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 2021 Annual N Removal/Harvest Report

February 26, 2022

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 Bay[2], 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 floating 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 achieving the TMDL without sewering within the Pond watershed. Monitoring during the demonstration project found significant removal of N and some water quality improvements due to shellfish growth and biodeposition.[3]

In 2018, the Town approved the Lonnie's Pond Aquaculture and Nitrogen Management Plan[4] to sustain the aquaculture N removal program by transitioning from a Town oyster aquaculture demonstration project to long-term commercial oyster aquaculture 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.

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

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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.

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 and through sediment processes. 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 a 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 beginning in 2020 that deployed 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. The program changes were successfully applied in 2020 and resulted in the removal (via denitrification and oyster harvest) of 108.6 kg N or an additional 33.6 kg N above the selected goal of 75 kg/yr. As a result, the program changes were extended to the 2021 project year.

The 2019 and 2020 Annual Reports were delivered to the Town in two parts, whereas the 2021 Annual Report is presented as a single document that contains the former Part 1 and 2 contents. The 2019 and 2020 Part 1 focused on N removal through oyster deployment, growth and N assimilation into biomass, and subsequent harvest; and Part 2 focused on the results of water quality monitoring, sediment nutrient fluxes, and N removal through sediment denitrification. The 2021 Annual Report includes the N removal attained during the 2021 season and initial N mass at deployment and at removal of YR2 oysters and subsequent replacement on harvest by new YR1 seed oysters throughout July and the N removed after these YR1 seed had grown and were removed in December 2021. YR2 oysters were deployed in March and a fraction (75 %) were removed and replaced over an extended period from June 1st through July 29th 2021 by the Aquaculture Contractor. Starting in July, as oysters were removed, oyster seed was put into the emptied bags and redeployed. All oysters were removed in December 2021. The CSP/SMAST team tracked all the oyster additions and removals, as well as water quality and sediment nutrient regeneration/denitrification throughout this period.

2.0 Water Quality Monitoring: Overview.

For the 2021 field season, CSP/SMAST staff began conducting biweekly water quality monitoring in Lonnie's Pond starting on March 16, 2021, using procedures detailed in the Lonnie's Pond QAPP. To date there have been thirteen (13) sampling events: March 16, April 20, May 19, June 2, June 17, June 30, July 14, July 28, August 17, August 31, September 13, September 28, and October 12 (Table 1.1). During each sampling event, water quality samples were collected at nine locations in Lonnie's Pond (Figure 1.1). In addition, two continuous monitoring instruments (sondes) were deployed April 20 0.30 m above the sediment surface directly east and west of southernmost oyster deployment area. The sondes support sensors that autonomously record chlorophyll-*a*, dissolved oxygen, salinity, temperature, and depth at 15-minute intervals (located at stations LP-5 and LP-6; Figure 1). On each monitoring event, water clarity (Secchi depth), temperature and dissolved oxygen profiles were collected with water samples. The water samples were transported to the CSP Analytical Facility and processed for nitrogen (nitrate+nitrite, ammonium, dissolved and particulate organic nitrogen), ortho-phosphate, particulate organic carbon, 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 cranberry bog outlet downstream of Crystal Lake (see Figure 1.1). A continuous stage meter was deployed in the stream from Pilgrim Lake to provide data for determining daily volumetric freshwater inflow. Flow from the bog outlet was sporadic and only instantaneous readings were made during each biweekly stream sampling event.

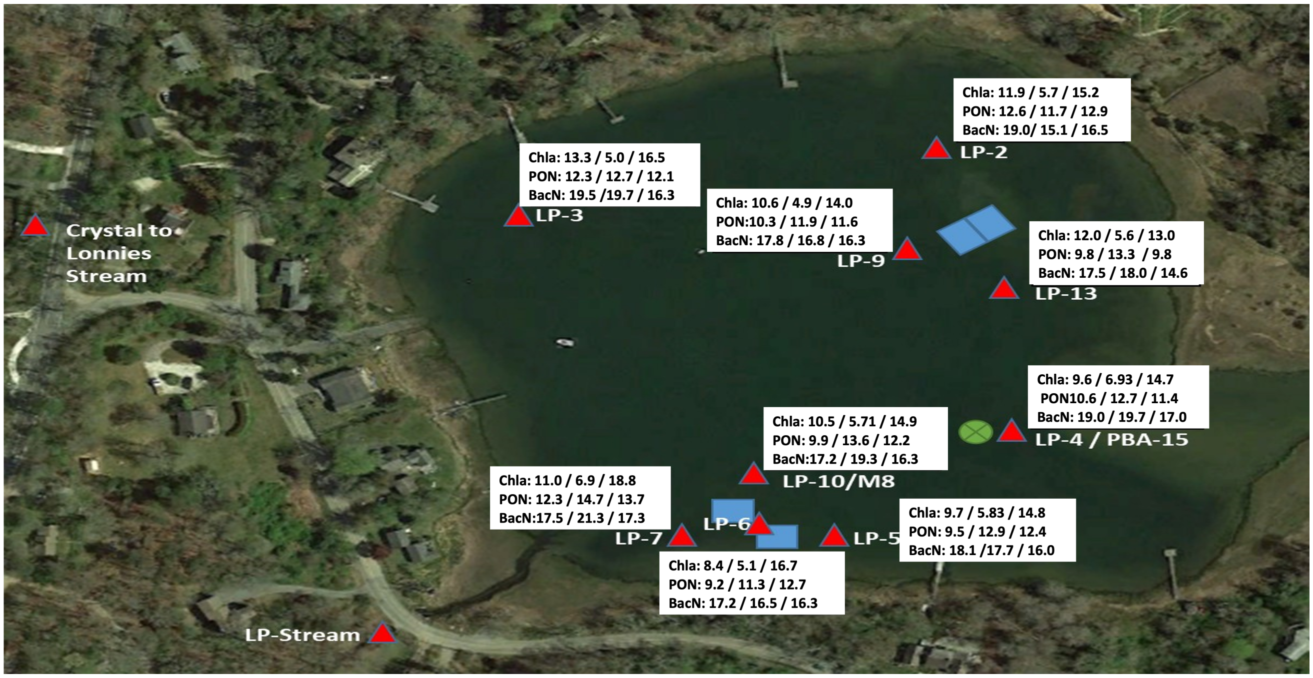

Figure 2.1 Map of Lonnie's Pond 2021 water quality sampling locations. The red triangles were sampled biweekly by CSP staff April 20 to October 12, 2021; green circle is sampled biweekly by Orleans citizen volunteers as part of the Pleasant Bay Alliance (PBA) monitoring program from July 15-September 15, 2021, and blue squares represent the oyster deployment areas. White boxes show mixed layer average concentrations of total-chlorophyll-*a* (µg L^{-1}), particulate organic N (µM), and bioactive nitrogen[5] (µM). Values represent averages of samples collected July-October for 2021, 2020, and 2019 (shown 2021/2020/2019), the active oyster growth period in those years. Water tends to flow from LP-3 to LP-7 to LP-5.

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

Table 2.1 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**
3-16-21	21	X	X	X	X	X	X	X	X
4-20-21	21	X	X	X	X	X	X	X	X
5-19-21	21	X	X	X	X	X	X	X	X
6-2-21	21	X	X	X	X	X	X	X	X
6-17-21	21	X	X	X	X	X	X	X	X
6-30-21	21	X	X	X	X	X	X	X	X
7-14-21	21	X	X	X	X	X	X	X	X
7-28-21	21	X	X	X	X	X	X	X	X
8-17-21	21	X	X	X	X	X	X	X	X
8-31-21	21	X	X	X	X	X	X	X	X
9-13-21	21	X	X	X	X	X	X	X	X
9-28-21	21	X	X	X	X	X	X	X	X
10-12-21	21	X	X	X	X	X	X	X	X
Total	**273**								

Water quality monitoring indicated that Lonnie's Pond in 2021 was generally horizontally mixed throughout where all stations follow the same trend although there was evidence of a spring bloom represented by a spike in chlorophyll-*a* concentration only at LP3 on 4/20/21 (12 ug/L) that the other stations did not experience (Figure 2.2). Interestingly, LP3 dropped to the lowest chlorophyll concentrations in the next spring sampling events and represented the highest of all the stations chlorophyll concentrations on 6/30/21 at 53 µg/L and 8/11/21(30 µg/L). All stations highest chlorophyll concentrations were recorded on 6/30/21 representing a pond-wide summer phytoplankton bloom (Figure 2.2). LP3 is not near either the northern or southern aquaculture areas and could be seen as what Lonnie's Pond chlorophyll-*a* concentrations might be in the without the oyster deployments (Figure 2.1). In addition, stations surrounding the northern aquaculture area (LP2, 9, 13) represented the lowest chlorophyll level of 24 µg/L during the 6/30/21 bloom and the southern oyster area the next lowest with 28 µg/L chlorophyll-*a*, which appears to be related to the filtering capacity of each oyster array (Figure 2.2).

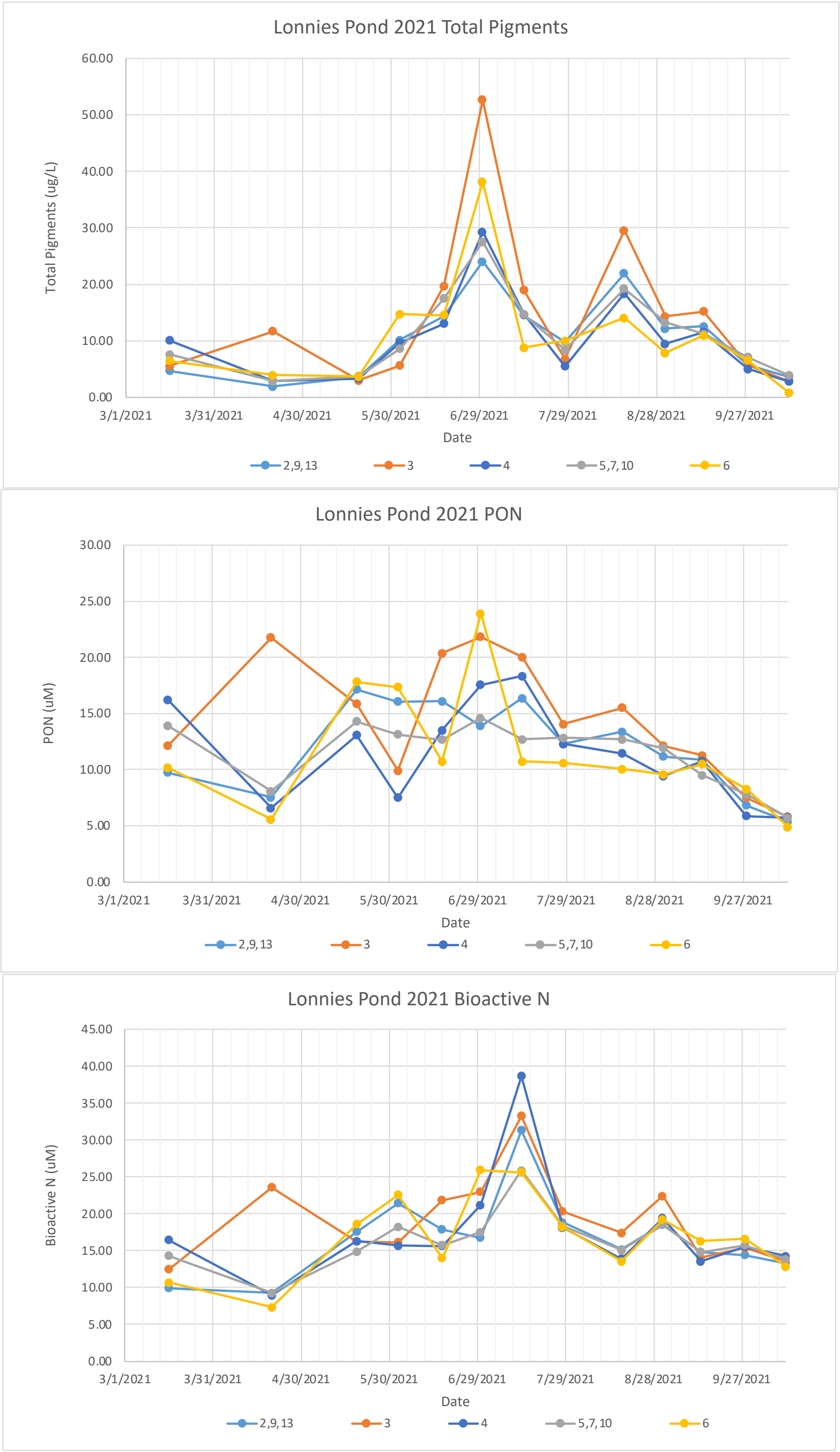

Figure 2.2 Time-series of mixed layer average total pigments, particulate organic nitrogen (PON), and bioactive N at stations LP3, LP4, LP6 and averages of the stations (LP2, 9, 13) and (LP5, 7, 10) associated with the North and South oyster deployment. Station numbers refer to locations in station map in Figure 2.1. The general trend was a lowering of particulate matter (PON, Chl-A) as water passed through the oyster arrays.

These trends and spikes in chlorophyll-*a* concentration were loosely mirrored in the PON data although the variability in PON concentrations between stations was more pronounced. LP3 had a spike in particulate organic nitrogen (PON) concentration on 4/20/21 (22 µM) while the other stations decreased from the earlier spring sampling events (Figure 2.2). It was LP6 though (instead of LP3) that had the highest POC and PON levels on 6/30/21, but not all stations had their highest recorded PON concentration on 6/30/21 unlike the chlorophyll record showed. LP6 is located within the southern oyster aquaculture area where particulate organic carbon and nitrogen is likely not reflective of general water column conditions but is elevated by biodeposits and can be affected when the oyster bags are disturbed.

Bioactive nitrogen is the combined pools of dissolved inorganic nitrogen (DIN) and particulate organic nitrogen (PON), and represents the most biologically labile or usable form of nitrogen. DIN is the sum of inorganic N components (nitrate (NOx) and ammonium (NH_4)) concentrations, and it was NH_4 that drove the bioactive nitrogen to have its highest spike on 7/14/21 opposed to 6/30/21 like the chlorophyll-*a* and PON spikes (Figure 2.2). Again, there was a lag in bioactive nitrogen where the station-wide spike occurred on 8/31/21 opposed to elevated chlorophyll and, to a lesser extent, PON on 8/17/21. LP3 still had its spike on 4/20/21 in bioactive nitrogen following the pattern in chlorophyll and PON.

During the non-bloom periods there was little variability in in chlorophyll and bioactive nitrogen concentrations across the pond on any given sampling day. However, nutrient concentrations did change over the period of oyster deployment, with the lowest concentrations in spring and fall and the highest during the warm summer (Figure 2.2). 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, yet despite an increase of 20,042 m^3 of water during April-June 2021 (April-June 2021 flow = 66,981 m^3) and an extra 23 kg N load (April-June 2021 N load 55 kg) from Pilgrim Lake compared to 2020 (46,939 m^3 and 32 kg N/3-months (April-June 2020)), nutrient concentrations were not elevated at LP7 the station closest to the stream (Figure 2.1). This is consistent with other observations of the generally well horizontally mixed nature of Lonnie's Pond.

Chlorophyll-*a* concentrations were lower at all sites in 2021 compared to 2020 and 8 out of the 9 sites had lower PON in 2021 compared to 2020 (Figure 2.1). Interestingly, bioactive nitrogen was lower in 2021 versus 2020 in the sites along the path of the stream input out to the channel (LP7, 10, 4, 13; Figure 2.1).

During the 2021 field season there appeared to be a summer and fall phytoplankton bloom as seen in the chlorophyll-*a* and PON records, particularly at station LP3, (Figure 2.2). It also appears that phytoplankton and PON levels declined by September and remained low through October. This follows the seasonal light and temperature cycle in Lonnie's Pond. Bioactive nitrogen was about 10 µM (0.14 mg N/L) in March when the oysters were deployed into Lonnie's and indicates that oyster growth was likely not limited by food (Figure 2.2). 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. During the 2021 sampling, June-mid July was the only bloom where all stations had greater than 20 µg/L of chlorophyll-*a* (Figure 2.2). This appears to show an improving trend in water quality of the pond, which may or may not be associated with the multi-year N removals by oyster aquaculture.

Water Quality Associated with Oyster Aquaculture

Oysters were deployed into Lonnie's Pond March 11th through March 30, 2021 by Ward Aquafarms staff while oyster weight and size metrics were collected by the Coastal Systems Program-SMAST staff. During the March deployments 2040 floating mesh bags filled with 2,843 kg of Year 2 oysters were placed into the 2 aquaculture arrays in Lonnie's Pond. Most of these bags of Year 2 oysters (75%; 1530 bags out of 2040 bags) were recovered in June and July of 2021, totaling 5159 kg of oysters (see Section 5; Table 5.5). Once emptied, these 1530 bags were refilled with 370 kg of Year 1 oysters starting July 5, 2021. The remaining Year 2 oysters were left in the northern aquaculture area surrounded by water quality stations LP2, 9, 13. In an effort to discover if the larger oysters deployed into Lonnie's Pond during the spring (March- June) filtered out more nutrients then the smaller Year 1 oysters deployed in the summer (July-October), the measured water quality during these 2 seasons was compared (Table 2.2).

Table 2.2 Lonnie's Pond mixed-layer water quality station averages from spring (March-June) and summer (July-October) 2021 for critical nutrient concentrations of chlorophyll-*a* (Total pigments), particulate organic nitrogen (PON), bioactive N, dissolved inorganic nitrogen (DIN), and total nitrogen (TN). From March-June Year 2 oysters were in northern and southern aquaculture areas. From July-October Year 1 oysters represented the majority of oysters in Lonnie's Pond along with the rest of the Year 2 oysters that were not removed during the June/July harvest.

March-June 2021						July-October 2021					
Stat #	Total Pigments µg/L	PON µM	Bioactive Nitrogen µM	DIN µM	TN µM	Stat #	Total Pigments µg/L	PON µM	Bioactive Nitrogen µM	DIN µM	TN µM
2	9.22	14.95	16.62	1.67	37.90	2	11.39	12.05	18.57	6.52	41.96
9	9.76	12.82	15.41	2.59	36.94	9	10.57	10.27	17.75	7.48	38.01
13	10.35	12.43	14.38	1.96	35.48	13	11.95	10.01	17.31	7.62	37.90
3	17.33	16.98	18.97	1.99	40.71	3	13.80	12.74	20.00	7.25	42.11
4	10.74	11.96	15.18	3.22	35.86	4	9.63	10.55	19.05	8.50	39.60
6	12.01	13.12	15.26	2.14	40.55	6	8.04	8.99	17.21	8.22	40.17
5	14.06	14.23	16.04	1.81	38.99	5	8.95	9.40	17.76	8.36	38.06
7	11.43	12.01	14.32	2.30	35.34	7	11.02	10.26	17.48	7.22	39.02
10	10.13	11.90	14.45	2.56	33.82	10	10.52	9.99	17.28	7.29	38.00

The spring of 2021 oyster grow out of the Year 2 oysters represents a larger filtering potential compared to the July through October grow out of mostly smaller Year 1 oysters in the aquaculture areas. Year 2 oysters when deployed averaged 15.1 g, while Year 1 oysters averaged 0.7 g (Table 5.8). Oysters filter surrounding water and assimilate nutrients into their biomass. In December 2021, 173,260 oysters were removed weighing 9804 kg (Table 5.6). Springtime water quality metrics show these larger oysters initially deployed into Lonnie's Pond reduced water column bioactive nitrogen, DIN, and TN significantly throughout Lonnie's Pond compared to the smaller Year 1 oysters that dominated the population from July-October (Table 2.3).

Table 2.3 Seasonal results from paired t-tests ($p < 0.05$) base on mixed-layer station averages sampled from Lonnie's spring (March-June) and July-October 2021 for key N constituents. T-tests were performed with all stations in Lonnie's Pond versus the oyster aquaculture area stations. Significant differences in red are seen in the PON (particulate organic nitrogen), DIN (dissolved inorganic nitrogen), and TN (total nitrogen) concentrations apparently due to pond-wide effects.

a)	**All stations compared**									
	Total Pigments µg/L		**PON µM N**		**Bioactive µM N**		**DIN µM N**		**TN µM N**	
	Mar-June	**July-Oct**	**Mar-June**	**July-Oct**	**Mar-June**	**July-Oct**	**Mar-June**	**July-Oct**	**Mar-June**	**July-Oct**
mean	11.67	10.65	13.38	10.47	15.63	18.05	2.25	7.61	37.29	39.43
variance	5.84	2.56	2.61	1.28	1.93	0.82	0.21	0.37	5.21	2.51
stdev	2.42	1.60	1.62	1.13	1.39	0.91	0.45	0.61	2.28	1.58
n	9	9	9	9	9	9	9	9	9	9
t	1.05		4.42		-4.38		-21.22		-2.31	
d.o.f	16		16		16		16		16	
critical value	2.12		2.12		2.12		2.12		2.12	
[t] > crital value	1.05<2.12		4.42>2.16		4.3>2.12		21.22>2.12		2.31>2.12	
	no significant diff.		**significantly diff.**		**significantly diff.**		**significantly diff.**		**significantly diff.**	

b)	**Only oyster stations compared**									
	Total Pigments µg/L		**PON µM N**		**Bioactive µM N**		**DIN µM N**		**TN µM N**	
	Mar-June	**July-Oct**	**Mar-June**	**July-Oct**	**Mar-June**	**July-Oct**	**Mar-June**	**July-Oct**	**Mar-June**	**July-Oct**
mean	11.20	10.32	13.26	10.16	15.34	17.68	2.08	7.57	37.56	39.12
variance	2.52	1.90	1.04	0.93	0.68	0.20	0.09	0.38	3.62	2.17
stdev	1.59	1.38	1.02	0.96	0.83	0.45	0.31	0.62	1.91	1.47
n	6	6	6	6	6	6	6	6	6	6
t	1.03		5.40		-6.10		-19.52		-1.66	
d.o.f	10		10		10		10		10	
critical value	2.23		2.23		2.23		2.23		2.23	
[t] > crital value	1.03<2.23		5.4>2.23		6.10>2.23		19.52>2.23		1.66<2.23	
	no significant diff.		**significantly diff.**		**significantly diff.**		**significantly diff.**		**no significant diff.**	

In 2020 oyster deployment occurred prior to the earliest planned water quality sampling on March 16th, but 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) ($p < 0.01$). The increase in PON and decrease in DIN with the presence of oysters in 2020 mirrors the same results in 2021 data and may reflect the feeding/excretion by the oysters and increased DIN release from sediments in summer.

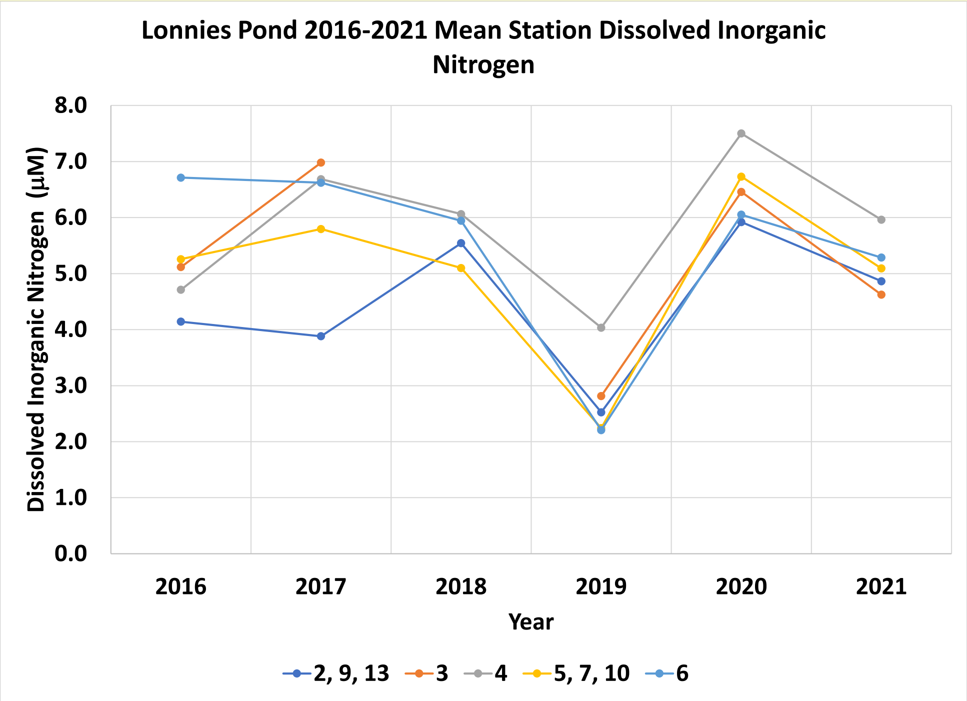

Figure 2.4 2016-2021 Interannual variation in mixed layer average of dissolved inorganic nitrogen (DIN) at stations LP 3, LP 4, LP 6 and averages of the stations (LP 5, 7, 10) and (LP 2, 9, 13) surround the oyster deployments. Station numbers refer to locations in station map in Figure 2.1.

We attempted to refine the oysters' effect on water quality by looking for changes in light extinction coefficient (light penetration) by utilizing Li-Cor light profile data collected on each sampling event at each of the water quality stations. We compared light profiles between stations upgradient and downgradient of the oyster aquaculture areas. The light extinction coefficient, k is calculated by taking the natural log of the light just below the water's surface, subtracted by the light on the bottom of the water column, divided by the depth difference between the two measurements ($k = \ln(I_O)-\ln(I_D)/d$). As the tide ebbs it pulls water out of Lonnie's Pond and out through the channel toward Little Pleasant Bay. Light was able to significantly ($p < 0.05$) penetrate deeper into the water column from station LP7 to LP5 (Figure 2.5). The light extinction coefficient considers the light measurements in relation to the water depth. The light extinction coefficient is a measure of light attenuation, or the amount of light absorbed within the water column. This means that a light extinction coefficient closer to zero represents clearer water and larger light extinction coefficients represent more turbid waters. As water moves through the southern oyster area (water from LP7 to LP5), it is significantly cleared (t-test, $p<0.05$); light extinction coefficient values were lower at LP5 vs LP7 on all but one sampling date in 2021 (Figure 2.5).

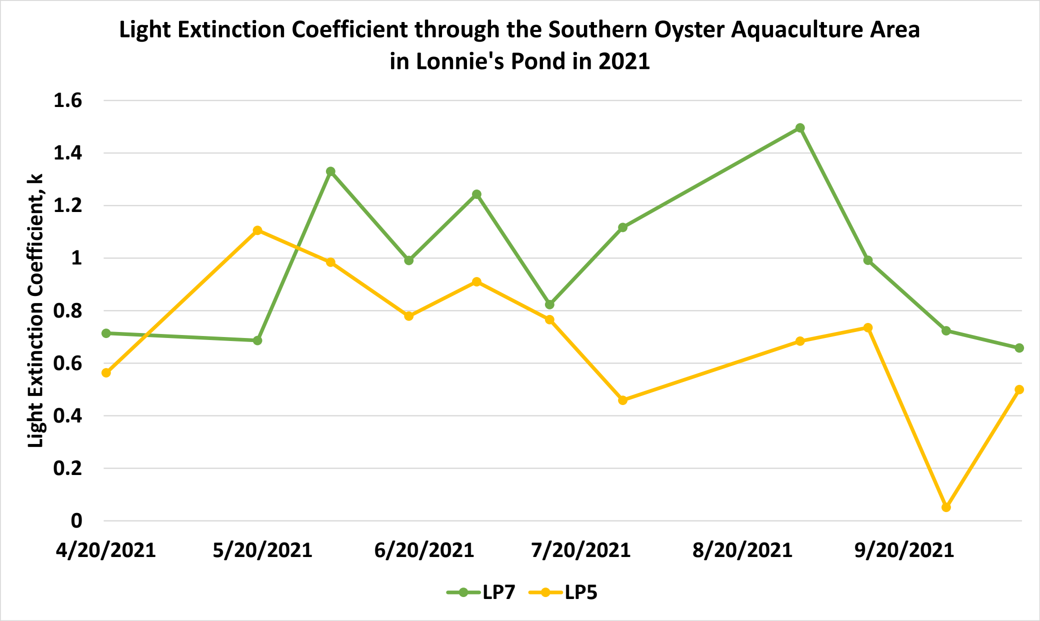

Figure 2.5. Time-series of watercolum light extinction coefficients, k during the 2021 water quality monitoring using the station light profiles in the southern oyster aquaculture area both upgradient (LP7) and downgradient (LP5). $k = \ln(I_{O)}-\ln(I_{D)}/d$

Water clarity could not be ascertained through the northern oyster area although tidal and wind driven flow velocities in the region of the oysters are very low and more variable, so that flow through the bags can follow significantly different tracks. Therefore, predicting the specific up-gradient and down-gradient sampling points through the northern array on any given day is uncertain, even though over the long term there is a general trend in particle tracks 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% (e.g., Bournes Pond, Falmouth).

CSP-SMAST has been quantifying the efficacy of using shellfish to reduce N load since 2016, so it is possible to investigate the water quality data for any trends over the past six years. The interannual variation in key water quality metrics, total chlorophyll-*a* pigments, PON, and bioactive nitrogen show increases in 2021 compared to 2020, but do not reach the highest concentrations recorded, which were seen in 2019 (Figure 2.6). 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 2.6). Chlorophyll, PON, and bioactive nitrogen pond-wide averages are lower in 2021 compared to the start of the study in 2016 and in 2019. All stations have lower chlorophyll-*a* concentrations in 2021 averaging 11.1 µg/L for the year compared to the start of the study in 2016 which averaged 13.6 µg/L in total pigments.

PON and bioactive N, like chlorophyll, reached peaks in 2019 and has declined to below the initial 2016 averages (PON 2016 average = 15.2 µM; 2021 PON average = 11.8 µM). Bioactive nitrogen in 2016 averaged 20.4 µM vs 16.9 µM in 2021. Increases in these metrics from 2020 could be attributed to the larger flow and nitrogen load entering Lonnie's Pond from the herring run stream as well as the increased total of precipitation recorded in 2021 compared to 2020 discussed in detail in the following section. Of the 6 years of data, 2017/2020/2021 are similar and significantly lower than 2016/2018/2019 in all metrics.

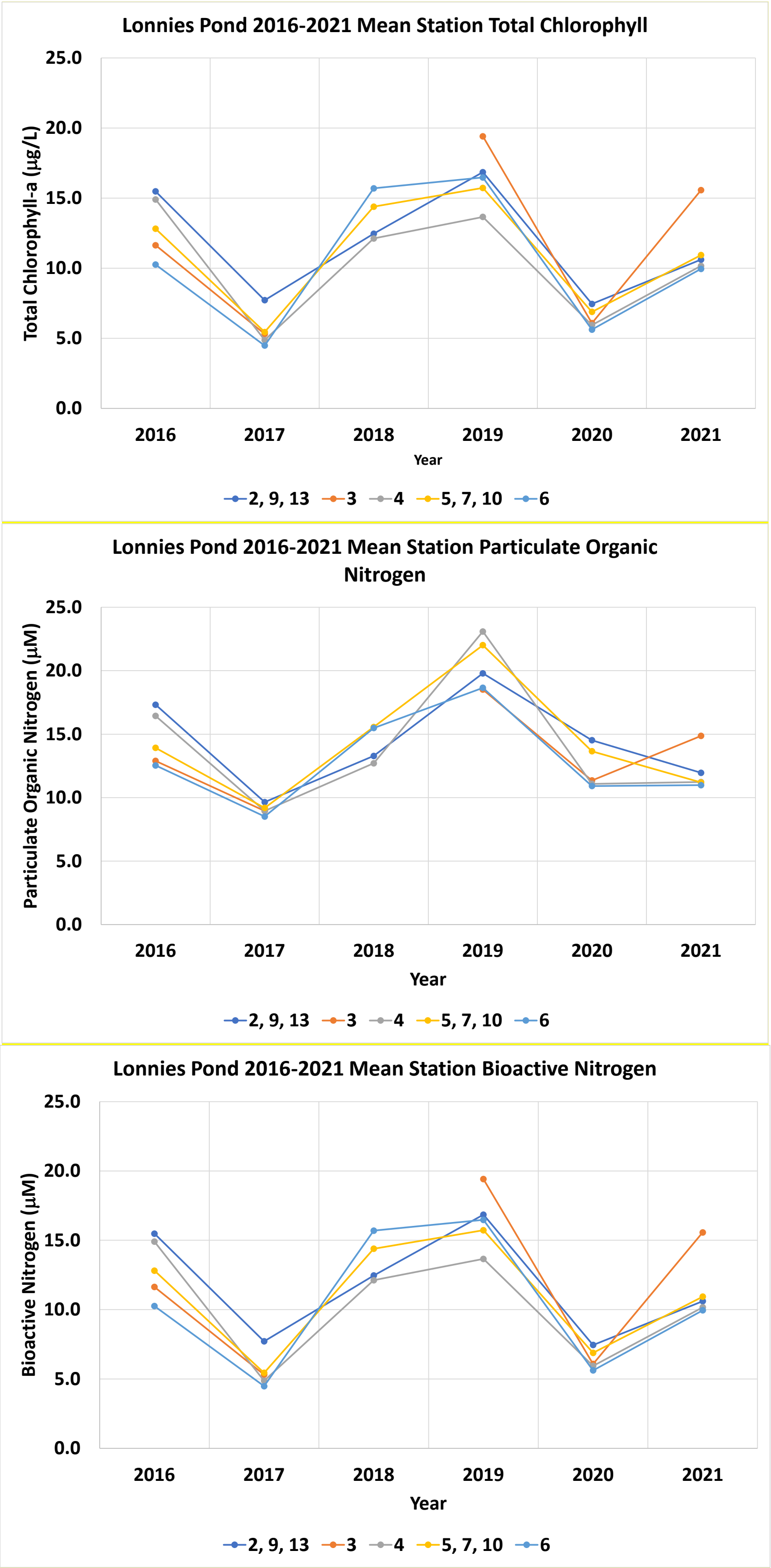

Figure 2.6 2016-2021 Interannual variation in mixed layer average total chlorophyll-*a (top)*, particulate organic nitrogen (middle), and bioactive N (btm) at stations LP-3, LP-4, LP-6 and averages of the stations (LP 5, 7, 10) and (LP 2, 9, 13) that surround the oyster arrays. Station numbers refer to locations in station map, Figure 2.1.

Total suspended solids, TSS, were measured in and around the southern oyster array consistently in 2016-2021. TSS represents the dry weight (mg) of suspended solids (particulates). Due to oysters filtering capacity, it is hypothesized that the TSS will be reduced as the tidal water ebbs out of Lonnie's Pond into Little Pleasant Bay. Station LP7 is up-gradient of the aquaculture area, LP6 is within the aquaculture area, and LP5 is down-gradient of the aquaculture area, (seestation map in Figure 2.1). All samples were collected on the ebb tide and the highest TSS was indeed found up-gradient of the oysters (LP7) and was reduced down-gradient of the oysters in each sampling from 2016-2020 except 2021 (Figure 2.7). 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. This is consistent with generally higher water clarity seen with the light extinction coefficient results. This finding is an important finding in areas where eelgrass restoration is being considered.

One source of TSS is the herring run (Lonnie's Stream) which discharges 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. During the duration of this study (2016-2021), the least amount of rain was recorded in 2020. Despite the general increase in measured TSS up-gradient of the oysters, it appears the aquaculture area has handled those increases and the filtration capacity for TSS has not been exceeded. Stream flow, load, and precipitation will be discussed further in the following section.

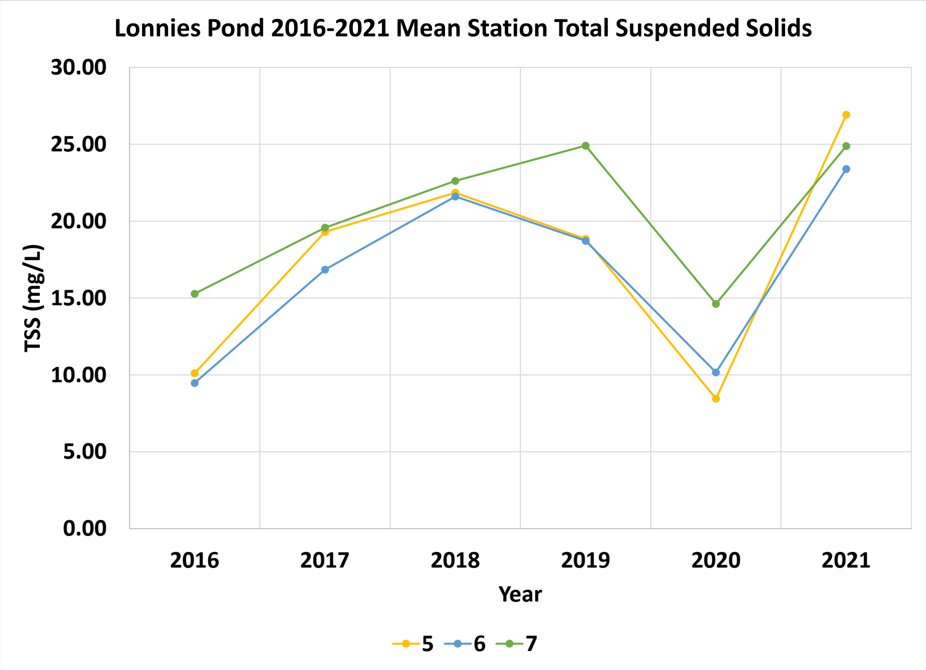

Figure 2.7 Compiled 2016-2021 averages for total suspended solids (TSS) at stations up-gradient (LP7), within (LP6), and down-gradient (LP5) of the Lonnie's Pond southern oyster aquaculture area. Station numbers refer to locations in station map in Figure 2.1.

The presence of oysters has affected the water quality in Lonnie's Pond by significantly reducing phytoplankton, represented by the total chlorophyll-*a* pigments (Figure 2.2), bioactive nitrogen (Figure 2.2), and TSS (Figure 2.7). Oysters have been shown to reduce water column particulates and increase water clarity (Figure 2.5), in Lonnie's Pond as well multiple other areas locally (e.g., Bournes Pond[6]) as well as regionally (Great Bay, New Hampshire[7] and Long Island Sound[8]). During 2021, we were able to quantify how the size class of oyster affects the water quality. It was found that larger oysters facilitated a greater reduction in bioactive nitrogen due to their greater filtration capacity. This supports the continued use of the 2020-2021 approach of deploying YR2 oysters in March and shifting to YR1 oysters in July.

Steam Inflows and Nutrient Loads:

Quantifying the effect of oyster aquaculture on the overall health of Lonnie's Pond must account for the stream inputs. Lonnie's Pond receives freshwater discharge from two streams, Pilgrim Lake to Lonnie's Pond and a small periodic discharge from Crystal Lake Bog to Lonnie's Pond. These nitrogen inputs to Lonnie's Pond play a role in setting the nutrient field in Lonnie's Pond as do the tidal flows and internal cycling.

Pilgrim Lake is the main source of surface water inflow and nutrient load and has been monitored since August 2016. Monitoring includes the placement of a gauge which records water level every 10 minutes. This gauge was placed at the herring run up-gradient of the culvert (Figure 2.8). While the gauge collects water level, Coastal Systems Program staff visit the site bi-weekly at low tide to collect water samples and volumetric discharge measurements.

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[6] 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.

[7] 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).

[8] 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.

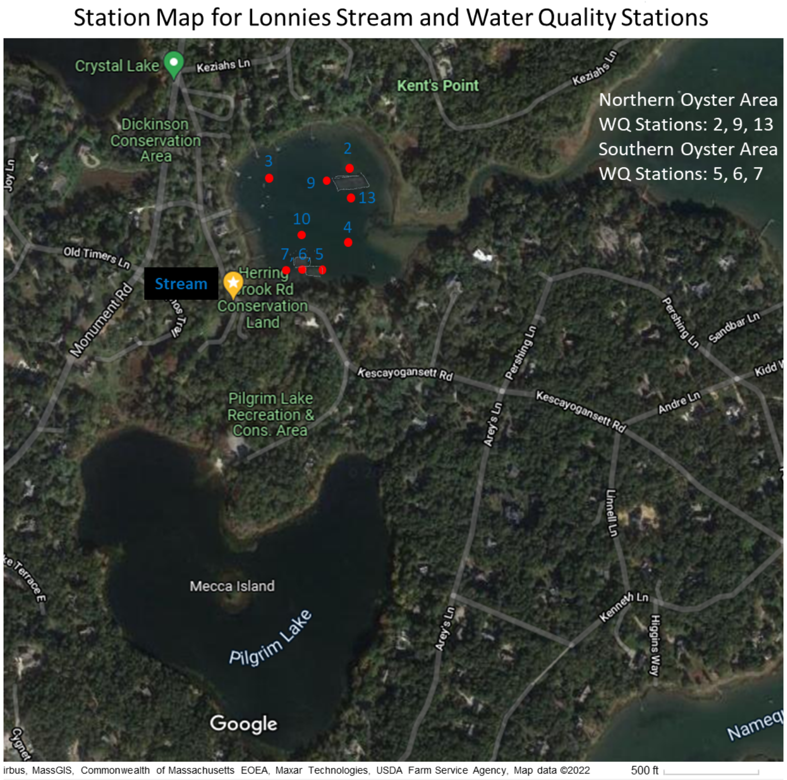


Figure 2.8 Location of the stream gauge (yellow star) deployed at the base of the herring ladder.

The water level data was averaged to obtain hourly water levels. However, since the gauge site experiences tidal influence on the highest tides, the diurnal low tide stage value was extracted on a day-by-day basis to obtain the daily stage value indicative of strictly freshwater flow. This low tide stage value for a given day was then entered into the updated MEP rating curve initially developed in 2003 that was refined with 2019 flow measurements to determine daily flows into Lonnie's Pond (Figure 2.9).

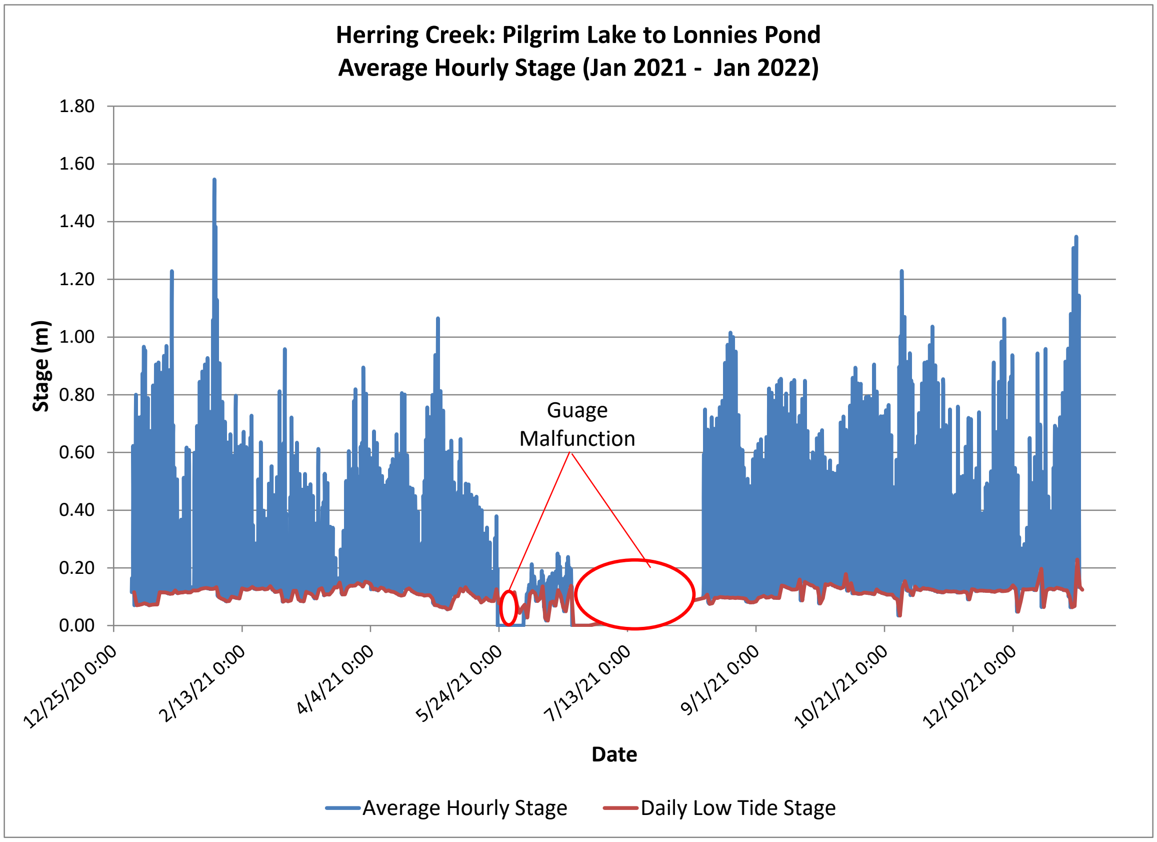


Figure 2.9 Average hourly stage record from Herring Creek discharge to Lonnie's Pond from Pilgrim Lake, January 2020 to Jan 2021 and associated daily low tide stage used to calculate freshwater flow and nitrogen load to Lonnie's Pond.

The surface water flow record from Herring Creek is then paired with the measured nitrogen concentration data to determine the mass input of nitrogen through the gauging site. Nutrient data was interpolated between data points in order to pair with daily volumetric flows with daily nutrient concentration. This data is expressed as mass of nitrogen per unit time (kg/d) and can be summed to obtain the weekly, monthly, and annual nutrient load to Lonnie's Pond. The "measured load" represents the nitrogen entering Lonnie's Pond directly from surface water as opposed to groundwater (recharge over area delineated by watershed, which was previously determined and is presented in the 2003 MEP report). Flow and load have been measured at this gauging location since August 2016.

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 when groundwater levels are typically low (Table 2.4). Water quality results from 2020 show that total nitrogen and flow inputs are significantly lower throughout the summer than in the preceding spring (Table 2.4). 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 18.8 kg N during the summer was observed in 2020 (Table 2.4). However, it should be noted that 2020 had very low groundwater levels. In contrast, in 2021 had more typical groundwater levels and summertime flow only decreased by about 1/5 of the volume of the preceding spring and total nitrogen loads remained at springtime levels during the summertime. Overall, springtime stream flow and nitrogen inputs to the pond via stream discharges was lowest in 2020 and highest in 2018 of the 5 years of measurement (Table 2.4). Stream inputs in spring 2021 had a nitrogen input 55.24 kg N/spring at the average of the 5 years, 53.16 kg N/spring, but because summer (60.38 kg N/summer) did not decline as is typical, it was significantly higher than the 5 year average (35.45 kg N/summer).

Table 2.4. Total nitrogen load (kg) entering Lonnie's Pond via Pilgrim Lake stream from 2017 - 2021. April – June 2019 was without oysters, whereas 2017, 2018, 2020 and 2021 had oysters during springtime freshwater flow conditions. July – September for all four years had oysters during these summertime 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	93,257	**Total Load (April-June 2017)**	3.86	1.92	5.79	32.59	12.08	50.46
Jul-Sept 2017	39,420	**Total Load (July-Sept. 2017)**	1.83	2.12	3.95	14.64	2.85	21.45
Apr-Jun 2018	137,888	**Total Load (April-June 2018)**	4.63	6.18	10.81	57.40	14.45	82.67
Jul-Sept 2018	100,995	**Total Load (July-Sept. 2018)**	5.08	9.91	15.00	39.52	8.11	62.62
Apr-Jun 2019	51,956	**Total Load (April-June 2019)**	6.09	2.66	8.74	25.81	11.19	45.74
Jul-Sept 2019	23,178	**Total Load (July-Sept. 2019)**	1.20	2.06	3.26	8.58	2.89	14.73
Apr-Jun 2020	46,939	**Total Load (April-June 2020)**	3.71	4.38	8.09	17.44	6.18	31.71
Jul-Sept 2020	12,238	**Total. Load (July-Sept. 2020)**	1.79	6.53	8.33	4.07	5.35	18.08
Apr-Jun 2021	66,981	**Total Load (April-June 2021)**	6.80	8.73	15.52	30.59	9.13	55.24
Jul-Sept 2021	54,308	**Total. Load (July-Sept. 2021)**	4.99	11.78	16.77	36.86	6.84	60.38

Viewing the monthly stream flow and nitrogen load inputs year-round illustrates just how much more water and nitrogen entered Lonnie's Pond in 2021 (Figure 2.10). The stream input of total nitrogen and corresponding stream flow discharging into Lonnie's Pond per month was totaled from January 2020 – January 2021. Results show the mass of nitrogen and stream input increased in 2021 compared to 2020 (Figure 2.10). Springtime flow and load doubled in 2021 compared to 2020 and September signaled a strong pulse of flow in load that continued through December in 2021 (Figure 2.10), possibly playing a role in the increased N levels at the southern array site in 2021 versus 2020 (likely dampened by the oysters). The increased flow can be explained by higher precipitation in the fall of 2021 compared to 2020 (Figure 2.11). Precipitation amounts were gathered by the Community Collaborative Rain Hail and Snow Network monitoring station in Orleans and the total precipitation in 2020 was 36.7 inches, while in 2021 the total precipitation was measured at 49.9 inches (https://www.cocorahs.org/ViewData/StationPrecipSummary.aspx).

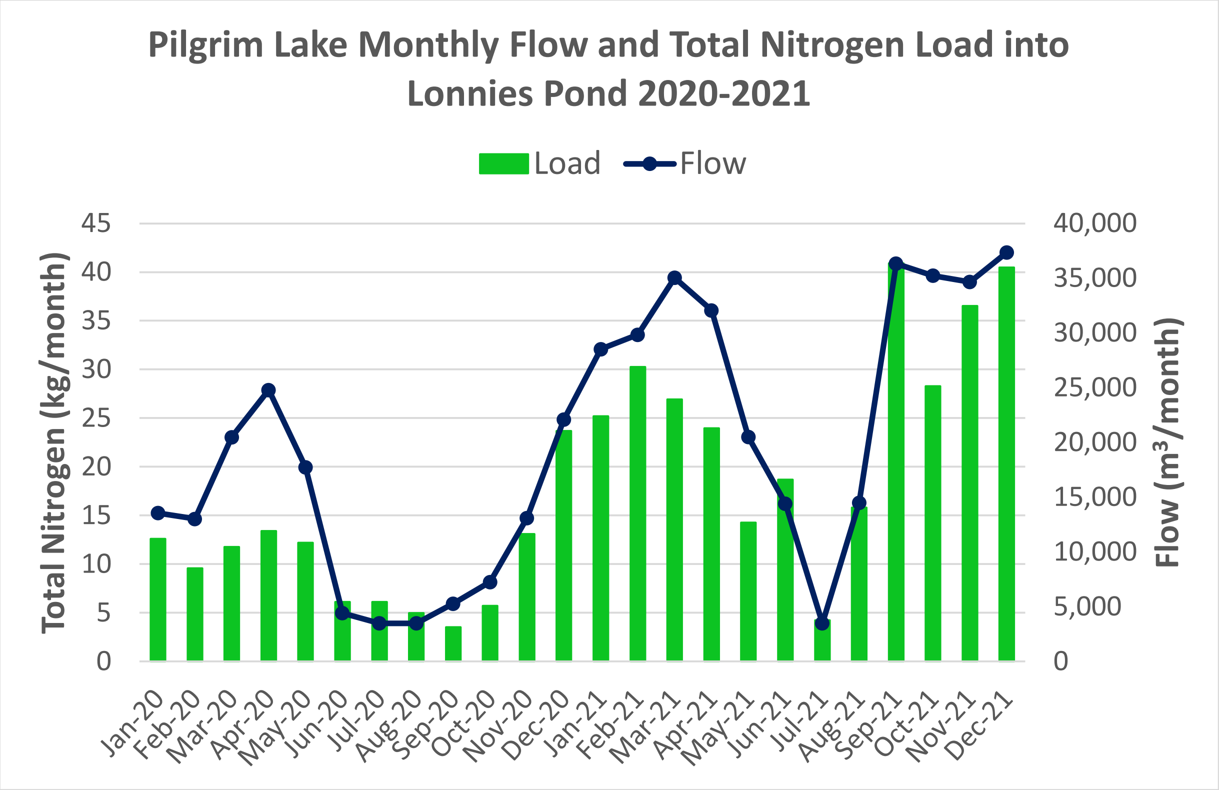


Figure 2.10. Monthly stream flow and monthly stream load of total nitrogen from Pilgrim Lake entering Lonnie's Pond from January 2020 to January 2021.

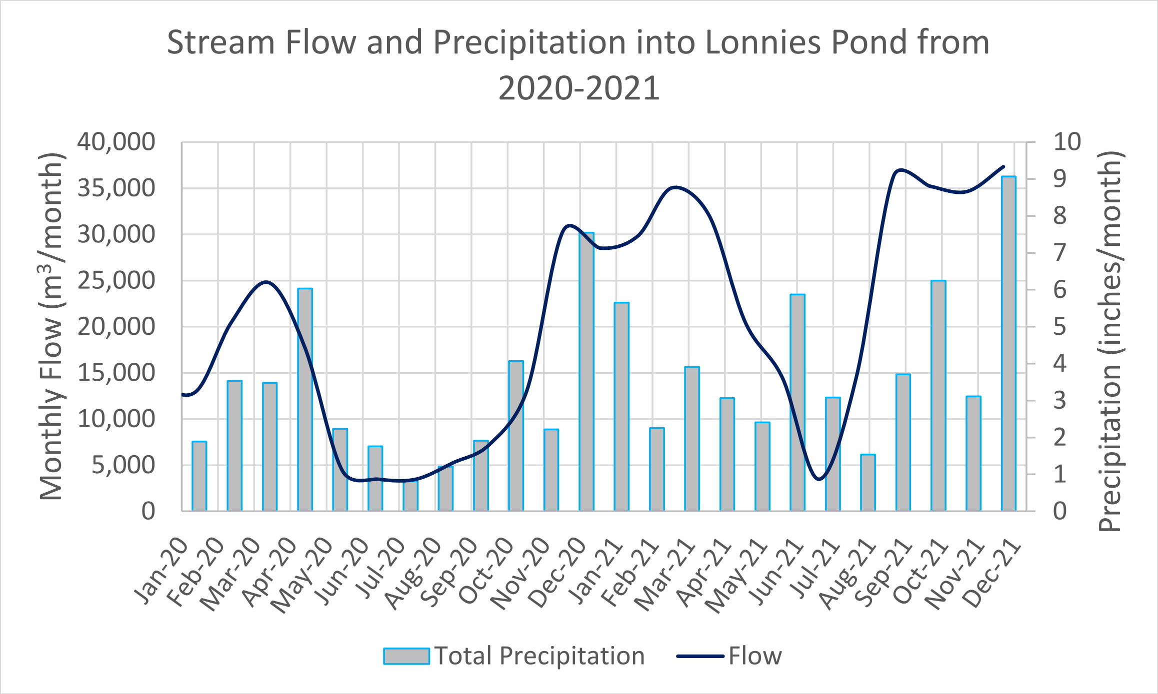


Figure 2.11 Monthly stream flow and corresponding precipitation totals for Jan 2020 – Jan 2021.

Precipitation totals from a site in Orleans were recorded by the Community Collaborative Rain Hail and Snow Network and summed to calculate precipitation totals for the duration of the oyster study (https://www.cocorahs.org/ViewData/StationPrecipSummary.aspx). The lowest amount of precipitation occurred in 2020 with 36.7 inches of precipitation, while 2017 had the most with 53.3 inches of precipitation (Figure 2.12). It is important to note that yearly precipitation in all years is very similar except for 2020 which was lower in that drought year.

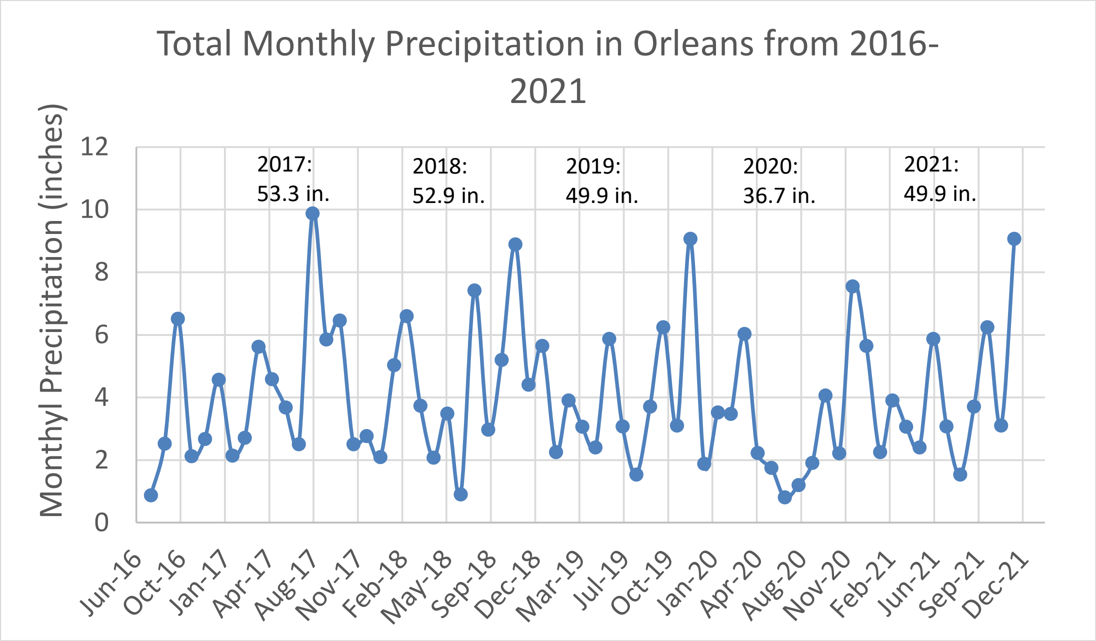


Figure 2.12 Monthly precipitation totals from summer 2016 through December 2021. Yearly precipitation totals are summed above the monthly totals.

An overview of how the latest 2021 stream input from Pilgrim Lake into Lonnie's Pond compared to previous years can be viewed by comparing the yearly nitrogen loads and flows from 2017 to present. The highest nitrogen load was recorded in 2021 with a total of 311 kg, while 2019 had the lowest mass of nitrogen recorded with 103 kg (Figure 2.13). Interestingly, 2019 and 2021 has the same yearly precipitation total (Figure 2.12).

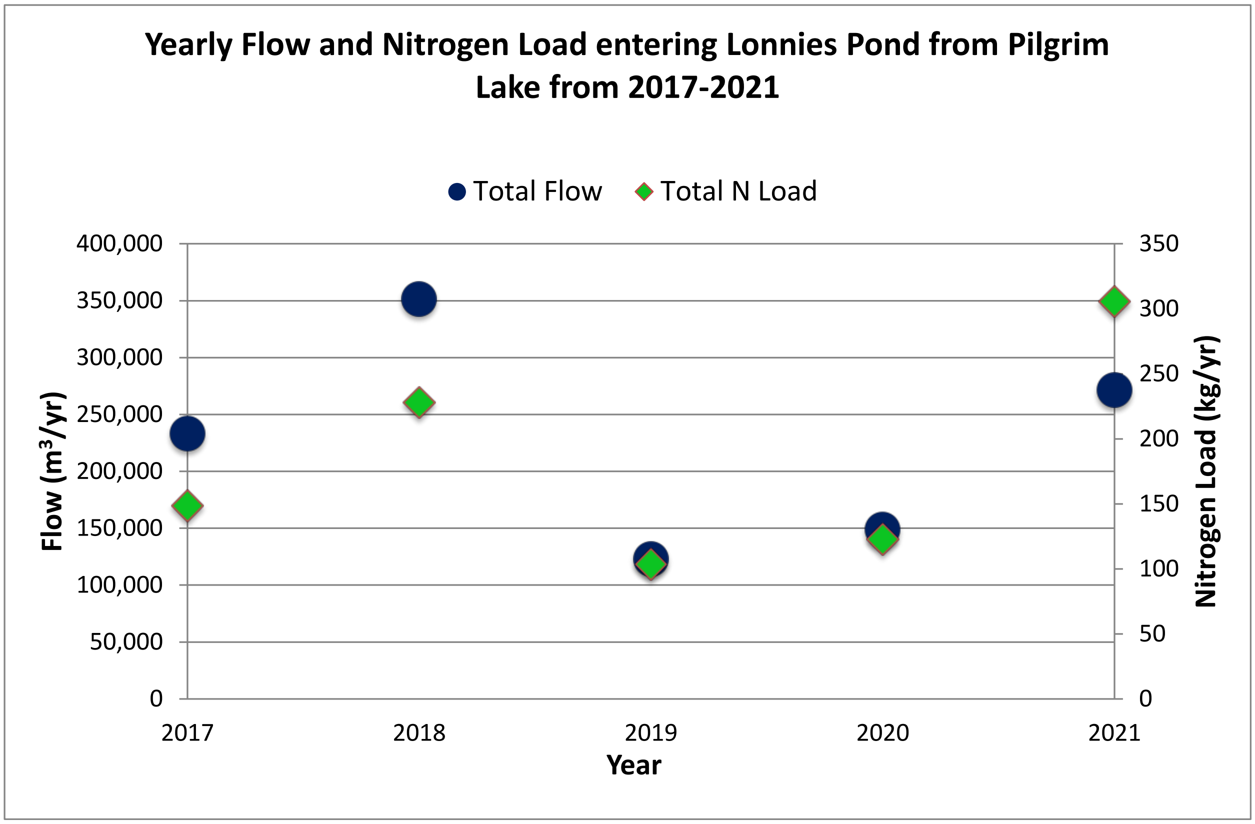


Figure 2.13 Yearly stream flow and nitrogen load from Pilgrim Lake into Lonnie's Pond from 2017 to 2021.

3.0 Dissolved Oxygen and Chlorophyll-*a* Continuous Monitoring

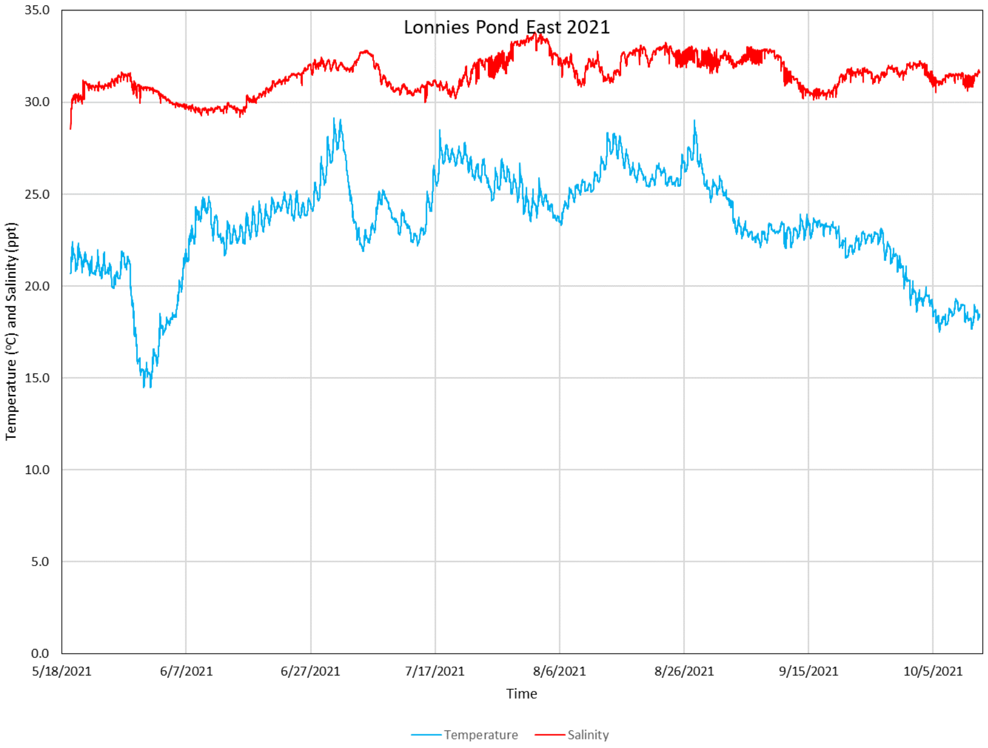
Two autonomous recording multiparameter sondes were deployed from April 19 through October 12, 2021. One sonde was deployed 30 cm from the bottom in approximately 1.5 meter of water along the eastern side of the aquaculture area (Lonnie's Pond East, see Figures 3.1, 3.2 and 3.3; top panel). The second sonde (Lonnie's Pond West, see Figures 3.1, 3.2 and 3.3; bottom panel) was deployed 30 cm from the bottom in approximately 2.0 meters of water slightly south of the where the two southern oyster arrays meet (4m south of LP6). Oysters filter large quantities of water and phytoplankton nutrients removed from the water that are not assimilated are deposited as feces and pseudofeces on the underlying sediments where they decompose releasing the 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 oyster growth and mortality, such as elevated temperatures or low salinity.

Average salinity and temperature were similar at the two mooring sites (Figure 3.1) and were not significantly different from the 2020 mooring data. In contrast, dissolved oxygen concentrations at the two mooring sites showed differences (Figure 3.2 top and Figure 3.3 top). The East mooring displayed higher average dissolved oxygen and increased diurnal variation compared to the West mooring. While at least six instances of anoxia were observed during the 2020 field season (West only), the 2021 field season displayed no bottom water anoxia and only three brief instances of dissolved oxygen under 2mg/L at each mooring location. The large dissolved oxygen excursions appeared to be directly related to the water column concentration of chlorophyll.

Bottom water oxygen, measured during water quality surveys, indicated that bottom water dissolved oxygen was generally higher in 2021 than in 2020. As seen in the 2020 data, the similarity between bottom water oxygen values at the moorings and the water quality monitoring sites suggests that factors that depressing oxygen concentrations were not related to a localized oyster effect, but instead were the result of the pond-wide effects of nitrogen enrichment.

Algal blooms, as determined from high chlorophyll concentrations, were generally associated with large rain events that were infrequent over the summer. Infrequent rain results in higher concentrations of nutrients entering the pond from the streams and overland flow as they have a longer time to accumulate on the soil and impermeable areas. As seen in 2020, the chlorophyll concentrations were higher at the East mooring than at the West mooring. The East mooring was moved farther from the mouth of Lonnie's Stream (moved to the east) to be adjacent to the edge of the 2021 oyster deployment, thus water column response to rain events should be diminished. It is likely that the historically (2017-2020), higher chlorophyll concentrations were consistently found to the east of the oyster deployment are due in part to the oyster filtration of phytoplankton.

An important finding has been a shift in the benthic communities as the oyster deployments have continued over the years. The observed shift from small oligachaete worm to dense amphipod mats is indicative of a lessening of ecological impairments in Lonnie's Pond. We have observed the increasing establishment of amphipod communities and their expanding population in recent years. Amphipod mats were absent prior to 2020 except in the deepest locations to the north of the deployment area, where patches were first noted. Habitat improvements by oyster removals of particulates and the high bottomwater oxygen in 2021 appears to have allowed expanded colonization of surficial sediments by amphipods. This was observed in Boston Harbor when sludge disposal ceased. Once sediments are colonized, the amphipods effectively mine carbon from the sediment, and improve the sediment oxidation status. Amphipods decrease sediment carbon, thereby decreasing sediment oxygen demand over time and ventilate the sediment potentially enhancing coupled nitrification-denitrification. As omnivore/detritivore, amphipods may also play a role in the clearance of chlorophyll from the water column contributing to the observed lower chlorophyll concentrations observed at the West vs. East moorings. Whereas the West mooring was in the middle of the oyster impact area, the East mooring was near the eastern edge of the impact area where amphipod densities were lower. Between oysters filtering the water column and expanding amphipod mats mining the deposited excess carbon (and possibly assisting in water clearance) the water quality was greatly improved over previous years. No anoxia was recorded in 2021, depression of dissolved oxygen below 2 mg/L only occurred three brief times, and the chlorophyll concentrations were greatly reduced. This will be tracked in future monitoring to see if this is a stable new healthier status for Lonnie's Pond



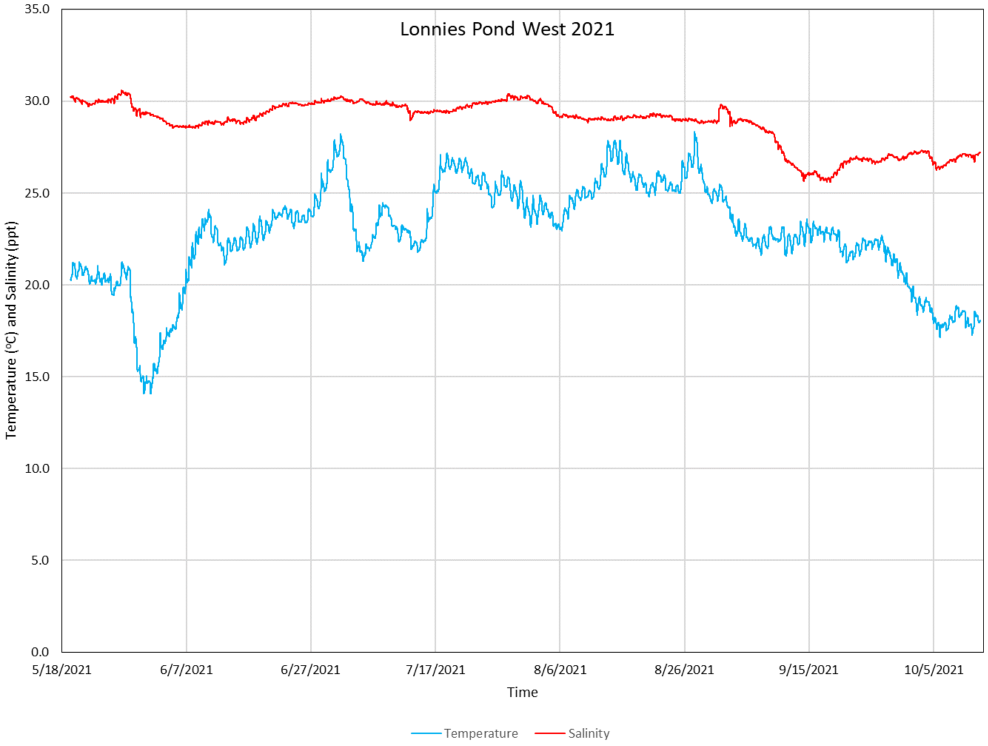
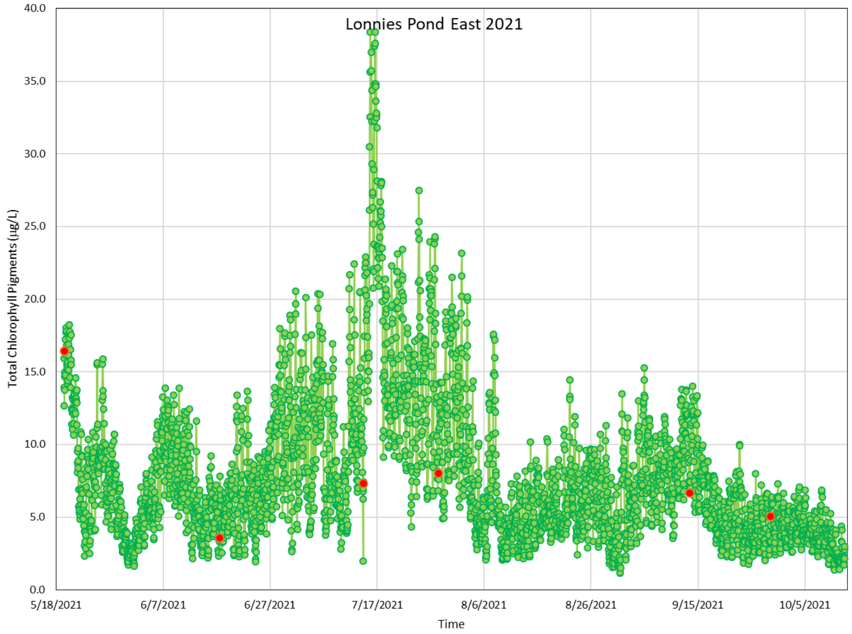


Figure 3.1 Time series temperature and salinity measurements for West (top) and East (btm) moorings. Decreased salinities in the West mooring record (September 15) were coincident with rainfall events.



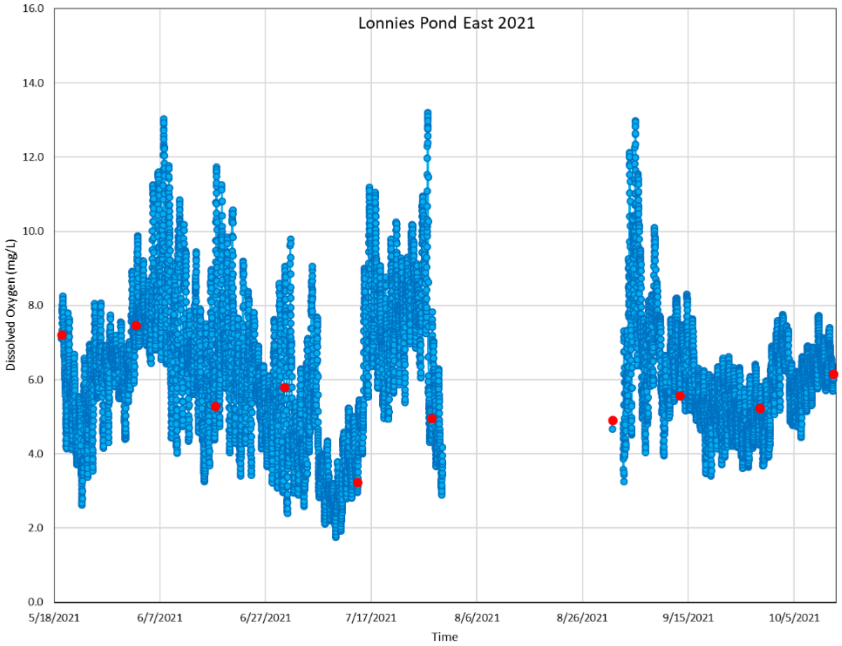
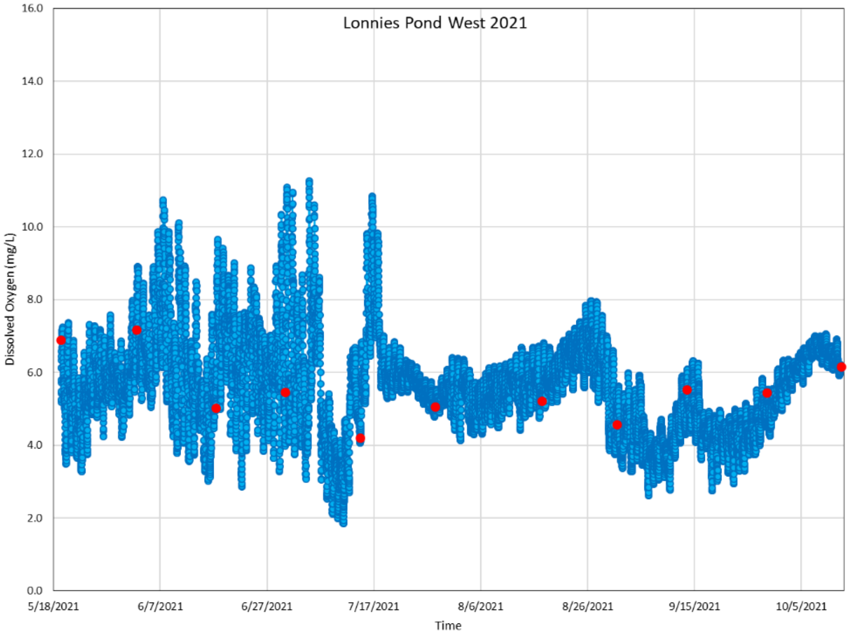


Figure 3.2 Time series oxygen (top) and chlorophyll (Bottom) measurements from East Mooring. Red dots indicate in situ calibration values obtained during monitoring activities.



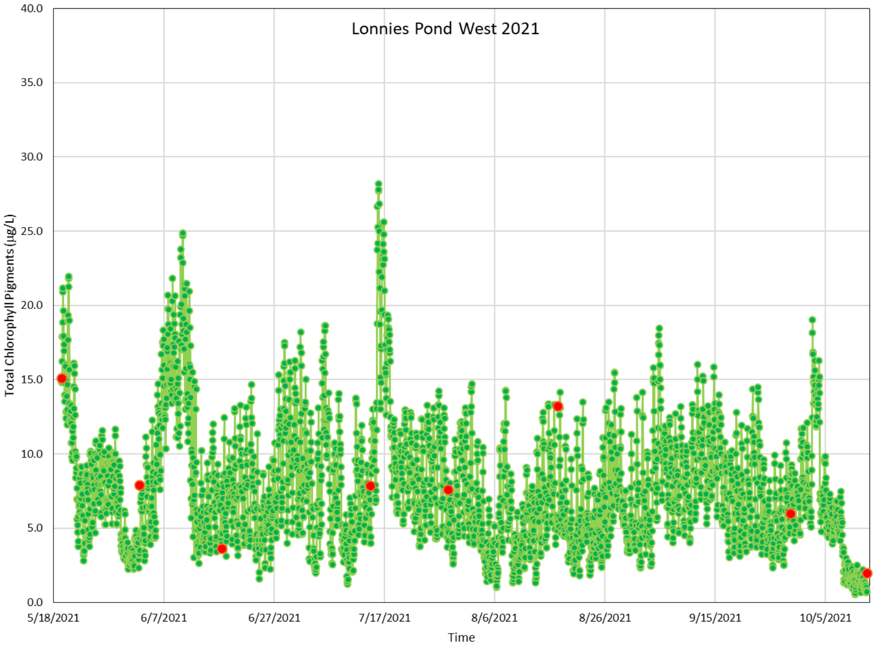


Figure 3.3 Time series oxygen (top) and chlorophyll (Bottom) measurements from West Mooring. Red dots indicate in situ calibration values obtained during monitoring activities.

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 enriched sediments. Particulate organic matter (POM) deposition to sediments drives biogeochemical cycling, 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 2021 (i.e., < 3-4 mg/L dissolved oxygen); however, anoxia did not occur and periodic hypoxia was less frequent and of shorter duration in 2021 compared to 2020. 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 materials, termed biodeposits. Shellfish aquaculture is projected to increase deposition and subsequently increase sediment respiration rates including denitrification. The recent finding of improved conditions allowing the major colonization by amphipods is also expected to have a significant positive effect sediment oxidation status and denitrification as has been indicated in other systems.[9]

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 19, August 8, and October 11, 2021 (April 2022 will also be captured 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

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[9] Howes, B.L. 1998. Sediment metabolism within Massachusetts Bay and Boston Harbor: relating to system stability and sediment-watercolumn exchanges of nutrients and oxygen in 1996. Mass. Water Resources Authority Environmental Quality Report pp.85.

4.2), this seasonal carryover of nutrients was measured in 2021 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.

Intact sediment cores were collected by SCUBA divers, held at in situ temperature, and returned to the field lab. The cores were then incubated at *in situ* temperatures with a mixed headspace such changes in headspace nutrient species (DON, NO_x^-, NH_4^+ and PO_4^-) and dissolved oxygen over time yield rates of sediment/watercolumn exchange. The rate of oxygen uptake was determined to: 1) evaluate the sediment's role in 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 or buried. Measurements of sediment regeneration were limited to oysters deployed in the southern oyster array area to allow comparison to prior years, especially as conditions can change (e.g., amphipods). Assays were performed on 12 cores collected throughout the southern deployment area, both in areas receiving oyster biodeposits (impact area) and areas receiving only background deposition (background).

An empirical model based upon measured water velocity and measured biodeposit settling rates were used to determine the specific area of bottom receiving biodeposits[10]. The distance of each core from the margin of the oyster array determined whether the core was within or outside the biodeposit impact area. The velocity data collected by an acoustic doppler current profiler (ADCP) in summers of 2016-2019 was used for the model. Additional inputs include: the mean sinking velocity of fecal material (8.14±5.01mm/s), 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 area assuming fecal pellet production was similar for all bags. The resulting biodeposit impact area was determined by taking the 95th percentile of biodeposit horizontal displacements during settling. The 2019 biodeposit impact area (2890 m^2) was applied to the 2020 and 2021 deployment because the 2020 and 2021 South deployment array area, location, and layout was unchanged from 2019 to 2021.

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 our estuaries.

The parallel determination of denitrification was based upon time series measurements of excess N_2 generation (compared to Argon; Ar) using isotope ratio mass spectrometry (IRMS). 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. Cryogenic traps remove water vapor and carbon dioxide (CO_2) gas. The remaining gas mixture is then analyzed by the mass spectrometer for masses 28 and 40 for determining N_2:Ar ratio. Calibration uses a certified reference gas of known composition. Finally, water column

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

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.

Sediment Nutrient Cycling Results (2020):

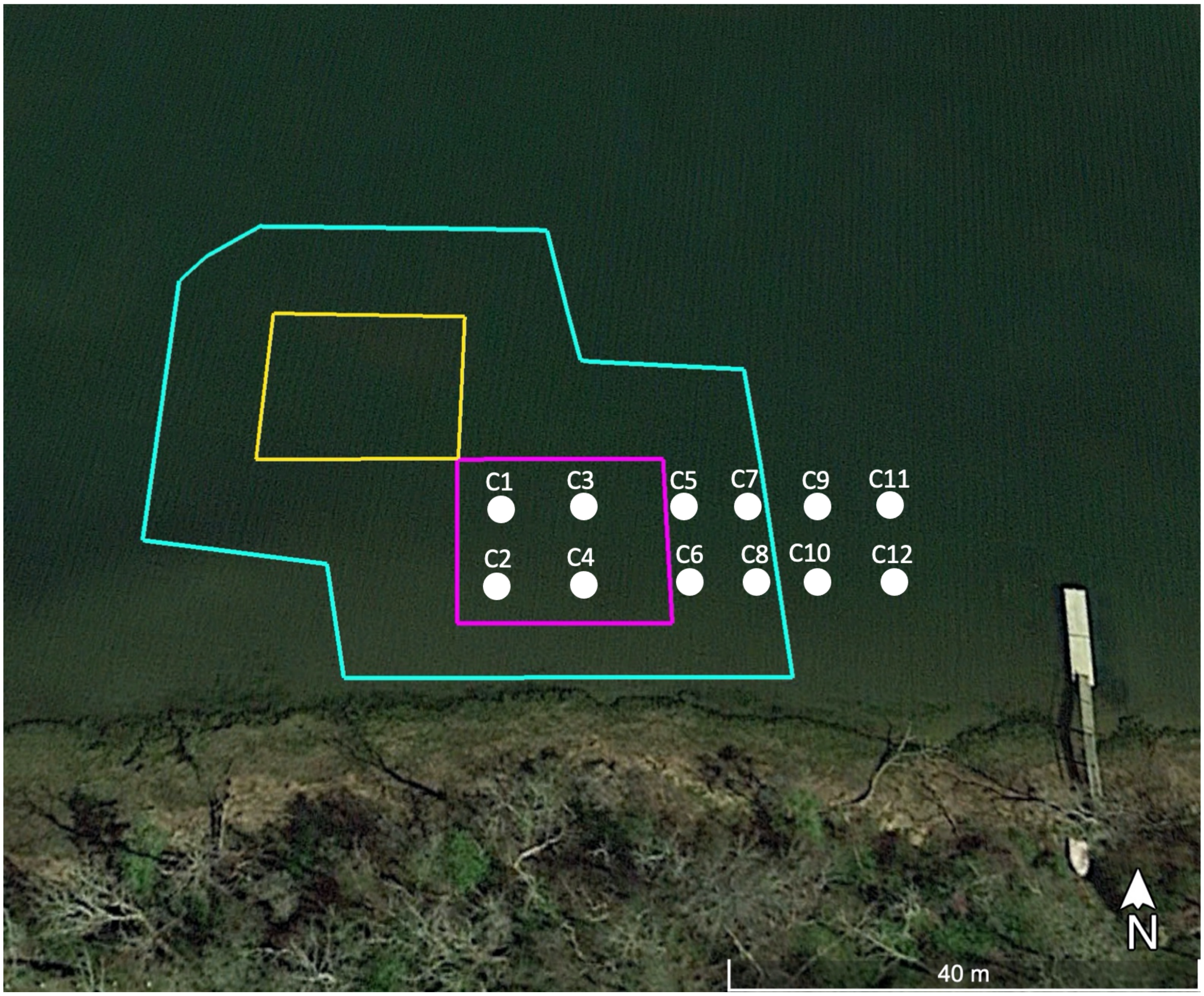

Figure 4.1 Aerial photograph showing the location of the oyster deployment area overlain with 2019-2021 oyster deployment area (yellow and purple polygons), and April, August, and October sediment core locations, C1 – C12 (white markers). Total sediment area receiving the oyster biodeposits as determined by fecal pellet distribution in 2019-2021 is shown as the outer bounded area (blue polygon).

Results of the April, August, and October 2021 sediment regeneration measurements are summarized in Table 4.1 below. The April 2021 survey was conducted to capture enhanced denitrification stimulated by the fall and overwinter storage of biodeposited organic nutrients, which accumulated during the 2020 oyster deployment. The gradual decrease in temperature during fall months reduces metabolic activity and decomposition and labile organic matter is "preserved" in the upper sediment layer until the following spring warming. Therefore, sediment cores were collected in April 2021 once water temperatures began to increase. April 2021 cores were collected to complete the assessment of the 2020 oyster deployment (Year 5; Figure 4.1).

The August 2021 sediment oxygen demand (SOD) and DIN regeneration rates showed small shifts in the sediments receiving biodeposits from previous years with lower exchange rates of SOD and NH_4^+ rates (2021 average treated SOD and NH_4^+ rates were 29% and 42% lower, respectively, than the 2016-2020 average rate) and greater treated NO_3^- release rates (average 2021 rate was 100% higher than 2016-2020 average). Like 2016-2020, 2021 oxygen uptake and DIN efflux was observed in all cores regardless of location relative to the impact area (e.g., treated vs. control). Release of DIN from the sediment to the water column is typical of estuarine sediments in summer. Organic matter deposition in the form of biodeposits increased sediment respiration and resulted in higher SOD and NH_4^+ flux rates in cores collected within the impact area. Despite lower overall NH_4^+ flux rates, on average, ammonium efflux from treated (biodeposit affected) cores was more than double the rate average of control cores collected outside the impact area. This finding indicates that biodeposit N settling within the impact area was being rapidly remineralized during summer as a result of peak biodeposition (i.e., peak annual oyster food availability) and temperature. The SOD rate difference between treated and control cores was less obvious. On average, SOD was higher within in the treated sediments; however, enhancement of sediment oxygen uptake over the control cores was less than that observed in previous project years (130% enhancement above background in 2020 vs. a 40% enhancement in 2021). Furthermore, the 2021 sediments within the core collection area have become colonized by amphipod mats, which were observed in nearly all cores. The presence and number of these bio-irrigating macrofaunal animals appear to have contributed to ammonium oxidation resulting in the nitrate release observed in all cores, an effect that was as seen in the control cores because of their now thicker oxic surface layer. In core C9 (control, not receiving biodeposition), the ammonium flux was below detection and nitrate was the primary component of nitrogen release. Similar high rates of nitrate release were observed in two treated cores (C3 and C8) that had similarly thick surface oxic layers. Shallow or patchy oxic surface sediment layers were observed other cores and phosphate efflux was recorded in all cores Although tube forming amphipod species tend to increase SOD rates through bio-irrigation and respiration[11], the decreased enhancement of sediment oxygen uptake may be attributed to the thinner oxic layer observed in treated cores versus control cores, and the presence of amphipod mats in nearly all cores. In other words, the amphipods bring sufficient oxygen into the sediments to support nitrification and an increase in surface sediment oxidation, but the rate of oxygen uptake is still higher in the treated sediments due to the greater organic matter remineralization versus control, non-biodeposit areas.

The October 11th cores were collected and incubated at 18.1°C, one degree warmer than cores collected on October 11, 2020 in nearly the same locations. October 2021 flux rates show similar patterns compared to October 2020 and to August, but with October rates were depressed as a result of a 5.7°C temperature decrease between the summer and fall flux dates. Ammonium was released from the sediments in all cores except C11 (control) and the average treated core NH_4^+ flux was double that of the control core efflux average, reflecting the N inputs in the biodeposits. Similar to October 2020 and in contrast to 2016–2019 October nitrogen flux rates, nitrate efflux occurred in all cores except C2, C5, and C6, due to the greater sediment oxidation by amphipods. On average, NO_3^- efflux made up only 4% of

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[11] Tucker, Jane, et al. "Response of benthic metabolism and nutrient cycling to reductions in wastewater loading to Boston Harbor, USA." *Estuarine, Coastal and Shelf Science* 151 (2014): 54-68.

DIN (ammonium + nitrate) fluxes out of the sediment in October 2020, whereas average NO_3^- efflux made up nearly 43% of DIN fluxes out of the sediment in October 2021. The average NH_4^+ flux rate of treated and control cores was comparable to Years 1–5; therefore, the increased fraction of NO_3^- in DIN efflux owes to a greater overall NO_3^- production/efflux rate. Amphipod mats were observed in all October cores. As in previous years, sediment heterogeneity exists among the treated cores, which leads to differences in sediment biogeochemistry. However, in 2021 the presence of amphipod mats was similar across treated and control cores collected on both August and October flux dates. Core descriptions indicate the presence of dense amphipod mats in almost all cores, the data is qualitative and cannot account for the true density and health of the mats. Nonetheless, the numerous organisms aerate surficial sediments through bio-irrigation activity and increase elemental cycling through mining of reduced compounds (organic matter, sulfides) [12].

The increased presence of amphipod mats in sediments within and outside of the biodeposit area in recent years is important and may help to explain biogeochemical differences observed in 2021. SCUBA diver observation and laboratory core descriptions indicate that amphipod mats were not present in coring areas in 2016 and 2017 but began to appear in 2018 with amphipod colonies identified in half of the August cores. By 2021, amphipod mats were widespread in both treated and control sediments associated with the southern oyster deployment area. Amphipods are considered a transitional species marking improving but not high quality habitat condition, which appears to be the case in Lonnie's Pond. Tube forming amphipods and other bio-irrigating macrofauna are known to deepen the sediment oxic layer, which increases the number of sites where nitrification and coupled nitrification-denitrification can occur. At the start of the Project in 2016, macrofauna (e.g., burrowing worms and clams) were present, but not at densities likely to have a major effect on nutrient cycling. Increased nitrate efflux and decreased ammonium efflux in August and October 2021 suggest that amphipod irrigation was injecting oxygen into the surficial sediments increasing nitrification. Although N is still being released from the sediments to the watercolumn, the efflux of NH_4^+ was half of the 2016-2020 average during August when water quality is most degraded. Furthermore, the amphipod colonization of both treated and control areas is expected to have major effect on nitrogen cycling and to the extent that nitrogen within surficial sediments is "mined out" by amphipod activities, the difference between control and treated sediments is projected to diminish, at least for 1-3 years.

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[12] Tucker, Jane, et al. "Response of benthic metabolism and nutrient cycling to reductions in wastewater loading to Boston Harbor, USA." *Estuarine, Coastal and Shelf Science* 151 (2014): 54-68.

Table 4.1 Summary of benthic flux rates from core incubations conducted April 19, August 8, and October 11, 2021. Rows shaded in gray indicate background (control) rates (e.g., core location outside area impacted by oyster deposition). N_2-N detection limits were determined for each analysis date.

Collection Date: April 19, 2021; Incubation Temperature 12.8 °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	57.83	4.50	-0.09	4.40	2.55	7.14	36%
C2	25.39	1.73	0.01	1.74	0.14	1.88	7%
C3	44.78	4.63	0.08	4.71	BDL	4.71	BDL
C4	36.25	3.41	-0.03	3.38	3.74	7.18	52%
C5	76.46	5.18	0.08	5.26	5.05	10.31	49%
C6	106.74	2.32	-0.08	2.25	4.96	7.36	67%
C7	95.32	4.59	-0.16	4.42	6.09	10.84	56%
C8	65.98	3.35	-0.04	3.31	8.83	12.22	72%
C9	73.20	0.74	-0.08	0.66	12.27	13.09	94%
C10	53.63	0.69	-0.04	0.65	1.41	2.14	66%
C11	84.21	7.37	0.01	7.38	3.80	11.18	34%
C12	46.14	1.05	-0.06	0.99	0.36	1.47	25%

Collection Date: August 8, 2021; Incubation Temperature 23.8 °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	55.45	3.38	0.63	4.01	4.17	8.19	51%
C2	86.47	8.64	0.59	9.23	6.06	15.29	40%
C3	117.31	3.99	4.18	8.17	3.64	11.81	31%
C4	199.15	12.13	0.09	12.22	4.84	17.06	28%
C5	84.66	8.33	0.15	8.48	4.90	13.38	37%
C6	123.17	10.02	0.47	10.49	1.32	11.81	11%
C7	51.09	6.59	0.24	6.84	1.70	8.54	20%
C8	109.93	2.30	5.59	7.89	4.07	11.95	34%
C9	81.58	-0.01	4.30	4.28	6.20	10.51	59%
C10	103.13	3.51	3.21	6.72	NS	6.72	NS
C11	70.07	5.70	0.66	6.37	4.83	11.20	43%
C12	99.68	2.67	3.99	6.66	7.44	14.10	53%

Collection Date: October 11, 2021; Incubation Temperature 18.0 °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	77.16	1.86	3.70	5.56	4.11	9.67	42%
C2	43.94	2.75	-0.49	2.26	0.95	4.20	23%
C3	54.86	4.71	0.68	5.39	1.18	6.57	18%
C4	18.84	2.73	2.03	4.76	2.55	7.32	35%
C5	25.15	5.60	-0.15	5.45	1.40	7.16	20%
C6	94.69	1.35	-0.26	1.09	0.59	2.20	27%
C7	83.29	0.27	1.47	1.73	12.95	14.68	88%
C8	104.29	6.01	1.25	7.26	2.02	9.28	22%
C9	49.40	0.40	1.25	1.65	2.21	3.85	57%
C10	62.98	3.49	2.29	5.78	2.04	7.82	26%
C11	68.54	-0.78	0.57	-0.21	2.24	3.59	62%
C12	80.65	1.75	2.28	4.03	3.69	7.72	48%

*Bubbles were observed in the 8/8/21 C10 N_2-N samples and could not be run. The corresponding Total N Cycled rate does not include contributions from N_2 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 2021 background rates and rates measured under the oyster treatment are shown in Table 4.2 alongside the 2016-2020 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 2021 flux were appended to the “Year 5 Mean Denitrification Rates” section in Table 4.2. The 2021 spring flux represents the end of the Year 5 oyster enhanced denitrification, as the enhanced denitrification rates in spring prior to the 2021 oyster deployment were the result of biodeposits overwintered from the 2020 oyster deployment.

The April 2021 “Oyster Effect” (2.6 mMoles/m^2/d; Table 4.2) is 1.5 (80%) and 1.3 (100%) greater than the Oyster Effect measured in April 2019 and 2020, respectively. Denitrification was observed in 11 out of the 12 April 2021 cores, with the rate for core C3 below detection level (BDL). The results of the April 2021 flux provide additional evidence that biodeposit organic matter deposited and stored in the fall is being remineralized and denitrified in the spring. There are now five years of April flux data indicating the need to track denitrification into the following spring in order to capture the full level of denitrification enhancement from oyster aquaculture. In addition, the spring fluxes provide further evidence of interannual variability. For example, the average ammonium efflux rate for treated cores was more than three times higher in 2021 compared to 2019 and 2020. The average ammonium efflux rate for control cores was nearly 40 times higher in 2021 compared to the average for 2019/2020 control cores. This difference cannot be attributed to interannual temperature differences (temperatures = 11.8, 10.6, and 12.8 in 2019, 2020, and 2021, respectively) nor biodeposit influence given that the core locations have never been within the biodeposit impact area. Similar to previous years, the 2021 average ammonium efflux from sediment receiving biodeposits was greater than the average background (control) rate, which suggests that the sediments were storing biodeposit N from the fall. However, the effect of amphipods was clear in the much greater ammonium flux from both control and treated sediments, as the amphipods have colonized both areas.

The October 2021 Oyster Effect (0.7 mMoles/m^2/d; Table 4.2) was comparable to previous years and equivalent to the Oyster Effect determined in October 2020. The August 2021 Oyster Effect (0.5 mMoles/m^2/d) was less than that measured in previous years and one-third the enhancement determined in August 2020. The combined Oyster Effect (treated minus control) for the 2021 oyster growing season was low compared to previous years; however, the overall denitrification rates (treated and control) were greater compared to previous Project years due to the stimulation of coupled nitrification-denitrification in both sediment areas due to the increased sediment oxidation. The 2021 denitrification rates were 1.8 and 4.9 times higher in treated and control cores, respectively, in August 2021 compared to August 2016-2020 averaged rates, and 1.3 and 3.8 times higher in treated and control cores, respectively, in October 2021 compared to October 2016-2020 averaged rates. The result being a larger N removal through denitrification, but a smaller net N removal through denitrification in sediments associated with oyster aquaculture. However, even though the control cores were not directly affected by oyster aquaculture through biodeposition, the gradual appearance of amphipod mats in the southern portion of the Pond since the start of the Project suggests that oyster aquaculture has had a positive effect on overall pond water and habitat quality, particularly in the region of the southern oyster deployment area (~20 m away from oyster bags). Oyster aquaculture is the only nitrogen reduction action that has been implemented to affect Lonnie’s Pond over the past decade. We hypothesize that the improvements due to oyster activities have been sufficient to shift the benthic community to amphipods and that the presence and bio-irrigation activities of mat forming amphipods have increased sites of coupled nitrification-denitrification within the core collection area leading to greater denitrification rates in all cores. It is possible that the amphipod effect has overwhelmed the Oyster Effect in Year 6 by increasing rates of nutrient cycling in all cores. However, this result is likely temporary as the reservoir of compounds (e.g., NH_4^+) being mined by the amphipod colonies from deeper sediment layers are finite. In other words, it appears that the continuing oyster deployments have resulted in enough improvement that extensive amphipod mats could develop which has enhanced denitrification in both sediments receiving biodeposits and adjacent areas. This should further increase improvements in nitrogen related water quality to the extent that the amphipod mats continue in coming years.

Table 4.2 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).

	Year 1 Mean Denitrification Rates (mMoles/m^2/d)				
	Treated		Background		Oyster Effect
Date	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

	Year 2 Mean Denitrification Rates (mMoles/m^2/d)				
	Treated		Background		Oyster Effect
Date	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

	Year 3 Mean Denitrification Rates (mMoles/m^2/d)				
	Treated		Background		Oyster Effect
Date	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

	Year 4 Mean Denitrification Rates (mMoles/m^2/d)				
	Treated		Background		Oyster Effect
Date	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

	Year 5 Mean Denitrification Rates (mMoles/m^2/d)				
	Treated		Background		Oyster Effect
Date	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
4/19/21	4.5	2.7	1.9	1.8	2.6

	Year 6 Mean Denitrification Rates (mMoles/m^2/d)

	Treated		Background		Oyster Effect
Date	Mean	Std. Dev.	Mean	Std. Dev.	
8/8/21	4.7	0.9	4.2	1.9	0.5
10/11/21	3.2	4.1	2.5	0.8	0.7
4/XX/22	TBD	TBD	TBD	TBD	TBD

A summary of the measured N removal from Lonnie's Pond via oyster harvest and enhanced denitrification during the 6 years of study is found in Table 4.3. 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, although if the amphipods continue, adding some additional denitrification might be justified (it is a shift from the MEP). Weighting of rates obtained during different parts of the season allowed the determination of annual nitrogen removal by denitrification (DeN_2). The Year 5 spring enhancement was the highest recorded over the course of the Project and removed an additional 6.48 kg N from the South deployment area (adding to the 55.6 kg N South deployment area removal reported for the 2020 oyster growing season). Total enhanced annual denitrification for Year 6 (2021) resulted in a net removal of 4.94 kg N. However, spring carryover related denitrification from the 2021 oyster deployment has not yet been measured; therefore, Year 6 net N loss is an underestimate at this date and cannot be directly compared with the previous five project years. Comparing only the oyster growing period, the total denitrification rates (treated plus background) in August and October 2021 (Table 4.2) were double the total denitrification rates determined for August and October 2020, and N removal from harvest of the South deployment area increased by 2.5 kg from 2020 to 2021.

Table 4.3 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 6 (2021) data. The Year 6 (2021) enhanced annual denitrification rate will be updated with spring 2022 carryover effects when the data is available and will almost certainly result in the greatest N removal by oyster aquaculture during the project period.

Year	**Year 1 (2016)**	**Year 2 (2017)**	**Year 3 (2018)**	**Year 4 (2019)**	**Year 5 (2020)**	**Year 6 (2021)**
Deployment Duration (days)	146	195	240	155	279	287
Enhanced Annual DeN₂ (mmol/m^2 N)	298	253.3	269.0	155.9***	383.8	122.1****
Enhanced Annual DeN₂ (g/m^2 N)	4.17	3.55	3.77	2.18***	5.38	1.71****
Impact area (m^2)	2287	2735	3250	2890	2890	2890
Total Annual Enhanced DeN₂ (g N)	9537	9709	12247	6309	15536	4944
Total Annual Enhanced DeN₂ (kg N)	9.54	9.71	12.25	6.31***	15.54	4.94****
Net Annual N removed by oysters[13] (kg N)	39.1	27.2	36.2 [57.3]*	30.8 [61.6]*	46.6 [93.1]*	49.1 [98.3]*
Enhanced DeN₂ as percent of N removed by oysters	24.4%	35.7%	33.8%**	20.5%**	33.4%**	10.1%**

* 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 2022 rates will be added once they become available.

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[13] 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. N removal in oyster harvest in sections below.

5.0 Oyster Aquaculture Deployment and Removal

Based upon refinements stemming from the findings of the 2020 Lonnie's Pond Annual Report and agreed upon by the Town, Aquaculture Contractor, and Monitoring Contractor, deployment of year two (YR2) oysters into Lonnie's Pond began on March 10th, 2021. Oysters were placed in floating bags to grow and increase biomass within the same footprint on the pond watersheet as in 2020.

Prior to deployment, oysters were sorted by the Aquaculture Contractor and classified into 3 size categories (by inches): YR-1 (< 1.25"), YR2-2 (1.25 – 1.75"), and YR-3 (> 1.75"). A subsample was collected of each size class for nitrogen analysis and to determine the initial mass of oysters entering Lonnie's Pond (Table 5.1). In addition, 17 floating bags from each size class were randomly tagged (51 bags total) to specifically monitor growth and time of removal for size classes. All 2,040 floating bags were marked by an identification tag containing a unique serial number. Identification tags were used by the Monitoring Contractor to facilitate an accurate count of the total bags deployed (input) or removed (output) from Lonnie's Pond on each event date. The Aquaculture Contractor approximated ~100 oysters per bag, regardless of size class; therefore, approximately 204,000 individual oysters were deployed in March 2021 with a total weight of 2843.46 kg (Table 5.2).

Oyster farm maintenance, including deployment, management and bag rotation, was led by the Aquaculture Contractor and continued through the growing season, 287 days. Twenty-five percent of the population deployed in March remained in Lonnie's Pond for the full period until December. The other 75% was harvested in June and July (83 to 139 days in pond) and replaced with YR1 oyster seed that continued to grow until December (129 to 170 days in pond). As floating bags were recovered and oysters harvested in summer and winter seasons, subsamples were taken from monitored bags, as well as randomly selected bags. Measurements were taken from bags for live and dead oyster weights, counts of live and dead oysters, and the empty bag mass to determine net oyster weight when filled bags were weighed. In addition, 20-30 oysters were collected for nitrogen content analysis. Total oyster weights were calculated through the combined use of weights from the scale at the Orleans Transfer Station and subsamples of individual bags during each input/output event date.

From June 1 through July 19, 2021, a total of 1,333 individual bags with identification tags were recovered. As bags were removed from Lonnie's Pond on a weekly basis, YR2 oysters were harvested from the bags and YR1 oyster seed was put in and the bags redeployed to maintain the oyster population and continued removal of carbon and nitrogen via oyster aquaculture. This removal and replacement process of YR2 and YR1 oysters continued over 9 events. Large quantities of YR1 oysters were bundled for transport and a subsample was measured from each event (Table 5.3). The total weight of YR1 seed deployed in Lonnie's Pond was 370.04 kg. In addition, a subsample of YR2 oysters was collected on each output date for oyster weight and counts per bag (Table 5.4) as well as nitrogen analysis. From the total number of bags removed and mean count per bag, an estimated 173,260 oysters were removed from Lonnie's Pond weighing a total of 5159.16 kg (Table 5.5).

Over December, with the exception of one Orleans Transfer Station date in October, all oysters (2040 bags) were removed over 6 event dates, which includes YR1 oyster seed deployed in July and the remaining YR2 oysters deployed in March (Table 5.6). Using the same methods in the March deployments and summer harvest and deployments, all December harvest dates were subsampled for oyster counts and weights per individual bag (Table 5.7). In addition, identification tags were counted to accurately assess the total number of bags removed from Lonnie's Pond on each harvest date. Most of the total mass of oyster aquaculture materials removed from Lonnie's Pond was as live oysters (93%), followed by dead oysters (empty valves; 7%), and fouling material on the oyster shells (<1%). Assuming only live oysters were deployed in March, the survival rate of YR2 oysters removed in the summer was 88% and the stocking density was approximately 113 oysters per bag. The survival rate of all oysters, YR2 and YR1, removed in December was 54%, which was comprised of small YR2 oysters deployed in March (about 126 individuals per bag) and YR1 oyster seed deployed in July (about 461 individuals per bag).

For each oyster group deployed, oyster subsamples were collected for % nitrogen content (whole oyster, shell, tissue, and fouling material) to allow determination of the mass of nitrogen removed in whole oysters and their components. The % nitrogen data, coupled with measured wet to dry weight ratios for oysters from each event date (input and output) was used to determine the total nitrogen in the oyster deployments and harvests when coupled with the total wet weights of oysters measured on the Transfer Station scale. Chemical analysis followed procedures specified in the QAPP to determine the initial nitrogen mass of the deployed oysters and cumulative mass in harvest (Tables 5.8-10 and Figure 5.1). Measuring the % nitrogen in shell and fouling material (amphipods, sediment deposition, algae etc.) collected on the shell during harvest allows for accounting nitrogen removal in dead oysters or shell hash separately from the live oysters. In general, %N content (dry weight) of oysters was not significantly different for YR1 (mean = 0.9 %N) and YR2 (0.7 to 1.0 %N; Table 5.9) at time of harvest. Collectively, the oysters (YR1 and YR2) in Lonnie's Pond incorporated a total of 98.25 kg of nitrogen in their tissues and shells during growth after their initial deployment.

All data collection followed the protocols in the MassDEP approved QAPP and guidelines, including modifications discussed prior to the 2020 growing season. Monitoring Contractor, CSP/SMAST, staff tracked all removals, redeployments, and new deployments of oysters throughout the 2021 season with the exception of 4 events (July 15, July 22, July 29, and October 25) conducted solely by the Aquaculture Contractor. Tables 5.2, 5.3, 5.5, and 5.6 summarize key tracking results during the 2021 growing season. Figure 5.1 summarizes the cumulative nitrogen removal in oyster harvest, including input and output data, throughout 2021.

Table 5.1 Oyster subsampling during March 2021 to estimate the deployed oyster population and verify mean oyster weight by size class and combined weights from the Transfer Station scale.

Age Class	Size Class	Totes Subsampled (n)	Mean Tote Weight by Size Class (kg)	Total Totes per Size Class (N)	Bags Subsample (n)	Mean Oyster Weight per Bag (kg)	Bags per Total Population (%)
YR2	SM	4	22.41	17	16	0.69	32.11
YR2	MD	12	23.73	50	34	1.38	49.90
YR2	LG	11	22.83	28	30	2.06	17.99

Table 5.2 Total deployment of oysters by weight to Lonnie's Pond in March 2021, including deployment events by date in accordance with weigh station records.

Date	Age Class	Bag#/Event	Oyster Wt. (kg)
3/11/2021	YR2	78	128.44
3/16/2021	YR2	185	202.16
3/19/2021	YR2	421	693.82
3/23/2021	YR2	501	810.66
3/26/2021	YR2	493	548.60
3/30/2021	YR2	362	459.78
Total:		**2040**	**2843.46**

Table 5.3 YR1 oyster seed input to Lonnie's Pond in July 2021. The same n-value of scoops (1 scoop per floating oyster bag) was weighed (kg) and counted for individuals (#). The total weight of YR1 seed as input to Lonnie's Pond was calculated from the mean.

Date	INPUT/ OUTPUT	YR1 Seed Bundle # (n)	Mean YR1 Seed Bundle Wt. (kg)	Bundles/Event (#)	Mean Wt./Scoop (kg)	Mean Count (#)
7/5/2021	INPUT	6	7.52	6	0.29	532
7/12/2021	INPUT	6	4.62	6	ND	ND
7/19/2021	INPUT	6	5.45	13	0.43	567
7/20/2021	INPUT	5	8.19	13	0.42	460
7/27/2021	INPUT	4	9.23	13	0.28	587
TOTAL		**27**	**7.28**	**51**	**0.35**	**535**

Table 5.4 Subsample measurements of oysters upon harvest from Lonnie's Pond in June and July 2021, showing the mean oyster count (#) and weight (kg) per bag recorded on each harvest event.

Date	Age Class	Size Class	Bag # (n)	Average Oyster Count/Bag/Event (#)			Average Oyster Weight/Bag/Event (kg)		
				Live	Dead	All Oysters	Live	Dead	All Oysters
6/1/2021	YR2	MD	10	100	7	107	1.88	0.04	1.93
6/7/2021	YR2	Random	5	120	3	123	2.46	0.05	2.56
6/14/2021	YR2	Random	5	106	12	118	3.50	0.04	3.54
6/21/2021	YR2	Random	10	102	4	106	2.76	0.04	2.80
7/5/2021	YR2	Random	8	73	39	112	1.71	0.17	1.89
7/12/2021	YR2	LG	6	77	4	81	4.81	0.10	4.98
7/19/2021	YR2	Random	7	115	12	128	5.98	0.13	6.12
7/27/2021	YR2	Random	7	121	19	140	6.02	0.20	6.24
Total:				**100**	**13**	**113**	**3.75**	**0.10**	**3.88**

Table 5.5 Total weight of YR2 oysters harvested from Lonnie’s Pond in June and July 2021 (5159.16 kg), as well as the number of bags removed and identification tags collected on each event date. Oyster weight (kg) was determined from Orleans Transfer Station truck scale weights (oyster filled truck weight minus empty truck weight); scale weights were provided by the Aquaculture Contractor.

Date	INPUT/OUTPUT	Bag#/Event	Oyster Wt. (kg)	Tags Collected (#)
6/1/2021	OUTPUT	170	521.41	180
6/7/2021	OUTPUT	170	433.09	154
6/14/2021	OUTPUT	170	583.14	169
6/21/2021	OUTPUT	170	581.65	170
7/5/2021	OUTPUT	170	449.48	155
7/12/2021	OUTPUT	170	329.34	157
7/15/2021	OUTPUT	105	333.64	ND
7/19/2021	OUTPUT	105	417.81	157
7/22/2021	OUTPUT	105	630.59	ND
7/26/2021	OUTPUT	105	459.55	101
7/29/2021	OUTPUT	90	419.46	90
TOTAL		**1530**	**5159.16**	**1333**

Table 5.6 Total weight of YR1 and YR2 oysters harvested from Lonnie’s Pond in December 2021, as well as the number of bags removed and identification tags collected on each event date. Oyster weight (kg) was determined from Orleans Transfer Station truck scale weights (oyster filled truck weight minus empty truck weight); weights were provided by the Aquaculture Contractor.

Date	INPUT/OUTPUT	Bag Count(#)	Oyster Wt. (kg)	Tags Collected(#)
10/25/2021	OUTPUT	85	553.38	ND
12/3/2021	OUTPUT	102	372.10	102
12/13/2021	OUTPUT	102	442.80	105
12/14/2021	OUTPUT	102	572.87	99
12/17/2021	OUTPUT	102	745.69	105
12/19/2021	OUTPUT	816	4070.97	691
12/21/2021	OUTPUT	731	3045.89	747
Total		**2040**	**9803.70**	**1849**

Table 5.7 Subsample measurements of oyster in December 2021 harvest from Lonnie’s Pond, showing the mean oyster count (#) and weight (kg) per bag per event.

Date	Age Class	Size Class	Bag # (n)	Mean Oyster Count/Bag/Event (#)			Mean Oyster Weight/Bag/Event (kg)		
				Live	Dead	All Oysters	Live	Dead	All Oysters
12/3/2021	YR2	SM	8	42	76	117	2.35	0.52	2.87
12/13/2021	YR2	SM	7	63	65	128	3.97	0.68	4.66
12/14/2021	YR2	Random	8	63	70	132	3.90	0.66	4.56
12/17/2021	YR2	Random	5	74	56	130	5.65	0.63	6.27
12/20/2021	YR1	Seed	20	286	206	492	3.72	0.30	3.96
12/22/2021	YR1	Seed	22	276	155	431	3.80	0.33	4.15
Total			**70**	**108**	**93**	**201**	**3.78**	**0.55**	**4.20**

Table 5.8 Carbon and nitrogen analysis of subsampled oysters on deployment using the length (mm) and weight (g) of individual oysters (n). Based on a percent by weight of the whole oyster (shell + tissue + fouling material), the total carbon (kg) and nitrogen (kg) input to Lonnie's Pond was calculated for each deployment date. The C/N content per gram dry weight was multiplied by the oyster dry/wet (harvest) weight ratio, and the mass of oysters harvested on each date (Table 5.2 and 5.3) to determine total C and N (kg) input by event. In total, 451.12 kg of carbon and 24.48 kg of nitrogen was in oyster when deployed into Lonnie's Pond.

Date	Input/ Output	Age Class	Whole Oyster					Event Input	
			Length (mm)	Total Wt. (g)	n	C by Wt. (%)	N by Wt. (%)	Total C (kg)	Total N (kg)
3/11/2021	INPUT	YR2						18.04	0.96
3/16/2021	INPUT	YR2						28.39	1.52
3/19/2021	INPUT	YR2	54.4	15.1	70	14.0	0.8	97.45	5.21
3/23/2021	INPUT	YR2						113.86	6.08
3/26/2021	INPUT	YR2						77.05	4.12
3/30/2021	INPUT	YR2						64.58	3.45
7/5/2021	INPUT	YR1	18.3	0.6	20	14.6	1.0	6.58	0.47
7/12/2021	INPUT	YR1	18.0	0.5	21	15.8	1.2	4.39	0.33
7/19/2021	INPUT	YR1	19.3	0.6	20	15.0	1.2	10.63	0.87
7/20/2021	INPUT	YR1	23.6	1.3	21	13.2	0.6	14.08	0.66
7/27/2021	INPUT	YR1	16.1	0.5	22	13.4	0.7	16.07	0.81
Total INPUT (YR1+YR2; kg)								**451.12**	**24.48**

Table 5.9 Carbon and nitrogen analysis of subsampled oyster data using the length (mm) and weight (g) of individual oysters (n). Based on a percent by weight of the whole oyster (shell + tissue + fouling biota), the total carbon (kg) and nitrogen (kg) output from Lonnie's Pond was calculated for each harvest date. The C/N content (dry weight) was multiplied by the oyster dry/wet (harvest) weight ratio, and the percent live/dead oysters and mass of oysters harvested on each date (Table 5.5 and 5.6) to determine total C and N (kg) removed on each harvest date. In total, 1972.06 kg of carbon and 122.73 kg of nitrogen was removed as gross output from Lonnie's Pond.

Date	Input/ Output	Age Class	Whole Oyster					Event Output	
			Length (mm)	Total W.t (g)	n	C by Wt. (%)	N by Wt. (%)	Total C (kg)	Total N (kg)
6/1/2021	OUTPUT	YR2	62.6	20.2	30	14.7	1.0	76.08	5.07
6/7/2021	OUTPUT	YR2	50.9	14.9	5	13.9	0.7	58.82	2.80
6/14/2021	OUTPUT	YR2	69.2	24.9	6	14.5	0.9	84.22	5.45
6/21/2021	OUTPUT	YR2	65.6	26.5	21	14.5	1.0	84.03	5.52
7/5/2021	OUTPUT	YR2						64.01	4.02
7/12/2021	OUTPUT	YR2	85.8	54.4	20	13.5	0.7	43.65	2.33
7/15/2021	OUTPUT	YR2						44.22	2.36
7/19/2021	OUTPUT	YR2	81.5	52.5	20	14.1	0.9	58.49	3.68
7/22/2021	OUTPUT	YR2						88.28	5.55
7/26/2021	OUTPUT	YR2	75.8	44.2	22	13.6	0.7	62.01	3.14
7/29/2021	OUTPUT	YR2						56.60	2.87
10/25/2021	OUTPUT	ND						74.02	3.36
12/3/2021	OUTPUT	YR2				13.6	0.7	49.77	2.26
12/13/2021	OUTPUT	YR2				14.6	0.9	62.99	3.39
12/14/2021	OUTPUT	YR2	80.9	57.4	19	14.5	0.9	81.38	4.81
12/17/2021	OUTPUT	YR2						106.55	6.46
12/19/2021	OUTPUT	YR1				12.4	0.9	506.61	34.51
12/21/2021	OUTPUT	YR1						370.33	25.15

Total OUTPUT (YR1+YR2; kg)	**1972.06**	**122.73**

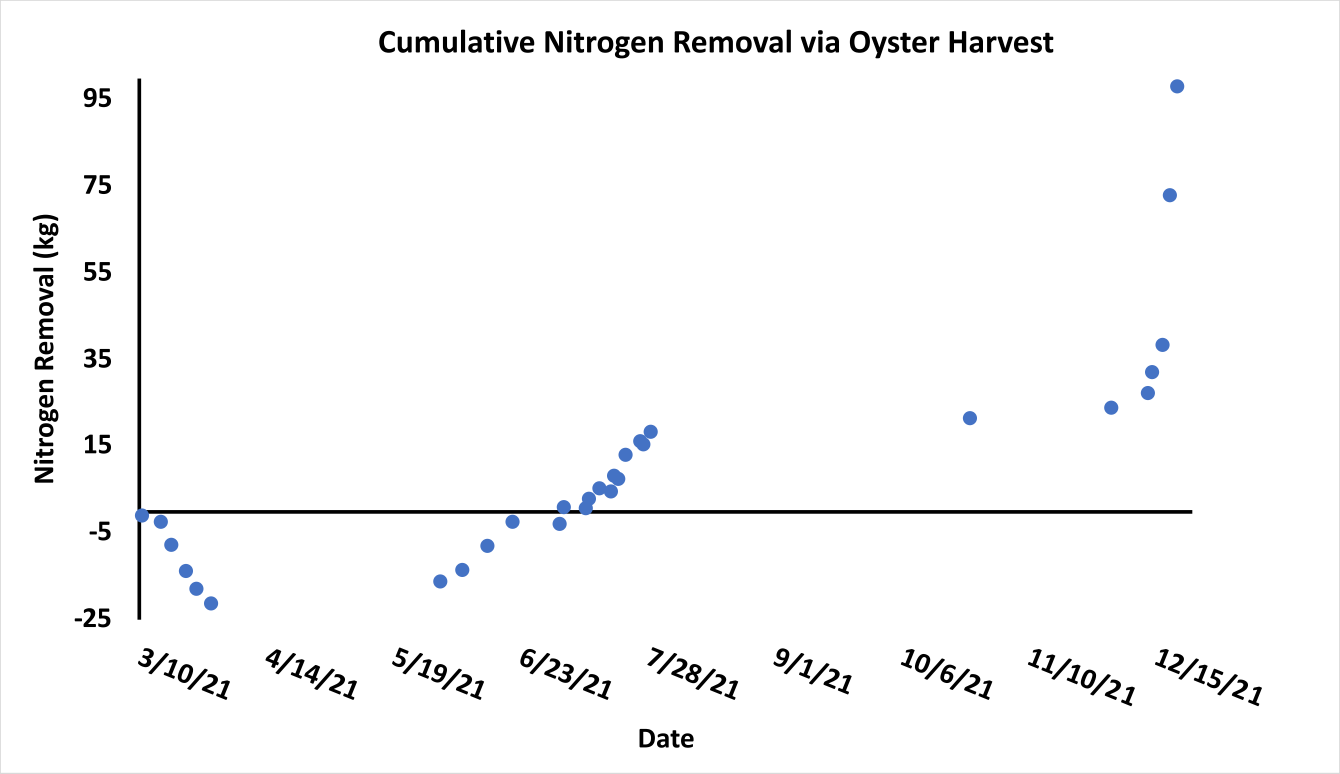

Figure 5.1 Cumulative 2021 Lonnie's Pond N removal via N assimilation into oyster biomass and subsequent oyster harvest. Nitrogen removal (kg) was determined using the total N mass of oysters deployed or removed from the Pond on each input/output event date. Total N mass was summed over the 287 day oyster growing season. Oyster deployments (input) represent a negative N removal and oyster harvest (output) represents a positive N removal. The total net nitrogen removal including live oysters, shell, and fouling material was 98.25 kg.

6.0 Key Findings and Future Considerations

As for the 2020 growing season, the 2021 oyster deployment exceeded the initial goal outlined in the Lonnie's Pond Aquaculture and Nitrogen Management Plan of 75 kg N removal in oyster harvest after accounting for N mass upon deployment. CSP/SMAST staff working with Ward Aquafarm and Town staff were able to document 98.25 kg N net removal, in the Lonnie's Pond 2021 oyster harvest. We also documented ways to make future deployment and monitoring more efficient. Key Project findings and considerations for future deployment include:

1. In 2021 the Orleans oyster aquaculture program removed a total net N mass from Lonnie's Pond by oyster harvest of 98.25 kg N (recovery (harvest)–deployment). The removal was equally divided between the southern and northern deployment areas. N removal by oyster enhanced denitrification within sediments receiving biodeposits was 4.94 kg N from the southern deployment area only and not including the April 2022 removals (not yet measured). *Therefore, total nitrogen removal from Lonnie's Pond associated with the 2021 deployment was at least 103.1 kg N and as high as 108.1 kg N if the removal of nitrogen through denitrification was the same at both deployment sites.*

2. In recent years amphipods have been colonizing the southern deployment region, almost certainly due to oyster activities that have been sufficient to shift the benthic community to amphipods. The presence and bio-irrigation activities of mat forming amphipods have increased sites of coupled nitrification-denitrification within the core collection area leading to greater denitrification rates in all cores. It appears that the continuing oyster deployments have resulted in enough improvement that now extensive amphipod mats have developed which has enhanced denitrification in both sediments receiving biodeposits and adjacent areas. This should further improve nitrogen related water quality throughout the pond to the extent that the amphipod mats continue in coming years. It is important to track these changes and possibly account for these changes in the overall N balance of Lonnie's Pond.

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 can lead to uncertainties in nitrogen removal. Procedures for removal of YR2 and reincorporation of YR1 stock changed over the season as 1,530 of 2,040 YR2 bags were replaced with YR1 oyster seed. The remaining 510 YR2 bags remained in the water until December.

4. Use of only the Orleans Transfer Station Scale "truck scale" for harvest wet weight determinations has been found to be the most accurate and reliable approach. Though manageable, transport to more distant scales has historically resulted in increased variability 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 truck weight loss from fuel usage or water loss as bags and oysters dry during transport (truck is weighed prior to adding bags, so that fuel usage results in under estimates of removals.

5. 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. Zip ties contained a unique serial number as well as sealed the bag opening to reduce oyster loss. Upon removal from Lonnie's Pond, zip ties are removed from bags and replaced with a new zip tie prior to redeployment. This reduces waste as the zip tie sealing the bag was also the identification tag. This procedure increases labor and material efficiency, and vigilance as removed tags are more likely to be collected as tags must be removed to open the bags.

6. Future monitoring of water quality can include less sites since the 2 deployment areas appear to be 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.

7. Coordination between the Town, aquaculture contractor and monitoring contractor is essential. The procedures developed over previous years are working very well and need to be codified by the Town and followed in future years. Written protocols will save both contractors time and streamline the effort.