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

2023 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, permeable reactive barriers, 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 for 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 for 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, 2022, and 2023 project years.

This document is the 2023 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[5] that was previously submitted to the Town. This 2023 Annual Report includes the N removal attained during the 2023 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.

Second year oysters (YR2) were deployed March 31, April 7, and April 12. The YR2 population was removed from Lonnie's Pond between July 13 and August 10 by the Aquaculture Contractor. YR2 oysters harvested in July 2023 were replaced with YR1 oyster seed. YR1 seed was removed December 18 and 20. The CSP/SMAST monitoring team tracked all the oyster deployments and removals, as well as water quality and sediment nutrient regeneration/denitrification throughout this period.

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[5] Coastal Systems Program, School for Marine Science and Technology (CSP/SMAST), University of Massachusetts-Dartmouth, Lonnie's Pond Aquaculture/TMDL Semi-Annual Status Update. July 2023. 10 pp.

2.0 Water Quality Monitoring

2023 Overview

Using procedures detailed in the Lonnie's Pond QAPP, CSP/SMAST staff began conducting water quality monitoring in Lonnie's Pond on March 28, 2023, to measure parameters prior to the oyster deployment starting March 31, 2023. Water quality samples were collected again in around the southern oyster aquaculture area during the benthic sediment flux on April 3, and the full-pond, bi-weekly sampling resumed on May 11, 2023. During 2023, staff completed thirteen (13) sampling events: March 28, May 11, May 25, June 12, June 26, July 10, July 26, August 10, August 24, September 9, September 21, October 5, and October 23, 2022 (Table 2.1). During each sampling event, CSP staff collected water quality samples at nine locations in Lonnie's Pond (Figure 2.1). In addition to the sampling event, staff deployed two continuous monitoring devices (sondes) June 12 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 31. 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**
3/28/2023	21	X	X	X	X	X	X	X	X
5/11/2023	21	X	X	X	X	X	X	X	X
5/25/2023	21	X	X	X	X	X	X	X	X
6/12/2023	21	X	X	X	X	X	X	X	X
6/26/2023	21	X	X	X	X	X	X	X	X
7/10/2023	21	X	X	X	X	X	X	X	X
7/26/2023	21	X	X	X	X	X	X	X	X
8/10/2023	21	X	X	X	X	X	X	X	X
8/24/2023	21	X	X	X	X	X	X	X	X
9/7/2023	21	X	X	X	X	X	X	X	X
9/21/2023	21	X	X	X	X	X	X	X	X
10/5/2023	21	X	X	X	X	X	X	X	X
10/23/2023	21	X	X	X	X	X	X	X	X
Total	**252**								

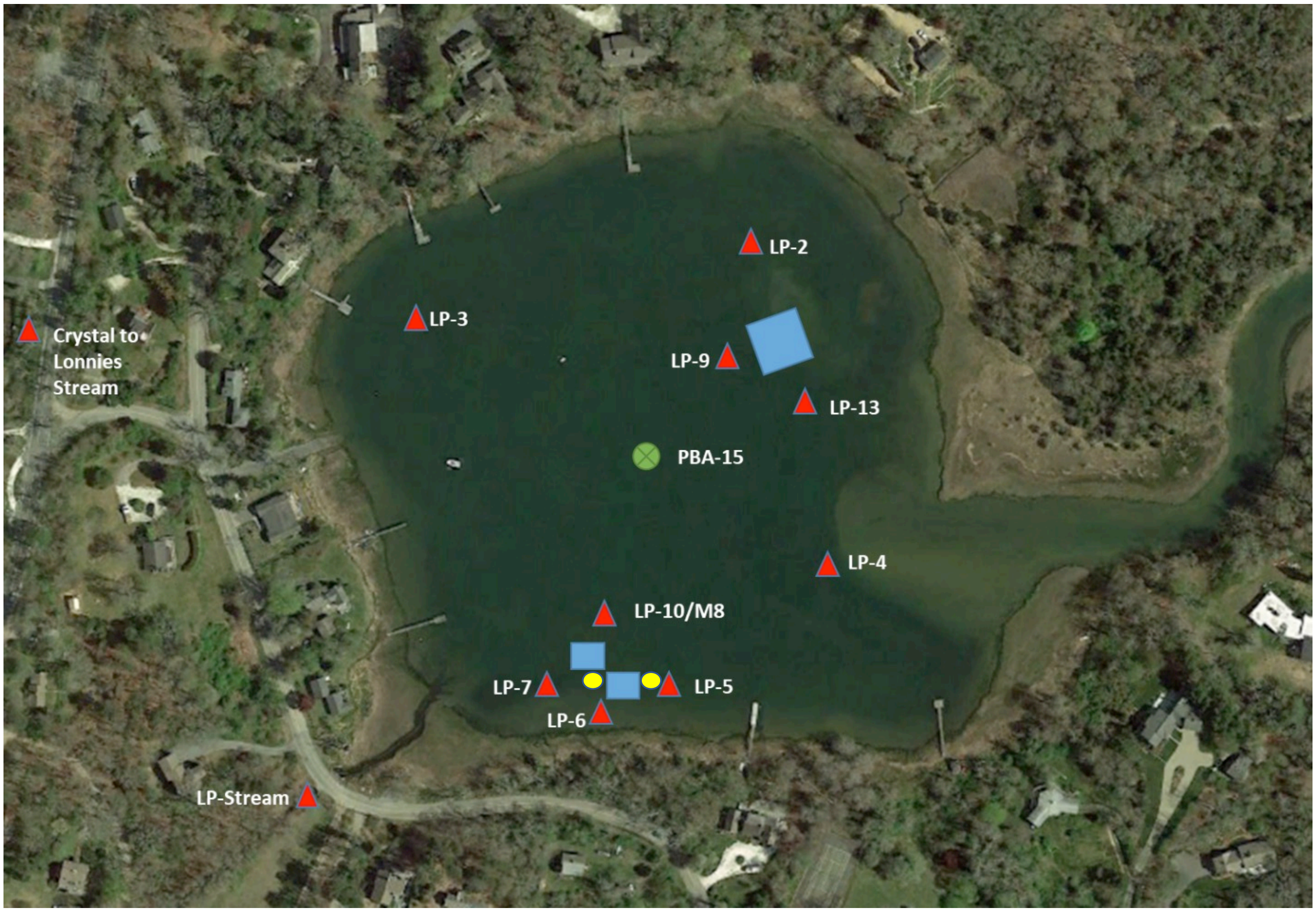

Figure 2.1 Map of Lonnie's Pond 2023 water quality sampling locations. Water column samples were collected biweekly by CSP staff March 31, 2023, to October 23, 2023, at the triangle locations. Dissolved oxygen and chlorophyll sondes were moored at the yellow circles. Samples were also collected biweekly by Orleans citizen volunteers as part of the Pleasant Bay Alliance (PBA) monitoring program from July 6-September 5, 2023, at the green circle location. The blue squares represent the oyster deployment areas (same areas as 2021). Water tends to flow from LP-3 to LP-7 to LP-5 on ebbing tides.

Water quality monitoring from 2023 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. A spring algae bloom was measured on May 25 seen by the spike in total chlorophyll pigments at all stations, with the stations surrounding the northern aquaculture area showing the highest concentration of 12.8 μg/L (Figure 2.2). The spring spike in chlorophyll was half the concentration compared to the spring 2022 bloom (average of 32.6 μg/L). By June 12, chlorophyll decreased by 38% in the northern aquaculture area which may be a result of the fast-growing oysters' filtering capability, which were redeployed into this area in March. Chlorophyll levels remained about 1/3 lower than the recorded spring bloom levels, but increased again from July 10 - August 10, which also coincided with the removal of the Year 2 oysters from the northern aquaculture area and the deployment of YR1 oyster seed throughout both aquaculture areas. 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 and is not near the northern or southern aquaculture areas. Chlorophyll levels measured at LP3 were higher than stations associated with oysters in 50% of the sampling events.

A noticeable increase in phytoplankton occurred on August 10 in the southern aquaculture area (LP5,7,10) following a 2.9-inch rain event, which increased stream input into Lonnie's Pond (see Figure 2.2). The stream discharges just west of LP7 and the southern aquaculture area (Figure 2.1). This input of storm water appears to affect the nitrogen constituents at all sites in Lonnie's Pond, seen as spikes PON, bioactive N, and TN on August 10. The presence of oyster aquaculture may have buffered the stormwater induced nutrients signaled by the lower total pigments (-32%), PON (-43%), bioactive N (-58%), and TN (-42%) measured within the oyster aquaculture area at station LP6 compared to the average nutrient concentrations at the rest of the stations on August 10. Oyster aquaculture's role as direct remediation of stormwater impacts highlights another valuable role this soft-solution can serve for embayment water quality. Chlorophyll concentrations reached their maximum values for sites without oyster aquaculture (LP3,4) on September 21 and then all stations had concentrations below 5 μg/L by the end of October. Overall, average chlorophyll pigments were 29% lower in 2023 than in 2022.

Nitrogen is the key water column proxy measure for ecosystem health in the Pleasant Bay TMDL. The MEP assessment of Pleasant Bay used total nitrogen, but focused on bioactive N, which is the sum of dissolved inorganic nitrogen (DIN) and particulate organic nitrogen (PON). DIN is the sum of inorganic N components [nitrate+nitrite (NOx) and ammonium (NH_4)] concentrations, while PON is typically N bound in phytoplankton or associated organic material. Bioactive N ranged from 13-21 μM (0.18-0.3 mg N/L) during the initial measurements in March when the oysters were deployed into Lonnie's Pond, and this indicates that oyster growth was likely not limited by food. Bioactive N was relatively stable with PON-driven spikes, with PON 9% higher in 2023 compared to 2022. This increase in PON may be due to the increase in oyster harvest and deployment days during the summer of 2023 (6 days) compared to 2022 (4 days). Rainfall and bag disturbance during sampling can also increase the amount of PON in the water column. Both bioactive N and PON showed concentration peaks within the southern aquaculture area, represented by station LP6, on June 26 and August 10 (Figure 2.2). All sites showed elevated concentrations on August 10 and to a lesser degree, on September 21. All sites decreased in PON concentration from September 21 until the end of the monitoring on October 31. DIN was still elevated within the oysters on October 5 represented by LP6.

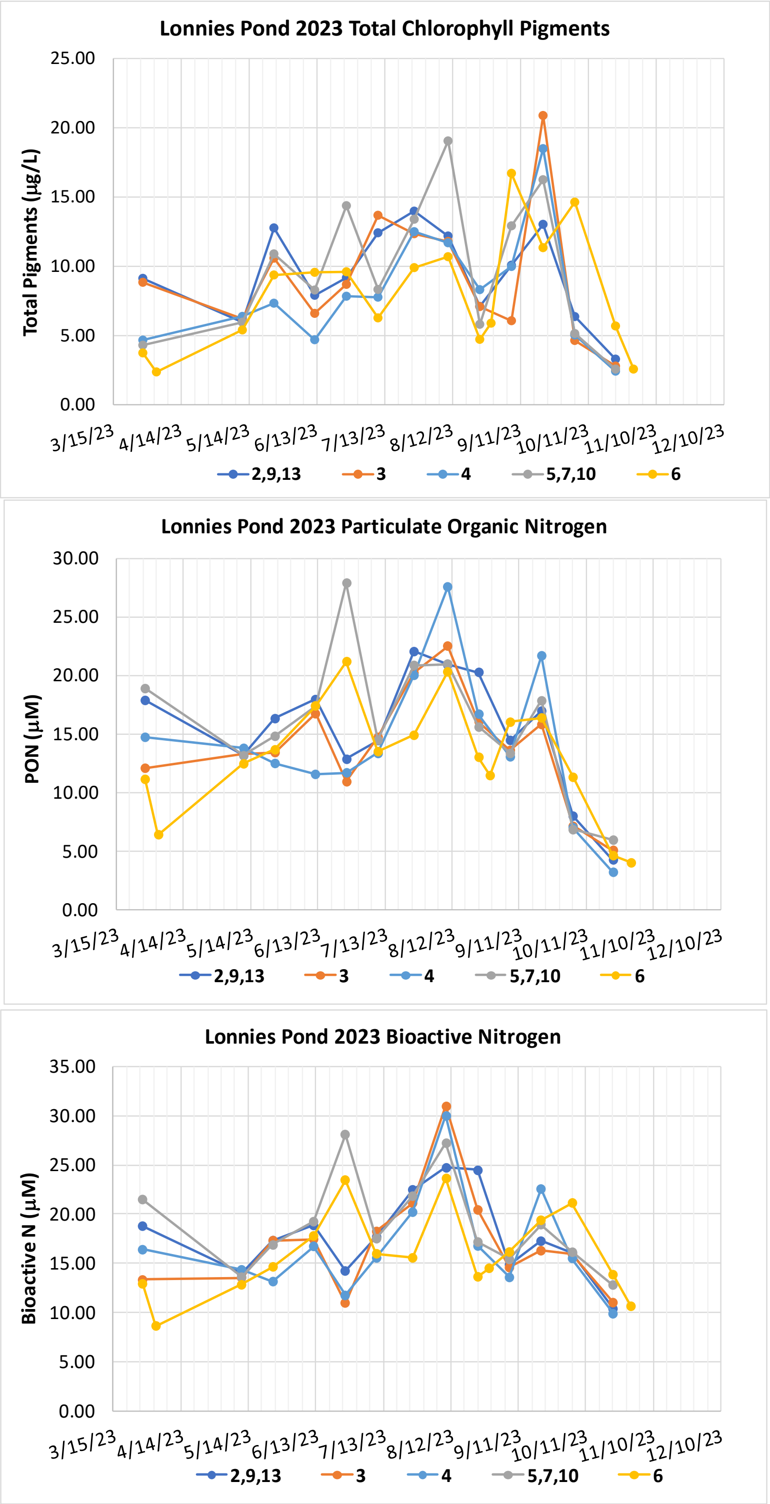

Figure 2.2 Lonnie's Pond 2023 Water Column Assay Results. Time-series of mixed layer average total pigments, particulate organic nitrogen (PON), and bioactive N at stations LP3, LP4, LP6 and averages of the stations and associated with the North (LP2, 9, 13) and South (LP5, 7, 10) oyster deployment.

2023 Water Quality Associated with Oyster Aquaculture

During review of the 2023 water column data, project staff reviewed a number of factors to see if the differences in the 2023 oyster deployment resulted in differing water quality results. The grow-out of the YR2 oysters during the spring of 2023 represents a larger filtering potential and clearing of water-column nutrients in the northern aquaculture area compared 1) the rest of the stations and 2) July-October nutrients in the same northern aquaculture area when YR1 oyster seed occupied the area. Another opportunity for comparison is to look for a signal between stations near and far from the aquaculture areas from July-October when smaller YR1 seed oysters occupied both aquaculture areas. A YR2 oyster averaged 16.7 g and 57.5 mm in length when deployed in March-April, while YR1 oyster seed deployed in July-August averaged 0.71 g and 19.3 mm. These weights and lengths are larger than they were in 2022.

Springtime water quality metrics showed larger oysters initially deployed into the northern aquaculture area (near stations LP2, 9, 13) in Lonnie's Pond in March coincided with lower water column bioactive N, DIN and TN compared to rest of the stations where there were no oysters (Table 2.3). In addition, spring pigment and DIN levels were notable lower than in 2022, though PON was approximately the same. Statistically, these lower 2023 spring values associated with the oysters were not significantly different from the measurements from the rest of the station in Lonnie's Pond (Table 2.4a). The northern aquaculture area was the grow-out site of YR2 oysters until July, when the oysters were removed and replaced by oyster seed. A comparison of water quality metrics associated with larger YR2 oysters in the spring and oyster seed in the summer found on average lower total pigments, bioactive nitrogen, and significantly lower DIN in the presence of larger oysters (Table 2.3 and 2.4b). This may be the result of the greater filtering capacity of these larger oysters compared to the smaller seed, but may also be due to greater DIN discharge from Pilgrim Lake in the later summer or some as yet undefined changes in the sediments.

During the summer both northern and southern aquaculture areas were filled with YR1 oyster seed, and the impact of the oysters seems to influence water quality throughout the pond. The water quality at stations in and around the aquaculture areas were compared to stations without oysters, LP3 and LP4. On average, PON, bioactive N, and TN were found to be lower in stations associated with the aquaculture areas in the summer but proved to not be significantly different (Table 2.3 and 2.4c). Another opportunity to compare water quality associated with oyster aquaculture was to examine if there was a difference between the empty southern aquaculture in spring vs. this same area in the summer when oyster seed was deployed. Results show total chlorophyll pigments and DIN were significantly higher in the southern aquaculture area during the summer compared to the spring (Tables 2.3, 2.4d). This may be indicative of the increased biodiversity and subsequent breakdown of organic matter that arises when placing oyster bags into the water.

Overall, results suggest that the larger YR2 oysters deployed in the northern aquaculture area reduced water column nutrients (Total pigments, Bioactive N, and DIN) in a greater capacity than oyster seed placed in the same area over the summer (Table 2.4b). The stations associated with the grow-out of oyster seed during the summer also showed lower concentrations of bioactive N, PON, and TN (Table 2.4c). These reductions of water column nutrients associated with oyster aquaculture are also reflected in the long-term water quality averages and their eutrophication index improvements (Figure 2.1; Table 2.2).

Table 2.3 Lonnie's Pond mixed-layer water quality station averages from spring (March-June) and summer (July-October) 2023. Average nutrient concentrations of total chlorophyll-*a* (Total pigments), particulate organic nitrogen (PON), bioactive N, dissolved inorganic nitrogen (DIN), and total nitrogen (TN) are shown. During spring, YR2 (~2 inch) oysters filled the northern aquaculture area only (LP 2, 9, and 13, highlighted in green). From July-October YR1 (~0.75 inch) YR1 oyster seed were placed in both the northern and southern aquaculture area (highlighted in blue). Total pigments and DIN were generally higher in the summer readings. Averages of stations with and without oysters are calculated for spring and summer.

		March-June 2023						**July-October 2023**				
Station #	**Location**	**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	Northern Aquaculture Area	8.28	14.60	15.06	0.46	36.05		9.27	17.62	20.47	2.85	38.83
9	Northern Aquaculture Area	7.81	13.87	14.61	0.74	35.29		10.25	14.80	18.52	3.72	36.47
13	Northern Aquaculture Area	10.87	18.40	20.23	1.83	41.05		9.90	13.07	16.50	3.43	35.32
Average with Year 2 Oysters		**8.99**	**15.63**	**16.64**	**1.01**	**37.46**	**Northern Ave. with Oyster Seed**	**9.81**	**15.16**	**18.50**	**3.33**	**36.87**
6	Southern Aquaculture Area	6.68	13.71	15.05	1.35	35.36		8.84	12.55	16.46	3.91	33.26
5	Southern Aquaculture Area	7.32	14.81	16.04	1.23	36.50		9.56	13.29	17.56	4.27	36.71
7	Southern Aquaculture Area	9.94	22.40	23.90	1.50	45.23		10.42	13.38	17.21	3.82	33.48
10	Southern Aquaculture Area	8.31	16.63	18.27	1.64	40.43		11.06	15.24	19.50	4.26	38.61
Average with NO oysters		**8.06**	**16.89**	**18.32**	**1.43**	**39.38**	**Southern Ave. with Oyster Seed**	**9.97**	**13.61**	**17.68**	**4.07**	**35.51**
							Ave. ALL with Oyster Seed	**9.90**	**14.28**	**18.03**	**3.75**	**36.10**
3	Boat Ramp	8.19	13.29	14.52	1.24	34.50		9.91	14.39	18.58	4.19	37.58
4	Channel	6.18	12.85	14.48	1.11	41.03		9.53	15.32	18.01	2.69	37.30
Average ALL with NO oysters		**7.77**	**15.61**	**17.04**	**1.34**	**38.84**	**Ave. with NO oysters**	**9.72**	**14.85**	**18.29**	**3.44**	**37.44**

Table 2.4 Comparison of 2023 Seasonal and Oyster Impacted Average Nitrogen and Pigment Concentrations. Paired t-tests ($\rho<0.05$) compared mixed-layer station averages for key N constituents sampled from Lonnie's Pond in four different settings : a) Spring (March-June) conditions comparing water quality at stations filled with YR2 oysters (northern aquaculture area) vs. the rest of the stations without oysters **b)** northern aquaculture area stations with YR2 oysters in spring vs. summer (July-October) YR1 oysters, **c)** Summer YR1 oyster seed stations vs. stations without oysters during the summer and **d)** Southern aquaculture area stations during spring when no oysters deployed vs. summer when YR1 oysters were deployed. Significant differences (in red) are seen with: 1) lower dissolved inorganic nitrogen (DIN) during YR2 oyster deployment vs. YR1 in the northern aquaculture area, and 2)higher total pigments and DIN in the southern aquaculture area during the summer when YR1 oysters were deployed vs. the spring when no oysters were deployed.

a) Comparing Northern Aquaculture Stations with Y2 oysters with rest of the pond without oysters in Spring

	Total Pigments µg/L		PON µM N		Bioactive µM N		DIN µM N		TN µM N	
	With Oysters	W/out	With	W/out	With	W/out	With	W/out	With	W/out
mean	8.99	7.77	15.63	15.61	16.64	17.04	1.01	1.34	37.46	38.84
variance	1.81	1.52	3.95	10.74	6.50	11.08	0.35	0.03	6.52	14.10
stdev	1.35	1.23	1.99	3.28	2.55	3.33	0.59	0.18	2.56	3.75
n	3	6	3	6	3	6	3	6	3	6
t	1.36		0.005		-0.21		-0.96		-0.65	
d.o.f	4		4		5		2		6	
critical value	2.78		2.45		2.57		4.3		2.45	
[t] > crital value	1.36<2.78		0.005<2.45		-0.21<2.357		-0.96<4.3		-0.65<2.45	
	no significant diff.		no significant diff.		no significant diff.		no significant diff.		no significant diff.	

b) Comparing Northern Aquaculture Stations with Y2 oysters in Spring vs. same area with Y1 oysters in Summer

	Total Pigments µg/L		PON µM N		Bioactive µM N		DIN µM N		TN µM N	
	March-June	July-Oct	March-June	July-Oct	March-June	July-Oct	March-June	July-Oct	March-June	July-Oct
mean	8.99	9.81	15.63	15.16	16.64	18.50	1.01	3.33	37.46	36.87
variance	1.81	0.16	3.94	3.51	6.50	2.62	0.35	0.13	6.52	2.13
stdev	1.34	0.40	1.99	1.87	2.55	1.62	0.59	0.36	2.55	1.46
n	3	3	3	3	3	3	3	3	3	3
t	-1.02		0.22		-0.81		-7.12		0.28	
d.o.f	2		2		2		2		2	
critical value	4.3		4.3		4.3		4.3		4.3	
[t] > crital value	-1.02<4.30		0.22<4.3		-0.81<4.3		[-7.12]>4.3		0.28<4.3	
	no significant diff.		no significant diff.		no significant diff.		significantly diff.		no significant diff.	

c) Comparing Stations with Y1 oysters with rest of the pond without oysters in Summer

	Total Pigments µg/L		PON µM N		Bioactive µM N		DIN µM N		TN µM N	
	With Oysters	W/out	With	W/out	With	W/out	With	W/out	With	W/out
mean	9.90	9.72	14.28	14.85	18.03	18.29	3.75	3.44	36.10	37.44
variance	0.48	0.04	2.65	0.22	2.01	0.08	0.21	0.56	4.26	0.02
stdev	0.69	0.02	1.76	0.66	1.53	0.40	0.50	1.06	2.23	0.20
n	7	2	7	2	7	2	7	2	7	2
t	0.61		-0.47		-0.25		0.76		-0.18	
d.o.f	7		7		7		7		7	
critical value	2.37		2.37		2.37		2.37		2.37	
[t] > crital value	0.61<2.37		-0.47<2.37		-0.25<2.37		0.76<2.37		-0.18<2.37	
	no significant diff.		no significant diff.		no significant diff.		no significant diff.		no significant diff.	

d) Southern Aquaculture Stations w/o oysters in spring vs with Y1 oysters in summer

	Total Pigments µg/L		PON µM N		Bioactive µM N		DIN µM N		TN µM N	
	March-June	July-Oct	March-June	July-Oct	March-June	July-Oct	March-June	July-Oct	March-June	July-Oct
mean	8.06	9.97	16.89	13.61	18.32	17.68	1.43	4.07	39.38	35.51
variance	1.52	0.71	11.23	0.98	11.76	1.26	0.02	0.04	14.96	5.07
stdev	1.23	0.78	3.35	0.99	3.43	1.12	0.15	0.20	3.87	2.25
n	4	4	4	4	4	4	4	4	4	4
t	-4.46		1.97		0.36		-20.32		1.66	
d.o.f	3		3		3		3		3	
critical value	3.18		3.18		3.18		3.18		3.18	
[t] > crital value	[-4.46]>3.18		1.97<3.18		0.36<3.18		[-20.32]>3.18		1.66<3.18	
	significantly diff.		no significant diff.		no significant diff.		significantly diff.		no significant diff.	

Project staff also measured water clarity during the oyster deployments by measuring light penetration of the water column. Li-Cor light profile data was collected during each sampling event at stations up-gradient (stations LP7, LP9) and down-gradient (LP5, LP13) of the southern and northern 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.*, $k = \ln(I_O)-\ln(I_D)/d))$). 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 2023, 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 passing from LP7 to LP5, light attenuation was lower, creating clearer water on the down-gradient side (LP5) 54% of the time in 2023 (Figure 2.3). In 2022, 55% of the LP5 readings were clearer. The northern aquaculture area showed a weaker water clearing effect with down-gradient LP13 allowing less light to the bottom and was clearer than up-gradient 38% of the time in 2023. This a lower percentage than in 2022, where 64% of LP13 readings were lower than LP9 readings. The differences at the northern aquaculture area are consistent with higher PON around the northern aquaculture area in 2023. It should be noted that water quality data from samples in defined flow fields typically show significant declines (~30%) from up-gradient to down-gradient samples (*e.g.*, Bournes Pond, Falmouth).

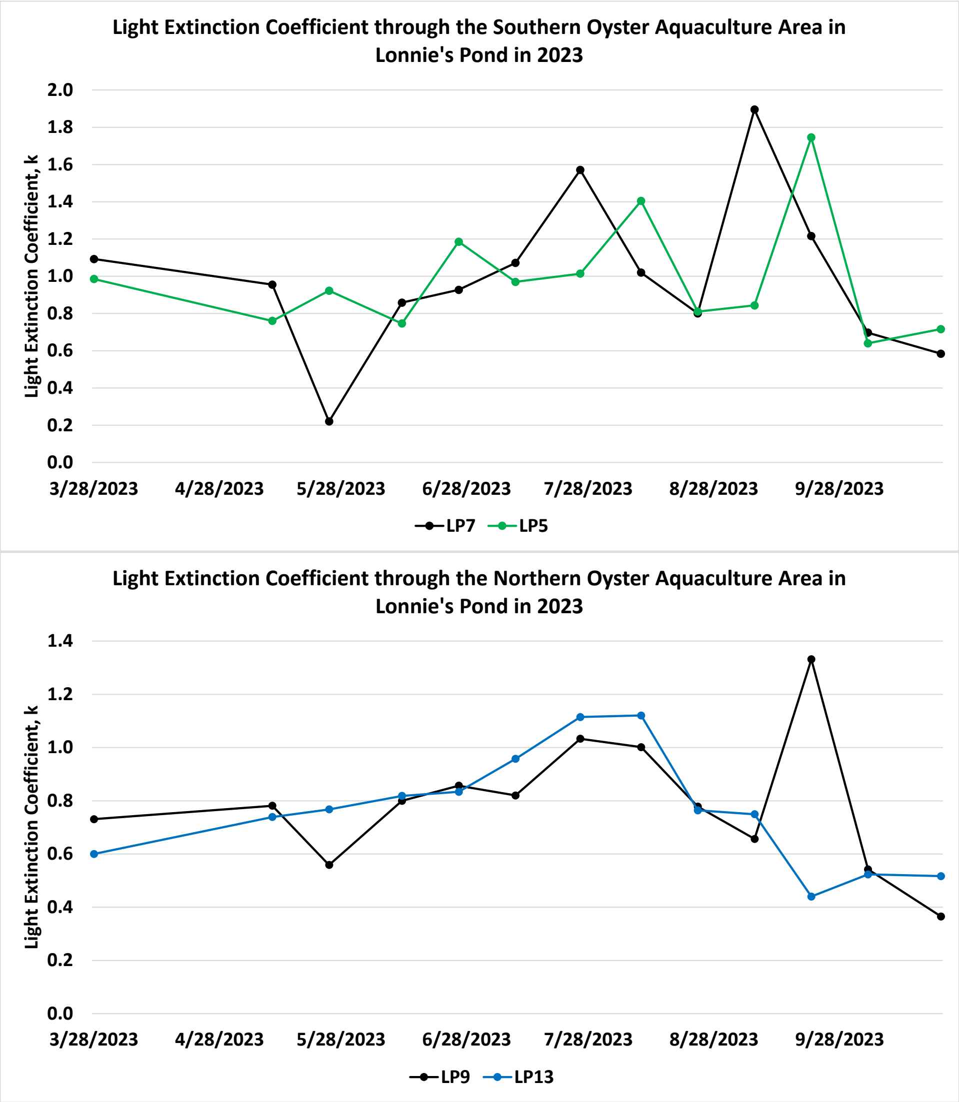

Figure 2.3. Lonnie's Pond 2023 time-series of water-column light extinction near aquaculture areas. Light extinction coefficients, k, were determined during the 2023 water quality monitoring using the station light profiles up-gradient (LP7) and down-gradient (LP5) of the southern oyster aquaculture area (top) and up-gradient (LP9) and down-gradient (LP13) of the northern aquaculture area (bottom). The light extinction coefficient, k represents attenuation so the higher the k value, the more turbid the water.

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 eight years. The inter-annual variation in total chlorophyll-*a* pigments show decreases in all stations in 2023 compared to 2022. 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 2.4). Chlorophyll levels were lower in 2023 than all years except 2017 and 2020. PON and bioactive nitrogen pond-wide averages were relatively stable with PON showing a slight increasing trend from 2020 to 2023 and bioactive N show a slight decreasing trend over the same period. Chlorophyll, PON, and bioactive N levels were highest in 2019 and lowest in 2017 (see Figure 2.4).

Dissolved inorganic nitrogen have decreased significantly since 2020, while total nitrogen has been relatively stable since 2019. LP6 is located within the southern aquaculture area and has decreased 56% since the installation of aquaculture (2016: 6.7 µM; 2023: 2.9 µM; Figure 2.5). All stations have DIN reductions of over 39% since 2016. Mean DIN results also show the stations associated with oysters are lower compared to sites without aquaculture and have been decreasing overall since 2020 (Figure 2.5). Station TN concentration has decreased 17-31% since 2016 with the stations associated with oyster aquaculture being reduced by approximately 25%.

Total suspended solids (TSS) were also measured in and around the southern aquaculture area consistently between 2016 and 2023. 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 up-gradient of the southern aquaculture area (station LP7), within the area (station LP6), and down-gradient 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 found up-gradient of the oysters (LP7) and was generally reduced down-gradient of the oysters in each of the years, but TSS has been increasing each year since 2020. This is consistent with higher water clarity seen with the light extinction coefficient results.

The cause of the increasing TSS concentrations since 2020 has not been resolved, but is likely related to changes in Pilgrim Lake (Figure 2.6). Total Flow from Pilgrim Lake was increasing steadily from 2019 until 2021 but has decreased in the last two years (see Figure 2.13 in stream section). 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. During the duration of this study (2016-2023), the least amount of rain was recorded in 2020 and outflows from Pilgrim Lake during the summer were exceptionally low (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

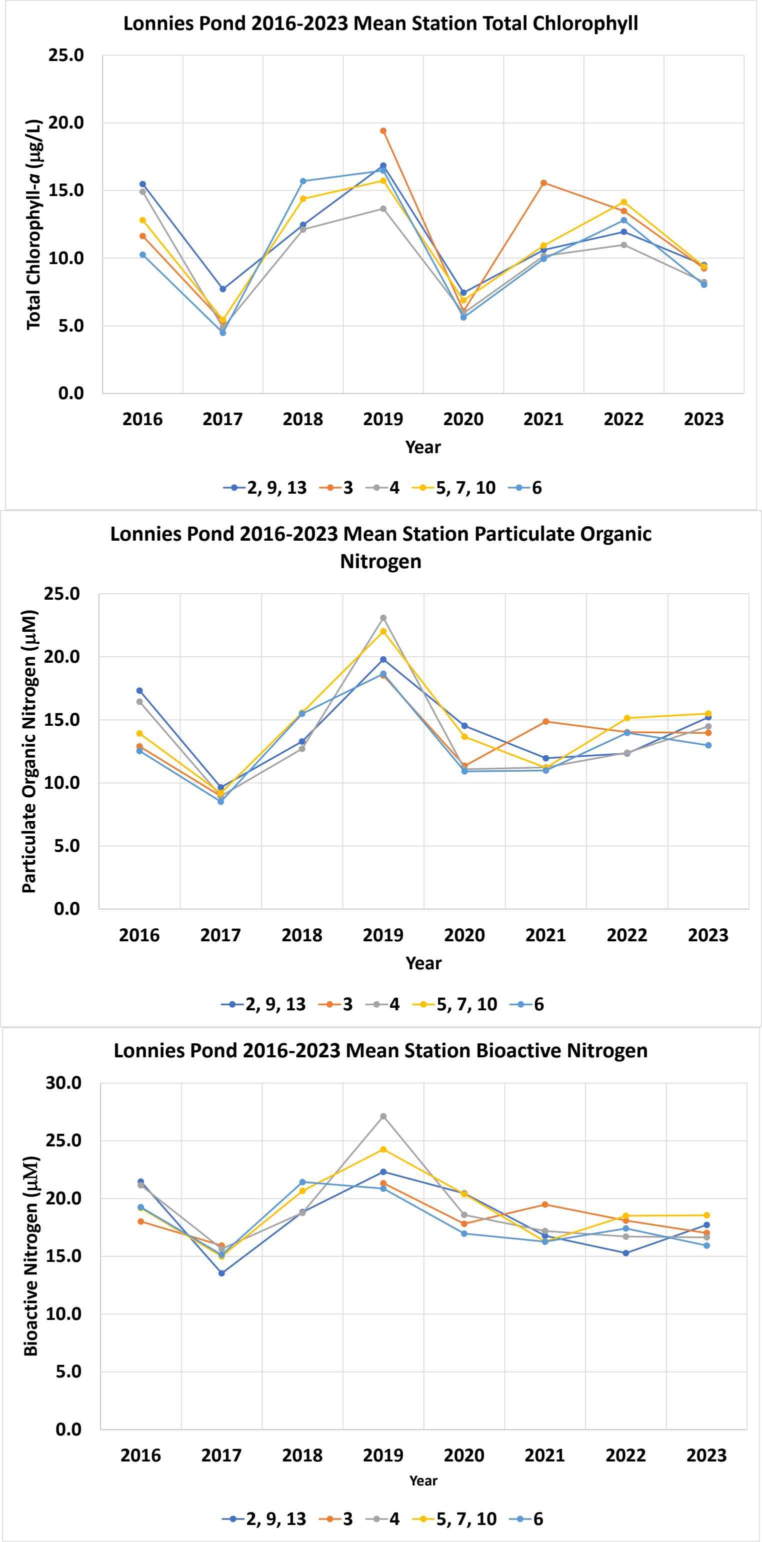

Figure 2.4 Historical average chlorophyll pigments, PON, and bioactive concentrations in Lonnie's Pond (2016-2023). Annual average concentrations of PON, and bioactive N generally show peak concentrations in 2019 and lowest concentrations in 2017. 2023 readings of PON and bioactive were generally consistent with 2020-2022 data, although there appear to be increasing and decreasing trends, respectively. Chlorophyll concentrations are more variable with 2023 readings lower than 2022 readings. Station numbers refer to locations in station map, Figure 2.1.

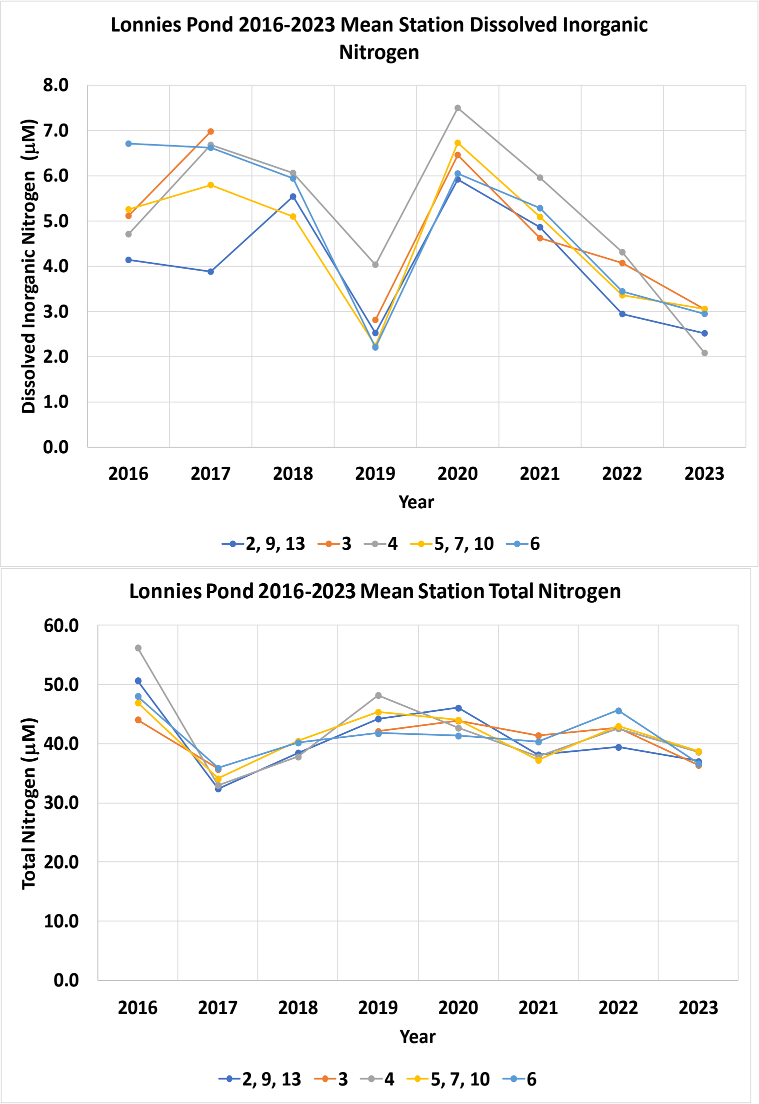

Figure 2.5 Historical average DIN and TN in Lonnie's Pond (2016-2023). Annual average concentrations in mixed layer of dissolved inorganic nitrogen DIN and total nitrogen (TN) at stations LP3, LP4, LP6 and averages of stations in the southern (LP5, 7, 10) and northern aquaculture area (LP2, 9, 13). Station DIN shows peak concentrations in 2020 and lowest concentrations in 2019 with a decreasing trend for all sites and new minima for LP4 in 2023. The 2023 pond-wide DIN average matched the previous minimum in 2019: 2.76 µM in 2019 and 2.73 µM in 2023. TN shows peak concentrations in 2016 and lowest in 2017, but generally station averages of 40 to 45 µM from 2018 through 2023. Station numbers refer to locations in station map, Figure 2.1.

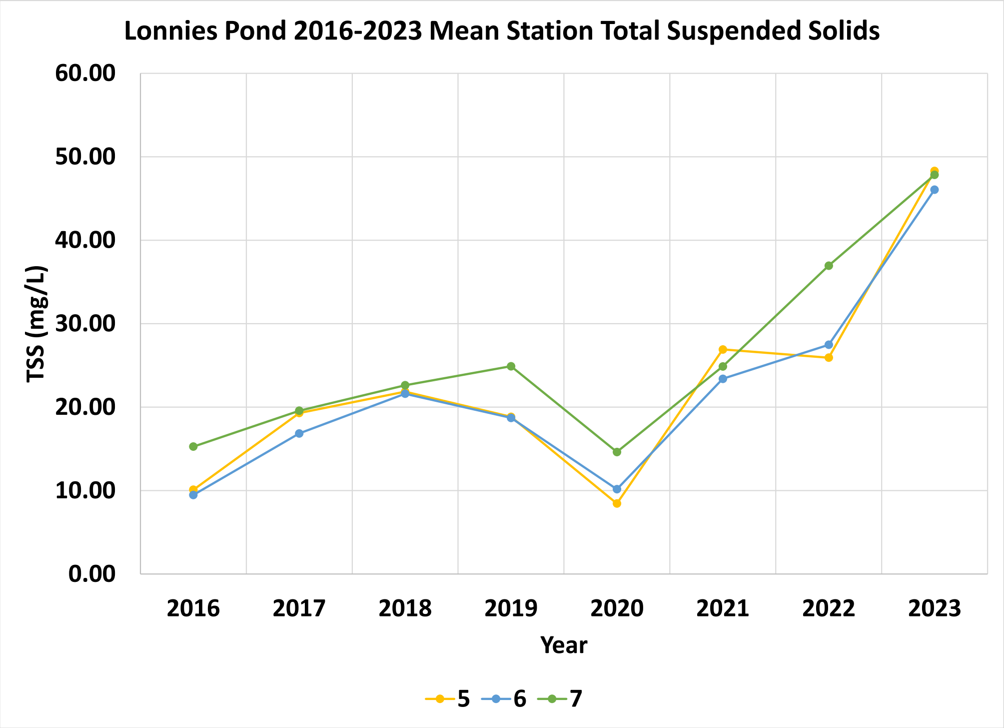

Figure 2.6 Average total suspended solids (TSS) concentrations at the southern aquaculture area: 2016-2023. Averages up-gradient (station LP7), within (station LP6), and down-gradient (station LP5) of the Lonnie's Pond southern oyster aquaculture area. Readings generally show removal of TSS within the aquaculture area: higher TSS concentrations up-gradient and lower TSS concentrations down-gradient. TSS concentrations have increased at all stations since 2020. Station numbers refer to locations in station map in Figure 2.

were occurring, most notably, increasing herring populations (2X between 2015 and 2017).[6] Herring counts have been higher than 2008 to 2015 counts, but relatively stable (*i.e.*, between 10,000 and 18,000 since peaking at approximately 28,000 in 2018).[7] Despite the general increase in measured TSS up-gradient of the oysters, it appears the aquaculture area has handled the TSS increases without significant changes in N uptake.

The presence of oysters in Lonnie's Pond has affected water quality by significantly reducing phytoplankton, bioactive nitrogen, and TSS. Oyster aquaculture has shown to reduce water column particulates and increase water clarity. These readings are consistent with readings in other areas locally (*e.g.*, Bournes Pond[8]) as well as regionally (Great Bay, New Hampshire[9] and Long Island Sound[10]). During 2023, there were a few notable events (*i.e.*, a May 25 phytoplankton bloom, which was later in the spring (5/10/2022) and half the concentration (32.6 ug/L) than 2022 and a large precipitation event on August 9, which increased the water quality metrics, but showed evidence of oyster sequestration of excess storm inputs, but once these temporary impacts passed, water quality conditions followed patterns seen in previous years. Seasonal patterns were similar, but present water quality conditions have improved in all stations except LP7 compared to the long-term averages.

With this extensive data set we can also track changes since the baseline Massachusetts Estuaries Project (MEP) assessment of Pleasant Bay, including Lonnie's Pond. [11] The MEP assessment determined Lonnie's Pond to be moderately to significantly impaired based on low DO, high chlorophyll, macroalgae presence, and stressed sediment infauna. The MEP recommended a bioactive nitrogen threshold of 0.210 mg/L based on the ecosystem health indicators and the overall water quality review and modeling. This threshold concentration was determined to restore the infauna community. The long-term water quality monitoring in Lonnie's Pond used by the MEP was based on monitoring from 2000-2005 at a single monitoring station (PBA15). The average TN concentration from 2000-2005 at PBA-15 was 0.777 mg/L, while the average bioactive N concentrations 0.281 mg/L.

The closest Lonnie's Pond aquaculture monitoring station to PBA-15 is LP4. The average 2016-2023 bioactive N concentration at LP4 was 0.266 mg/L. This concentration is only a 5% decrease from the MEP baseline and this indicates that additional nitrogen reductions will be required to attain the TMDL. However, the limited 2022 sampling of the benthic infauna indicated that the population appeared to have improved and the water quality data shows that there are changes occurring within the ecosystem.

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[6] 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.

[7] https://apcc.org/wp-content/uploads/2023/11/Cape-Cod-Herring-Run-Summary-2007-2023-Final.pdf (accessed 3/25/24)

[8] 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.

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

[10] 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.

[11] 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.

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 to Lonnie's Pond and its discharge has been monitored continuously 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 up-gradient of the culvert (Figure 2.7). 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.

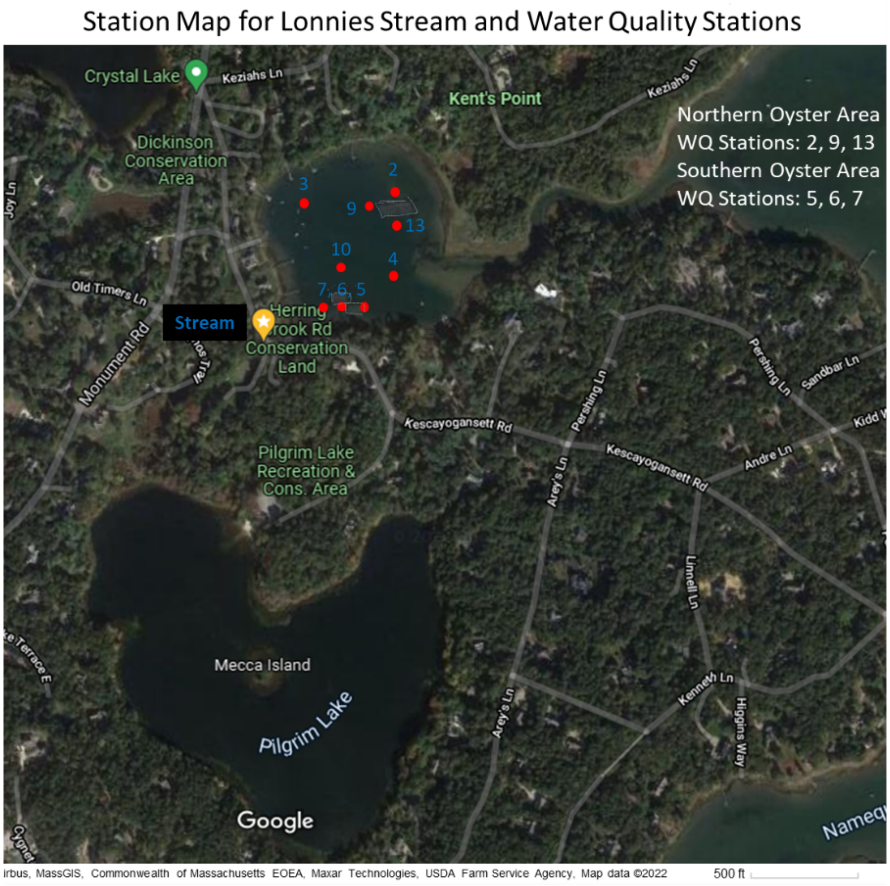

Figure 2.7 Location of the Pilgrim Lake herring run stream gauge (yellow star) deployed at the base of the herring ladder.

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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 2.8).

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.

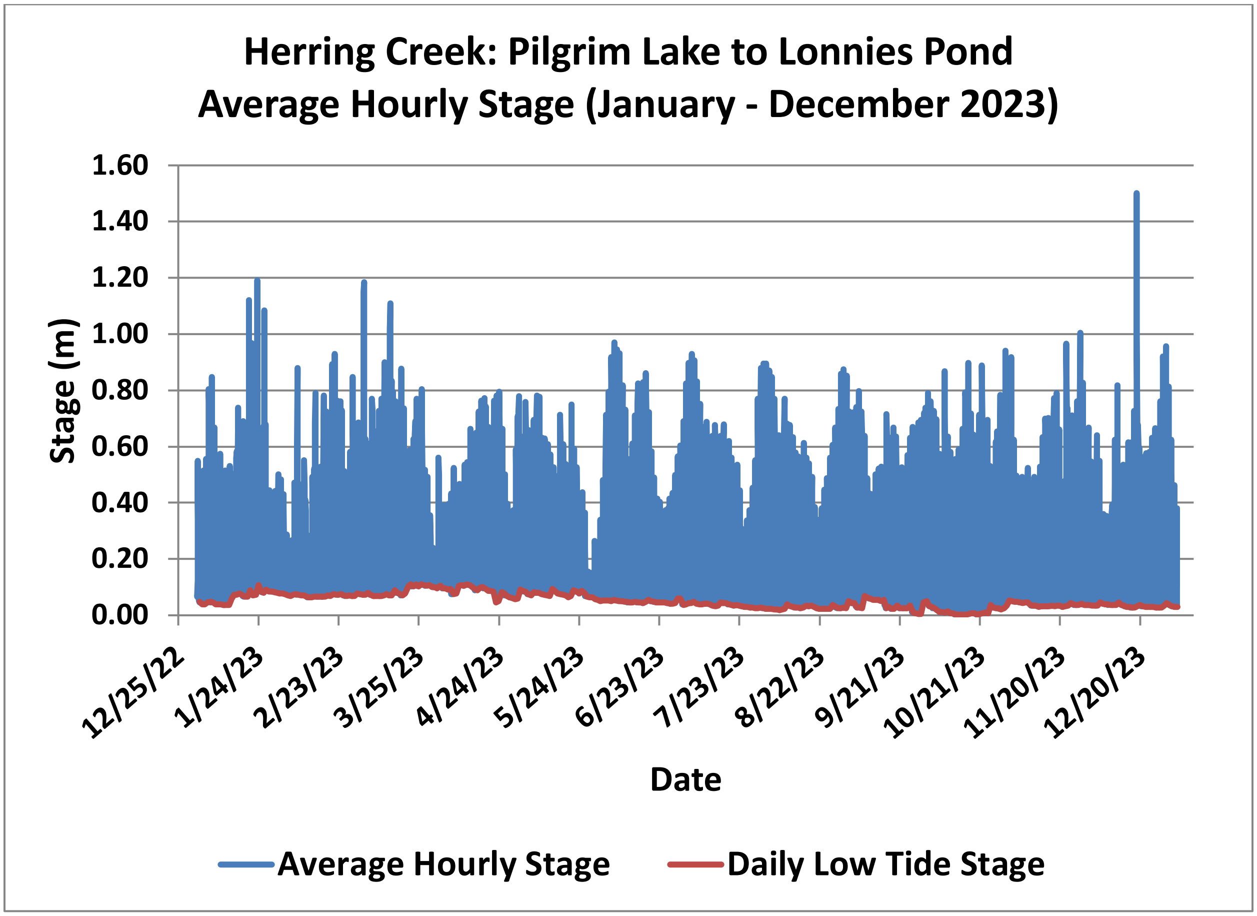

Figure 2.8 Average hourly 2023 stage record from discharge to Lonnie's Pond from Pilgrim Lake. Stage elevations were scrubbed to remove daily tide impacts and elevations were compared to stage-discharge relationship based on previous data (2003, 2016-2022) to develop continuous flow readings from January to December 2023. These continuous flow readings were 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 2.6). Water quality results from 2023 show that spring total nitrogen and flow inputs to Lonnie's Pond are approximately half those of 2022, while summer inputs are slightly higher than 2022 summer inputs. In previous years summer (July-September) stream inflows were 26% to 81% of corresponding spring (April-June) inflows, and in 2023, the summer inflow was 33% of spring inflow. Summer TN input (18.0 kg) from Pilgrim Lake was 57% of spring input (31.8 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] It is also notable that some the nitrogen constituents generally decrease from the spring to summer; this is a consideration for figuring out the seasonal variations in the Lonnie's Pond water column measurements. For example, summer DIN significantly increased in Lonnie's Pond (see Table 2.4) and also increased in the 2023 Pilgrim Lake outflow, but decreased in 2022 outflow. 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 2.6 Seasonal total nitrogen load (kg) entering Lonnie's Pond via Pilgrim Lake stream from 2017 - 2023. Summer (July-September) inflow has been 26%-81% of spring (April-June) inflow but in 2023 was only 14% of spring 2022 inflow. The summer flow in 2023 was 33% of spring inflow. Summer 2023 inflow also represented 33% of the average inflow over the seven years (36,189 m^3), while spring 2023 inflow was half (51%) of the seven-year spring average (73,384 m^3). Summer TN input for 2023 was 57% of spring TN input. Summer 2023 TN input of 18.01 kg was 60% of the average summer input (30.7 kg). Spring 2023 TN input was 38% lower than the seven-year average (50.9 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)
93,257	Total Load (April-June 2017)	3.86	1.92	5.79	32.59	12.08	50.46
39,420	Total Load (July-Sept. 2017)	1.83	2.12	3.95	14.64	2.85	21.45
137,888	Total Load (April-June 2018)	4.63	6.18	10.81	57.40	14.45	82.67
100,995	Total Load (July-Sept. 2018)	5.08	9.91	15.00	39.52	8.11	62.62
51,956	Total Load (April-June 2019)	6.09	2.66	8.74	25.81	11.19	45.74
23,178	Total Load (July-Sept. 2019)	1.20	2.06	3.26	8.58	2.89	14.73
46,939	Total Load (April-June 2020)	3.71	4.38	8.09	17.44	6.18	31.71
12,238	Total. Load (July-Sept. 2020)	1.79	6.53	8.33	4.07	5.35	18.08
66,981	Total Load (April-June 2021)	6.80	8.73	15.52	30.59	9.13	55.24
54,308	Total. Load (July-Sept. 2021)	4.99	11.78	16.77	36.86	6.84	60.38
79,129	Total Load (April-June 2022)	6.39	7.19	13.58	34.68	10.58	58.83
10,940	Total. Load (July-Sept. 2022)	1.39	9.24	10.63	3.49	1.13	15.24
37,539	Total Load (April-June 2023)	4.06	3.42	7.48	19.50	4.82	31.80
12,243	Total. Load (July-Sept. 2023)	4.65	5.02	9.67	7.05	1.30	18.01

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 less water and nitrogen entered Lonnie's Pond in 2023 compared to 2022 (Figure 2.9). Results show the annual mass of nitrogen and stream input decreased in 2023 compared to

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

2022, but with significantly different seasonal patterns (Figures 2.9 and 2.10). Annual volumetric flow of water entering Lonnie's Pond in 2023 totaled 101,344 m^3, which was a decrease of 60% from 2022 (2022: 254,660 m^3). The 2023 annual nitrogen load entering Lonnie's Pond was 105 kg N/yr., which represented a 63% decrease from the previous year (2022: 283 kg N/yr.). The notably higher March-April 2023 flows and loads compared to the rest of the year may have played a role in the May phytoplankton bloom. Peak flows of precipitation in late 2022 (Figure 2.10) may have stored N in the Pilgrim Lake sediments over the winter and allowed its release to Lonnie's during the comparative warmth in spring 2023.

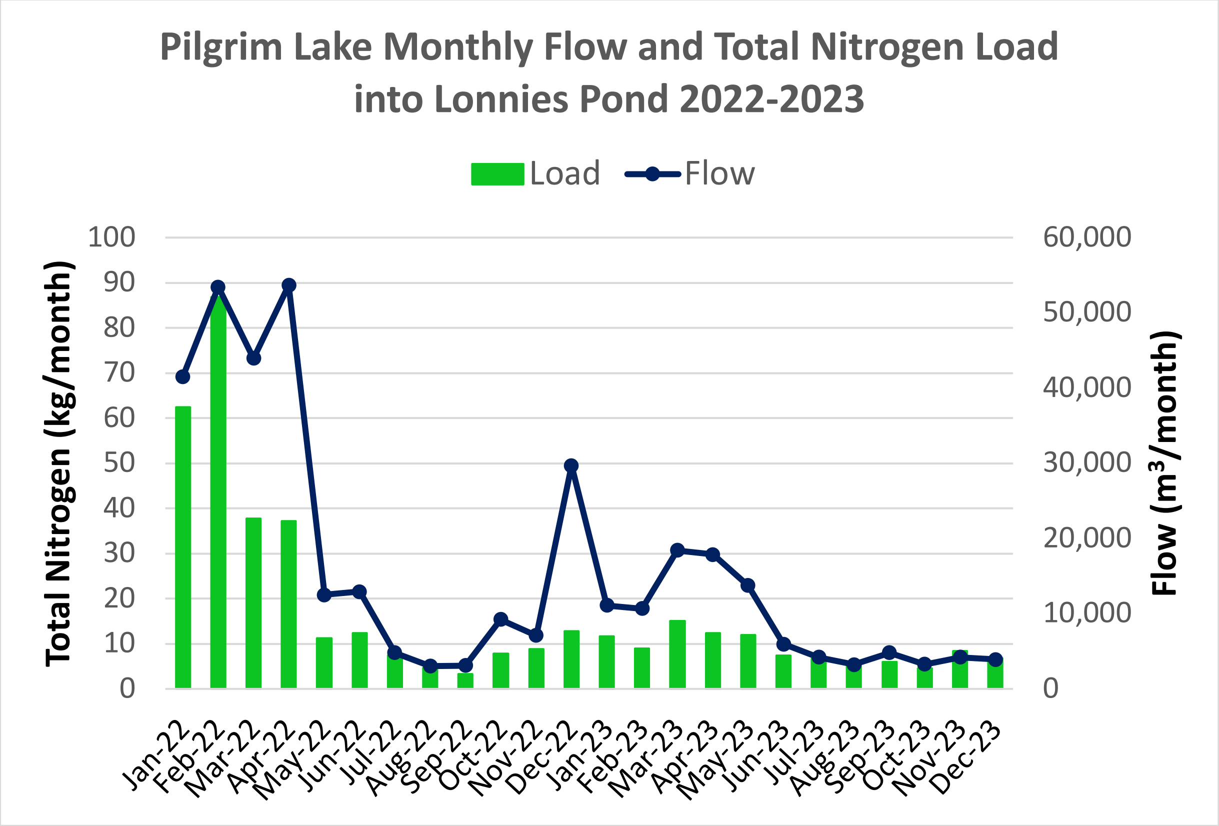

Figure 2.9 Pilgrim Lake monthly stream flow and total nitrogen load (2022-2023). Flows and loads are based on monthly measurements, sample collection, and stage-discharge relationship for readings at the bottom of herring run prior to discharge into Lonnie's Pond. There was a large decrease in annual flow (60%) from 2022 to 2023. 2023 flows were concentrated in March-April and then were much lower than 2022 throughout the rest of the year. TN loads were generally higher in months with higher flows.

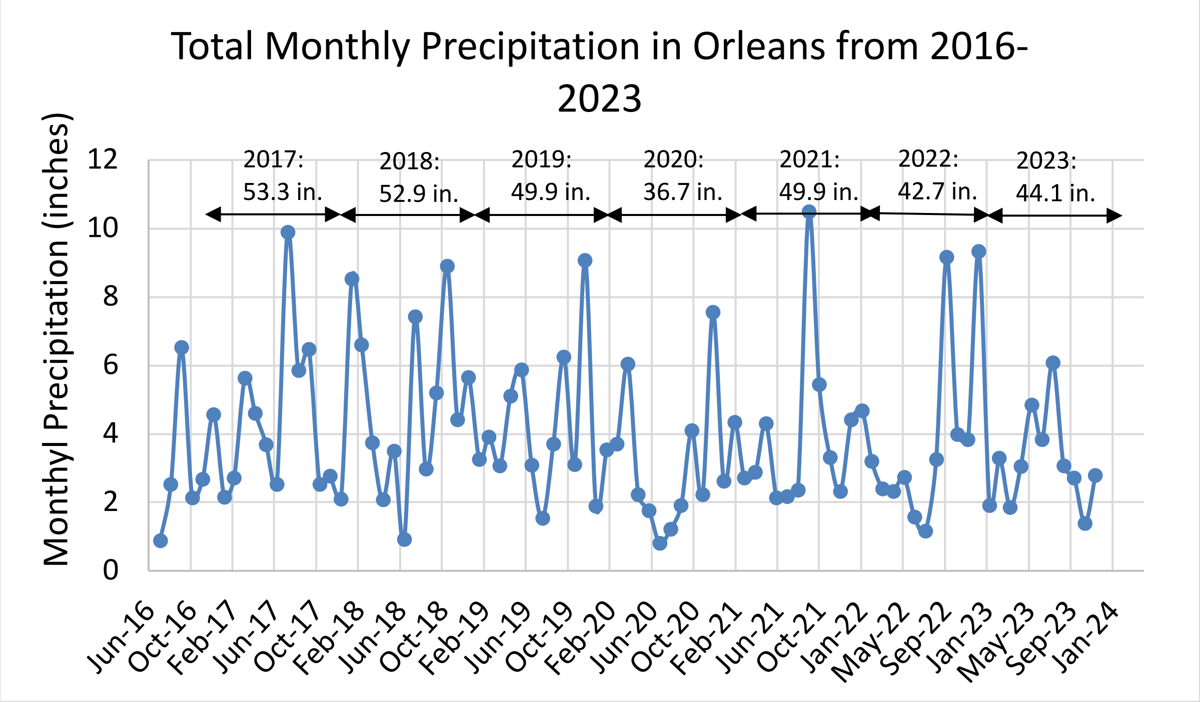

Figure 2.10 Total monthly precipitation record from a meterological station maintained by the Community Collaborative Rain, Hail & Snow Network located in Orleans, Ma. https://www.cocorahs.org/ViewData/StationPrecipSummary.aspx

Comparison of annual stream flow and nitrogen loads from Pilgrim Lake into Lonnie's Pond show that flows and nitrogen loads fluctuate over relatively large ranges (Figure 2.11). Annual flow range is ~200,000 m^3/yr., which is 550 m^3/d or 120% of the summer average flow (445 m^3/d), while annual loads fluctuate over approximately a 200 kg range, which is approximately 0.6 kg/d or 100% of the average spring N load. These differences reinforce that in addition to the temporary impacts associated with large rain events or seasonal changes, there are also long-term annual changes. All of these changes will impact the conditions in Lonnie's Pond.

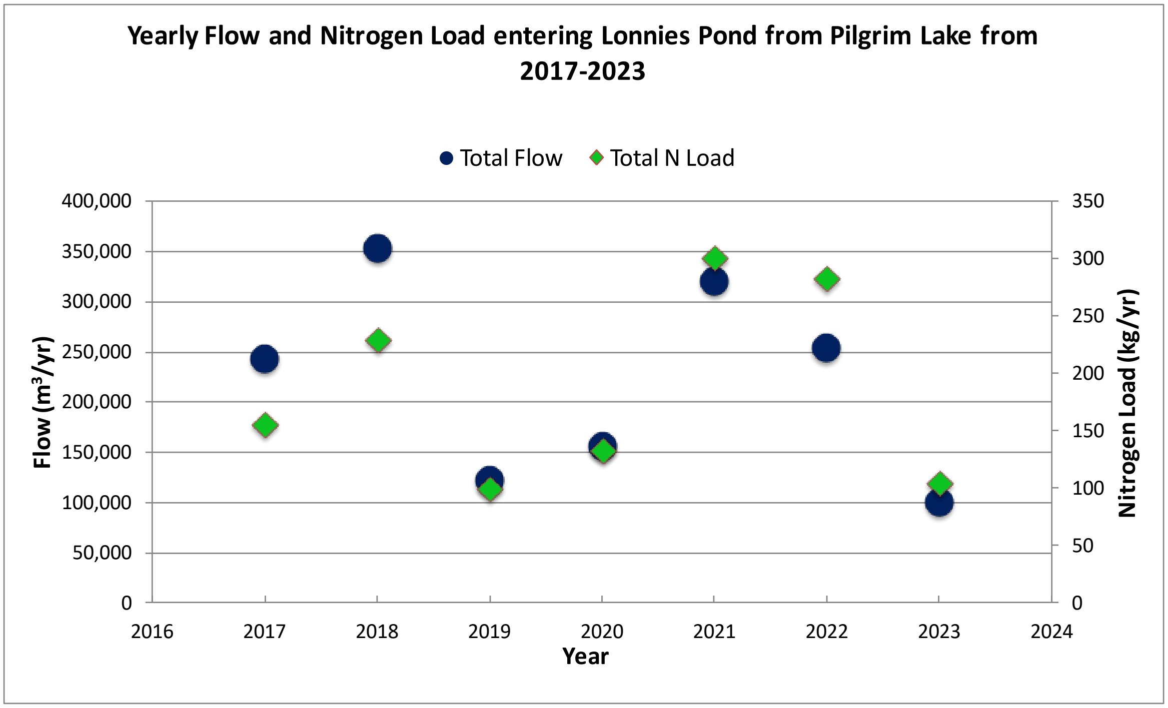

Figure 2.11 Annual stream flow and nitrogen load from Pilgrim Lake into Lonnie's Pond from 2017 to 2023. On an annual basis, flow varies over a relatively small range (~19.4 m^3/d or <5% of the summer average flow), while nitrogen loads vary over a relatively large range (~0.6 kg/d or 100% of the average spring N load).

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

Two autonomous recording multiparameter sondes were deployed from June 12 through October 5, 2023. 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) 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) (see figure 2.1).

Continuous readings varied depending on the parameter. Average salinity and temperature were similar at the two mooring sites based upon point measurements during profiling. (Figure 3.1). Salinity and temperature were generally lower throughout the 2023 season than in 2022 and more similar to 2021 because of a return to near average rainfall. Lower temperatures result in higher oxygen solubility in water and decreased respiration rates, both processes lead to improvements in water column oxygen concentrations. The DO records from the two mooring sites showed a significant decrease in the duration of hypoxia and no incidence of anoxia (Figure 3.2) in contrast to 2022 when persistent hypoxia and near daily anoxia (15-20 minute max duration) occurred during July and early August when water temperatures were elevated above 25°C. The DO readings also appear to be an improvement from MEP mooring data, which had 73% of the readings less than 4 mg/L.[14] This improvement in near-sediment DO suggests that less oxygen demand is occurring in the sediments.

Comparison of the continuous mooring record to the regular water column monitoring results showed that bottom water DO concentrations were very similar and did not show steep gradients between stations. Continuous DO measured beneath the oysters were more similar to those observed at far field water quality monitoring sites than in 2022. This suggests that while the depressed oxygen concentrations seen last year could be related to localized oyster effects, meteorology also probably played a significant role.

Algal blooms, as determined from high chlorophyll concentrations, occurred three times during 2023 (Figure 3.3). Beginning on June 13, a bloom appears to be collapsing. Another bloom appears during the last half of July and a final larger bloom centered around September 13. Each of these bloom events was preceded by a rain event.

The continuous data indicates improved water quality compared to earlier years. Reduced duration of hypoxia, no instances of anoxia, and lower chlorophyll-a concentrations all imply better water quality conditions in 2023.

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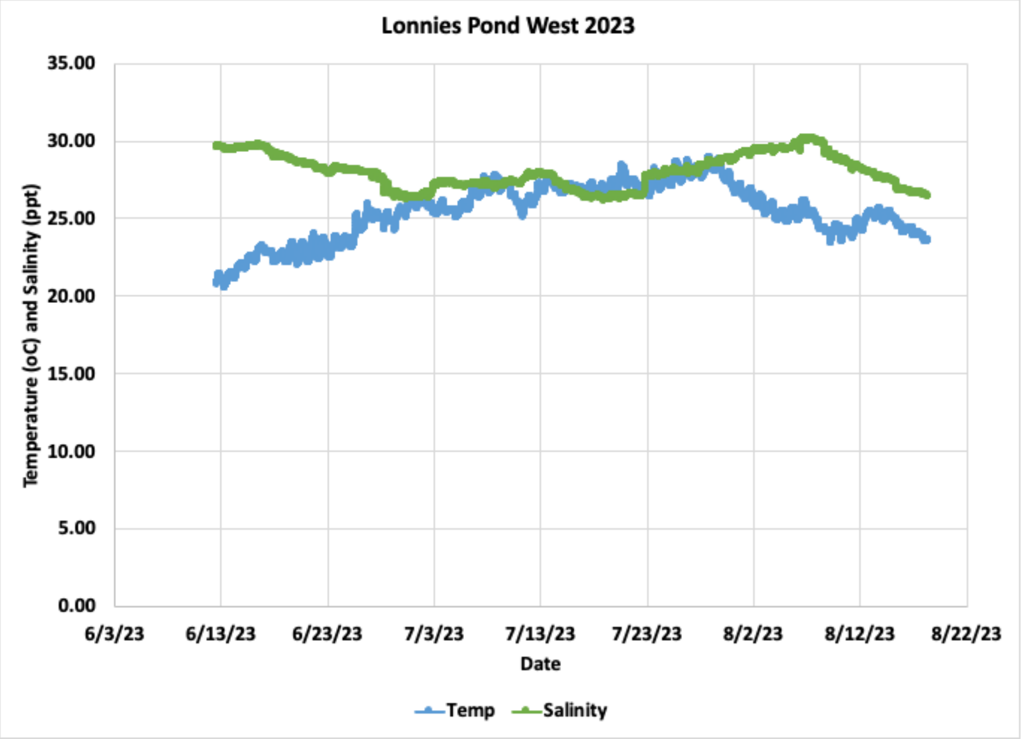
[14] Table VII-1 in the Pleasant Bay MEP report.



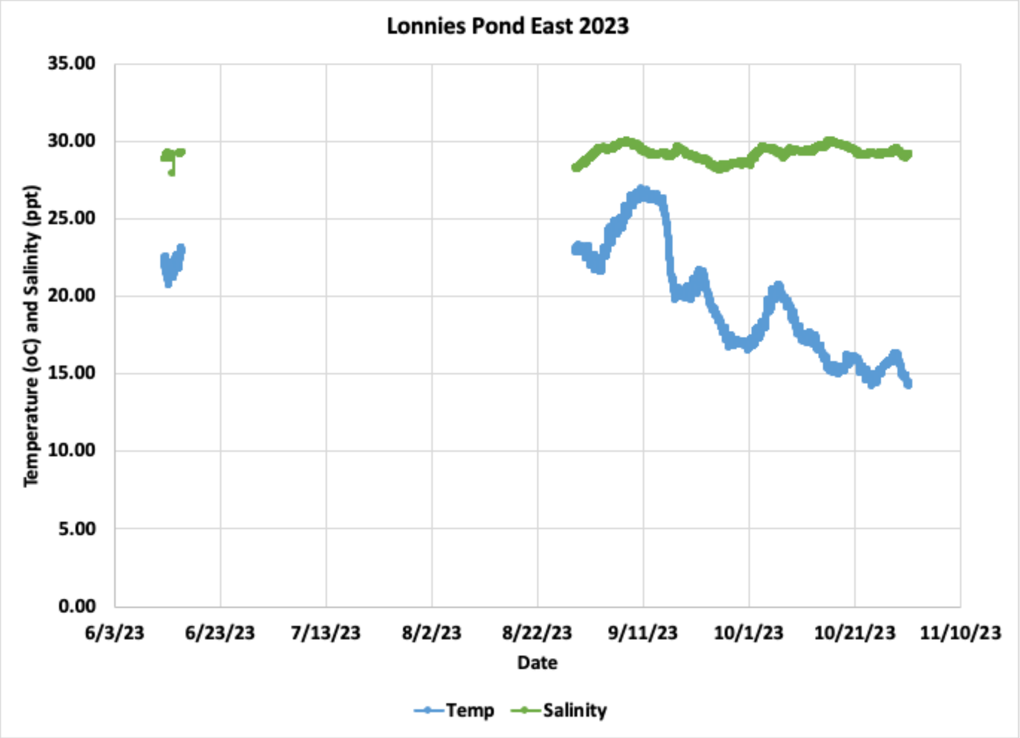


Figure 3.1 Lonnie's Pond 2023 continuous temperature and salinity readings at West (top) and East (bottom) moorings. Sensors were programmed to collect readings every 15 minutes. Readings were similar at both mooring locations. Note: readings at West were from June 12 to August 18, while East readings were primarily from August 29 to October 31. Gaps in the data are due to sensor failure.

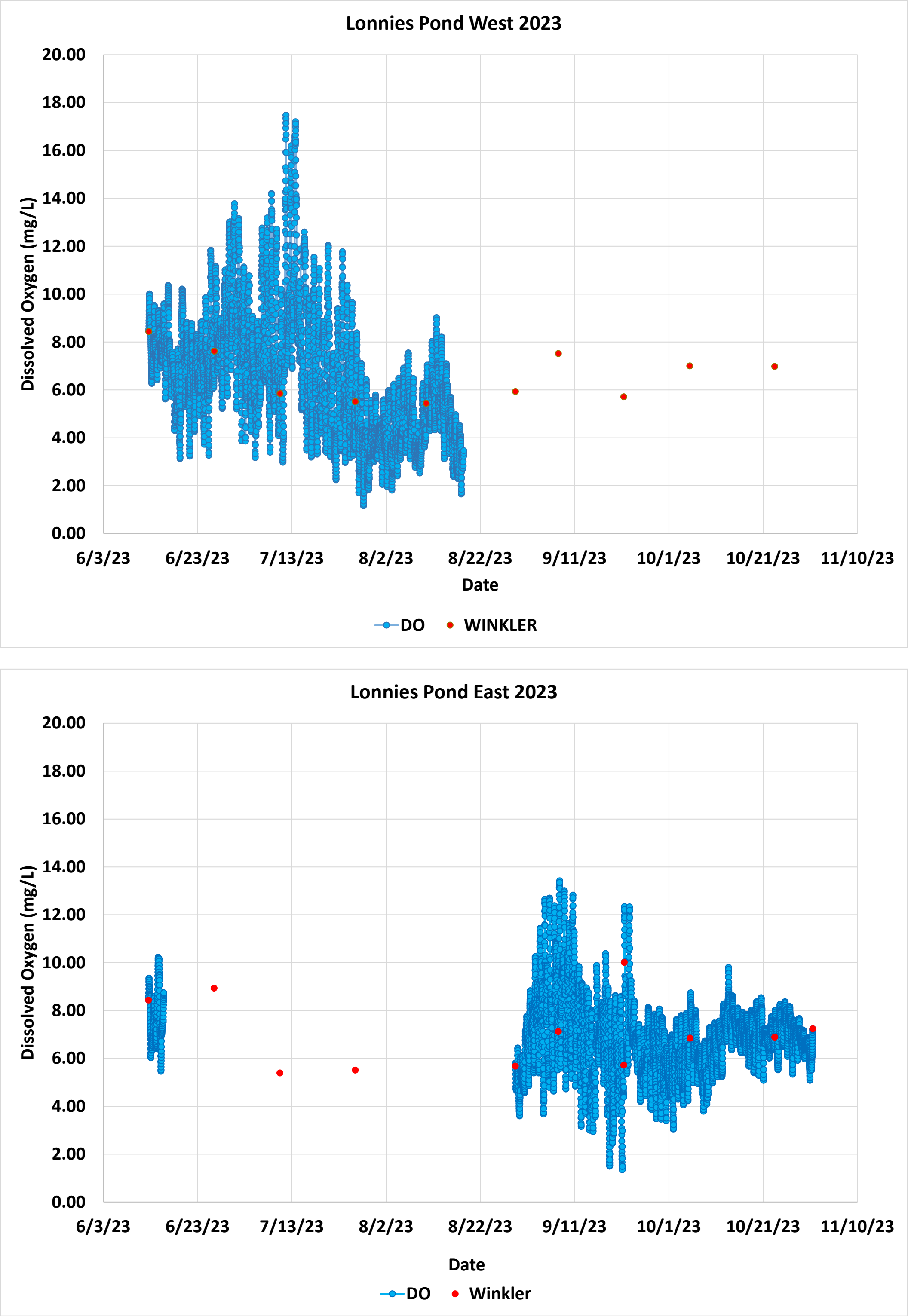


Figure 3.2 Lonnie's Pond 2023 continuous dissolved oxygen readings at West (top) and East (bottom) moorings. Red dots indicate *in situ* calibration values measured during water column monitoring activities.

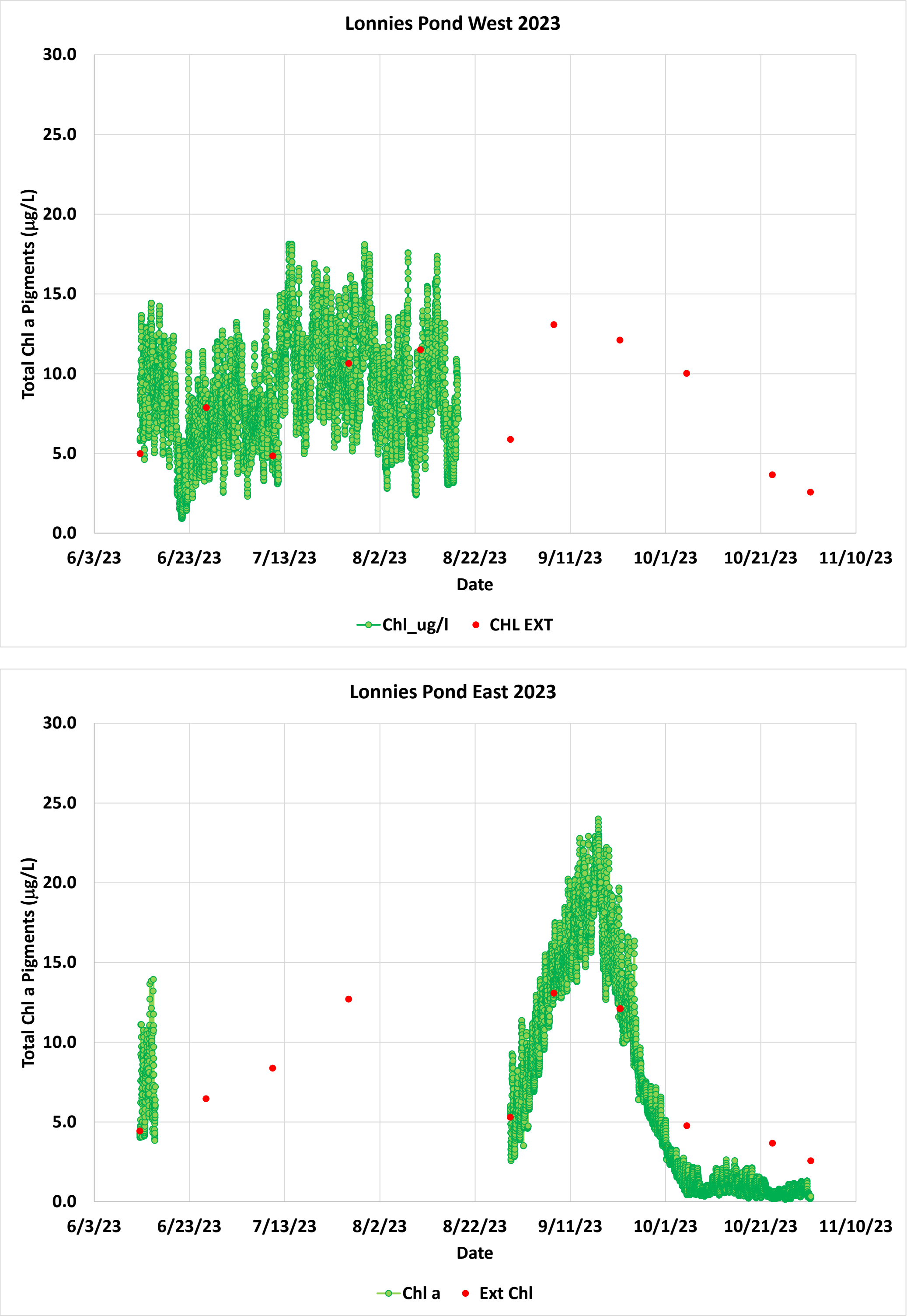


Figure 3.3 Lonnie's Pond 2023 continuous chlorophyll *a* pigment readings at West (top) and East (bottom) moorings. Red dots indicate *in situ* calibration values measured during water column monitoring activities.

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 short periods of hypoxia and no anoxia during mid-summer. 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 2022 finding of improved sediment 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 2023: April 5, August 29, and October 31. Sediment samples will also be collected in April 2024 to capture the complete 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

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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.

are also important, as higher temperatures increase bacterial respiration and carbon and N decay rates. April sediment surveys are conducted because N rich, POM depositions from oysters (biodeposits) are "preserved" in the upper sediment layer December to April 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 2023 dates, twelve intact sediment cores were collected by SCUBA divers in core tubes (15 cm interior diameter, 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.*, 2022 amphipods). It should be noted that in 2023, oysters were not deployed in the southern portion of the pond until July 2023. 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 on the sediment surface; 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

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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

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 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 (*e.g.*, the MEP benthic flux of N was 65% of the watershed N load to Lonnie's Pond[18]). 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.

2023 Sediment Nutrient Cycling Results
Spring (April), summer (August), and fall (October) 2023 sediment nutrient flux rates are summarized in Table 4.1. April 2023 cores were collected to complete the assessment of the 2022 oyster deployment (see inclusion in Year 7; Table 4.2). August 2023 core incubations captured warmer summer water conditions, peak temperatures, and biodeposition rates. The cores were collected in late August to allow time for the YR1 oyster biodeposits to accumulate on sediment surface of the southern deployment area. August 2023 cores were collected and incubated at 22.8°C, which is consistent with the 2017-2022 summer flux temperature average (24.0°C, std dev = 0.4 °C) allowing direct comparison of the rates. Late October 2023 cores were collected and incubated at 12.9°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 biodeposits, rather than temperature and ambient particle settling, was the primary driver of sediment respiration. Cores will be collected in April 2024 to complete the 2023 sediment nutrient flux rates.

As expected, the highest sediment oxygen demand (SOD) rates were found in treated cores collected in summer. The average August 2023 SOD rates for treated (106.1 mmol O_2 m^{-2} day^{-1}) and control cores (62.3 mmol O_2 m^{-2} day^{-1}) were 25% and 20% lower than the respective 2016-2022 averages: treated,136.3 mmol O_2 m^{-2} day^{-1}); control,77.5 mmol O_2 m^{-2} day^{-1}. Since the August 2023, SOD was enhanced by 43.8 mmol O_2 day^{-1}above background, biodeposit settling and decomposition within surface sediment layer increased sediment oxygen uptake 70% above the background rate. These results are equivalent to 2022 measurements despite the later deployment of oysters in the southern portion of the Pond in 2023. The seasonally high SOD rates combined with lower oxygen solubility during summer and lower water column mixing can depress bottom water oxygen levels leading to anoxia or hypoxia. During the first years of the

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[17] Ibid.

[18] Table VI-2 in Pleasant Bay MEP report

Project (2016-2018), high organic matter loading and low bottom water oxygen levels led to periodic loss of the oxidized surficial sediments and included observations of black sulfidic mud (associated with anoxia). However, in recent years, including August 2023, treated and control cores were characterized by oxic surficial sediments and oxidized layers extending down to 2-7 cm depth.

Release of DIN, particularly ammonium, from the sediments to the water column is typical of estuarine sediments in summer. The rate of DIN (ammonium + nitrate) release during the 2023 summer was lower than that measured in previous summers (2016-2021), but remained above the minimum values measured in 2022. In 2020-2023, the composition of DIN has shifted to include a higher proportion of nitrate relative to ammonium. This shift was likely driven by changes in surface sediment redox status, transitioning from anoxic to oxic conditions. The thicker oxidized layer facilitated enhanced nitrification within the sediments. Despite the dominance of NH4+ release overall, 2022 was the only year with a higher NO3- proportion. Interestingly, treatment effects were observed in 2023, with higher NH4+ fluxes measured in treated cores (6.21 mmol NH4+-N m-2 day-1) compared to controls (4.96 mmol NH4+-N m-2 day-1). Notably, despite a shorter oyster deployment period in the southern area, ammonium and nitrate efflux rates remained comparable to 2021 levels.

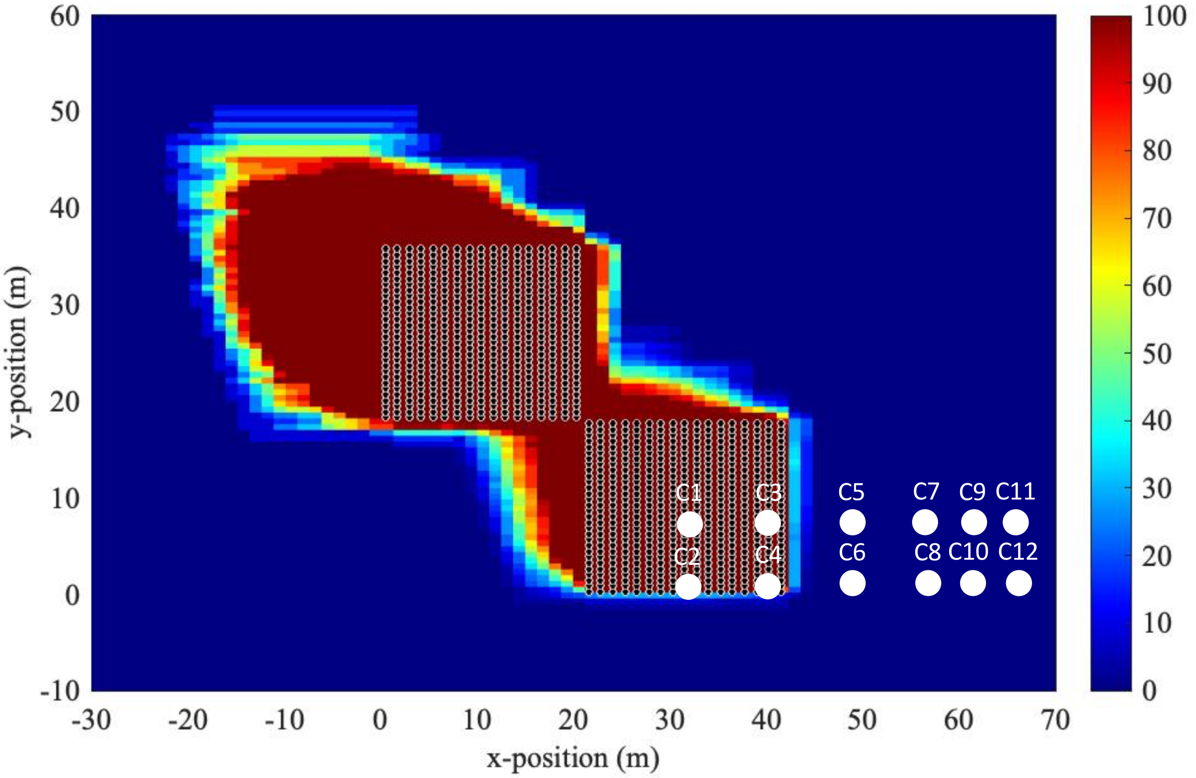

Figure 4.1 Sediment Biodeposit Deposition Areas in Lonnie's Pond. High to low deposition areas are shown with a color temperature scale: high deposition areas are colored dark red and received 100% of the average areal fecal pellet production and deposition, while areas colored dark blue received 0% of the average areal fecal pellet production and were not directly impacted by the oyster deployment. The biodeposit deposition map is overlain with 2019-2023 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 2023 benthic flux rates from Lonnie's Pond core incubations. Cores were collected April 5, August 29, and October 31. Gray shaded rows indicate background (control) rates (*e.g.*, cores outside area impacted by oyster deposition).

Collection Date: April 5, 2023; Incubation Temperature 10.6°C							
Site ID	**SOD**	**NH_4^+**	**NO_3^-**	**DIN**	**N_2-N**	**Total N Cycled**	**Denitrified**
	mMol/m^2/d	**mMol/m^2/d**	**mMol/m^2/d**	**mMol/m^2/d**	**mMol/m^2/d**	**mMol/m^2/d**	**% Total Cycled N**
C1	65.02	0.07	-0.14	-0.07	9.83	10.05	98%
C2	50.93	0.04	-0.02	0.02	2.67	2.73	98%
C3	52.99	0.32	-0.03	0.29	5.94	6.29	94%
C4	8.41	0.12	0.33	0.45	0.12	0.57	21%
C5	23.75	0.77	-0.05	0.72	1.27	2.09	61%
C6	67.06	0.16	-0.18	-0.02	12.05	12.39	97%
C7	48.55	0.45	-0.05	0.41	0.07	0.57	12%
C8	57.87	0.42	-0.04	0.38	1.90	2.36	80%
C9	55.74	0.19	-0.03	0.16	3.60	3.83	94%
C10	50.47	0.06	-0.02	0.04	0.00	0.09	0%
C11	45.98	0.02	-0.12	-0.10	0.39	0.53	73%
C12	45.01	0.25	-0.05	0.20	0.79	1.09	73%

Collection Date: August 29, 2023; Incubation Temperature 22.8°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	124.88	7.32	1.94	9.27	2.91	12.18	24%
C2	68.79	0.21	0.62	0.83	2.80	3.63	77%
C3	134.79	8.17	1.78	9.95	4.67	14.63	32%
C4	96.87	7.52	0.38	7.90	0.51	8.41	6%
C5	96.08	2.28	3.47	5.74	3.81	9.56	40%
C6	115.22	11.79	0.52	12.31	5.29	17.60	30%
C7	94.53	11.74	-0.02	11.73	2.97	14.73	20%
C8	83.59	1.30	0.06	1.36	0.78	2.14	36%
C9	12.32	5.69	1.22	6.91	3.07	9.97	31%
C10	49.53	5.74	0.10	5.84	3.06	8.91	34%
C11	59.06	4.32	0.62	4.94	0.40	5.33	7%
C12	74.83	0.99	2.13	3.12	3.09	6.21	50%

Table 4.1 Summary of 2023 benthic flux rates from Lonnie's Pond core incubations (continued). Cores were collected April 5, August 29, and October 31. 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 31, 2023; Incubation Temperature 12.9°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	77.47	2.37	1.55	3.93	2.38	6.31	38%
C2	68.85	2.78	0.67	3.44	4.25	7.69	55%
C3	49.76	1.38	0.09	1.47	0.70	2.17	32%
C4	54.61	3.92	0.25	4.17	0.62	4.79	13%
C5	47.86	1.12	-0.39	0.72	0.53	2.04	26%
C6	23.72	0.30	0.28	0.58	1.20	1.77	67%
C7	25.80	0.48	0.70	1.17	1.36	2.53	54%
C8	26.42	0.61	0.35	0.96	0.79	1.75	45%
C9	49.02	-0.69	-0.39	-1.08	1.05	2.13	49%
C10	34.67	0.49	1.00	1.49	0.74	2.24	33%
C11	42.93	0.02	-0.09	-0.07	1.04	1.15	91%
C12	34.05	1.33	-0.18	1.16	1.06	2.57	41%

The October 31, 2023 flux rates were depressed compared to those in August as a result of reduced primary productivity in response to cooling waters (temperature decreased 9.9°C since August 29) and decreasing light availability. Average treated and control sediment oxygen demand rates decreased by approximately half over a 10°C suggesting the change in rates are primarily driven by temperature and the direct effect on microbial metabolism. DIN rates decreased by 70% and 90% from August to October in treated and control cores, respectively. The seasonal decrease in DIN rates is likely the effect of cooling temperatures, as well as the decrease in N loading to the sediments from both ambient particulates and N-rich biodeposits. 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. Similar to October 2021-2022, 2023 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. Nitrate and ammonium release from the 2023 treated and control cores were statistically similar to the 2016-2022 rates. Overall, our findings suggest a generally stable state of nitrogen cycling during fall, as evidenced by consistent ammonification and nitrification rates across the study years.

Historical and 2023 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 measurements, and most years include measurements the following spring to capture impacts from overwintering from the previous summer.

The April 2023 Oyster Effect rate (2.6 mmol N_2-N m^{-2} d^{-1}), which represents the overwintered impacts from 2022 (Year 7), was higher than the average enhanced dentification rates determined for the 2016-2022 spring fluxes. The April 2023 Oyster Effect rate follows a lower than average April 2022 Oyster Effect rate measured in 2022. The observed interannual variability suggests the importance of spring measurements if the goal is to determine annual N removal via sediment denitrification. Similar to previous years, the 2023 average ammonium efflux from sediment receiving biodeposits was greater than the average background (control) rate, which suggests that the sediments were storing biodeposit N from the fall. However, the spring 2023 ammonium efflux was 18% and 24% of the 2016-2022 treated and control core averages, respectively. Nonetheless, the results of the April 2023 flux provide continued evidence that biodeposit organic matter deposited and stored in the fall is being remineralized and denitrified in the spring.

The summed summer and fall Oyster Effect measured in 2023 was consistent with the 2016-2022 averages. Previous reports noted that the Oyster Effect seemed to be diminishing in 2021, but the sediment denitrification rates throughout the pond seem to have increased and stabilized in 2022 and 2023. The August 2023 Oyster Effect (1.1 mmol N_2-N m^{-2} d^{-1}) was equivalent to the 2016-2021 average enhanced rates. Similarly, the October 2023 Oyster Effect (0.6 mmol N_2-N m^{-2} d^{-1}) was consistent with the Oyster Effect determined in October 2020-2022 (0.7 mmol N_2-N m^{-2} d^{-1}). The more consistent Oyster Effect in 2022 and 2023 is likely due to more consistent oyster deployment methods (*i.e.*, same oyster deployment areas, timing of age cohort deployment and removal, oyster stocking density) and an improved, oxidized sediment surface layer. Overall denitrification rates (treated and control) were greater compared to previous years due to the stimulation of coupled nitrification-denitrification in both sediment areas due to the increased sediment oxidation. For example, average denitrification rates measured in control cores were over five times higher for 2021-2023 compared to 2016-2020. 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 (see *2023 Amphipod Colonization Results* section below). 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 nitrification-denitrification within the core collection area leading to greater denitrification rates in all cores.

A summary of the measured N removal from Lonnie's Pond via oyster harvest in the southern aquaculture area and enhanced denitrification during the eight 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 (*i.e.*, it is a shift from the MEP conditions). 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 2023 oyster deployment <u>has not yet been measured</u>; therefore, Year 8 net N loss is an <u>underestimate at this date</u> and cannot be directly compared with the previous seven project years.

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 2024 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
4/5/23	4.2	4.6	1.6	1.8	2.6
	Year 8 Mean Denitrification Rates (mMoles/m^2/d)				
	Treated		Background		Oyster Effect
Date	Mean	Std. Dev.	Mean	Std. Dev.	
8/29/23	3.3	1.7	2.2	1.3	1.1
10/31/23	1.6	1.5	1.0	0.2	0.6
4/1/24	TBD	TBD	TBD	TBD	TBD

Table 4.3 Annual Nitrogen Removal Budget for the Lonnie's Pond southern 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 8 (2023) data; 2023 enhanced annual denitrification rate will be updated with spring 2024 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)**	**Year 8 (2023)**
Time Deployed (days)	175	195	241	155	279	287	260	167[f]
Enhanced Annual DeN_2 (mmol/m^2 N)	308.7	269.7[c]	317.4	155.9[b]	383.8	145.2	326.9	143.9
Enhanced Annual DeN_2 (g/m^2 N)	4.32	3.78	4.45	2.18[b]	5.38	2.04	4.58	2.02
Impact Area (m^2)	1574.6	1330.7	2717.4	2122.4	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	9.72	4.28[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	31.5
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]	13.6[a]

[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 2024 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)

[f] Days deployed in Year 8 (2023) reflects the number of days oysters were deployed in the Southern deployment area only.

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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.

2023 Amphipod Colonization Survey

Amphipods are considered a transitional species marking improving, but not high-quality benthic habitat conditions. 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. The presence of amphipods has also been shown to correspond to increased populations of quahogs since the tube mats reduce water siltation and encourage the settlement of quahog larvae[21].

The increased presence of amphipod mats in sediments inside and outside of the Lonnie's Pond biodeposit impact area in recent years is a notable shift in the benthic community and may help to explain biogeochemical differences observed in 2021-2023 (*e.g.*, lower sediment DIN release, ammonium oxidation). A photo taken from a sediment core collected in August 2023, shows dense amphipod tubes had created a mat on the sediment surface (Figure 4.2). 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 4.3). The presence of amphipod mats associated with the southern oyster deployment areas was first noted in the 2020 Annual Report.

Figure 4.2 Sediment core collected under the southern aquaculture area on August. 29, 2023 showing the abundant amphipod tube mat covering the oxidized sediment surface.

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[21] Mackenzie, C.L., Pilkanowski R., McMillan, D.G. "*Ampelisca* Amphipod Tube Mats May Enhance Abundance of Northern Quahogs *Mercenaria Mercenaria* in Muddy Sediments." *Journal of Shellfish Research*. 25(3) (2006): 841-847.

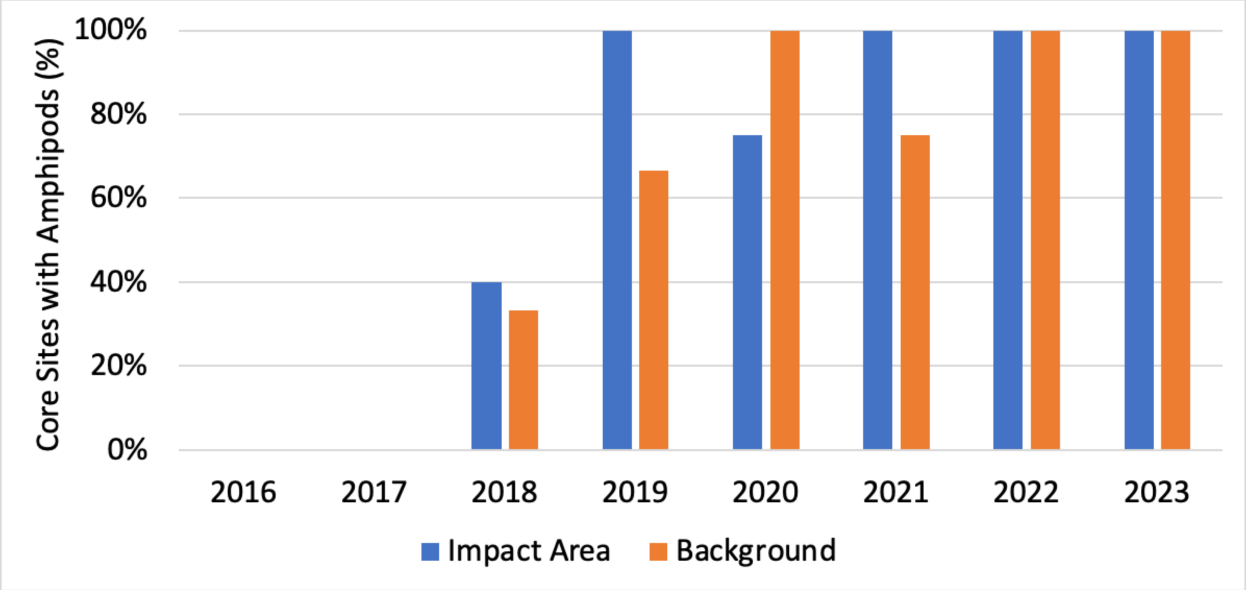

Figure 4.3 Amphipod Presence in Lonnie's Pond. Percentage of cores containing amphipods (2016-2023) inside (blue) and outside (orange) biodeposit impact areas. For each core, field and laboratory observations were recorded and compared to confirm the presence of amphipods.

Following the October 2022 core flux, a core was sieved to collect amphipods for species identification. Two species, *Eolbrolgus spinosus and Byblis serrata,* were identified (Figure 4.4). 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 sediment organic loads decrease, most notably in Boston Harbor when sewage sludge disposal ceased. Once sediment conditions favor the colonization by amphipods, 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.

During the MEP, all of the species in the infauna population in Lonnie's Pond were identified and the number of individuals were counted; this type of characterization has not been requested by the Town to date, but staff observations have driven curiosity about the extent of amphipod mats. In order to further quantify the changes in Lonnie's Pond, project staff completed a diver survey in 2023 to determine amphipod spatial distribution in the southern portion of Lonnie's Pond. The survey was completed by establishing a transect layout and determining the bottom coverage of amphipod mats along the transects. The transect survey was completed in the southern oyster aquaculture area on September 28, 2023. The survey area was concentrated along the southern aquaculture area where the sediment cores are collected in the spring, summer, and fall. Divers started at the western edge of the marsh (41.76912° N°, -69.97750° W°) and laid a measured transect line 109 m until it intersected with a dock (Figure 4.5). A second transect line was surveyed, 30 meters north and parallel to transect 1. A third transect was surveyed running south to north bisecting the two oyster aquaculture arrays. A Go-Pro Hero 5 was fixed to a 0.5 m^2 quadrat and photos were captured in 1-meter increments along each transect.

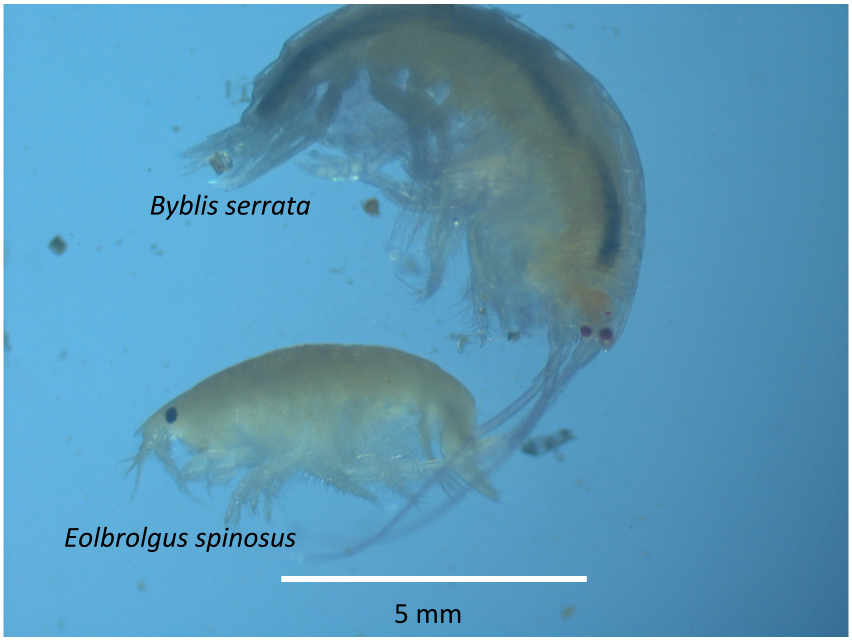

Figure 4.4 Amphipod Species in Lonnie's Pond. Following the October 2022 core collection, a core was collected and sieved for species identification. Two amphipod species were identified by Russ Winchell (Ocean Taxanomic) in this October core: *Eolbrolgus spinosus* and *Byblis* serrata (bottom figure).

The transect survey found widespread, dense amphipod mat coverage within and extending outside of the southern oyster deployment area (Figure 4.6). The presence of amphipod mats inside and outside of the biodeposit impact area strengthens the evidence that oyster aquaculture has a wider positive impact on the health of the benthic community. The transect survey also noted small areas of macrophyte/macroalgae coverage directly below the western portion of the northernmost oyster plot. The macroalgae have the highest percent bottom coverage at the margins of the transect area and within the noted area of the northernmost oyster plot. While opportunistic macroalgae are typical in enclosed basins with high organic matter, in this case, full coverage was limited to a small, deep area of the pond. Continued monitoring of benthic communities is recommended to gain a deeper understanding of the interactions between oyster aquaculture and overall ecosystem health.

Overall, it is recommended that the town complete a benthic infauna survey similar to the MEP. This survey would be best completed on a regular basis, at least every 3 to 5 years and more frequently if water column nitrogen levels decrease. The MEP threshold concentration and subsequent MassDEP TMDL was based on the restoration of the benthic community in Lonnie's Pond and the recovery of this community will be the gauge of whether restoration goals have been achieved.

Figure 4.5 Amphipod Survey in Lonnie's Pond. Map of transects used for an amphipod mat bottom coverage in Lonnie's Pond in Orleans, MA. The survey was completed September 28, 2023. Grey markers show the current footprint of the oyster aquaculture area, while the orange lines show the survey transects (end points shown by blue markers). The length of each transect is shown by the blue markers. Transects were completed by SCUBA diver capturing 0.5 m^2 quadrat pictures using a GoPro camera in 1-meter increments. Pictures were reviewed individually to measure the percent coverage of amphipod tube mats.

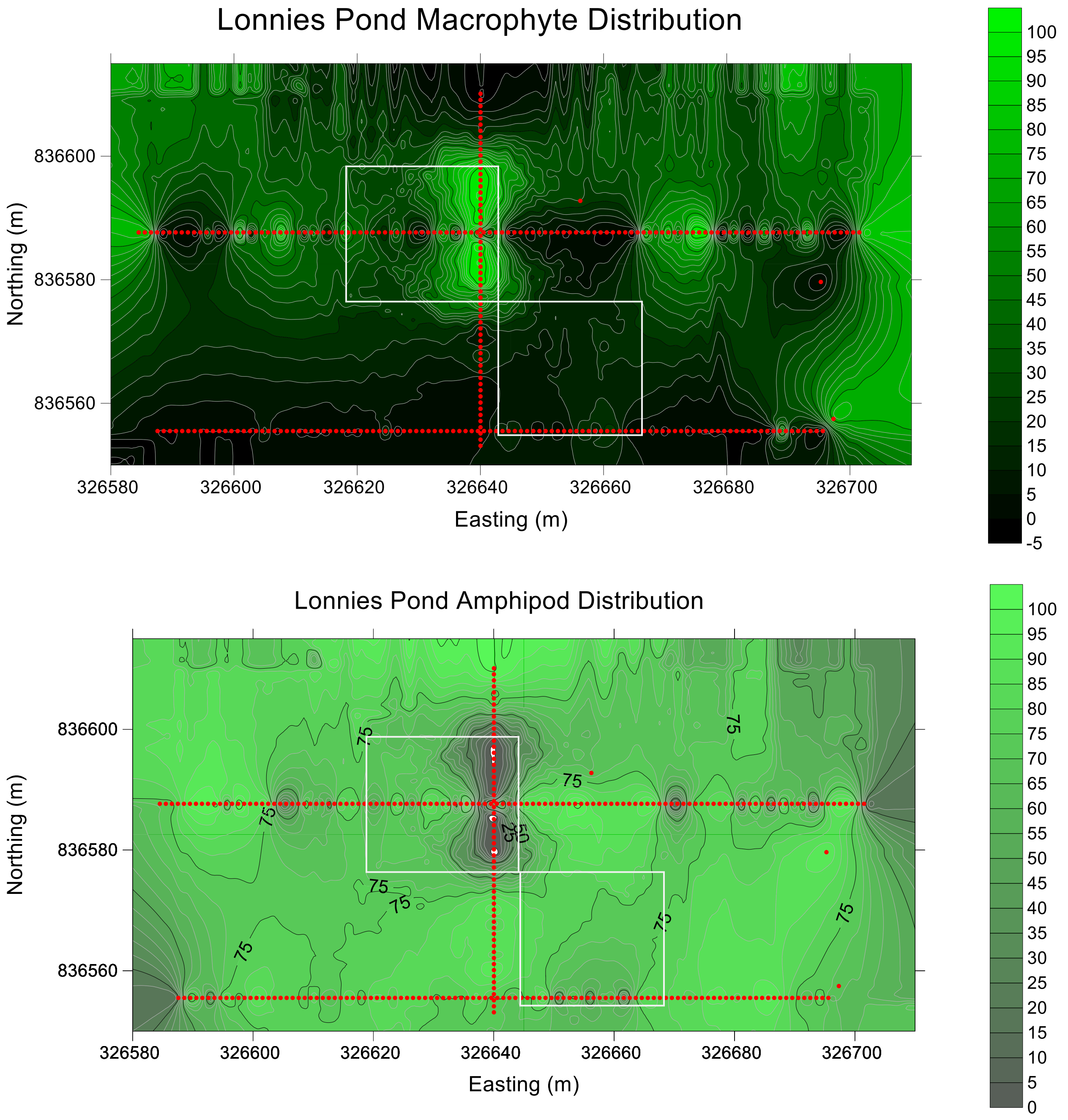

Figure 4.6 Contour Maps of Macrophyte and Amphipod Survey Results. Red dotted lines represent the survey transects, two parallel transects 30 m apart running east to west and one transect running south to north. Color bars represent percent coverage within the 0.5 m^2 quadrat of macrophyte coverage (top) and amphipod mat (bottom). Contour maps were created based on recorded GPS location and diver observed and GoPro photographed percent coverage.

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 March 31-April 12, 2023, and July 5-August 10, 2023, 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 2019-2022 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 deployments and removals occurring from March through December 2023 when all shellfish was removed from the Pond. Oyster aquaculture site maintenance, including deployment, management and bag rotation, was conducted by Ward Aquafarms throughout the 264-day growing season. Oysters were deployed for 4 additional days in 2023 compared to 2022, and 109 additional days compared to 2019.[22] A subpopulation of 2022 YR1 oysters deployed in Lonnie's Pond were overwintered in cold storage and redeployed as 2-inch YR2 oysters in March/April 2023.

CSP/SMAST staff subsampled oysters from bags during deployment and harvest events, collecting live oyster and shell weight, and empty bag weights (tare weights) for replicate bags (n = 6-54, Tables 5.1 and 5.3). In addition, 20-70 oysters were collected to determine individual oyster shell heights and weights and nitrogen content. The sample size was determined based on population size. Total oyster wet weights (total mass of live oysters and shell 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 deployment/harvest event. During the March and April 2023 deployments, 976 floating mesh bags filled with 4,859 kg of YR2 oysters were placed into the northern aquaculture areas in Lonnie's Pond (Table 5.2). The southern aquaculture area remained unoccupied until July when YR1 seed oysters were deployed. In July and August, YR1 seed oysters were deployed in both northern and southern aquaculture areas in 1,978 bags (Table 5.4). These YR1 seed oyster bags replaced 964 YR2 oyster bags, which were harvested over the same July-August time period. In December 2023, all YR1 oyster bags (1,969) were removed from Lonnie's Pond. All oysters were either relayed to another site or overwintered in dry storage with no redeployments in Lonnie's Pond until Spring 2024. YR2 oysters were deployed for 92 to 132 days, while YR1 oyster were deployed for 130 to 167 days. Net increase in oyster weight after accounting for input weights was 17,132 kg (Table 5.5). Average size and length of YR2 and YR1 oysters are listed in Tables 5.6 and 5.7, respectively.

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[22] Growing season length will depend on when deployment starts and ends. The 2022 and 2023 deployments were 260 days and 264 days, respectively. Deployments from 2020-2023 were much longer than 2019 since they started earlier in the year.

Table 5.1 2023 second year (YR2) oyster deployment and harvest subsamples. Weights were determined for a subsample of bags that were deployed and harvested from Lonnie's Pond during spring and summer, respectively. Subsampled bags were emptied to separately measure the weights of the live oysters and shells. SD = standard deviation of the sample average.

Event Type	Date	Age Class	Oyster Wt. per Bag (kg)	SD	n	Live Oyster Wt. per Bag (kg)	SD	n	Shell Wt. per Bag (kg)	SD	n
Deployment	3/31/23	YR2	4.69	0.26	14	4.05	NA	1	0.11	NA	1
	4/7/23	YR2	5.01	0.5	21	4.64	0.51	21	0.13	0.04	4
	4/12/23	YR2	5.45	0.34	10	5.21	0.39	10	0.16	0.08	9
	Average		**5.01**	**0.48**	**45**	**4.80**	**0.55**	**32**	**0.15**	**0.07**	**14**
Harvest	7/13/23	YR2	9.96	2.22	15	7.27	1.89	15	2.69	1.17	15
	7/20/23	YR2	11.07	1.16	24	10.77	1.15	24	0.3	0.12	24
	7/27/23	YR2	11.26	1.04	21	10.93	1.03	21	0.33	0.13	21
	8/3/23	YR2	13.07	0.72	16	12.82	0.71	16	0.25	0.05	16
	8/10/23	YR2	14.24	0.91	12	14.00	0.90	12	0.24	0.08	12
	Average		**11.72**	**1.87**	**88**	**11.02**	**2.37**	**88**	**0.70**	**1.03**	**88**

Note: The field-scale battery malfunctioned on 3/31/23 and limited subsampling of live oyster and shell to one bag. Subsequently, the field-scale battery was replaced.

Table 5.2 2023 second year (YR2) oyster deployment and harvest total weight and bag count. The total weight of the YR2 oysters was determined from the average oyster weight per bag and the total number of bags deployed or harvested on each date. The total weights determined by the CSP staff are compared to the weights measured at the Orleans Transfer Station truck scale.

Event Type	Date	Age Class	No. of Bags	Total Wt. (est. from bag wt. and count; kg)	Net Weight from Truck Scale (kg)	Percent Difference
Deployment	3/31/23	YR2	352	1650.88	1760.28	6.41%
	4/7/23	YR2	439	2199.39	2055.09	-6.78%
	4/12/23	YR2	185	1008.25	1198.88	17.27%
	Total		**976**	**4,858.52**	**5,014.25**	**3.15%**
Harvest	7/13/23	YR2	216	2151.36	2324.41	7.73%
	7/20/23	YR2	243	2690.01	2672.98	-0.64%
	7/27/23	YR2	226	2544.76	2413.18	-5.31%
	8/3/23	YR2	161	2104.27	2115.28	0.52%
	8/10/23	YR2	118	1680.03	1926.35	13.66%
	Total		**964**	**11,170.43**	**11,452.19**	**2.49%**

Table 5.3 2023 first-year (YR1) oyster deployment and harvest subsamples. Weights were determined for a subsample of bags deployed in Lonnie's Pond during July and August. The number of YR1 oysters added to each bag was determined by the volume in a container. The oyster-filled containers were subsampled immediately before the bags were filled to determine the average weight of the oysters per bag. SD = standard deviation of the sample average.

Event Type	Date	Age Class	Oyster Wt. per Bag (kg)	SD	n	Live Oyster Wt. per Bag (kg)	SD	n	Shell Wt. per Bag (kg)	SD	n
Deployment	7/6/23	YR1	0.69	0.04	26	NA	NA	NA	NA	NA	NA
	7/13/23	YR1	0.53	0.10	6	NA	NA	NA	NA	NA	NA
	7/20/23	YR1	0.15	0.02	20	NA	NA	NA	NA	NA	NA
	7/27/23	YR1	0.21	0.02	47	NA	NA	NA	NA	NA	NA
	8/3/23	YR1	0.25	0.01	54	NA	NA	NA	NA	NA	NA
	8/10/23	YR1	0.39	0.01	49	NA	NA	NA	NA	NA	NA
	Average		**0.33**	**0.17**	**202**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
Harvest	12/18/23	YR1	5.08	0.59	28	5.06	0.59	28	0.02	0.01	28
	12/20/23	YR1	5.71	1.23	28	5.54	1.06	28	0.17	0.18	28
	Average		**5.39**	**0.81**	**56**	**5.30**	**0.78**	**56**	**0.09**	**0.09**	**56**

Note: 0.5% of the subsampled oysters had empty valves (shells). Empty oyster valves contributed negligible weight to the bags being deployed, and were not separated and weighed.

Table 5.4 2023 first-year (YR1) oyster deployment and harvest total weight and bag count. The total weight of the YR1 oysters was determined from the average oyster weight per bag and the total number of bags deployed each date. The total weights determined by CSP staff will be compared to weights measured at the Orleans Transfer Station truck scale for YR1 bag harvest only.

Event Type	Date	Age Class	No. of Bags	Total Wt. (est. from oyster wt. and count; kg)	Net Weight from Truck Scale (kg)	Percent Difference
Deployment	7/6/23	YR1	130	90.2	NA	NA
	7/13/23	YR1	37	19.61	NA	NA
	7/20/23	YR1	487	71.35	NA	NA
	7/27/23	YR1	479	99.47	NA	NA
	8/3/23	YR1	500	126.2	NA	NA
	8/10/23	YR1	345	134.69	NA	NA
	Total		**1,978**	**541.52**	**NA**	**NA**
Harvest	12/18/23	YR1	984	5332.07	6032.88	12%
	12/20/23	YR1	985	5930.71	7464.81	23%
	Total		**1,969**	**11,262.78**	**13,497.69**	**18%**

Table 5.5 Monthly summary of total oyster weight deployed and harvested from Lonnie's Pond during the 2023 season. Weights are calculated from estimated bag weight and count.

2023	Oyster Weight (kg)	
	Deployment	Harvest
March	1,651	0
April	3,208	0
July	181	7,386
August	261	3,784
December	0	11,263
Total	**5,301**	**22,433**
Net (Harvest-Deployment)	**17,132**	

Table 5.6 2023 second-year (YR2) oyster physical measurements. The average shell height and whole oyster wet weight are shown for replicate oyster samples subsampled at the time of deployment and harvest during spring and summer, respectively. SD = standard deviation of the sample average.

Event Type	Date	Age Class	Shell Height (mm)	SD	n	Whole Oyster Wt. (g)	SD	n
Deployment	3/31/23	YR2	55.38	6.20	50	14.90	7.61	50
	4/7/23	YR2	58.69	7.90	67	18.05	6.50	67
	4/12/23	YR2	58.13	7.46	30	16.87	4.98	30
	Average		**57.45**	**7.34**	**147**	**16.74**	**6.74**	**147**
Harvest	7/13/23	YR2	85.84	5.50	26	51.92	11.93	26
	7/20/23	YR2	85.24	8.20	30	43.48	9.33	30
	7/27/23	YR2	86.58	10.69	19	52.17	9.98	19
	8/3/23	YR2	73.04	22.93	29	53.97	12.24	29
	8/10/23	YR2	88.42	7.67	25	52.74	10.99	25
	Average		**83.43**	**14.00**	**129**	**50.61**	**11.53**	**129**

Table 5.7 2023 first-year (YR1) oyster physical measurements. The average shell height and whole oyster wet weight are shown for replicate oyster samples subsampled at the time of deployment. SD = standard deviation of the sample average.

Event Type	Date	Age Class	Shell Height (mm)	SD	n	Whole Oyster Wt. (g)	SD	n
Deployment	7/6/23	YR1	23.10	5.56	70	1.05	0.38	70
	7/13/23	YR1	17.31	3.49	68	0.63	0.39	68
	7/20/23	YR1	15.91	2.58	50	0.30	0.16	50
	7/27/23	YR1	17.96	3.11	50	0.50	0.21	50
	8/3/23	YR1	18.42	3.93	50	0.61	0.47	50
	8/10/23	YR1	22.03	3.58	50	1.04	0.30	50
	Average		**19.26**	**4.71**	**338**	**0.71**	**0.44**	**338**
Harvest	12/18/23	YR1	55.73	10.74	82	16.72	7.98	82
	12/20/23	YR1	61.61	11.41	48	21.30	9.20	48
	Average		**57.90**	**10.99**	**130**	**18.41**	**8.43**	**130**

Subsamples were also collected to determine C and N content analysis of whole oyster, shell, tissue, and fouling material were also completed (Table 5.8 and 5.9). 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 (shell) 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 deployment/harvest event, was used to determine the total mass of nitrogen as oyster biomass 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 5.8) and cumulative mass in harvest (Table 5.9). At time of harvest, average percent nitrogen content (dry weight) of YR2 and YR1 oysters was 0.6% N (0.5% to 0.8%) and 0.8%, 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 72.8 kg of nitrogen in their tissues and shells during the growing season.

Table 5.8 2023 Combined Oyster Deployment Characteristics. Combined March, April, and July oyster deployment average carbon and nitrogen contents and physical measurements. Average shell height and whole oyster weight are shown for replicate oyster samples subsampled prior to March, April, July 2023 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 5.2 and 5.4) to determine total C and N (kg) input by event. In total, 524.89 kg of carbon and 19.36 kg of nitrogen was deployed into Lonnie's Pond in March/April and July/August 2023. This is about 4-5 greater compared to the 96.34 kg of carbon and 5.03 kg of nitrogen of oyster deployed in spring and summer 2022.

Event Type	Date	Age Class	Whole Oyster					Event Input	
			Shell Height (mm)	Whole Oyster Wt. (g)	%C	%N	n	Total C (kg)	Total N (kg)
Deployment	3/31/23	YR2	57.82	18.18	17.2	0.6	5*	156.16	5.42
	4/7/23	YR2	64.30	21.05				219.99	7.48
	4/12/23	YR2	60.32	21.02				99.52	3.46
	7/6/23	YR1	27.65	1.24	16.4	1.0	32	8.25	0.50
	7/13/23	YR1	21.06	1.04				1.86	0.11
	7/20/23	YR1	16.67	0.37				7.42	0.45
	7/27/23	YR1	18.83	0.53				8.74	0.53
	8/3/23	YR1	16.95	0.43				9.65	0.59
	8/10/23	YR1	21.91	1.01				13.30	0.81
	Deployment Total (YR1+YR2 oysters: 5,400.04 kg)							**524.89**	**19.36**

* n = 5 oysters subsampled for %C and %N. Shell, tissue, and fouling material C/N contents were measured separately (n = 11).

Table 5.9 2023 Combined Oyster Harvest Characteristics. Combined July, August, and December oyster harvest average carbon and nitrogen contents and physical measurements. Average shell height and whole oyster weight are shown for replicate oyster samples subsampled during harvest. Total carbon and nitrogen (kg) removed from Lonnie's Pond was calculated for each harvest date using a percent by weight of the whole oyster (shell + tissue). 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 5.2 and 5.4) to determine total C and N (kg) removed on each harvest date. In total, 2,004.46 kg of carbon and 92.21 kg of nitrogen was removed as gross output from Lonnie's Pond. Five oysters were subsampled (n = 5) for %C and %N. Shell, tissue, and fouling material C/N contents were measured separately (n = 11).

Event Type	**Date**	**Age Class**	**Whole Oyster**					**Event Input**	
			Shell Height (mm)	**Whole Oyster Wt. (g)**	**%C**	**%N**	**n**	**Total C (kg)**	**Total N (kg)**
Harvest	7/13/23	YR2	81.41	55.77	16.8	0.8	5	238.04	8.53
	7/20/23	YR2	82.81	46.26	14.5	0.6	5	226.66	8.43
	7/27/23	YR2	88.26	52.89	16.2	0.6	5	237.19	9.09
	8/3/23	YR2	87.30	48.01	13.1	0.6	5	198.31	8.68
	8/10/23	YR2	87.45	52.71	14.8	0.5	5	184.84	6.78
	12/18/23	YR1	62.80	20.24	14.1	0.8	5	443.81	24.65
	12/20/23	YR1	60.55	21.03	14.1	0.8	5	475.62	26.04
	Harvest Total (YR1+YR2 oysters: 22,433.21 kg)							**2,004.46**	**92.21**

6.0 Key Findings and Future Considerations

CSP/SMAST staff working with Ward Aquafarm and Town staff were able to measure and document 77.1 kg of net N removal in the Lonnie's Pond 2023 to date without including additional denitrification from overwintering sediment deposits that will be measured in April 2024. Of the 77.1 kg of N removed, 72.8 kg was in oyster biomass generated by oyster growth in the pond. The remaining 4.3 kg was in sediment denitrification, but it does not yet include the full year. Even without the additional April 2024, the Lonnie's Pond Aquaculture and Nitrogen Management Plan nitrogen removal goal of 75 kg removal has been attained in 2023.

Key Project findings and considerations for future monitoring and oyster deployment in Lonnie's Pond include:

1. Total Lonnie's Pond 2023 oyster aquaculture nitrogen removal was at least 77.1 kg N. Total net N 2023 mass removal in Lonnie's Pond from oyster harvest – deployment was 72.8 kg N, which is a 4.3 kg higher removal than in 2022 (Figure 6.1). Oyster enhanced denitrification within sediments receiving biodeposits was an additional 4.3 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 2024. If denitrification at the northern deployment area was the same as the southern deployment area, the total 2023 removal (pending additional sediment removal in April 2024 measurements) was 82.6 kg.

2. Aquaculture in Lonnie's Pond has caused significant additional nitrogen removal by sediment denitrification. Sediment nitrogen removal has been directly measured every year from 2016 through 2023 at the southern oyster deployment area and has varied between 4.3 and 12.1 kg of nitrogen removed from the Pond. This load is equivalent to the wastewater flow from approximately 1 to 3 houses.

3. Lonnie's Pond sediments are improving, but the extent of the improvement has not been quantified. The oyster aquaculture in Lonnie's Pond is improving the observed sediment infauna. Amphipods have been colonizing the southern deployment area, almost certainly due to improvements caused by oyster activities that have shifted the benthic community from species associated with significantly impaired conditions (oligochaetes) to species associated with slightly less impaired conditions (amphipods). Although infauna characterization was not part of the Lonnie's Pond QAPP, CSP/SMAST conducted limited species identification in 2022 and completed a diver survey of the extend of bottom coverage by amphipod mats in and around the southern oyster deployment area in September 2023. The survey indicated the continued proliferation of dense amphipod mats on the sediments both within and outside of the aquaculture oyster biodeposits areas. Since the MassDEP TMDL is based on restoration of the benthic infaunal community in Lonnie's Pond, it is recommended that the Town consider collecting benthic infauna samples on a frequent basis (every 3 years) using Massachusetts Estuaries Project procedures so that a more quantitative comparison can be done to baseline Lonnies's Pond MEP infauna data.

4. The Town may reduce water quality monitoring in Lonnie's Pond, but reduced sampling should be done in a way that preserves the Town's ability to document TMDL compliance, water quality improvements, and benthic infaunal changes. CSP/SMAST staff has successfully tracked aquaculture mediated water quality improvements in Lonnie's Pond since 2016. Over that time, Lonnie's Pond grab samples were collected at a shallow and deep

depth at nine (9) stations every two weeks throughout each year's oyster growing season. CSP/SMAST staff have sufficiently documented water quality results in Lonnie's Pond over the last eight (8) years to warrant less frequent and less extensive water quality monitoring during the 2024 oyster growing season. Statistical analysis of the 2023 water quality data indicated no difference in seasonal averages and standard deviations when data was compiled bi-weekly versus monthly. It is recommended that the Town consider the following ecosystem monitoring program:

a. Reduce the monitoring sites from 9 to 3: LP-4 (sentinel station), LP-6 (immediately adjacent to the southern deployment area), and LP-9 (immediately adjacent to northern deployment area)
b. Reduce the monitoring frequency from 13 events [March (once) and May through October (every two weeks)] to 7 events (monthly March, May through October)
c. Reduce the deployment of sondes from 2 to 1: continue the deployment in the southern aquaculture area; this will measure the impact of the aquaculture at a much higher frequency than can be done in monthly readings
d. Reduce the annual stream monitoring and sediment core denitrification surveys from every year to every three years
e. Add a comprehensive benthic infauna sampling every three years

If the Town chooses to alter monitoring procedures, the Lonnie's Pond Quality Assurance Project Plan (QAPP) will also need to be altered to ensure continued compliance with MassDEP regulatory requirements.

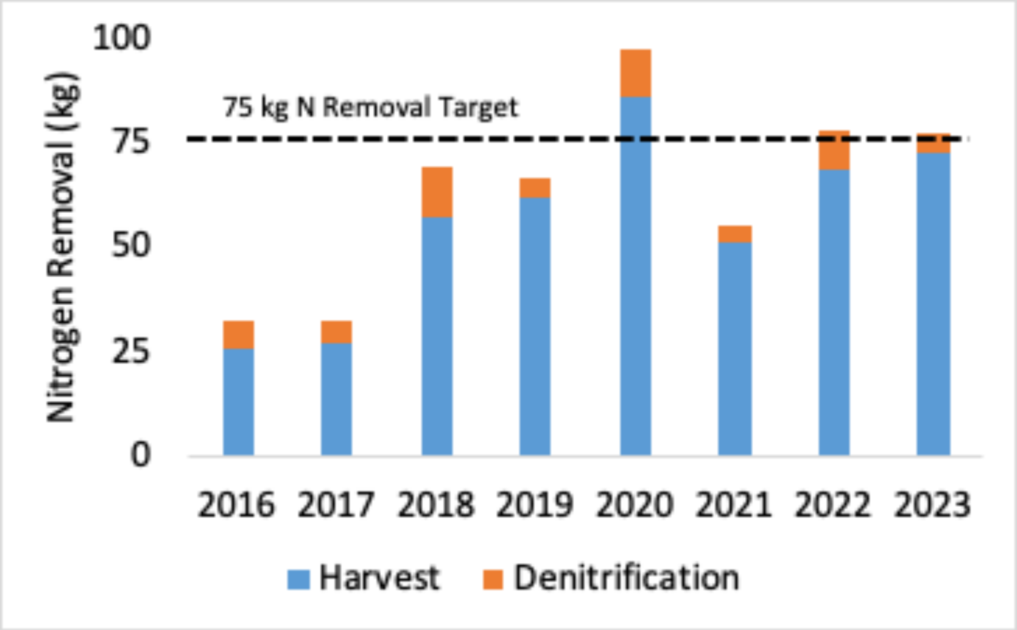

Figure 6.1 Total nitrogen removal in Lonnie's Pond over the 2016-2023 growing seasons separated by harvest and denitrification. The 2023 total will be revised when overwintered denitrification is accounted for in April 2024 sediment sampling.

5. Continue to deploy YR1 and YR2 oysters in a similar fashion to 2022 and 2023. YR1 oysters have the greatest potential for N removal due to their growth and biomass increase compared to the YR2 oysters, while early season/longer deployment of YR2 oysters results in increased the market value of harvestable oysters and a sustainable aquaculture operation. Figure 6.2 shows that YR1 oysters generally have greater net weight increase and are generally the predominant portion of biomass increase in 2020-2023. 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. The early deployment of YR2 oysters in 2023 maximized the length of the oyster growing season and increased the market value of harvestable oysters. Average shell heights of YR2 oysters deployed on two dates in March/April 2023 were 55.4-58.7 mm. With a market requirement of shell heights ≥63.5 mm, live YR2 oysters harvested in July and August 2023 had shell heights ranging from 81.4 mm to 88.3 mm. Continued deployment of YR2 oysters in early spring will also provide a benefit of reduced YR1 oyster mortality and interannual nitrogen removal variability. Given a reduced sediment testing, it is also recommended that YR2 oysters be deployed in the southern deployment area first to allow sediment data collection consistent with previous years.

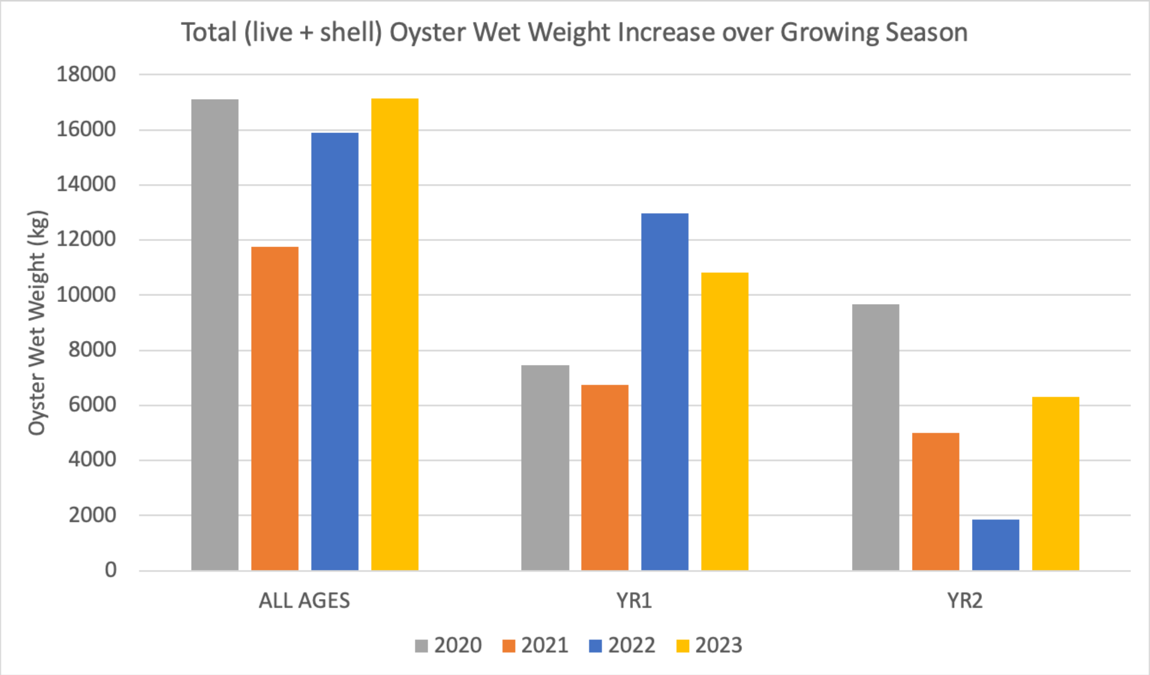

Figure 6.2 Increase in oyster biomass in Lonnie' Pond over the 2020-2023 growing seasons. YR1 oysters have greater increase in biomass than YR2 oysters.

6. Continue exclusive use of the Orleans Transfer Station Scale "truck scale" for measuring harvest wet weight. 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 due to fuel use. Transfer Station Scale receipts with truck license plate numbers should be photocopied and shared with the Monitoring Contractor in addition to records of any truck contents when determining truck tare weights.

7. Continue to use zip ties to identify bags designated for Lonnie's Pond. In order to optimize tracking of oysters and accounting of N entering and leaving the Pond, CSP/SMAST and the Ward Aquafarm use zip ties to identify bags. In previous years, zip ties were black and included a unique serial number. Moving forward, it is recommended that CSP/SMAST acquire brightly colored zip ties that are easily distinguishable from the standard black zip

ties used to secure oyster bags. Zip ties will continue to be used to seal 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. Zip ties should also be removed, counted, and redeployed as YR1 oysters grow and are redeployed at lower stocking densities.

8. Continued coordination between the Town, Aquaculture Contractor (Ward Aquafarm) and Monitoring Contractor (CSP/SMAST) is essential for the continued success of the nitrogen removal accounting associated with the Lonnie's Pond aquaculture program. 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 the Aquaculture and Monitoring 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, the Monitoring Contractor needs to be on-site when the Aquaculture Contractor is adding or removing oysters. 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 required water quality sampling dates. In 2022, the Aquaculture Contractor reduced the number of oyster inputs and outputs to 13 dates. In 2023, the Aquaculture Contractor slightly increased the number of oyster inputs and outputs to 16 dates. In order to have reliable accounting of nitrogen, the Aquaculture Contractor and Monitoring Contractor have to have in-step communications especially when different age oysters are being used. It is recommended that opportunities to streamline monitoring and aquaculture regularly be pursued.