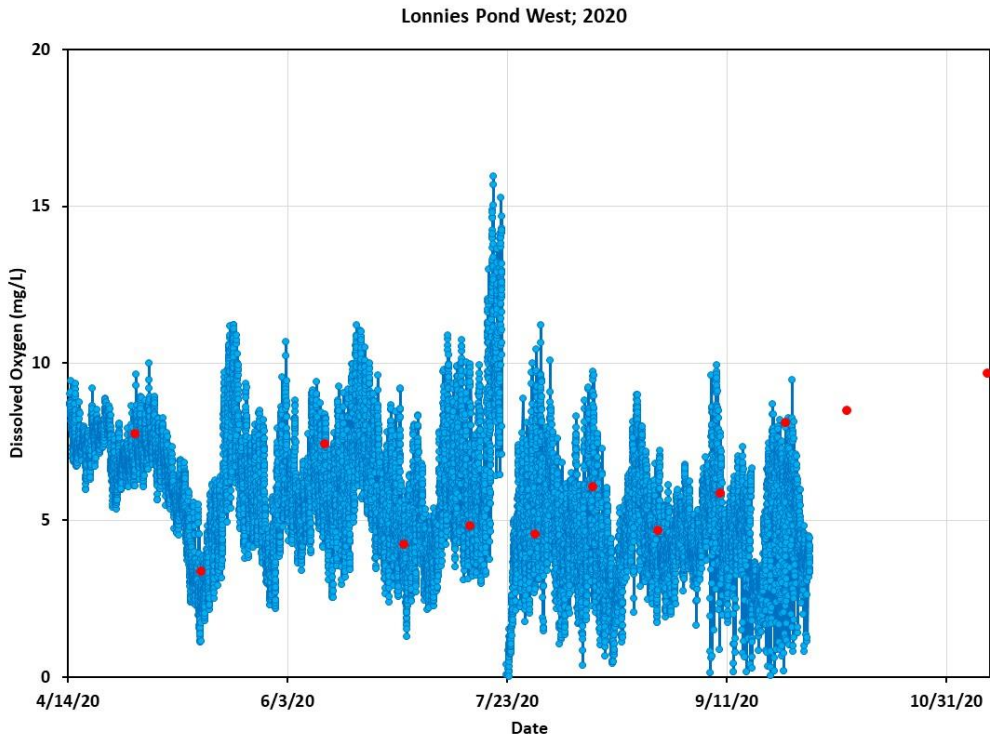


Figure 7. Time series oxygen measurements for West (top) and East (btm) moorings. Red dots indicate in situ calibration values obtained during monitoring activities.

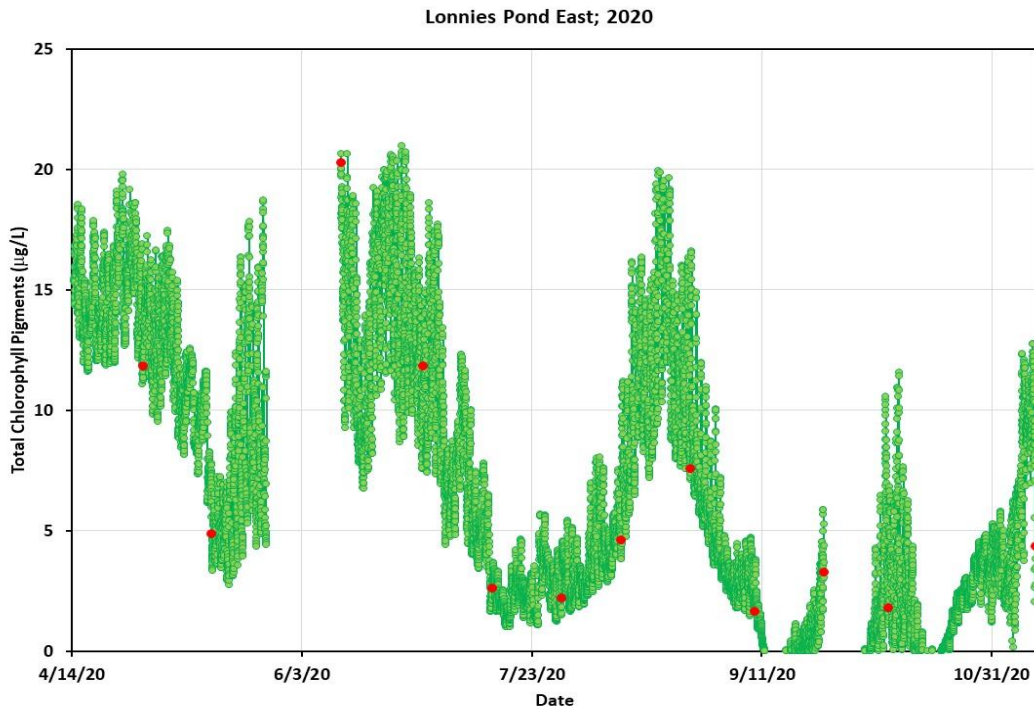
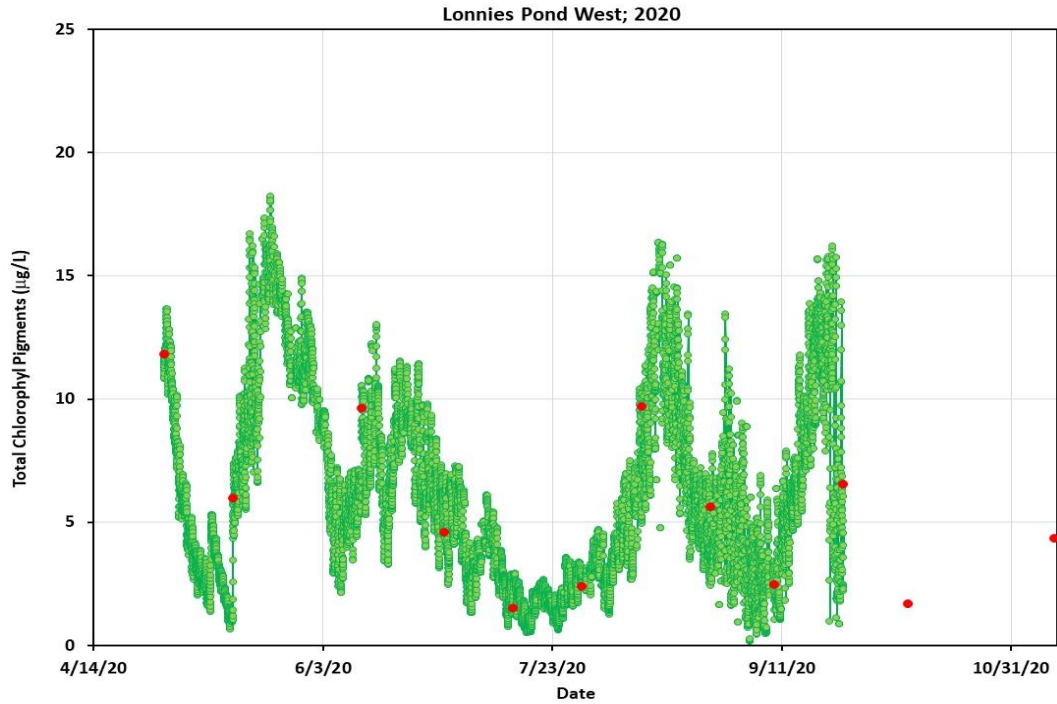


Figure 8. Time series chlorophyll measurements for West (top) and West (btm) moorings. Red dots indicate in situ calibration values obtained during monitoring activities. These time-series measurements clearly show the 2 major blooms during summer that were indicated in the water quality program. These blooms and their declines are consistent with the observed bottom water dissolved oxygen levels which likely result in part from their settling and decay.

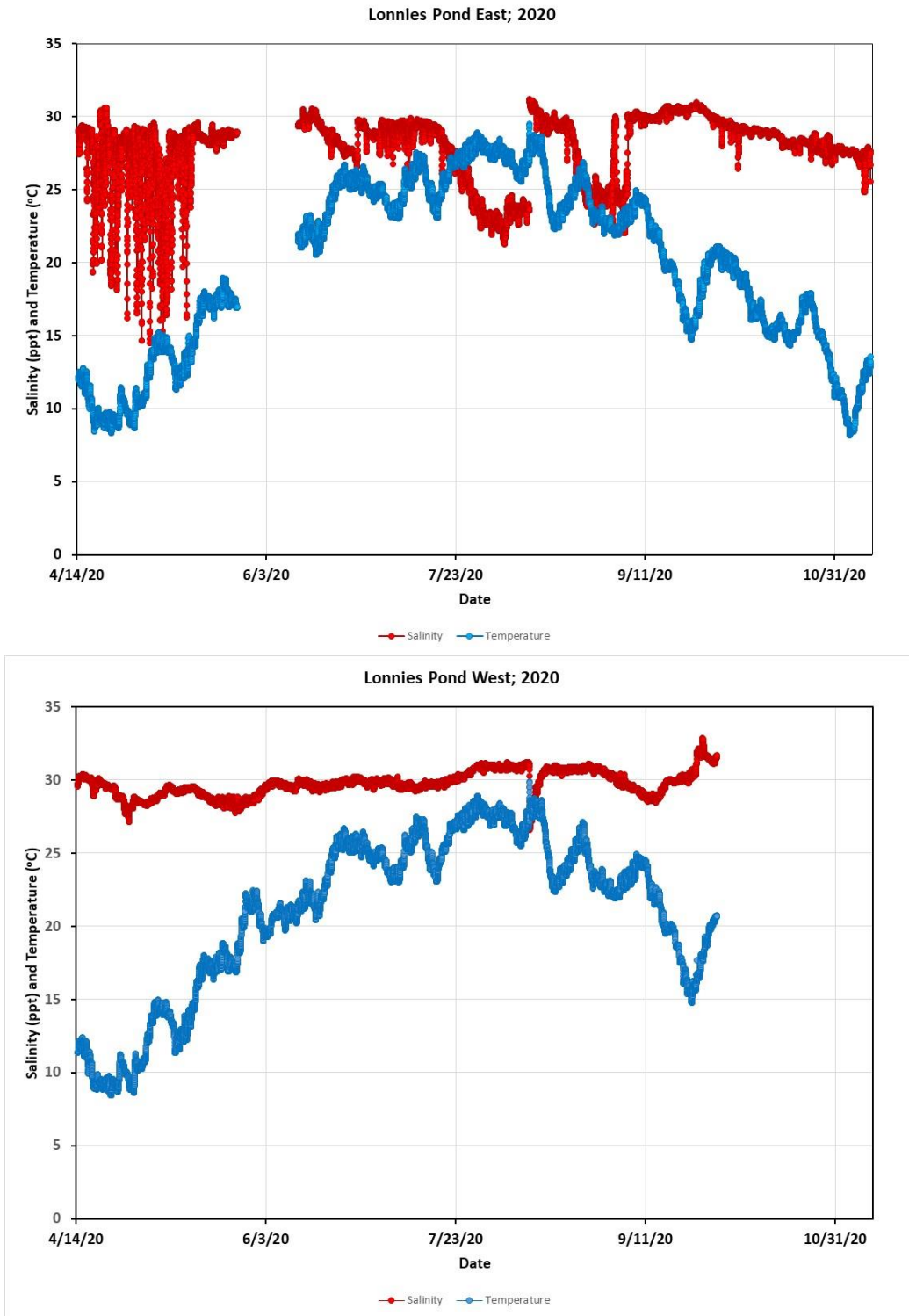


Figure 9. Time series salinity and temperature measurements for East and West moorings. Decreased salinities in the East mooring record were coincident with rainfall events.

4.0 Effects of Oysters on Nutrient Regeneration and Denitrification in Sediments

In estuarine basins such as Lonnie's Pond, N is transformed and recycled within the sediments and water column. This recycled N 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 N accounts for nearly half the total supply available to phytoplankton blooms, during the warmer months. Failure to account for this recycled N generally results in significant errors in determination of the effects of watershed N loadings, the overall balance of the system, and how oyster aquaculture may affect nutrient dynamics at the sediment-water interface.

The Lonnie's Pond basin, and other similar shallow water basins tributary to Pleasant Bay and around Cape Cod, contain organically enrich sediments. Particulate organic matter (POM) deposition to sediments drives biogeochemical cycles, including benthic respiration, which is secondarily controlled by temperature. Residing within the water column, heterotrophic processes intercept labile organic matter and lower the quantity of POM reaching the benthos. Thus, embayment respiration rates in shallow coastal systems are typically significantly higher than those found in adjacent deeper and colder offshore waters. Sediment oxygen uptake rates play a major role in bottom water oxygen levels and declines in this ecosystem structuring parameter effect habitat quality. During the warmer summer months, Lonnie's Pond periodically shows short-term periodic hypoxia and did again in 2020 (i.e., < 3-4 mg/L dissolved oxygen). Although water depth is an important factor in POM deposition dynamics, the addition of filter feeders like oysters have the potential to overwhelm the "depth effect" through the concentrated emission of large packaged fecal material. Shellfish aquaculture is projected to increase deposition and subsequently increase sediment respiration rates including denitrification.

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

In order to determine any enhancement of benthic carbon and nitrogen cycling, nitrogen regeneration and removal through denitrification associated with oyster aquaculture activities in Lonnie's Pond, sediment samples were collected and incubated under *in situ* conditions on three dates, April 14, August 16, and October 11, 2020 (April 2021 will also be captured for to complete the annual cycle). The effect of oysters on nitrogen cycling and oxygen availability was most significant during periods of maximum oyster activity (July-September) and maximum oyster biomass (October-December). Temperature changes between sampling periods is also important, as higher temperatures increase bacterial respiration while lower temperatures decrease carbon and nitrogen decay rates. Early spring rates are important to capture since as early as the April 18, 2017 flux it was found that N rich, POM depositions from oysters (biodeposits) from the prior late summer and fall oysters were stored in surficial sediments over-winter and fuel a high rate of cycling the following early spring. Similar to previous years (Table 5), this seasonal carryover of nutrients was measured in 2020 and indicates that biodeposits can be stored and failure to account for denitrification associated with them results in a large underestimate of this pathway of N removal.

The SCUBA diver collected intact sediment cores were incubated at in situ temperatures with a mixed headspace such that the time-course of changes in regenerated nutrient species (DON, NO_x^- , NH_4^+ and PO_4^-) and dissolved oxygen can be determined. The rate of oxygen uptake was determined to: 1) evaluate the oxygen depletion in response to oyster aquaculture alone, 2) examine the response to organic matter deposition rates, and 3) determine the amount of the oyster biodeposited N that is regenerated back to the watercolumn versus removed through denitrification. Measurements of sediment regeneration were limited to oysters deployed in the South oyster deployment area to allow comparison to prior years. Assays were performed on 10-12 cores from sites distributed throughout the South deployment area and outside the area on each date. Cores were collected directly in areas receiving oyster biodeposits (impact area) and at distances outside of the aquaculture area (background).

An empirical model of measured water velocity and measured biodeposit settling rate was used to determine the area of bottom receiving biodeposits⁸. The distance of cores from the margin of the oyster array determined whether the core was within the biodeposit impact area. The velocity data collected by acoustic doppler current profiler (ADCP) in summers of 2016-2019, the prior data was used in the model. with the mean sinking velocity of fecal material (8.14 ± 5.01 mm/s), with the mean depth around the margin of the oyster deployment area, and the tidal range. Fecal material settling was modeled step-wise over the entire bag array assuming fecal pellet production was similar among all bags. The 2019 biodeposit impact area (2890 m²) was applied to the 2020 deployment because the 2020 South deployment array area, location, and layout was unchanged from 2019 to 2020.

The results allowed determination of the spatial pattern of oyster aquaculture biodeposition and how nutrient exchange rates, oxygen uptake and denitrification are affected by the oyster arrays. From our experience, sediment regeneration during the summer is a large and important source of nutrients for both phytoplankton and macroalgal production in embayments throughout southeastern 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.

The parallel determination of denitrification was based upon time series measurements of excess N_2 (compared to Argon) using membrane injection mass spectrometry (MIMS). N_2 produced by denitrification is precisely detected by ratio analysis 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 is pumped at ml/min rates through a gas permeable membrane in order to extract gas into the mass spectrometer inlet. The inlet has cryogenic traps to remove water vapor and carbon dioxide (CO_2) gas. Sample gas was analyzed by the mass spectrometer for masses 28 and 40 for determining N_2 :Ar ratio. Calibration was made by comparison with a reference gas of known composition. Water column respiration was determined on water samples collected east and west of the South deployment area at Lonnie's water quality monitoring stations 5 and 7. These results with the sediment oxygen uptake support assessment of potential low oxygen events.

⁸ The model has been both calibrated and verified with field measurements and has been used previously (M. Labrie, Ph.D. Dissertation May 2021).

Sediment Nutrient Cycling Results (2020):

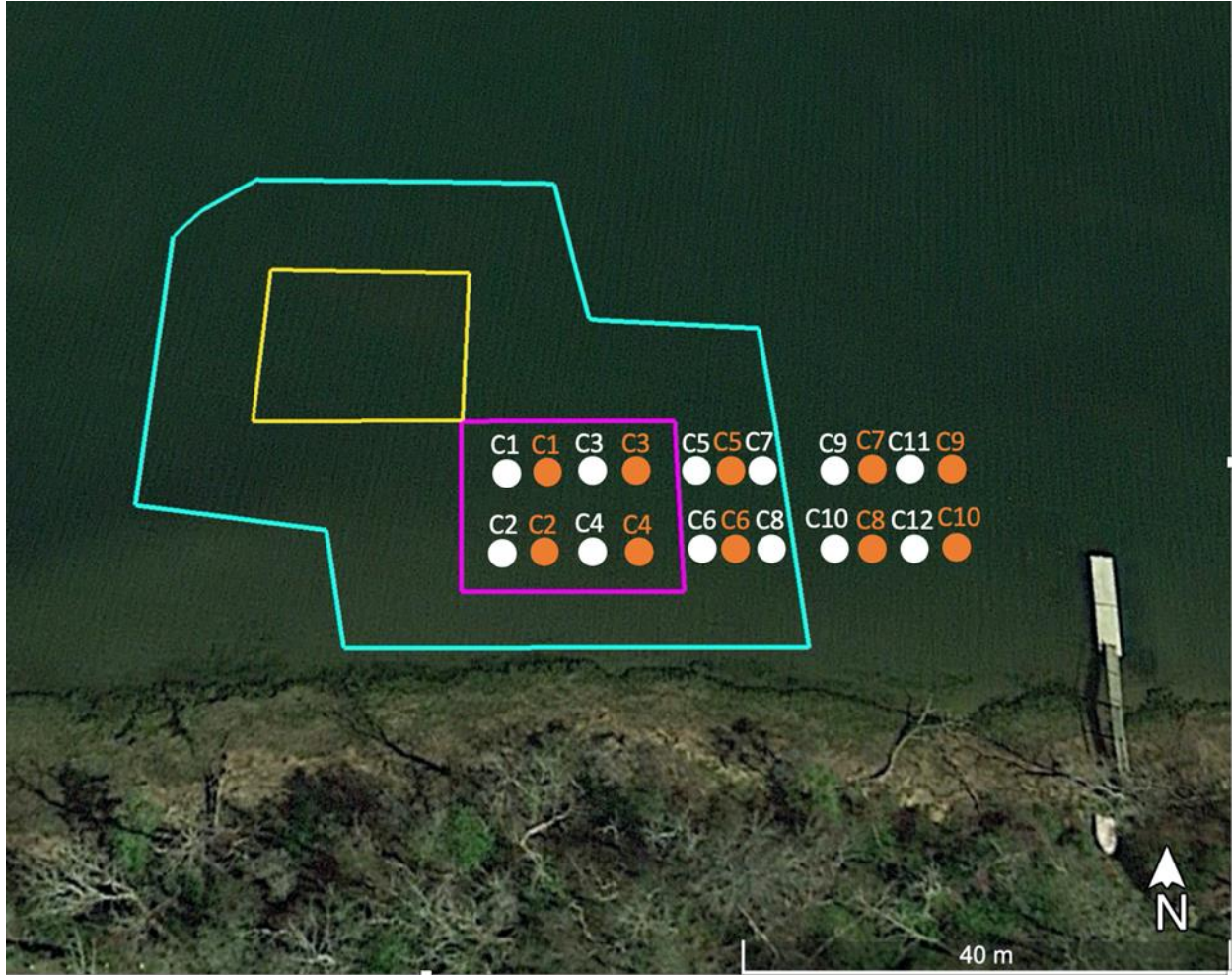


Figure 8. Aerial photograph showing the location of the oyster deployment area overlain with 2019-2020 oyster deployment area (yellow and purple polygons), and April (orange markers) and August/October (white markers) sediment core locations. Total area impacted by the oyster deployment as determined by fecal pellet distribution in 2019-2020 is shown as the outer bounded area (blue line).

Results of the 2020 sediment regeneration measurements are summarized in Table 4 below. The April 2020 survey was conducted to capture enhanced denitrification stimulated by the overwinter storage of biodeposited nutrients, which accumulated during the 2019 oyster deployment. Gradual decreases in temperature during fall months reduces metabolic activity from heterotrophs and labile organic matter is “preserved” until the following spring. Therefore, sediment cores were collected in April 2020 once water temperatures began to increase. April 2020 cores were collected to complete assessment of the 2019 oyster deployment (Figure 8).

The August 2020 sediment oxygen demand (SOD) and DIN regeneration rates are comparable to rates from cores collected within the biodeposit impact area in July and August of previous project years. With the exception of a small NO_3^- uptake rate in C6, net NO_3^- and NH_4^+ fluxes were outward from the sediments and DIN was released to the overlying water column as is the case for

most estuarine sediments in summer. The effect of the biodeposits is apparent when comparing sediment oxygen uptake rates (SOD) within the biodeposit impact area (enriched) and SOD rates from the background cores (outside of the impact area). The SOD rate (495.5 mMoles/m²/day) in C8 (biodeposits area) was the highest SOD rate measured in Lonnie’s Pond since the start of the project and an extreme value (2.4 standard deviations above average treated SOD rate). The extreme SOD resulted from the presence of an amphipod mat, which have been found to support very high rates due to the number of organisms and their associated bio-irrigation of the sediments. Even excluding C8, SOD rates in biodeposited area were approximately 2 times the background SOD rates. The increased availability of labile organic matter in the biodeposit impact area generally supports greater bacterial activity and greater oxygen demand. In August, ammonium flux out of the sediment was the dominant pathway of nitrogen release. Sediment temperature (set to *in situ* temperature) during the August flux was 24.5°C and within 1°C of 2017 – 2019 July and August incubations. Due to the similar temperatures between years (2017-2020) in August, inter-annual differences in the measured SOD and NH₄⁺ release rates are attributable to differences in biodeposition and/or background POM deposition rates.

On October 11, 2020, 12 cores were collected and incubated at 17.0°C for the same assays. Core locations were nearly the same between August and October and cores can be directly compared. In contrast to 2016 – 2019 October nitrogen release rates, nitrate efflux occurred in all cores except C6, C8, and C12; however, rates were small overall. On average, NO₃⁻ efflux made up only 4% of DIN (ammonium + nitrate) fluxes out of the sediment, typical of organic rich sediments. Furthermore, net NH₄⁺ fluxes were outward from the sediments in all cores regardless of collection location and the average NH₄⁺ flux rate of treated and background cores was comparable to Years 1 - 4. As in previous years, sediment heterogeneity exists among the treated cores, which leads to differences in sediment biogeochemistry; however, obvious patterns of N cycling are clear throughout the biodeposit impact area.

Table 4 - Summary of benthic flux rates from core incubations conducted April 14, August 16, and October 11, 2020. Rows shaded in gray indicate background rates (e.g., core location outside area impacted by oyster deposition). N₂-N detection limits were determined for each analysis date based on the consistency of seawater standards measured after every eighth sample.

Collection Date: April 14, 2020; Incubation Temperature 11.0 °C							
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	19.16	0.85	-0.06	0.79	1.55	2.47	63%
C2	43.57	1.95	-0.21	1.73	1.73	3.89	45%
C3	68.25	0.62	-0.19	0.43	4.45	5.27	85%
C4	38.14	3.37	-0.04	3.33	3.14	6.56	48%
C5	54.39	-0.12	-0.11	-0.23	1.08	1.31	83%
C6	70.47	-0.10	-0.10	-0.21	2.77	2.98	93%
C7	53.41	0.22	-0.10	0.12	2.87	3.19	90%

C8	93.27	0.20	-0.13	0.08	0.66	0.99	67%
C9	37.59	-1.78	-0.86	-2.64	1.42	4.06	35%
C10	81.24	-0.89	-0.43	-1.32	3.68	5.00	74%

Collection Date: August 16, 2020; Incubation Temperature 24.5 °C							
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	183.16	8.77	4.84	13.62	3.45	17.06	20%
C2	96.78	9.28	0.55	9.83	1.24	11.07	11%
C3	166.07	9.31	2.58	11.90	3.34	15.24	22%
C4	188.97	11.25	2.52	13.78	5.16	18.94	27%
C5	105.69	9.69	0.43	10.12	0.56	10.68	5%
C6	165.91	23.62	-0.26	23.36	1.56	25.44	6%
C7	128.54	13.21	0.90	14.10	0.55	14.65	4%
C8	495.50	19.19	0.36	19.55	NS	19.55*	NS
C9	60.79	6.36	0.42	6.78	0.31	7.09	4%
C10	115.94	6.96	4.23	11.19	1.79	12.98	14%
C11	22.24	8.67	1.17	9.84	0.62	10.46	6%
C12	60.80	4.09	1.63	5.72	0.39	6.11	6%

Collection Date: October 11, 2020; Incubation Temperature 17.0 °C							
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	88.64	6.80	0.44	7.24	2.30	9.54	24%
C2	40.98	3.67	0.08	3.75	0.58	4.34	13%
C3	54.14	4.12	0.10	4.22	1.91	6.13	31%
C4	46.50	4.17	0.18	4.35	1.58	5.93	27%
C5	20.33	1.40	0.02	1.42	0.87	2.29	38%
C6	75.24	4.96	-0.02	4.94	3.13	8.12	39%
C7	61.85	4.74	0.20	4.94	1.13	6.07	19%
C8	60.69	0.45	-0.02	0.43	0.48	0.94	50%
C9	38.74	1.32	0.09	1.40	0.11	1.51	7%
C10	67.14	1.63	0.04	1.67	1.04	2.71	38%

C11	57.63	5.89	0.22	6.11	0.77	6.89	11%
C12	57.02	0.87	-0.01	0.86	1.14	2.02	56%

*Bubbles were observed in the 8/16/20 C8 N₂-N samples and could not be run. The corresponding Total N Cycled rate does not include contributions from N₂ production.

A conservative estimate of the total N removed by oyster enhanced denitrification can be made using our measurements of background denitrification rates (outside biodeposit impact area) with the rates within the biodeposit impact area. The background rates may be a slight overestimate as advection and dispersion of the biodeposits by water currents as they sink were the only processes examined and the particles may have spread over a wider area due to storm resuspension. The 2020 background rates and rates measured under the oyster treatment are shown in Table 5 alongside the 2016-2019 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 biodeposit 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 2020 flux were appended to the “Year 4 Mean Denitrification Rates” section in Table 5. The 2020 spring flux represents the end of the Year 4 oyster enhanced denitrification, as the enhanced denitrification rates in spring prior to the 2020 oyster deployment were the result of biodeposits from the 2019 oyster deployment. The April 2020 “Oyster Effect” (1.3 mMoles/m²/d; Table 5) is 0.6 and 0.4 mMoles/m²/d less than the Oyster Effect measured in April 2017 and 2019, respectively. All of the spring surveys underscore the need to tracking denitrification into the following spring in order to capture the full level of denitrification enhancement. The October 2020 oyster enhanced denitrification was more than double the enhanced denitrification in October 2018 and 2019, and was nearly the same as the Oyster Effect measured in 2017. Water column NO₃⁻ availability, sediment redox conditions, and the presence/absence of bioturbators are factors controlling October enhanced denitrification and interannual differences.

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

Date	Year 1 Mean Denitrification Rates (mMoles/m ² /d)				Oyster Effect
	Treated		Background		
	Mean	Std. Dev.	Mean	Std. Dev.	
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
Date	Year 2 Mean Denitrification Rates (mMoles/m ² /d)				Oyster Effect
	Treated		Background		
	Mean	Std. Dev.	Mean	Std. Dev.	
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
Date	Year 3 Mean Denitrification Rates (mMoles/m ² /d)				Oyster Effect
	Treated		Background		
	Mean	Std. Dev.	Mean	Std. Dev.	
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
Date	Year 4 Mean Denitrification Rates (mMoles/m ² /d)				Oyster Effect
	Treated		Background		
	Mean	Std. Dev.	Mean	Std. Dev.	
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
4/14/20	2.4	1.2	1.0	0.5	1.3
Date	Year 5 Mean Denitrification Rates (mMoles/m ² /d)				Oyster Effect
	Treated		Background		
	Mean	Std. Dev.	Mean	Std. Dev.	
8/16/20	2.3	1.7	0.8	0.7	1.5
10/11/20	1.5	0.9	0.8	0.5	0.7
4/XX/21	TBD	TBD	TBD	TBD	TBD

A summary of the measured N removal from Lonnie's Pond via oyster harvest and enhanced denitrification during the 5 years of study is found in Table 6. In all years and seasons, sediments receiving oyster biodeposits show higher rates of denitrification than background sites. The mass of N removed from the system through enhanced denitrification can be calculated by multiplying this enhanced N removal rate by the biodeposit impact area. Weighting of rates obtained during different parts of the season allowed the determination of annual nitrogen removal by denitrification (DeN₂). Enhanced Annual DeN₂ calculated for Year 5 (2020) resulted in a net loss of 9.06 kg N. However, spring carryover related denitrification from the 2020 oyster deployment has not yet been measured; therefore, Year 5 net N loss is an underestimate and cannot be directly compared with the previous four project years. Nevertheless, Year 5 net N loss is nearly double the net N loss determined for Year 4 and is 5% and 7% less than Year 1 and Year 2 losses. It is almost certain that this apparently higher denitrification loss is due to the higher amounts of filtration and biodeposition in 2020 due to the sustained oyster biomass over a longer portion of the growing season. Furthermore, net N removed by oyster harvest in the overall deployment area is approximately 7 kg greater than in Year 1 (previously the highest N loss through oyster harvest in Lonnie's Pond). As a result, total net N losses from denitrification and oyster harvest in Year 5 will be the highest measured over the 5 project years. Lastly, Year 5 spring enhanced denitrification will add to the 55.6 kg N removed from the South deployment area in 2020. Assuming Year 5 spring enhancements are consistent with that of Year 4 (1.3 mMoles/m²/d), an additional 3 kg N could be removed through denitrification before the close of Year 5, increasing enhanced DeN₂ as percent of N removed by oysters from 19.5% to 26.5%.

Table 6. Annual Nitrogen Removal Budget for the oyster impact area showing contributions from enhanced denitrification and oyster harvest. Note that the values are for the southern deployment area only and that spring carryover denitrification is not yet included in Year 5 (2020) data. Year 5 (2020) enhanced annual denitrification rate will be updated with spring carryover effects when the data is available and will almost certainly result in the greatest N removal by oyster aquaculture during the combined 5-year project period.

Year	Year 1 (2016)	Year 2 (2017)	Year 3 (2018)	Year 4 (2019)	Year 5 (2020)
Deployment Duration (days)	146	195	240	155	279
Enhanced Annual DeN₂ (mmol/m ² N)	298	253.3	269.0	155.9***	223.7****
Enhanced Annual DeN₂ (g/m ² N)	4.17	3.55	3.77	2.18***	3.13****
Impact area (m ²)	2287	2735	3250	2890	2890
Total Annual Enhanced DeN₂ (g N)	9537	9709	12247	6309	9056
Total Annual Enhanced DeN₂ (kg N)	9.54	9.71	12.25	6.31***	9.06****
Net Annual N removed by oysters⁹ (kg N)	39.1	27.2	36.2 [57.3]*	30.8 [61.6]*	46.6 [93.1]*
Enhanced DeN₂ as percent of N removed by oysters	24.4%	35.7%	33.8%**	20.5%**	19.5%**

* Includes the northeast deployment area that did not have denitrification measurements.

** Based on denitrification and harvest data from South 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.

**** April rates are not included. April 2021 rates will be added once they become available.

⁹ 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 Lonnie's 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

Based upon refinements stemming from the findings of the 2019 Lonnie's Pond Annual Report and agreed upon by the Town and the Aquaculture and Monitoring Contractors, deployment of year two (YR2) oysters into Lonnie's Pond began on March 12th, 2020. These oysters were placed in floating arrays within the same footprint used in 2019 to grow and increase their N biomass.

In order to monitor growth of the YR2 oysters, they were sorted before deployment and classified by size into three size categories (by inches): YR2-1 inch, YR2-2 inch, and YR2-3 inch. A subsample for N analysis from of each size class was collected to determine the N mass in the deployed oysters. In addition, 17 grow-out bags were filled with individual sizes classes (51 bags total) to monitor growth. As each grow-out bag was recovered, subsamples were collected and measured for weight of live and dead oysters per bag, count of live and dead oysters per bag, and the empty bag weight. Because the deployment was earlier in the year, the 2020 growing season was projected to have an additional 115 days for growth.¹⁰ Overall, 230,343 YR2 oysters were deployed in March 2020 in 2,040 bags, with a cumulative oyster weight of 3,747 kg (Table 2).

Beginning in July 2020, 1,309 bags of the total 2,040 YR2 oysters were gradually replaced by YR1 oysters (Tables 2 and 5). The YR2 oysters began to be removed on July 9, with weekly removals continuing to August 27, 2020. (Tables 3, 4). As YR2 oysters were removed from the deployed bags, new YR1 seed oysters were placed in the now empty bags and redeployed to sustain the overall oyster population and continue removal of N by oyster aquaculture (Table 5).

During September, the remaining 731 YR2 oyster bags were removed. The Aquaculture Contractor sorted the oysters by size in a facility in Wareham, MA and removed oysters larger than 76.2 mm (3 inches) for market sale. Smaller YR2 oysters were redeployed to Lonnie's Pond until November (Tables 8 and 9). The total oyster weight redeployed and removed, including dead removal, were recorded at the Wareham facility by CSP/SMASST (Table 6). Comparison of oyster weights at the Wareham facility and the Orleans Transfer Station Scale were within 0.5% for all September removal and sort days.¹¹ This indicates again that the truck scale is an accurate way to determine total mass of oysters removed, when appropriate corrections for the weight of the empty grow out bags are included. During November and December, all oysters remaining in Lonnie's Pond were removed (*i.e.*, the new YR1 seed deployed in July and August and redeployed YR2s) (Table 7).

For all oyster groups deployed, oyster subsamples were collected for % N content (whole oyster, shell, tissue, and fouling biota). These fractions allowed determination of N removed in each fraction and in the whole oysters. The N data when coupled with measured wet to dry weight ratios for oysters from each collection and deployment date was used to determine the total N from the harvest and deployment wet weight measurements (truck scale and totes). Chemical analysis followed procedures specified in the QAPP to determine the initial N mass of the deployed oysters and cumulative mass at removal (Tables 8-9 and Figure 2). Measuring the shell %N and fouling material (amphipods, sediment deposition, algae, etc.) collected on the shell, has the added benefit

¹⁰ Because 2019 deployment started later in the year (July), a total of only 145 days was available for growth.

¹¹ The Orleans Transfer Station scale was used for weighing oyster in 2019.

of being able to also include N removal in dead oysters or shell hash separately from the live oysters.

All data collection followed the protocols in the approved QAPP and guidelines, including modifications discussed prior to the 2020 growing season. CSP/SMASST staff tracked all removals, redeployments, and new deployments throughout 2020 growing season. Tables 4, 6 and 7 summarize key tracking results during the 2020 growing season. Table 10 summarizes the total wet weight inputs and outputs of oyster by month throughout 2020.

Table 2. Total wet weight and counts from the initial oyster deployment of YR2 in March and YR1 oysters in July and August. Oyster subsamples were collected from each size category for percent nitrogen determination. All values (weights, numbers, %N) relate to live oysters.

Date	Size Class	Category	Bags Deployed	Total Wet	Live Oysters	N Content	Total N
	(Year)	ID	#	Weight (kg)		Whole Oyster %	Input (kg)
3/11/2020	YR2	1 in (25.4 mm)	17	7.1	1746	0.25	0.02
3/11/2020	YR2	2 in (50.8 mm)	1677	2775.0	192424	0.32	9.01
3/11/2020	YR2	3 in (76.2 mm)	346	965.0	36173	0.34	3.33
7/9-7/28/2020	YR1	A	289	90.6	226672	0.37	0.34
8/12/2020	YR1	B	340	135.2	205190	0.49	0.66
8/20/2020	YR1	C	340	135.1	223380	0.50	0.68
8/25/2020	YR1	D	340	165.9	220745	0.44	0.73
Total:			3349	4274	1106330		14.8

Table 3. Subsampled bags classified by size classes 1 inch, 2 inch, and 3 inch now YR2 oysters upon initial deployment in March 2020 (A). On August 13 through August 27, 2020 a subset was measured by size class with growth measurements of each size class. An additional set of random bags was subsampled to improve wet weight estimates for the remaining 731 bags (B). Recovered YR2 oysters deployed in March. This data was used to compare growth from March through August, but does not represent the entirety of the July-September removals.

A

Date	Size Class	Category	Deployed	Bag	# Live	Weight Live		# Dead	Weight Dead			
	(Year)	ID	Bag #	Sample n=	Oyster/Bag	Oyster/Bag (kg)		Oyster/Bag	Oyster/Bag (kg)			
				#	Average	S.E.	Average	S.E.	Average	S.E.		
3/11/2020	YR2	1 in (25.4 mm)	17	17	120	0.0	0.53	0.0	7.80	0.9	0.02	0.0
3/18/2020	YR2	2 in (50.8 mm)	1677	10	120	0.0	1.75	0.0	3.70	0.8	0.02	0.0
3/18/2020	YR2	3 in (76.2 mm)	346	10	120	0.0	3.36	0.0	2.60	0.7	0.02	0.0

B

Date	Size Class	Category	Relayed	Bag	# Live	Weight Live		# Dead	Weight Dead		% Mortality			
	(Year)	ID	Bag #	Sample n=	Oyster/Bag	Oyster/Bag (kg)		Oyster/Bag	Oyster/Bag (kg)					
				#	Average	S.E.	Average	S.E.	Average	S.E.	Average	S.E.		
8/13/2020	YR2	1 in (25.4 mm)	11	15	59	3.3	2.33	0.1	52	3.0	0.24	0.0	46.72	2.6
8/21/2020	YR2	2 in (50.8 mm)	1076	16	103	2.6	6.68	0.3	22	3.4	0.34	0.1	17.59	2.5
8/25/2020	YR2	3 in (76.2 mm)	222	17	115	1.5	10.04	0.2	6	0.6	0.14	0.0	4.96	0.5
8/27/2020	YR2	Random	N/A	3	77	11.2	5.10	0.1	24	5.5	0.37	0.0	23.56	3.9

Table 4. July-August 2020 removal dates and the confirmed weight (kg) of YR2 removed oysters. Removal amounts to 64% of the total YR2 population in Lonnie’s Pond and was replaced with YR1 oysters. Weights at initial deployment of YR1 stock and YR2 removal are shown. YR2 oysters removed from Lonnie’s Pond in July were relayed to market by aquaculture contractor (approx. 29,574 individual oysters). YR2 stock in August was relayed to an approved Pleasant Bay site (approx. 107,263 individual oysters).

Date	YR2 Removal	YR1 Deploy	Bags Relayed
	(kg)	(kg)	#
7/9/2020	290.30	23.29	68
7/16/2020	371.95	23.29	68
7/28/2020	861.82	43.99	153
8/12/2020	1433.35	74.78	170
8/13/2020	1206.55	60.40	170
8/20/2020	1288.20	70.50	170
8/21/2020	1651.07	64.60	170
8/25/2020	1342.63	96.05	170
8/27/2020	1124.91	69.85	170
Total:	9570.79	526.74	1309

Table 5. July and August deployment of YR1 seed was measured for estimating initial total nitrogen through oyster count per bag and cumulative wet weight at deployment to the Pond.

Date	Length per Oyster (mm)			Wet Weight per Bag (kg)			Count per Bag (#)			Total Bags	Estimated	Tot. Wet
	Average	S.E.	n=	Average	S.E.	n=	Average	S.E.	n=	#	Total Count	Weight (kg)
July	16.64	0.24	90	0.29	0.03	4	759.25	39.47	4	289	226672	90.6
Week 8/12/2020	18.14	0.30	80	0.36	0.01	20	610.25	11.53	4	340	205190	135.2
Week 8/20/2020	20.12	0.29	50	0.44	0.01	3	644.25	3.21	3	340	223380	135.1
Week 8/25/2020	18.09	0.58	55	0.32	0.03	16	616.17	151.48	6	340	220745	165.9

Table 6. September 2020 removal dates and confirmed weight (kg) of YR2 oysters. The remaining 36% of the total YR2 population was sorted at the Wareham facility for market size oysters, and undersized oysters redeployed back to Lonnie’s Pond on the listed dates in September. Weights of YR2 oysters removed for sale and weight of YR2 oysters redeployed to the pond are shown.

Date	Size Class	YR2 Removal	YR2 Re-deployed	Bags Relayed
	(Year)	(kg)	(kg)	#
9/2/2020	YR2	226.00	122.12	51
9/8/2020	YR2	376.44	175.15	85
9/9/2020	YR2	393.81	191.03	85
9/15/2020	YR2	474.92	226.26	85
9/15/2020	YR2	380.75	213.99	85
9/16/2020	YR2	485.56	169.62	85
9/22/2020	YR2	668.10	404.13	170
9/29/2020	YR2	466.17	180.87	85
Total:		3471.75	1683.17	731

Table 7. November (A) and December (B) removal dates and the confirmed weight (kg) of YR2 and YR1 oysters. November included the removal of the remaining YR2 oysters categorized as undersized during sorting in September. December dates included the removal of all YR1 oysters placed in Lonnie's Pond over July and August 2020.

A

Date	Size Class	Category	Total Removal (kg)	Bags
	(Year)	ID		Removed (#)
11/9/2020	YR2	Cull (< 76.2 mm)	313.99	85
11/10/2020	YR2	Cull (< 76.2 mm)	259.56	102
11/12/2020	YR2	Cull (< 76.2 mm)	315.63	170
11/16/2020	YR2	Cull (< 76.2 mm)	304.92	102
11/17/2020	YR2	Cull (< 76.2 mm)	259.56	85
11/18/2020	YR2	Cull (< 76.2 mm)	312.04	85
11/19/2020	YR2	Cull (< 76.2 mm)	266.68	102
Total:			2032.37	731

B

Date	Size Class	Category	Total Removal (kg)	Bags
	(Year)	ID		Removed (#)
12/14/2020	YR1	A-D	3625.64	705
12/16/2020	YR1	A-D	4352.30	595
Total:			7977.94	1300

Table 8. Cumulative measurements of oysters removed from Lonnie's Pond by month. This allowed for truck scale weights and oyster counts to be estimated per bag. The percent difference between the truck scale weight and estimated wet weight through subsamples of oyster bags removed from Lonnie's Pond over each month is shown.

Date	Length per Oyster (mm)			Wet Weight per Bag (kg)			Count per Bag (#)			Mortality %	Total Bags #	Estimated Oyst. Count #	Truck Wet Weight (kg)	Estimated Wet Wt. % Diff
	Average	S.E.	n=	Average	S.E.	n=	Average	S.E.	n=					
July	ND	ND	ND	ND	ND	ND	ND	ND	ND	12.8	289	29574	1524	ND
August	86.34	1.61	25	6.78	0.25	19	98.74	3.44	19	18.5	1020	107264	8047	4.7
September	83.12	0.59	180	ND	ND	ND	ND	ND	ND	19.0	731	65790	3492	0.6
November	91.08	0.96	85	2.37	0.17	20	29.80	2.10	20	35.1	731	23192	2032	9.3
December	49.55	0.84	123	6.01	0.64	20	540.80	50.20	20	15.3	1309	707907	7978	1.4

Table 9. Nitrogen removal measurements based on the percent nitrogen content of whole oysters in accordance with size class and category ID. The total wet weight removed is found using confirmed truck scale weights (kg). Given the oyster removal methods and collection of weight data, YR2 oysters collected in July and August were grouped together to extrapolate live oyster counts. The same action was taken in December with YR1 oysters, which were collectively weighed. These values need to be adjusted for the nitrogen mass in each grouping at deployment to determine the net mass of N removed from Lonnie’s Pond.

Date	Size Class	Category	Bags Removed	Total Wet Weight	Live Oysters	N Content	Total N
	(Year)	ID	#	(kg)	#	%	(kg)
July	YR2	1 in (25.4 mm)	11	9570.8	136837	0.522	43.41
&	YR2	2 in (50.8 mm)	1076			0.386	
August	YR2	3 in (76.2 mm)	222			0.426	
September	YR2	Cull (> 76.2 mm)	731	3491.8	65790	0.498	17.40
November	YR2	Cull (< 76.2 mm)	731	2032.4	23192	0.499	10.15
December	YR1	A	289	7977.9	707907	ND	36.88
December	YR1	B	340			0.433	
December	YR1	C	340			0.391	
December	YR1	D	340			0.492	
Total			4080	23073	933727		107.83

Table 10. Monthly summary of total oyster wet weight deployed (input) and removed (output) from Lonnie’s Pond during 2020 season.

	INPUT (kg)	OUTPUT (kg)
MARCH	3747	0
JULY	91	1524
AUGUST	436	8047
OCTOBER	0	0
SEPTEMBER	1683	3472
NOVEMBER	0	2032
DECEMBER	0	7978
SUM (kg)	5957	23053
NET (kg)	17096	

Table 11. Days of deployment in Lonnie’s Pond of either YR1 or YR2 oysters and the net mass of nitrogen removed (harvest minus initial N mass) over the 2020 field season. Overall, the average net removal of nitrogen is determined using an average nitrogen content per whole oyster at harvest determined from monthly samples. Total net N removed by aquaculture harvest in 2020 was 93.1 kg N.

Days Deployed	Size Class (Year)	Net Removal Nitrogen (kg)
139	YR2	58.6
to		
253		
114	YR1	34.46
to		
161		
Total Removal (kg)	93.1	

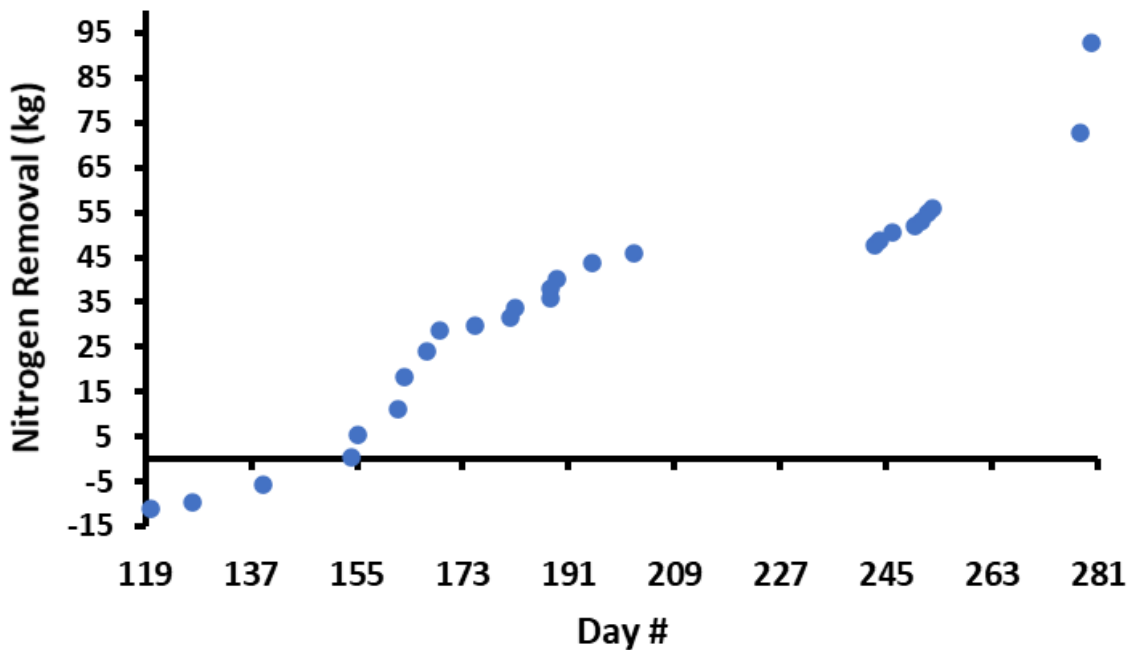


Figure 2. Cumulative 2020 Lonnie’s Pond Nitrogen Removal. N removal (kg) was determined using the total N mass of N removed on each oyster removal date. Total removal is accumulated over 160 days and 26 events. After calculating the N input (shown as a negative) from oysters deployed in March (12.36 kg), total net N removal was 93 kg. Also, the N mass in the deployment of YR1 seed from July 9 to August 27 was accounted for in these data.

4.0 Key Findings and Future Considerations

Overall, the 2020 oyster deployment exceeded the initial goals outlined in the Lonnie's Pond Aquaculture and Nitrogen Management Plan of 75 kg N removed in oyster harvest per year. CSP/SMASST staff working with Ward Aquafarm and Town staff were able to document 93.1 kg N net removal, in the 2020 oyster harvest (Table 11). We also documented ways to make future deployment and monitoring more efficient. Considerations for future deployment include:

- 1) In 2020 the Orleans oyster aquaculture program removed a total net N mass from Lonnie's Pond by oyster harvest of 93.1 kg N (recovery – deployment). The removal was equally divided between the southern and northern deployment areas. N removal by oyster enhanced denitrification within sediments receiving biodeposits was 9.06 kg N from the southern deployment area only and not including the April 2021 removals (not yet measured). *Therefore, total nitrogen removal from Lonnie's Pond associated with the 2020 deployment was at least 102.2 kg N and as high as 111 kg N if the removal of nitrogen through denitrification was the at both deployment sites.*
- 2) For comparison purposes, 111 kg N/yr removal is equal to the full wastewater N discharge reaching the estuary from 21.2 houses. At a cost of \$30,000 cost per dwelling, this has an installation (excluding operation and maintenance) cost of ~\$640,000. The costs are constantly being refined, so these calculations are only approximate.
- 3) In a complex logistical environment with complex monitoring needs, communication between Aquaculture Contractor and Monitoring Contractor on the deployment and removal processes of oyster stock needs to be as rigorous and reliable as possible. Moving and redeploying different age oysters over extended periods creates uncertainties in nitrogen removal. Procedures for removal of YR2 and reincorporation of YR1 stock changed over the season as 1,309 of 2,040 YR2 bags were replaced with YR1 oyster seed. The remaining 731 YR2 bags were culled and undersized YR2 oysters were returned and remained in the water until November. Some uncertainties occurred during summer YR2 re-deployment and cull methods in September when wet weight and counts per bag showed the highest variation.
- 4) Use of only the Orleans Transfer Station Scale “truck scale” for harvest wet weight determinations is desirable. Though manageable, transport to more distant scales increase variation in factors necessary for determining N removal, such as changes in truck weights full of oysters and empty tare weights due to potential changing empty bag weights and need to determine weight loss from fuel usage.
- 5) Crop rotation of YR2 oysters for YR1 seed is important during a time period of critical growth for both N removal from the system and the grower. With over 99% of bags containing YR2 oysters at a size over 50.8 mm (2 inches) in March, oysters will become too large for ideal market value. However, long distance transportation for culling and reincorporation of only undersized YR2 oysters is a laborious and time-consuming effort amounting to only 10.9% of the total N removal. In addition, estimates on the 23,192 live YR2 oysters show minimal gains in overall growth (+ 7.9 mm and + 14.2 g per individual oysters) and highest mortality by removal date (Table 8). The exception is when we find in Table 3 that the smallest sized YR2 oysters also have highest mortality, which is expected of an r-strategist species. Complete

extractions of oyster size classes would retain more reliable input/output data sets while also keeping mortality to a minimum.

- 6) As a new approach to optimize monitoring, both CSP/SMAST and the Ward Aquafarm used zip ties to identify bags designated for only Lonnie's Pond and sealing the opening to the pillow bag to reduce oyster loss. Upon removal from Lonnie's Pond, both sets of zip ties are removed from the bag and replaced with a new zip tie prior to redeployment. To reduce waste of plastic in future processing monitoring of the aquaculture farm, the zip tie sealing the bag should also include an identification tag. Through tag collection, CSP/SMAST accounted for 97% of all oyster bags deployed and removed by Ward Aquafarm. This procedure change would require both collaborative parties to focus on a single objective when relaying oysters, improving confirmation in bag collection and identification.
- 7) Future monitoring of water quality should focus on fewer sites since having 2 deployment areas is impacting a larger area of the pond. Instead, water quality monitoring should increase around the oyster arrays and be coupled with flow velocity and direction measures by ADCP. This should be done by a reallocation of existing effort, with no additional funds required.
- 8) Continue to specify coordination between the Town, aquaculture contractor and monitoring contractor. The procedures developed during 2019/20 worked very well and need to be codified and followed in future years. Written protocols will save both contractors time and streamline the effort.