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

2022 Lonnie’s Pond Annual Report: Aquaculture N Removal/TMDL Goal

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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.*, oyster aquaculture, PRB's, etc.). These efforts reflect an informed community and economic links to healthy ecosystems and clean water, and a goal 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 within the Town of Orleans, including Lonnie's Pond, as being impaired by nitrogen enrichment.[1] The Massachusetts Estuaries Project (MEP) analysis for Pleasant Bay[2], which is the technical basis for the TMDLs, suggested that the nitrogen load to Lonnie's Pond would need to be lowered by 300 kg N/yr to mitigate the impairments as the remainder of the bay is restored.

In 2016, the Town began a demonstration project in Lonnie's Pond to evaluate a non-traditional, nitrogen reduction approach using oyster aquaculture. The Lonnie's Pond Demonstration Project was planned as a three-year effort to evaluate the water quality impacts and determine any implementation issues associated with enhanced aquaculture for nitrogen reduction as part of achievement of the TMDL without sewering within the Pond watershed. Monitoring during the demonstration project found significant removal of nitrogen and some water quality improvements due to shellfish growth and biodeposition. However, it was noted that full restoration of Lonnie's Pond will require nitrogen mitigation within the larger upper Pleasant Bay/The River watershed as well.[3]

In 2018, the Town approved the Lonnie's Pond Aquaculture and Nitrogen Management Plan[4] to transition from an oyster aquaculture demonstration project to long-term implementation of nitrogen removal by 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 nitrogen management program for its estuaries, including two regular reports on Plan implementation to be prepared by the Monitoring Contractor: a Semi-Annual Status Update and Annual Report. A Quality Assurance Project Plan (QAPP) reflecting the Management Plan was submitted and approved by MassDEP in May 2019 to ensure regulatory acceptance of collected data for TMDL compliance.

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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.

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

Based on 2019 monitoring and synthesis, CSP/SMAST determined that oyster harvest removed 60 kg of N from Lonnie's Pond between July 15 and December 9, 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 100.2 kg N or an additional 25.2 kg N[5] above the selected goal of 75 kg/yr. As a result, the program changes were extended to the 2021 and 2022 project years.

This document is the 2022 Annual Report. It is the second of two regular reports on the Lonnie's Pond Aquaculture Management Plan prepared by the CSP/SMAST, the first being the Semi-Annual Status Update[6] that was previously submitted to the Town. This 2022 Annual Report includes the N removal attained during the 2022 growing season, determined through oyster tracking and subsampling data during oyster deployment and harvest. It also details water column conditions, sediment impacts, and stream inflows for Pilgrim Lake.

YR2 oysters were deployed April 5 and 7, which is approximately one month later than in 2021. Approximately half of the YR2 population was removed July 11 and 12 by the Aquaculture Contractor and the remaining half was removed over a month period from November 8 through December 5, 2022. YR2 oysters harvested in July 2022 were replaced with YR1 oyster seed. All YR1 seed was removed from the Pond by December 21. The CSP/SMAST monitoring team tracked all the oyster additions and removals, as well as water quality and sediment nutrient regeneration/denitrification throughout this period.

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[5] 2020 Nitrogen removal via incorporation into oyster soft tissue and shell, and subsequent harvest from Lonnie's Pond has been corrected and updated since the 2020 Annual Report.

[6] CSP/SMAST. 2022. Lonnie's Pond Aquaculture/TMDL Semi-Annual Status Update. 8 pp.

2.0 Water Quality Monitoring

2022 Overview

Using procedures detailed in the Lonnie's Pond QAPP, CSP/SMAST staff began conducting biweekly water quality monitoring in Lonnie's Pond starting on April 6, 2022 (<24 hrs after the initial YR2 oyster deployment). During 2022, staff completed twelve (12) sampling events: April 6, May 10, May 24, June 8, June 21, July 7, July 20, August 4, August 18, August 31, September 19, and October 18, 2022 (Table 2.1). During each sampling event, water quality samples were collected at nine locations in Lonnie's Pond (Figure 2.1). In addition to the sampling event, staff deployed two continuous monitoring devices (sondes) May 24 at 0.30 m depth directly east and west of southernmost oyster deployment area (located at stations LP-5 and LP-6; Figure 2.1). These sondes were programmed to record chlorophyll-*a*, dissolved oxygen, salinity, temperature and depth every 15 minutes and were deployed until October 18. At the time of each sampling event, water clarity (Secchi depth), temperature, light and dissolved oxygen profiles were also collected. Collected water samples were transported to the CSP Analytical Facility to be 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, staff also collected stream flow and water quality measurements biweekly at the herring run from Pilgrim Lake (see Figure 2.1; "LP-Stream"). A continuous stage meter is deployed in the stream at the same location to provide data for determining daily volumetric freshwater inflow.

Table 2.1 Sampling dates for water quality and laboratory assays performed on samples. Note that NH4 is ammonium-nitrogen, PO4 is ortho-phosphorus, TDN is Total Dissolved Nitrogen, POCN is particulate organic carbon and nitrogen (mainly phytoplankton), TSS is total suspended solids, CHLA is total chlorophyll-*a* pigments.

		Assays							
Sample Date	**# of samples**	**NH4**	**PO4**	**NO3/NO2**	**TDN**	**POCN**	**TSS**	**CHLA**	**Salinity**
4-6-22	19	X	X	X	X	X	X	X	X
5-10-22	21	X	X	X	X	X	X	X	X
5-24-22	21	X	X	X	X	X	X	X	X
6-8-22	21	X	X	X	X	X	X	X	X
6-21-22	21	X	X	X	X	X	X	X	X
7-7-22	21	X	X	X	X	X	X	X	X
7-20-22	21	X	X	X	X	X	X	X	X
8-4-22	21	X	X	X	X	X	X	X	X
8-18-22	21	X	X	X	X	X	X	X	X
8-31-22	21	X	X	X	X	X	X	X	X
9-19-22	21	X	X	X	X	X	X	X	X
10-18-22	21	X	X	X	X	X	X	X	X
Total	**250**								

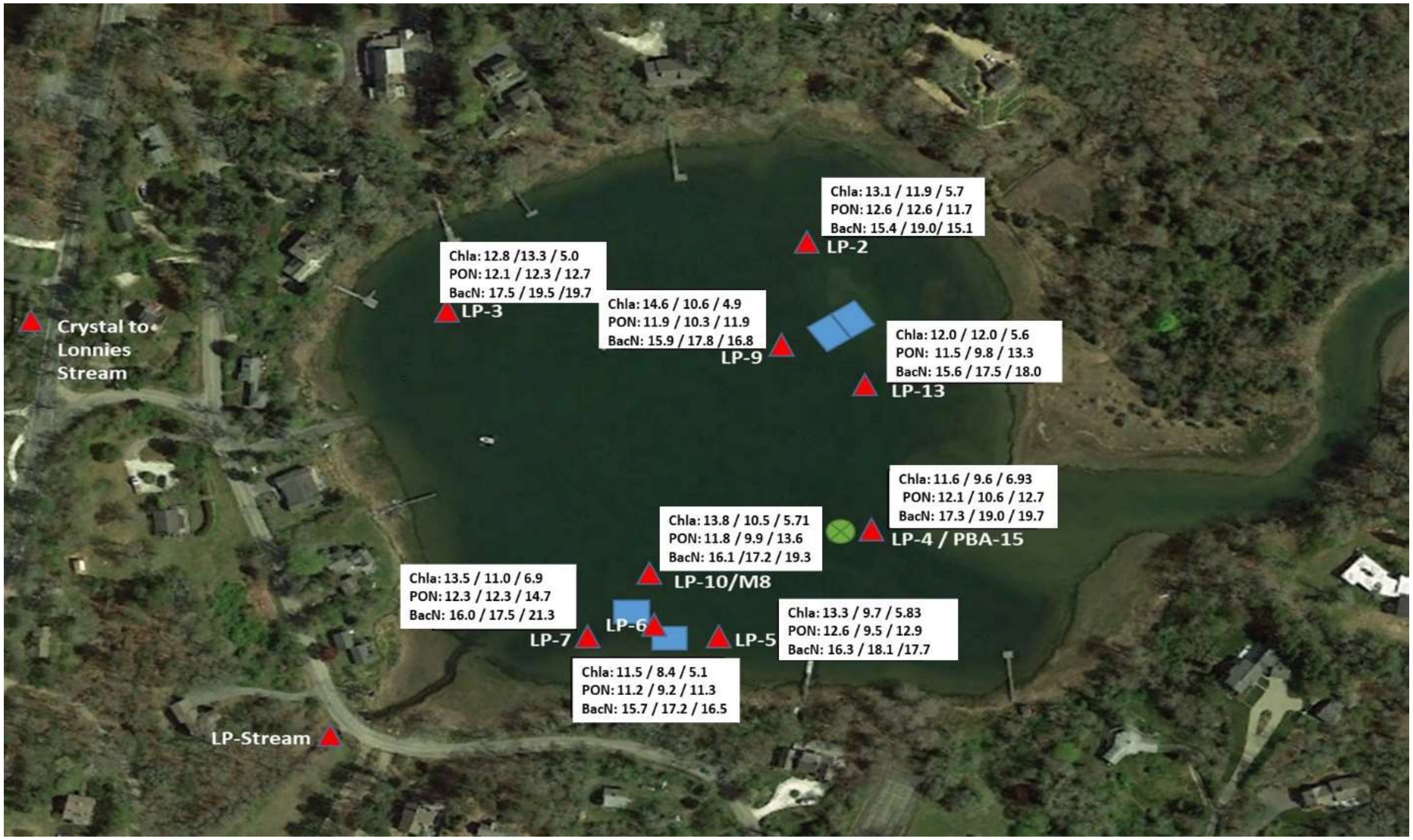

Figure 2.1 Map of Lonnie's Pond 2022 water quality sampling locations and 2020-2022 Average concentrations for Chlorophyll a, PON, and bioactive N. Water column samples were collected biweekly by CSP staff April 6, 2022, to October 18, 2022, at the red triangle locations. Samples were also collected biweekly by Orleans citizen volunteers as part of the Pleasant Bay Alliance (PBA) monitoring program from July 18-September 1, 2022, at the green circle location. The blue squares represent the oyster deployment areas (same areas as 2021). White boxes show mixed layer average concentrations of total-chlorophyll-*a* (µg L^{-1}), particulate organic N (µM), and bioactive nitrogen (µM). Bioactive nitrogen consists of dissolved inorganic nitrogen and particulate organic nitrogen, representing the most biologically active nitrogen within the total nitrogen pool. Values represent averages of samples collected July-October for 2022, 2021, and 2020, the active oyster growth period in those years. Water tends to flow from LP-3 to LP-7 to LP-5 on ebbing tides.

Water quality monitoring indicated that Lonnie's Pond water column was generally horizontally mixed throughout with similar concentrations at all depths. However, differences were noted in each station's proximity to the aquaculture areas with differences between each of the areas, as well as notable spikes in concentrations on May 10 and June 8. Bioactive N was about 10 µM (0.14 mg N/L) in the initial readings in April when the oysters were deployed into Lonnie's Pond, and this indicates that oyster growth was likely not limited by food. The May 10 spike in pigments shows there was a spring phytoplankton bloom that spread across the western half of the pond [*i.e.*, the southern aquaculture area (LP5,6,7,10) and the control station (LP3), but not the northern aquaculture area (LP2,9,13) or the channel station (LP4)] (Figure 2.2). The average pigment concentration for the impacted stations was 32.6 µg/L. Chlorophyll pigment levels generally decreased below 20 µg/L for the rest of the 2022 water quality sampling, except for a smaller spike in concentrations on July 20 that impacted all of the stations throughout the pond (average = 21 µg/L). This July spike was similar, but at a lower peak, to a pigment spike on June 30, 2021. Overall, average chlorophyll pigments were higher in 2022 than in 2021 but followed a similar pattern to 2021 with concentrations fluctuating, but generally decreasing following the mid-summer peak.

Another peak that was different from 2021, was the large spike in PON, but not chlorophyll, on June 8, 2022. PON, which as a component of bioactive N, also created a spike in bioactive N at the southern aquaculture stations (LP5,7,10)(see Figure 2.2). Driving the elevated PON concentration in the southern aquaculture stations, was elevated concentrations at LP7. LP7 is closest to the stream outflow from Pilgrim Lake and review of precipitation records showed that 0.66 inches of rain was recorded on June 8. Storms of this magnitude occur approximately 10% - 14% of all storms in Orleans[7] and is the likely cause for elevated PON concentrations on June 8. These early season spikes in chlorophyll pigments and PON were not measured in 2021.

Station LP3 was initially established as a control site to measure what Lonnie's Pond chlorophyll-*a* concentrations might be in the absence of aquaculture (see Figure 2.1); it is not near the northern or southern aquaculture areas. Chlorophyll levels measured at LP3 generally had the highest recorded chlorophyll in Lonnie's Pond (9 out of 12 sampling events), but there were occasions where its results matched those collected from within the southern aquaculture area (LP6) that may be occasions where wind mixing events overcame tidal-driven mixing.

After early season peaks and the mid-summer pigment peak, water column concentrations of pigments, PON, and bioactive N fluctuated but generally decreased for the rest of sampling season. Following the June 8 spike, all sites showed a sharp decline in PON on June 21 (averaging around 10 µM), with station LP3 having the highest concentration (11.5 µM). PON rose slightly on July 7, but then generally decreased during each remaining 2022 sampling events and remained below 20 µM. PON was typically higher in stations without oysters, LP3 and LP4 (7 out of 12 sampling events). Oysters have been shown to remove nitrogen by consuming PON and phytoplankton, represented as chlorophyll pigments here, from the water column. The occasional higher PON results in stations in and around the aquaculture areas may be a result of bag disturbance while sampling.

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[7] Eichner, E., B. Howes, and D. Schlezinger. 2022. Baker Pond Management Plan and Diagnostic Assessment. Town of Orleans, Massachusetts. Coastal Systems Program, School for Marine Science and Technology, University of Massachusetts Dartmouth. New Bedford, MA. 106 pp.

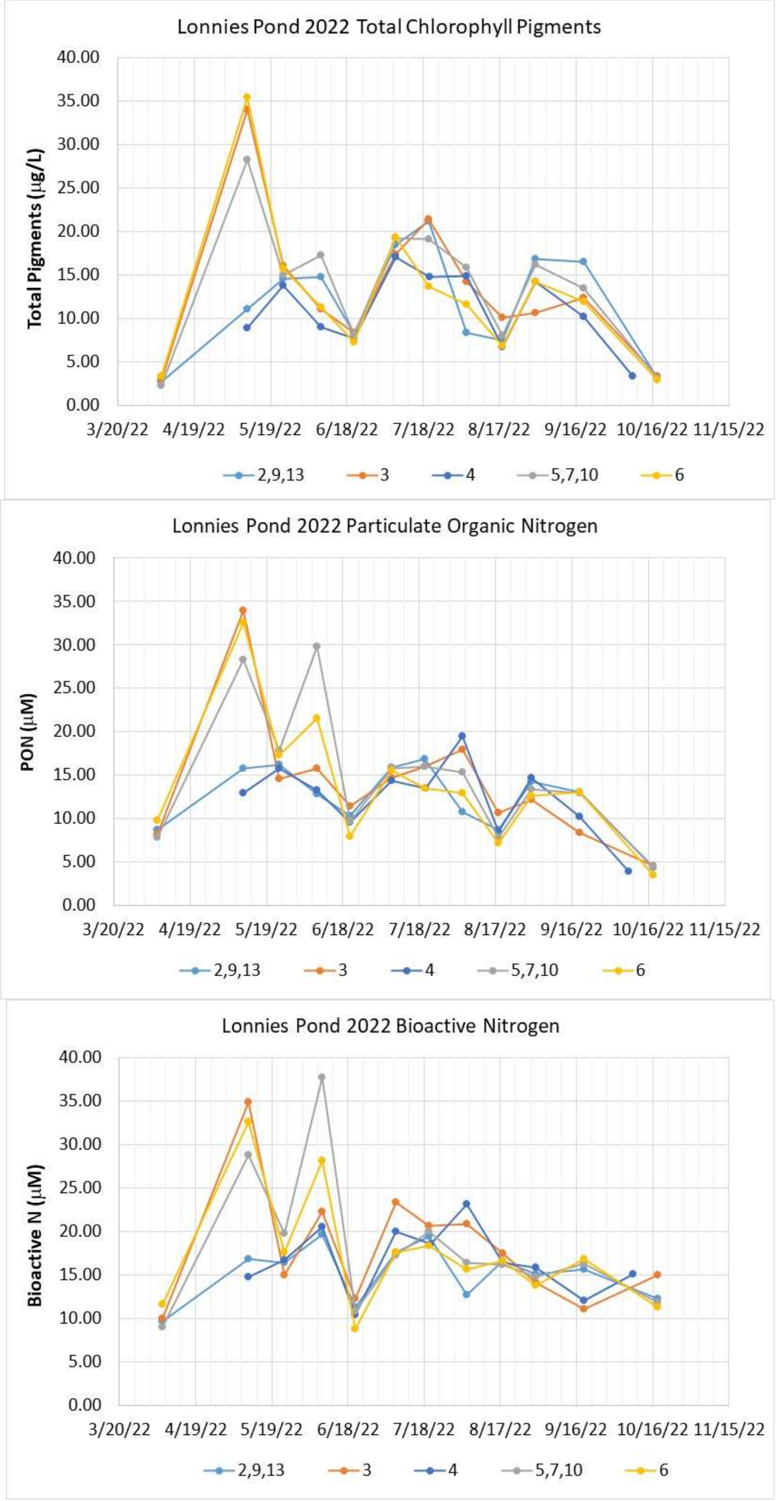

Figure 2.2 Lonnie's Pond 2022 Water Column Assay Results. Time-series of mixed layer average total pigments, particulate organic nitrogen (PON), and bioactive N at stations LP3, LP4, LP6 and averages of the stations and associated with the North (LP2, 9, 13) and South (LP5, 7, 10) oyster deployment. The general trend in 2022 was a lowering of particulate matter (PON, Chl-*a*) as water passed through the oyster deployment areas.

Bioactive N is the combined pools of dissolved inorganic nitrogen (DIN) and PON and represents the most biologically labile or usable form of nitrogen. DIN is the sum of inorganic N components [nitrate+nitrite (NOx) and ammonium (NH_4)] concentrations. Combined high NH_4 and PON drove the bioactive N to have its highest 2022 concentration on June 8, although it also had a spike on May 10 (see Figure 2.2). As with PON, bioactive N concentrations tended to be highest in the stations without oysters (LP3 and LP4); highest bioactive N concentrations in 9 of the 12 sampling events were at LP3 and LP4.

The freshwater stream inputs from Pilgrim Lake and, to a lesser degree, Crystal Lake have been shown in prior monitoring to be key determinants of water column N and their variations in these flows have impacts on water quality measurements. As was noted in the discussion above, streamflow changes from individual precipitation events can impact water quality, but year-to-year changes also have season-long impacts. An increased flow and load from Pilgrim Lake were recorded in spring 2022. April-June inflow from Pilgrim Lake increased 12,147 m^3 compared to 2021 (2021, 66,981 m^3 vs. 2022 flow, 79,129 m^3). This increase in flow translated into an extra 3.59 kg of N load from Pilgrim Lake. Higher flow and load would cause higher TN, bioactive N, and total chlorophyll concentrations at LP7, the station closest to the stream (see Figure 2.2).

Yearly trends in key metrics over the peak growing season for oysters show trends of decreasing bioactive nitrogen, increasing chlorophyll, and stable PON concentrations (see Figure 2.2). The bioactive nitrogen concentrations are also consistently lower in sites associated with aquaculture, apart from LP7 in 2022, which is likely due to stream output. The chlorophyll concentrations have increased yearly over the same time period in all stations except for LP3,

Water Quality Associated with Oyster Aquaculture

During review of the 2022 water column data, project staff reviewed a number of factors to see if the differences in the 2022 oyster deployment resulted in differing water quality results. The grow-out of the YR2 oysters during the spring of 2022 could potentially represent a larger filtering potential in the southern aquaculture area compared to July-October when smaller YR1 seed oysters occupied aquaculture areas. A YR2 oyster averaged 7.38 g and 45.9 mm in length when deployed in April, while YR1 oyster seed deployed in July averaged 0.47 g and 18.1 mm. Comparison of results was also complicated by the high percentage (40%) of dead YR2 oysters deployed (see Table 5.3) and seasonal changes in sediment interactions. In an effort to discover if the larger oysters deployed into Lonnie's Pond during the spring (April- June) filtered more nutrients than the smaller YR1 seed deployed in the summer (July-October), the measured water quality results during these two seasons were compared.

Overall, springtime nutrients were generally higher in the southern aquaculture area compared to the rest of the sites, although some of the increase may have been impacted by the May 10 phytoplankton bloom and the significant June 8 Pilgrim Lake inflow. Springtime water quality metrics showed larger oysters initially deployed into the southern aquaculture area (near stations LP5, 6, 7, 10) in Lonnie's Pond in April coincided with lower water column DIN and TN compared to July through October when Y1 seed deployed and half of the Y2 oysters deployed in the spring were rotated out (Table 2.2, in red). LP5 had higher average spring TN and DIN concentrations than LP3, while LP7 had a higher TN concentration, but a similar DIN. The third southern aquaculture station (LP10) had a higher spring DIN concentration than LP3, but lower TN concentration (see Table 2.2). It is notable that LP2 had the lowest spring DIN and LP13 had the lowest spring TN. Spring TN concentrations at LP5,7,10 were all higher in 2021, but the largest change (+41%) was at LP7, which, as previously noted, had significant impacts from Pilgrim Lake.

Summer water quality showed a different pattern, but most importantly a notable decrease in bioactive N at the southern aquaculture site stations. TN and DIN concentrations increased throughout the system in the July through October measurements, but bioactive N concentrations around the southern aquaculture site (LP5,7,10 stations) were generally lower than the spring averages and comparatively similar to readings from the other stations throughout the system. The highest summer bioactive N concentration was at the background station (LP3). As expected, the decrease was largely driven by decreases in PON concentrations. Not surprisingly, total chlorophyll pigment concentrations also decreased at the LP5,7,10 stations and were comparable to the other stations in the pond.

Statistical comparison of the pigments, PON, bioactive N, DIN, and TN concentrations confirmed that the seasonal changes in PON were statistically significant, but also showed that year-round DIN concentrations were significantly lower at stations impacted by oysters. Paired t-tests showed that there were no significant differences between the average spring and summer pigment, bioactive N or TN at all stations in 2022, but the average summer PON concentration was significantly lower (15.68 µM vs 12.01 µM; $\rho<0.05$) (Table 2.3a). DIN concentrations, on the other hand, were significantly higher during the summer (2.28 µM vs 4.19 µM; $\rho<0.05$) (Table 2.3b). A similar comparison of stations with and without oyster impacts showed that although DIN increased in the summer, the influence of oysters significantly lowered DIN concentrations (3.12 µM with oysters vs 4.04 µM without oysters; $\rho<0.05$). This type of grouping did not show any significant difference between average concentrations for total pigments, PON, bioactive N, or TN. The decrease in DIN with the presence of oysters also occurred in 2019, 2020, and 2021. Mean 2022 DIN results also show the stations associated with oysters are lower compared to sites without aquaculture and have been decreasing overall since 2020 (Figure 2.4).

Table 2.2 Lonnie's Pond mixed-layer water quality station averages from spring (April-June) and summer (July-October) 2022. Average nutrient concentrations of chlorophyll-*a* (Total pigments), particulate organic nitrogen (PON), bioactive N, dissolved inorganic nitrogen (DIN), and total nitrogen (TN) are shown. During spring, YR2 runts (1 inch) oysters filled the southern aquaculture area (LP5,7,10, and LP6; bolded) and only minimally occupied the northern areas array (LP2,9,13). From July-October YR1 oyster seed represented most oysters in Lonnie's Pond along with the rest of the YR2 oysters that were not removed during the July harvest. DIN and TN were generally higher in the summer readings. Nutrients which were lower in April-June vs. July-October in the southern aquaculture area are highlighted.

	April-June 2022					July-October 2022				
Station #	Total Pigments (µg/L)	PON µM	Bioactive Nitrogen µM	DIN µM	TN µM	Total Pigments (µg/L)	PON µM	Bioactive Nitrogen µM	DIN µM	TN µM
2	9.09	12.86	14.69	1.84	38.04	13.07	12.62	15.45	2.84	42.09
9	11.09	12.71	14.73	2.02	36.05	14.35	11.90	15.87	3.97	41.17
13	10.63	12.87	14.93	2.06	35.48	12.03	11.47	15.58	4.11	41.31
3	14.50	16.78	18.89	2.10	42.79	12.77	12.05	17.53	5.48	42.56
4	9.86	12.88	15.63	2.75	39.80	11.62	12.12	17.33	5.20	44.16
6	**14.61**	**15.68**	**19.76**	**1.93**	**40.62**	**11.52**	**11.21**	**15.74**	**4.53**	**49.17**
5	**13.44**	**17.03**	**20.04**	**3.00**	**44.86**	**13.31**	**12.64**	**16.26**	**3.63**	**42.22**
7	**18.50**	**26.40**	**28.54**	**2.14**	**49.82**	**13.54**	**12.31**	**15.95**	**3.64**	**39.85**
10	**12.69**	**13.92**	**16.62**	**2.70**	**38.53**	**13.83**	**11.79**	**16.11**	**4.32**	**43.13**

Table 2.3 Comparison of 2022 Seasonal and Oyster Impacted Average Nitrogen and Pigment Concentrations. Paired t-tests (ρ<0.05) compared mixed-layer station averages sampled from Lonnie's Pond for key N constituents a) during the spring (April-June) and summer (July-October) and b) stations associated with and without oyster aquaculture. Significant differences (in red) are seen in the particulate organic nitrogen (PON) and dissolved inorganic nitrogen (DIN) for pond-wide seasonal station data and in DIN concentrations due to aquaculture effects.

a) All stations compared

	Total Pigments µg/L		PON µM N		Bioactive µM N		DIN µM N		TN µM N	
	Apr-June	July-Oct	Apr-June	July-Oct	Apr-June	July-Oct	Apr-June	July-Oct	Apr-June	July-Oct
mean	12.71	12.89	15.68	12.01	18.20	16.20	2.28	4.19	40.67	42.85
variance	7.67	0.88	17.07	0.21	17.49	0.48	0.16	0.59	18.49	6.33
stdev	2.77	0.94	4.13	0.45	4.18	0.70	0.40	0.77	4.30	2.52
n	9	9	9	9	9	9	9	9	9	9
t	-0.19		2.65		1.42		-6.63		-1.32	
d.o.f	16		16		16		16		16	
critical value	2.12		2.12		2.12		2.12		2.12	
[t] > crital value	-0.19<2.12		2.65>2.16		1.42<2.12		-6.63>2.21		-1.32<2.12	
	no significant diff.		**significantly diff.**		**no significant diff.**		**significantly diff.**		**no significant diff.**	

b) Stations associated with and without oysters

	Total Pigments µg/L		PON µM N		Bioactive µM N		DIN µM N		TN µM N	
	With Oysters	Without	With Oysters	Without	With Oysters	Without	With Oysters	Without	With Oysters	Without
mean	12.95	12.63	13.70	13.00	16.81	17.03	3.12	4.04	41.47	42.23
variance	1.70	1.36	4.10	0.53	4.25	0.59	0.10	0.06	6.67	0.30
stdev	1.3	1.16	2.02	0.53	2.06	0.77	0.31	0.24	2.58	0.55
n	7	3	7	3	7	3	7	3	7	3
t	0.37		0.57		-0.17		-4.49		-0.49	
d.o.f	8		8		8		8		8.00	
critical value	2.31		2.31		2.31		2.31		2.31	
[t] > crital value	0.37<2.31		0.57<2.31		-0.17<2.31		-4.49>2.31		-0.49<2.31	
	no significant diff.		**no significant diff.**		**no significant diff.**		**significantly diff.**		**no significant diff.**	

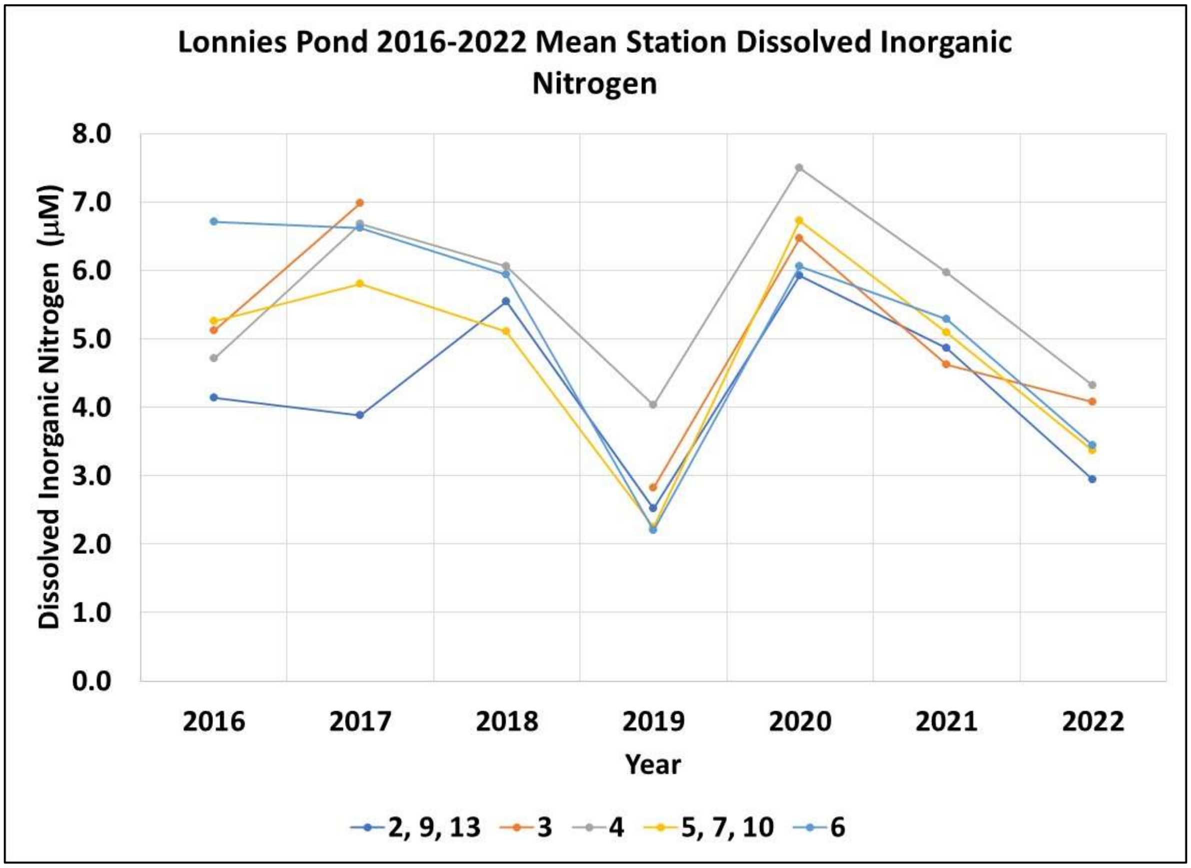

Figure 2.4 2016-2022 Interannual DIN in Lonnie's Pond. Average concentrations in mixed layer of dissolved inorganic nitrogen (DIN) at stations LP3, LP4, LP6 and averages of the stations (LP 5, 7, 10) and (LP 2, 9, 13) surrounding the oyster deployments. These concentrations show variability from year to year with the minima in 2019, but 2022 readings approached this minima. Station numbers refer to locations in station map in Figure 2.1.

Project staff also attempted to refine the oysters' effect on water quality by looking for changes in light penetration (light extinction coefficient) by utilizing Li-Cor light profile data collected during each sampling event at stations upgradient and downgradient of the aquaculture areas. As the tide ebbs, it pulls water out of Lonnie's Pond out through the channel toward Little Pleasant Bay and creates the upgradient to downgradient flow. 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 (*i.e.*, $k = \ln(I_{O)}\text{-}\ln(I_{D)}/d))$. The light extinction coefficient considers the light measurements in relation to the water depth and 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.

In Lonnie's Pond in 2022, light was generally able to penetrate deeper into the water column after water passed through the aquaculture areas. At the southern aquaculture area with flow from LP7 to LP5 passing through the southern aquaculture area, light extinction was higher on the upgradient side (LP7) 55% of the time in 2022 (Figure 2.5). The northern aquaculture area showed a stronger water clearing effect with downgradient LP13 allowing more light to the bottom 64% of the time in 2022. It should be noted that water quality data from samples in defined flow fields typically show significant declines (~30%) from upgradient to downgradient samples (*e.g*., Bournes Pond, Falmouth).

Historical Water Quality Comparisons

CSP/SMAST has been quantifying the efficacy of using shellfish to reduce N loads in Lonnie's Pond since 2016, so it is possible to also investigate the water quality data for any trends over the past seven years. The interannual variation in total chlorophyll-*a* pigments and PON show slight increases in 2022 compared to 2021, but do not reach the highest concentrations recorded, which were seen in 2019 (Figure 2.6). The increases in phytoplankton and PON seen in 2022 apply for all station except LP3, which is located away from either aquaculture area. This may be indicative of oyster's effect to clear the water throughout the pond or may be due to the usually high concentration spike in chlorophyll and PON at the LP3 station in May 2022. As for the long-term bioactive N, there were decreases since 2021 in the northern aquaculture area (LP2,9,13) as well as away from the aquaculture areas (LP3 and LP4). Also, there were only slight increases in bioactive N measured in 2022 for the southern aquaculture area, which are affected by the increased flow and load coming in from the stream from Pilgrim Lake. The bioactive N pool is a source of food for the oysters and can be seen as being assimilated into their shells and tissues.

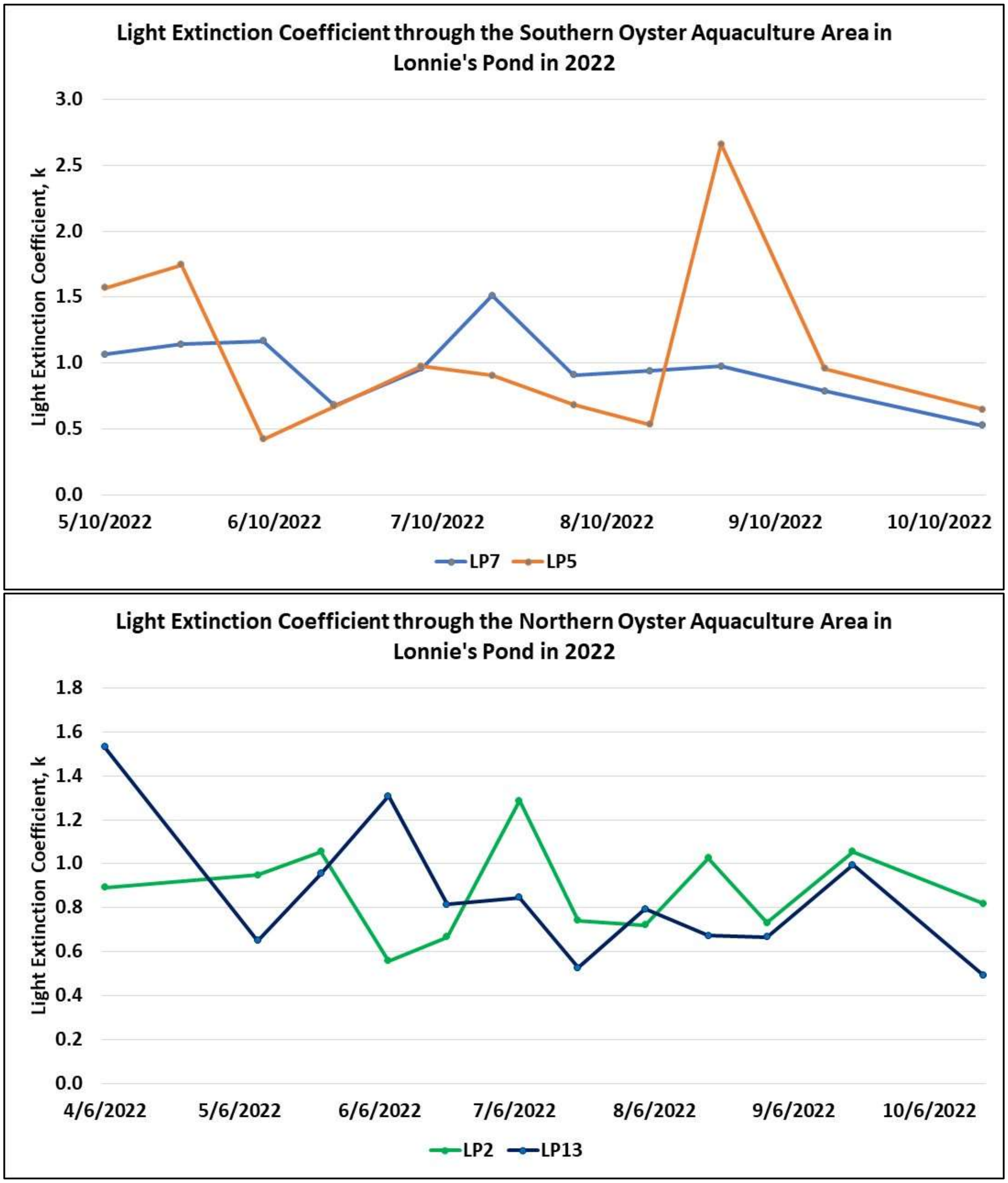

Figure 2.5. Lonnie's Pond 2022 time-series of water-column light extinction near aquaculture areas. Light extinction coefficients, k, were determined during the 2022 water quality monitoring using the station light profiles upgradient (LP7) and downgradient (LP5) of the southern oyster aquaculture area (top) and upgradient (LP2) and downgradient (LP13) of the northern aquaculture area (bottom).

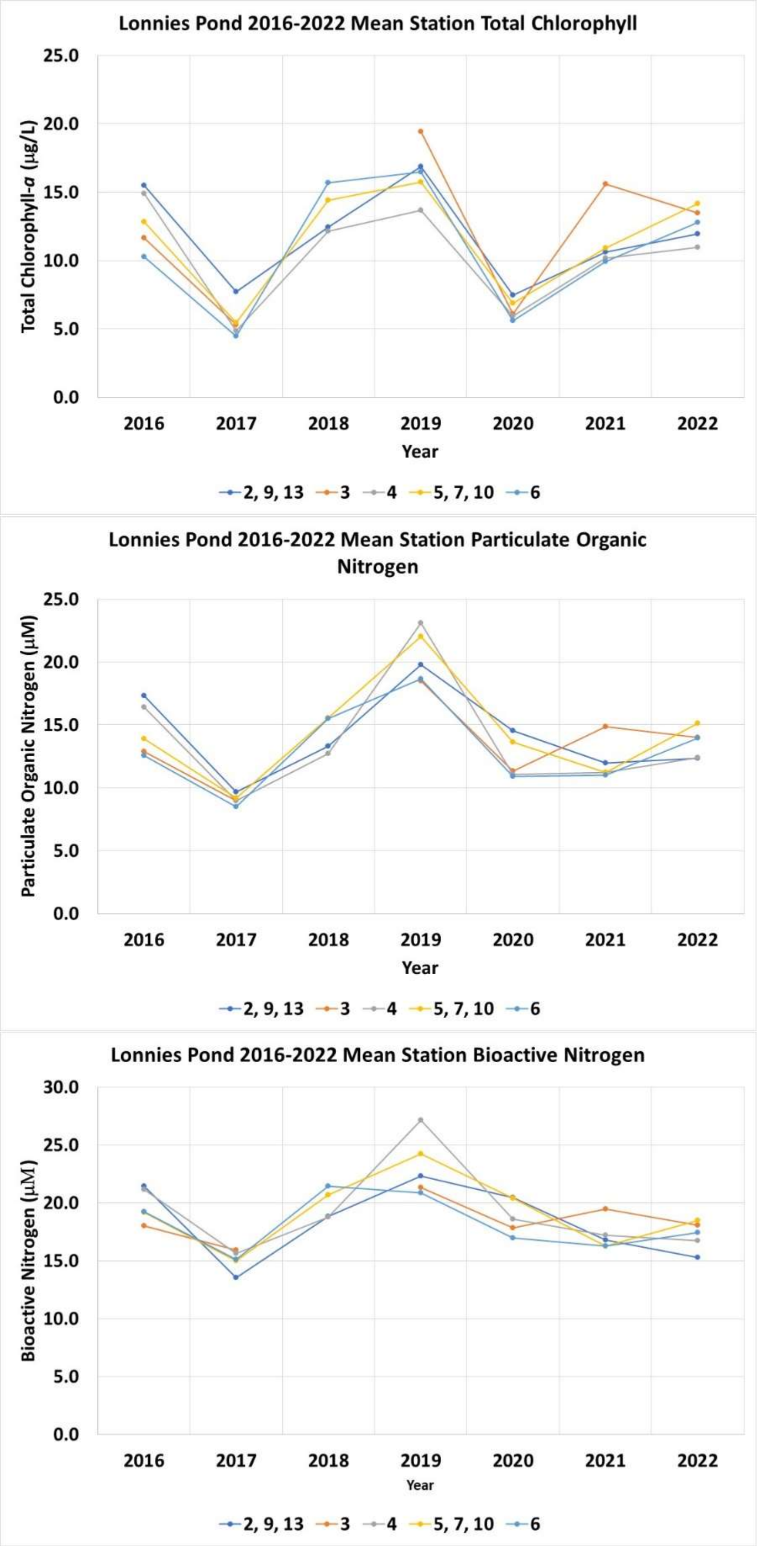

Figure 2.6 Historical average chlorophyll pigments, PON, and bioactive concentrations in Lonnie's Pond (2016-2022). Annual average pigment concentrations generally show peak concentrations in 2019 and lowest concentrations in 2017. Bioactive N concentrations have generally decreased in 2020 from the maximum in 2019 and have been relatively stable in 2021 and 2022. 2022 averages were slightly higher or lower than 2021 averages, depending on the station. Station numbers refer to locations in station map, Figure 2.1.

Based on the compiled pond-wide results, average annual 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 or comparable in 2022 as to the start of the study in 2016 (Figure 2.6). All stations have lower chlorophyll-*a* concentrations in 2022 averaging 12.8 µg/L for the year compared to the start of the study in 2016 which all stations averaged 13.6 µg/L in total pigments. Average chlorophyll for 2022 was only slightly higher from last year: 2021, 11.1 µg/L vs. 2022, 12.8 µg/L.

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; PON 2022 average = 13.6 µM). Bioactive N in 2016 averaged 20.4 µM vs 17.0 µM in 2022. The slight increases in these metrics from 2021 could be attributed to the larger flow and nitrogen load entering Lonnie's Pond from Pilgrim Lake, but precipitation recorded in 2022 was 7.2 inches less compared to 2021 (2021: 49.9 inches vs. 2022: 42.7 inches) and will be discussed in detail in the following section pertaining to stream inputs.

Total suspended solids (TSS) were also measured in and around the southern aquaculture area consistently between 2016 and 2022. TSS represents the dry weight (mg) of suspended solids (particulates). Due to oysters filtering capacity, it was hypothesized that the TSS will be reduced as the tidal water ebbs out of Lonnie's Pond into Pleasant Bay. As with the light measurements, TSS samples were collected upgradient of the southern aquaculture area (station LP7), within the area (station LP6), and downgradient of the aquaculture area (station LP5) (see Figure 2.1 for station locations). All samples were collected on the ebb tide and the highest TSS was indeed found upgradient of the oysters (LP7) and was reduced downgradient of the oysters in each sampling from 2016 through 2022 except 2021 (Figure 2.7). Interestingly, TSS was lowest within the aquaculture area (LP6) every year except 2020 and 2022 and was significantly reduced compared to LP7 ($p = 0.01$) showing a clear reduction of suspended solids by the oysters. This is consistent with generally higher water clarity seen with the light extinction coefficient results. This finding is important in areas where eelgrass restoration is also being considered.

One additional finding from the TSS sampling is that Pilgrim Lake TSS outflow has been increasing since 2020. LP7 is just to the east of the Pilgrim Lake stream outflow, so its water quality is impacted by outflow characteristics, especially during ebb tide when flow is from the stream toward the Lonnie's Pond outlet. Since TSS measurements were collected during ebb tides, changes in TSS at LP7 also reflects changes in Pilgrim Lake outflow. TSS concentrations at LP7 have been increasing from 2016-2022, with the exception of 2020, but certainly since 2020 (Figure 2.7). During the duration of this study (2016-2022), the least amount of rain was recorded in 2020 and outflows from Pilgrim Lake were the lowest (discussed below). But it is also notable that the 2016 TSS concentrations approximate the 2020 TSS concentrations. Review of groundwater levels in the Pilgrim Lake Management Plan suggested that 2016/2017 conditions were representative of long-term average conditions, but there were changes in the lake system that were occurring, most notably, increasing herring populations (2X between 2015 and 2017).[8] Despite the general increase in measured TSS upgradient of the oysters, it appears the aquaculture area has handled the TSS increases and the filtration capacity for TSS has not been exceeded.

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[8] Eichner, E., B. Howes, and D. Schlezinger. 2019. Pilgrim Lake Management Plan and Diagnostic Assessment. Town of Orleans, Massachusetts. Coastal Systems Program, School for Marine Science and Technology, University of Massachusetts Dartmouth. New Bedford, MA. 114 pp.

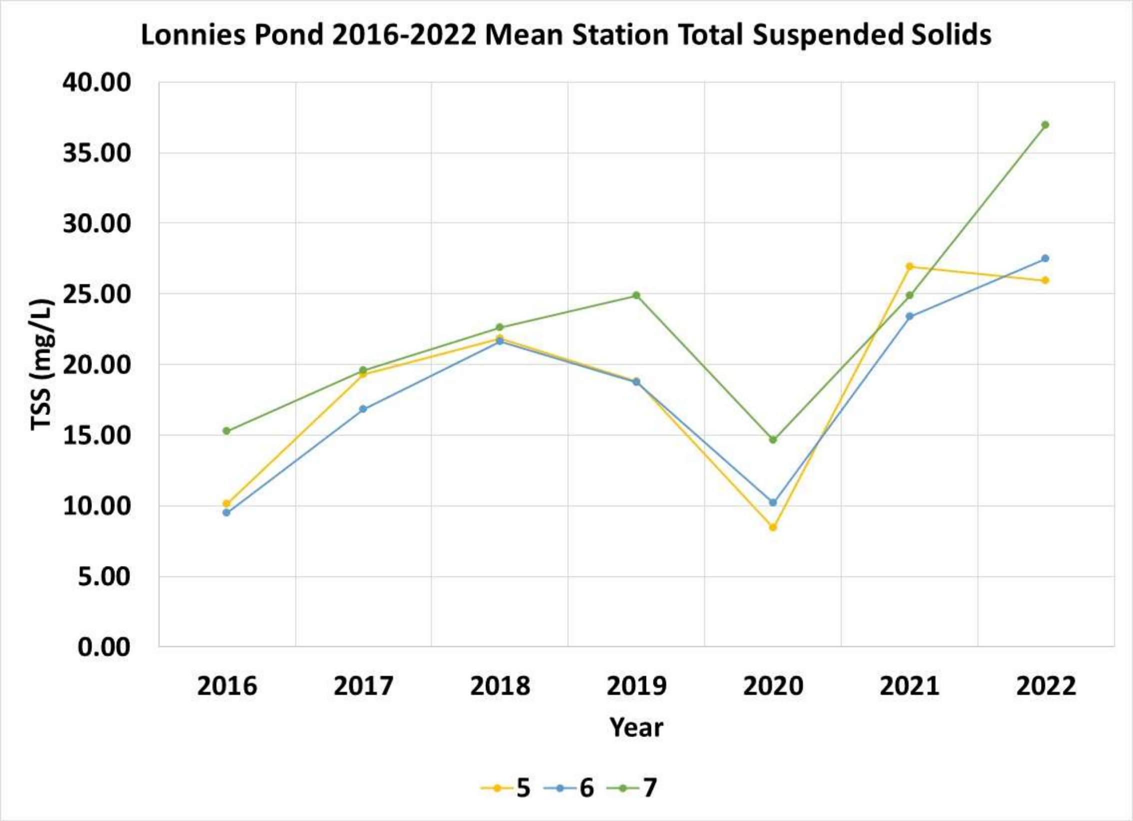

Figure 2.7 Average total suspended solids (TSS) concentrations at the southern aquaculture area: 2016-2022. Averages upgradient (station LP7), within (station LP6), and downgradient (station LP5) of the Lonnie's Pond southern oyster aquaculture area. Readings consistently show removal of TSS within the aquaculture area: higher TSS concentrations upgradient and lower TSS concentrations downgradient. TSS concentration have increased at all stations since 2020. Station numbers refer to locations in station map in Figure 2.1.

The presence of oysters in Lonnie's Pond has generally affected water quality by significantly reducing phytoplankton, bioactive nitrogen, and TSS. Oysters have been shown to reduce water column particulates and increase water clarity. These readings are consistent with readings in other areas locally (*e.g.*, Bournes Pond[9]) as well as regionally (Great Bay, New Hampshire[10] and Long Island Sound[11]). During 2022, there were a number of events that have not been measured before (*i.e.*, a May 10 phytoplankton bloom and a June 8 large precipitation event), but once these impacts passed, water quality conditions generally followed patterns seen in previous years.

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[9] 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.

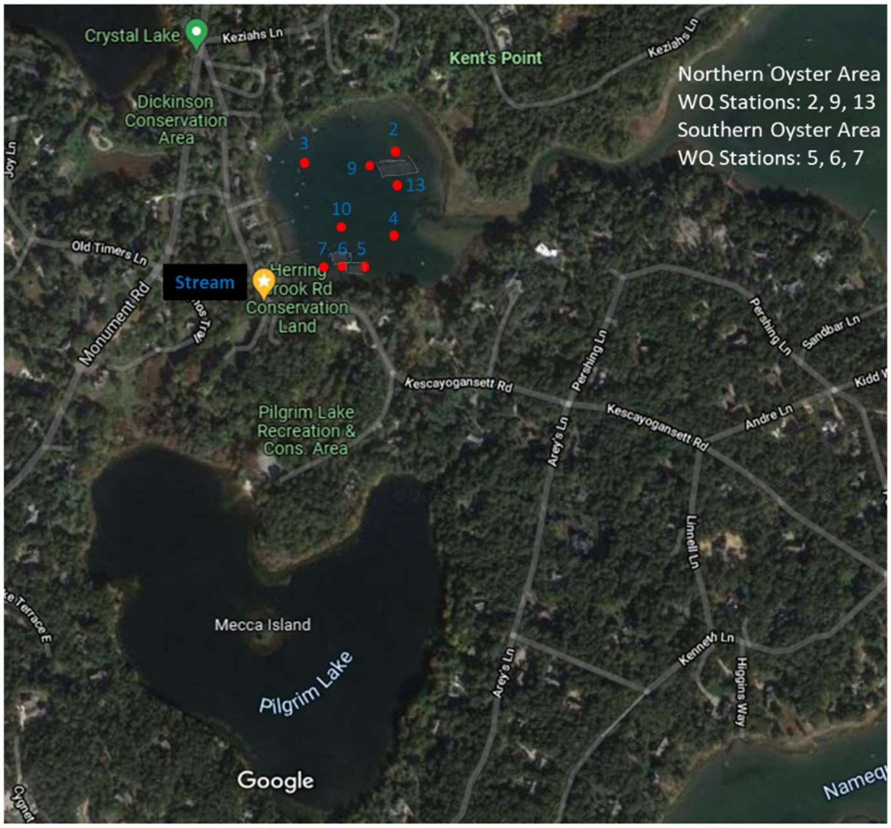
[10] Bricker, S.B., Grizzle, R.E., Trowbridge, P. et al. 2020. Bioextractive Removal of Nitrogen by Oysters in Great Bay Piscataqua River Estuary, New Hampshire, USA. *Estuaries and Coasts* 43, 23–38).

[11] 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.

Steam Inflows and Nutrient Loads

Quantifying the effect of oyster aquaculture on the overall health of Lonnie's Pond must include the nitrogen 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 through a cranberry bog to the west of 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.[12] Monitoring includes the placement of a gauge which records water level every 10 minutes. This gauge was placed at the herring run upgradient of the culvert (Figure 2.8). While the gauge collects water level, CSP/SMAST project staff visit the site bi-weekly at low tide to collect water samples and volumetric discharge measurements.



Station Map for Lonnies Stream and Water Quality Stations

irbus, MassGIS, Commonwealth of Massachusetts EOEA, Maxar Technologies, USDA Farm Service Agency, Map data ©2022 500 ft

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

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[12] Monitoring also occurred during 2002/03 for the Pleasant Bay MEP report (Howes, *et al*, 2006).

Collected 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 an 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).

The surface water flow record from the herring run 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 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 result is the measured nitrogen load from the Pilgrim Lake herring run to Lonnie's Pond. Flow and load have been measured at this gauging location since August 2016.

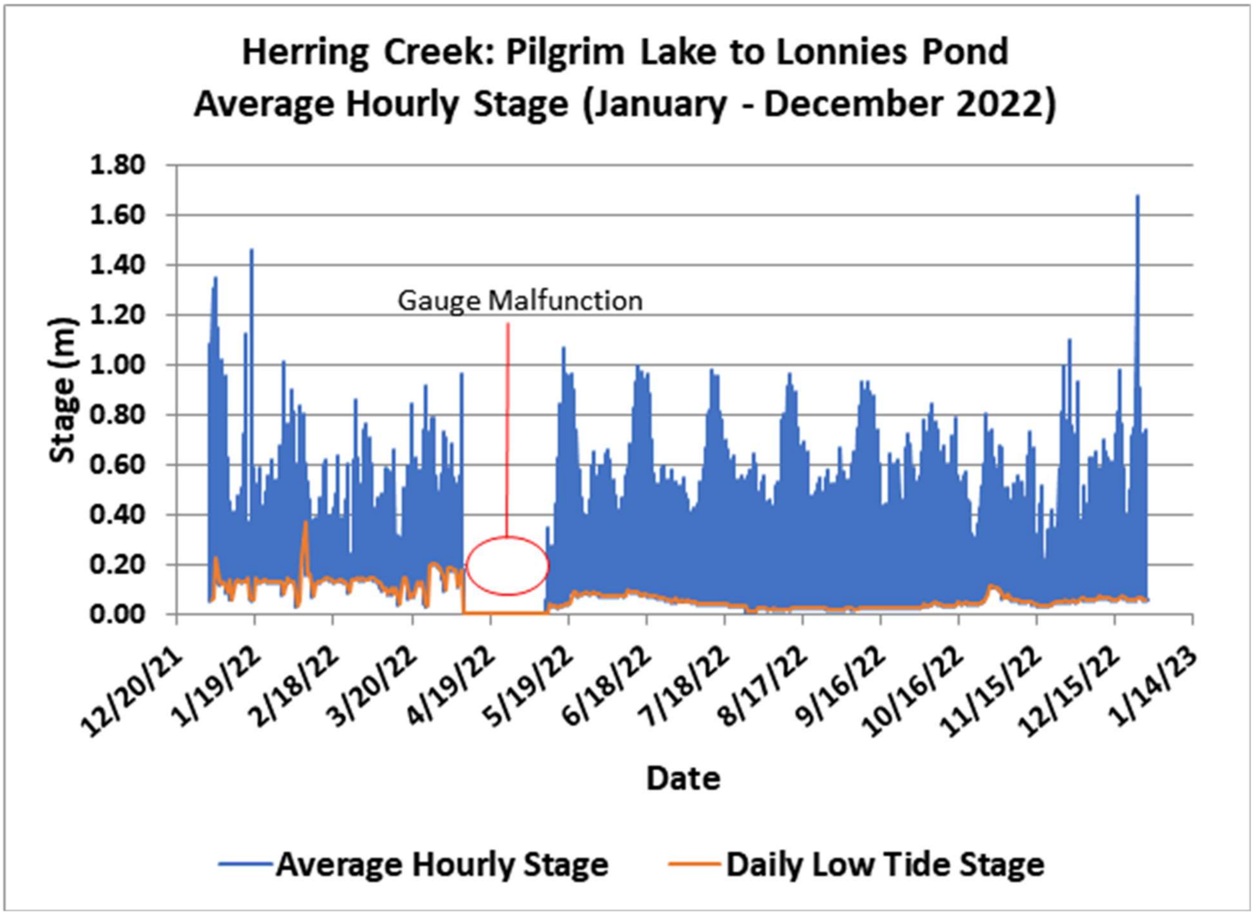

Figure 2.9 Average hourly 2022 stage record from discharge to Lonnie's Pond from Pilgrim Lake. Stage elevations were scrubbed to remove daily tide impacts and elevations were compared to regular flow readings from January to December 2022 to develop stage-discharge relationship. This relationship was used to develop a refined annual flow and, with water quality data, nitrogen load to Lonnie's Pond from the herring run.

The stream flow varies seasonally with highest flows and nitrogen load generally occurring in the spring and lower flow and load occurring in the summer months when groundwater levels are typically lower (Table 2.4). Water quality results from 2022 show that spring total nitrogen and flow inputs to Lonnie's Pond are bit higher than 2021, but summer inputs are notably lower and notably lower than spring 2022 inputs. In previous years summer (July-September) stream inflows were 26% to 81% of corresponding spring (April-June) inflows, but in 2022, the summer inflow was only 14% of spring inflow. Summer TN input (15.2 kg) from Pilgrim Lake also decreased to only 26% of spring input (58.8 kg), after having summer input in 2021 of 60.4 kg that was 9% greater than the spring 2021 input (55.2 kg). The average seasonal loss of nitrogen from the Pilgrim Lake herring run (57%; 2017-2022) approximates the 50% nitrogen attenuation rate determined in the water column measurements and watershed assessment in the diagnostic assessment portion of the Pilgrim Lake Management Plan.[13] The natural variations in N export from Pilgrim Lake mean that Lonnie's Pond and the southern aquaculture area, which is nearest the herring run, have nitrogen and freshwater loads that vary with the seasons and year-to-year.

Table 2.4 Seasonal total nitrogen load (kg) entering Lonnie's Pond via Pilgrim Lake stream from 2017 - 2022. Summer (July-September) inflow has been 26%-81% of spring (April-June) inflow but was only 14% of spring 2022 inflow. Summer 2022 inflow was 27% of the average inflow over the six years (40,180 m^3), while spring 2022 inflow was approximately the same as the six year spring average (79,358 m^3). Summer TN input has been 32%-109% of spring TN input, but was only 26% of spring TN input in 2022. Summer 2022 TN input of 15.2 kg was the second lowest TN summer input of the six years and was 48% of the average summer input (32.1 kg). Spring 2022 TN input was close to the average of six years (54.1 kg).

	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
Apr-Jun 2022	79,129	**Total Load (April-June 2022)**	6.39	7.19	13.58	34.68	10.58	58.83
Jul-Sept 2022	10,940	**Total. Load (July-Sept. 2022)**	1.39	9.24	10.63	3.49	1.13	15.24

Although seasonal flows and loads are important for assessing the aquaculture impacts, the assessment of the Lonnie's Pond ecosystem requires an understanding of the annual flows and loads entering from Pilgrim Lake and how they vary throughout the year. Viewing the monthly stream flow and nitrogen load inputs year-round, especially in January-March, illustrates just how much more water and nitrogen entered Lonnie's Pond in 2022 (Figure 2.10). The stream input of total nitrogen and corresponding stream flow discharging into Lonnie's Pond per month was totaled from January 2021 – December 2022. Results show the annual mass of nitrogen and stream input decreased slightly in 2022 compared to 2021, but with significantly different seasonal

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[13] Eichner, E., B. Howes, and D. Schlezinger. 2019. Pilgrim Lake Management Plan and Diagnostic Assessment.

patterns (Figure 2.10 and 2.13). Annual volumetric flow of water entering Lonnie's Pond in 2022 totaled 254,824 m^3, which was a decrease of 6% from 2021 (2021: 271,271 m^3). The 2022 annual nitrogen load entering Lonnie's Pond was 283 kg N/yr, which represented a 11% decrease from the previous year (2021: 301 kg N/yr). The notably high January-March 2022 flows and loads may have played a role in the May phytoplankton bloom. The low precipitation from spring 2022 through the winter will likely to translate into lower 2023 flows and nitrogen loads to start the year, but recent precipitation records have shown wider swings in annual precipitation and more frequent significant monthly totals (*e.g.*, 10.48 inches in September 2021; 23% of the annual total and 9.16 inches in October 2022; 21% of the annual total[14]). With large portions of the annual precipitation occurring in single months, groundwater levels have been decreasing (Figure 2.11) and this created drought conditions throughout Cape Cod during 2022 (Figure 2.12).

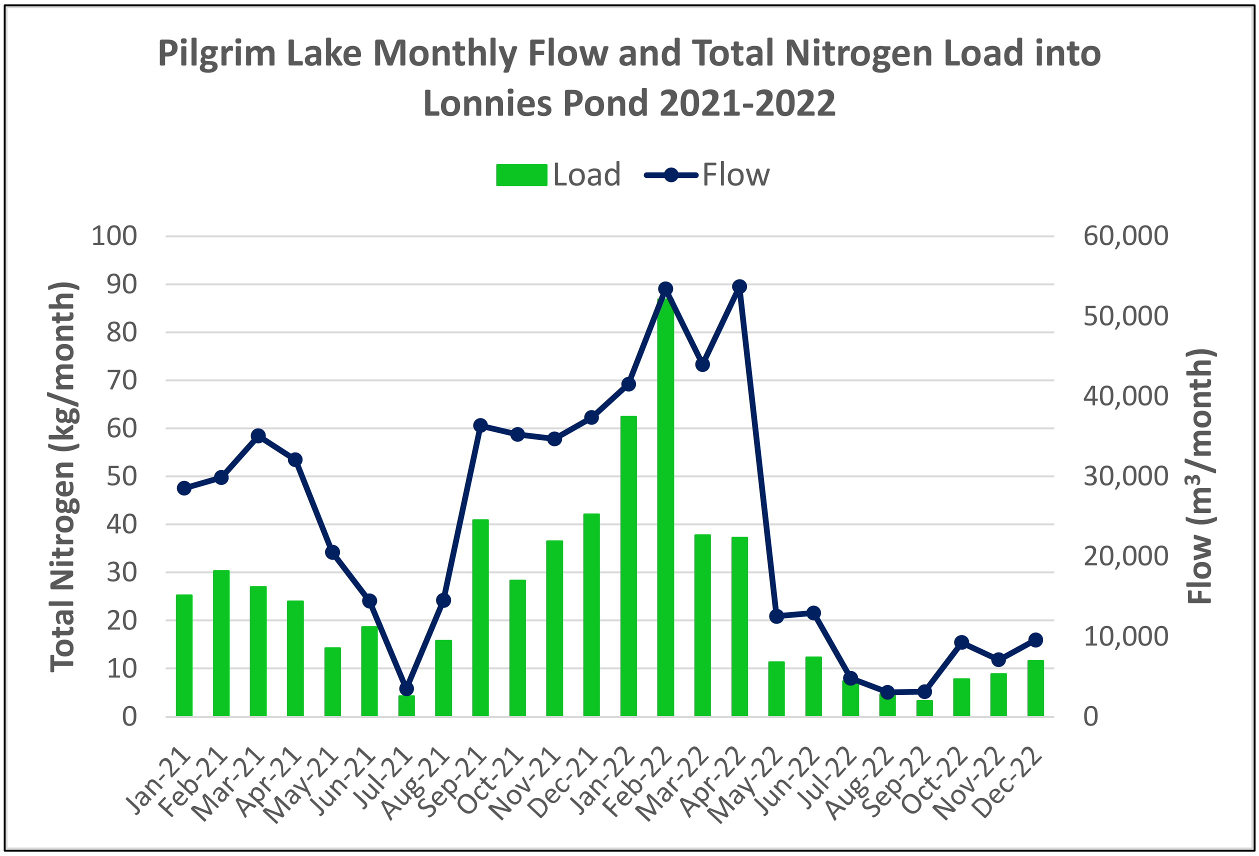

Figure 2.10 Pilgrim Lake monthly stream flow and total nitrogen load (2021-2022). Flows and loads are based on monthly measurements, sample collection, and stage-discharge relationship for readings at the bottom of herring run prior to discharge into Lonnie's Pond. There was a small decrease in annual flow (6%) from 2021 to 2022, but the pattern of monthly flows was notably different. 2022 flows were concentrated in January-April and then were generally lower than 2021 throughout the rest of the year. TN loads were generally higher in months with higher flows, but similar monthly flows often produced varying loads.

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[14] Readings at station MA-BA-12 (near Town Cove); https://www.cocorahs.org/; readings recorded since 2011.

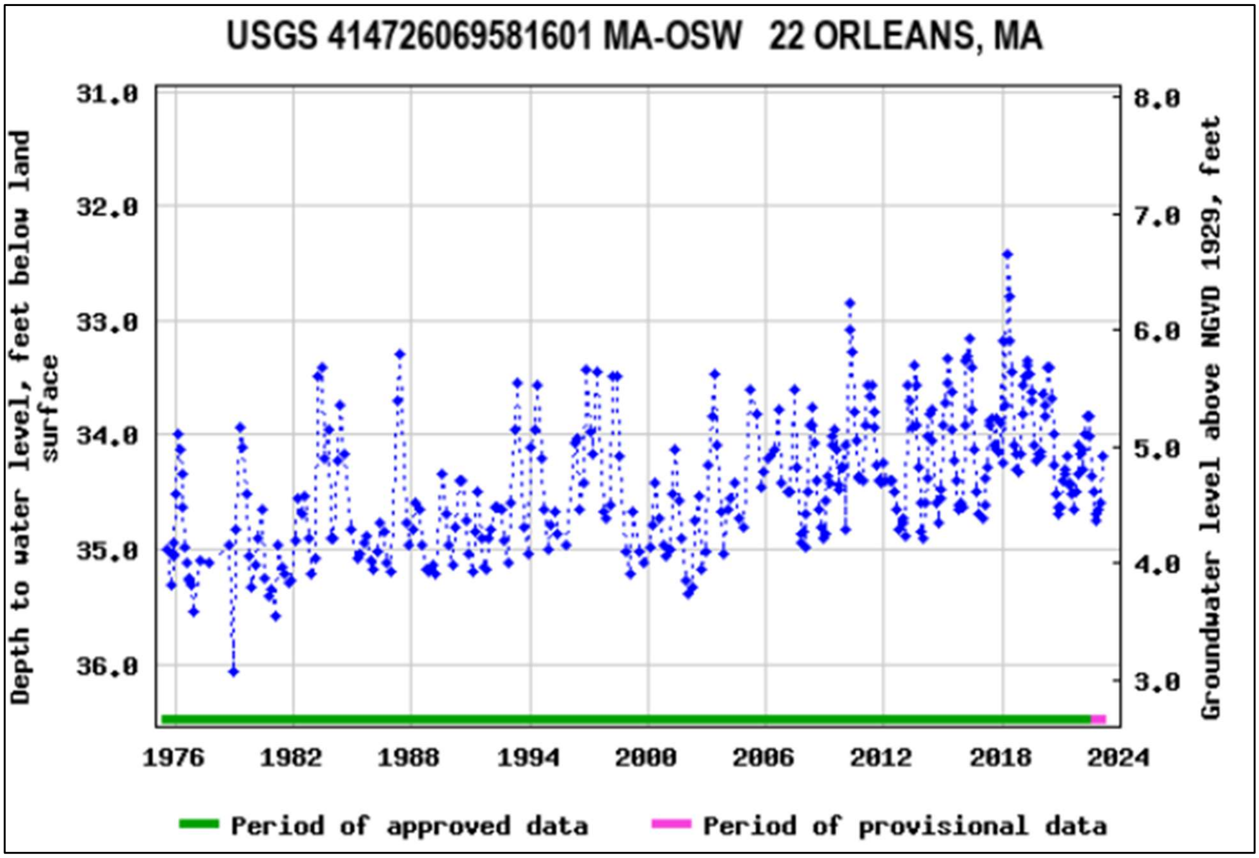


Figure 2.11 Orleans Groundwater Levels (1976-2023). Groundwater levels generally decreased in 2022 largely due to concentration of large portions of the annual precipitation in single months (*i.e.*, September 2021 and October 2022) and these large amounts occurring when evapotranspiration is higher and less of the precipitation is recharged. Graph from US Geological Survey, NWIS (nwis.waterdata.usgs.gov; accessed 2/9/23).

Comparison of annual streamflow and nitrogen loads from Pilgrim Lake into Lonnie's Pond show that the flow fluctuates over relatively small range while the nitrogen loads vary over a large range (Figure 2.13). These differences reinforce that the seasonal aspects are more important for how flows and loads impact Lonnie's Pond. Annual flow range is ~200,000 m3/yr, which is 19.4 m3/d or <5% of the summer average flow (445 m^3/d). In contrast, annual loads fluctuate over approximately a 200 kg range, which is approximately 0.6 kg/d or 100% of the average spring N load.

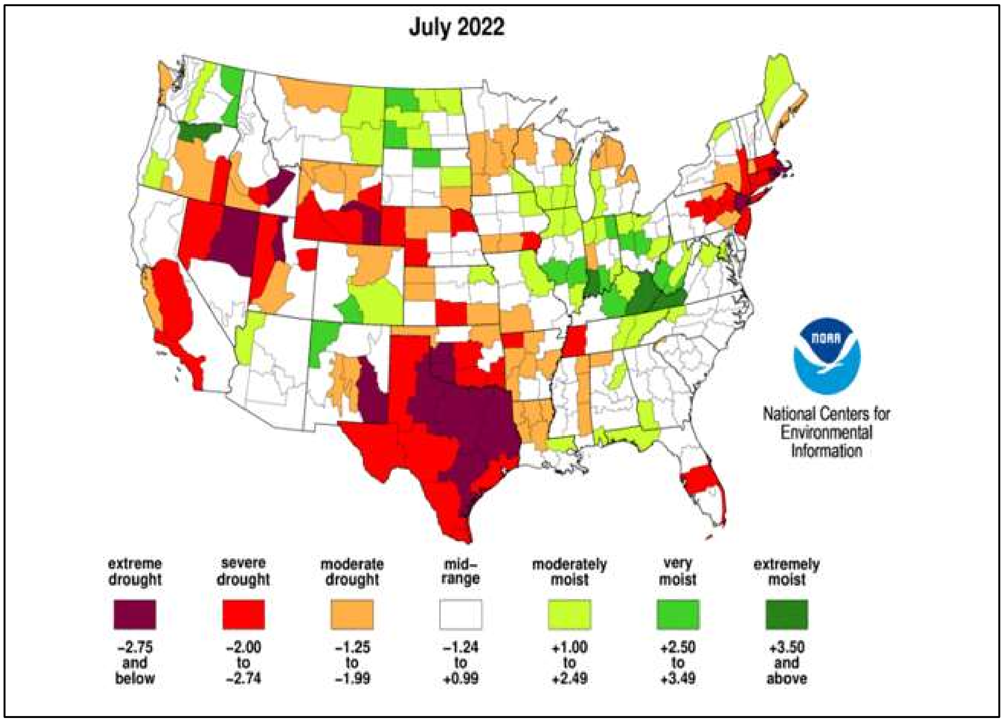


Figure 2.12 United States drought condition status July 2022. Cape Cod was in the extreme drought category. Map from: https://www.ncei.noaa.gov/access/monitoring/monthly-report/drought/202213.

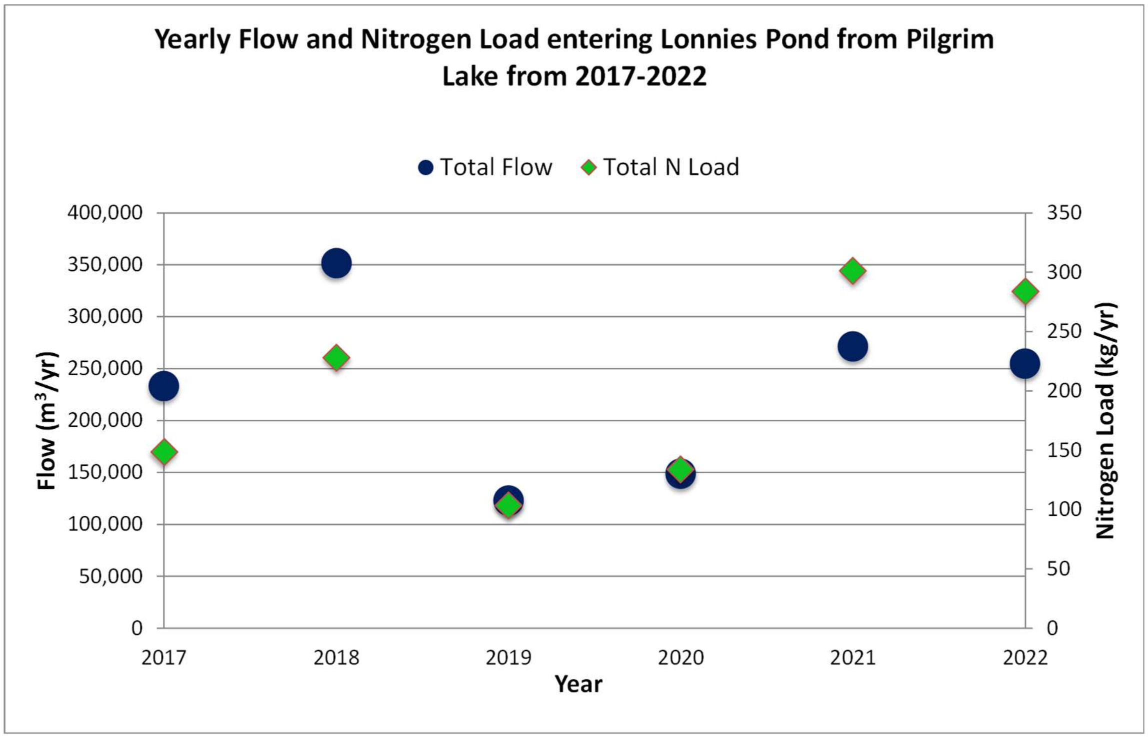


Figure 2.13 Annual stream flow and nitrogen load from Pilgrim Lake into Lonnie's Pond from 2017 to 2022. On an annual basis, flow varies over a relatively small range (~19.4 m3/d or <5% of the summer average flow), while nitrogen loads vary over a relatively large range (~0.6 kg/d or 100% of the average spring N load).

3.0 Continuous Water Column Monitoring: Dissolved Oxygen and Chlorophyll-*a*

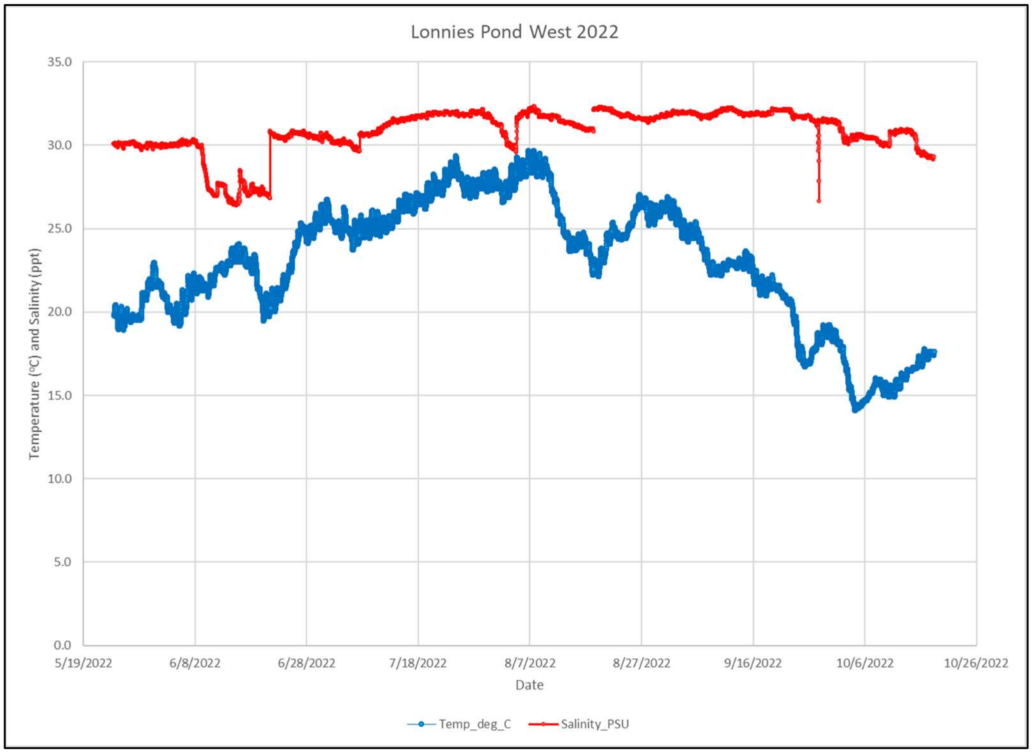
Two autonomous recording multiparameter sondes were deployed from May 24 through October 18, 2022. Autonomous instrumentation was deployed to measure dissolved oxygen, chlorophyll, temperature, salinity and depth at 15-minute intervals. These high frequency measurements provide a means to determine whether 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. 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). The second sonde (Lonnie's Pond West, s) 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 (4 m south of LP6).

Continuous readings varied depending on the parameter. Average salinity and temperature were similar at the two mooring sites (Figure 3.1). Salinity varied less in 2022 than 2021 because of below average rainfall. Water temperatures were significantly higher in 2022 than in 2021, exceeding 25°C throughout most of July and into early August. Higher temperatures result in lower oxygen solubility in water and increases respiration rates, both processes lead to declines in water-column oxygen concentration. DO concentrations at the two mooring sites showed similar temporal patterns in concentration (Figure 3.2). The East mooring displayed higher average DO and increased diurnal variation compared to the West mooring. Water column DO concentrations beneath the oysters in 2022 showed persistent hypoxia and near daily anoxia (15-20 min max duration) during July and early August when water temperatures were elevated above 25°C. In contrast, the 2021 field season displayed no bottom water anoxia and only three brief instances of dissolved oxygen under 2 mg/L at each of the mooring locations.

Comparison of the continuous mooring record to the regular water column monitoring results show the benefits of continuous monitoring, including getting a better understanding of the regular anoxia. Bottom water DO concentrations measured during water quality surveys did not show large spatial gradients between stations. However, continuous DO was generally lower beneath the oysters, with hypoxic and anoxic events occurring during plankton blooms (*i.e.*, large diurnal oxygen excursions, short duration, early morning DO minima) and bloom collapse (*i.e.*, moderate diurnal oxygen excursion, extended duration). Unlike previous years, bottom water DO values observed at the moorings were lower than those observed at far field water quality monitoring sites. This suggests that the depressed oxygen concentrations could be related to a localized oyster effect.

Algal blooms, as determined from high chlorophyll concentrations, occurred at different times at the two stations in 2022 (Figure 3.3). Both stations had a bloom near 6/8 that persisted for a number of days and coincided with three days of rain (total=1.65 inches). Another bloom occurred at the West station, but not the East station, from early July to approximately 7/7; 1.37 inches of rain during this period. The East station, but not the West station that began on 7/7 and peaked around 7/20. Negligble rain was recorded during this period. Additional blooms occurred in early

August and late August. From the pattern of blooms it appears that multiple factors are causing the blooms. In 2020 and 2021 the chlorophyll concentrations were higher at the West mooring than at the East mooring. The West mooring was moved farther from the mouth of Lonnie's Stream (moved to the east) to be adjacent to the edge of the 2022 oyster deployment, thus decreasing water column response to rain events. Comparison of the 2022 chlorophyll data showed higher chlorophyll concentrations at the East mooring. This is consistent with moving the mooring further from the stream but also suggests that changes in the benthic community may be contributing to water filtration.



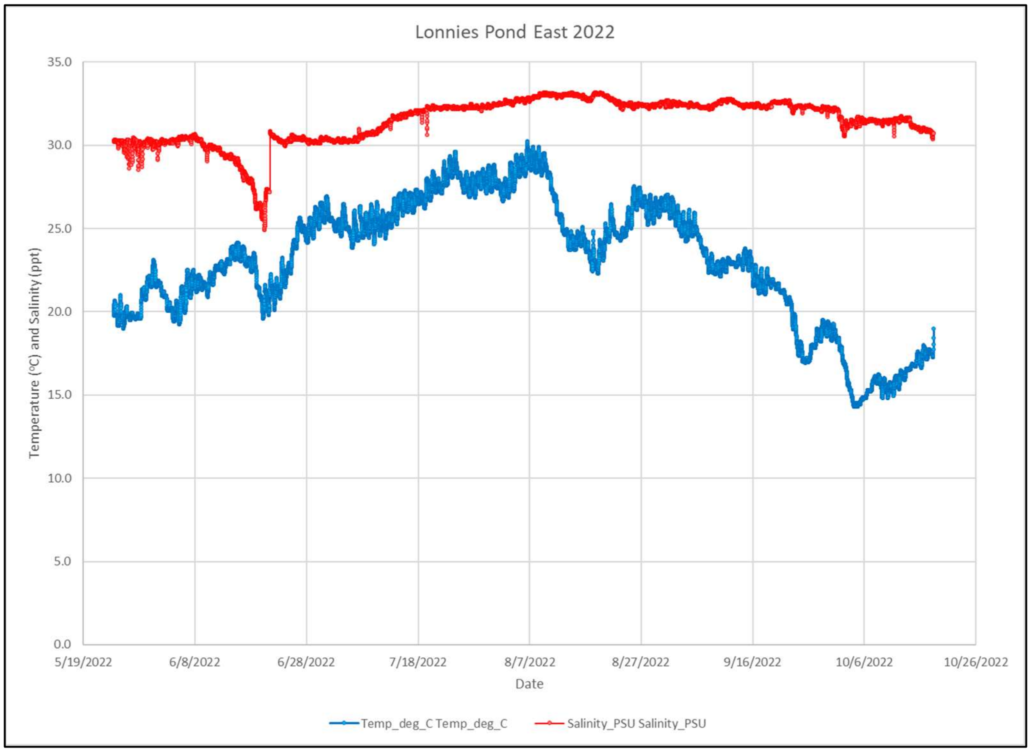
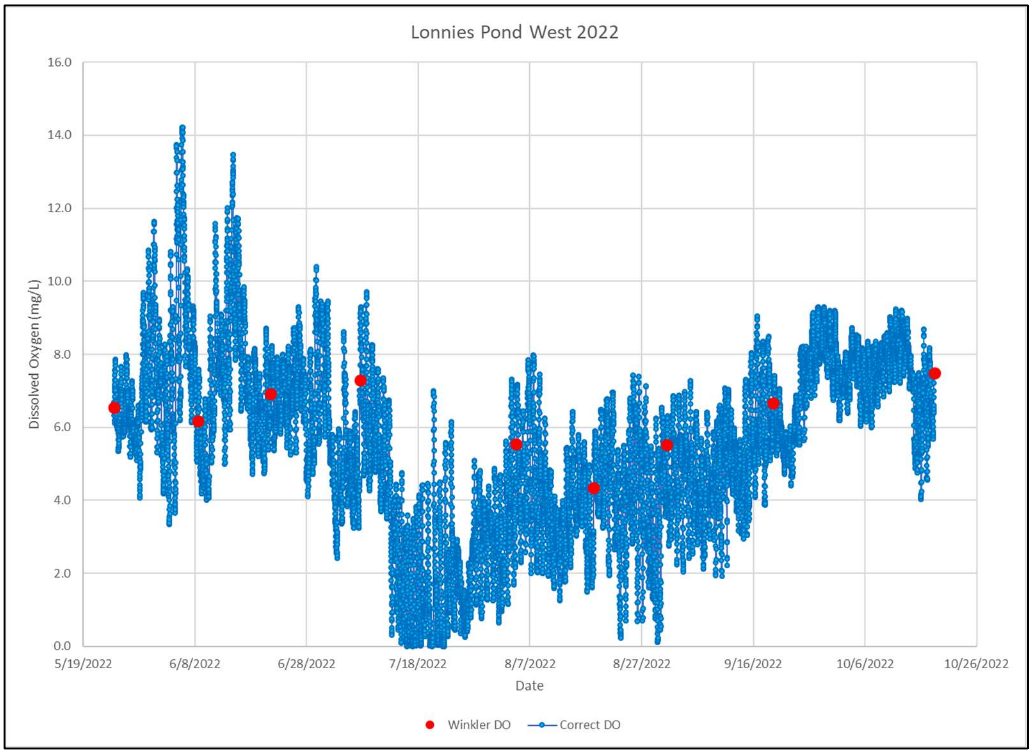


Figure 3.1 Lonnie's Pond 2022 continuous temperature and salinity readings at West (top) and East (bottom) moorings. Sensors were programmed to collect readings every 15 minutes. Readings were similar at both mooring locations.



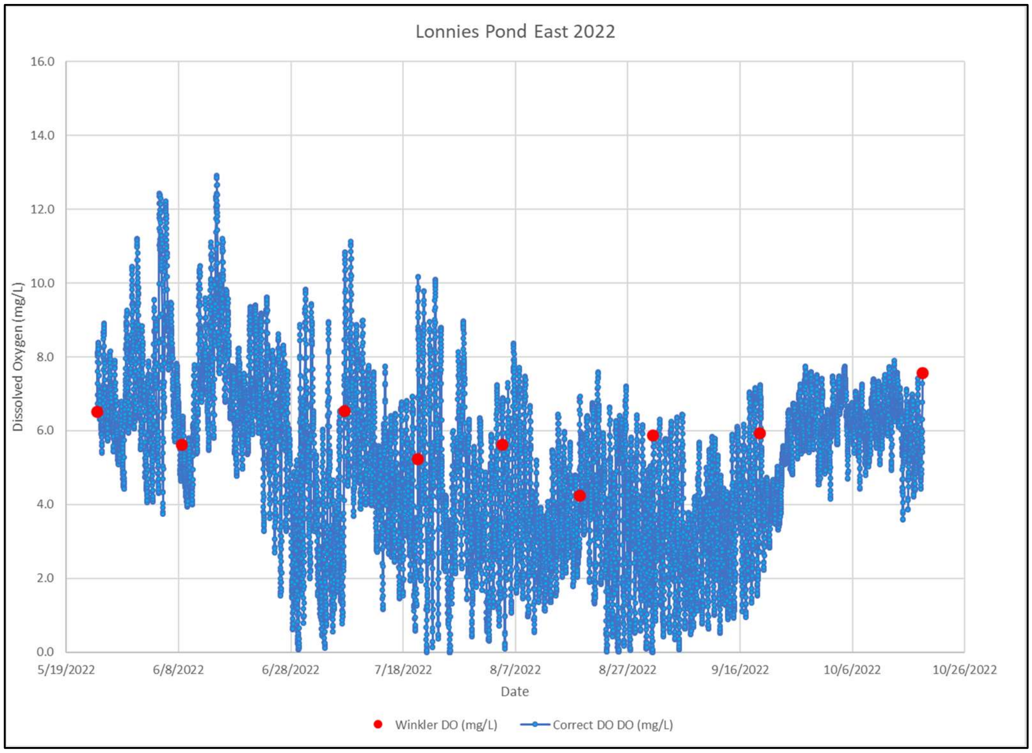
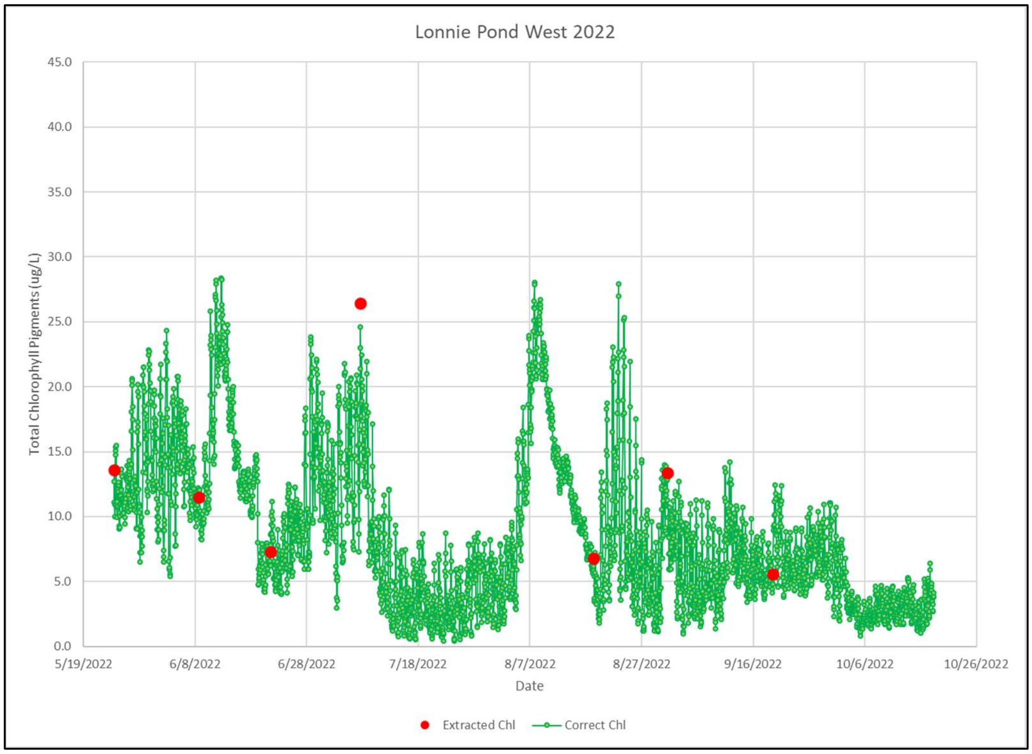


Figure 3.2 Lonnie's Pond 2022 continuous dissolved oxygen readings at West (top) and East (bottom) moorings. Red dots indicate *in situ* calibration values measured during water column monitoring activities. Readings at both stations indicate persistent hypoxia, especially when temperatures were >25°C during most of July and into early August. Reading also indicated near daily anoxia persisting for 15-20 minutes at both moorings.



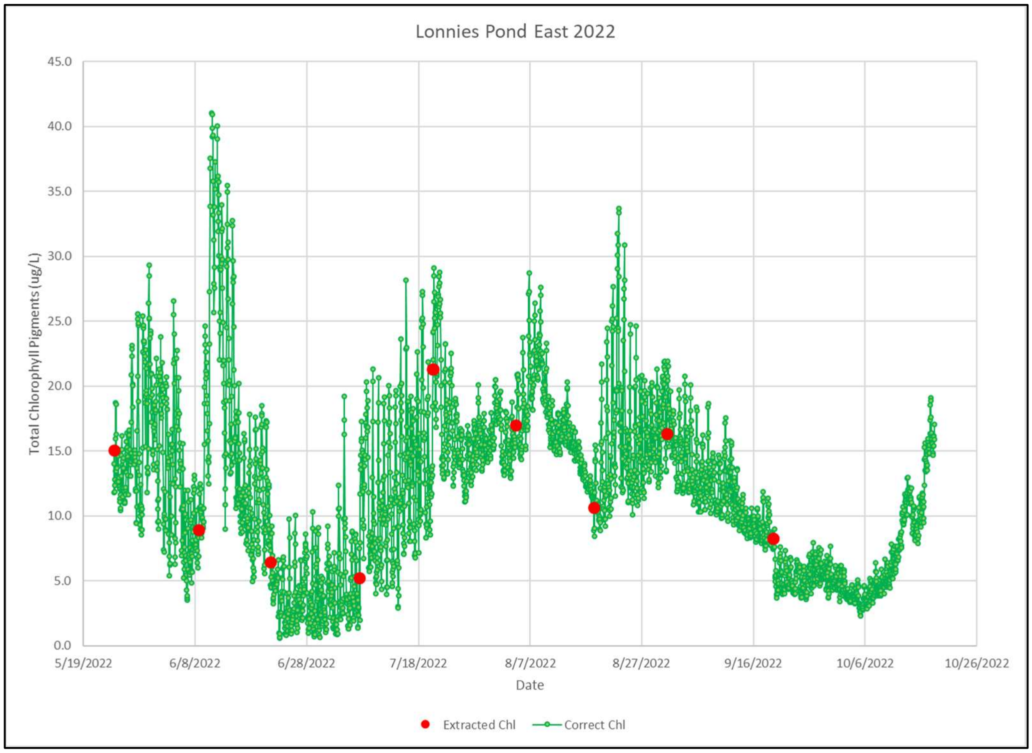


Figure 3.3 Lonnie's Pond 2022 continuous chlorophyll a pigment readings at West (top) and East (bottom) moorings. Red dots indicate *in situ* calibration values measured during water column monitoring activities.

4.0 Effects of Oysters on Nutrient Regeneration and Denitrification in Sediments

In eutrophic shallow-water depositional systems 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 assessed through the Massachusetts Estuaries Project, recycled N accounted for nearly half the total supply available to phytoplankton 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 and the overall N balance of the system. In Lonnie's Pond, it is a significant factor that must be addressed to understand 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. This deposition is tempered somewhat by heterotrophic processes in the water column that intercept labile organic matter, recycle it into new growth, and lower the quantity of POM reaching the sediments. The deposition of POM and the associated respiration in the sediments are typically higher in shallow coastal systems 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 affect habitat quality.

During the warmer summer months, Lonnie's Pond can experience low DO levels. In 2022, water column DO concentrations beneath the oysters showed persistent hypoxia and near daily anoxia during mid-summer when water temperatures were elevated above 25°C. Although water depth is an important factor in POM deposition dynamics, the addition of filter feeders like oysters has 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 associated denitrification. The recent finding of improved conditions allowing the major colonization by amphipods is also expected to have a significant positive effect on sediment oxidation status and denitrification as has been indicated in other systems.[15]

Benthic Nutrient Regeneration, Denitrification and Sediment Oxygen Uptake

In order to determine any enhancement of benthic carbon and N cycling, N 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 in 2022: April 10, August 14, and October 30. Sediment samples will also be collected in April 2023 to capture the complete the annual N cycle. The effect of oysters on N cycling and oxygen availability was most significant during periods of maximum oyster activity (July-September) and maximum oyster biomass (October-December). Seasonal temperature variations between sampling periods are also important, as higher temperatures increase bacterial respiration while

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[15] 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.

lower temperatures decrease carbon and nitrogen decay rates. April sediment surveys are conducted because N rich, POM depositions from oysters (biodeposits) are "preserved" in the upper sediment layer over the winter until the following spring and these fuel a high rate of cycling the following early spring. April surveys capture residual effects of accumulated biodeposits from the latter half of the year as temperatures began to warm and sediment metabolism increased. Previous years' measurements found that failure to account for denitrification associated with overwintered biodeposits results in a large underestimate of this pathway of N removal.

On each of the three 2022 dates, twelve intact sediment cores were collected by SCUBA divers in core tubes (15 cm i.d., 30 cm height, 15 cm of sediment) and immediately transported at *in situ* temperature by boat to a nearby field laboratory for incubation in pre-equilibrated insulated water baths. Headspace water was replaced with filtered water from the core site, aerated and mixed at speeds that replicated *in situ* conditions without inducing resuspension. Water baths and core headspace water were held within 1-2°C of *in situ* temperatures. Time series measurements of nutrient species (TDN, NO_x^-, NH_4^+ and PO_4^-) and dissolved oxygen were conducted over a 16-18 hr period under dark conditions, mimicking the low bottom water light levels at the coring site. Sediment oxygen uptake was determined in order to: (1) evaluate sensitivity to oxygen depletion of the oyster deployment areas of Lonnie's Pond, (2) rank sediments as to organic matter deposition rates (not possible using organic content) and (3) develop a general nitrogen model for how the oysters may be affecting the nitrogen cycle in the sediments associated with oysters. Coring sites were constrained to the oyster deployment areas in the southern portion of Lonnie's Pond to allow comparison to prior years, especially as sediment conditions can change (*e.g.*, amphipods). Core locations were distributed to capture potential variation in nutrient flux rates throughout the biodeposit impact area and in background sediments (*i.e.*, outside the biodeposit impact area).

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 measured by ratio analysis with the naturally occurring inert gas (Ar). 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 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.

An analytical/numerical model was developed for suspended aquaculture systems to predict the spatial distribution of biodeposits; input parameters include water depth, tidal elevation, biodeposit settling rate, and wind and tidally driven current velocity.[16] The distance of each core from the margin of the oyster array determined whether the core was within or outside the biodeposit impact area. 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±5.01 mm/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

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[16] Labrie, M.S., Sundermeyer, M.A. & Howes, B.L. Modelling the Spatial Distribution of Oyster (Crassostrea virginica) Biodeposits Settling from Suspended Aquaculture. *Estuaries and Coasts* 45, 2690–2709 (2022). https://doi.org/10.1007/s12237-022-01096-4

by taking the 95th percentile of biodeposit horizontal displacements during settling (Figure 4.1). The 2019 biodeposit impact area (2,122 m^2; updated since the 2021 Annual Report) was applied to the 2020 and 2021 deployment because the 2020 and 2021 South deployment array area, location, and layout were unchanged from 2019 to 2021. The 2016-2021 impact areas have been updated to be consistent with Labrie *et al*. (2022)[17].

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

2022 Sediment Nutrient Cycling Results

Spring (April), summer (August), and fall (October) 2022 sediment nutrient flux rates are summarized in Table 4.1. April 2022 cores were collected to complete the assessment of the 2021 oyster deployment (see inclusion in Year 6; Table 4.2). August incubations captured warmer summer water conditions, peak temperatures, and biodeposition rates. August 2022 cores were collected and incubated at 24.1°C, which is also within one degree of the 2017-2021 summer flux temperature average (24.0 °C, std dev = 0.5 °C) allowing direct comparison of the rates. Late October 2022 incubations were collected and incubated at 15.5°C and capture conditions after the main summer conditions have passed. Sediment oxygen demand (SOD) followed seasonal patterns of temperature and organic matter availability. Within the impact area, organic matter delivery to the sediments as biodeposition overwhelmed the effect of temperature and ambient particle settling on sediment respiration.

The highest sediment oxygen demand (SOD) rates were found in treated cores collected in summer. The average August 2022 SOD rates for treated (126.0 mmol O_2 m^{-2} day^{-1}) and control cores (80.6 mmol O_2 m^{-2} day^{-1}) were within 10% of the 2016-2021 average treated (138.0 mmol O_2 m^{-2} day^{-1}) and control (77.0 mmol O_2 m^{-2} day^{-1}) SOD rates. The highest SOD recorded from Lonnie's Pond cores was 191.1 mmol O_2 m^{-2} day^{-1} in August 2020. In 2022, SOD within the impact area was enhanced 96.2 mol O_2 day^{-1} (3.1 kg O_2 day^{-1}) above background; therefore, biodeposit settling and decomposition within surface sediment layer increased sediment oxygen uptake 56% above the background rate. Seasonally high SOD rates, lower solubility of oxygen during summer, and low water column mixing limiting re-aeration of bottom water can depress bottom water oxygen levels leading to anoxia or hypoxia. However, August 2022 treated, and control cores were characterized by oxic surficial sediments and oxidized layers extending down to 2-7 cm depth even in spite of regular hypoxia and anoxia recorded in the continuous moorings (see Figure 3.2).

The composition and rate of DIN (ammonium + nitrate) release during the 2022 summer was inconsistent with that measured in previous summers (2016-2021). Release of DIN from the sediments to the water column is typical of estuarine sediments in summer. The 2016-2021 average ammonium flux accounted for 93% (treated) and 85% (control) of average DIN release.

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[17] Ibid.

However, in August 2022 cores, ammonium accounted for only 13% and 12% of DIN release for treated and control cores, respectively, with nitrate-N becoming the predominant DIN release. In addition, the August 2022 ammonium fluxes were the lowest summer rates recorded during Project Years 1-7. The 2022 ammonium flux did not vary between treated and control cores with rates of 0.62 and 0.63 mmol NH_4^+-N m^{-2} day^{-1}, respectively. The average 2016-2021 ammonium efflux rates were 18 times higher (11.1 mmol NH_4^+-N m^{-2} day^{-1}) in treated cores and 9 times higher in control cores (11.1 mmol NH_4^+-N m^{-2} day^{-1}) compared to those measured in August 2022. A similar, but less drastic decrease in 2021 ammonium efflux and greater treated NO_3^- efflux compared to the 2016-2020 average, was observed and documented in the 2021 Annual Report. In 2022, nitrate release rates did not vary by treatment and rates were 375% and 345% higher than the 2016-2021 treated and control rate average, respectively.

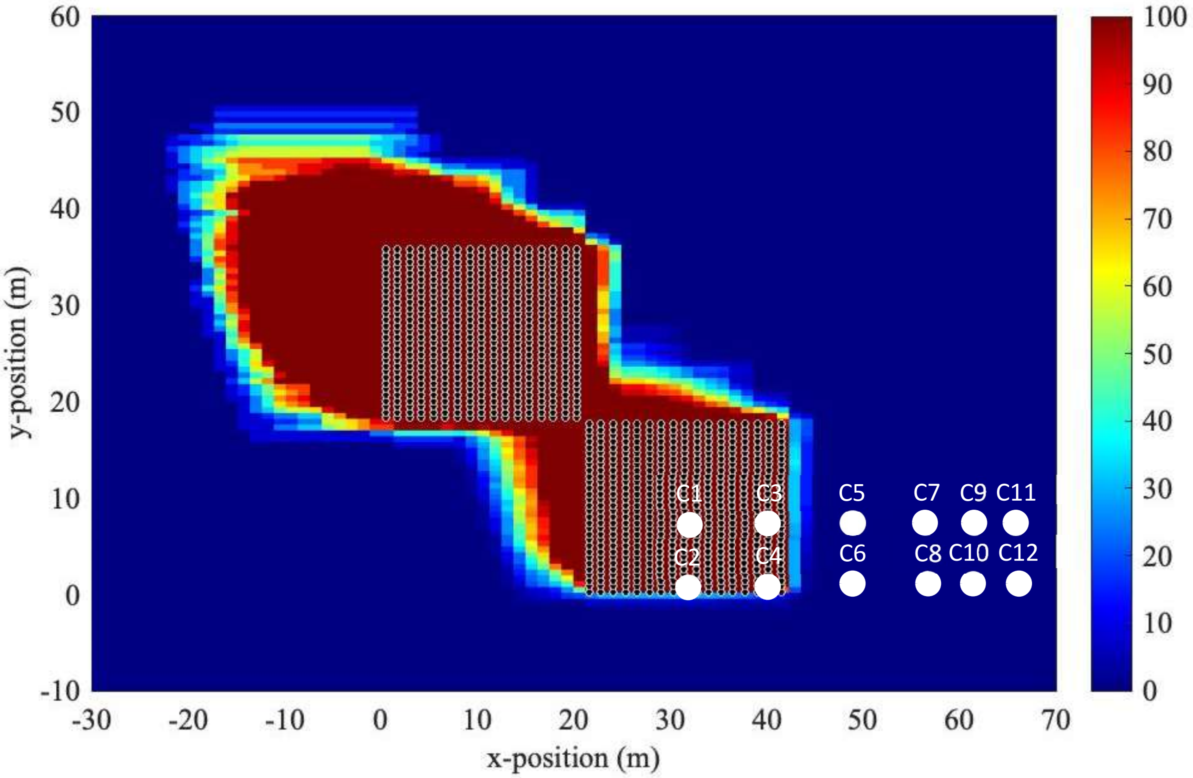

Figure 4.1 Sediment Biodeposit Deposition Areas in Lonnie's Pond. High to low deposition areas are shown with a color temperature scale: high deposition areas are colored dark red and received 100% of the average areal fecal pellet production and deposition, while areas colored dark blue received 0% of the average areal fecal pellet production and were not directly impacted by the oyster deployment. The biodeposit deposition map is overlain with 2019-2022 oyster deployment areas (two square areas with black filled circles representing individual floating bags), and April, August, and October sediment core locations, C1 – C12 (white markers).

Table 4.1 Summary of 2022 benthic flux rates from Lonnie's Pond core incubations. Cores were collected April 10, August 14, and October 30. Gray shaded rows indicate background (control) rates (*e.g.*, cores outside area impacted by oyster deposition). N_2-N detection limits were determined for each analysis date.

Collection Date: April 10, 2022; Incubation Temperature 11.9 °C							
Site ID	**SOD**	**NH_4^+**	**NO_3^-**	**DIN**	**N_2-N**	**Total N Cycled**	**Denitrified**
	mM/m^2/d	mM/m^2/d	mM/m^2/d	mM/m^2/d	mM/m^2/d	mM/m^2/d	% Total Cycled N
C1	52.76	2.34	0.03	2.36	1.46	3.82	38%
C2	33.53	0.12	-0.02	0.10	1.11	1.25	89%
C3	53.57	1.22	0.05	1.27	3.03	4.29	71%
C4	49.52	0.64	0.21	0.84	0.30	1.15	27%
C5	53.03	4.82	0.05	4.87	2.02	6.89	29%
C6	41.87	1.55	-0.01	1.54	1.27	2.83	45%
C7	8.80	2.61	0.02	2.63	1.06	3.69	29%
C8	58.15	0.62	-0.04	0.58	1.65	2.31	71%
C9	73.32	0.65	-0.03	0.62	2.62	3.31	79%
C10	46.10	0.20	-0.01	0.19	1.36	1.56	87%
C11	55.83	0.31	-0.01	0.30	1.57	1.89	83%
C12	62.56	0.08	-0.01	0.07	1.25	1.35	93%

Collection Date: August 14, 2022; Incubation Temperature 24.1 °C							
Site ID	**SOD**	**NH_4^+**	**NO_3^-**	**DIN**	**N_2-N**	**Total N Cycled**	**Denitrified**
	mM/m^2/d	mM/m^2/d	mM/m^2/d	mM/m^2/d	mM/m^2/d	mM/m^2/d	% Total Cycled N
C1	37.45	0.80	5.53	6.33	3.97	10.30	39%
C2	132.31	0.67	3.46	4.13	9.70	13.83	70%
C3	136.52	0.62	2.13	2.75	7.13	9.88	72%
C4	53.28	0.34	3.66	4.00	5.18	9.18	56%
C5	345.65	0.93	4.82	5.74	9.48	15.22	62%
C6	50.62	0.38	5.04	5.42	5.99	11.41	52%
C7	42.48	0.42	4.59	5.02	6.01	11.02	54%
C8	25.15	0.42	3.43	3.85	8.98	12.83	70%
C9	114.33	0.81	7.99	8.81	2.42	11.23	22%
C10	114.03	0.66	5.28	5.94	7.15	13.10	55%
C11	92.52	0.94	3.65	4.59	7.16	11.75	61%
C12	95.32	0.52	3.32	3.84	4.45	8.29	54%

Table 4.1 Summary of 2022 benthic flux rates from Lonnie's Pond core incubations (continued). Cores were collected April 10, August 14, and October 30. Gray shaded rows indicate background (control) rates (*e.g.*, cores outside area impacted by oyster deposition). N_2-N detection limits were determined for each analysis date.

Collection Date: October 30, 2022; Incubation Temperature 15.5 °C							
Site ID	**SOD**	**NH_4^+**	**NO_3^-**	**DIN**	**N_2-N**	**Total N Cycled**	**Denitrified**
	$mM/m^2/d$	$mM/m^2/d$	$mM/m^2/d$	$mM/m^2/d$	$mM/m^2/d$	$mM/m^2/d$	% Total Cycled N
C1	54.40	1.40	2.71	4.11	2.30	6.41	36%
C2	35.51	0.91	0.34	1.25	1.99	3.24	61%
C3	57.51	0.58	1.53	2.11	2.33	4.44	52%
C4	37.34	5.52	0.11	5.63	0.78	6.42	12%
C5	26.98	2.04	0.05	2.09	2.11	4.20	50%
C6	43.76	2.17	-0.12	2.05	0.64	2.93	22%
C7	36.72	0.48	0.39	0.87	1.43	2.29	62%
C8	37.36	-1.63	-0.58	-2.21	1.81	4.02	45%
C9	49.61	-0.34	0.38	0.03	2.24	2.96	76%
C10	26.21	-1.02	-0.19	-1.21	0.86	2.07	42%
C11	24.47	1.40	0.24	1.64	1.25	2.89	43%
C12	34.02	0.83	-0.10	0.73	1.07	2.01	53%

The October 30, 2022 flux rates were depressed compared to those in August as a result of reduced primary productivity in response to cooling waters (temperature decreased 8.6°C since August 14) and decreasing light availability. Similar to October 2021, 2022 nitrate fluxes were into and out of sediments with averaged rates resulting in a net release of nitrate to the water column, due to the greater sediment oxidation by amphipods. On average, nitrate efflux accounted for 37% and 43% of DIN fluxes out of the sediment in October 2022 and 2021, respectively, whereas average nitrate efflux accounted for only 4% of DIN fluxes out of the sediment in October 2020. Nitrate flux in 2022 was 509% higher than the 2016-2021 average rate for treated cores, but 25% lower than the 2021 treated core rate. Nitrate release from the 2022 control cores was 99% and 86% lower compared to the 2016-2021 and 2021 average rate, respectively. Ammonium flux increased from 0.62 to 2.10 mmol NH_4^+-N m^{-2} day^{-1} in treated cores between August and October, while control cores had a decrease in output of 0.63 mmol NH_4^+-N m^{-2} day^{-1} to an input of -0.05 mmol NH_4^+-N m^{-2} day^{-1}. Higher fluxes within treated cores reflect the N inputs in the biodeposits as oysters continue to filter particulate organic matter from the water column and release it to the impact area sediments.

The increased presence of amphipod mats in sediments inside and outside of the biodeposit impact area in recent years is a notable shift in the benthic community and may help to explain biogeochemical differences observed in 2021 and 2022. SCUBA diver observation and laboratory core descriptions indicate that amphipod mats were not present in coring areas in 2016 and 2017 and began to appear in 2018 with amphipod colonies identified in half of the August cores (Figure 4.2). The presence of amphipod mats associated with the southern oyster deployment areas was first noted in the 2020 Annual Report. The observed shift from small oligochaete worm to dense amphipod mats is indicative of a lessening of ecological impairments in Lonnie's Pond. This type of transition from oligochaetes to amphipods has been noted in other areas where organic loads decrease, most notably in Boston Harbor when sewage sludge disposal ceased. Once sediments are colonized, the amphipods effectively mine carbon from the sediment, and improve the sediment oxidation status and potentially enhancing coupled nitrification-denitrification. As omnivore/detritivore, amphipods may also play a role in the clearance of chlorophyll from the water column; they may play a role in the observed lower chlorophyll concentrations at the East moorings where amphipod densities have expanded since 2021.

Amphipod mats have continued to colonize the southern portion of the Pond (inside and outside the biodeposit impact area) and affect N cycling. 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 (averaged rates, mmol NO_3^--N m^{-2} day^{-1}; treated = 4.11; control = 4.71), an effect that was also seen in the control cores because of their now thicker oxic surface layer. Elevated rates of nitrate release and observations of thick surface oxic layers have increased from five cores in 2021 to all cores in 2022. The increased fraction of nitrate in DIN efflux likely owes to a greater overall nitrate production/efflux rate as a result of burrowing and mat forming amphipods that remove ammonium from the sediment and stimulate nitrification.

Amphipods or amphipod mats were observed in 67% of October 2022 cores; amphipod mats were observed in all April and August cores, and most October cores. Cores without amphipods (and few burrows) were characterized by variable surficial sediments (diffuse redox potential depths and mottling). Higher rates of nitrate efflux were measured in treated cores with greater amphipod mat surface coverage (>50%). 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) .[18]

Following the October 2022 flux, a core was sieved to collect amphipods for species identification. Two species, *Eolbrolgus spinosus and Byblis serrata,* were identified by Russ Winchell (Ocean Taxanomic). 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 and burrowing 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 Lonnie's Pond 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 and 2022 suggest that amphipod irrigation was injecting oxygen into the surficial sediments increasing nitrification. Although N is still being released from the sediments to the water column, the efflux of NH_4^+ was 6% (treated) and 11% (control) of the 2016-2021 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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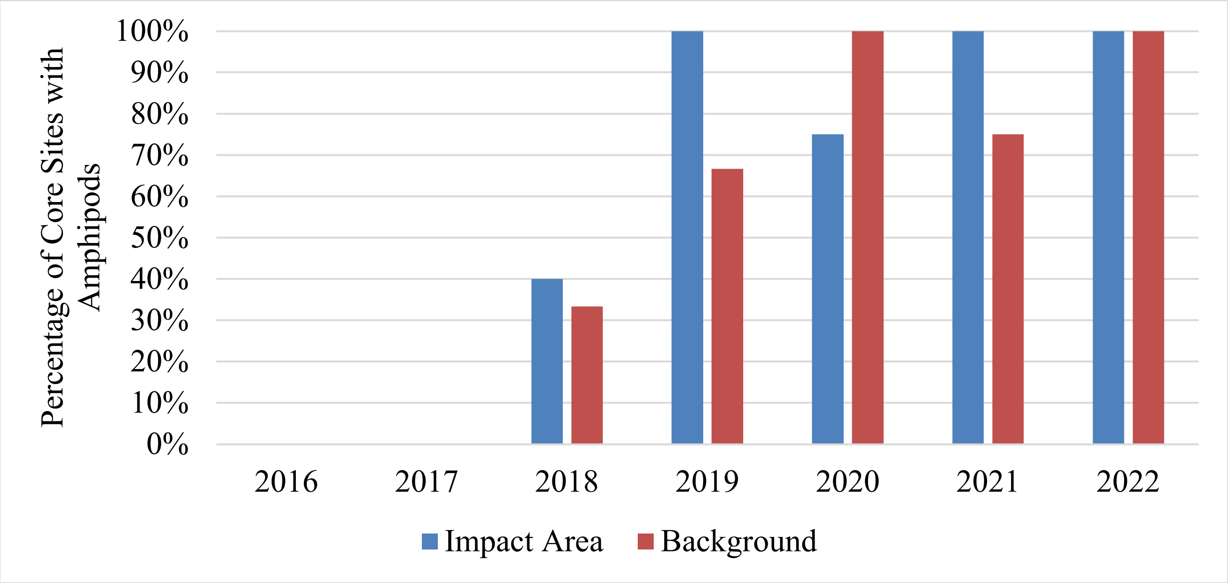
[18] Tucker, Jane, et al. "Response of benthic metabolism and nutrient cycling to reductions in wastewater loading to Boston Harbor, USA." *Estuarine, Coastal and Shelf Science* 151 (2014): 54-68.



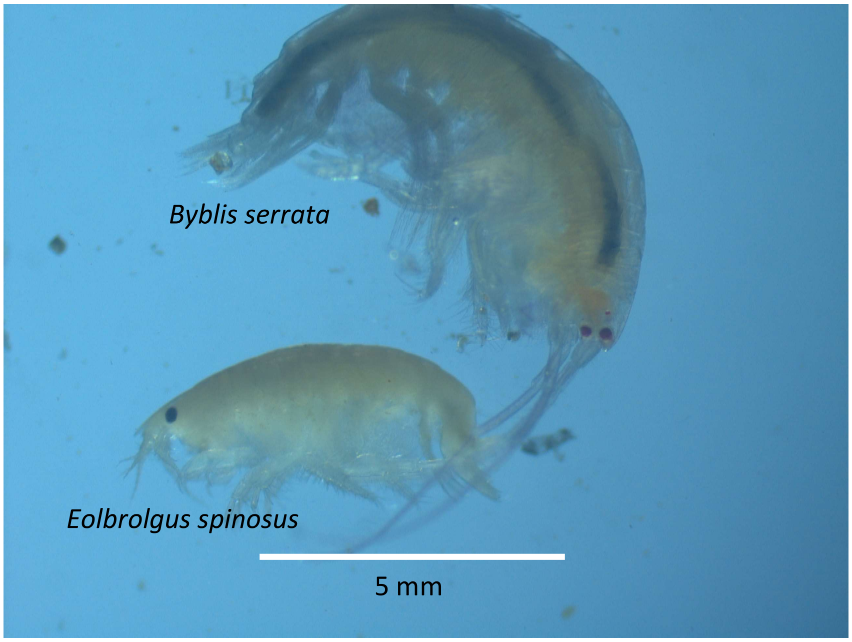


Figure 4.2. Amphipod Presence in Lonnie's Pond. Percentage of cores containing amphipods (2016-2022, top) in (blue) and outside (red) biodeposit impact areas. For each core, field and laboratory observations were recorded and compared to confirm the presence of amphipods. Following the October 2022 core collection, a core was collected and sieved for species identification. Two amphipod species were identified by Russ Winchell (Ocean Taxanomic) in this October core: *Eolbrolgus spinosus* and *Byblis* serrata (bottom figure).

Historical and 2022 sediment denitrification rates are shown in Table 4.2. 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 (*i.e.*, the "Oyster Effect'). Average denitrification rates measured in control cores outside the impact area were considered to represent background rates. Background rates may be a slightly overestimated as advection and dispersion of sinking biodeposits by water currents were the only processes examined and the particles may have spread over a wider area due to storm resuspension. Each year of denitrification includes summer readings and most years include readings the following spring to capture impacts from overwintering from the previous summer.

The April 2022 Oyster Effect rate (0.4 mmol N_2-N m^{-2} d^{-1}), which represents the overwintered impacts from 2021 (Year 6), was lower than the average enhanced dentification rates determined for the 2016-2021 spring fluxes. However, denitrification was observed in all of the cores, and rates were greater than 1.0 mmol N_2-N m^{-2} d^{-1} for all cores except C4 (0.30 mmol N_2-N m^{-2} d^{-1}). The control core Oyster Effect (1.4 N_2-N m^{-2} d^{-1}) is 34% higher than the 2016-2021 Oyster Effect average. Similar to previous years, the 2022 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. The results of the April 2022 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 six years of April flux data indicating the need to track denitrification into the following spring in order to capture the full extent of denitrification enhancement from oyster aquaculture.

In general, the difference between treated and background impacts (*i.e.*, the Oyster Effect) seems to be diminishing, but the sediment denitrification rates throughout the pond are increasing. The August 2022 Oyster Effect (0.9 mmol N_2-N m^{-2} d^{-1}) was within the range of 2016-2021 average enhanced rates. Similarly, the October 2022 Oyster Effect (0.7 mmol N_2-N m^{-2} d^{-1}) was equivalent to the Oyster Effect determined in October 2020 and 2021. Comparing the last two years, the August 2022 Oyster Effect increased 69% relative to the 2021 Oyster Effect and decreased 41% relative to the 2020 Oyster Effect. The combined Oyster Effect (treated minus control) for the 2022 oyster growing season (1.6 mmol N_2-N m^{-2} d^{-1}) was 80% of the 2016-2021 Oyster Effect average. In contrast, the August 2022 mean treated and background denitrification rates were the highest measured during the Lonnie's Pond. The August 2022 total denitrification rate increased 325% and 45% over August 2020 and 2021, respectively. 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 result being a larger N removal through denitrification, but a smaller net N removal through denitrification in sediments associated with oyster aquaculture.

Another sign of impacts of the aquaculture on other parts of the pond, is the gradual appearance of amphipod mats in the southern portion of the Pond outside of the primary biodeposition areas. Amphipod mats have appeared 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

Table 4.2 Mean denitrification rates for *Lonnie's Pond* cores. Cores were collected within and outside of the biodeposit impact area (Treated *vs.* Background, respectively). The difference in these two values should represent the contribution made by the ongoing oyster aquaculture (*i.e.,* Oyster Effect). An April 2023 sampling will occur to complete 2023 impacts. Note: 2016-2018 rates have been refined to be consistent with the rates published in Labrie *et al.* (2022).

	Year 1 Mean Denitrification Rates (mMoles/m^2/d)				
	Treated		Background		Oyster Effect
Date	Mean	Std. Dev.	Mean	Std. Dev.	
8/16/16	3.0	1.1	1.7	0.3	1.2
10/5/16	2.8	1.1	1.7	0.7	1.1
4/18/17	2.7	1.7	0.9	0.3	1.8
	Year 2 Mean Denitrification Rates (mMoles/m^2/d)				
	Treated		Background		Oyster Effect
Date	Mean	Std. Dev.	Mean	Std. Dev.	
6/27/17	1.3	0.4	0.3	0.4	1.0
8/1/17	2.1	0.9	1.6	0.8	0.5
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	3.3	2.5	1.2	0.4	2.2
10/2/18	0.5	0.3	0.2	0.3	0.4
4/22/19	1.8	1.2	0.4	0.5	1.4
	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
4/10/22	1.8	0.7	1.4	0.2	0.4
	Year 7 Mean Denitrification Rates (mMoles/m^2/d)				
	Treated		Background		Oyster Effect
Date	Mean	Std. Dev.	Mean	Std. Dev.	
8/14/22	6.9	2.3	6.0	2.3	0.9
10/30/22	2.2	0.2	1.4	0.5	0.7
4/XX/23	TBD	TBD	TBD	TBD	TBD

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 7 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 and expand in coming years.

A summary of the measured N removal from Lonnie's Pond via oyster harvest in the southern aquaculture area and enhanced denitrification during the seven 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). Total enhanced annual denitrification for 2022 resulted in a net removal of 4.94 kg N. However, spring carryover related denitrification from the 2022 oyster deployment has not yet been measured; therefore, Year 7 net N loss is an underestimate at this date and cannot be directly compared with the previous six project years.

Table 4.3 Annual Nitrogen Removal Budget for the Lonnie's Pond oyster impact area. Budget calculations focus exclusively on the southern aquaculture area showing contributions from enhanced denitrification and oyster harvest. Note that spring carryover denitrification is not yet included in Year 7 (2022) data; 2022 enhanced annual denitrification rate will be updated with spring 2023 carryover effects when the data is available. The model used to determine the impact areas was refined, and the Impact areas and Total Annual Enhanced Denitrification have been updated to be consistent with published values.[19]

Year	**Year 1 (2016)**	**Year 2 (2017)**	**Year 3 (2018)**	**Year 4 (2019)**	**Year 5 (2020)**	**Year 6 (2021)**	**Year 7 (2022)**
Time Deployed (days)	175	195	241	155	279	287	260
Enhanced Annual DeN$_2$ (mmol/m^2 N)	308.7	269.7[c]	317.4	155.9[b]	383.8	145.2	166.1
Enhanced Annual DeN$_2$ (g/m^2 N)	4.32	3.78	4.45	2.18[b]	5.38	2.04	2.33
Impact Area (m^2)	1574.6	1330.7	2717.4	2122.4	2122.4	2122.4	2122.4
Total Annual Enhanced DeN$_2$ (kg N)	6.81	5.03	12.08	4.63[b]	11.41	4.33	4.94[d]
Net Annual N Removed by Harvest (Southern Area only)[20] (kg N)	25.9[e]	27.2	36.2	30.8	42.3[e]	25.5[e]	30.0
Enhanced DeN$_2$ as a Percent of N Mass Removed by Harvest (%)	26.3%[e]	18.5%	33.4%[a]	15.0%[a]	27.0%[a e]	16.9%[a e]	14.4%[a d]

[a] Based on denitrification and harvest data from Southern deployment area only.

[b] 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.

[c] Year 2 (2017) spring carryover additions were estimated from Year 1 and Year 3 April enhanced denitrification measurements; the Year 2 enhanced denitrification estimate accounted for differences in biodeposition between years

[d] April rates are not included. April 2023 rates will be added once they become available.

[e] Net Annual N removed by oysters updated or corrected since previously reported (2016, N based on Science Wares Inc. report; 2020 and 2021, corrected by CSP/SMAST staff)

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[19] Labrie, M. S., M. A. Sundermeyer, and B. L. Howes. 2022. Quantifying the effects of floating oyster aquaculture on nitrogen cycling in a temperate coastal embayment. *Estuaries and Coasts*. https://doi.org/10.1007/s12237-022-01133-2

[20] 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 Aquaculture Oyster Deployment and Harvest

Following the strategies developed and implemented in 2020, the Aquaculture Contractor (Ward Aquafarms, LLC) deployed second year (YR2) and first year (YR1) oysters in Lonnie's Pond during April 5-7, 2022, and July 11-28, 2022, respectively. Oysters were placed in floating bags to filter and consume water column particulate N and incorporate that consumed N into their soft tissue and shell as they grow. Floating bags were deployed at two sites (north and south) and secured within four oyster deployment areas (see Figure 2.1, blue squares) that are consistent (surface area and location) with 2019-2021 deployments. All data collection followed the protocols in the Lonnie's Pond QAPP and guidelines, including modifications, established in collaboration with the Town of Orleans and Ward Aquafarms in January 2020. Ward Aquafarms and CSP/SMAST staff tracked all removals, redeployments, and new deployments of oysters throughout the 2022 season except for four events (July 11-12, July 21, and July 28), which were conducted solely by Ward Aquafarms. Tables 5.1-5.13 present key tracking results.

CSP/SMAST staff subsampled bags during deployment and harvest events, collecting live and dead (empty valves) oyster weight, empty bag weights (tare weights), and live and dead oyster counts. In addition, 20-30 oysters were collected to determine individual oyster shell heights and weights and nitrogen content. Replicate bags (n = 5-20) collected from each age class were sampled for oyster weights. Total oyster wet weights (total mass of live and dead oysters deployed or removed) were determined using net weights from the Orleans Transfer Station scale (oyster-filled truck weight minus empty truck weight) and individual bags weights measured during each input/output event. During the April deployments 1,109 floating mesh bags filled with 741 kg of YR2 runt oysters and the bags were placed into the north and south aquaculture areas in Lonnie's Pond. The southern aquaculture area was filled first, occupying all 1,020 of available array, while the remaining 89 bags were deployed in the northern aquaculture area. A large portion of oysters input into Lonnie's Pond in April were dead shells (42% on April 5; 37% April 7 by weight; Table 5.1).

Oyster aquaculture site maintenance, including deployment, management and bag rotation, was conducted by the Aquaculture Contractor throughout the 260-day growing season. Oysters were deployed for 27 fewer days in 2022 compared to 2021, but 105 additional days compared to 2019.[21] A subpopulation of 2021 YR1 oysters deployed in Lonnie's Pond were overwintered in cold storage and redeployed as 1-inch YR2 oysters in April 2022. Totes used to transport these YR2 oysters to the pond were weighed to determine an initial total oyster wet weight. Approximately, 190,210 YR2 oysters (live + dead) were deployed in 1,109 bags, with a cumulative oyster weight of 741 kg (see Table 5.1). Total YR2 oyster biomass deployed was lower in 2022 compared to 2021 because 2022 YR2 oysters were smaller (1-inch shell length in 2022 vs. 1-, 2-, and 3-inch shell lengths in 2021) and had a high initial mortality (40%). Oysters were culled to less than 1.5 inches prior to deployment in 2022 and CSP/SMAST staff collected tote subsamples to determine average oyster weight per bag (Table 5.2), live and dead counts and mass per bag (Table 5.3), and determined average size, weight, % carbon and % nitrogen of deployed oysters (Table 5.4). The total mass of initial N estimated for the initial oyster deployment and the two April dates was 4.08 kg. Of the 1,109 bags filled in April 2022, 597 remained in Lonnie's Pond for the full growing season until November/early December.

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[21] Growing season length will depend on when deployment starts and ends. The 2021 deployment was slightly longer than 2022 because it started on March 11, 2021. Deployments in 2021 and 2022 were much longer than 2019 since they started earlier in the year.

Table 5.1 April 2022 second year (YR2) oyster deployments weight and bag count. Total weight of YR2 oysters was determined by summing individual tote weights; tote measurements captured the entire population of April YR2 oysters deployed.

Date	Age Class	No. of Bags Deployed/Event	Total Weight (kg)	Live Oyster Weight (kg)	Dead Oyster Weight (kg)
4/5/22	YR2	587	360	207	153
4/7/22	YR2	522	382	241	141
TOTAL		**1,109**	**741**	**451**	**294**

Table 5.2 April 2022 YR2 oyster deployment tote subsamples. Subsamples of the April 2022 deployment were completed to determine average weights of bags and totes. Weights were determined for all totes containing oysters to be deployed and oysters were deployed in at total of 1,109 floating bags.

Date	Age Class	Totes Subsampled (n)	Average Tote Weight (kg)	Bags Subsampled (n)	Average Oyster Weight per Bag (kg)
4/5/22	YR2	16	22.5	29	0.50
4/7/22	YR2	17	22.4	25	0.50

Table 5.3 April 2022 YR2 oyster input subsamples weight and bag count. Subsample means with standard deviations in parentheses for replicate bag samples. Random sets of bags were subsampled during every event CSP/SMAST staff were present to capture the full oyster population deployed.

Age Class	Bags (n)	Average Oyster Count/Bag			Average Oyster Weight/Bag (kg)		
		Live	Dead	Total	Live	Dead	Total
YR2	21	79 (15)	89 (22)	172 (33)	0.30 (0.06)	0.20 (0.05)	0.50 (0.04)

Table 5.4 April 2022 YR2 oyster deployment/input average carbon and nitrogen contents and physical measurements. Average shell length and whole oyster wet weight are shown with standard deviations in parentheses for replicate oyster samples subsampled prior to April 2022 deployment. Total carbon and nitrogen (kg) input to Lonnie's Pond was calculated for each deployment/input date using a percent by weight of the whole dry oyster (shell + tissue + fouling material). The C/N content per gram dry weight was multiplied by the oyster dry/wet (harvest) weight ratio, and the mass of oysters deployed on each date (Table 5.1) to determine total C and N (kg) input by event.

Date	Age Class	Whole Oyster					Event Input	
		Length (mm)	Total Wet Wt. (g)	n	%C	%N	Total C (kg)	Total N (kg)
4/5/22	YR2	43.5 (3.17)	6.08 (0.55)	5	14.93	1.25	34.81	2.51
4/7/22	YR2	48.2 (5.14)	8.68 (1.24)	5	13.89	0.88	31.89	1.56
Total INPUT (741 kg total deployed weight)							**66.70**	**4.08**

On July 11 and 12, a portion of the initial April oyster deployment was removed and YR1 oyster replaced them between July 11 and 13. A total of 512 floating bags containing YR2 oysters (46% of the April total) were removed after 96 and 97 days in Lonnie's Pond, respectively (Table 5.5). These bags totaled approximately 11,264 Year 2 oysters weighing 489 kg. Subsamples of these bags showed that 43% of the mass in each bag were dead oysters (Table 5.6).

Once emptied, these 512 bags plus 152 more were refilled with 390 kg of YR1 oysters and deployed starting July 11. The remaining YR2 oysters (597 bags) were left in the northern aquaculture area. On July 21 and 28, the YR1 oysters were split into an additional 687 bags to decrease the seed stocking density. No additional YR1 oysters were deployed on July 21 or 28. These additional bags meant a total of 1,351 bags with 390 kg YR1 oyster were present in the pond beginning on July 28 (Table 5.7). All floating bags were initially marked by an identification tag containing a unique serial number, but split bags were not. Identification tags were used by the CSP/SMAST staff to facilitate an accurate count of the total bags deployed (input) and removed (output) from Lonnie's Pond on each event date.

Table 5.5 July 2022 Measured weight of YR2 oyster removal. A portion of the April 2022 deployment was removed in July 11 and 12. Total mass removed was determined in two ways: 1) from subsampled bag weights and 2) from measurements of total mass measured at the Orleans Transfer Station truck scale. Total Output was calculated by taking the number of bags multiplied by average oyster weight.

Date	Size Class	No. of Bags Removed/Event	Total Output (live + dead) (kg)	Net Weight from Truck Scale (kg)	% Difference
7/11/22	YR2	240	187	197	5%
7/12/22	YR2	272	212	291	32%
TOTAL		**512**	**398**	**489**	**20%**

Note: the number of bags was calculated using field notes provided by Ward Aquafarms, as well as verbal communication.

Table 5.6 July 2022 YR2 oyster removal subsamples weight and bag count. Subsample means with standard deviations in parentheses for replicate bag samples. Random sets of bags were subsampled during every event CSP/SMAST staff were present to capture the full oyster population deployed.

Age Class	Bags (n)	Average Oyster Count/Bag			Average Oyster Weight/Bag (kg)		
		Live	Dead	Total	Live	Dead	Total
YR2	5	22 (12)	146 (66)	168 (65)	0.57 (0.37)	0.39 (0.17)	0.96 (0.45)

Table 5.7 Subsample data from July 2022 deployment/input of YR1 oysters. YR1 oysters were deployed in Lonnie's Pond in 1,351 bags on between July 11-13, then a portion of these were split to decrease stocking density on July 21 and July 28 (no new oyster seed was deployed on these two later dates). Bag count was confirmed upon bag removal and tracking of marked zip ties. CSP/SMAST staff counted bags and collected tote subsamples on selected days; mean tote weights with standard deviations in parentheses are shown. Weights were determined for all totes containing YR1 seed oysters on the three deployment dates.

Date	Age Class	Totes (n)	Tote Weight (kg)	Total Weight (kg)	Bags Subsampled (n)	Count/ Bag	Weight/Bag (kg)	Total Bags
7/11	YR1	8	9.0 (1.7)	72.3	ND	ND	0.33*	221
7/12	YR1	9	10.0 (0.6)	89.8	ND	ND	0.31*	291
7/13	YR1	23	9.9 (1.1)	227.9	5	816	0.43	152
7/21	YR1	NA	NA	0	ND	ND	ND	381
7/28	YR1	NA	NA	0	ND	ND	ND	306
YR1 Event Total:				**390.1**				1,351

* Values were determined using total weight and the total number of bags deployed per day.

Ward Aquafarms removed all oysters by December 2022 with YR2 deployed in April removed on four dates (11/8, 11/14, 11/15, and 12/5) and YR1 oyster that were deployed in July were removed in December (12/19 and 12/21). All oysters were either relayed to another site or overwintered in dry storage with no redeployments in Lonnie's Pond until Spring 2023. YR2 oysters were deployed for 215 to 244 days, while YR1 oyster were deployed for 160 to 163 days. Net increase in oyster weight after accounting for input weights was 15,888 kg (Table 5.8).

YR1 and YR2 oyster counts and subsamples for oyster weights and counts per bag were collected during the removal events (Tables 5.9 and 5.10). Subsamples were also collected to determine C and N content analysis of whole oyster, shell, tissue, and fouling material were also completed (Table 5.11). Collection and nutrient analysis of fouling material (primarily encrusting calcareous organisms, biodeposits, and algae) were conducted to quantify its contribution to whole oyster N mass. Empty valves (dead oysters) were counted, collected and analyzed for N content to allow a full accounting of oyster biomass. Oyster N content as the percent N of an individual whole oyster or empty valve, coupled with measured wet to dry weight ratios and total wet weight determined for each input/output event, was used to determine the total mass of nitrogen as live/dead oysters going into or coming out of the Pond. Chemical analysis followed procedures specified in the QAPP to determine the cumulative initial nitrogen mass of the deployed oysters (see Table 5.11) and cumulative mass in harvest (Table 5.12). At time of harvest, average percent nitrogen content (dry weight) of YR2 and YR1 oysters was 0.91% N (0.87% to 1.02%) and 0.75% N (0.63% to 0.83%) respectively. Nitrogen contents were determined for whole oysters and include N contributions from fouling material scrubbed from the shell. Collectively, the oysters (YR1 and YR2) in Lonnie's Pond incorporated a net total of 68.5 kg of nitrogen in their tissues and shells during the growing season.

Table 5.8 Monthly summary of total oyster weight deployed (input) and removed (output) from Lonnie's Pond during the 2022 season. Weights are calculated from net truck weight.

2022	Oyster Weight Input (kg)	Oyster Weight Output (kg)
April	741	0
July	390	489
November	0	1,924
December	0	14,606
Total	**1,131**	**17,019**
NET:	**15,888 kg**	

Table 5.9 December 2022 YR1 oyster removal: floating bag counts and total weights. These oysters were deployed for 160 to 163 days (July to December). Number of bags was calculated using field notes provided by Ward Aquafarms, as well as verbal communication.

Date	Size Class	No. of Bags Removed/ Event	Average # live Oysters/ bag	Total Output (live + dead) (kg)	Net Weight from Truck Scale (kg)	% Difference
12/19/22	YR1	676	391	6,614	6,037	9%
12/21/22	YR1	675	662	7,312	8,398	15%
TOTAL		**1,351**		**13,926**	**14,705**	**6%**

Table 5.10 November/December 2022 YR2 and YR1 oyster removal subsamples. Oyster counts and weights were determined by taking an average per event (standard deviation presented in parentheses for bags measured), and then an overall average of the total number subsampled.

Date	Age Class	Bags (n)	Average Oyster Count/Bag			Average Oyster Weight/Bag (kg)		
			Live	Dead	Total	Live	Dead	Total
11/8	YR2	20	24 (11)	133 (36)	157 (33)	1.75 (0.81)	0.52 (0.28)	2.28 (0.90)
11/14	YR2	20	25 (13)	156 (51)	181 (42)	1.88 (1.13)	0.40 (0.11)	2.29 (1.12)
11/15	YR2	20	35 (12)	165 (42)	200 (39)	2.84 (1.22)	0.49 (0.08)	3.33 (1.20)
12/5	YR2	10	30 (7)	168 (45)	197 (45)	2.54 (0.55)	0.49 (0.10)	3.03 (0.56)
YR2 Event Average			**28 (12)**	**156 (43)**	**184 (39)**	**2.25 (1.01)**	**0.48 (0.17)**	**2.78 (1.02)**
12/19	YR1	10	391 (56)	31 (16)	422 (66)	8.62 (0.88)	0.16 (0.08)	8.78 (0.87)
12/21	YR1	10	662 (98)	32 (11)*	705 (84)	10.76 (1.05)	0.06 (0.04)	10.82 (1.04)
YR1 Event Average			**526 (80)**	**31 (14)**	**563 (76)**	**9.69 (0.97)**	**0.11 (0.07)**	**9.80 (0.96)**

*n=1 bag replicate was determined to be a statistical outlier and was not included in the presented data

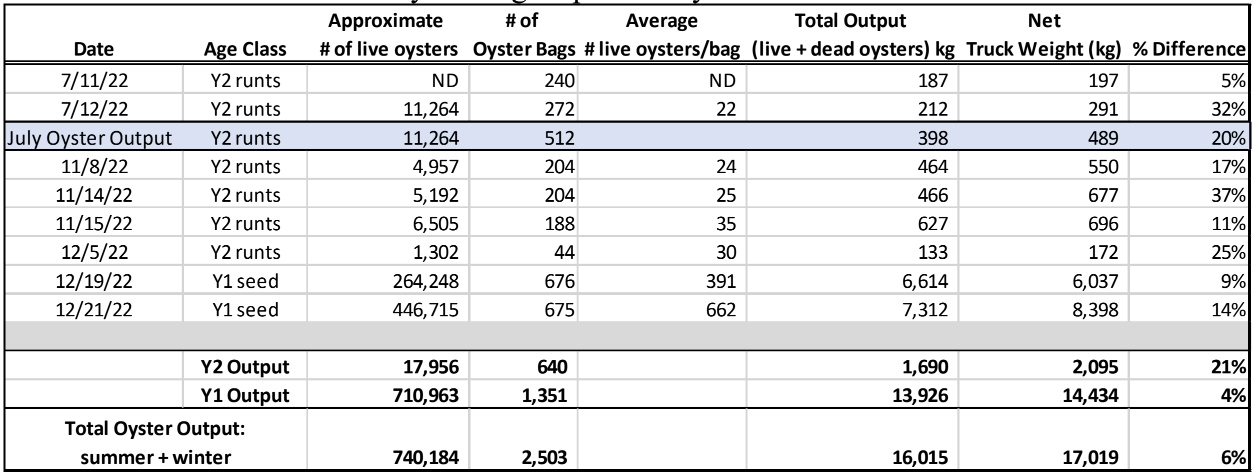
Table 5.11 Combined April and July oyster input average carbon and nitrogen contents and physical measurements. Average shell length and whole oyster wet weight are shown with standard deviations in parentheses for replicate oyster samples subsampled prior to April 2022 and July 2022 deployments. Total carbon and nitrogen (kg) input to Lonnie's Pond was calculated for each deployment date using a percent by weight of the whole dry oyster (shell + tissue + fouling material). The C/N content per gram dry weight was multiplied by the oyster dry/wet (harvest) weight ratio, and the mass of oysters deployed on each date (Table 5.1 and 5.6) to determine total C and N (kg) input by event. In total, 96.34 kg of carbon and 5.03 kg of nitrogen was deployed into Lonnie's Pond in April and July 2022. This is much less compared to the 451.12 kg of carbon and 24.48 kg of nitrogen of oyster deployed in April 2021.

Date	Input/ Output	Age Class	Whole Oyster					Event Input	
			Length (mm)	Total Wet Wt. (g)	n	%C	%N	Total C (kg)	Total N (kg)
4/5	INPUT	YR2	43.5 (3.17)	6.08 (0.55)	5	14.93	1.25	34.81	2.51
4/7	INPUT	YR2	48.2 (5.14)	8.68 (1.24)	5	13.89	0.88	31.89	1.56
7/11	INPUT	YR1	18.13 (1.58)	0.47 (0.18)	15	12.72	0.41	5.49	0.18
7/12	INPUT	YR1						6.82	0.22
7/13	INPUT	YR1						17.31	0.56
7/21	INPUT	YR1						0.00	0.00
7/28	INPUT	YR1						0.00	0.00
Total INPUT (YR1+YR2 oysters: 1,131 kg)								**96.34**	**5.03**

Table 5.12 Combined July, November, and December oyster removal average carbon and nitrogen contents and physical measurements. Average shell length and whole oyster wet weight are shown with standard deviations in parentheses for replicate oyster samples subsampled during harvest. Total carbon and nitrogen (kg) input to Lonnie's Pond was calculated for each harvest/output date using a percent by weight of the whole oyster (shell + tissue + fouling material). The C/N content (dry weight basis) 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 5.4 and 5.9) to determine total C and N (kg) removed on each harvest date. In total, 1,250 kg of carbon and 68.5 kg of nitrogen was removed as gross output from Lonnie's Pond.

Date	Input/ Output	Age Class	Whole Oyster					Event Output	
			Length (mm)	Total Wet Wt. (g)	n	%C	%N	Total C (kg)	Total N (kg)
7/11/22	OUTPUT	YR2	68.7 (10.5)	32.3 (5.5)	5	14.34	1.02	16.16	0.85
7/12/22	OUTPUT	YR2						23.86	1.25
11/8/22	OUTPUT	YR2						49.88	2.58
11/14/22	OUTPUT	YR2	89.6 (6.4)	82.35 (22.4)	5	15.69	0.92	60.75	3.24
11/15/22	OUTPUT	YR2						62.09	3.36
12/5/22	OUTPUT	YR2	107.4 (10.6)	102.6 (14.1)	5	15.77	0.87	18.95	0.95
12/19/22	OUTPUT	YR1	78.8 (5.4)	36.2 (4.1)	5	14.10	0.83	501.55	29.22
12/21/22	OUTPUT	YR1	69.7 (4.7)	24.1 (4.8)	5	11.85	0.63	516.83	27.06
Total OUTPUT (YR1+YR2; 15,932.5 kg)								**1250.06**	**68.51**

Table 5.13 Summary table of Lonnie's Pond 2022 oyster harvest output. Total output is calculated by taking the average weight of oysters multiplied by the number of bags using subsampled data. Truck weights were measured at the Orleans Transfer Station before and after harvest and subtracts the final scale weight from tare weight and weight of the oyster bags. *Year 2 bag output sums to 640 bags, but only 597 Y2 bags remained from July; likely 43 bags harvested from November were mistakenly Y1 bags input in July.



Date	Age Class	Approximate # of live oysters	# of Oyster Bags	Average # live oysters/bag	Total Output (live + dead oysters) kg	Net Truck Weight (kg)	% Difference
7/11/22	Y2 runts	ND	240	ND	187	197	5%
7/12/22	Y2 runts	11,264	272	22	212	291	32%
July Oyster Output	Y2 runts	11,264	512		398	489	20%
11/8/22	Y2 runts	4,957	204	24	464	550	17%
11/14/22	Y2 runts	5,192	204	25	466	677	37%
11/15/22	Y2 runts	6,505	188	35	627	696	11%
12/5/22	Y2 runts	1,302	44	30	133	172	25%
12/19/22	Y1 seed	264,248	676	391	6,614	6,037	9%
12/21/22	Y1 seed	446,715	675	662	7,312	8,398	14%
	Y2 Output	**17,956**	**640**		**1,690**	**2,095**	**21%**
	Y1 Output	**710,963**	**1,351**		**13,926**	**14,434**	**4%**
Total Oyster Output: summer + winter		**740,184**	**2,503**		**16,015**	**17,019**	**6%**

6.0 Key Findings and Future Considerations

CSP/SMAST staff working with Ward Aquafarm and Town staff were able to document 63.5 kg net removal in the Lonnie's Pond 2022 oyster growth and harvest. Nitrogen removed from Lonnie's Pond via incorporation into oyster biomass and subsequent harvest did not exceed the goal outlined in the Lonnie's Pond Aquaculture and Nitrogen Management Plan of 75 kg N removal in harvest after accounting for N mass upon deployment. Additional N removal will be added via sediment denitrification once April 2023 sediment sampling is completed. The reasons for the reduced net removal are discussed below.

During the preparation of the 2022 review, CSP/SMAST staff noted that the 2020 and 2021 removals were calculated incorrectly, but for two different reasons. In 2020, a project staffer accounting for 1,683 kg YR2 oysters that were removed in September, culled and redeployed resulted in a N removal overestimate. In 2021, project staff accounting of N content in oyster wet and dry weight relationship was erroneous. The corrected N removals for 2020 and 2021 are 85 kg and 51 kg, respectively.

In order to ensure that these types of issues are avoided in the future, CSP/SMAST will appoint a single project manager to oversee and review data processing, require that all data processing files are reviewed by all members of the Project team, and enforce the use of a template to calculate N removal. CSP/SMAST also suggests that some of this issue may be due to the tracking complexities and streamlining of growing and monitoring could avoid these issues in the future. Further discussion of these and other issues are described in the Key Project findings below:

Key Project findings and considerations for future deployment include:

1. Total net N 2022 mass removal in Lonnie's Pond from oyster aquaculture program was 63.5 kg N (recovery/harvest – deployment) or approximately the same as in 2019. The removal was equally divided between the southern and northern deployment areas. In addition, oyster enhanced denitrification within sediments receiving biodeposits was an additional 4.9 kg N removal from the southern deployment area only without accounting for additional sediment removal that will occur in overwintering biodeposits; these will be measured in April 2023. Therefore, total Lonnie's Pond aquaculture nitrogen removal from associated with the 2022 deployment was at least 68.3 kg N. If denitrification at the northern deployment area was the same as the southern deployment area, the total 2022 removal (pending additional sediment removal in April 2023 measurements) was 73.2 kg.

2. The regular reductions in the amount of N in Lonnie's Pond due to the aquaculture is improving the sediments throughout the whole ecosystem and reducing sediment N additions to the water column. 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 from oligochaetes to amphipods. In 2022, amphipods were observed throughout the pond in all August sediment cores. The presence and bio-irrigation activities of mat-forming amphipods have increased coupled nitrification-denitrification and greater denitrification rates within all collected cores. August 2022 denitrification rates in sediments impacted by the aquaculture installations and control sediments unimpacted by the installations were the highest measured in Lonnie's Pond. August ammonium efflux rates were less than 10% of the 2016-2021 ammonium rate average.

It would be expected that continued aquaculture should further improve nitrogen-related water quality throughout the pond to the extent that the amphipod mats continue in coming years. In 2022, two species of amphipods inhabiting Lonnie's Pond sediments were identified. It is important to continue tracking these changes and possibly account for these changes in the overall N balance of Lonnie's Pond. It is recommended that a video survey be conducted to determine the spatial extent of the amphipod mat coverage and its relationship to the biodeposit impact areas.

3. YR1 oysters have the greatest potential for N removal due to their growth and biomass increase compared to the YR2 oysters. Figure 6.1 shows that YR1 oysters generally have greater net weight increase (*i.e.*, biomass increase) and are generally the predominant portion of biomass increase in 2020-2022. Given that the percent N content of a whole oyster is relatively consistent, net N removal is dependent on the mass of YR2 or YR1 oysters deployed and the biomass created.

4. Early season/longer deployment of YR2 results in greater N removal. 2020 had the greatest mass of oysters deployed in March/April (Table 6.1) and the largest TN removal (Figure 6.2). In March 2020, 3,747 kg of oysters were deployed as opposed to 741 kg deployed in March/April 2022. The early deployment of YR2 oysters in 2020 maximized the length of the oyster growing season and increased the market value of harvestable oysters. Average shell lengths of YR2 oysters deployed on two dates in April 2022 were 43.5-48.2 mm. With a market requirement of shell length ≥63.5 mm, live YR2 oysters harvested in July 2022 had an average shell length of 68.2 mm. Continued deployment of YR2 oysters in early spring will also dampen the effect of YR1 oyster mortality and reduce interannual variability in nitrogen removal through oyster harvest.

5. Reducing oyster mortality and culling oysters prior to deployment is another key to N removal. March 2020 deployment had 3-7% mortality, while April 2022 deployment had 40% dead oysters deployed. So in addition to a lower mass of oysters deployed in 2022, less of the deployed oysters were alive to add biomass and remove N. YR1 oyster inputs in 2022 and 2021 were nearly equal, but mortality was 6% and 39%, respectively, leading to a reduction in the N removed in 2021 compared to 2022.

6. Continued exclusive use of the Orleans Transfer Station Scale "truck scale" for harvest wet weight determinations should be maintained. The success of oyster aquaculture as an N management approach and its continued success requires a simple and reproducible approach. Use of the Town scale 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, including changes in truck weights (fuel use) and changes in tare weights of the truck and bags and oyster weights due to water loss. Consistent use of the Orleans Transfer Station Scale should continue in 2023 to determine the weight of oysters removed from the Pond on all dates. Transfer Station Scale receipts should be photocopied and shared with the Monitoring Contractor in addition to records of any truck contents when determining truck tare weights.

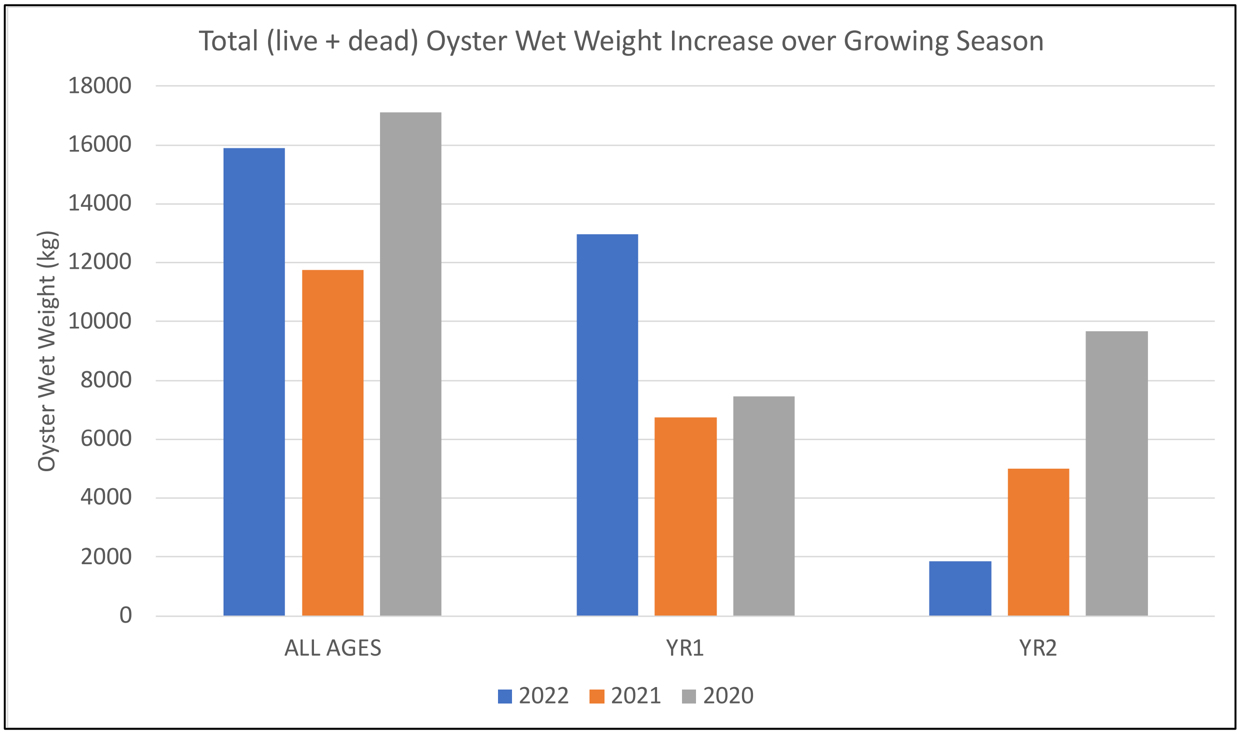


Figure 6.1 Increase in oyster biomass in Lonnie' Pond over the 2020-22 growing seasons.

Table 6.1 Oyster input and output weights by month for 2020-22.

Month	2022		2021		2020	
	Oysters Weight (kg) IN	Oysters Weight (kg) OUT	Oysters Weight (kg) IN	Oysters Weight (kg) OUT	Oysters Weight (kg) IN	Oysters Weight (kg) OUT
MARCH			2,843		3,747	
APRIL	741					
JUNE				2,119		
JULY	390	489	370	3,040	91	1,524
AUGUST					436	8,047
SEPTEMBER					1,683	3,492
OCTOBER				553		
NOVEMBER		1,924				2,032
DECEMBER		14,606		9,250		7,978
OVERALL	1,131	17,019	3,213	14,963	5,957	23,073
NET	15,888		11,749		17,116	

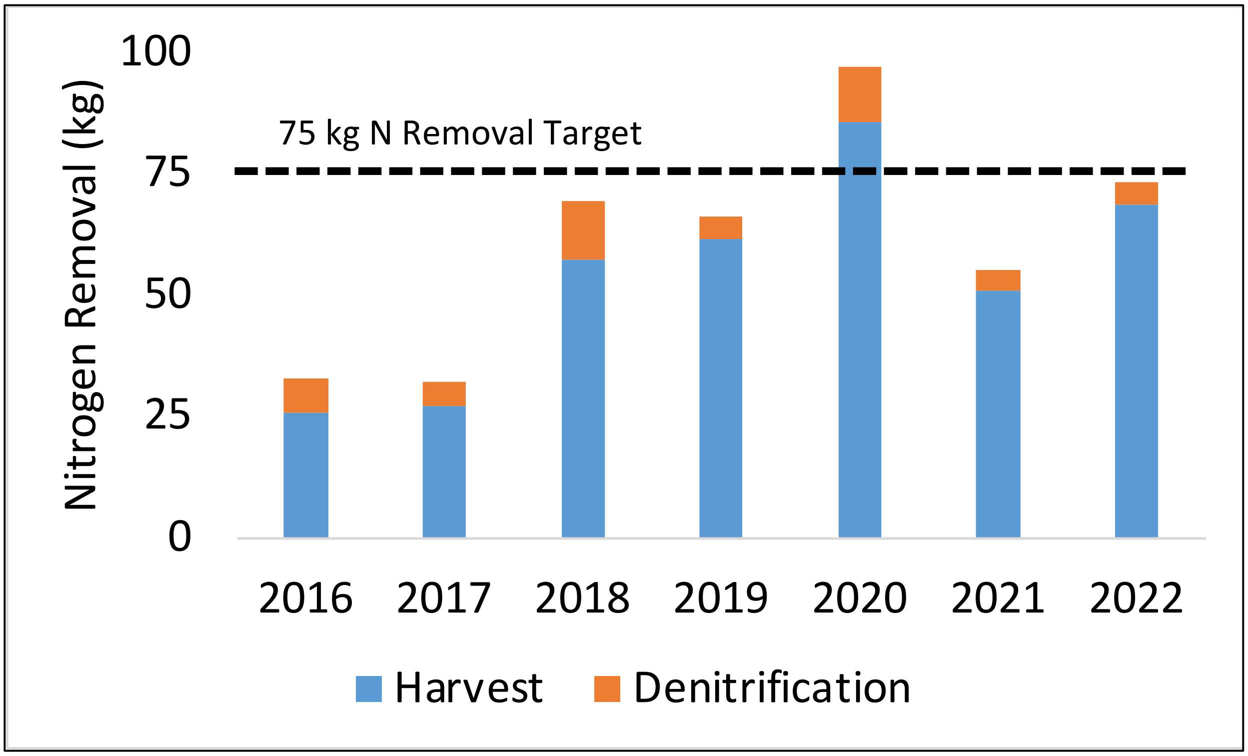


Figure 6.2 Total nitrogen removal in Lonnie's Pond over the 2016-2022 growing seasons separated by harvest and denitrification. 2022 total will be revised when overwintered denitrification is accounted for in April 2023 sediment sampling.

7. The used of zip ties to identify bags designated for Lonnie's Pond should continue. In order to optimize tracking of oysters (and N accounting), CSP/SMAST and the Ward Aquafarm use zip ties to identify bags. Zip ties include a unique serial number and sealed bag openings 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 approach increases efficiency of labor and material use and weight/N accounting, as tags must be removed to open the bags. If necessary, zip ties should be replaced for the 2023 growing season to ensure serial number readability. Zip ties should also be removed, counted, and redeployed as YR1 oysters grow and are redeployed at lower stocking densities.

8. 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. Streamlined oyster deployment simplifies N accounting. In order to account for all N entering and leaving Lonnie's Pond during oyster deployment, CSP/SMAST as the Monitoring Contractor needs to be on-site. In 2021, CSP/SMAST needed to be on-site to account for oyster inputs and outputs on 26 dates, which was in addition to the 13 water quality sampling dates required to monitoring water quality changes in the system. In 2022, the Monitoring Contractor reduced the number of oyster inputs and outputs to 13 dates. As the moving and redeploying of different age oysters over extended periods can lead to uncertainties in documenting of nitrogen removal (as was seen in 2020), in-step communications between Aquaculture Contractor and Monitoring Contractor on the deployment and removal processes of oyster stock needs to be as rigorous and reliable as

possible. The CSP/SMAST staff members overseeing November and December 2022 YR1 and YR2 oyster removal are now well versed in the Aquaculture contractor's procedures. For continuity, the same CSP/SMAST staff members will oversee the Spring 2023 deployment of YR2 oysters. It is recommended that opportunities to streamline monitoring and aquaculture regularly be pursued.