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

Assessment of Bournes Pond Oyster Aquaculture Effects on Water Quality and Nutrient Cycling

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I. <u>Summary</u>

Scientific staff of the Coastal Systems Program (CSP) at the University of Massachusetts-Dartmouth (UMD) School for Marine Science and Technology (SMAST), in collaboration with the Town of Falmouth's Marine and Environmental Services, have been collecting data to complete a detailed assessment of water quality and nutrient cycling in bottom sediments of Bournes Pond, specifically related to suspended oyster aquaculture. The initial assessment of oyster aquaculture monitoring in Bournes Pond was conducted during the 2018 growing season, which built on several other on-going CSP joint efforts. Work continued in 2019 and spring 2020 with monitoring of a new oyster deployment site in upper Bournes Pond; this assessment covers monitoring completed during 2019 and spring 2020. Oyster aquaculture monitoring conducted in Bournes Pond has benefited from other on-going CSP joint efforts, which provided pond-wide water quality data for the period 2012-2019 (Falmouth PondWatch Water Quality Monitoring Program) from sites above and below the Town's main oyster culture area in 2018 and 2019. An oyster habitat suitability survey, which had 2 sites in Bournes Pond (2017-2018) and relevant PondWatch water quality results have been integrated to provide this assessment of potential nitrogen and water quality effects of oyster culture in Bournes Pond.

II. <u>Introduction</u>

Scientific staff of the Coastal Systems Program (CSP) at the University of Massachusetts-Dartmouth (UMD) School for Marine Science and Technology (SMAST), in collaboration with the Town of Falmouth's Marine and Environmental Services, conducted a detailed assessment of water quality and nutrient cycling in bottom sediments of Bournes Pond, specifically related to suspended oyster aquaculture. The majority of work on Bournes Pond for this assessment was conducted during 2019-2020 (Figure II.1). Several towns across southeastern Massachusetts have been investigating oyster deployments/reefs as a means to improve nitrogen related water quality in their estuaries and have been quantifying the removal of water column nitrogen into harvested oysters and the associated enhancement of sediment denitrification (NO_3^-→N_2) to gauge total nitrogen removal efficiency by this management alternative. This report is part of the overall effort to assess the use of shellfish as an *in situ* water quality management tool to attain estuarine specific nitrogen thresholds developed by the Massachusetts Estuaries Project (MEP) and associated USEPA/MassDEP TMDLs. Determination of the rates, mechanisms and controls on oyster associated nitrogen removal in Bournes Pond is to help guide the Town of Falmouth in planning estuarine restoration, which includes the use of oysters as a low cost *in situ* nitrogen remediation approach.

This report is to provide the Town of Falmouth's Water Quality Management Committee with a summary of the current oyster/nitrogen related work in Bournes Pond based mainly on pilot deployments and data sources developed by CSP scientists in partnership with the Town through the PondWatch program long-term monitoring of nitrogen related water quality.

Figure II.1: Bournes Pond oyster aquaculture site for duration of deployment in 2019.

III. <u>Data Collection</u>

The goal of this study was to quantify nitrogen (N) regeneration and removal associated with suspended oyster aquaculture in Bournes Pond. Water quality measurements and analysis of sediment N cycling were linked to water filtration by oysters, seasonal rates of biodeposition and remineralization and transformation of benthic N due to microbial activity. Data was collected to determine:

- Changes in overall Bournes Pond water quality (WQ) due to oyster aquaculture in 2019 compared to prior observed WQ conditions in summers 2014-2018, with all data collected using directly comparable protocols;
- Effects of oysters on water quality (total and particulate N, dissolved oxygen, and total chlorophyll-*a* pigments) due to filtration and biodeposition within the oyster deployment area;

- Seasonal transformation and regeneration of sediment N and denitrification in response to suspended oyster aquaculture.

Water quality samples were collected upgradient and downgradient of the oyster aquaculture site, on the ebb tide, to assess changes in bioactive N (dissolved inorganic N + particulate organic N), total N, total chlorophyll-a pigments, and dissolved oxygen through the oyster aquaculture site from June through November 2019, and April 2020. Moored autonomous instrumentation (YSI 6600) were deployed directly upgradient and downgradient (0 m North and South) of the oyster aquaculture site to monitor dissolved oxygen and chlorophyll-a at 15-minute intervals, 30 cm above the sediment surface in July and September (2019). Intact sediment cores were collected on 4 occasions (July, August, October 2019, and April 2020) and incubated at *in situ* temperatures for quantifying sediment oxygen uptake (community respiration), nutrient regeneration and denitrification. Cores were collected within the oyster site (treatment cores) and outside the oyster site (control cores) to identify changes in N cycling due to shellfish biodeposits. The Coastal Systems Program (CSP) also conducted measurements of the oysters themselves for total number of live individuals, growth rate, and mortality from June through November (harvest). The biomass of oysters throughout the plot was also determined on removal of the oysters from the aquaculture site. The biomass of contributed feces and pseudofeces to the underlying sediments was also measured to assess changes in sediment oxygen demand, nutrient regeneration and denitrification. In addition, oysters can significantly alter water quality parameters, primarily particulate constituents. Therefore, these parameters were examined using the July/August Falmouth PondWatch Program sampling data augmented by CSP monitoring at the aquaculture site, to assess the overall water quality changes in Bournes Pond.

IV. <u>Results</u>

1. Oyster Growth in Bournes Pond

Town of Falmouth Oyster Grow-Out Area: Oyster Numbers, Growth, and Mortality

Over the growing-season, oyster biomass increases and the amount of biodeposits delivered to the sediments also increases due to increasing filtration rates at higher temperatures and increased phytoplankton availability. Given the mean water depth of the aquaculture site (~ 0.70 m), the small tidal excursion (0.5 m) and the low flow velocities, most of the biodeposition hits the sediments in the immediate vicinity of the oysters.

On May 23, 2019, the Town of Falmouth Marine and Environmental Services (MES) deployed 35,568 oysters in floating 9 mm mesh bags in an 83 m^2 array in Bournes Pond (Figure IV.1). Oysters were second year; from 2018-seed. Oyster metrics were collected at deployment and periodically throughout the growing season. Metrics included oyster wet weight, shell height, and numbers of live/dead. Measurements were conducted on six occasions over the deployment period on samples from 10 representative bags, 5 from the margins and 5 from the center of the array.

Figure IV.1: View of Bournes Pond aquaculture array in summer 2019 from boat (left) and shore (right).

Salinity was relatively constant averaging 23.6 PSU, in contrast temperature in the oyster aquaculture area varied over the deployment, ranging from 23-29.8°C in summer (July-August), 26-15°C in fall (September-October), 14.5-4°C in winter (November-December), with warming the following spring to 12.5°C (April). All oysters were deployed on May 23, 2019 and were removed 207 days later on December 16, 2019.

Growth rates were determined from May 18 – November 14 based upon periodic standard measures of shell height and oyster weight. In order to establish a scale for biomass, size characteristics were analyzed based on initial measurements of second year oysters by CSP in collaboration with the Falmouth MES. Measurements between the May 2019 initial deployment and July 2019 showed a very low to negligible growth rate based upon size and on weight respectively: 5/23/2019 deployment (48.20 ± 6.71 mm and 12.80 ± 3.93 g) and 7/18/2019 (53.01 ± 2.20 mm and 11.98 ± 1.56 g)[1]. However, after this initial low growth period oysters grew normally (e.g. from July – November). Based upon these later measures, oysters in Bournes Pond appeared to grow at an average rate of 0.37 g day^{-1} (Table IV.1), reaching an average wet weight of 48.78 ± 2.35 grams oyster^{-1} nearly 4 times their initial weight (Figure IV.2) and reaching an average shell height of 81.75 ± 1.44 mm. This floating culture system did not show noticeable differences in growth rates between bags from the center and edges of the rectangular array. The size of the aquaculture site allowed for relatively good access to food resources compared to larger propagation sites which sometimes experience reduced food availability to oysters near the center of the array, resulting in differential growth rates unless the bags are regularly rotated during the season. Similar patterns in growth can be found in oyster reefs, where oysters near the margins grow faster than in the central reef areas due to higher food availability.

The localized water quality data collected over the growing season in the nearfield to the oyster array Figure (IV.3), reveals the most likely cause of the negligible growth of the oysters in the early season from May 23 to early July 2020 (Figure IV.4). It is clear from the temporal record of chlorophyll-a pigments, a surrogate for phytoplankton biomass, that during the early season

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[1] This observation of low growth in May/June was also observed in Orleans Lonnies Pond Oysters in 2019.

plankton levels (food) were very low. In fact, the levels were very low compared to the prior 5 years. It is not clear what caused this low level of production, but it is clear that the growth of the oysters tracked the availability of their food source as would be expected.
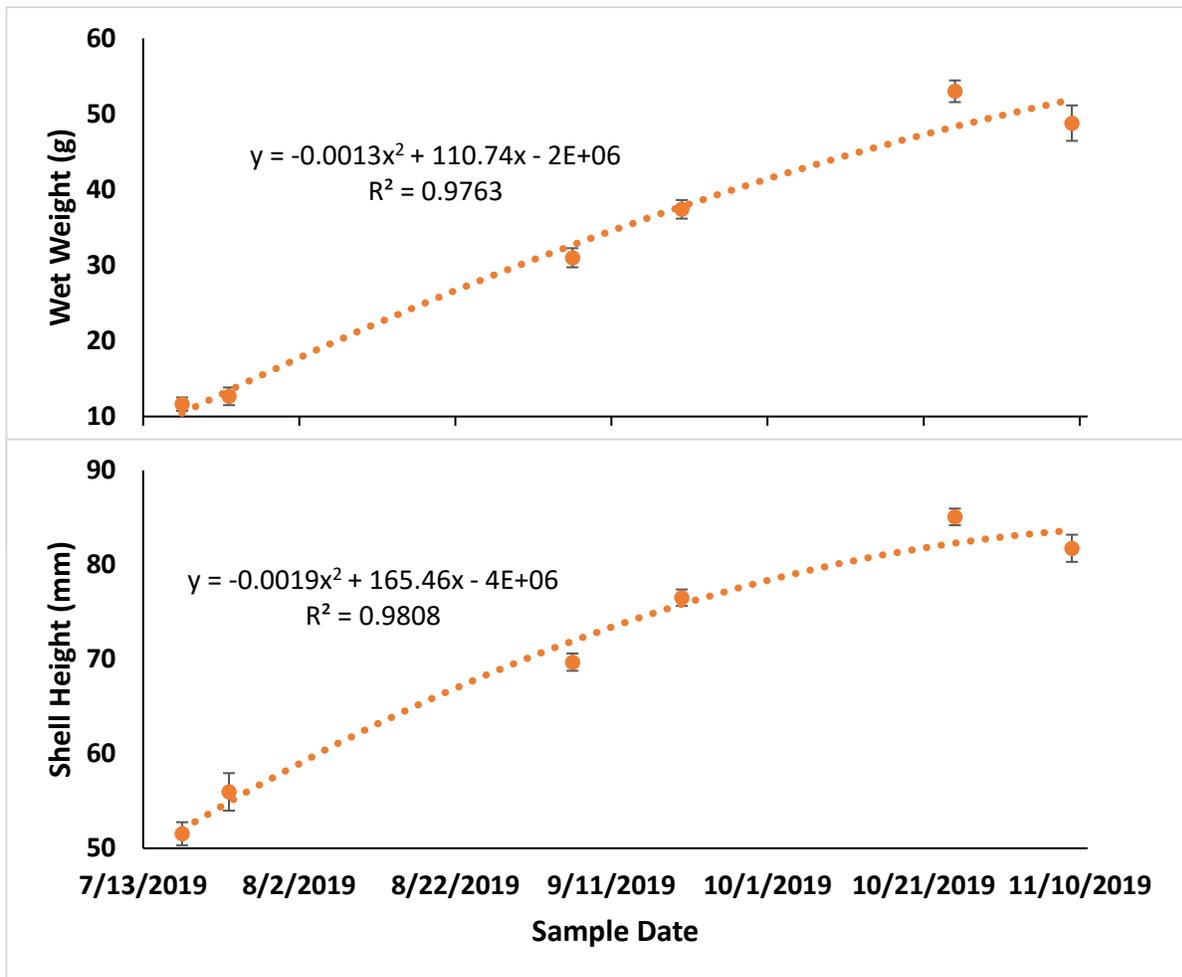

Figure IV.2: 2019 Bournes Pond oyster aquaculture mean wet weight (top) and mean shell height (bottom) over growing season. A best-fit 2nd order polynomial regression is shown for both data sets. Error bars represent standard error of the sample population.
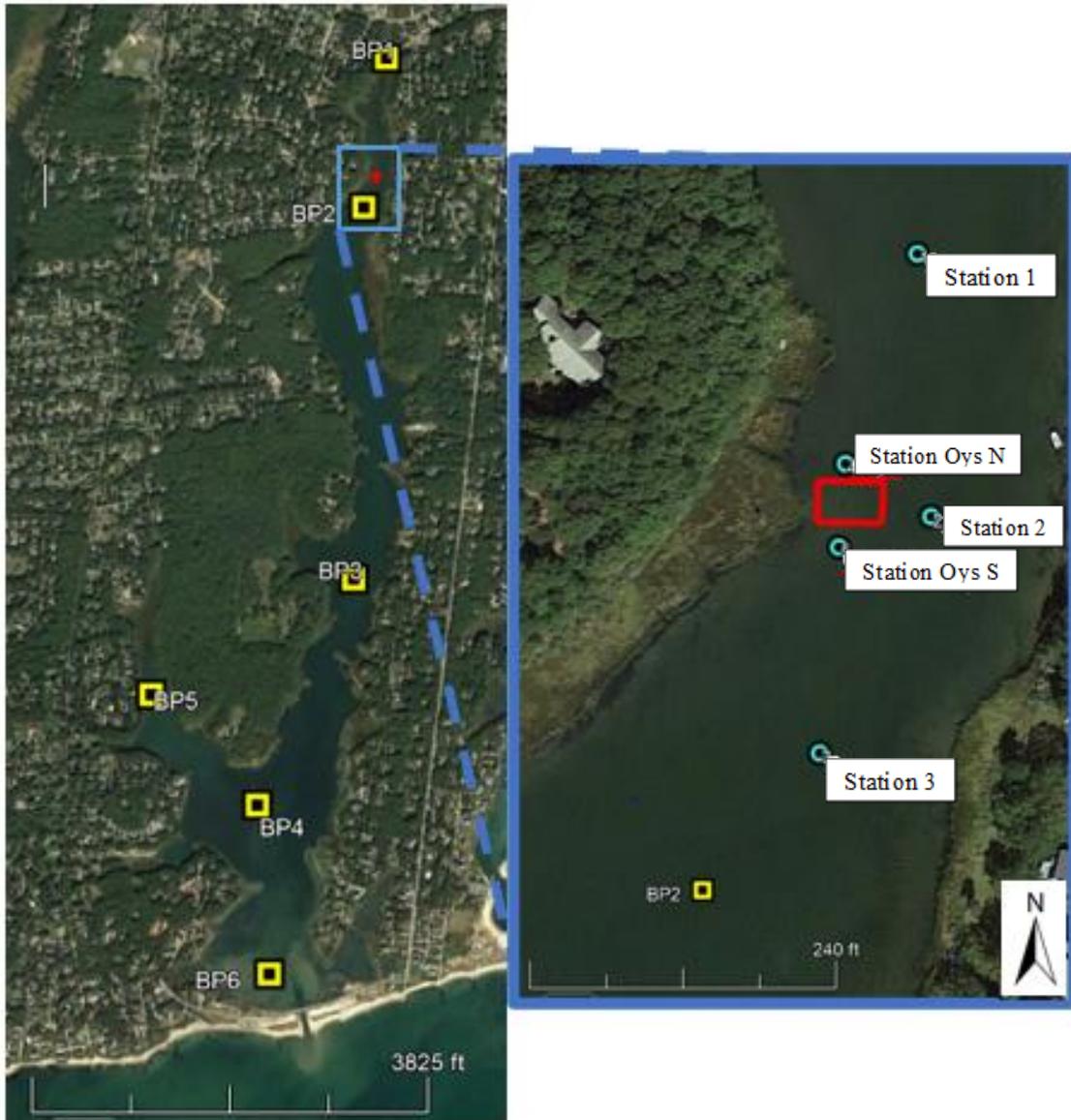
Figure IV.3: Satellite imagery (Google Earth 2019) of Bournes Pond indicating established PondWatch water quality sampling locations (yellow boxes). Of these stations, BP1 and BP2 are the closest upgradient and downgradient stations, respectively, to the oyster site. Five additional water quality stations surrounding the aquaculture site (red box) were sampled in 2019 (blue circles). Specifically localized to the study site, station Oys N is directly 0 m north of the aquaculture array and station Oys S at 0 m south, while station 1 was upgradient, station 2 was adjacent, and station 3 was downgradient, relative to an ebbing tide.

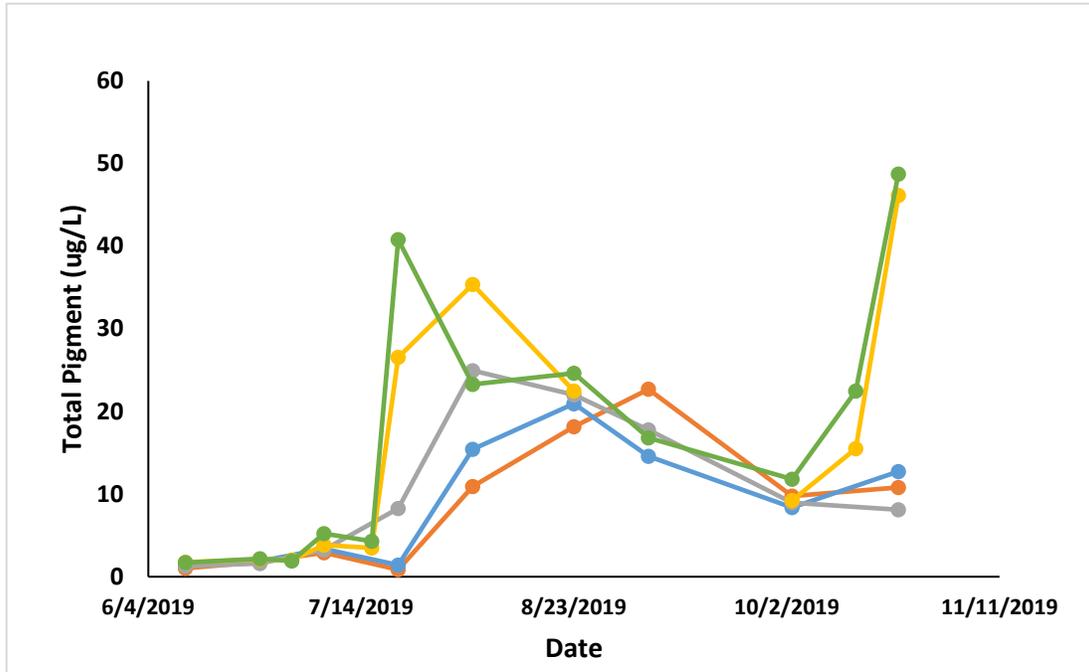Figure IV.4: Total pigments (chlorophyll-*a* + pheophytin-a), during the 2019 oyster aquaculture deployment. All stations local to the aquaculture array (Figure IV.2.1), station 1 50 m upgradient (red), station 3 50 m downgradient (grey), station 2 mid-channel (blue) and border sites 0m north (orange) and south (green) of the suspended culture array. Note the low phytoplankton biomass during the initial deployment period.

Table IV.1: Average oyster growth rates in Bournes Pond based on 10 bags sampled from the total array (30 oysters measured per bag) over the latter part of the 2019 oyster deployment (July-December) 2019 after an initial negligible growth period from late May – June. Growth rate variability is reported with the mean and standard error (mean ± SE).

Bournes Pond 2019 Oyster Growth Rates					
Wet Weight per Oyster (g/day)		Shell Height per Oyster (mm/day)		Stocking Density (oysters/bag)	
0.37	± 0.04	0.28	± 0.04	329.33	± 20.92

In addition to oyster growth, oyster survival was assessed over the growing season by counting the number of live and dead oysters per bag. The average mortality from the periodic bag counts over the growing season was 10.6% which compares well with the mortality based upon the total live oysters deployed and recovered, 16.8% (Table IV.2). It should be noted that there was no significant difference in mortality rated when comparing bags sampled from the middl of the array compared to bags from the outer edges of the array. These mortality rates are similar to the results from the 2018 pilot study in Bournes Pond at the same location (BP2)[2]. The total number of live oysters removed in December 2019 was estimated at 29,610 compared to 35,568 deployed (83.2% survival). The average wet weight oyster biomass per bag was approximately 13.53 kg, for a combined total of 1,462 kg of oysters harvested from the aquaculture array.

[2] 2018. Howes, B.L. Ph.D. A. Unruh, M.S., David R. Schlezinger, Ph.D., J. Benson, M.S., M. Labrie, Ph.D. Candidate with Falmouth MES C. Martinsen and C. Lovely. Preliminary Assessment of Bournes Pond Oyster Aquaculture Effects on Water Quality and Nutrient Cycling. Coastal Systems Technical Memorandum, School for Marine Science and Technology UMassD. For the Town of Falmuth Water Quality Management Committee, pp. 23.

Table IV.2: Assessment of cumulative oyster mortality from tracked bag locations. The total number of live oysters were tabulated by bag and monitored for mortality over the growing season of 2019. Bags were not rotated, thus bags on the edges of the array (outside) were counted along with bags from the center (inside).

Date	Total Oyster Counts									
	Outside Bags					Center Bags				
	1	2	3	4	5	1	2	3	4	5
7/18/2019	279					415				
7/24/2019									374	
9/20/2019		292	258	279	445					
10/5/2019							305	317		278
10/25/2019		256	225	241	412				295	
11/9/2019							270	288		
Mortality	18	36	49	53	35	31	36	43	71	15
Rate (%)	6.5	12.3	19.0	19.0	7.9	7.5	11.8	13.6	19.0	5.4

Upon removal (December 2019) of the oysters from Bournes Pond the Town of Falmouth MES relayed them to West Falmouth Harbor. Of the relayed oysters, 10 individual oysters were collected, 1 from each of the 10 bags sampled, on December 16, 2019 for elemental analysis (PerkinElmer 2400 Series II CHN Elemental Analyzer) to determine the total N and carbon (C) mass of the shell and tissue biomass. Additionally, 10 individual oysters were collected June 25, 2019 for determining the shell and tissue N and C content per oyster at deployment in Bournes Pond. From these data the nitrogen removal by oyster harvest from the aquaculture site can be determined. On average, in June individuals were 11.34 ± 0.72 g oyster^{-1} wet weight and 55.58 ± 1.93 mm oyster^{-1} in shell height comparable to independent measures by the Falmouth MES in May and June (13.68 ± 0.73 g oyster^{-1} and 51.22 ± 1.02 mm oyster^{-1}). The average wet weight and shell height of individuals collected for elemental analysis in December were 78.74 ± 7.86 g oyster^{-1} and 92.08 ± 3.76 mm oyster^{-1}, respectively. The June and December per whole oyster total N content averaged 45.79 ± 3.10 mg N oyster^{-1} upon deployment and averaged 311.11 ± 35.29 mg N oyster^{-1} and harvest (Figure IV.5). From this data it was determined that 1.35 kg N was in the oysters (total bags in the array) at deployment and 9.21 at removal, therefore the total N removed solely from harvesting oysters in Bournes Pond was determined to be 7.86 kg.
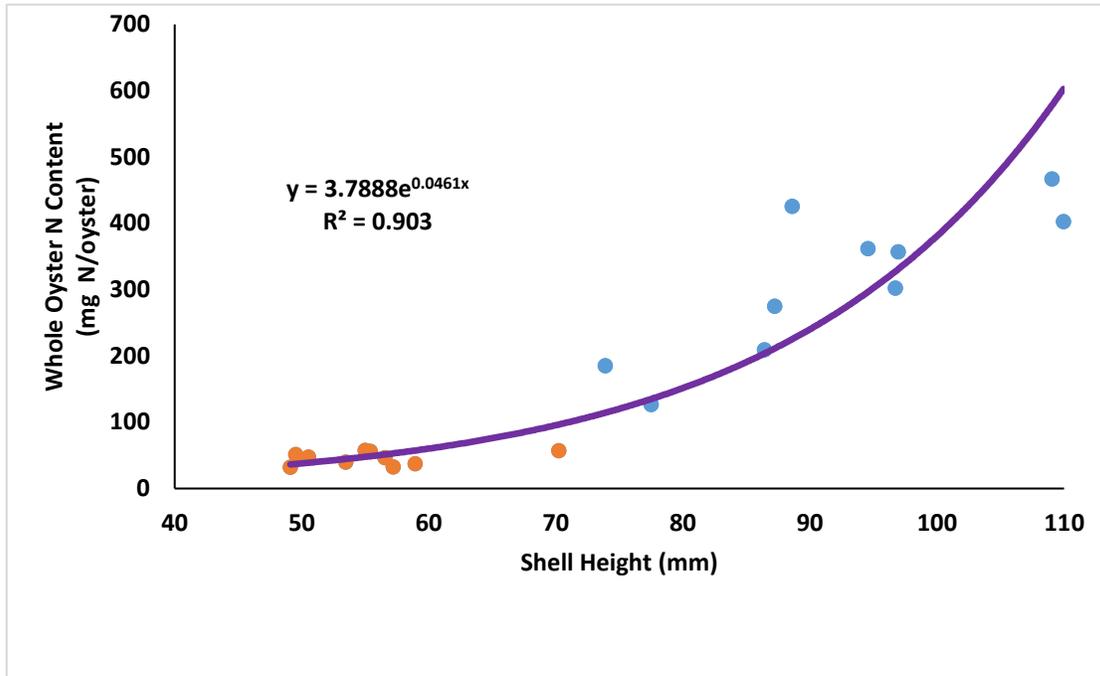
Figure IV.5: Relationship between N content of oyster biomass (soft tissue + shell) and shell height for a sub-sample of oysters harvested in June (orange) and December (blue). A best-fit exponential regression model of the overall dataset is shown. While oysters were initially deployed on May 23, there was no measurable growth from deployment in May to June measurement date.

2. Overall Water Quality in Bournes Pond Prior (2014-2018) vs. Present (2019)

Water quality monitoring in Bournes Pond has been conducted as part of the Falmouth PondWatch Program for over three decades, to gauge long-term water quality trends in this water body. From stations located throughout Bournes Pond (Figure IV.3 above) grab samples from 2014-2018 were compared to the water quality conditions of 2019. The 2014-2018 "baseline" was used as a basis of comparison to identify any potential large-scale effects of the 2019 oyster deployment on water quality. Because oysters are filter feeders, water quality metrics associated with primary production and particulate matter were examined. Therefore, phytoplankton abundance assayed as total chlorophyll-*a* pigments, components of total N (TN), inorganic and organic dissolved N (DIN, DON) and particulate organic N (PON) were examined. Potential effects on bioactive N, which includes PON removed by oysters and the DIN excreted by oysters were also assessed. Water clarity can also be used as a metric to assess oyster impact, which PondWatch measures using a Secchi disk. Unfortunately, the shallow water depths in the region of the aquaculture site allowed light to reach the sediment surface so a true Secchi depth could not be determined.

Nutrient related water quality from the PondWatch Monitoring Program was averaged for each station in Bournes Pond by year. Stations were ordered from the head of Bournes Pond (BP1) to the tidal inlet (BP6), noting station BP5 is located within the tributary basin of Israels Cove (Figure IV.3). The suspended oyster aquaculture farm installed in May 2019 was in the upper pond between stations BP1 and BP2. Based on data from 2014-2018, there is a natural gradient in total chlorophyll-*a*, TN, and bioactive N moving from the head of the system to the mouth (inlet), with the highest levels at the headwaters and lowest at the mouth of Bournes Pond. As a tributary basin between BP-4 and BP-6, Israels Cove (BP-5) has higher levels (lower water quality) due to inputs from its own subwatershed. Significantly, while the gradient seen in 2014-2018 was relatively consistent, 2019 showed a different pattern in all 3 constituents (CHLA, TN, Bioactive N) mainly in the uppermost stations (BP1, BP2 and BP3, Figure IV.6). When compared to 2018, chlorophyll-*a*, TN, and bioactive N showed the greatest reductions at station BP1 (68.3%, 76.6%, and 81.5%) followed by station BP2 (52.9%, 47.0%, and 51.8%).

PondWatch Monitoring Results:

Figure IV.6: Total chlorophyll-*a* pigments, total nitrogen, and bioactive nitrogen (dissolved inorganic nitrogen + particulate organic nitrogen) were averaged yearly using PondWatch monitoring data in Bournes Pond from 2014-2019 to track changes in water column nutrient concentrations. Stations are listed spatially from upgradient to downgradient and the purple arrow illustrates the position of the aquaculture site.

As seen in annual water quality results above, 2019 had lower chlorophyll a pigments, TN and bioactive N when compared to the previous 5 years at the stations adjacent the aquaculture site (Figure IV.6). In addition, the system-wide gradual decreasing gradient in each constituent from the headwaters (BP-1) to lower pond (BP-4) was disrupted in 2019 and showed more variation (Figure IV.7) on a scale that cannot be ascribed to the oysters except possibly in the uppermost tidal reach. Comparing BP-1 in 2019 to the average over the previous 5 years for each of the 3 key constituents there appear to be reductions of 68.5 % (chlorophyll-*a*), 69.2% (TN), and 75.5% (bioactive N). In addition, BP2 also displayed significant reductions by 39.4%, 49.6%, and 56.1%. It is likely that some portion of these reductions can be attributable to the newly installed oyster aquaculture site, but other factors are also likely involved, such as a change in freshwater flow or tidal flushing due to dredging in the area of the tidal inlet.

The 2005 Massachusetts Estuaries Project (MEP) report identified Bournes Pond is exceeding the total daily maximum load (TMDL), resulting in impaired water quality and suggested channel dredging and inlet widen, amongst other management options, to reduce nutrient concentrations in the water column.   The Army Corps of Engineers received a permit application for an inlet increase from 50 feet to 90 ft. and dredging of 11,000 cubic yards of sediment in October 2017 [1]. The dredging project may have increased the current flow and temporarily altered and increased the amount of sediment and nutrients flowing out and in over a tidal cycle. [3]

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[3] https://www.nae.usace.army.mil/portals/74/docs/regulatory/PublicNotices/NAE-2017-1057_Falmouth.pdf
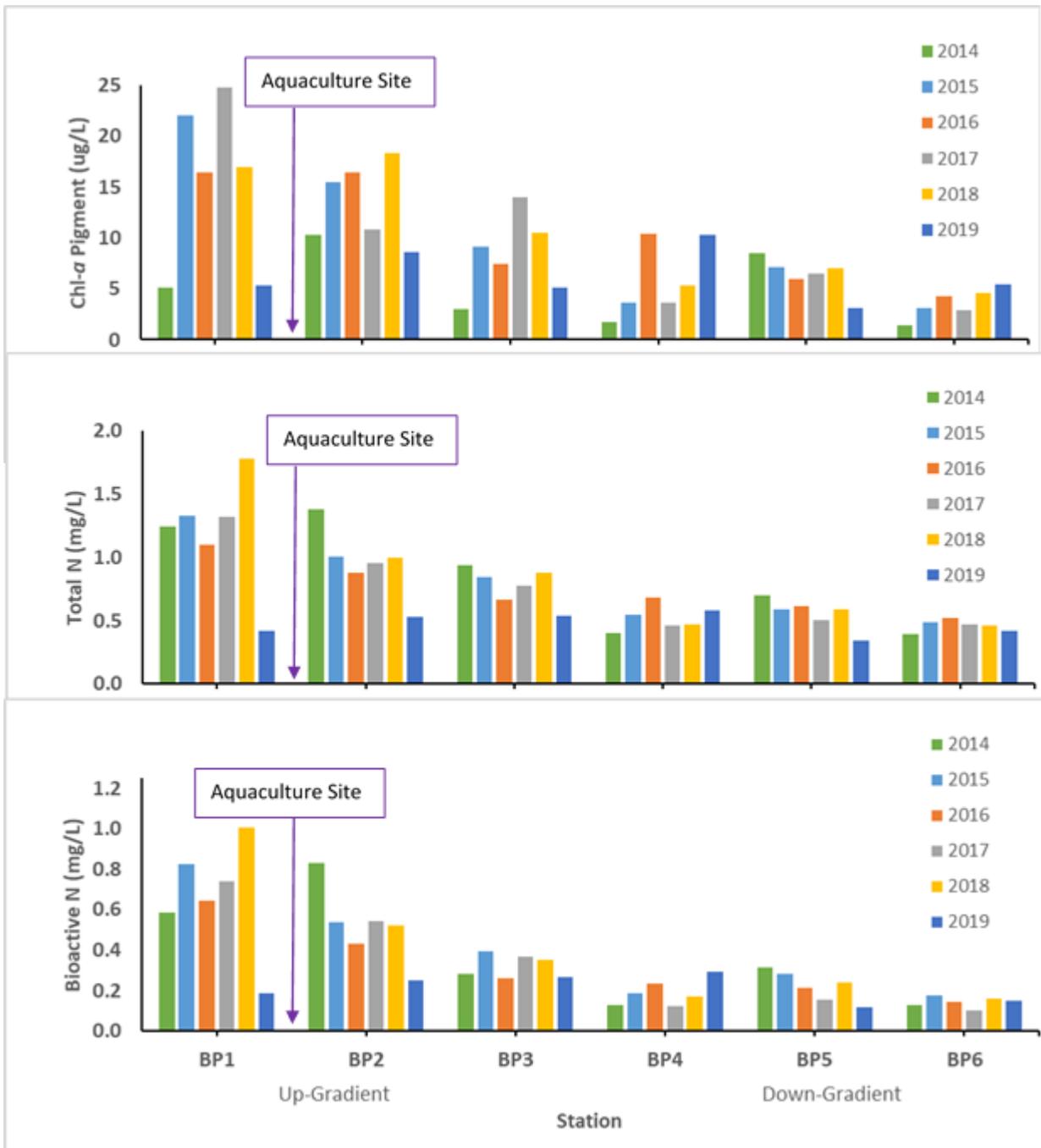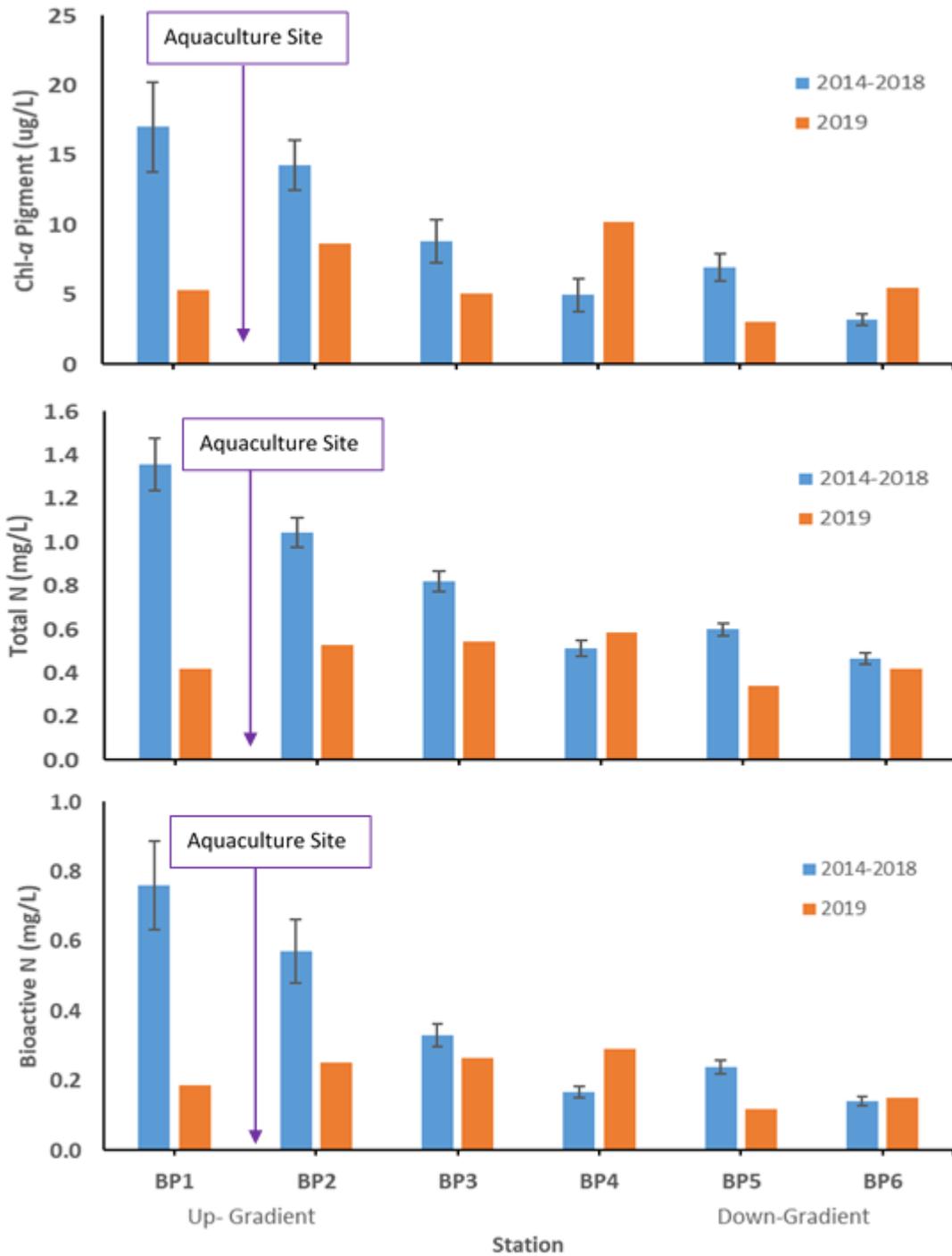
Figure IV.7: Total chlorophyll-*a* pigments, total nitrogen, and bioactive nitrogen (dissolved inorganic nitrogen + particulate organic nitrogen) were averaged yearly using PondWatch monitoring data in Bournes Pond from 2014-2018 and compared to 2019 data to assess water quality with the addition of the oyster aquaculture area. Stations are listed spatially from upgradient to downgradient and the purple arrow illustrates the position of the aquaculture site.

Water Quality in the near-field of the Oyster Aquaculture Site:

In order to identify localized impacts of suspended oyster aquaculture, a set of near-field (relative to oyster array) stations were established to make comparisons of stations directly associated with the aquaculture area. A total of five (5) stations were established at the: upgradient (North) and downgradient (S) borders of the propagation site and a mid-channel site (site 2) and additional stations a further 50 m to the N (site 1) and S (site 3) of the site (Figure IV.2.1). The five near-field stations were all located between BP-1 and BP-2 and were sampled from June-November 2019.

The results showed a clear spatial pattern in TN and bioactive nitrogen (Figure IV.8). Levels of both TN and bioactive N were lower at the north and south stations directly adjacent the array, while the station offshore of the site in the mid channel and the more distant sites were higher and generally showed the same levels. The samples directly associated with the array showing lower TN and bioactive N levels than the farther away stations is consistent with filtration and deposition of nitrogenous materials by the oysters in the array and has been observed in prior Bournes Pond oyster deployments. While the results from the near-field stations are consistent with the system-wide results (BP-1,2,3,4,6), they are more directly related to oyster activities than the system-wide analysis.
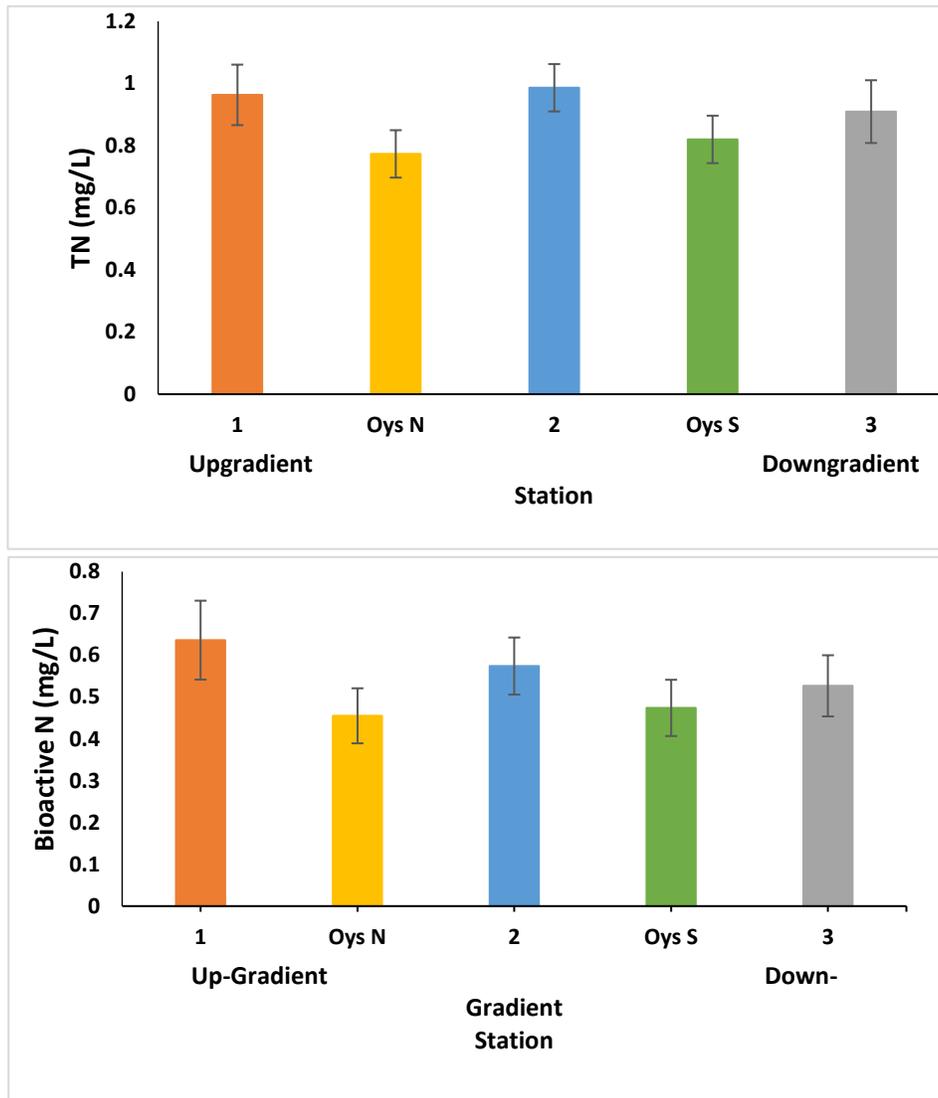
Figure IV.8: Total nitrogen (TN), and bioactive nitrogen (dissolved inorganic nitrogen + particulate organic nitrogen) results from sampling of the nearfield stations at the 2019 oyster aquaculture site. Stations are listed spatially from upgradient to downgradient with Oys N and Oys S at 0 m from the suspended culture array and station 2 being in the mid channel adjacent the site. Stations 1 and 3 are 50 m north and south of the site.

Oyster Effect on Dissolved Oxygen and Total Chlorophyll-a (time-series sensors):

YSI 6600 Multi-parameter Autonomous Water Quality Monitors were deployed to record bottom water constituents immediately upstream and downstream of the oyster culture area 30 cm above the sediment surface. The instruments captured dissolved oxygen, chlorophyll a, temperature, salinity and water depth data every 15 minutes over the deployment period (IV.2.5). The instruments provide the high frequency measurements required to observe both transient events, such as early morning oxygen minima, and synchronous paired events such as changes in chlorophyll concentrations in water passing through the the oyster culture site. The latter is

crucial to quantifying filtration and uptake of phytoplankton by the oysters as tides push water through the culture area.
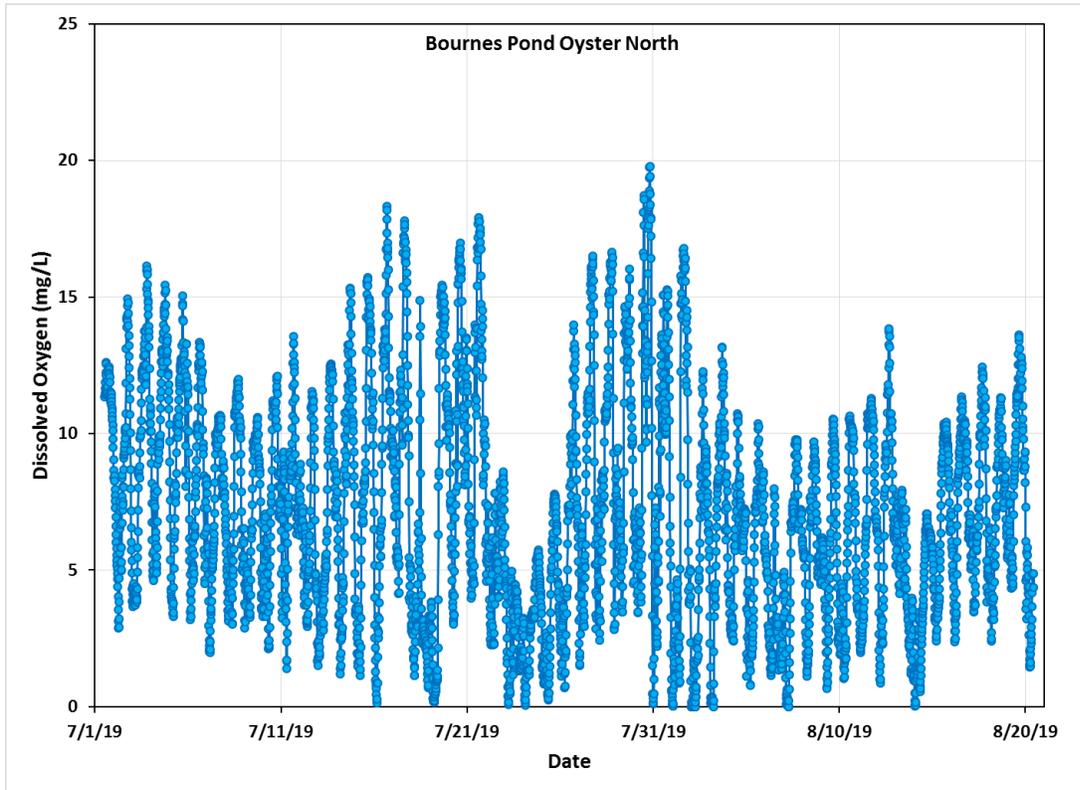

Figure IV.9: Dissolved oxygen time series from North meter above the oyster array.

Dissolved oxygen displayed large diurnal variations typical of a eutrophic estuary. Daily dissolved oxygen excursions were large during the deployment period approaching 20 mg/L on several occasions (Figure IV.9). During the 50 day deployment oxygen dropped below 3 mg/L at least once each day, typically in the early morning before the onset of photosynthesis. Excursions below 3 mg/L were usually of short duration averaging less than 3 hours; the shortest time period was 15 min and the longest excursion was 11.5 hours. The meter recorded 12 times that the water column became anoxic, the majority of events occurred after July 31 coincident with a phytoplankton bloom that increased chlorophyll concentrations above 15 ug/L. Phytoplankton blooms appear to be a dominant factor driving hypoxic events.

Chlorophyll concentrations upstream and downstream of the oyster culture were relatively low between July 1 and July 17 averaging less than 10 ug/L (Figure IV.10). After July 17 chlorophyll concentrations increased rapidly reflecting a phytoplankton bloom which continued until the end of the deployment record. This bloom represented a 4-5 fold increase in the average chlorophyll concentration, and appeared to influence the frequency of anoxia.

By comparing the upstream (North) and downstream (South) chlorophyll concentrations, an estimate of plankton removal by the oysters may be made (Figure IV.11). This approach was

very successful in 2018 at a pilot site further downstream within Bournes Pond. The present data was more difficult to analyze, however. When chlorophyll concentrations were below 10-15 ug/L apparent uptake by the oysters was not detected. This is consistent with previous studies using a similar approach in Lower Bournes Pond (2018) and in the Mashpee River. In both cases ebb tides showed the highest chlorophyll concentrations.

The difference between chlorophyll concentrations measured at the North and South meter locations (Figure IV.11) should be related to chlorophyll uptake by the oysters during an ebb tide. No consistent difference was seen between July 1 and July 21. When chlorophyll concentrations increased during the bloom between July 21 and the end of the deployment the difference in chlorophyll concentrations between the North sensor and the South sensor became more consistent. The consistency was limited to ebb tides occurring at night however. Bloom conditions suggest that this may have been due to active photosynthesis.

Looking at a three day window between July 27 and July 30 (Figure IV.11) during which time chlorophyll concentrations exceeded 10-15 ug/L, uptake of chlorophyll by the oysters accounted for the removal of 8.9 and 7.0 ug/L chlorophyll on July 28 and July 29, respectively. This removal represented over 60% removal by oyster uptake.

Using mean current velocity data (0.02m/s), mean depth (0.7m) and the dimensions of the oyster culture site (7.5m N-S, 11.0m E-W) an estimate of the total mass of chlorophyll uptake by the oysters. The total volume of water under the floating oyster bags was 58 m^3. Using 2cm/s average water velocity, the amount of water passing under the oyster bags was 4524m^3 and 4620m^3 for the shaded areas on July 28 and July 29, respectively. The observed 6.9 and 8.9 ug/L chlorophyll deficit translates to roughly 40 g Chl removed on July 28 and 32 g Chl removed on July 28.
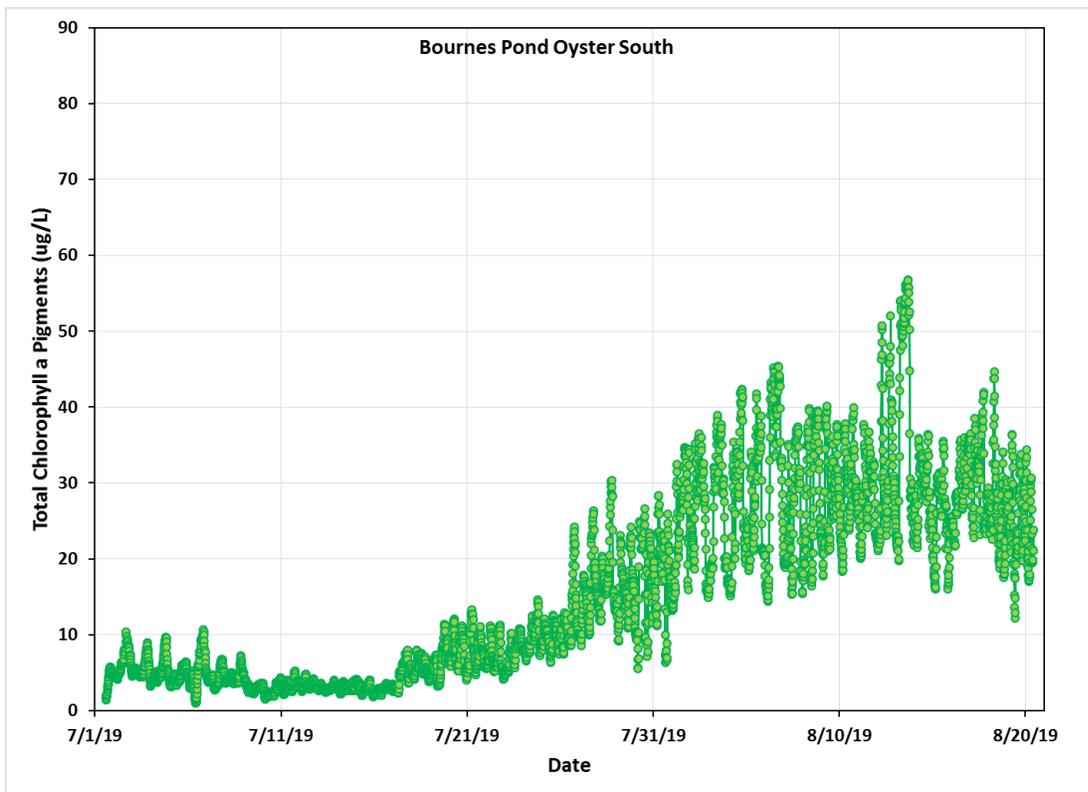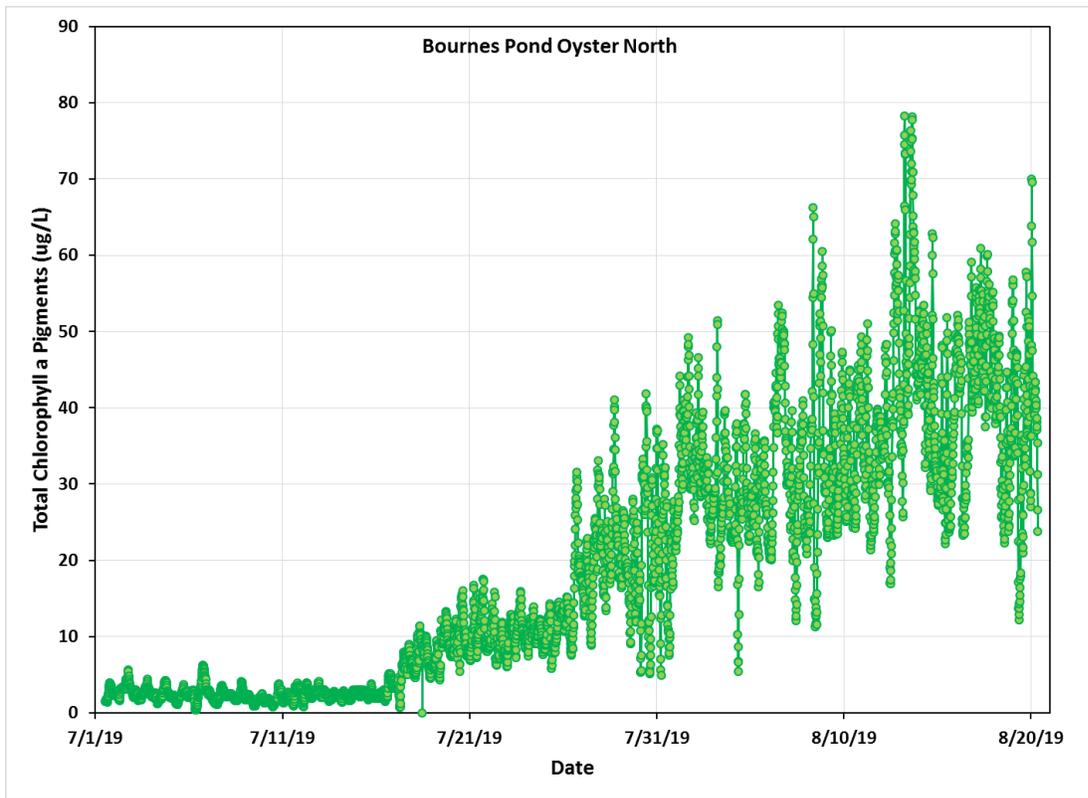
Figure IV.10: Chlorophyll data from the North meter above the array (top panel) and South meter below the array (bottom panel).
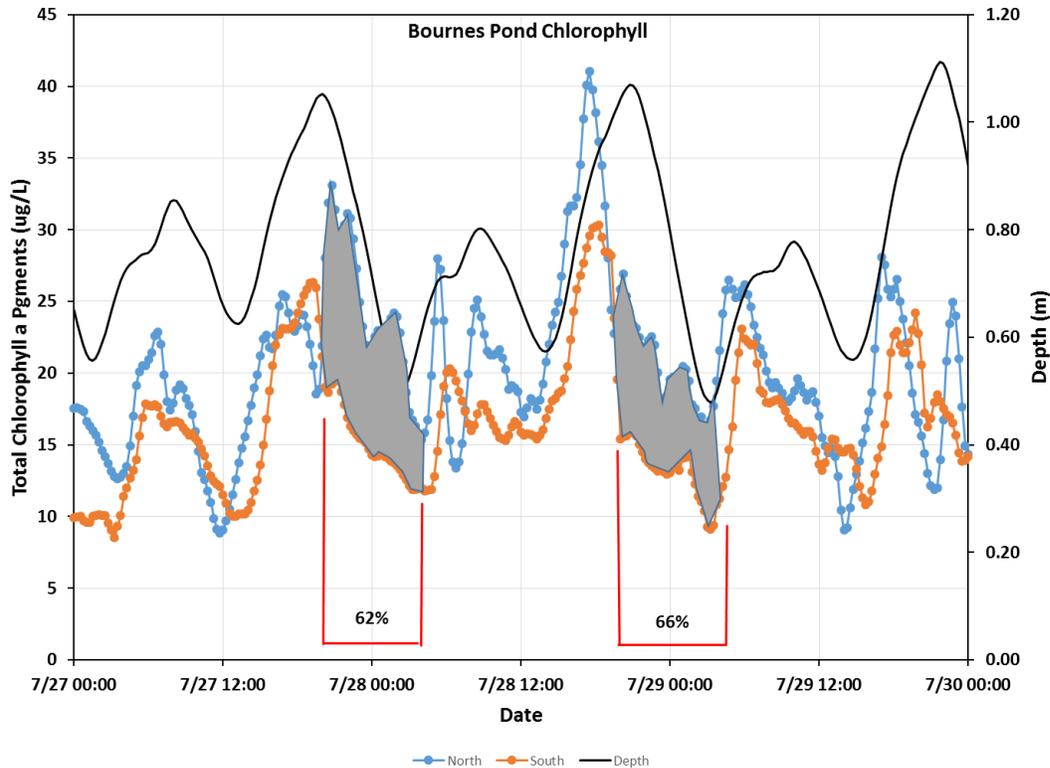
Figure IV.11: Magnified two day view of chlorophyll concentrations recorded at the northern and southern margins of oyster farm. Chlorophyll uptake is shown in gray and represents the difference between the upstream and downstream measurements. Average percent uptake is shown for each day in the red brackets.

Tidal Flux Related Water Quality Results:

In order to measure the change in depth and water quality associated with tidal flows passing through the oyster array samples were collected of the watercolumn at an upstream and multiple downstream sites during ebb and flood tidal cycle. Taken at one hour intervals, beginning low tide at 7:10 on September 16, 2019, integrated water column samples at stations Oys N, Oys S, and station 2 during both the ebb or flood stages of the tidal cycle. Additional samples were collected of water that had passed through the array at 4, 8 ,16, and 32 m distance downstream from the array (respective to tidal flow). A total of 4 flood and 4 ebb tide sampling events were performed (Figure IV.12). Station selection was based on capturing any effect of the aquaculture beds and with parallel flow velocities measured by Acoustic Doppler Current Profiler (ADCP). Depth was used as a record to calculate the volume of water captured at each grab sample. Examination of the depth record indicates a tidal range of approximately 0.34 m between the max ebb and flood, using stations Oys N and Oys S since station locations do not change through the tidal cycle.

In order to capture water quality differences as tides transport nutrients and plankton through the oyster aquaculture site and oysters produce biodepostion to be transported by the tide, entire water column grab samples were analyzed for N constituents (NH_4, NO_x, TDN, and PON), PO_4, chlorophyll-*a*, TSS and salinity. During both ebb and flood tide, noticeable reductions in chlorophyll-*a*, bioactive N, TN, and TSS were observed in the 0-4 m on the downgradient side of the array compared to the water upgradient that had yet to pass through the oyster array. But nutrients show little to no difference further than 4 meters from the downgradient edge of the array due to mixing and dispersion with the higher concentration waters that had not been effected by the oyster activities, such as the water in the East Channel. Flood tide results are constrained to reductions between Oys N and Oys S at 0 m, then also show some mixing >4 m from the border of the array. Under both ebb and flood tide conditions, chlorophyll-a, bioactive N and TSS was lower after passing through the array by 30%-40% (compare the N and S stations).
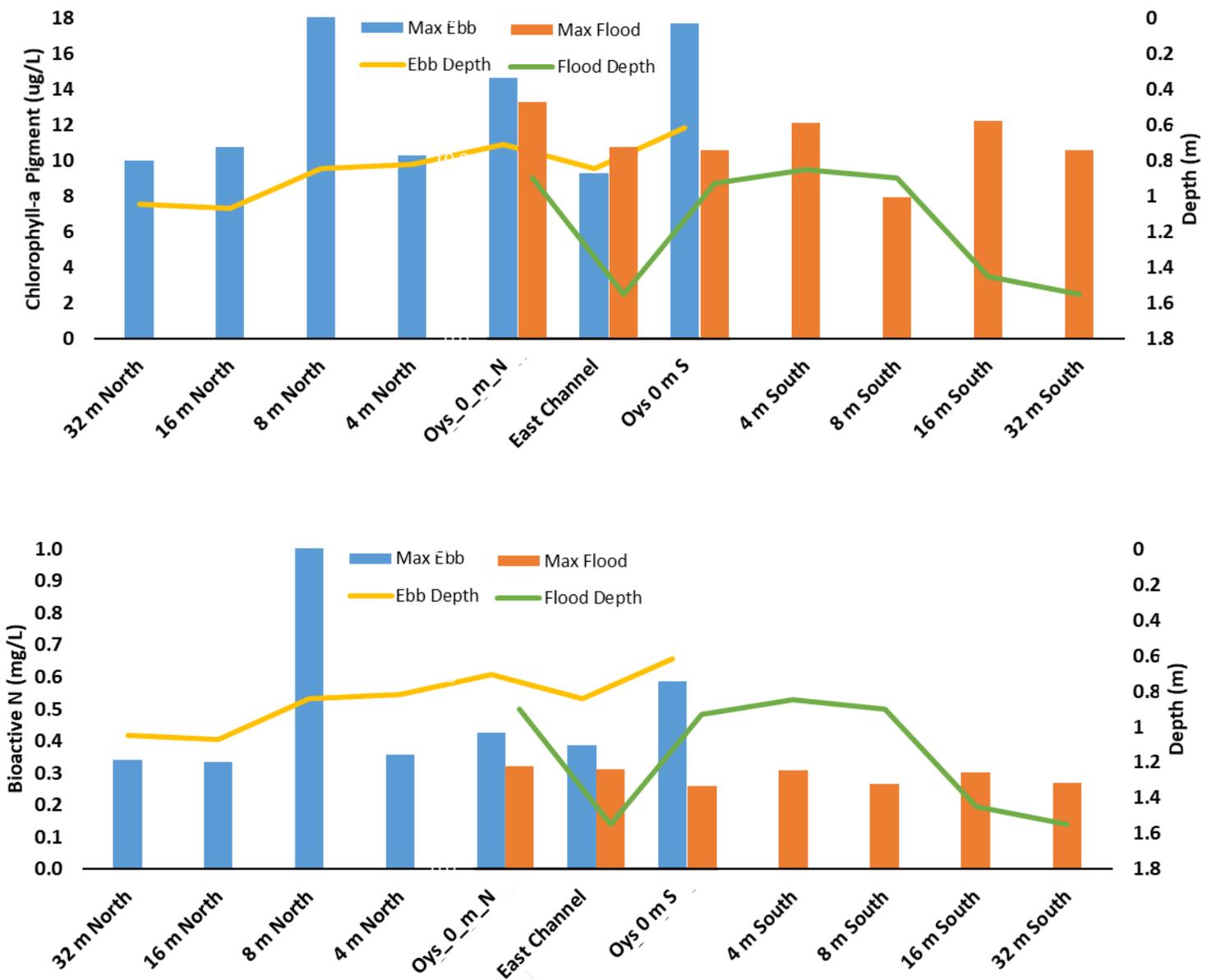
Figure IV.12: Tidal record of nutrient constituents chlorophyll-*a*, bioactive N (NH_4+NO_x+PON), during max ebb (blue) and max flood (orange) events on September 16, 2019 across stations at 0 m, 4 m, 8 m, 16 m, 32 m, and adjacent (East Channel) to oyster aquaculture site. Depth was recorded at each station on ebb (yellow line) and flood (green line) cycles.
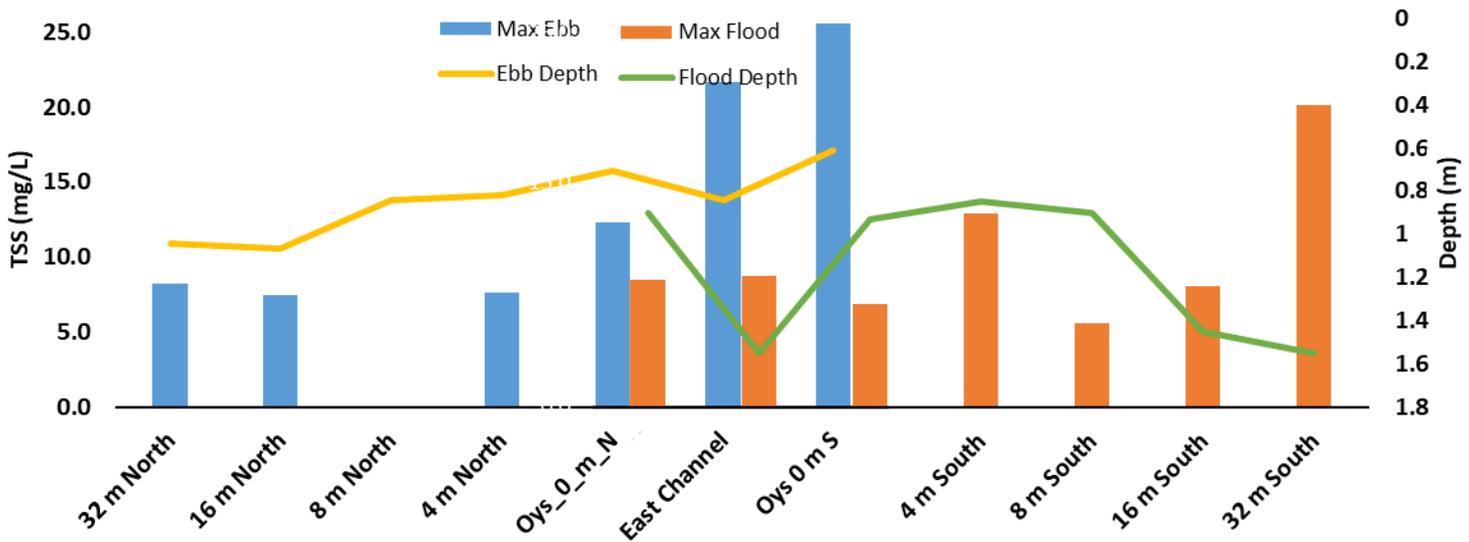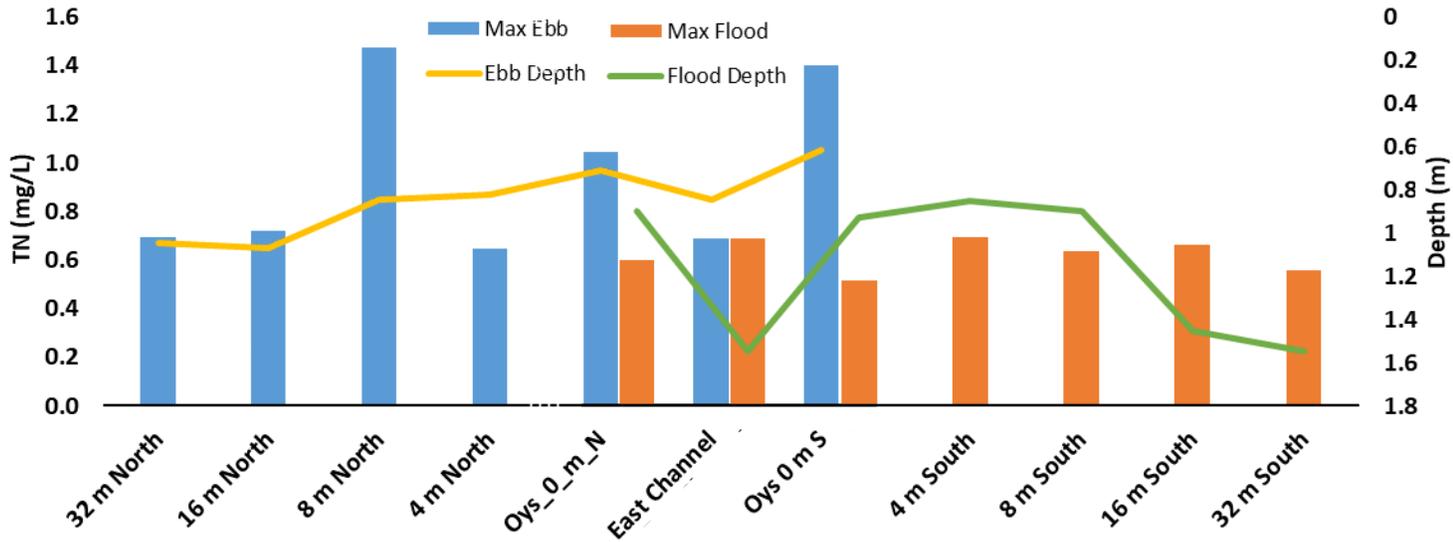
Figure IV.12 cont'd: Tidal record of nutrient constituents total nitrogen (TN), and total suspended solids (TSS) during max ebb (blue) and max flood (orange) events on September 16, 2019 across stations at 0 m, 4 m, 8 m, 16 m, 32 m, and adjacent (East Channel) to oyster aquaculture site. Depth was recorded at each station on ebb (yellow line) and flood (green line) cycles.

3. Biodeposition by Oysters

Biodeposit traps were deployed every 3 weeks in 24-hour intervals to evaluate seasonal changes in oyster biodeposition (feces+pseudofeces) to the sediments over the grow-out period. Samples were weighed and analyzed for the rate of total biodeposits dry weight per m2/d and for N mass per m2/d with rates expressed as grams dry weight per m2 and total deposition for the 54 m2 array (Figure IV.13)[4]. The total amount of biodeposit produced per bag over 207-day deployment was estimated 1.47 ± 0.06 kg dry weight, which amounts to 158.85 ± 6.68 kg of biodeposits for the entire propagation array (9.25 g dry wt./m2/day). The effect of biodeposits on the sediments from the oyster farm contributed an estimated total of 4.71 ± 0.21 kg of N to the sediments over the deployment period.

Figure IV.13: Total biodeposits (top) and N (bottom), by dry weight, captured on each date from four individual bags (orange) and the mean (blue) on that date.

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[4] The area of the array of the bags only (e.g. no spaces) was 54 m^2, the area inside the outline of the array was 83 m^2 including gaps between bags, and the impact area receiving biodeposits, 200 m^2.

4. Biodeposit Impact Area

Although biodeposits settle relatively rapidly through the water column, factors such as biodeposit density, current velocity and water depth can affect t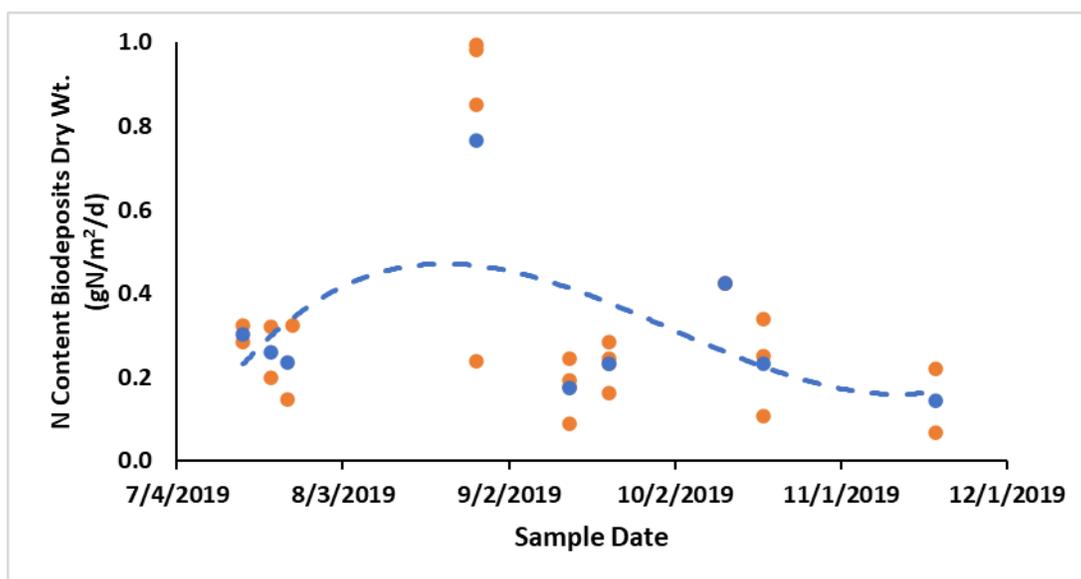he dispersion of biodeposits before they settle to the sediment surface. During the July 2019 sediment regeneration and September tidal flux measurements, an upward facing Acoustic Doppler Current Profiler (ADCP, Aquadopp Profiler HR 2 MHz ADCP, Nortek USA Inc., Boston, MA) was deployed near the sediment surface in the center of the oyster aquaculture area. Velocity measurements were made at 10-minute intervals and conducted at the highest possible sensitivity. Velocities measured acoustically track particle movement using sound reflected off of particles suspended within the water column and give water velocity based upon the assumption that the particles move passively with the flowing water. The particles in the vicinity of the oyster bags are comprised of a mixture of biodeposits and phytoplankton, with the latter being numerically dominant. Therefore, measured horizontal velocities are probably biased towards lower velocities as the larger sized biodeposits tend to settle faster and move less laterally. However, direct measures of biodeposit fall rates using lazers suggests relatively good agreement with ADCP measurements, but also supports that the ADCP measured horizontal velocities are conservative.

ADCP velocity data suggest a shear at the surface and the bottom (no-slip bottom velocity condition) of the water column with peak velocities approximately twenty centimeters below the bottom of the floating bags. Horizontal velocity measurements completed in September 2019 were consistent and generally confirmed measurements made in July with RMS velocities for July of 1.78 ± 0.02 and September of 2.17 ± 0.04 cm/s. As seen in the July and September time series plots (Figure IV.14), the highest depth averaged velocities were recorded during ebb tides. During both velocity surveys the current direction was primarily south-southwest, possibly due to the influence of fresh water inputs to the pond headwaters. Also, flow was primarily tidally driven in the aquaculture area as no significant wind driven currents were observed.

Using the previously determined mean biodeposit vertical settling velocity (8.14 ± 5.01 mm/s) and the mean depth around the border of the oyster deployment area, the horizontal displacement of biodeposits from oyster bags was modeled step-wise assuming using the average biodeposit production per bag across the array (Figure IV.15). The September model results largely confirm the results from July concerning the extent of the spatial distribution of the impact area of surficial sediments. For example, 2 meters downgradient from the southwest corner of the deployment area deposition is 15% of that directly under the deployment area and moving across the array to the southeast corner deposition increases to 30% of the deposition rate directly under the array as depth increases from 0.5 to 1.0 meters.

Integrating the results from biodeposition over the entire array yields a total impact area of 200 m^2, an expansion of 117 m^2 over the oyster deployment area footprint of 83 m^2 (Figure IV.16). These spatial estimates for biodeposition represent direct settling to sediments, but do not include

bordering areas that may be affected by particle transport along the sediment surface post-deposition. These direct deposition areas and extended areas of deposition were used to determine the enhanced particulate nitrogen loading to Bournes Pond sediments from the deployed oyster bags and to target areas to assess potential enhancements of sediment nitrogen regeneration and denitrification.
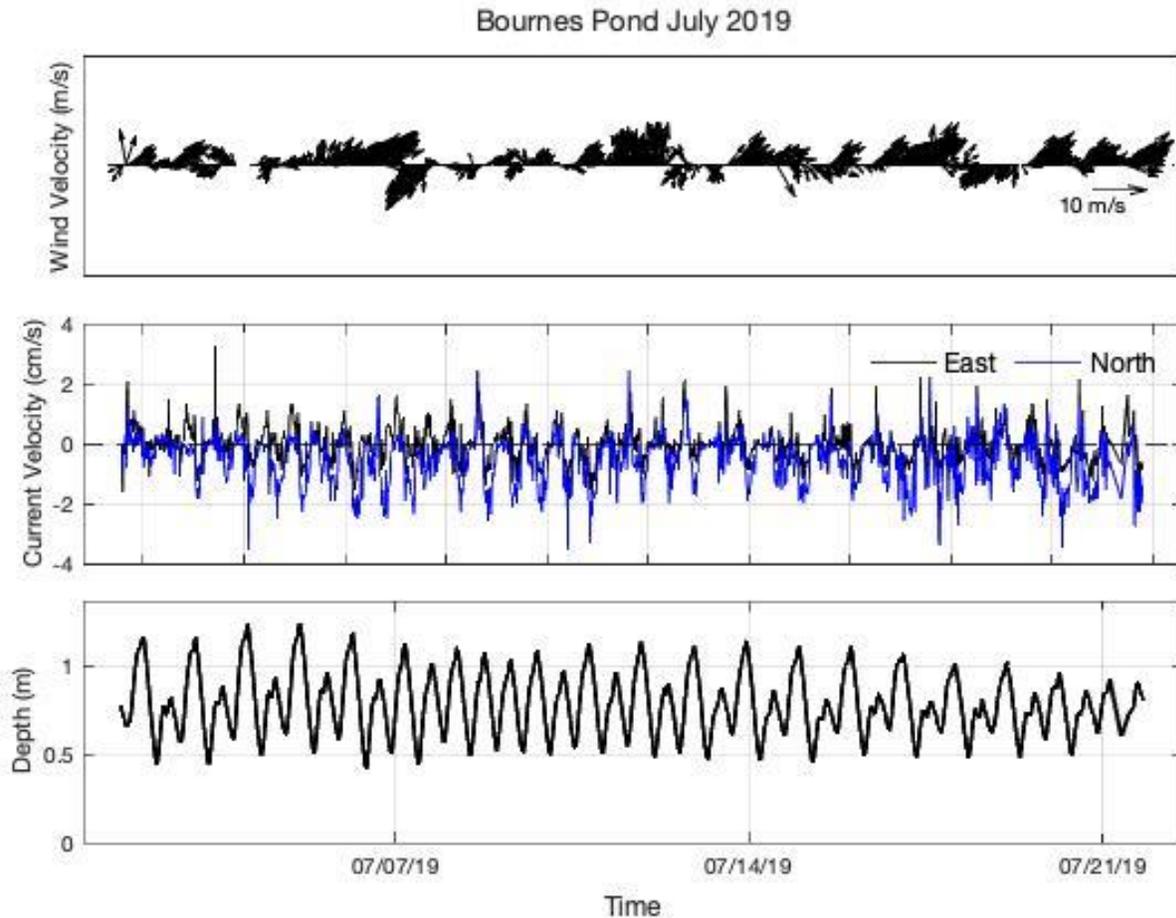
Figure IV.14: July 2019 Bournes Pond time series plots showing (top) wind velocity (m/s), (middle) depth integrated current velocity (cm/s), and (bottom) depth for each month. Wind direction is the direction from which the wind is blowing and direction is relative to true north. Wind velocity data are from Falmouth Otis National Guard Airport (NOAA local climate data) and current velocity and depth data are from the bottom deployed ADCP in the center of the Bournes Pond oyster deployment area.
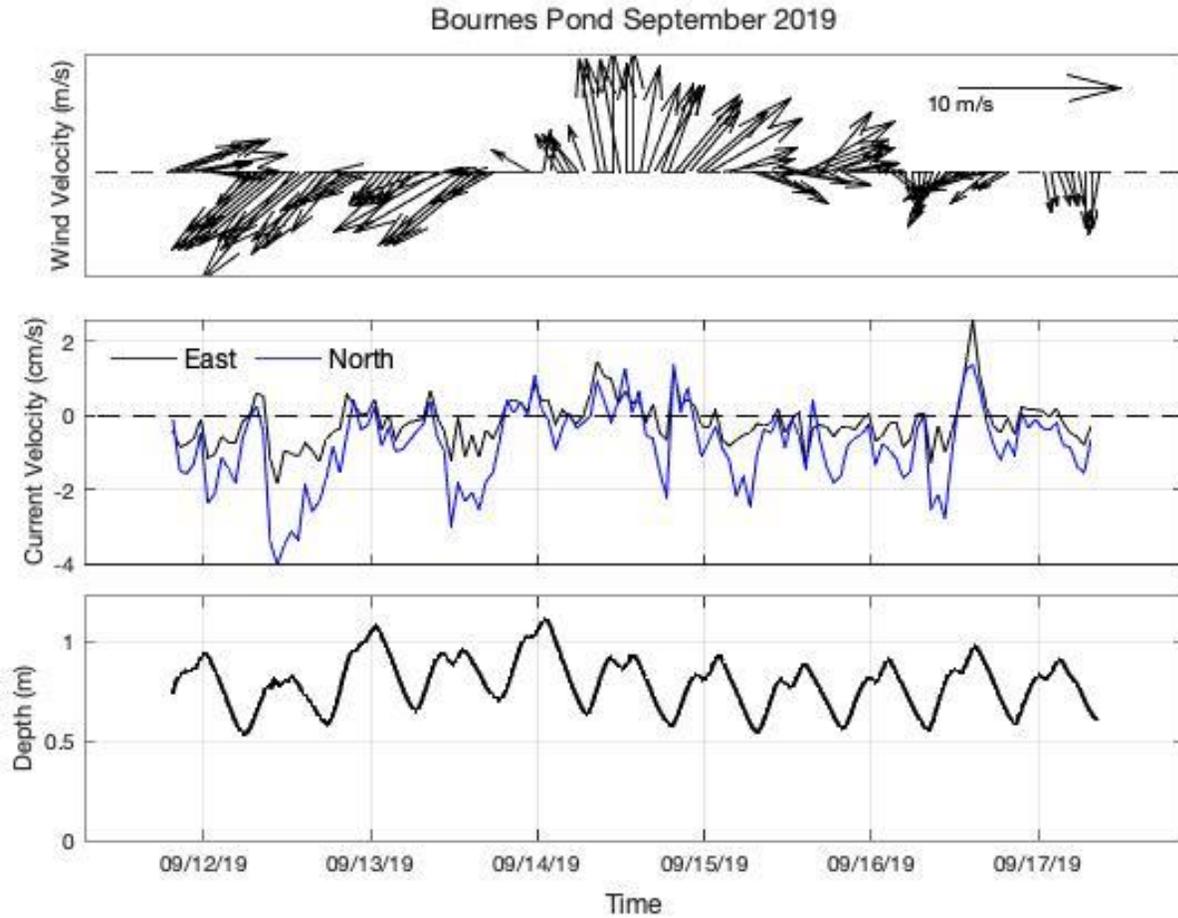
Figure IV.14 cont'd: September 2019 Bournes Pond time series plots showing (top) wind velocity (m/s), (middle) depth integrated current velocity (cm/s), and (bottom) depth for each month. Wind direction is the direction from which the wind is blowing and direction is relative to true north. Wind velocity data are from Falmouth Otis National Guard Airport (NOAA local climate data) and current velocity and depth data are from the bottom deployed ADCP in the center of the Bournes Pond oyster deployment area.
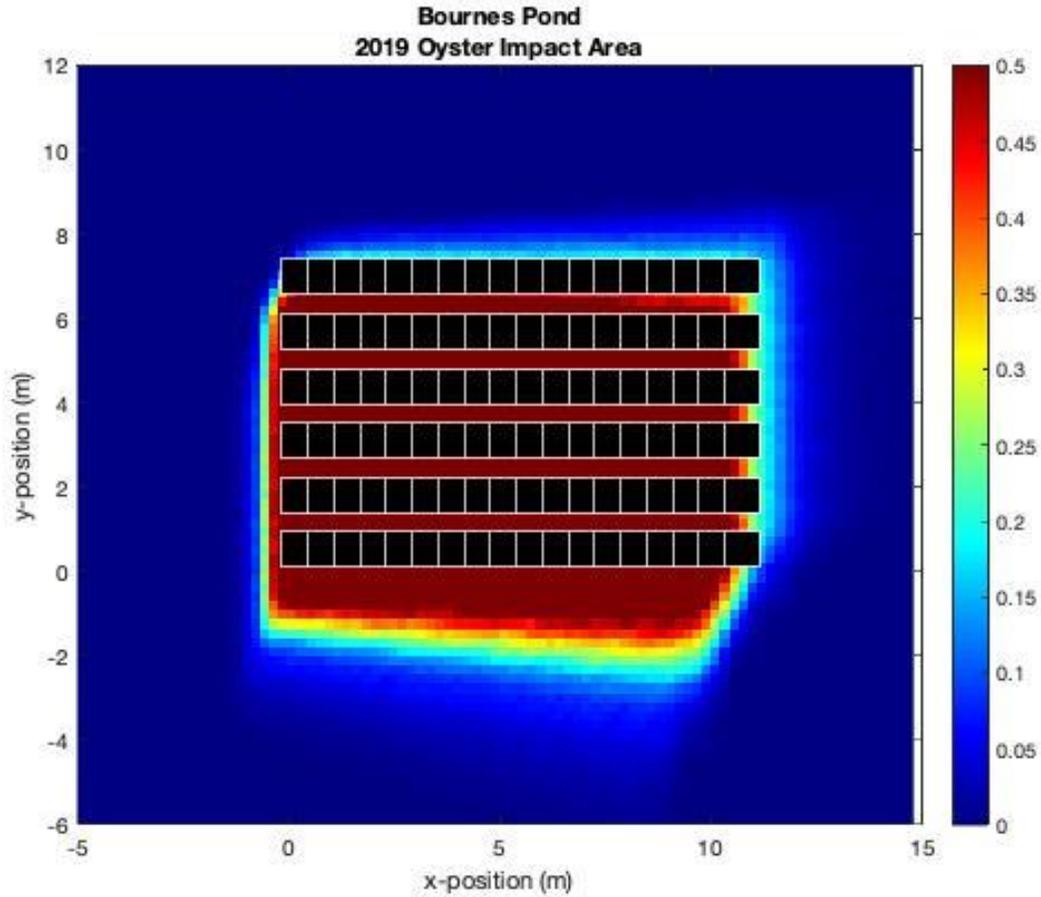
Figure IV.15: Projected area of sediment receiving biodeposits from the array of floating oyster bags. Biodeposit production was given as 1 and results are shown in the contours as a fraction of the biodeposit production. Areas colored dark red received 100% of the average areal rate of biodeposit production to the sediment surface; areas colored dark blue received 0% of the biodeposit production and not directly impacted by the oyster deployment.
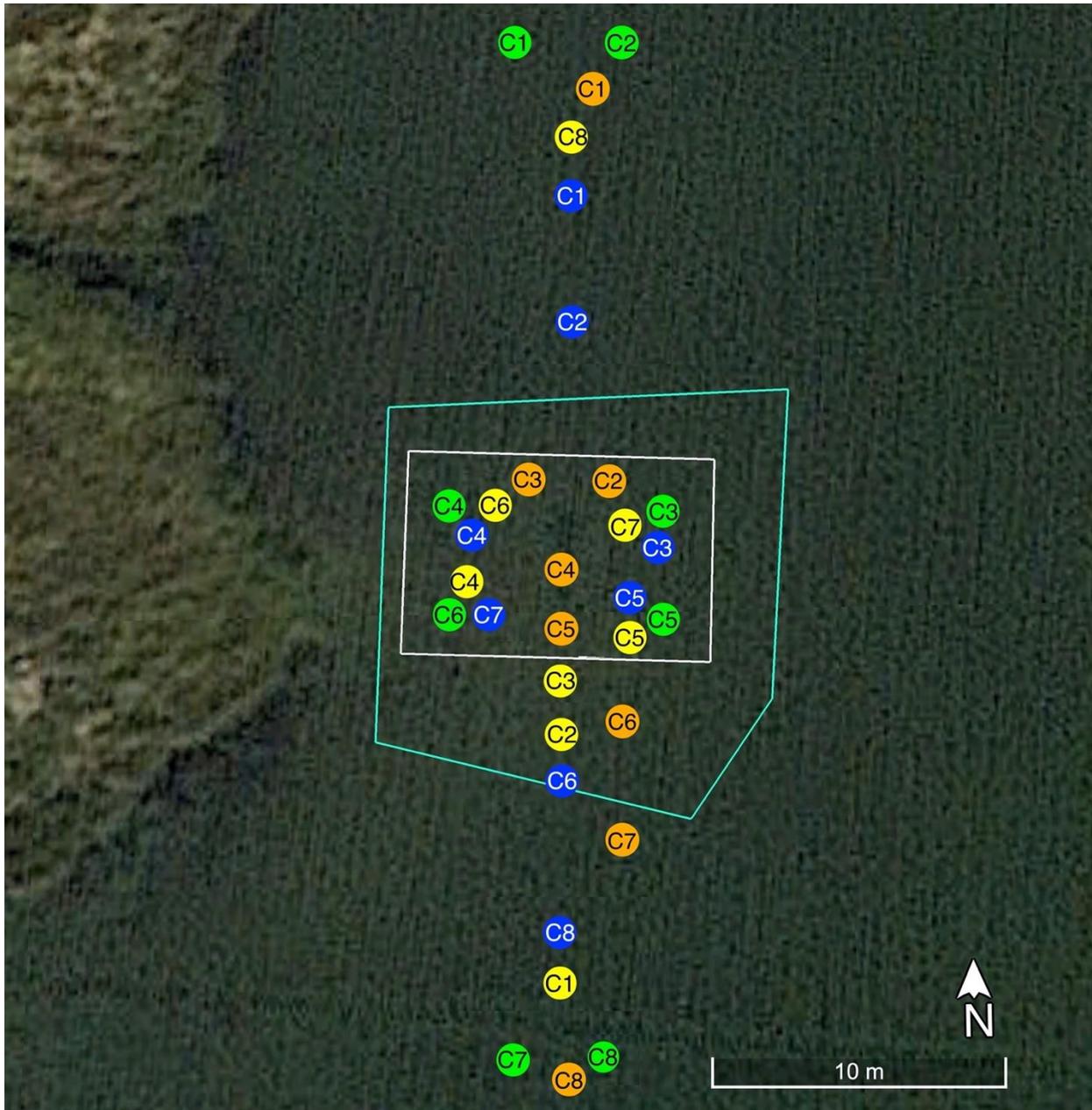
Figure IV.16: Aerial photograph showing the location of the 2019 oyster deployment area (white polygon) and summer/fall sediment flux locations (July: green markers, August: blue markers, October: yellow markers, April: orange markers).). Total area impacted by the oyster deployment as determined by biodeposit displacement in 2019 is shown as the outer (teal) bounded area.

5. Oyster Effects on Sediment Nitrogen Cycling: Regeneration and Denitrification

To determine the contribution of sediment regeneration to nutrient levels within the oyster aquaculture portion of Bournes Pond and the effect the oysters may have on nitrogen recycling rates and oxygen levels, sediment samples were collected and incubated under in situ conditions. In July, August, October of 2019, as well as, April of 2020, intact sediment cores were collected within and outside of the oyster aquaculture site to determine the effect of enhanced deposition to the sediment surface from biodeposition (Figure IV.16). Sediment cores were collected from outside the oyster biodeposition impact area to serve as "controls" and inside the impact area to determine if oyster aquaculture makes a measurable difference in the nitrogen flux rates or level of denitrification in the sediments underlying the deployment area. It will also provide sediment nitrogen regeneration rates to determine how oysters potentially effect overall water column nitrogen levels in Bournes Pond during the critical summer management period, when impairment is greatest.

Benthic nutrient regeneration results are summarized in Table IV.3 below. Both the July and August cores were collected to coincide with peak growing season. The effect of biodeposits on the sediments was apparent in the summer months of July and August when primary productivity and oyster growth are greatest. In comparison to control cores, the highest release of organic N was found in August. Sediment oxygen demand (SOD) was nearly double the rate for cores below the oyster array (Table IV.3). The greatest remineralization of N was in July with the greatest contrast being between incubation cores extracted from the treatment area and control sites. Incubation cores showed more than 20 fold higher NH4+ release from the impact area (Table IV.4). In contrast, control sediment cores displayed more removal through NO3- and DON uptake with August resulting in significantly different rates (Table IV.5).

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 mud with some mixed sand in the treatment cores. Overall, it appears that the rate of sediment metabolism was enhanced by oyster biodeposits within the deployment site in Bournes Pond. Both carbon mineralization (organic matter decay as measured as SOD) and nitrogen remineralization and release as ammonium were consistently higher in sediments receiving biodeposition compared to "background" areas at distance (Table IV.3). This was clear on all dates except during April 2020.

In order to assess the residual effects of oyster biodeposition after harvest and after the winter period of very low metabolism, as early spring warming coincides with an increases in primary productivity, sediment flux rates were analyzed again in April 2020 (Table IV.6). Results from the April sediment core incubations indicated uniformity between control and treatment sites, which infers sediment erosion and biodeposit redistribution likely attributable to high energy hydrodynamics caused by winter storms having redistributed the surficial biodeposits.

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In other oyster aquaculture study locations, such as Lonnies Pond in Orleans, MA. sediment cores were collected underneath the suspended floating oyster aquaculture array 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. The SOD rates in treatment cores in Bournes Pond were similar with average SOD being 173 mmol m-2 day-1 with a low of 73 mmol m-2 day-1 in April. Cores collected under the oyster bags in Bournes Pond do not appear to have received the same amount of biodeposition as seen in Lonnies Pond, likely due to the significantly smaller aquaculture footprint and greater flows. Yet the SOD rates in Bournes Pond were higher when compared to the Mashpee River's oyster aquaculture site. The Mashpee River's sediment regeneration rates in and around bottom mounted trays of oysters showed treatment SOD rates of 69.9 mmol m-2 day-1 and control rates of 62.1 mmol m-2 day-1 averaged over three growing seasons showing the variability in grow-out technique and effects on biodeposition.

July, August, and October 2019 control sediment cores had a distinct contrast from treatment sediment cores, where the oyster biodeposits increased the rates of SOD uptake and ammonium release. Of all the flux dates, July 2019 had the greatest rates of denitrification, as measured by N_2 production across both treatment and control cores, followed by August and then October. Where significant denitrification has been observed, it is primarily related to the increased deposition of labile organic matter. The high control and treatment SOD rates seen in July support this hypothesis (Table IV.3). By summing the product of the measured rates of denitrification by the intervals between the denitrification measurements and extending measurements forward to mid-November when temperatures begin to become too cold for significant oyster activity and microbial activity is slowed, it is possible to obtain an annual mass of nitrogen that was denitrified, which ca the be compared to the total mass of nitrogen removed from the system by incorporation into oyster biomass. The annual enhanced denitrification resulted in a net loss of 2.87 kg N in 2019. Compared to the net annual nitrogen removed by oysters (7.86 kg N), enhanced denitrification accounted for 36.5% of the mass of nitrogen removed by oyster harvest. Therefore, the total mass of nitrogen removed by enhanced denitrification and oyster harvest was 10.73 kg for 2019. However, given the temporal uncertainty, for planning purposes, a % denitrification of 25% might be considered reasonable as it reaches across several aquaculture/denitrification assessments in s.e. Massachusetts, including the new results from Bournes Pond in 2019.

Table IV.3: Average sediment oxygen demand (SOD) and nutrient flux rates for control and treatment (under oysters) sediment cores.

		SOD		NH4		NO3		DON		N$_2$-N	
		Rate	S.E.	Rate	S.E.	Rate	S.E.	Rate	S.E.	Rate	S.E.
		(mmol/m^2/d)		(mmol/m^2/d)		(mmol/m^2/d)		(mmol/m^2/d)		(mmol/m^2/d)	
July	Control	-213.5	129.7	0.07	1.02	-0.60	0.33	-8.12	2.84	32.18	28.26
	Treatment	-298.7	155.5	18.73	5.67	-0.91	0.26	-5.12	7.22	43.35	7.13
August	Control	-118.4	4.5	-0.47	0.11	-1.32	0.18	-4.72	3.07	3.29	0.08
	Treatment	-235.3	5.0	10.03	0.77	-0.57	0.13	15.77	17.24	6.29	2.21
October	Control	-57.5	2.4	1.75	0.13	-0.69	0.11	-0.38	1.09	1.06	0.75
	Treatment	-93.9	1.9	11.84	0.96	-0.65	0.11	2.26	1.90	2.6	0.2
April	Control	-75.2	2.2	0.98	0.34	-0.46	0.17	-0.44	0.15	ND	ND
	Treatment	-73.1	3.6	0.85	0.33	-0.42	0.15	-0.32	0.36	ND	ND

Table IV.4: July sediment incubation SOD and nutrient flux rates (negative values = influx, positive values = efflux) for control (white rows) and treatment (red rows) sediment cores in the study area.

Jul-19	SOD			NH4			NO3			DON			Denite Rate		
	Rate	S.E.	n	Rate	S.E.	n	Rate	S.E.	n	Rate	S.E.	n	Rate	S.E.	n
Core ID	(mMoles/m^2/d)			(µMoles/m^2/d)			(µMoles/m^2/d)			(µMoles/m^2/d)			(µMoles/m^2/d)		
C1	-101	3	7	-1205	119	5	-803	96	6	-9434	2932	5	5025	1940	3
C2	-156	9	5	770	41	3	-884	131	6	-3863	2253	5	35413	0	2
C3	-470	5	4	19713	681	5	-1211	262	5	-10219	5194	4	59629	14255	4
C4	-107	4	6	16596	501	5	-1025	140	5	-3588	2132	6	48612	0	2
C5	-365	4	5	12578	2302	5	-794	57	6	4455	8442	5	58839	0	2
C6	-253	17	5	26016	5051	5	-627	68	5	-11136	1737	4	6304	0	2
C7	-198	0	5	-299	67	5	-150	33	6	-9778	1992	5	18055	3363	3
C8	-399	2	5	1015	570	5	-582	138	4	-9412	2881	5	70244	0	2

Table IV.5: August sediment incubation SOD and nutrient flux rates (negative values = influx, positive values = efflux) for control (white rows) and treatment (red rows) sediment cores in the study area.

Aug-19	SOD			NH4			NO3			DON			Denite Rate		
	Rate	S.E.	n	Rate	S.E.	n	Rate	S.E.	n	Rate	S.E.	n	Rate	S.E.	n
Core ID	(mMoles/m^2/d)			(∝Moles/m^2/d)			(∝Moles/m^2/d)			(∝Moles/m^2/d)			(∝Moles/m^2/d)		
C1	-77	9	5	-622	19	4	-1003	224	6	-8142	2633	5	2223	747	3
C2	-178	1	5	-802	234	5	-1973	237	5	-2226	384	5	4357	634	3
C3	-313	1	6	6267	1106	5	-475	107	6	34628	6879	5	7683	5524	4
C4	-215	7	5	14064	359	6	-875	328	4	-3323	1926	5	5040	3727	3
C5	-183	0	5	1424	1492	4	-194	46	4	24192	4665	6	6158	1123	3
C6	-141	5	7	5253	492	6	-325	95	6	-1812	2694	5	ND	ND	ND
C7	-324	12	6	23123	395	4	-970	92	6	25171	2726	5	ND	ND	ND
C8	-100	3	5	20	66	6	-977	85	6	-3789	1730	6	ND	ND	ND

Table IV.6: October sediment incubation SOD and nutrient flux rates (negative values = influx, positive values = efflux) for control (white rows) and treatment (red rows) sediment cores in the study area.

Oct-19	SOD			NH4			NO3			DON			Denite Rate		
	Rate	S.E.	n	Rate	S.E.	n	Rate	S.E.	n	Rate	S.E.	n	Rate	S.E.	n
Core ID	(mMoles/m^2/d)			(μMoles/m^2/d)			(μMoles/m^2/d)			(μMoles/m^2/d)			(μMoles/m^2/d)		
C1	-31	4	5	2116	120	6	-895	87	6	-1483	351	6	788	106	3
C2	-62	3	5	783	34	6	-768	140	6	-114	473	5	486	164	4
C3	-77	0	5	1552	72	6	-583	98	5	1022	711	6	-1984	656	4
C4	-102	2	5	9623	273	6	-485	124	6	4005	1171	5	4086	0	2
C5	-169	2	10	25741	2542	6	-1181	181	6	3613	1157	6	3737	562	3
C6	-55	1	8	3750	175	6	-426	87	6	1467	683	5	540	199	4
C7	-49	2	6	8240	863	6	-515	57	5	-47	1011	6	1961	226	4
C8	-60	3	7	2542	310	6	-517	132	6	-949	389	6	1910	612	4

Table IV.7: April sediment incubation SOD and nutrient flux rates (negative values = influx, positive values = efflux) for control (white rows) and treatment (red rows) sediment cores in the study area.

Apr-20	SOD			NH4			NO3			DON			Denite Rate		
	Rate	S.E.	n	Rate	S.E.	n	Rate	S.E.	n	Rate	S.E.	n	Rate	S.E.	n
Core ID	(mMoles/m^2/d)			(μMoles/m^2/d)			(μMoles/m^2/d)			(μMoles/m^2/d)			(μMoles/m^2/d)		
C1	-80	4	6	1343	423	5	-486	185	6	-479	152	5	ND	ND	ND
C2	-76	5	7	1560	621	6	-384	143	6	-70	39	5	ND	ND	ND
C3	-74	2	5	999	302	5	-463	174	6	-955	292	5	ND	ND	ND
C4	-67	5	6	904	385	6	-333	124	6	-176	153	5	ND	ND	ND
C5	-80	2	5	523	219	6	-444	155	6	-178	63	5	ND	ND	ND
C6	-69	4	5	258	98	5	-482	176	6	-204	82	5	ND	ND	ND
C7	-76	3	5	860	341	5	-494	182	6	-568	171	4	ND	ND	ND
C8	-70	0	5	744	268	6	-392	150	6	-268	102	6	ND	ND	ND

6. Major Findings

The 2019 oyster aquaculture pilot in Bournes Pond significantly lowered chlorophyll a, TN, bioactive N and TSS in water passing through the array, as seen in previous oyster deployments. The removal of organic matter by filtration resulted in significant oyster biodepostion to the sediments in the region of the aquaculture array. The tidal measurements on ebb and flood tides showed a nearfield reduction of 30%-40% in chlorophyll-a, bioactive N and TSS after passing through the array (compare the N and S stations). A larger reduction was seen in chlorophyll by the continuous recording sensors placed at the margins of the array mainly on ebbing tides with levels >10 ug/L where chlorophyll a levels dropped by as much as 60 % in water from immediately before and after passing through the array.

Given the high rate of particle removals it was possible to track the fall trajectory of the biodeposits to map the area of the bottom sediments impacted by organic matter enrichment associated with the biodepostion associated with oyster activities which had a foot print of 200

m^2 compared to the 83 m^2 of the array alone. Nitrogen regeneration, denitrification and oxygen uptake were all enhanced in the enrichment area compare to surrounding sediments.

Direct nitrogen removal by the oysters (growth and harvest) and indirect removal by enhanced denitrification were both observed with N uptake by the oysters through removal at harvest being the major pathway. In addition to physical removal of oyster N, the oyster activities, mainly filtration/biodepositon, resulted in enhancement of the sediment N cycle and denitrification and specifically it resulted in important additional N removal. Given the available denitrification measurements over the annual cycle, it was determined that this N removal pathway may have added to the total N removal by the oyster array as much as 36%. While this denitrification removal is in line with several aquaculture/denitrification assessments throughout the region it is within the upper range. It is recommended that a more conservative value of 25% be used for management projections as it would allow for areas that do not show these moderate-high levels of aquaculture enhanced denitrification that were found in Bournes Pond in 2019. It should be noted that the high rates of sediment associated denitrification in this study compared to the 2018 Bournes Pond oyster deployment were likely due to the siting of the aquaculture array in a low velocity depositional area to allow organic enrichment of the sediments compared to the previous deployment. Equally important the observed moderate to high rates were also likely enhanced by the generally oxygenated bottom waters compared to measurements in other field deployments in the region with similar rates of biodeposition.