University of Massachusetts Dartmouth
The School for Marine Science and Technology

Technical Report

Final

Water Quality Improvements Associated with Mashpee Shellfish Aquaculture: Effectiveness in Reducing Water Column Nutrient Concentrations in Popponesset and Waquoit Bays

To:
The Town of Mashpee

From:
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Section I. Introduction

Background:

Estuarine water quality in Mashpee is impaired due to excessive nitrogen inputs from development within their watersheds and has possibly been magnified by the loss of key filter feeders (oysters, quahogs) determined by the detailed estuarine analyses completed under the Massachusetts Estuaries Project, which set the embayment specific nitrogen thresholds supportive of habitat restoration across all southeastern Massachusetts. To date the Town of Mashpee has received, through the Massachusetts Estuaries Project, the target nitrogen thresholds for both the Popponesset Bay and the Waquoit Bay estuaries that need to be achieved to restore their impaired estuarine habitats. In this study, we are measuring the effectiveness of filter feeders as a form of in-estuary treatment to reduce the concentration of water column nutrients. The filter feeders generally considered are quahogs and oysters, as they grow well in the shallow warm estuaries of southeastern Massachusetts, and the mechanics of seeding and aquaculture are well established regionally.

Oysters have documented pumping and filtering rates, as has been recorded by a recent analysis for the Town of Falmouth pilot test. Less clear, however, is how the water quality benefits of oyster culture should be incorporated into TMDL compliance and development of Comprehensive Wastewater Management Planning (CWMP) development. The main policy/regulatory consideration relative to these issues are to be able to quantify the nitrogen removal by an oyster deployment, both through harvest and enhancement of sediment microbial processes resulting in conversion of organic nitrogen to nitrogen gas (N_2). This latter process (denitrification) can result in several times the nitrogen removal than harvest alone, greatly decreasing the cost of each pound of nitrogen removed thus creating a significant cost savings to the towns. In addition, through their filtering shellfish remove particulates from the water column increasing water clarity and allowing for re-establishment of eelgrass beds, possibly accelerating the restoration process, and again reducing the time/cost needed to address the impairment. The goal of this comprehensive monitoring of ongoing oyster aquaculture is to quantify these processes for the Town of Mashpee to determine the proper "credit" towards compliance with the TMDLs established for these impaired estuaries and lowering the need for other nitrogen reduction approaches (hence cost).

Year 1 data collection included monitoring of water column parameters (nutrient concentrations, dissolved oxygen, chl-*a*, turbidity), and benthic properties (denitrification, nutrient regeneration) in and around a specified oyster aquaculture deployment. The oyster aquaculture areas studied where in the Mashpee River and Shoestring Bay. Year 2 repeated the field activities of Year 1; however, the water quality sampling frequency and sampling stations were increased to determine any nutrient gradients around the oyster deployment area. Year 3 was a continuation of the higher intensity water quality as well as a focus on data synthesis, reporting / presenting results and developing strategies for refining the actual aquaculture deployment to maximize the positive effects of shellfish filtration on water quality and nitrogen removals.

Section II. Water Quality Sampling of the Mashpee River and Shoestring Bay Aquaculture Deployments

Sampling Program:

A sampling program was implemented by the Coastal Systems Program (CSP), University of Massachusetts-Dartmouth, School for Marine Science and Technology (SMAST) in collaboration with the Mashpee Department of Natural Resources (DNR) to quantify nitrogen (N) removal and changes in water quality associated with the "long-term" shellfish aquaculture operations in the Mashpee River and Shoestring Bay. Initial sampling at the Little River aquaculture area determined that the site was too shallow and too heavily trafficked to yield unconfounded data and it was replaced by the Shoestring Bay aquaculture area. Six (6) water quality sampling locations were monitored in 2016, 3 each in at each site (MR1-3; SB1-3), building upon pre-existing water quality data from the Mashpee Collaborative Water Quality Monitoring Program (MCWQMP) in each basin (Figure II.1). The MCWQMP is a collaboration of the Mashpee Wampanoag Tribe, the Town of Mashpee, and the University of Massachusetts Dartmouth, SMAST, CSP. Stations were selected up-gradient, within, and down-gradient of each aquaculture deployment location (Mashpee River: MR1, 2, 3 and Shoestring Bay: SB1, 2, 3 respectively), to determine the effect of the oyster aquaculture on water quality (Figure II.1). The near field stations (MR1 and 3 and SB1 and 3) were positioned immediately up-gradient and down-gradient (respectively) of the aquaculture deployment areas to quantify the potential effect of the oysters filtering particulates (nitrogen and chlorophyll a) from the water column. A third station at each location (MR2 and SB2) was positioned within each aquaculture deployment area to quantify the immediate effect of shellfish filtration on water quality as well as to support quantification of nutrient cycling (denitrification, regeneration, deposition). To better evaluate water column constituent gradients within the Mashpee River and how these may be affected by the presence of oyster culture, 2 more sampling locations (MR0, MR3E) were added for the 2017-2018 seasons (Figure II.1).

Water sampling occurred bi-weekly during mid-ebb tide conditions and usually in the early morning as in the estuary-wide monitoring effort. Samples were collected at mid-water and analyzed for: temperature, salinity, total nitrogen (nitrate + nitrite, ammonia, dissolved organic nitrogen, particulate organic nitrogen), chlorophyll-a (Chl-a), pheophytin-*a*, orthophosphate, dissolved oxygen, transparency (secchi depth), and alkalinity. Samples were collected according to protocols outlined for the Massachusetts Estuaries Project (MEP) and which are followed by all other water quality monitoring undertaken by the SMAST-Coastal Systems Program across the southeastern Massachusetts region. Weather, tide-status, and results of water quality monitoring were documented. Quality Assurance samples (field duplicates) were collected (10% of total number of samples collected) with the goal of gaining acceptance of study results by MassDEP and USEPA.   Dissolved oxygen, temperature, and light intensity profiles (surface to bottom at 0.5m increments) were completed at each sampling location using a YSI-55 handheld dissolved oxygen meter and a LI-193 Spherical Quantum Sensor (LI-COR) following standard protocols. Winkler samples were collected in triplicate at the continuously recording DO/CHLA moorings at the sensor depth.

Aquaculture:

The Mashpee Comprehensive Watershed Nitrogen Management Plan (CWNMP, Mashpee Sewer Commission 2015) was written to reduce the nitrogen load and restore water quality in the Town's estuaries. It calls for new wastewater infrastructure (sewering and treatment plants), and increased shellfish aquaculture and fisheries as a major component (fertilizer management, and stormwater runoff control are other components). Mashpee's estuaries are currently nitrogen enriched and need to reduce nitrogen levels to meet their MassDEP and USEPA TMDL's. This can be accomplished through reducing inputs from their watersheds or removal of nitrogen within their waters (e.g. enhanced shellfish populations). Shellfish filter algae from blooms and assimilate nitrogen from the algae. Also, shellfish deposit indigestible components of the particles they filter (pseudo-feces) to the sediments where they are stored, some of the nitrogen is permanently removed (burial, denitrification) or nitrogen is held until after the sensitive summer season. These activities have an immediate in situ positive effect on water quality to the extent that they occur. To take advantage of the positive effects of shellfish on estuarine waters, the Mashpee Department of Natural Resources (DNR) is implementing the shellfish component of the Town's nitrogen management plan in phases. In addition, the Mashpee Wampanoag Tribe is also expanding oyster aquaculture at their oyster farm in the main basin of Popponesset Bay and has started an oyster bed restoration project in Shoestring Bay.

The Town oyster aquaculture project in the Mashpee River started in 2004 a decade before the CWNMP was approved. The oyster fishery that was lost in the 1980s was restored, and water quality in the river improved to the point that there were no fish kills after the oyster population was re-established. Each year in late June/early July up to 2 million oysters in plastic mesh bags of remote-set oyster seed (newly set oysters on clam shell) from the ARC hatchery (Dennis, MA) are distributed into the trays. Each bag contains up to 1,000 seed oysters at a size of about 1mm. The average number of seed per bag is variable year to year and has ranged from 309-762 individual oysters per bag from 2014-2018. After growth to a larger size (> 1 cm), the bags are opened, and the seed is spread out in trays. The seed is grown to harvest in 330 bottom mounted trays (Aquamesh trays 1.5m wide x 3.0m long) installed just below the main area of fringing salt marsh in the mid Mashpee River. Most grow to a harvestable size of 3 inches in about 1.5 years and are typically harvested from November through March, and survival of oyster seed from the previous year is around 50%.

The Mashpee Wampanoag Tribe's Natural Resources Department began an oyster reef restoration project in 2016 in Shoestring Bay initially a near shore site was seeded with 2,000 bags of remote set 1mm oyster seed from the ARC hatchery containing less than 1,000 oyster seed/bag. These bags were placed in the shallows of the bay margin directly on the bottom sediments. After growth to a larger size, the bags were opened, and the seed with attached shell was spread out on the bottom. Survival was exceptionally low possibly due to the excessive fouling and tunicate growth on the bags which restricted water flow-through. In 2017, another 2,000 bags of oyster seed were placed in an adjacent site on piles of shell (cultch) to keep them from being buried in the sediments. Survival was better, and the oysters were left to grow and become a reef.

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Figure II.1: Mashpee Aquaculture Monitoring station map showing the newly added near field aquaculture water quality monitoring stations in the Mashpee River (MR) and Shoestring Bay (SB) as well as existing long-term water quality stations maintained by the town of Mashpee. PB08 is sentinel station with a MEP established nitrogen threshold to restore water quality of 0.38 TN mg/L.

In summary, a total of 23 sampling events were completed in the Mashpee River and Shoestring Bay associated with oyster aquaculture sites from 2016 through 2018. Given the shallow depths in the shellfish aquaculture deployment areas, all stations were sampled at the mid depth only. The overall effort yielded total of 221 samples inclusive of 43 QA/QC samples (~15% of total,). After the first year of monitoring, the effort nearly doubled for the subsequent two years to include 9 sampling events in both 2017 and 2018 (Table II.1).

Table II.1: Sampling dates and assays performed for the Mashpee Aquaculture Monitoring Project

Date	Number of Samples	Assays Performed						
		Salinity/ Conductivity	Ortho Phosphate (PO^4)	Ammonium (NH^4)	Nitrate-Nitrite (NO$_x$)	Total Suspended Solids (TSS)	Particulate Organic Carbon and Nitrogen (POC/N)	Chlorophyll-*a*
8/18/16	8	✓	✓	✓	✓	✓	✓	✓
8/31/16	6	✓	✓	✓	✓	✓	✓	✓
9/13/16	8	✓	✓	✓	✓	✓	✓	✓
10/11/16	6	✓	✓	✓	✓	✓	✓	✓
10/24/16	3	✓	✓	✓	✓	✓	✓	✓
6/20/17	13	✓	✓	✓	✓	✓	✓	✓
7/5/17	13	✓	✓	✓	✓	✓	✓	✓
7/18/17	10	✓	✓	✓	✓	✓	✓	✓
8/2/17	11	✓	✓	✓	✓	✓	✓	✓
8/17/17	11	✓	✓	✓	✓	✓	✓	✓
8/30/17	10	✓	✓	✓	✓	✓	✓	✓
9/14/17	9	✓	✓	✓	✓	✓	✓	✓
9/28/17	11	✓	✓	✓	✓	✓	✓	✓
10/19/17	10	✓	✓	✓	✓	✓	✓	✓
6/7/18	11	✓	✓	✓	✓	✓	✓	✓
6/21/18	11	✓	✓	✓	✓	✓	✓	✓
7/8/18	10	✓	✓	✓	✓	✓	✓	✓
7/20/18	10	✓	✓	✓	✓	✓	✓	✓
8/6/18	10	✓	✓	✓	✓	✓	✓	✓
9/4/18	10	✓	✓	✓	✓	✓	✓	✓
9/18/18	10	✓	✓	✓	✓	✓	✓	✓
10/16/18	10	✓	✓	✓	✓	✓	✓	✓
11/5/18	10	✓	✓	✓	✓	✓	✓	✓
Total	**221**							
QC	**43**							

Description of Findings and Conclusions from Water Quality 2016-2018:

The water quality samples from three monitoring efforts have been consolidated: The ~~Town of~~ Mashpee summer water quality program, ~~the MCWQMP~~ Mashpee year-round sampling, and now the Mashpee Aquaculture monitoring effort. The year-round monitoring began in 2010 and includes stations PBM on the opposite side of the Mashpee River near the aquaculture area, PBG in Popponesset Bay near the mouth of the Mashpee River, and the newly added station in 2016, PBN up-gradient of the oyster aquaculture area in Shoestring Bay (Fig. II.1). A selection of the ~~Town's~~ summer water quality program in Popponesset Bay also represent far-field up-gradient as well as down-gradient stations in both aquaculture areas (Fig. II.1). ~~The Town of~~ Mashpee's summer program conducts monitoring four times in July and August, while the ~~Wampanoag monitoring group samples monthly~~ year-round sampling is conducted monthly. These stations' water quality results were compiled and calculated for yearly means for comparison.

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Spatial distribution of the major water quality constituents was more variable within the Mashpee River sub-estuary compared to Shoestring Bay and appears to be associated with the freshwater discharge from the Mashpee River. Salinity generally increased as station location moved down the estuary toward Popponesset Bay but showed much more variability in the Mashpee River (Figure II.2). It is interesting to note station PB04, which is down-gradient of the Mashpee River aquaculture area, has a lower salinity than the aquaculture sites (MR) (Fig. II.2). This could be the result of sampling differences from partial mixing of freshwater input through storm run-off since all eight summer sampling dates in 2016 and 2017 had rain 24 hours prior to sampling. Inversely related to increasing salinity were the decreasing concentrations of total nitrogen and total chlorophyll pigments found as station locations moved down the sub-estuaries (Figures II.2, II.3, II.4). When narrowing the focus on the newest stations positioned in and around the aquaculture areas, the near-field, down-gradient station MR3 had decreased concentrations of key water quality constituents compared to the up-gradient stations MR1 (Figures II.5). The Shoestring Bay sites also had decreasing concentrations passing over the aquaculture site except for the total suspended solids and total chlorophyll pigments from 2016 (Figure II.6). This could be due to the scattered distribution of oysters set on the sediment surface as opposed to inside discreet bottom cages.

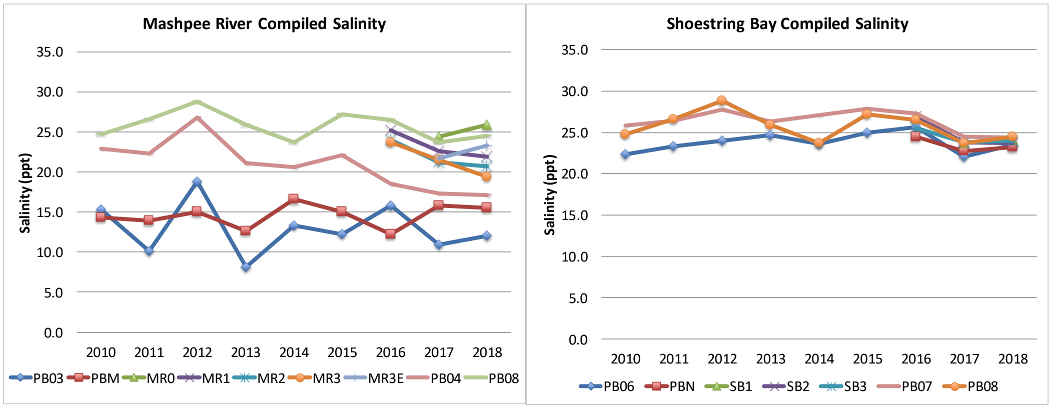

Figure II.2: Compiled yearly average salinity for all sampling stations around the Mashpee River and Shoestring Bay inclusive of the MCWCMP summer and year-round sampling, and Mashpee Aquaculture monitoring programs. Station names are listed from up-gradient to down-gradient.

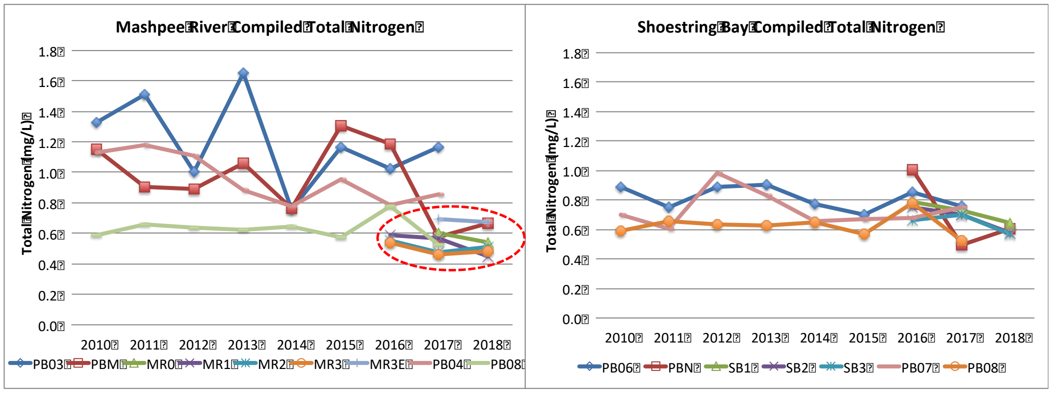

Figure II.3: Compiled yearly average total nitrogen for all sampling stations around the Mashpee River and Shoestring Bay inclusive of the MCWQMP summer and year-round sampling, and Mashpee Aquaculture monitoring program. Station names are listed from up-gradient to down-gradient. Mashpee Oyster stations are circled in red to show how they have lower concentrations compared to the bracketing water quality stations PB03 and PB04.

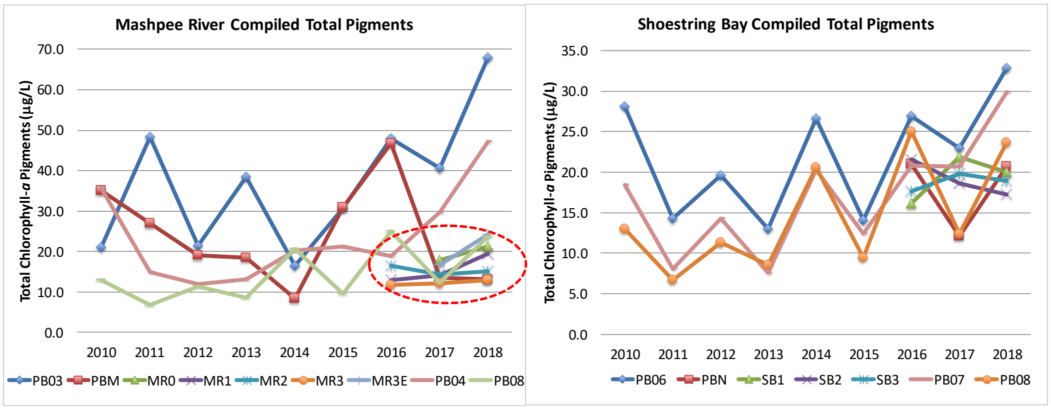

Figure II.4: Compiled yearly average total chlorophyll-a pigments for all sampling stations around the Mashpee River and Shoestring Bay inclusive of the MCWQMP summer and year-round sampling, and Mashpee Aquaculture monitoring program. Station names are listed from up-gradient to down-gradient. Note the scale on the Shoestring Bay chlorophyll-a concentration is half that of the Mashpee River sites. Mashpee Oyster stations are circled in red to show how they have lower concentrations compared to the bracketing water quality stations PB03 and PB04.

The Mashpee River aquaculture area had two new sites added in 2017 to capture the far-field up-gradient (MR0) and down-gradient channel site (MR3E), which showed the aquaculture area had reduced concentrations of total suspended solids, total chlorophyll pigments, and total nitrogen compared to the down-gradient MR3E site (Figure II.5). Spatially the key nutrients would decrease as station location moved down the estuary towards Popponesset Bay and the inlet connecting to the open ocean, but directly comparing MR3 with MR3E, shows higher concentrations down-gradient and away from the oysters suggesting oysters' effectiveness of removing nutrients from the water column (Figure II.5). Another example of the Mashpee River oyster aquaculture reducing surrounding nutrients is the Mashpee Water Quality data PB03 (above oysters) and PB04 (down-gradient) compared to the MR0-MR3 sites concentrations (Figure II.3 and II.4). Near-field oyster stations have improved water quality compared to surrounding water quality.

Interannual differences in water quality could be seen in spatial gradients, again more pronounced variability existing in the Mashpee River compared to the Shoestring Bay sub-estuary (Figures II.2, II.3, II.4). It should be noted that the MEP derived sentinel station PB08 is still averaging 0.63 mg/L total nitrogen from 2010-2017, nearly double the MEP established nitrogen threshold of 0.38 mg/L (Howes et al. 2004). Using the newest station data located in and around the aquaculture areas, the lowest total suspended solids and total pigments were measured in 2016 and are likely due to the low rainfall during 2016 (Figure II.7, II.8). Rainfall in 2016 measured 38.45 inches, while 2017 and 2018 had 60 and 57 inches of rain respectively (Figure II.9). Lower rainfall causally relates to the discharge of the Mashpee River and could lead to less nitrogen and fewer particles transported downstream.

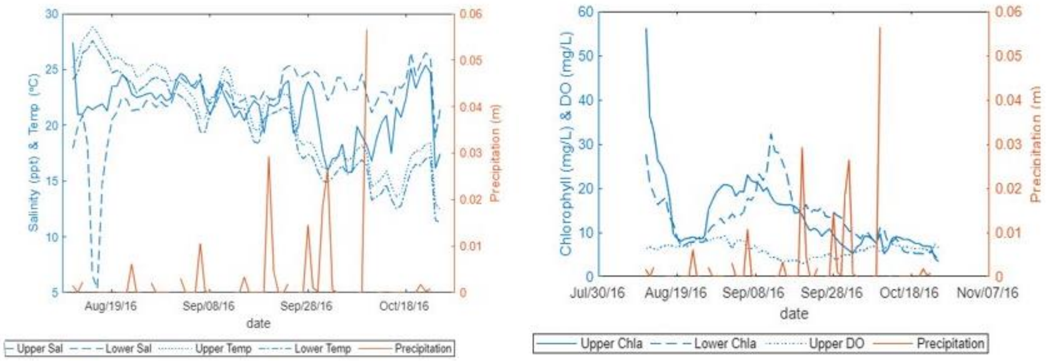

Figure II.5: Comparison plots of salinity and temperature to daily precipitation recorded (left graph) for the data from both the upper and lower sondes. Comparison of upper and lower sonde Chl-a data with the addition of the D.O. data recorded at the upper sonde (right ~~figure~~ graph).

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Precipitation data corresponds to changes in salinity and temperature recorded in the data (Figure II.5). While the precipitation was low for this study it does account for increases and decreases in the data. As salinity is a conservative tracer it is vital that its variability can be explained. There is an increase in precipitation in October, as well as, a decrease in temperature as it shifts from the Summer to the Fall. A decline in T can be explained by these factors and dips in salinity correspond to rises in precipitation (except the initial dip as the sonde was experiencing its initial calibration). The similarities expressed in the left graph ensure that the data compared in the right graphic are true values.

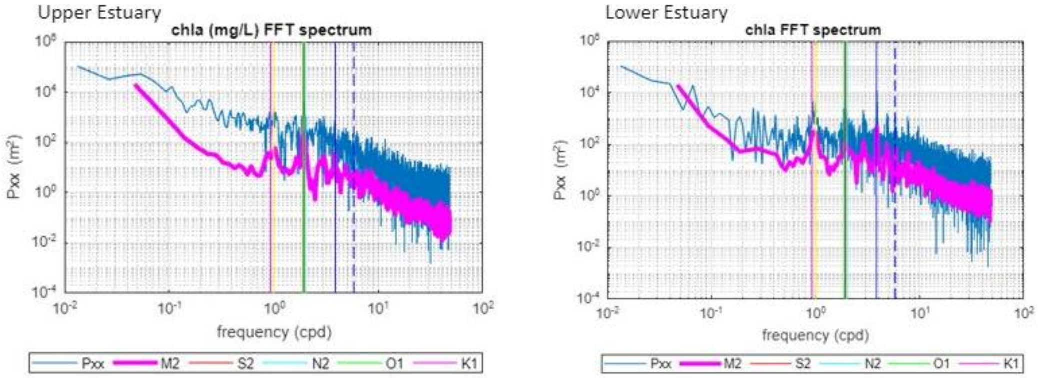

Figure II.6: Upper estuary sonde (left) and lower estuary sonde FFT analysis for Aug-Oct 2016 in the Mashpee River.

A Fast Fourier Transform (FFT) was performed on the different monitored constituents, to determine frequency components within the data, with chl-a showing both a diurnal and semi-diurnal signal peak (Figure II.6). A diurnal peak is influenced by sunlight, while a semi-diurnal peak is controlled by tides. This suggests that the estuary has a chl-a gradient that is advected up and down estuary that will pass through the oyster location.

Oysters have been shown to filter as much as 50 gallons of water/day and reduce as much as 50% of the water column particle organic nitrogen (Newell & Jordan 1983) and up to 28% of chlorophyll *a* (Grizzle et. al., 2008). Stations were positioned in a way to capture the nutrient concentrations up-gradient, within, and down-gradient of the aquaculture areas. Key indicators of the oysters filtering capacity may be seen in total suspended solids, total chlorophyll pigments, and total nitrogen concentrations. Results of the Mashpee River aquaculture area monitoring show a reduction in all three indicators when comparing the near-field up-gradient station, MR1 relative to the near-field down-gradient station, MR3 (Figure II.7). The total chlorophyll showed the strongest signal of removal from up-gradient to down-gradient in the Mashpee River and it is interesting to note how much higher the chlorophyll concentration was in surrounding water both far-field up-gradient, MR0 and along-side the down-gradient edge, MR3E in both the 2017 and 2018 seasons (Figure II.7). Total nitrogen has also been decreasing yearly in the Mashpee River aquaculture area over the 2016-2018 sampling.

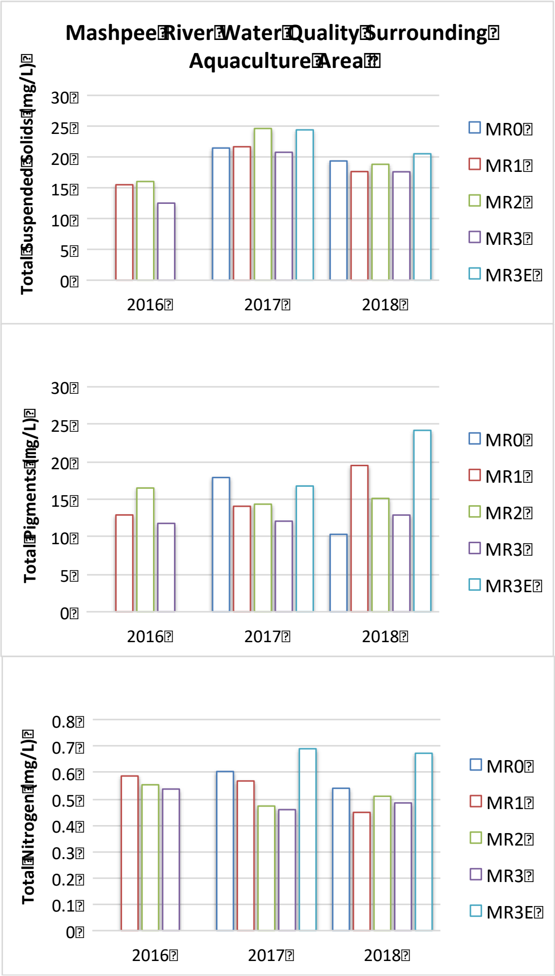

Figure II.7: Yearly station averages for total suspended solids, total chlorophyll-*a* pigments, and total nitrogen in the Mashpee River aquaculture area. Stations are listed from up-gradient to down-gradient. Both chlorophyll a pigments and total nitrogen were significantly lower than the upgradient stations due to passage through the oyster area (MR3) in all years

The Shoestring Bay aquaculture area showed a reduction in total nitrogen (TN) concentration from up-gradient (SB1) to down-gradient (SB3) for all three years the study was conducted and showed a consistent overall reduction in TN from 2016-2018 (Figure II.8). The phytoplankton concentration, represented by the chlorophyll-*a* pigments, was reduced from up-gradient to down-gradient in both 2017 and 2018 and also showed a yearly reduction in concentration, while the total suspended solids only showed a reduction 2018 possibly due to the 2 year class oysters that now inhabit the area as of August 2018 (Figure II.8).

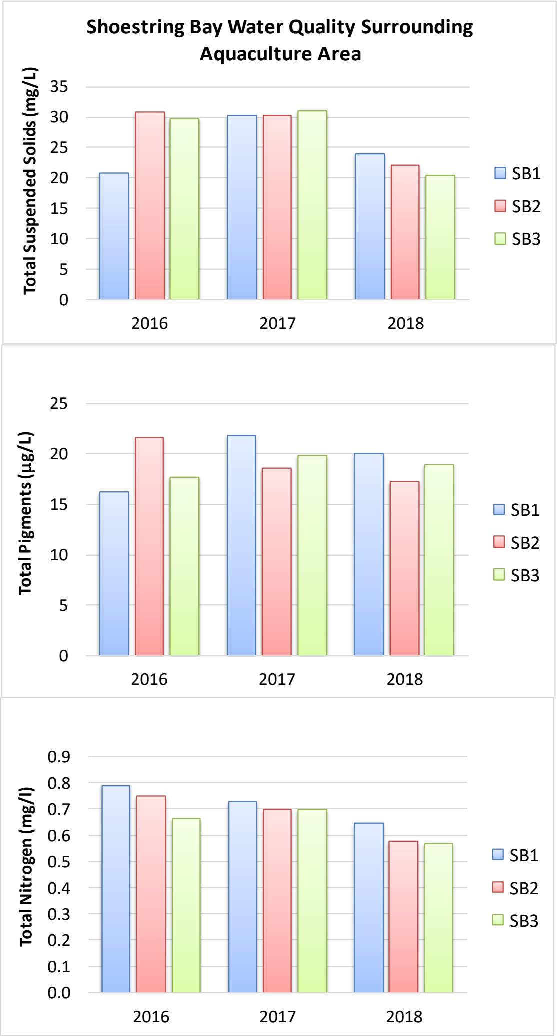

Figure II.8: Yearly averages for total suspended solids, total chlorophyll-*a* pigments, and total nitrogen in the Shoestring Bay aquaculture area. Stations are listed from up-gradient to down-gradient. Both chlorophyll a pigments and total nitrogen were significantly lower at the downgradient than the upgradient station (SB1 vs SB3) due to passage through the oyster area in 2017 and 2018. The nearshore placement of the shellfish did not allow for a clear flow path through the deployment area, like causing the more variable results, however the more established “reef” in 2018 did show the clearest reductions.

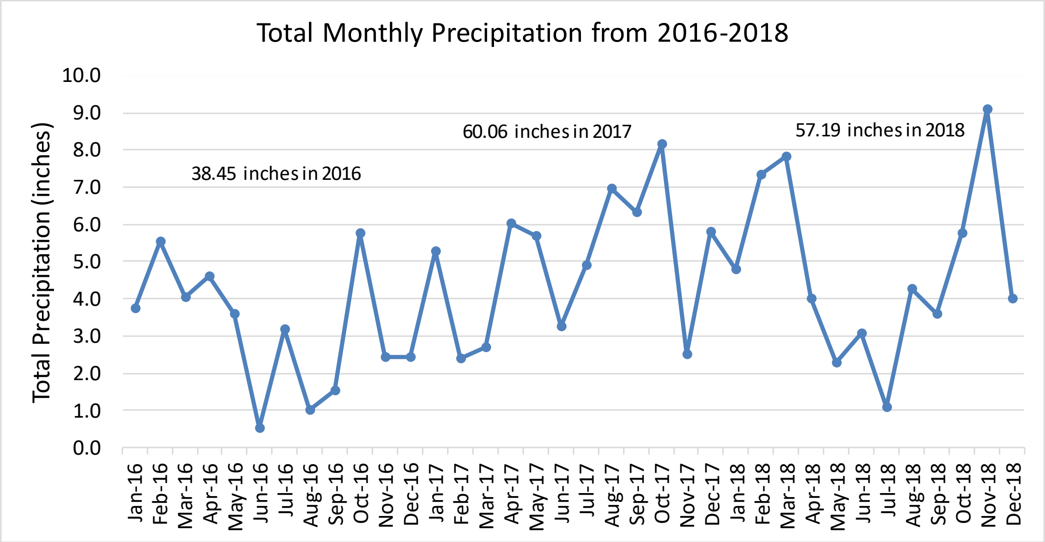

Figure II.9: Monthly precipitation totals from Hyannis, Ma from 2016-2018. Corresponding yearly precipitation totals are listed.

A more comprehensive yearly water quality data set shows higher concentrations of total nitrogen (TN), and chlorophyll-*a* pigments (total pigments), as well as total suspended solids (TSS) in Shoestring Bay compared to the Mashpee River (Table II.2). However, there is a stronger signal of reduction in nutrients from up-gradient relative to down-gradient in the Mashpee River, likely due to the much higher number of oysters in the river, and the hydrodynamic processes of unidirectional flow during ebb tide over the oysters in Mashpee River compared to Shoestring Bay (Table II.2 and 3). It appears that the concentrated aquaculture area in the Mashpee River attributed to the stronger “oyster effect” on water quality compared less aggregated aquaculture area of Shoestring Bay. However, a reduction in dissolved (DON) and total nitrogen (TN) was shown in both the Mashpee River and the Shoestring Bay aquaculture areas (Table II.3).

Table II.2 Mean 2016 water quality results for Mashpee River (upper) and Shoestring Bay (lower) and the relative "oyster effect" or difference between the up-gradient and the down-gradient stations. Decreases of concentrations from up-gradient to down-gradient locations are noted in red, with generally a 10% to 15% reduction in PON and TN.

2016 Mean values

Location Relative to Oysters	Sta. ID	PO4 (uM)	NH4 (uM)	NOX (uM)	DIN (uM)	DON (uM)	TDN (uM)	PON (uM)	TN (uM)	TSS (mg/L)	Total Pigments (ug/L)
above oysters	MR1	0.3	1.3	2.7	3.9	18.8	22.8	19.2	41.9	15.5	12.9
in oysters	MR2	0.4	2.5	2.4	5.0	16.8	21.8	17.7	39.6	16.1	16.5
below oysters	MR3	0.3	1.4	1.9	3.3	18.3	21.6	16.8	38.4	12.5	11.8
oyster effect		-0.1	0.1	-0.8	-0.6	-0.6	-1.2	-2.4	-3.5	-3.0	-1.1
above oysters	SB1	0.3	2.2	1.3	3.5	24.5	28.0	28.3	56.3	20.8	16.2
in oysters	SB2	0.2	1.8	1.5	3.2	24.4	27.6	25.9	53.5	30.9	21.6
below oysters	SB3	0.3	2.3	1.5	3.8	19.5	23.3	24.2	47.5	29.7	17.6
oyster effect		0.0	0.1	0.2	0.3	-5.0	-4.7	-4.1	-8.8	8.8	1.5

Table II.3 Compiled 2016-2018 mean water quality results for the Mashpee River and Shoestring Bay stations and the relative "oyster effect" or difference between up gradient and down-gradient stations. Decreases of concentration from up-gradient locations are noted in red. Note the large average reductions in Mashpee River and Shoestring Bay for PON (47%, 16%) and TN (24%. 11%).

2016-2018 Mean values

Location Relative to Oysters	Stn ID	PO4 (uM)	NH4 (uM)	NOX (uM)	DIN (uM)	DON (uM)	TDN (uM)	PON (uM)	TN (uM)	TSS (mg/L)	Total Pigments (ug/L)
above oysters	MR1	0.4	1.8	2.4	4.2	18.2	22.4	23.2	44.4	18.3	15.5
in oysters	MR2	0.4	2.2	2.3	4.5	16.0	20.4	15.7	36.6	19.9	15.3
below oysters	MR3	0.7	2.3	3.1	9.8	18.0	18.7	12.4	33.7	16.8	21.1
oyster effect		0.3	0.5	0.7	5.6	-0.2	-3.7	-10.8	-10.7	-1.6	5.6
above oysters	SB1	0.4	2.3	1.3	3.6	22.9	27.0	25.9	51.5	25.1	19.3
in oysters	SB2	0.4	2.1	1.3	3.5	22.3	25.4	22.4	48.2	27.7	19.1
below oysters	SB3	0.4	2.8	1.7	4.5	19.7	24.0	21.7	45.9	27.0	18.8
oyster effect		0.0	0.5	0.4	0.9	-3.1	-3.0	-4.2	-5.6	2.0	-0.6

Key Findings from Water Quality Sampling Results 2016-2018:

Oysters were found to significantly remove phytoplankton (chlorophyll a) and particulate nitrogen in both the more spatially distributed deployment in the Mashpee River and the more confined shoreline deployment in Shoestring Bay. Unexpectedly, it appears that the oyster biodeposits are stimulating direct denitrification (nitrate → nitrogen gas) in the Mashpee River site. This was seen by the decline in nitrate in overlying waters passing over the oyster arrays in 2016, but not 2017-2018, which needs to be resolved to enhance this process.

The Mashpee River site appeared to have a greater level of water quality improvement than the Shoestring Bay site, most likely due to the higher number of oysters and more distributed nature (more water contact) in the River. It may be that distributing oysters across a basin allows better water flow for oyster filtration than in a confined shoreline reef. Similar findings have been found in floating oyster aquaculture where the density of oysters per bag can result in decreased filtration of particulates as the oyster density is increased. This needs to be resolved, but if more reef areas are to be put in-place it would be prudent to consider more open areas or central areas for reef deployment (such as large shallow area at mouth of Shoestring Bay).

Section III. 2016 – 2018 Near Field Time-Series Dissolved Oxygen (DO)/Chl-*a* Moorings (High Frequency Sampling)

Time-series Mooring Deployment and Sampling:

Continuous monitoring of key water quality parameters to assess the impact of the oyster aquaculture is having on the ambient water column was completed using a mooring program. The oyster aquaculture mooring program utilized YSI sondes and HOBO light sensors to collect time-series measurements of 5 parameters (below). The same protocols and procedures established as part of the time-series mooring (D.O., chlorophyll-*a*) program for the MEP analysis of the Popponesset Bay and Waquoit Bay systems was used such that data collected through this effort will be comparable to long term DO/chl-*a* data collected from 2000-2014. Additionally, the DO/chl-*a* data will be comparable to that generated from the Town of Mashpee established monitoring locations in the vicinity of the aquaculture area in the Mashpee River (year-round water quality station PBM). Three moorings were deployed in and around the Mashpee River oyster deployment area were set to make measurements at 15-minute intervals, averaged to hourly to even out short-term spikes (Fig.2). Sensors were placed 30 cm above the sediment surface, same as in the MEP, and recorded the following:

- Dissolved oxygen (DO)
- Light Attenuation (as an indicator of water clarity)
- Chlorophyll-*a* (via fluorescence)
- Salinity
- Water temperature

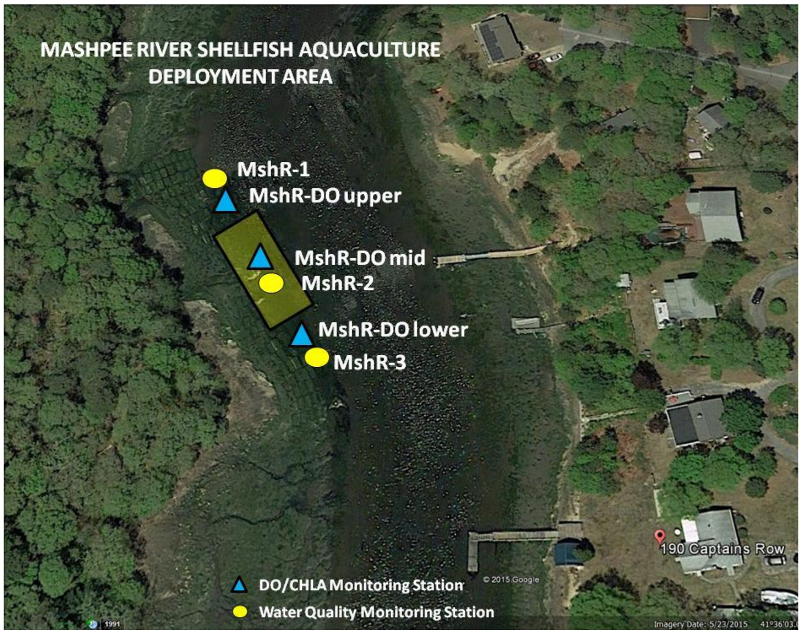

Figure III.1: Location of Dissolved Oxygen (DO) and Chlorophyll-*a* (CHLA) sondes and light meter profiles relative to oyster aquaculture installation paired water quality monitoring stations. Moorings were deployed 30 cm above the sediment surface.

Time Series Results and Discussion:

The time-series mooring dissolved oxygen mooring data was collected from August 10-October 24, 2016. The dissolved oxygen results from the mooring location up-gradient of the oyster deployment area show hypoxic conditions (<3 mg/L) occurred 8% of the time of deployment (Table III.1). Instrument failures at the down-gradient location prevented the acquisition of time series dissolved oxygen data from that location.

Table III.1 Frequency and duration of dissolved oxygen incrementally at the Upper Mashpee River Mooring located up-gradient of the oyster deployment footprint.

			Deployment	Duration with DO Below Value				Number of Times Below Value				% of Deployment Duration Below Value			
Mooring Location	Start Date	End Date	(Days)	<6 mg/L	<5 mg/L	<4 mg/L	<3 mg/L	<6 mg/L	<5 mg/L	<4 mg/L	<3 mg/L	<6 mg/L	<5 mg/L	<4 mg/L	<3 mg/L
Mashpee River Upper	8/10/2016	10/24/2016	75.0	40.90	25.81	13.52	6.20	91	72	56	33	55%	34%	18%	8%
			Mean	0.46	0.36	0.24	0.19								
			Min	0.02	0.01	0.01	0.02								
			Max	6.89	1.81	0.66	0.48								
			S.D.	0.75	0.36	0.21	0.15								

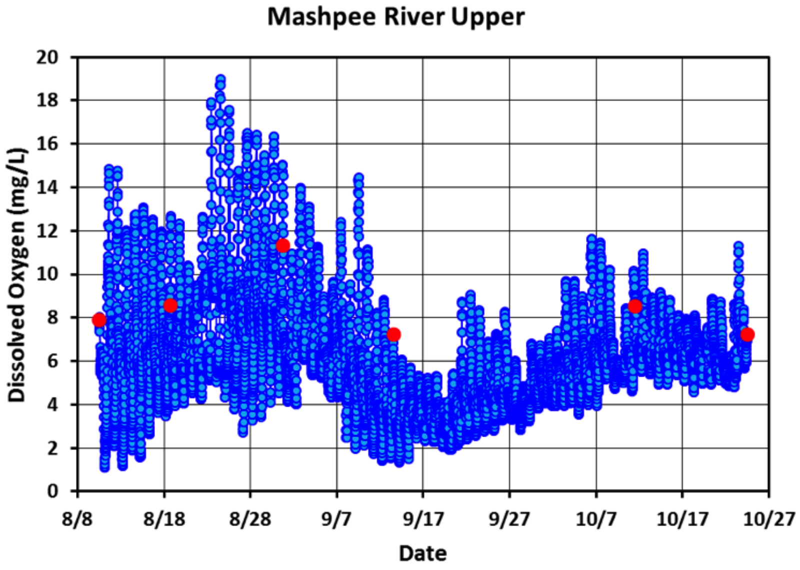

Figure III.2. Time series dissolved oxygen at the Upper Mashpee River Mooring located up-gradient of the oyster deployment footprint. Red markers indicate Winkler titration calibration points.

The chlorophyll sensors at both the up and down-gradient sites provided complete time series data records (Table III.2 and Figure lll.3). The records show predominantly higher chlorophyll concentrations up-gradient of the oyster deployment area compared to the down gradient location except for a brief period between September 10 and September 17 which suggested a bloom a bloom occurring in the outer bay extending up the Mashpee River on flood tides. A detailed analysis of the time series chlorophyll data was performed to determine the forces driving the differences observed in chlorophyll concentrations.

Table III.2 Frequency and duration of chlorophyll incrementally at the Mashpee River moorings located up-gradient and down-gradient of the oyster deployment area.

			Deployment	Duration with Chlorophyll Exceeding Value					Number of Times Value Exceeded					% Deployment Duration Value Exceeded				
Mooring Location	Start Date	End Date	(Days)	>5 ug/L	>10 ug/L	>15 ug/L	>20 ug/L	>25 ug/L	>5 ug/L	>10 ug/L	>15 ug/L	>20 ug/L	>25 ug/L	>5 ug/L	>10 ug/L	>15 ug/L	>20 ug/L	>25 ug/L
Mashpee River Lower	8/10/2016	10/24/2016	69.4	15.16	13.66	13.79	7.09	3.16	14	50	45	37	25	22%	20%	20%	10%	5%
Mean Chl Value = 12.9 ug/L			Mean	1.08	0.27	0.31	0.19	0.13										
			Min	0.01	0.01	0.01	0.01	0.01										
			Max	7.19	4.61	2.78	1.29	0.59										
			S.D.	1.85	0.66	0.45	0.27	0.15										
Mashpee River Upper	8/10/2016	10/24/2016	73.8	13.46	9.97	12.48	12.76	5.83	9	20	30	54	46	18%	14%	17%	17%	8%
Mean Chl Value = 13.5 ug/L			Mean	1.50	0.50	0.42	0.24	0.13										
			Min	0.01	0.01	0.01	0.01	0.01										
			Max	7.54	6.00	5.05	2.76	0.72										
			S.D.	2.31	1.31	0.90	0.39	0.16										

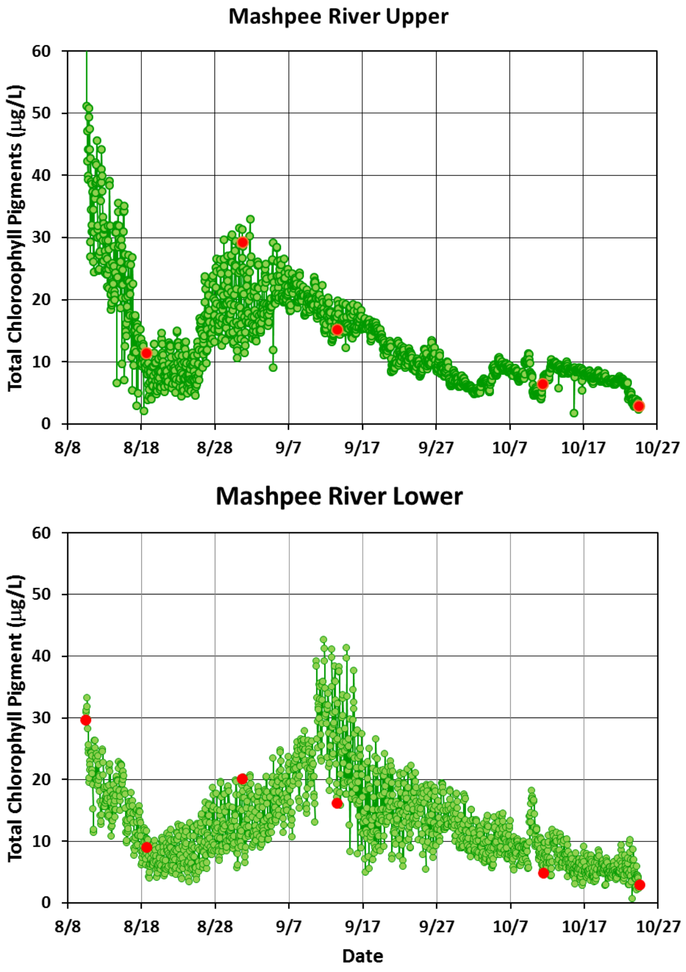

Figure III.3. Time series chlorophyll measurements at the Mashpee River Moorings comparing the up-gradient and down-gradient concentrations of the oyster deployment footprint. Red markers indicate chlorophyll extraction calibration points.

The time series chlorophyll data for the up and down gradient sonde locations was compared for discrete time periods representative of: Daytime, Nighttime, Ebb tide, Flood tide, Daytime ebb, Daytime flood, Nighttime ebb and Nighttime flood. All these comparisons yielded diffuse scatter of values, both above and below the 1:1 line, except for the two conditions of Daylight Ebb Tide and Nighttime Flood Tide shown below (Figures III.4 & III.5).

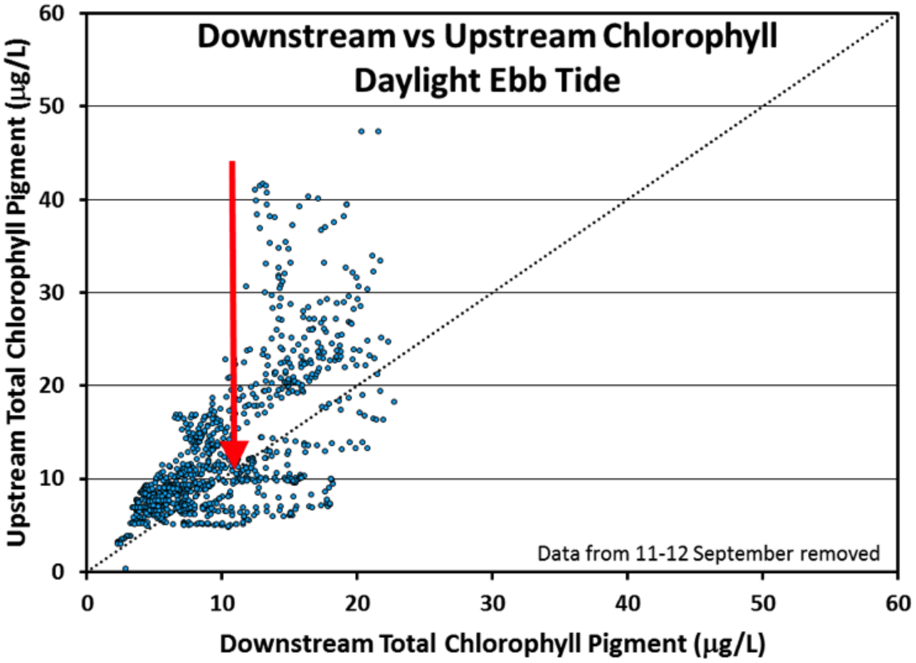

Figure III.4 Mashpee River summer chlorophyll levels during daylight hours (phytoplankton biomass) as water passed through the oyster aquaculture area on the ebbing tides (data in Figure III.3). Oysters remove a significant fraction of the phytoplankton in tidal water passing over the bottom racks, also removing particulate nitrogen, much of which is deposited in sediments increasing water clarity (see below) and improving water quality. Values above 10 ug/L (arrow) are considered an indication of high nitrogen conditions.

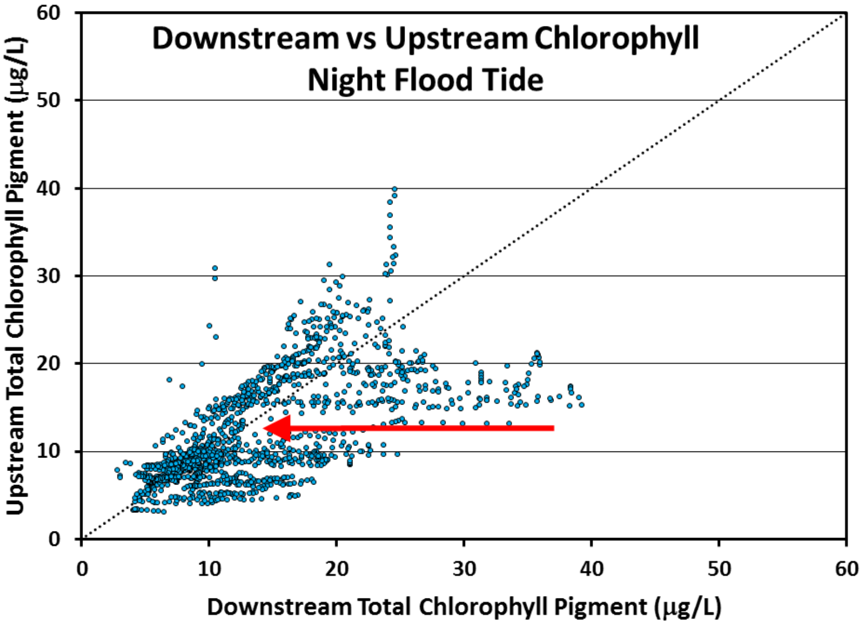

Figure III.5: Mashpee River summer chlorophyll levels during nighttime hours (phytoplankton biomass) as water passed through the oyster aquaculture area on the incoming flooding tides (data in Figure III.3). Oysters remove a significant fraction of the phytoplankton in tidal water passing over the bottom racks, also removing particulate nitrogen, much of which is deposited in sediments increasing water clarity (see below) and improving water quality. Values above 10 ug/L (arrow) are considered an indication of high nitrogen conditions.

We believe the observed pattern reflects the oyster feeding response to the increased presence of phytoplankton within the water column. While oysters pump water across their gills they can detect higher concentrations of particles and preferentially feed when concentrations are highest. The Mashpee River had higher concentrations of phytoplankton generally, however, during the day light hours photosynthesis caused these concentrations to increase; during ebb tides the highest concentrations of chlorophyll pass over the oysters stimulating feeding. Conversely, during the night, photosynthesis does not occur and the mixing of bay water and river water results in a smaller gradient less likely to stimulate active feeding. Flood tide differences were observed only during the nighttime and were confined the to the time period between September 10-17 when down-gradient chlorophyll concentrations were higher than the up-gradient concentrations reflecting a bloom in the bay. Of note is the apparent break from the 1:1 line in Figures III.4 and III.5 at a concentration of ~10ug/L chlorophyll. This suggests that feeding rate can increase rapidly to exploit a concentrated water column phytoplankton population and the threshold concentration for increased feeding rates is ~10ug/L chlorophyll, a threshold that is also considered to be diagnostic of eutrophic water quality conditions.

The time-series measures of transparency or water clarity of the water-column surrounding and within the aquaculture sites were assessed using a separate mooring system found to be effective in the Falmouth Oyster Pilot study. This assay determines the ability of oysters to clear the water and allow increased light penetration, an essential part of eelgrass restoration. Turbidity is a measure of the water clarity and was determined at the up-gradient and down-gradient locations in the Mashpee River (MR1 & 3) (Fig. 1) using HOBO® Temperature/Light Pendant Data Loggers (UA-002-64). These light pendants were permanently positioned just below the water's surface (0.2m) and close to the bottom at a depth of approximately 1m at each station.

The light pendants measured light intensity in units of μE/m^2/s every 10 minutes and profiles were used to calculate the percent surface irradiance and the light extinction coefficient (k) within the water column. The light extinction coefficient, k was calculated using the Beer-Lambert Law which describes the logarithmic decay of light through a medium; the larger the light extinction coefficient, the more rapid the loss of light through the water column and represents a greater degree of turbidity. In contrast, small light extinction coefficients reflect greater light transmission through the water column and less turbidity.
The results of the light meters showed the surface meters were exposed to the air during low tide and a subset of this data was used to calculate irradiance through the water column. When comparing the up-gradient station, MR1, relative to the down-gradient station, MR3, the down-gradient site had a higher surface and bottom light intensity on average (Table 2) indicating higher water clarity (same surface light with higher bottom light). The higher bottom light down-gradient of the oysters at MR3 suggests water clarity is improved as it travels through the oyster aquaculture area due to the filtration of particulates from the tidal waters (Table 2).

Table III.3 Light meter profile results using a sub-sample of the 2016 season mooring deployment for the Mashpee River sites MR1 and MR3.

MR1 (up-gradient)	**Surface Mean (uE/m^2/s)**	**Bottom Mean (uE/m^2/s)**	**% Light Penetration**	**k^{-m}**
average	449.9	269.9	60%	0.83
minimun	13.4	5.8	42%	2.46
maximum	1282.7	728.2	94%	0.00
standard deviation	500.3	283.2	0.2	0.59
n	24	24	24	24
standard error	102.1	57.8	0	0.12
MR3 (down-gradient)	**Surface Mean (uE/m^2/s)**	**Bottom Mean (uE/m^2/s)**	**% Light Penetration**	**k^{-m}**
average	458.9	334.3	73%	1.5
minimun	12.6	4.2	17%	3.58
maximum	1370.4	1335.3	99%	0.01
standard deviation	500.6	484.6	0.3	1.07
n	24	24	24	24
standard error	102.2	98.9	0%	0.22

The mean temperature using the subset of data from the light meters showed surface and bottom temperatures to be consistent with one another and the surface water to be only slightly cooler than the bottom water (Table 3).

Table III.4 Mean temperature profile results using a sub-sample of the 2016 season mooring deployment for the Mashpee River sites MR1 and MR3

Sample ID	Mean Temperarture (°C)
MR1S	24.83
MR1B	25.08
MR3S	24.75
MR3B	25.09

The shallow waters at the Mashpee River aquaculture site did pose a problem in collecting a consistent light profile and future efforts would have to have light meters positioned only 0.25m apart between surface and bottom, in which case may not represent a viable measurement of light attenuation. Point measurements of light intensity using a Li-Cor were used in the 2017 and 2018 sampling events to produce more accurate results.

Because of oysters filtering capacity it is expected that water clarity should improve in areas where there are oysters. To measure an effect on water clarity, light profile measurements using a Li-Cor from 2017 and 2018 were compiled and averaged for each station. Results for the Mashpee River aquaculture area show light penetration was highest surrounding the aquaculture area (MR1, 2, 3) (Fig. III.6). The additional stations added in 2017 and 2018 in the Mashpee River aquaculture area, MR0 and MR3E elucidate the spatial difference in water clarity surrounding the aquaculture area. The water clarity above the oysters at the MR0 station is much lower at 42% light penetration, while the down-stream station MR3E has 50% light penetration showing the aquaculture area indeed had improved water clarity compared to surrounding waters (Fig. III.6).

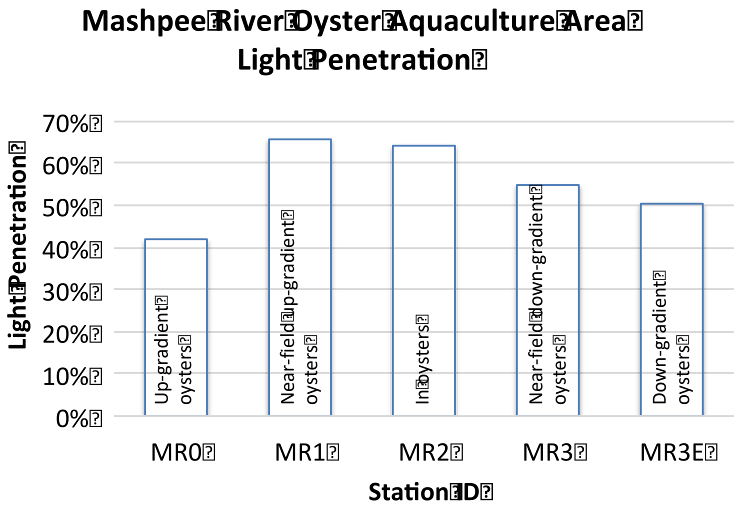

Figure III.6: Compiled 2017 and 2018 Li-Cor light intensity measurements of the surface and bottom water column at each station in the Mashpee River aquaculture area were calculated and averaged to represent the percentage of light penetration. Stations are listed from up-gradient (above oysters) to down-gradient (below oyster area). Higher percentages indicate clearer water, with greatest clarity within oyster area where filtration effects are greatest and by down gradient mixing with unaffected water lowered the clarity. Data indicates that event the relatively small area of bottom positioned oysters significant increased water clarity over a large area.

Shoestring Bay shows consistent water clarity throughout the aquaculture area represented by the small range of light penetration with the three stations SB1, 2, and 3 having a light penetration of 53.9, 53.2, and 53.1% respectively from up-gradient to down-gradient (Fig III.7).

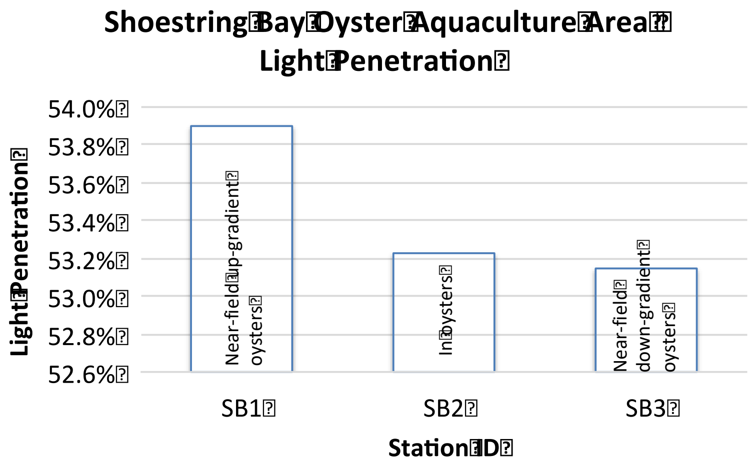

Figure III.7: Compiled 2017 and 2018 Li-Cor light intensity measurements of the surface and bottom water column at each station in the Shoestring Bay aquaculture area were calculated and averaged to represent the percentage of light penetration. Stations are listed from up-gradient to down-gradient.

Findings:

Time-series continuous measurements of total chlorophyll-*a* concentration above and below the Mashpee River aquaculture area showed a clear significant decrease in phytoplankton biomass as water passed through the oyster site on both ebb and flood tides. This filtration of particulates (phytoplankton) also resulted in a small decrease in nitrogen levels in the grab sampling data, which is a less sensitive measure. However, the time-series chlorophyll a data is unequivocal and compelling when especially when coupled with the significant improvement in water clarity at both the Mashpee River and Shoestring Bay sites. Water clarity is fundamental to re-establishing eelgrass, which is a key metric for gauging the restoration of Popponesset Bay under the established TMDL. To improve the measure of nitrogen removal a field flume study could be performed where mixing of the effluent with waters from outside of the oyster impact area is prevented. However, the improved water clarity at both aquaculture sites and large removal of phytoplankton at the Mashpee River site indicate that expanded shellfish deployments, especially if spatially distributed, will result in positive impacts on the water and habitat quality of Mashpee's estuaries.

Section IV. Particle Capture and Biodeposit Production by Oysters

While the results from the water quality monitoring (Section II) and mooring tasks (Section III) show clear positive effects of oyster deployments, they were conducted under conditions of natural flow and were slightly confounded by mixing of post-oyster water with waters that had not passed through the shellfish sites (e.g. some mixing of oyster treated and untreated waters). That water quality improvements were clear under these conditions supports the contention that shellfish can be key players in restoration. Based upon these results and to more clearly gauge the actual particle removals by oysters a more focused study of oyster filtration/biodeposition was conducted as part of a companion SMAST study (M. Labrie, Ph.D. research).

Oysters, as well as other sessile filter feeders (e.g., barnacles, sponges), increase water column clarity by filtering out particulates, which are later, released in biodeposition (Newell et al. 2005). The suspended particulate matter consists of photosynthesizing microscopic organisms (phytoplankton), dead particulate organic matter (detritus), and bacteria, which typically colonize the phytoplankton and detritus (Newell et al. 2002). Oysters selectively digest nitrogen-rich particles and reject the less-nutritious and inorganic particles as pseudofeces (Newell et al. 2004; Newell and Jordan 1983). Nutrients from captured foods may be assimilated into biomass (Higgins et al. 2011). The particulates passing through the digestive system are finally deposited as feces and the rejected material deposited as pseudofeces, which together are termed biodeposition (Figure IV.1).

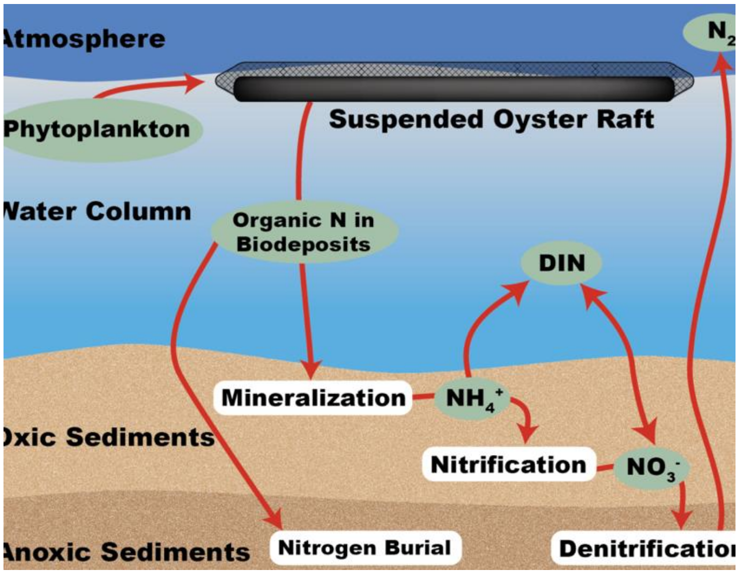

Figure IV.1– Diagram of oyster's role in the marine nitrogen cycle in a shallow water estuary with oxygenated (oxic) and non-oxygenated (anoxic) sediments. White rectangles indicate microbial processes; green ovals indicate nitrogen species (Diagram adapted from Kellogg et al 2013).

Particulate Capture and Biodeposition by Oysters:

In situ biodeposit traps were used to determine individual oyster biodeposition rates. Biodeposit traps were deployed in Shoestring Bay at Bosun's Mashpee Neck Marina at a depth of approximately one meter, at low energy site not affected by boat traffic. Biodeposit traps were deployed in Shoestring Bay on the following dates: 9/21/16, 10/12/16, 10/27/16, 7/27/17, 8/28/17, 9/14/17, 9/25/17, and 10/19/17. Oysters used in the biodeposit traps were allowed to acclimate to the environment for a period of three to five days before deployment. The traps were deployed for 24 hours to span a full day/night cycle and two tidal cycles. We positioned four oysters onto a rectangular PVC platform (30 cm x 20 cm; 4 mm thickness). Eight holes were drilled into the platform with eight traps beneath the holes. Traps allowed independent capture of feces and pseudofeces (Figure IV.2). To account for any ambient particle settling into the traps, we deployed a control apparatus (with oyster shells) alongside the treatment group. Additionally, we fixed small mesh plastic screen over the biodeposit trap to prevent shrimp and small fish from entering the traps.

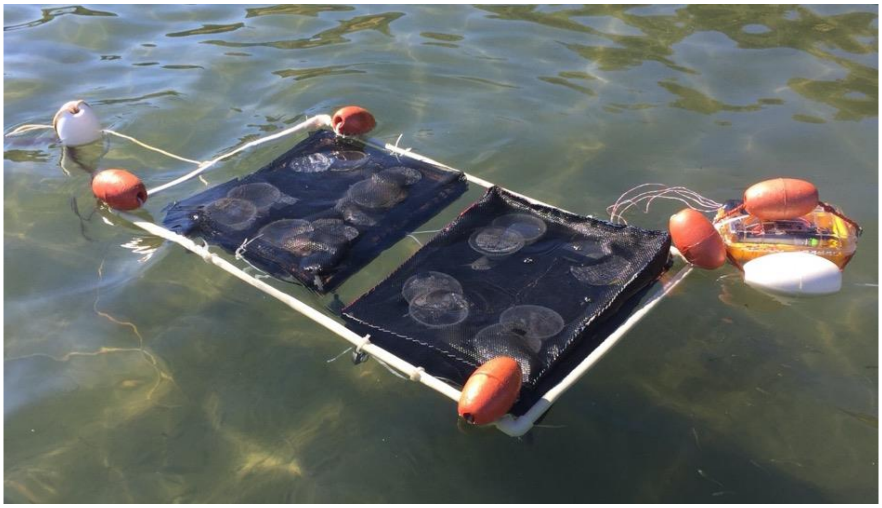

Figure IV.2. Biodeposit trap deployed in Shoestring Bay, Mashpee, MA.

Surface water temperature measurements and water samples were collected upon trap deployment and removal. These water samples were processed for total pigment (TPig; chlorophyll *a* + pheophytin *a*), total suspended solids (TSS) and particulate organic carbon (POC) and nitrogen (PON). 2017 water grab samples were further analyzed for size fractionated total pigment using 5 μm pore size nitrocellulose filters and a 30 μm Nitex mesh filter. TSS filters were analyzed for particulate organic carbon and nitrogen using a Perkin Elmer 2400 elemental analyzer.

Biodeposit traps were weighed before deployment and upon return to the laboratory the biodeposits were allowed to settle and then the top water layer siphoned off. Biodeposit samples were then filtered, weighed, dried to a constant weight, and then processed for particulate organic carbon and nitrogen. Oysters deployed in the biodeposit traps were measured to determine height, whole oyster weight, wet tissue / shell weight, and dry tissue / shell weight.

Biodeposition rates were calculated based on the collected mass of feces and pseudofeces minus the mass of ambient particulates collected in the control traps and the deployment duration. The biodeposition rates were standardized to 1-g (dry tissue weight) individual based on an allometric relationship with an exponent of 0.58 (Cranford et al. 2011). By standardizing the individual biodeposition rates, we were able to expand the biodeposition rate to include the total oyster biomass within the aquaculture study sites. The number of oysters deployed and growth and mortality information relative to the 2016-2018 Shoestring Bay and Mashpee River aquaculture deployments were provided by the Town of Mashpee (Rick York and Ashley Fisher). Live oyster weights were converted to dry tissue weights using the following relationship:

Dry tissue weight (g) = $0.05 \times whole\ oyster\ (g)^{0.89}$; $r^2 = 0.84$ for n = 91

We determined the fitted coefficients using a least-squares regression of previously collected whole oyster and dry tissue weight data.

The biodeposit measurements (mass collected per day and particulate organic carbon and nitrogen) and surface water parameters (total suspended solids and particulate organic carbon and nitrogen) were used to determine the mass of particulate organic nitrogen settling from the oysters.

Additional feeding measurements were calculated based on the "biodeposition method" outlined in Iglesias et al. (1998). The biodeposition method requires separate quantification of feces and pseudofeces and water column food concentrations to calculate feeding measurements, e.g. clearance and filtration rate. The fraction of inorganic matter available as food and deposited as feces and pseudofeces is used to calculate clearance rate and assumes that inorganic matter serves as a quantitative tracer as it is not assimilated by the oyster (Iglesias et al. 1998). Clearance rate (CR) is defined as the volume of water cleared of particles per unit time and was estimated by:

$$CR = \frac{IER}{PIM}$$

where IER is the inorganic egestion rate and PIM is the concentration of particulate inorganic matter (Iglesias et al 1998).

Valve Activity (active filtration):

Valvometry experiments occurred alongside biodeposit trap deployments to determine when the oysters were actively filtering. The valvometers consist of a Hall effect sensor and a small magnet. Valvometer fitted oysters were allowed to rest/acclimate at the study site in a modified suspended bag for 48 to 72 hours before experimentation. The valvometer continuously records whether the oyster is open (filtering) or shut (quiescent).

Water Quality and Oyster Feeding Rates Associated with Biodeposit Trap Deployment:

Total pigment (TPig), total suspended solids (TSS) and particulate organic carbon (POC and nitrogen (PON) are indicators of the amount of food available for oyster feeding. Chemical analyses of these water quality parameters suggest sufficient food concentrations to support oyster biodeposition and consequently, growth. The fraction of total pigment of particle size less than five microns (< 5 μm: total pigment) was assessed for grab samples from 2017 trap deployment events. For 7/27/17, 8/28/17, 9/14/17, 9/25/17, and 10/19/17 the fractions of total phytoplankton <5 um were 0.37, 0.10, 0.12, and 0.14, respectively. Riisgård (1988) reported that the filtration of particles smaller than 5 μm decreased gradually to 50% for a 2 μm size. Results of tank experiments in this study shows plankton as small as 3 μm being filtered by oysters. TSS, total pigment, POC and PON (Table IV.1) were greatest on 7/27/17. However, 7/27/17 water quality samples had the largest fraction of total pigment smaller than 5 μm.

Food availability was greater overall in 2017 compared to 2016; TSS in July, September, and October 2017 is more than double that of September and October 2016. These high TSS values may be partially attributable to rainfall, which typically leads to increased TSS in the form of phytoplankton blooming from increased N, and particulates are washed into the estuary from terrestrial sources and sediments may be resuspended. Rain was recorded on biodeposit trap deployment and/or recovery dates except for 8/28/17. Additionally, the increase in POC in 2017 is not proportional to the increase in TSS from 2016 to 2017, which suggests that there was a large fraction of particulate inorganic matter in the water column including the inorganic silicon valves of diatoms. Low clearance rates for July, September, and October 2017 estimated from the above CR equation are the result of low inorganic matter content in the biodeposits compared to the water column.

Despite low oyster clearance rates estimated from 2017 data, clearance rates and biodeposition rates from the present study are comparable to rates reported by similar laboratory (Riisgård 1988) and field biodeposition studies (Grizzle et al. 2008; Hoellein et al. 2015). Hoellein et al (2015) reported mean clearance rates for oysters in two estuaries in New Hampshire with dissimilar water quality were 84.2 and 175.4 l/g dry tissue weight/day. Oyster feeding rates determined in the present study using the biodeposition traps are likely conservative rates as some biodeposits settling from the oysters are subject to turbulence and may be lost from the trap's collection vials.

Greater carbon content is associated with higher oyster food quality, but oyster feeding rates appear to stabilize once food concentrations reach ca. 300 μg C/L seawater (Tenore and Dunstan 1973). Observed food concentrations were greater than 300 μg C/L seawater in 2017 with a surface water maximum of 2050 μg C/L during the 7/27/17 biodeposit trap deployment and a minimum of 454 μg C/L seawater during the 10/27/16 biodeposit trap deployment. Pseudofeces are produced when food concentrations are too high or when filtered particles have low nutrient content (Newell and Jordan 1983) Given that Shoestring Bay particulates contain sufficient N, then pseudofeces production results from food particles in greater concentration than needed to meet the nutritional needs of the oyster (Hawkins et al. 1998, Grizzle et al. 2008). Pseudofeces production ranged from 41.3 %to 60.2% (Table IV.2) of total biodeposit production for all trap deployments with October 2016 and 2017 having the greatest fraction of pseudofeces production.

Table IV.1. Mean (± SE) values for water quality parameters relevant to oyster feeding at the Shoestring Bay biodeposit trap deployment site.

Deployment Date	Temperature (°C)	Salinity	Total Pigment (ug l^{-1})	TSS (mg l^{-1})	POC (uM)	PON (uM)	C:N
9/21/16	22.8 (0.8)	28.6 (0.9)	8.67 (0.46)	7.51 (0.64)	55.54 (2.53)	7.62 (0.53)	7.34 (0.26)
10/12/16	15.3 (0.3)	25.7 (0.7)	4.45 (0.21)	3.51 (0.47)	39.62 (0.76)	5.20 (0.03)	7.62 (0.10)
10/27/16	9.9 (0.4)	28.7 (0.2)	3.89 (0.16)	5.49 (1.08)	37.79 (2.77)	4.47 (0.17)	8.49 (0.68)
7/27/17	23.5 (NA)*	24.8 (1.5)	18.55 (0.84)	34.03 (1.05)	170.65 (6.89)	23.53 (1.84)	7.32 (0.34)
8/28/17	22.8 (0.5)	26.3 (0.1)	13.43 (3.13)	9.51 (1.78)	101.95 (13.52)	15.44 (1.66)	6.55 (0.17)
9/14/17	23.6 (0.1)	26.2 (0.4)	6.84 (1.47)	15.70 (10.0)	80.78 (24.21)	9.34 (2.01)	8.36 (0.66)
9/25/17	23.3 (0.0)	22.0 (1.1)	8.27 (0.68)	29.48 (1.50)	97.91 (13.11)	12.45 (1.47)	7.82 (0.16)
10/19/17	17.0 (0.5)	27.5 (0.9)	6.85 (3.18)	30.65 (0.76)	73.39 (8.05)	9.06 (1.29)	8.22 (0.28)

* Not enough data available to calculate standard error.

Table IV.2. Oyster feeding measurements from Shoestring Bay biodeposit trap deployments. Oyster valve activity (duration of valve gape) was determined as the % (± SE) of time oysters remained open over the trap deployment (approximately 24 hours). Mean (± SE) clearance, biodeposition, and nitrogen deposition rates are standardized to a 1 g dry tissue weight. Deposition rates are expressed as the mass of dry biodeposits per day.

Deployment Date	Total Time Open (% of Record)	Clearance rate (l g^{-1} day^{-1})	Biodeposition rate (mg g^{-1} day^{-1})	Pseudofeces Deposition (% of Biodeposition)	N deposition rate (mg g^{-1} day^{-1})
9/21/16	82.5 (3.5)	82.5 (10.3)	565.1 (56.1)	48.8%	2.9 (0.5)
10/12/16	85.7 (1.3)	51.9 (10.0)	172.1 (12.3)	48.0%	1.6 (0.3)
10/27/16	90.0 (1.9)	20.5 (3.6)	115.1 (23.2)	59.5%	1.1 (0.3)
7/27/17	92.8 (1.7)	10.6 (1.1)	406.1 (39.9)	48.8%	6.8 (0.7)
8/28/17	90.1 (1.0)	46.1 (7.1)	435.9 (47.5)	41.3%	6.7 (0.9)
9/14/17	83.8 (2.6)	18.5 (3.4)	311.6 (50.4)	51.7%	5.5 (0.8)
9/25/17	90.2 (0.6)	7.0 (NA)*	247.1 (23.6)	40.8%	3.9 (0.4)
10/19/17	91.0 (2.4)	7.5 (0.1)	264.9 (11.7)	60.2%	4.0 (0.3)

* Not enough data available to calculate standard error.

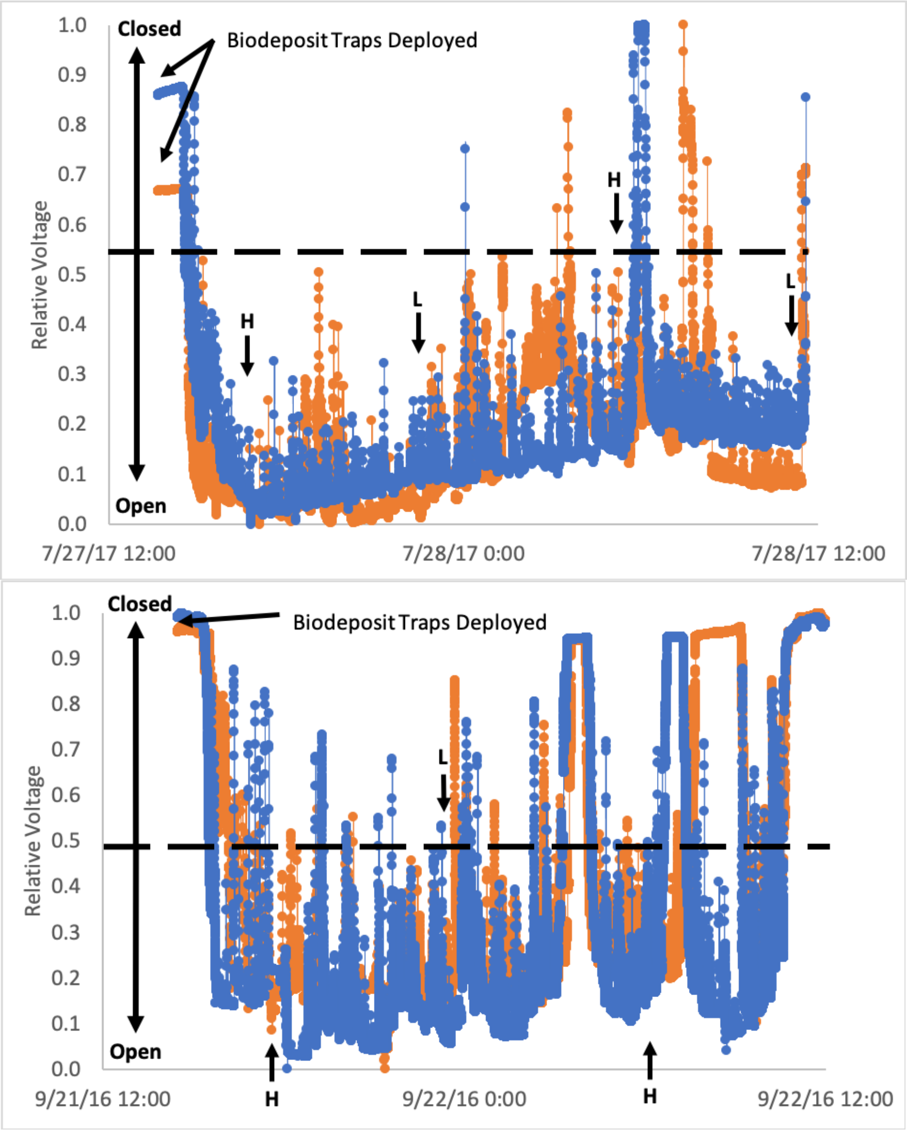

Figure IV.3. Time series plots of valve activity of oysters deployed in biodeposit traps in Shoestring Bay. Top: relative voltage of two oysters deployed in traps from 7/27/17 – 7/28/17; sunrise was at 5:10 and sunset was at 20:21. Bottom: relative voltage of two oyster deployed in traps from 9/21/16 – 9/22/16; sunrise was at 6:30 and sunset was at 18:40. The voltage threshold for counting an oyster as open is 50% percent of the peak voltage (0.5 of the relative voltage). High (H) and low (L) tides are marked with black arrows.

Valve activity of oysters deployed in biodeposit traps in Shoestring Bay was similar to that of oysters fed continuously in an experiment by (Higgins et al. 1980). Higgins et al. (1980) found that *C. virginica* subjects fed continuously on an algal suspension (50,000 cells/ml) were open 94.3% of feeding experiment duration, whereas, oysters fed discontinuously (fed continuously for 12 hours and then not fed for 12 hours) were open 78.9% of time, and unfed oysters were open only 35.1% of time. On average, oysters were open 92.8% and 82.5% of time during trap deployment on 7/27/17 and 9/21/16, respectively. The decrease in valve gape duration may be attributable to the decrease in food availability from July to September. Additionally, without further correlation analysis, oyster valve activity does not appear to be affected by diurnal or tidal cycles. The absence of environmental signals in the data, as well as, the percent of time oysters spent open agrees with findings by Higgins et al. (1980).

Expanding oyster feeding rates to full oyster biomass:

To determine the mass of nitrogen deposited to the sediments from the oyster deployment areas, SMAST scientists developed a biodeposition regression model to predict 2016-2018 biodeposition rates with measured 2016-2018 water quality data. Linear regression analysis was conducted on the compiled dataset with feces deposition and pseudofeces deposition regressed separately as response variables and water quality parameters (TSS, POC, PON, total chlorophyll-*a*, salinity, and temperature) serving as predictor variables. Multiple linear regression was not applicable because multicollinearity exists between the predictor variables (Figure IV.4). For example, POC and total chlorophyll-*a* are positively correlated because they are both present in phytoplankton. Use of a model with multiple related predictor variables can lead to erratic results. Therefore, a single predictor variable was determined for feces and pseudofeces based on simple linear regression model evaluation. Feces deposition was best fitted to POC and pseudofeces deposition is best fitted to particulate inorganic matter (PIM; water quality parameter derived from TSS) (Figure IV.5). A linear relationship between daily pseudofeces production and PIM is consistent with findings by Haven and Morales-Alamos (1972).

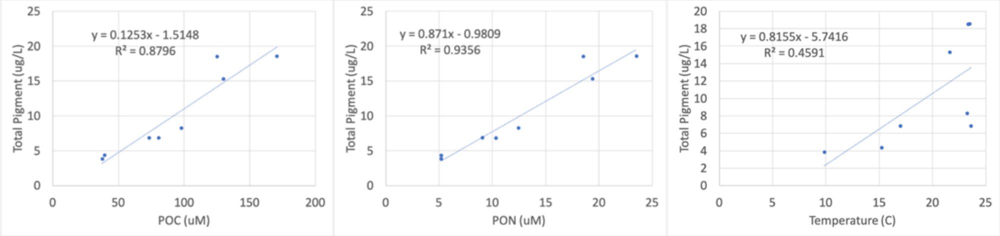

Figure IV.4. Simple linear regression of TPig and POC, PON, and temperature demonstrating collinearity of the predictor variables. Left plot: TPig vs. POC with an R-squared of 0.8796. Middle plot: TPig vs. total pigment with an R-squared of 0.9356. Right plot: TPig vs. temperature with an R-squared of 0.4591

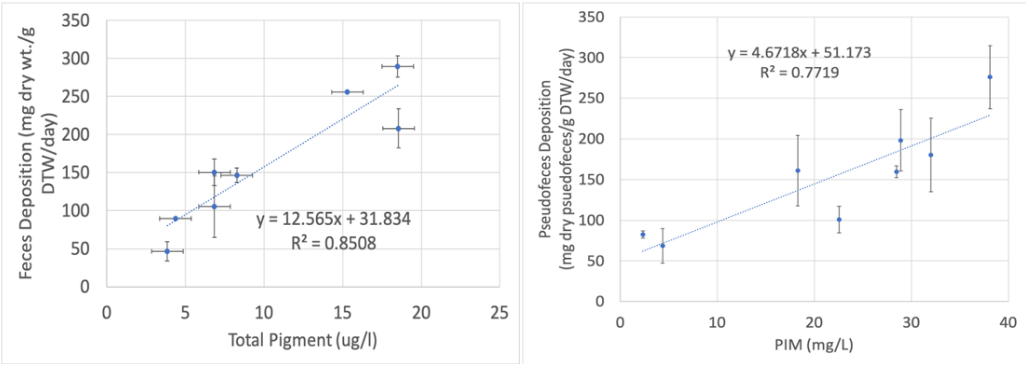

Figure IV.5. Left plot: linear regression of feces deposition as the response variable and total pigment as the predictor variable; Number of observations: 8, Error degrees of freedom: 6, Root Mean Squared Error: 35, R-squared: 0.851, Adjusted R-Squared 0.826, p-value = 0.0011. Right plot: linear regression of pseudofeces as the response variable and PIM as the predictor variable; Number of observations: 8, Error degrees of freedom: 6, Root Mean Squared Error: 35.3, R-squared: 0.772, Adjusted R-Squared 0.734, p-value = 0.00408.

The linear regression models for feces and pseudofeces were used to predict new response values for 2016-2018 average monthly feces and pseudofeces deposition rates standardized to a 1 g DTW oyster given average monthly TPig (feces) and PIM (pseudofeces) values (Table IV.3 and Table IV.4). The standardized individual oyster (1 g DTW) monthly biodeposition rates were multiplied by the average monthly dry tissue biomass determined for the Shoestring Bay and Mashpee River oyster deployment area to expand the biodeposition rate to include the total oyster biomass deployed at both aquaculture sites (Table IV.5).

Table IV.3. Predicted response values for feces and pseudofeces given monthly averages for total pigment (TPig) and PIM measurements in Shoestring Bay. The biodeposition column values were determined by summing the feces and pseudofeces values for each month. Measured Monthly Averages were calculated from water quality sites SB1, SB2, and SB3; only mid water column grab samples were included in averages as the oysters were bottom planted.

Month	Measured Monthly Averages		Predicted Responses		
			Feces	Pseudofeces	Biodeposition
	TPig (ug/L)	PIM (mg/L)	(mg dry biodeposit/g DTW/day)		
2016					
August	19.26	15.8	273.8	155.7	429.6
September	28.13	20.6	385.3	187.1	572.4
October	6.26	11.5	110.5	128.0	238.5
2017					
June	11.81	11.8	180.3	129.5	309.8
July	16.23	16.6	235.7	160.7	396.4
August	32.37	30.4	438.6	250.8	689.4
September	11.76	14.1	179.5	145.0	324.5
October	13.26	10.7	198.4	122.8	321.3
2018					
June	9.60	13.7	152.5	142.1	294.5
July	18.67	12.0	266.5	130.9	397.4
August	52.08	20.8	686.2	188.2	874.5
September	24.17	7.2	335.5	99.7	435.3
October	11.79	18.2	179.9	171.0	351.0
November	5.42	13.7	99.9	142.3	242.2

Table IV.4. Predicted response values for feces and pseudofeces given monthly averages for total pigment (TPig) and PIM measurements in Mashpee River. The biodeposition column values were determined by summing the feces and pseudofeces values for each month. Measured Monthly Averages were calculated from water quality sites MR1, MR2, and MR3; only mid water column grab samples were included in averages as the oysters were bottom planted.

Month	Measured Monthly Averages		Predicted Responses		
			Feces	Pseudofeces	Biodeposition
	POC (uM)	PIM (mg/L)	(mg dry biodeposit/g DTW/day)		
2016					
August	18.0	11.6	257.7	128.5	386.2
September	20.7	10.8	291.7	123.4	415.1
October	5.0	4.7	94.2	83.7	178.0
2017					
June	4.8	11.7	92.0	128.9	220.9
July	13.8	10.4	204.8	120.9	325.7
August	22.9	13.0	319.7	137.7	457.3
September	7.7	11.6	128.2	128.2	256.4
October	4.5	12.2	88.8	132.4	221.2
2018					
June	8.1	9.9	133.7	117.5	251.1
July	11.0	3.8	169.6	77.8	247.3
August	25.4	19.1	351.1	177.0	528.2
September	27.0	5.8	371.2	90.8	461.9
October	7.0	11.1	120.1	125.3	245.4
November	4.7	15.4	91.0	152.8	243.8

Table IV.5. Mass of dry biodeposits deposited to the sediments by aquaculture site and month. Shoestring Bay oyster biomass was estimated assuming the deployment of 850,000 seed oysters in August 2016, a 50% mortality rate during the first growing season, and a 50% mortality rate during winter quiescence. No data (ND) indicates that no water quality data was available.

Plot ID	Mass of Dry Biodeposits (kg) Deposited by Month					
	June	July	Aug	Sept	Oct	Nov
Shoestring Bay						
2016	ND	ND	2721	3237	1291	ND
2017	3613	4197	10481	5328	6032	ND
2018	5257	7856	18523	9537	8458	6012
Mashpee River						
2016	ND	ND	7966	8369	3778	ND
2017	2863	3326	11029	6369	6052	ND
2018	1856	2123	7269	6547	3831	3929

Table IV.6. Mass of nitrogen (dry weight; kg) deposited to the sediments by aquaculture site and month. No data (ND) indicates that no water quality data was available.

Plot ID	Mass of Nitrogen (kg) Deposited by Month					
	June	July	Aug	Sept	Oct	Nov
Shoestring Bay						
2016	ND	ND	35	42	17	ND
2017	47	54	135	69	78	ND
2018	68	101	239	123	109	77
Mashpee River						
2016	ND	ND	103	108	49	ND
2017	37	43	142	82	78	ND
2018	24	27	94	84	49	51

Key Findings (2016 - 2018):

The filtration of particulates from Shoestring Bay and Mashpee River waters and its packaging into feces and pseudofeces appears to support a large amount of biodeposition to bottom sediments. This feature supports potential enhanced nitrogen removal as oyster biodeposition accelerates the transport of organic nitrogen to the sediments and enhances the coupling of benthic-pelagic processes. In Shoestring Bay, the mass of dry biodeposits and biodeposit PON deposited to the sediments increased from 2016 to 2018 coinciding with increasing oyster biomass over the three years. The reduction in oyster biomass as the result of harvesting and mortality from 2016 – 2018 was compensated by increases resulting from oyster growth. Inter-annual differences in biodeposition assessed for Mashpee River primarily results from year to year differences in the amount of seed oysters deployed. In addition, it is notable that deposition of PON continues into the fall months (directly measured in 2016 and 2017 and included in 2018 modeled results). Oyster food availability starts to decrease in September but is still more than sufficient phytoplankton in Shoestring Bay for oysters (as indicated above, ca. 300 ug C/L).

A key finding that can be derived from the biodeposition studies and the volume of water exchanged through tidal forcing relates to the fraction of each tidal volume that is filtered by oysters. A regression model was not created for estimated clearance rates; however, the maximum estimated oyster clearance rate (82.5 l g^{-1} day^{-1}; Shoestring Bay; 9/21/16) was used to estimate the average volume of water cleared of particles over each day's tidal flow (2 tides/day). The estimated volume of water cleared of particles can then be compared to the Shoestring Bay and Mashpee River average tidal prism (the volume of tidal water exchanged in each tidal cycle) from the Massachusetts Estuaries Report for Popponesset Bay (614,700 and 194,700 m^3, respectively). Given the estimated biomass in Shoestring Bay for 9/21/16 (186 kg dry tissue weight); oysters can clear approximately 2% of the water exchanged per day. Furthermore, given the estimated biomass in Mashpee River for 9/21/16 (674 kg dry tissue weight); oysters can clear approximately 29% of the water exchanged per day.

Section V. Nitrogen Cycling and Oyster Culture: Regeneration and Denitrification

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

The organic rich nature and relatively shallow waters of coastal systems like the Popponesset Bay Estuary and others on Cape Cod result in sediments playing a significant role in system biogeochemical cycles. Organic matter deposition to sediments, hence benthic metabolism, tends to decrease with increasing depth of overlying waters due to interception by water column heterotrophic processes resulting in lower deposition of labile (decomposable) organic matter. The result is that embayment respiration rates are typically many-fold higher than in the adjacent offshore waters. With potential stratification of embayment waters, sediment metabolism plays a major role in bottom water oxygen declines (an ecosystem structuring parameter). This applies particularly to Popponesset Bay, which has periodically gone hypoxic (i.e. low D.O) during the summer months (Figure III.2). It should be noted that while water depth is important in altering the deposition of labile organic matter to sediments, filter feeders and especially large filter feeders like oysters can overwhelm the "depth effect" due to the large amount of packaged feces that they emit. In these situations, oysters are projected to increase deposition several fold and alter sediment respiration rates.

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

To determine the contribution of sediment regeneration to nutrient levels within the oyster aquaculture portion of the Mashpee River system and the effect the oysters may have on nitrogen recycling rates and oxygen levels, sediment samples were collected and incubated under *in situ* conditions during October 24, 2016, August 31, 2017 & September 12, 2018. The August 31 and September 12 sampling dates were during the period of maximum oyster activity in the summer interval (July-September) and the October 24 sampling was during the period of maximum oyster biomass (October-December).

Time series measurements of total dissolved nitrogen, nitrate+nitrite, ammonium and ortho-phosphate were made on each incubated core sample. The rate of oxygen uptake was also determined to: (1) evaluate sensitivity to oxygen depletion of the oyster aquaculture area of the Mashpee River, (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. On each date, assays were performed on 8 cores from sites distributed throughout the oyster aquaculture area. Cores were collected directly within the oyster aquaculture racks and at distances north (up-gradient) and south (down-gradient) of the aquaculture area. (Fig. IV.1)

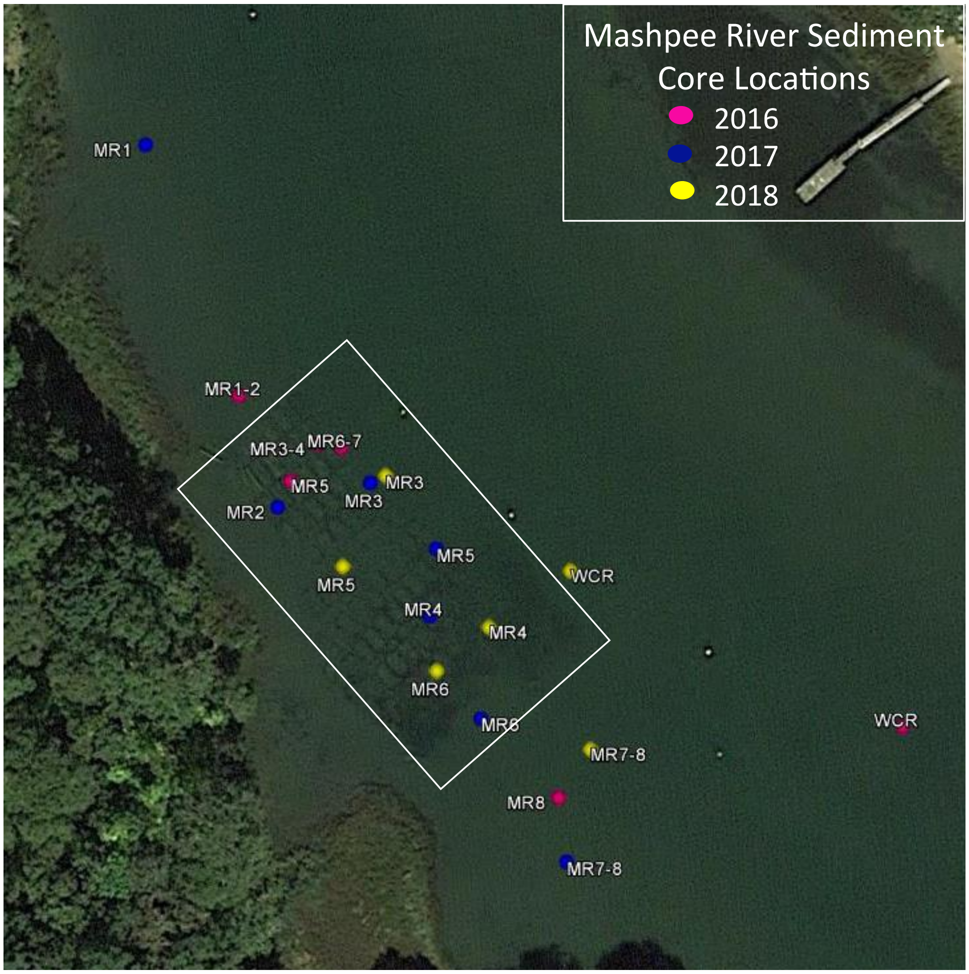

Figure IV.1: Station map showing the sediment coring location for 2016 (pink markers), 2017, (blue markers) and 2018 (yellow markers) as well as the aquaculture border in white. Station MR1 and 2 were the same location for both 2017 and 2018.

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 the Mashpee River. 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 S.E. Massachusetts and 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.

N_2 excess, a measure of denitrification, was measured using membrane-inlet mass spectrometry (MIMS). N_2 produced by denitrification is precisely detected by analysis of its ratio with the inert gas Argon. Water samples were collected and stored to prevent gas exchange or bubble formation until assay. In the laboratory, sample water was pumped at mL/min rates through a gas permeable membrane in order to extract gas into the mass spectrometer inlet. The inlet was fitted with cryogenic traps to remove water vapor and CO_2 gas. Sample gas was analyzed by the mass spectrometer for masses 28 and 40 for determining the N_2 to Argon ratio. Calibration was made by comparison with a reference gas of known composition. A quadrupole mass spectrometer (e.g. Pfeiffer 422) was used for its sensitivity and speed of analysis and the analysis of the samples conforms to the same methods as was utilized during a comprehensive survey undertaken by the CSP in 2008. Benthic cores were collected on October 24, 2016, August 31, 2017 and September 12, 2018. Water column respiration measurements were collected within and bracketing of the oyster area.

Sediment Nutrient Cycling Results:

Benthic results are summarized in Table IV.1 below. Both the August and September cores were collected to coincide with peak growing season and the time during which the most stressed water quality conditions occur. October cores were collected near the end of the growing season when the full effects of oyster culture could be determined. The sediments were characterized as dark brown soft muds with some mixed in sand. To compensate for the temperature difference between fluxes, Table IV.2 shows the sediment flux rates adjusted using a Q10 temperature coefficient of 2. Higher temperatures increase bacterial respiration while lower temperatures decrease respiration.

Cores collected within the oyster aquaculture area show greater variation in rates than the control cores for all dates. Nonetheless specific patterns reveal average sediment oxygen demand (SOD) decreased with temperature along with ammonium release (Table IV.1). When incubation rates are adjusted to 22 ˚C for comparison, the SOD rates within and surrounding the aquaculture area increase through the years, with on average higher SOD rates within the treatment area (Table IV.2). This is also seen with the ammonium release, where the treatment area has higher ammonium release (higher organic nitrogen mineralization) in the 2017 and 2018 than in 2016 (first year oyster deployment). In contrast to SOD rates over the years, the ammonium release in the control cores for all years was relatively unchanged. In 2016, denitrification rates were the highest of all years and similar for both the treatment and control cores, with 1.2 and 1.1 mmol m^{-2} day^{-1} N_2-N production, respectively. Where the fraction of nitrogen used in denitrification was 54% of all nitrogen cycled through the sediments in the control cores and 37% in the treatment cores. In 2017 and 2018, denitrification rates were significantly lower throughout the sediment collection area ranging from an average of 0.1 to 0.5 mmol m^{-2} day^{-1} N_2-N production. The percent of nitrogen used in denitrification of the total nitrogen cycled in the sediments decreased from 54% to 21% for control cores and 36% to <5% for the treatment cores from 2016 to 2018.

Overall, it appears that the rate of sediment metabolism was enhanced by oyster biodeposits within the deployment site in the Mashpee River. Both carbon mineralization (organic matter decay as measured as SOD) and nitrogen remineralization and release as ammonium were consistently higher in the immediate oyster areas than at distance. This was clear on all dates. Mashpee River oyster aquaculture in bottom trays caused an increase in regeneration of NH_4^+ in the immediate oyster area and an increase the total N cycled in the sediments, but there was no clear increase of denitrification compared to the control sediments. There are multiple potential factors causing his finding. First and foremost is the generally low oxygen in bottom waters at the site which inhibits the major process needed to support denitrification, nitrification of ammonium to nitrate. Second, the bottom trays are set on the sediment surface creating very low oxygen conditions in the surface sediments and inhibiting denitrification. Higher rates are common in sediments below suspended aquaculture systems. Finally, the “control” areas appear to have also been influenced by oyster biodeposits due to the tidal flow, the short distance from the edge of the aquaculture trays and the multi-year deployment of the aquaculture system (years). Further, the increasing rate of SOD in the control cores from year to year suggests that the control cores were possibly within the impact area of biodeposits transported by current to the surrounding sediments, decreasing the difference between control and treated sites.

In other oyster aquaculture study locations, such as Lonnies Pond in Orleans, MA sediment cores were collected underneath the suspended floating oyster aquaculture with oxic bottom waters. These cores impacted by oyster biodeposition revealed SOD rates as high as 200 mmol m^{-2} day^{-1} on average, with a low of 100 mmol m^{-2} day^{-1} on average after three years of oyster aquaculture. Cores collected between bottom cages in Mashpee River do not appear to have received the same amount of biodeposition as seen in Lonnies Pond, possibly due to biodeposits being transported from the trays in tidal waters

Table IV.1 Summary of benthic flux rates from core incubations conducted in 2016, 2017, and 2018. Sites within the aquaculture area are shaded.

10/24/2016 - Incubation Temperature 14 ˚C

	SOD	NH4	NOx	DIN	TDN	N2-N	Total N cycled	Denitrified
Site ID	(mMoles/m^2/d)	(mMoles/m^2/d)	(mMoles/m^2/d)	(mMoles/m^2/d)	(mMoles/m^2/d)	(mMoles/m^2/d)	(mMoles/m^2/d)	% Total Cycled N
MR1	30.54	0.8	-0.4	0.4	0.3	0.9	1.2	75%
MR2	24.67	1.2	-0.5	0.6	1.2	0.5	1.6	29%
MR3	25.76	2.0	-0.5	1.5	1.5	0.6	2.1	28%
MR4	53.02	2.4	-0.5	1.8	1.7	0.7	2.4	29%
MR5	31.37	1.8	-0.6	1.2	1.8	1.0	2.7	35%
MR6	17.40	0.9	-0.1	0.8	0.3	0.6	0.9	65%
MR7	34.41	-0.3	-1.3	-1.6	-1.9	0.8	2.7	28%
MR8	25.22	-0.1	0.3	0.2	-0.4	0.5	0.9	58%

8/31/2017 - Incubation Temperature 22 ˚C

	SOD	NH4	NOx	DIN	TDN	N2-N	Total N cycled	Denitrified
Site ID	(mMoles/m^2/d)	(mMoles/m^2/d)	(mMoles/m^2/d)	(mMoles/m^2/d)	(mMoles/m^2/d)	(mMoles/m^2/d)	(mMoles/m^2/d)	% Total Cycled N
MR1	62.07	1.3	-0.4	1.0	1.1	0.4	1.4	27%
MR2	79.51	7.6	-0.3	7.3	6.7	0.3	7.0	5%
MR3	61.85	4.3	-0.3	4.0	4.5	0.3	4.8	6%
MR4	63.90	12.8	-0.3	12.5	11.5	-0.1	11.6	-1%
MR5	87.48	11.9	-0.4	11.4	10.9	0.3	11.2	2%
MR6	35.93	0.8	-0.5	0.3	-0.4	-0.1	0.4	-14%
MR7	57.90	0.9	-0.5	0.4	-0.2	0.3	0.5	61%
MR8	63.76	0.2	-0.7	-0.5	-1.5	0.4	1.9	20%

9/12/2018 - Incubation Temperature 20.5 ˚C

	SOD	NH4	NOx	DIN	TDN	N2-N	Total N cycled	Denitrified
Site ID	(mMoles/m^2/d)	(mMoles/m^2/d)	(mMoles/m^2/d)	(mMoles/m^2/d)	(mMoles/m^2/d)	(mMoles/m^2/d)	(mMoles/m^2/d)	% Total Cycled N
MR1	72.64	1.3	-0.1	1.2	-1.2	0.1	1.3	5%
MR2	127.64	8.4	-0.1	8.3	8.7	0.8	9.5	8%
MR3	54.87	0.4	-0.1	0.3	-1.3	0.3	1.6	17%
MR4	77.12	6.4	0.0	6.4	4.7	0.2	4.9	4%
MR5	70.34	1.1	0.0	1.1	-0.7	0.0	0.7	-3%
MR6	64.94	1.8	0.0	1.7	1.8	0.0	1.8	0%
MR7	71.82	0.6	-0.1	0.5	-2.2	0.3	2.5	12%
MR8	67.39	0.6	-0.2	0.5	-1.1	1.0	2.1	46%

Table IV.2 Summary of benthic flux rates from core incubations conducted in 2016, 2017, and 2018 averaged by control versus treatment location. The bottom table shows sediment flux rates adjusted using a Q10 factor of 2 to allow direct comparison to the August 2017 cores, which were incubated at a temperature of 22 ˚C.

Mean Treatment and Control Area Sediment Flux Rates						
Date	2016	2017	2018	2016	2017	2018
	Oct 27	Aug 31	Sept 12	Oct 27	Aug 31	Sept 12
Area	Control			Treatment		
Temperature (˚C)	14	22	20.5	14	22	20.5
Rate (mMol/m^2/d)						
SOD	26.8	61.2	70.6	32.4	65.7	79.0
NH4	0.6	0.8	0.9	1.3	7.5	3.6
NO3	-0.2	-0.5	-0.1	-0.6	-0.4	0.0
DIN	0.4	0.3	0.7	0.7	7.1	3.6
TDN	0.4	-0.2	-1.5	0.7	6.7	2.6
N$_2$-N	0.6	0.3	0.4	0.7	0.1	0.2
Total N Cycled	1.3	1.3	1.9	2.2	7.0	3.7
Denitrified % Total N	54%	36%	21%	37%	0%	5%

Mean Treatment and Control Area Sediment Flux Rates - Q10 Reference Temperature 22 ˚C						
Date	2016	2017	2018	2016	2017	2018
	Oct 27	Aug 31	Sept 12	Oct 27	Aug 31	Sept 12
Area	Control			Treatment		
Temperature (˚C)	14	22	20.5	14	22	20.5
Rate (mMol/m^2/d)						
SOD	46.7	61.2	78.4	56.4	65.7	87.6
NH4	1.1	0.8	0.9	2.3	7.5	4.0
NO3	-0.3	-0.5	-0.1	-1.0	-0.4	0.0
DIN	0.7	0.3	0.8	1.3	7.1	4.0
TDN	0.6	-0.2	-1.7	1.2	6.7	2.9
N$_2$-N	1.1	0.3	0.5	1.2	0.1	0.3
Total N Cycled	2.2	1.3	2.2	3.8	7.0	4.1
Denitrified % Total N	54%	36%	21%	37%	0%	5%

Key Findings from Sediment Nutrient Cycling Results 2016-2018:

The major sediment nutrient cycling results of the 3-year study of the effect of oyster aquaculture on water quality in the Mashpee River are summarized below.

1. The nitrogen and chlorophyll a removal observed as water passes through the aquaculture deployment site was distributed to the sediments enhancing both oxygen uptake (carbon mineralization, SOD) and nitrogen cycling.
2. Much of the nitrogen deposited to sediments was not regenerated to the water column or denitrified based upon the determined rates of biodeposition. It appears that much of the summer biodeposition is either transported from the site in tidal action or is “stored” in the sediments for release during the less sensitive parts of the year.
3. Low oxygen beneath the oyster trays and at the site generally appears to be inhibiting nitrification and therefore coupled nitrification-denitrification. However, denitrification was active at the site as seen in the consistent uptake of nitrate from the overlying waters (direct denitrification). Improving oxygen conditions as nitrogen mitigation actions are undertaken should improve oxygen levels and greatly increase denitrification rates in both oyster areas and background sediments.
4. Given the apparent migration of biodeposits in tidal flows, future sediment denitrification surveys should be conducted over a wider control area.
5. It appears that the oyster aquaculture site is improving water quality locally and having significant delivery of particulate nitrogen to the associated sediments lowering TN delivery to the downgradient estuarine waters during the nitrogen sensitive summer months.

Section VI. Oyster Growth, Nitrogen Assimilation, and Harvest

Oysters assimilate nutrients (e.g. N) into their soft tissue and shell as they grow (Kellogg et al. 2013). Oyster harvest represents a potential pathway for permanent N removal from estuaries (Grizzle et al. 2016). Previous findings suggest that N assimilation capacities differ between wild and aquaculture oysters, as well as, between cultured oysters raised in locations with unique water quality conditions (Newell 2004, Higgins et al. 2011, Kellogg et al. 2013). Significant differences in N assimilation capacities could affect water quality management plans that implement oyster restoration to reduce N levels in estuaries.

Oyster sub-samples from Mashpee River (Figure VI.1) and Shoestring Bay were collected and transferred to SMAST, UMass Dartmouth on ice in June and October 2016. Shell height and whole wet weight measurements were determined upon arrival to SMAST. Live oysters were scrubbed to remove epibiotic growth and detritus with a wire brush and razor blade. Scrubbed materials were rinsed with deionized water into a single large tared weigh pan. Oysters were then placed in filtered seawater for approximately 24 hours to allow biodeposits to be released. Biodeposits were collected using a transfer pipette and transferred to pre-weighed centrifuge tubes. Oysters were opened at the hinge with an oyster knife and soft tissue and shell were separated and weighed. Tissue, shell, biodeposits and scrubbed materials were dried in a 60°C oven, then re-weighed to determine dry weights. Dried samples were ground separately using a mortar and pestle. Organic carbon and nitrogen content of the processed samples were determined using a Perkin Elmer 2400 elemental analyzer. The mass of nitrogen (per oyster estimate) contained in the biodeposits and scrubbed materials was incorporated into the calculation of the total mass of nitrogen contained in a whole dried oyster (Figure VI.5 and VI.6).

Figure VI.1: Satellite image (Google Earth 2016) of the oyster deployment sites within the Mashpee River.

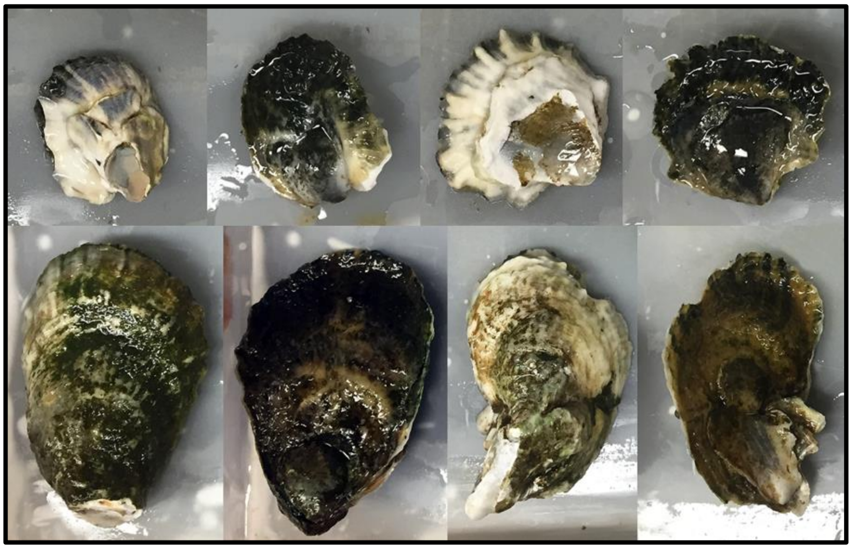

Figure VI.2: Images of oysters sub-sampled for nutrient content analysis showing (top) 2016 seed oysters harvested from the western side of the Mashpee River 10/17/16 and (bottom) adult oysters harvested from the western side of Mashpee River 10/17/16.

Results:

The oysters sampled from the Mashpee River and Shoestring Bay represented varying age classes and show a strong relationship between age and wet weight (r^2=0.84; Figure VI.3). Data also suggests that you can predict an oyster's wet weight based on shell height (r^2=0.85; Figure VI.4). This information may prove useful when estimating the mass of oysters removed during harvest since a harvestable oyster needs to be a minimum of 3 inches (76.2mm) if wild and 2.5 inches (63.5 mm) if aquaculture reared on commercial licensed farms in Massachusetts (https://www.mass.gov/service-details/commercial-shellfish-sea-urchin-regulations). The mass of nitrogen contained in an oyster was measured and averaged from the oysters collected in the Mashpee River and Shoestring Bay and shows a strong relationship with whole wet weight (r^2=0.95; Figure VI.5) and shell height (r^2=0.88; Figure VI.6).

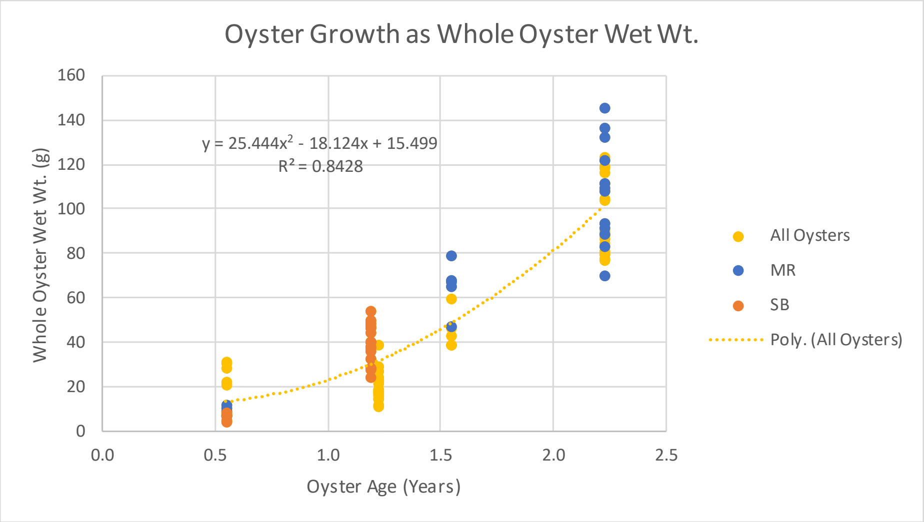

Figure VI.3: Relationship between oyster age in years and the wet weight of a whole oyster using oysters from varying age classes from the Mashpee River (MRW, MRE) and Shoestring Bay (SB).

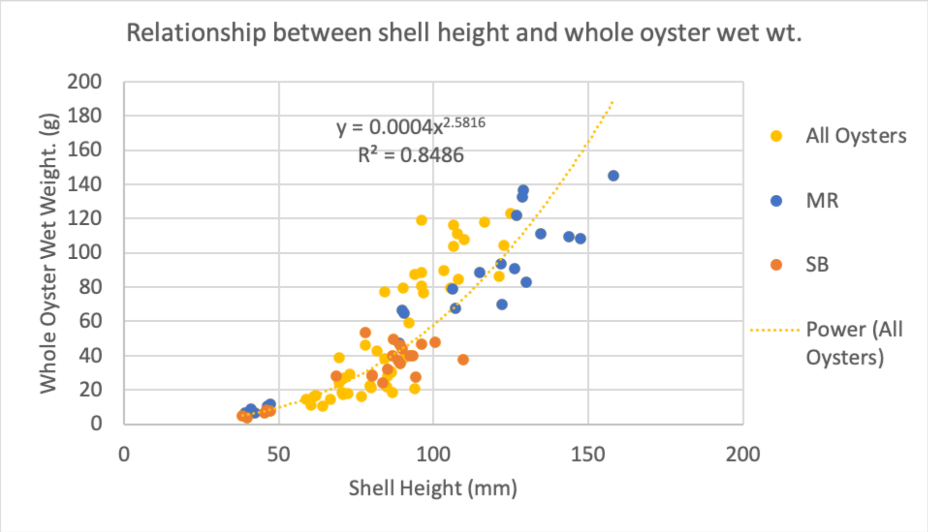

Figure VI.4: Relationship between oyster shell height and the wet weight of a whole oyster using oysters from varying age classes from the Mashpee River (MR) and Shoestring Bay (SB).

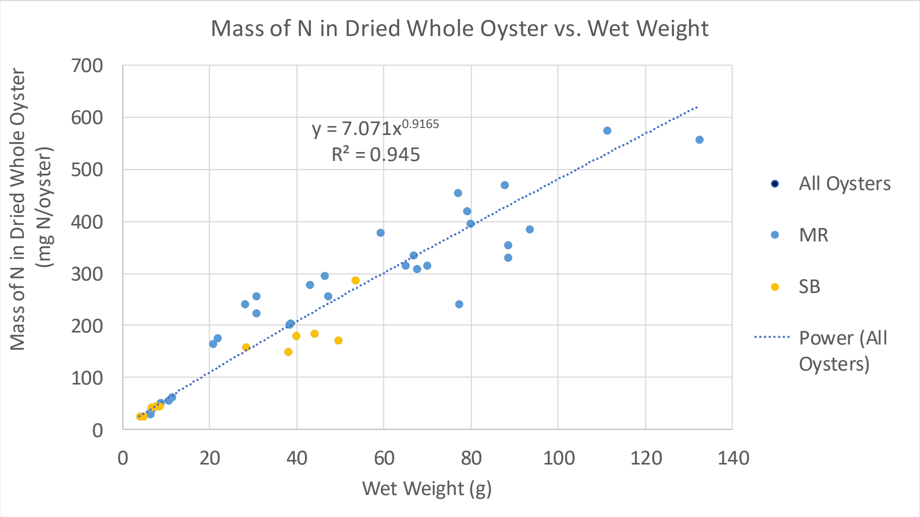

Figure VI.5: Relationship between oyster wet weight and the mass of nitrogen of a whole dried oyster using oysters from varying age classes from the Mashpee River (MRW, MRE) and Shoestring Bay (SB).

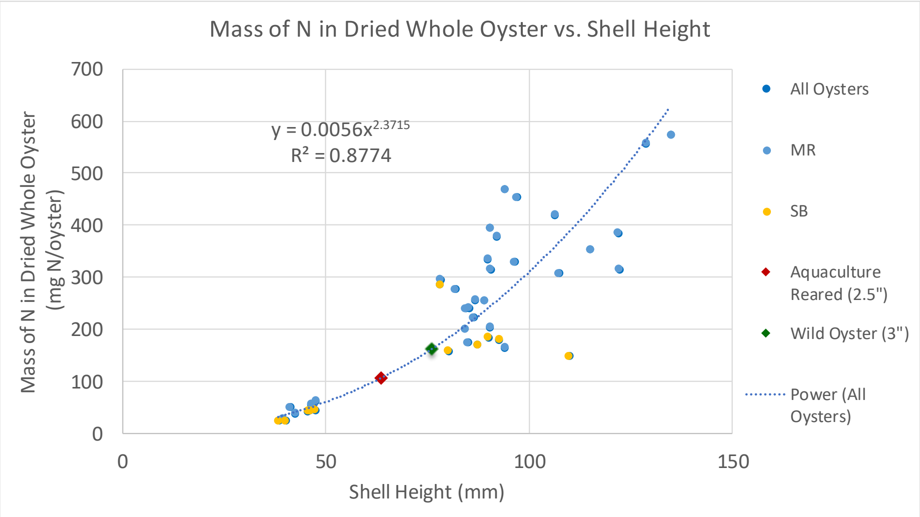

Figure VI.6: Relationship between oyster shell height and the mass of nitrogen using oysters from varying age classes from the Mashpee River (MR) and Shoestring Bay (SB). Harvestable oyster shell height for aquaculture reared (red diamond) and wild (green diamond) oysters are also shown.

Table VI.1 Oysters harvested June 2016. Percent nitrogen and carbon per gram dry tissue and shell of oysters collected from Shoestring Bay (SB) and Mashpee River (MR).

Jun-16	Site	n	Nitrogen		Carbon		C/N
			%	Range	%	Range	
Tissue							
Regular to Jumbo	SB	5	8.71 ± 0.14	8.04 - 9.03	38.75 ± 0.13	37.16 - 39.95	5.18
	MR	5	9.61 ± 0.25	9.33 - 10.50	40.69 ± 0.26	39.03 - 44.24	4.94
	MR	5	9.21 ± 0.15	8.67 - 9.95	38.10 ± 0.18	34.31 - 41.88	4.82
Shell							
Regular to Jumbo	SB	5	0.20 ± 0.03	0.13 - 0.29	11.99 ± 0.13	11.94 - 12.07	
	MR	5	0.29 ± 0.04	0.20 - 0.41	12.30 ± 0.26	12.16 - 12.50	
	MR	5	0.44 ± 0.09	0.24 - 0.60	12.34 ± 0.16	11.89 - 12.62	

Oct-16	Site	n	Nitrogen		Carbon		C/N
			%	Range	%	Range	
Tissue							
Sub-Market	SB	5	8.85 ± 0.25	8.15 - 9.82	41.25 ± 0.27	40.80 - 41.60	5.44
	MR	5	8.88 ± 0.22	8.48 - 9.42	42.06 ± 0.22	39.33 - 43.07	5.52
Regular	MR	5	7.17 ± 0.12	6.05 - 6.96	42.43 ± 0.11	41.16 - 45.91	6.90
	MR	5	7.51 ± 0.19	7.03 - 7.77	42.80 ± 0.19	39.29 - 45.63	6.64
Shell							
Sub-Market	SB	5	0.20 ± 0.03	0.15 - 0.30	11.98 ± 0.26	11.80 - 12.13	
	MR	5	0.20 ± 0.02	0.18 - 0.26	12.06 ± 0.23	11.97 - 12.23	
Regular	MR	5	0.30 ± 0.02	0.26 - 0.33	12.22 ± 0.11	12.09 - 12.31	
	MR	5	0.36 ± 0.04	0.27 - 0.46	12.31 ± 0.18	12.22 - 12.57	

Oysters from the Mashpee River are harvested from fall to early spring and are accounted for by the town Department of Natural Resources. The results of previous studies of oysters from the Mashpee River at the times of year that they are harvested were that the average 100 g oyster (>3”) contained 500 mg N (Mashpee CWNMP, Mashpee Sewer Commission 2015). The nitrogen content of the oysters in this study is comparable to the previous study results, this study found that a 100 g oyster (>3”) contained approximately 481 mg N. The harvested totals for 2017 were 416,600 oysters and a significantly reduced amount of 151,437 in 2018. Using the Mashpee CWNMP estimate, 208 kg N was removed in 2017, and 75.7 kg N in 2018. In the present study, using the relationship between oyster whole wet weight and mass of nitrogen in a whole dried oyster (Figure VI.5) we can estimate the nitrogen removed by each year’s harvest. We do not know the exact shell height of each harvested oyster, but if we assume that the average individual oyster weighs 100 g (> 3 inches or 76.2 mm) upon harvest, then each oyster will remove approximately 481.37 mg N. Using this estimate, 201 kg N were removed through harvest in 2017 and 73 kg N removed in 2018 (Table VI.2).

The present watershed load into the Mashpee River is 27.67 kg/day and the target load to meet the TMDL embayment threshold is 13.95 kg/day (Table VI.3; Howes et al. 2004). This represents a 49.5% reduction in nitrogen load entering the Mashpee River and over 5,000 kg N/year. At this rate, 29,000 oysters would need to be harvested daily to remove 13.95 kg N/day. Using the CWNMP N content 27,900 oysters would contain the 13.95 kg N.

The role of aquaculture is only to supplement an overarching plan of reducing nitrogen. If aquaculture were increased and able to sustain a harvest of about 1.5 million oysters per year, this would effectively reduce the mass of nitrogen by~~)~~ 15%.

Table VI.2 Mashpee River oyster harvest data in 2017 and 2018 from the town of Mashpee and the estimated nitrogen removed. Projections of number of oysters required to remove the full nitrogen reduction required to meet the TMDL, 10% and 5% estimates are also shown.

	Number of Oysters	Nitrogen in Harvestable Oyster (mg N/ 100 g wet weight oyster)	Nitrogen Removed (mg N)	Nitrogen Removed (kg N)
2017 Harvest	416,600	481.37	200,538,742	201
2018 Harvest	151,437	481.37	72,897,229	73
Full N Reduction	10,403,640	481.37	5,008,000,187	5,008
10% N Reduction	1,046,364	481.37	503,688,239	504
5% N Reduction	520,182	481.37	250,400,009	250

Table VI.3 Excerpt from the Massachusetts Estuaries Project Reports for Waquoit Bay (Howes et al. 2011) and Popponesset Bay (Howes et al. 2004) showing the sub-embayments discussed in this report and their present watershed nitrogen loads, the threshold load to reach the total maximum daily load to restore the system, and the mass of nitrogen required to reach these loads.

Estuary	Sub-embayment	Present Watershed (kg N/day)	Threshold Watershed (kg N/day)	Reduction Required (kg N/day)	Reduction Required (kg N/year)
Waquoit Bay	Hamblin Pond	4.381	0.953	3.428	1,251
	Little River	1.096	0.211	0.885	323
	Jehu Pond	3.912	1.025	2.887	1,054
	Great River	3.671	0.997	2.674	976
Popponesset Bay	Mashpee River	27.67	13.95	13.72	5,008
	Shoestring Bay	30.77	19.71	11.06	4,037

Section VII: Changes in water quality with the addition of seeded aquaculture in Waquoit Bay

Background:

The Mashpee Water Quality Monitoring Program is an on-going collaborative effort between the Mashpee Wampanoag Tribe, the Town of Mashpee and the Coastal Systems Program (CSP) within the University of Massachusetts – Dartmouth, School of Marine Science and Technology (SMAST). The project has a two-fold goal: 1) sustain a continuing assessment of the nutrient related water quality of the Waquoit Bay and Popponesset Bay Estuaries relative to regulatory standards (TMDL's) and 2) monitor improvements in water quality resulting from restoration efforts (e.g. shellfish propagation, dredging, N removals by freshwater systems, wastewater treatment, etc.) as undertaken by the Town, Tribe and others. The program goals are achieved through the collection and analysis of water samples and associated field parameters relevant to assessing the health of estuarine habitats within the Waquoit Bay and Popponesset Bay Systems, Cape Cod, MA. These data form the basis for gauging short and long-term trends in water quality, validating the Massachusetts Estuaries Project threshold modeling approach for Waquoit Bay and Popponesset Bay, and determining compliance with USEPA and MassDEP nitrogen targets set under the Clean Water Act by TMDL analysis that has been previously formalized for all of Mashpee's estuarine waters.

Protection and restoration of coastal embayments from nitrogen overloading has resulted in a focus on determining the assimilative capacity of these aquatic systems for nitrogen. While this effort is ongoing (e.g. USEPA TMDL studies), southeastern Massachusetts has been the site of intensive efforts in this area (Eichner et al., 1998, Costa et al., 1992, Howes and Taylor, 1990, Falmouth Coastal Overlay Bylaw). These efforts resulted in the 2002 implementation of the Massachusetts Estuaries Project (MEP). The goal of the MEP has been to determine the nitrogen thresholds for each of the estuaries in southeastern Massachusetts to support TMDL development by the USEPA and MassDEP and to set estuary specific targets for nitrogen management plans aimed at restoring/protecting these systems. MEP assessments and threshold development have been completed for both Popponesset Bay and Waquoit Bay, including the eastern sub-embayments of Waquoit Bay (Howes et al. 2004, 2011).

MEP analyses indicated that almost all the estuarine reaches within the Popponesset Bay and Waquoit Bay Systems are near or beyond their ability to assimilate additional nutrients without impacting their ecological health. Nitrogen levels are elevated throughout both systems and as watershed development continues, estuarine conditions are projected to decline further until nitrogen management is implemented.

The result is that nitrogen management of these estuaries is aimed at restoration, not protection or maintenance of existing conditions. Nitrogen management within Popponesset Bay has already begun with the consistent annual maintenance of the flow through the tidal inlet, propagation of oysters within the system and capping of the Town of Mashpee landfill. In addition, watershed nitrogen management planning has been completed (CWNMP, Mashpee Sewer Commission 2015) with the goal of reducing the major sources of nitrogen (primarily septic system discharges), conducting "in estuary" N removal by shellfish, and possibly enhancing nitrogen removed during transport from sources to the estuary by enhancing natural attenuation through pond and stream restoration.

In this study, the water quality results in Waquoit Bay, specifically Little River and Great River, are being utilized to assess nitrogen removal by shellfish (Figure VII.1). Because of the continued effort by the Mashpee Water Quality Monitoring Program, we can track changes in water column nutrients.

Figure VII.1: Station map of the quahog seeding locations from 2014-2018 and the site-specific, long-term water quality sampling stations within the Waquoit Bay System (2001-2018).

Aquaculture in Waquoit Bay:

Implementation of quahog seed aquaculture and planting started in the Great River, Little River, Hamblin Pond and Jehu Pond area (SC-16) with planting of approximately 10 million quahog seed from 2014 to 2017 and continues with annual planting (Figure VII.1). Small seed (~ 2 mm) from the ARC hatchery is grown in upweller tanks at the Little River Town dock. After growing larger, the seed is transferred to trays in the river, and then planted in fall at larger sizes. Larger seed has higher survival. Reductions of total nitrogen (TN) were recorded in the Water Quality Monitoring data from 2017 and 2018 in areas that were heavily seeded relative to previous years while TN other areas increased (Figure VII.3).

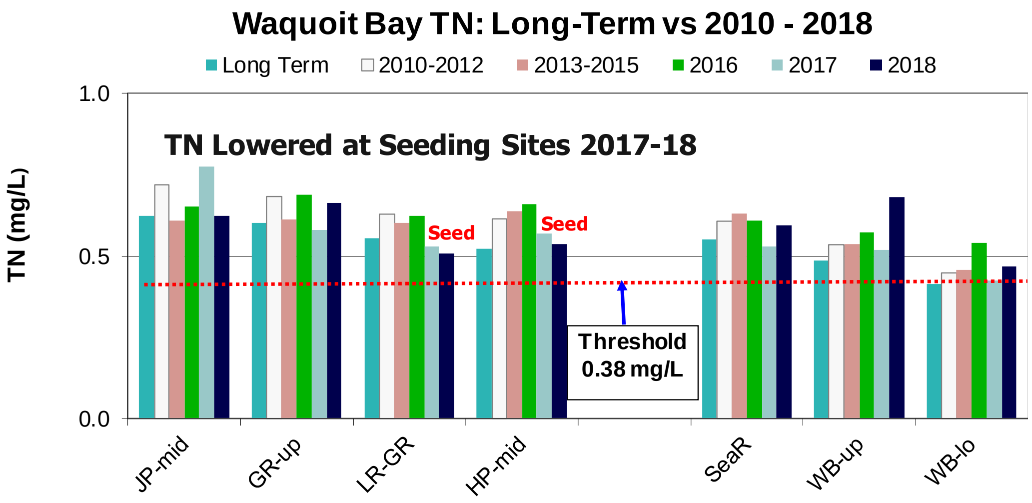

Figure VII.3: Distribution of total nitrogen within Waquoit Bay. The total nitrogen was averaged for each station for long-term and summers of 2010 through 2018. JP - Jehu Pond (WB01), GR - Great River (WB02), LR-GR - Little River-Great River confluence (WB03), HP - Hamblin Pond (WB04), SeaR - Seapit River, WB - Waquoit Bay; up - uppermost reach, mid - middle reach, lo - lower basin near mouth or inlet. The red line shows the TMDL (total maximum daily load), calculated for Waquoit Bay.

Key Findings:

To assess the effect of the addition of aquaculture into the Little and Great River systems of Waquoit Bay the water quality monitoring stations up-gradient and down-gradient of aquaculture were investigated. In the Little River the stations used were WB04 located in Hamblin Pond and WB03 at the mouth of Great River/Little River (Figure VII.1). The percent difference between up-gradient and down-gradient sites was calculated and ranged from 5.2 in 2016, increasing to 6.8 in 2017, to 5.3 in 2018, but an overall reduction in water column nitrogen has been occurring over those years (Figure VII.2). The water column nitrogen concentration determined to restore habitat quality is 0.38 mg/L determined by the MEP (Howes et al. 2011).

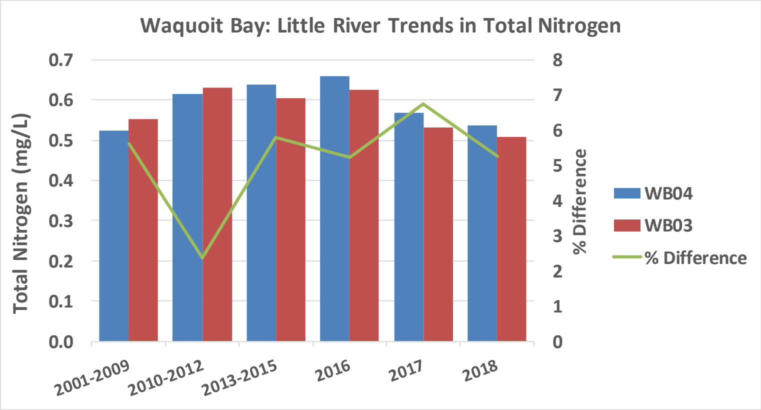

Figure VII.2: Water quality station averages of total nitrogen for long term (2001-2009) to 2018 of Hamblin Pond (WB04) to the mouth of Little River (WB03). Each year comprises of four early morning, ebb tide, summer sampling events. Spatial changes of total nitrogen can be seen from up-gradient (WB04) to down-gradient (WB03) and the calculated percent difference is represented by the green line.

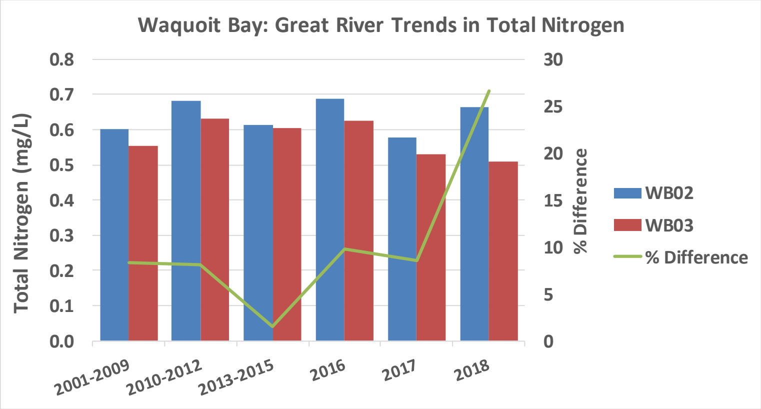

Figure VII.3: Water quality station averages of total nitrogen for long term (2001-2009) to 2018 of upper Great River (WB02) and to the mouth of Great River (WB03. Each year comprises of four early morning, ebb tide, summer sampling events. Spatial changes of total nitrogen can be seen from up-gradient (WB02) to down-gradient (WB03) and the calculated percent difference is represented by the green line.

The average total water column nitrogen concentration in the upper Great River (WB02) is higher compared to the Little River (ebb flow from Hamblin Pond WB04), but also shows a larger percent difference (Figure VII.3). The percent difference in nitrogen concentration went from 8.6 to 26% from 2017 to 2018. This uptake of nitrogen may be attributed to the quahogs seeded in between these two monitoring stations. Average total chlorophyll-*a* pigments were also examined, but there has been a significant increase in chlorophyll from 2017 to 2018 along with higher chlorophyll concentrations at the mouth of Great/Little Rivers (WB03) causing a negative percent difference between up and down gradient concentration for Little River (Figure VII.4). The higher chlorophyll concentrations at WB03 relative to WB04 appears to have been the result of sampling more of the Great River water (WB02) at WB03 where the rivers converge and mix. Chlorophyll concentration also was elevated in 2018 in the Great River but showed a 37% difference between the upper station (WB02) and mouth of Great River (WB03) (Figure VII.5).

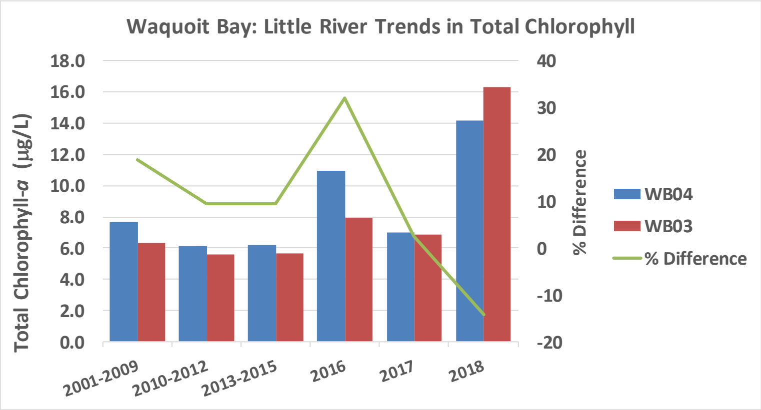

Figure VII.4: Water quality station averages of total chlorophyll-*a* pigments for long term (2001-2009) to 2018 of Hamblin Pond (WB04) to the mouth of Little River (WB03). Each year comprises of four early morning, ebb tide, summer sampling events. Spatial changes of total nitrogen can be seen from up-gradient (WB04) to down-gradient (WB03) and the calculated percent difference is represented by the green line.

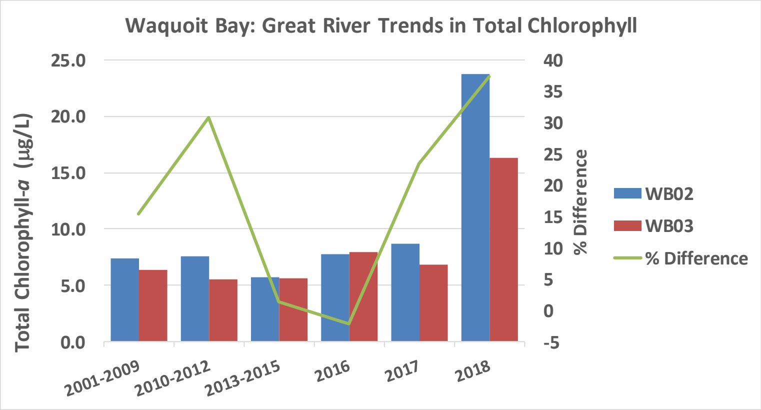

Figure VII.5: Water quality station averages of total chlorophyll-*a* pigments for long term (2001-2009) to 2018 of upper Great River (WB02) to the mouth of Great River (WB03). Each year comprises of four early morning, ebb tide, summer sampling events. Spatial changes of total chlorophyll can be seen from up-gradient (WB04) to down-gradient (WB03) and the calculated percent difference is represented by the green line.

Using the same analysis, we can use the Mashpee Water Quality Monitoring Program data in Popponesset Bay. The stations PB03 (up-gradient) and PB04 (down-gradient) bracket the Mashpee River oyster aquaculture area. The total nitrogen has been increasing at both stations, but the removal of TN from PB03 to PB04 is increasing (Figure VII.6).

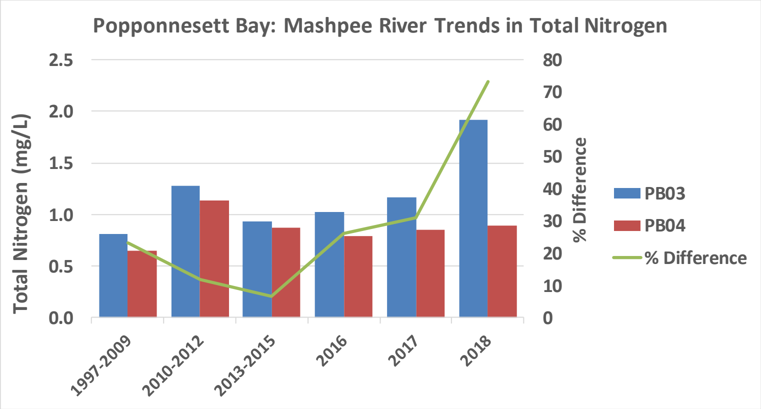

Figure VII.6: Water quality station averages of total nitrogen for long term (1997-2009) to 2018 of upper Mashpee River (PB03) and to the mouth of Mashpee River (PB04). Each year comprises of four early morning, ebb tide, summer sampling events. Spatial changes of total nitrogen can be seen from up-gradient (PB03) to down-gradient (PB04) and the calculated percent difference is represented by the green line.

The stations PB03 and PB04 did not show a significant percent difference in chlorophyll concentrations spatial over the oyster aquaculture area (VII.7). The increased sample stations added for this specific study did however prove lower TN and chlorophyll concentrations around the oyster area showing near-field reduction in nutrients (See Section II: Figure II.3, II.4).

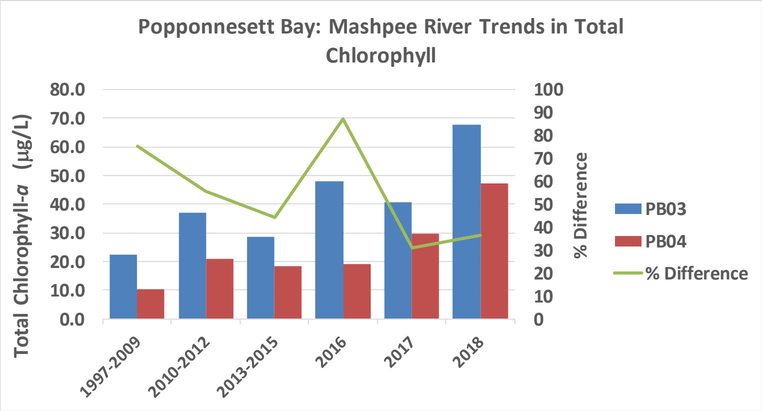

Figure VII.7: Water quality station averages of total chlorophyll for long term (1997-2009) to 2018 of upper Mashpee River (PB03) and to the mouth of Mashpee River (PB04). Each year comprises of four early morning, ebb tide, summer sampling events. Spatial changes of total chlorophyll can be seen from up-gradient (PB03) to down-gradient (PB04) and the calculated percent difference is represented by the green line.

Section VIII. Tank Experiments of Nitrogen Removal by Oysters and Quahogs

Background:

The Mashpee Department of Natural Resources ~~is~~ was directed by Rick York with Ashley Fisher serving as the Shellfish Constable during this study. The Department of Natural Resources conducted tank experiments at the Little River Town Dock located in Waquoit Bay. The goal of these experiments was a preliminary step to quantify the nitrogen removed by shellfish using the natural waters of the Little River.

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The nitrogen removal experiments described below were conducted in 2 different systems. One was conducted in upweller tanks supplied with water from the Little River continuously pumped through screens (silos) containing quahog seed. Water samples were collected from the input water entering the tank, and outflow from each silo. The other experiment took place in tanks that were filled with river water, but no additional water flow following (static). The tanks were mixed and aerated by bubbling air through diffusers (air stones). Adult oysters were added to the static tanks. Water samples were collected from the initial river water, and at intervals after the shellfish were added to determine the removal of algae/N over time.

Upweller Experiment September 19, 2018:

On September 19, 2018, an experiment in one of the 2 land based upweller tanks (5’ x 24’) at the Little River Town dock was conducted. Each fiberglass tank holds up to 22 fiberglass 61 cm (2’) diameter silos with screen mesh (1.5 mm) bottoms (0.2835 m^2) to hold the seed. River water pumped into the tank, flows up through the silo and seed, and exits through a 2” drain at the top of the silo (Figure VIII.1). The flow rate was 19 liters/min (5 GPM) through each silo. At 1719 hours EDT, water samples were collected in the tank before flowing through the silos, and from the outflow from the silos. The water temperature was approximately 23 Celsius. The salinity was 30 PPT. The plankton content of the river water was 35,000 cells/ml dominated by 33,000/ml small (3 to 5 micron) diatoms (*Chaetoceros tenuissimus*), plus 1,000/ml flagellates (10 microns), and 1,000/ml of a different species of flagellate (15 microns). These species are sizes that are filtered by shellfish and support effective growth. No harmful algae were observed. Water samples were sent to the SMAST lab for analysis of nitrogen and other parameters. The number of quahog seed and weight in each silo is listed in Table VIII.1. In this experiment at a temperature of 23 C, the quahog seed removed an average of 0.005 mg N/kg live quahog/liter/minute (Table VIII.2).

Table VIII.1 Quahog seeds weight and number in each silo of the tank experiment conducted on the Little River Town dock on Sept. 19, 2018.

Silo	Quahog seed: Total Live Weight (kg/silo)	Total Number (number/silo)	Average Individual Weight (g)
6	2.756	7,800	0.353
7	3.239	9,000	0.36
8	2.813	27,000	0.104
9	2.814	36,000	0.079
10	2.955	21,620	0.137
11	3.097	22,400	0.138

Table VIII.2: Nitrogen removal by quahog seed in each silo of the tank experiment conducted at the Little River dock on Sept. 19, 2018.

Water Sample	TN (mg/L)	TN Removal (mg/L)	TN Removed/kg quahog (mg N/kg quahog live wt.)	TN Removed/kg quahog/L min^{-1} (mg N/kg quahog live wt./L min-^{1})
River Water IN	0.470		-	-
Silo 6 Out	0.390	0.080	0.029	0.004
Silo 7 Out	0.350	0.120	0.037	0.006
Silo 8 Out	0.360	0.110	0.039	0.006
Silo 9 Out	0.380	0.090	0.032	0.005
Silo 10 Out	0.390	0.080	0.027	0.004
Silo 11 Out	0.360	0.110	0.036	0.006
Average				**0.005**

Figure VIII.1: Upweller tanks with silos

Tank experiment September 11, 2019:

At 1400 hours EDT (t_o) on September 11, 2019, oysters were placed in 2 tanks (55-gallon translucent polyethylene drums), 49 oysters in Tank 1, and 51 oysters in Tank 2 (Figure VIII.2). Each tank was filled with 200 liters of river water. Both tanks were aerated and mixed with air from diffusers (air stones) connected to aquarium air pumps. The water temperature was 23 C. Initial water samples collected at 1400 hrs. (t_o), and a second set of samples collected at 1700 hrs. (t_1) were analyzed for plankton content and sent to the SMAST lab for analysis of nitrogen and other parameters. The initial plankton content was dominated by small (3 to 5 micron) diatoms (*Chaetoceros tenuissimus*) at a count of 60,000 cells/ml (over 98% the total count that included 1,000 cells/ml of 4-micron size flagellates). No harmful algae were observed. The weights of oysters in the tanks is listed in Table VIII.3. Nitrogen concentrations and removal are in Table 4. In this experiment at a temperature of 23 C, the oysters removed an average of 0.01 mg N/kg live oyster/hour (Table VIII.4).

Table VIII.3 Oyster weight and number in each tank during the experiment conducted at the Little River Town dock on Sept. 11, 2019.

Tank	Species	Total Live Weight (kg)	Number of Oysters	Average Live Weight (g)
1	Oyster	2.858	49	58
2	Oyster	2.975	51	58

Table VIII.4 Nitrogen removal by shellfish in each tank during the experiment conducted at the Little River Town facililty on Sept. 11, 2019.

Water Sample	TN (mg/L)	TN Removal (mg/L)	TN Removed/kg oyster (mg N/kg oyster live wt.)	TN Removed/kg oyster/hr (mg N/kg live oyster/hr)
Initial (t_o)	0.566			
Tank 1 (t_1)	0.524	0.042	0.015	0.005
Tank 2 (t_1)	0.427	0.138	0.047	0.016

Figure VIII.2: Oysters in tank for the experiment conducted on Sept. 11, 2019 at the Little River dock.

Section VIIII. Mashpee River and Shoestring Bay Oyster Study 2016-2018: Conclusions

The major results of the 3-year study of oysters in the Mashpee River and Shoestring Bay are summarized below.

1) Overall, the results indicate oysters reduce near-field total suspended solids, total chlorophyll and total nitrogen. These measurable reductions are evidence that oysters can improve water quality even in nitrogen-enriched waters.

2) Time-series continuous measurements of total chlorophyll-*a* concentration above and below the Mashpee River aquaculture area showed a clear significant decrease in phytoplankton biomass as water passed through the oyster site on both ebb and flood tides. This filtration of particulates (phytoplankton) also resulted in a small decrease in nitrogen levels in the grab sampling data, which is a less sensitive measure. This particle removal is seen more clearly when focusing on ebb tide, daytime chlorophyll-*a* concentration sonde results.

3) Times-series light measurements show there is more light on the bottom down-gradient of the oyster aquaculture area compared to up-gradient suggesting water clarity is improved as it travels through the oysters. Point measurements of light showed there is more light penetration in the oyster area compared to the surrounded area. The time-series light results are even more compelling when coupled with the significant reduction in chlorophyll-a at the Mashpee River site. Water clarity is fundamental to re-establishing eelgrass, which is a key metric for gauging the restoration of Popponesset Bay under the established TMDL. The improved water clarity at both aquaculture sites and large removal of phytoplankton at the Mashpee River site indicate that expanded shellfish deployments, especially if spatially distributed, will result in positive impacts on the water and habitat quality of Mashpee's estuaries.

4) Sediment core incubations showed no measured enhanced denitrification by oyster aquaculture in bottom cages. It is possible the "control" sediment cores may have been within the oyster biodeposit impact area. Future studies should increase the distance of control cores from the aquaculture area. The area of deposition could be determined using an ADCP to measure the speed and direction of water flow.

5) Dissolved oxygen was less than 3 mg/L 8% of the deployment time from August to October at the Mashpee River site. Low bottom water dissolved oxygen appears to be reducing the amount of coupled nitrification-denitrification, as oxygen is required for nitrification in surficial sediments.

6) Biomass data of oysters placed in the aquaculture area will profoundly improve the functionality of water quality monitoring and be the solution to calculating oyster filtration and biodeposition rates.

7) Maintaining the oyster trays so that the supporting pipes keep them above the sediments may increase denitrification. Deploying oysters in floating surface bags may increase the nitrogen removal by denitrification as seen by similar benthic regeneration studies in Lonnies Pond, Orleans, MA. Fouling of

floating gear by algae mats moving with tides in the Mashpee River was unmanageable when tried in the past.

8) In this study, a 100 g (> 3”) oyster has an approximate mass of 481 mg of nitrogen. In the CWNMP estimate, a 100 g (> 3”) harvest size oyster contained approximately 500 mg N. Increasing the number of oysters harvested, while also harvesting larger oysters will maximize the nitrogen removal

9) The role of aquaculture is only to supplement an overarching plan of reducing nitrogen. If aquaculture were increased in the Mashpee River and able to sustain a harvest of about 1.5 million oysters per year, this study estimates that would effectively reduce the present watershed nitrogen load by 15%. Using the CWNMP estimate, the load would also be reduced by 15%. Higher removals are clearly possible, based upon comparisons to other aquaculture sites.

10) Waquoit Bay shellfish seeding has had a positive effect on water quality in the Little River/Great River basins. This is based upon analysis of water quality monitoring data (all ebb tides) and would require tidal studies for a more robust assessment of improvement (and likely show larger impacts).

11) Overall, the combination of results clearly indicates that shellfish deployments in Popponesset and Shoestring Bays are currently having a localized positive effect on water quality. Expanded shellfish aquaculture and seeding programs should expand the positive effects and provide a feasible mechanism for localized restoration in the tributary basins and a supplement to the Town’s overall nitrogen management program. It is almost certain that as nitrogen levels decline that improved bottom water dissolved oxygen in the Mashpee River will result in improved nitrogen removal by denitrification.

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