RIBBED MUSSEL NUTRIENT BIO-EXTRACTION PILOT PROJECT

Final Report
Southern New England Coastal Watershed Restoration Program
Nutrient Management Grant

EPA Grant Number: CE96184201

June 6, 2016

Robbie Hudson
Thomas E. Kutcher
Julie M. Rose
Mark S. Dixon

This project was funded by an agreement (CE96184201) awarded by the Environmental Protection Agency to the New England Interstate Water Pollution Control Commission on behalf of the Narragansett Bay Estuary Program.
Table of Contents

Acknowledgements .................................................................................................................. 2
Disclaimer ................................................................................................................................. 2
List of Acronyms ..................................................................................................................... 2
Abstract .................................................................................................................................. 2
Introduction ............................................................................................................................. 3
Methods ..................................................................................................................................... 4
  Growth Rates .......................................................................................................................... 4
  Tissue Mass and Nitrogen Content ......................................................................................... 7
  Real-time Bioextraction using Biodeposition ......................................................................... 7
  Statistical Analysis ............................................................................................................... 9
Results ...................................................................................................................................... 10
  Mussel Dimension and Mass Analysis ................................................................................. 10
  Growth ................................................................................................................................. 11
  Nutrient Analysis ................................................................................................................ 12
  Water Quality ...................................................................................................................... 13
  Real-time Bioextraction ...................................................................................................... 13
Discussion ............................................................................................................................... 15
Literature Cited ........................................................................................................................ 19
Appendix I ................................................................................................................................ 22
Appendix II ............................................................................................................................ 24
Appendix III ........................................................................................................................... 25
Acknowledgements
We would like to thank the funders of this project who made this research possible: Environmental Protection Agency (EPA), New England Interstate Water Pollution Control Commission (NEIWPCC), Narragansett Bay Estuary Program (NBEP), and Senators Jack Reed and Sheldon Whitehouse. Thanks to Dr. Graham Forrester for guidance during the initial study design. We also appreciate all the work by NOAA Northeast Fisheries Science Center Staff, BioProcesses H2O, our coworkers at Save The Bay, and the interns and volunteers that assisted in this project.

Disclaimer
This project was funded by an agreement (CE96184201) awarded by the Environmental Protection Agency to the New England Interstate Water Pollution Control Commission on behalf of the Narragansett Bay Estuary Program. Although the information in this document has been funded wholly or in part by the United States Environmental Protection Agency under agreement CE96184201 to NEIWPCC, it has not undergone the Agency’s publications review process and therefore, may not necessarily reflect the views of the Agency and no official endorsement should be inferred. The viewpoints expressed here do not necessarily represent those of the NBEP, NEIWPCC, or U.S. EPA nor does mention of trade names, commercial products, or causes constitute endorsement or recommendation for use.

List of Acronyms
- EPA: Environmental Protection Agency
- NBEP: Narragansett Bay Estuary Program
- NEIWPCC: New England Interstate Water Pollution Control Commission
- NOAA: National Oceanic and Atmospheric Administration
- STB: Save The Bay (Providence, RI)

Abstract:
The upper portions of Narragansett Bay have high levels of anthropogenic nitrogen. Excess nitrogen can lead to overproduction of algae, which reduces light infiltration, can directly smother benthic organisms, and can reduce local oxygen levels in the water when it decomposes. Filter feeding bivalves can indirectly reduce water-column nitrogen by consuming plankton, but edible bivalves can pose a human health threat in polluted waters, making their restoration controversial. Ribbed mussels are not commonly eaten and thus could be useful for bioextraction in polluted systems. Ribbed mussels can effectively consume nutrient-rich seston from the water column while in their natural intertidal settings or while continually submerged. We grew adult ribbed mussels in three settings: (1) a fringing salt marsh, (2) hanging continually submerged in shallow water from a floating raft, and (3) in a shellfish aquaculture upweller that continually forced water past the animals to theoretically increase feeding rate. We used total mass and dimensions (primarily shell length) to estimate tissue mass growth, which we used as a proxy for net seasonal bioextraction efficiency. We additionally measured the bioextraction efficiency of a small number of mussels in real time using the biodeposition method. Mean
seasonal growth in length of typical-size adult mussels (mean initial length = 6.23 cm) ranged from 0.15 cm to 0.28 cm among the settings during the growth season, whereas smaller animals grew more rapidly (0.27 to 1.2 cm over a similar period). We estimate an average net nitrogen bioextraction of 7 to 13 mg per animal over the period using growth data compared with 0.03 to 0.09 mg per hour per g of dry weight using the biodeposition method. Continually submerged and intertidal animals appeared to grow similarly; whereas animals subjected to increased seston delivery by an aquaculture-style upweller appeared to grow slowest, indicating that factors associated with aquaculture grow-out equipment may have suppressed growth. Problems we encountered with the grow-out equipment during this project likely contributed to these results. Our findings suggest that ribbed mussels were able to successfully acclimate to constant submersion, and that our field site had sufficient quantity and quality of food to support good growth. Our bioextraction estimates may be useful to inform projects aimed at nitrogen mitigation of eutrophic estuarine waters using ribbed mussels.

Introduction:
The upper portions of Narragansett Bay have high levels of anthropogenic nitrogen from various sources. Excess nitrogen leads to overproduction of algae, which reduces important light infiltration, can directly smother benthic organisms, and can reduce local oxygen levels in the water when it decomposes. Widespread turbidity, macroalgae growth, and low oxygen have been documented in the upper bay, and state environmental regulatory agencies and their partners in Massachusetts and Rhode Island have reacted by taking steps to reduce nitrogen inputs (Narragansett Bay Commission, unpublished data). These partners have made great progress in reducing municipal wastewater inputs, the largest source of nitrogen to the bay (Pryor et al. 2007). However, substantial inputs of anthropogenic nitrogen remain from treatment limitations, on-site wastewater treatment systems, and urban stormwater (Greene and Deacutis, 2000; Pryor et al. 2007; Industrial Economics Inc. 2012). It has become clear that restoring healthy levels of nutrients to upper Narragansett Bay will require a multi-prong approach that includes not only mitigation at the various sources, but additionally the restoration of ecosystem-based feedback processes that reduce nitrogen while enhancing or restoring the ecological function of the system.

Filter-feeding animals can reduce nitrogen concentrations in the water column by converting plankton into fleshy biomass and shell, thus removing it from the water column (Furnas et al. 1976; Sournia 1978). Therefore, changes in the nitrogen mass within the biomass of an animal can be measured to estimate the net amount of nitrogen (intake minus waste) that animal has extracted from the water column over a given period of time, assuming any nitrogen not incorporated into tissue is returned to the system. Shellfish have been extensively studied to assess their effectiveness in extracting nutrients from the water column through this process known as bioextraction (Galimany et al. 2013a, Galimany et al. 2013b, Galimany et al. 2015), and their abundance has been implicated in the restoration of eutrophic systems (Kuenzler 1961). However, the restoration of shellfish to polluted waters is problematic in that it creates a situation where their value as a food source may cause the local community to harvest and eat
the shellfish at the risk of contamination. The use of non-edible shellfish for bioextraction circumvents this problem (Leonard and Macfarlane, 2011).

Ribbed mussels (*Geukensia demissa*) are not commonly eaten by people, are able to withstand periods of drought and extreme fluctuations in temperature and salinity, are able to filter both in their natural intertidal niche and while fully submerged (Galimany et al. 2013b), and play a critical role in the health of salt marshes by exhibiting a cooperative relationship with marsh plants and animals (Bertness 1999). As a noncommercial species, ribbed mussels are suitable for bioextraction in both bacteria- and nutrient-impaired waters where public health and commercial shellfishing concerns make use of other shellfish species impractical. Preliminary findings of an ongoing NOAA study suggest that ribbed mussels may be equally or more efficient than oysters at bioextraction and are capable of filtering finer particles including pathogenic bacteria (M. Dixon, personal communication). Galimany et al. (2015) found that differential makeup of suspended nutrient sources in the water column among study sites can strongly affect ribbed mussel feeding and assimilation rates, making site selection an important factor in determining the efficacy of using ribbed mussel for nutrient bioextraction. It is therefore important to test ribbed mussel bioextraction in the specific water body of concern.

We assessed the capacity and efficiency of ribbed mussels for bioextraction in the Providence River, an urbanized eutrophic sub-embayment of Narragansett Bay, in Rhode Island. We assessed differences in growth among animals with access to different rates of exposure to the same ambient water over a growing season by measuring dimensional and mass change before and after deployment. We subjected adult ribbed mussels to three growing settings to determine how growth, as a proxy for nutrient bioextraction, varies under natural intertidal, continually submerged, and force-fed continually-submerged (i.e. an aquaculture-style grow-out setting) water regimes. We also developed models to estimate tissue mass, nitrogen content, and net bioextraction rate from the measurements. We then compared our findings to real-time bioextraction rates measured during the same time period in the same ambient waters. Our findings will help to determine whether there are advantages among scaled-up bioextraction enhancement strategies: (1) restoration to a natural setting, (2) suspending ribbed mussels from in-water infrastructure, and (3) creating ribbed mussel growing infrastructure that increases flow.

**Methods:**

**Growth Rate**

Ribbed mussels were placed in three distinct environmental settings (one natural and two in-situ experimental treatments) to detect and document any differences in growth rates, used as a proxy for net seasonal bioextraction efficiency, among the groups. We examined the nutrient bioextraction rates using two settings where the mussels were continuously submerged, compared with mussels returned to an adjacent salt marsh - their natural setting. Specifically, we tested whether ribbed mussel bioextraction was most effective from a passive aquaculture-style raft (hereafter Passive), an increased flow apparatus (hereafter Active), or in a natural
setting (hereafter Control). The study was conducted off the Save The Bay Center at Fields Point in the lower Providence River (Fig 1).

Figure 1. Locations of treatments in upper Narragansett Bay, Rhode Island; red box represents Active treatment, green box represents Passive treatment, and stars represent Control.

The Passive treatment mussels ($n=100$) were grown in flexible tubular mesh media suspended from a floating raft structure (similar to those used in Galimany et al. 2013). Ribbed mussels were placed inside of these mesh “socks” (supplied by Coastal Aquacultural Supply, App. 1), which are specifically designed to secure and maintain shellfish for aquaculture grow-out. The socks were hung underneath the raft, continuously submerging the mussels for the duration of the experiment, except when removed once weekly for scrubbing off biofouling organisms with a stiff nylon brush, a method commonly used in blue mussel aquaculture.

Active treatment mussels ($n=100$) were grown in a floating upweller system, or “FLUPSY,” that used an electric motor and pump to constantly move ambient sea water from below across the animals as they remained submerged while contained in a chamber attached to a floating dock (the silo). The FLUPSY theoretically increased the opportunity to for the animals to filter nutrients from the water (App. 1). Regular maintenance of the FLUPSY was conducted on a once-weekly basis to prevent fouling and to minimize algal growth.

For both of the above treatments, ribbed mussels were harvested from the nearby Control site, a fringing marsh along the lower Providence River ($41°47.128’$ N, $71°22.807’$ W). We selected average sized adults from the salt marsh to represent the characteristic population. The mussels were cleaned of biofouling organisms, measured, bagged, and relocated to the raft and FLUPSY at each replicate location. Control mussels were cleaned, measured, and returned to their salt marsh locations. We used 100 animals per treatment and 100 control animals ($50$ animals per treatment X $2$ replicates X $3$ settings = $300$). Dimensions and mass of all animals
were measured at the beginning (May, 2015) and end (October, 2015) of the experiment. Shell dimensions of all animals were measured to the nearest millimeter using scientific calipers. Length (L) was measured along the longest axis of the animal approximately parallel to the axis of hinge, width (W) was measured across the widest point of the shell, approximately perpendicular to the axis of the hinge, and depth (D) was measured as the widest point approximately perpendicular to the intersection of the L and W measurements (App. 2). Total animal mass (M) was measured by removing all biofouling organisms from the shells of the animals, drying the shells with a cotton cloth, and weighing each entire animal individually on a Denver Instrument’s electronic scale.

Treatments were compared within replicates and across replicates to determine the extent by which differences in nitrogen bioextraction were related to treatments versus localized conditions or other potentially confounding circumstances. Dimensions and total mass of all whole animals were recorded at the onset and at the end of the experiment, pre and post-growing season, respectively

Survival of animals in each treatment was determined by quarterly counts of deceased animals, by observing whether the shells had separated, indicating the release of the abductor muscle that accompanies the death of this species. Dead animals were returned to the treatments.

Note: Two mussels that were already harvested and were to be used in the control died prior to deployment into the salt marsh at the beginning of the experiment; these were replaced by two animals of similar shell size.
**Tissue Mass and Nitrogen Content**

An additional sample of animals \((n = 60 = 10 \times 6\) replicates) was gathered from the Control marsh site at the onset of the experiment to determine nitrogen content of the fleshy tissues. Nitrogen content of the shell was disregarded, as nitrogen content in the shell is low (e.g. \(\approx 0.2\%\) dry weight for oysters) and mussel shell to flesh mass ratio is low relative to shellfish, such as oysters, in which shell nitrogen is an important consideration (Rodhouse et al. 1984, Higgins et al. 2011). The shell dimensions \((L, W, D)\) and mass of these animals were measured as described above. The animals were then flash frozen on dry ice and all fleshy tissues were removed from the shells and weighed while frozen. We used these measurements to develop best-fit regression models to estimate fleshy tissue mass from the dimensions and total animal mass of our experimental samples. The tissue samples were then delivered frozen to University of Maine Darling Marine Center in Walpole, Maine for nitrogen content analysis, following Zimmermann et al. (1997). In the laboratory, mussel tissues of each replicate were homogenized prior to nitrogen content analysis.

At the end of the growing season, a subset of experimental animals from Passive, Active, and Control treatments were measured and analyzed for nitrogen content, as above, to determine whether fleshy tissue nitrogen content varied among treatments or compared with animals analyzed at the experiment onset. Results were generated for each of the three treatments \((n = 20 \times 3\) replicates \(\times 3\) treatments = 180). We then used the nitrogen content, by proportion of fleshy biomass, to estimate net nitrogen assimilation rate over the growing (experiment) period.

**Real-time Bioextraction using the Biodeposition Method**

Additional growth rates of ribbed mussels were determined from two-point measurements of a separate sample of labeled individuals. Mussels for growth measurements were collected in May 2014 from a fringe salt marsh adjacent to Save The Bay, and suspended in mesh bags from the Save The Bay dock. On 6/11/14, 25 mussels were cleaned of epiphytes and other encrusting organisms, shell length (defined as the longest length of the shell approximately parallel to the hinge) was determined using calipers, numbered plastic tags were attached to their shells using epoxy, and mussels were returned to the mesh bags for the remainder of the growing season. Bags were cleaned of tunicates and other fouling organisms approximately every two weeks. Mussels were individually checked and cleaned once at the end of July, then final collection and shell length measurements were completed in the field on 11/4/14.

Mussel filtration rate and nitrogen absorption rate were measured using the biodeposition method (Hawkins et al., 1996; Iglesias et al., 1998). Mussels for biodeposition measurements were collected in May 2014 and May 2015 from a fringe salt marsh adjacent to Save The Bay, suspended in mesh bags from the Save The Bay dock, and allowed to acclimate for >1 month. Biodeposition measurements were conducted monthly using adult mussels with lengths ranging from 42.3 to 64.8 mm. Biodeposition measurements were conducted at Save The Bay using two portable, flow-through devices designed to quantify mussel feeding on natural seston (Galimany et al. 2011). Briefly, an underwater pump suspended at 1m depth continuously pumped seawater into a PVC “reservoir” tank. This tank was aerated to maintain suspension of
particles in the water. Seawater flowed from the lower part of the reservoir tank through 20 tubes, each connected to an individual, flow-through chamber. Flow rates to each chamber were calibrated before each measurement. Sixteen of the chambers each contained a single live mussel; four were control chambers that each contained one empty mussel shell.

Sixteen mussels were selected randomly from the suspended mesh bag at the Save The Bay dock for each measurement, and epiphytes and other encrusting organisms were removed from the shells. A small, plastic, velcro fastener was glued to each mussel and used to affix the mussel to the flow-through chamber. Each mussel was exposed to a constant flow rate of 12 L h\(^{-1}\) of ambient water; this flux was shown in previous laboratory experiments to result in homogeneous distribution of particles between chambers with no water recirculation or lateral flow between the chambers (Galimany et al., 2011). Mussels were allowed to recover for 2 hours in the chambers from any stress associated with handling.

To synchronize the seston available with the corresponding biodeposits produced by the mussels, it was necessary to estimate the gut transit time (GTT). Gut transit time was defined as the minimum time for an organic particle to pass through the digestive tract of a mussel after ingestion. This variable was determined for each experiment using a method adapted from Hawkins et al. (1996). Five ribbed mussels from the same suspended mesh bag were placed in individual beakers containing a mixture of local seawater and cultured Tetraselmis chui (PLY 429). The time that elapsed between the mussel opening to feed on cultured T. chui and the first deposition of green-colored feces was considered to be the minimum gut transit time. The average GTT of all feeding mussels was used to synchronize the collection of seawater and corresponding biodeposits.

All samples collected during the biodeposition measurement (water, feces, and pseudofeces) were filtered separately through Whatman GF/C filters (25 mm diameter). Prior to the biodeposition measurements, filters were washed with Milli-Q water, dried for 24 hours at 65°C, ashed in a muffle oven for four hours at 450°C, and weighed on an analytical balance. After sample collection, filters were rinsed with either isotonic ammonium formate (filtration rate) or Milli-Q water (nitrogen absorption), kept on ice, and transferred to the laboratory.

Total particulate matter (TPM) was determined by drying ammonium formate-rinsed sample filters at 65°C for 48 hours and re-weighing on the analytical balance. Particulate inorganic matter (PIM) then was determined by ashing samples at 450°C for 4 hours and re-weighing. Particulate organic matter (POM) was calculated as the difference between TPM and PIM. The organic content of the water \((f)\) was calculated as the mean organic fraction of total particulates \((f=POM/TPM)\). In 2015 only, particulate nitrogen of the seston was also determined for fresh water-rinsed sample filters using a Costech ECS 4010 CHNS elemental analyzer (Valencia, CA). All samples were dried in an oven (60°C) overnight. With all runs a standard reference material (SRM 8704 Buffalo River Sediment) was analyzed with the samples, with a reported total carbon value of 3.351%. Our recovery for total carbon was 3.150% ± 0.327% (n=50) with our sample analysis, which is within the reported value range.
During the biodeposition measurements, 300 ml seawater was collected every 15 minutes from the intake pump and from the exit flow of each control chamber. Collection of feces and pseudofeces was delayed by the length of the gut transit time to synchronize the seawater collection with the feces production. Individual flow-through chambers were cleaned immediately before beginning feces and pseudofeces collection, then each biodeposit type was collected separately from each individual mussel with a pipette as soon as produced. The time period of collection of biodeposits was between 1 and 2 hours; length was determined based on collection of sufficient biodeposits by the end the measurement for accurate quantification. Feces and pseudofeces produced by each mussel \((n=16)\) were filtered separately and processed for organic and inorganic matter measurements and nitrogen content as described above to compute the rates of total, organic, inorganic, and nitrogen egestion and rejection, respectively. Data from the few mussels that produced no feces or pseudofeces (i.e., did not open) during the measurement period were not included in subsequent analyses. Filtration rate and nitrogen absorption rate then were calculated according to the biodeposition method (Iglesias et al., 1998; Hoellein et al., 2014).

All mussel variables were standardized to 1 g of dried mussel flesh using the following equation:

\[
Y_s = Y_e x \left(\frac{1}{W_e}\right)^b
\]

Where, for the physiological variables, \(Y_s\) is the standardized physiological rate, \(Y_e\) is the experimentally-determined rate, and \(W_e\) is the dry body mass measured for each mussel. We used a \(b\) value of 0.83 as determined by Riisgård (1988) for \(G. demissa\).

**Statistical Analysis**

Data were analyzed using statistical software (SAS OnDemand and WinSTAT, R.K. Fitch 2016). Laboratory and field measurements were used to generate simple regressions showing the relationships among dimension (length, width, and depth), biomass, and nitrogen content in pre-growing-season animals. Similar regressions were generated at the end of the experiment to show the relationships for post-growing-season animals using animals from the experiment. The regressions were used to estimate biomass and nitrogen content from the dimensional measurements. Regression analysis outcomes were then applied to estimate the bioextraction capacity of ribbed mussels under the various conditions. Analysis of Variance (ANOVA) was used to compare treatment parameters and control parameters to determine differences in growth and nutrient content. During the ANOVA analysis, each variable \((L, W, D, M)\) was looked at separately, and a Levin’s Test was conducted to test for homogeneity (after which the variances for the variables were assumed to be the same). QQ-Plots were run to test for normality and Tukey HSD tests were used in multiple comparisons of each variable separately across the three treatments. All statistics were tested at a 0.05 significance level.
Results:

*Mussel Dimension and Mass Analysis*

Using simple regression (power function for dimensions and linear for mass), all dimensions and total animal mass strongly predicted tissue mass at the onset of the experiment, i.e. before the growing season ($n = 59$; Fig. 1): length ($R^2 = 0.98$, $P < 0.01$), width ($R^2 = 0.95$, $P < 0.01$), depth ($R^2 = 0.95$, $P < 0.01$), mass ($R^2 = 0.99$, $P < 0.01$). We removed a single data point (of 60) that was identified as an outlier in these regressions, likely resulting from an erroneous tissue mass measurement. Direct measurements were less effective for predicting tissue mass at the end of the experiment, i.e. after the growing season ($n = 276$): length ($R^2 = 0.63$, $P < 0.01$), width ($R^2 = 0.58$, $P < 0.01$), depth ($R^2 = 0.50$, $P < 0.01$), total mass ($R^2 = 0.76$, $P < 0.01$).

![Graphs showing tissue mass predictions](image)

Figure 1. Best-fit models predicting ribbed mussel soft tissue mass from direct measurements at the onset of the experiment.

Using length as a representative measure, replicates of the treatments did not differ at the onset or end of the experiment (Table 1); we therefore pooled replicates to increase statistical power. Initial length ranged from 4.30 to 8.13 cm ($\bar{X} = 6.81 \pm 0.74$ cm). Survival among treatments was: Active = 83%, Passive = 92% and Control = 93%. We detected no differences in ribbed mussel size among the treatments at the onset of the growing period for the variables length (ANOVA; $F = 0.86$, $P = 0.43$), width ($F = 0.35$, $P = 0.71$), depth ($F = 1.36$, $P = 0.26$), and mass ($F = 0.11$, $P = 0.89$) at the 5% confidence level (Table 2, App. 3). Likewise, no differences were detected among treatments at the end of the growing period for length ($F = 1.89$, $P = 0.15$), width ($F = 2.33$, $P = 0.10$), depth ($F = 0.51$, $P = 0.60$), and mass ($F = 2.09$, $P = 0.13$) (Table 3, App. 3.)
Table 1. Discrimination of homogeneous subsets of replicates × treatments of ribbed mussel length (n = 50 per replicate = 300 total) using ANOVA and Tukey test, where A = Active, P = Passive, C = Control, W = westward replicate, E = eastward replicate, S = start of the experiment, and E = end of the experiment.

<table>
<thead>
<tr>
<th>Homogeneous subsets</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>AWS</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>PWS</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>CWS</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>PES</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>AES</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>AEF</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>CWF</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>AWF</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>PWF</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>CES</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>PEF</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>CEF</td>
<td></td>
<td>*</td>
</tr>
</tbody>
</table>

Table 2. Size ranges for ribbed mussels at the onset of the growing period

<table>
<thead>
<tr>
<th>Confidence Interval</th>
<th>Length (cm)</th>
<th>Width (cm)</th>
<th>Depth (cm)</th>
<th>Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active (n = 100)</td>
<td>5.95-6.24</td>
<td>2.52-2.71</td>
<td>1.96-2.07</td>
<td>15.62-18.11</td>
</tr>
<tr>
<td>Passive (n = 100)</td>
<td>5.92-6.23</td>
<td>2.50-2.64</td>
<td>1.94-2.06</td>
<td>15.20-17.72</td>
</tr>
<tr>
<td>Control (n = 102)</td>
<td>6.06-6.33</td>
<td>2.53-2.64</td>
<td>2.01-2.11</td>
<td>15.55-17.67</td>
</tr>
</tbody>
</table>

Table 3. Size ranges for ribbed mussels at the end of the growing period

<table>
<thead>
<tr>
<th>Confidence Interval</th>
<th>Length (cm)</th>
<th>Width (cm)</th>
<th>Depth (cm)</th>
<th>Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active (n = 100)</td>
<td>6.10-6.39</td>
<td>2.58-2.71</td>
<td>2.14-2.25</td>
<td>16.73-19.33</td>
</tr>
<tr>
<td>Passive (n = 100)</td>
<td>6.21-6.50</td>
<td>2.67-2.80</td>
<td>2.17-2.29</td>
<td>18.32-21.16</td>
</tr>
<tr>
<td>Control (n = 102)</td>
<td>6.31-6.58</td>
<td>2.67-2.78</td>
<td>2.13-2.25</td>
<td>17.01-19.32</td>
</tr>
</tbody>
</table>

**Growth**

According to ANOVA and Tukey HSD tests at a significance level of 0.05, evidence of growth over the growing period was detected in Depth for animals across all treatments (Active, Passive, Control), in Width for Passive and Control treatments, and in total Mass for the Passive treatment (Tables 2 and 3, Fig. 2, App. 3). Our regression models (Fig.1) suggest that changes in total Mass and Length best represent net growth of tissue mass over the period and thus would best serve as proxies for bioextraction rate.

We found seasonal growth to be similar among treatments. Although we did not detect significant differences among treatment samples at the end of the experiment for any of our measures, all measures indicated the same trend: mean growth was highest for the Passive treatment, followed by Control, and lastly Active (Fig. 2, Table 4).
Ribbed Mussel Nutrient Bio-Extraction Pilot Project
EPA Grant Number: CE96184201

Figure 2. Mean total mass and length of ribbed mussels before and after a single growing season, where C = Control, P = Passive, A = Active, S = Start (i.e. before the growth period), and F = Finish (i.e. after the growth period).

Table 4. Net growth of ribbed mussels over the growing season in 2015

<table>
<thead>
<tr>
<th></th>
<th>Length (cm)</th>
<th>Width (cm)</th>
<th>Depth (cm)</th>
<th>Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change Active</td>
<td>0.15</td>
<td>0.03</td>
<td>0.18</td>
<td>1.17</td>
</tr>
<tr>
<td>Change Passive</td>
<td>0.28</td>
<td>0.17</td>
<td>0.23</td>
<td>3.28</td>
</tr>
<tr>
<td>Change Control</td>
<td>0.25</td>
<td>0.14</td>
<td>0.13</td>
<td>1.56</td>
</tr>
</tbody>
</table>

Nutrient Analysis
Nitrogen content did not significantly differ with time or treatment (ANOVA; $F = 1.16$, $P = 0.18$), and pooled nitrogen content was 9.78% nitrogen by dry weight. (Table 5, App. 3).

Table 5. Nitrogen content (% dry weight) of ribbed mussel soft tissue at the onset of the experiment, after the growing period by treatment, and pooled; N is number of replicates of six-animal aggregate samples measured

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean %</th>
<th>Conf. (±)</th>
<th>Std. Error</th>
<th>Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset</td>
<td>10</td>
<td>9.82</td>
<td>1.16</td>
<td>0.51</td>
<td>1.62</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>10.23</td>
<td>1.33</td>
<td>0.59</td>
<td>1.85</td>
</tr>
<tr>
<td>Passive</td>
<td>9</td>
<td>8.67</td>
<td>1.96</td>
<td>0.85</td>
<td>2.55</td>
</tr>
<tr>
<td>Upweller</td>
<td>10</td>
<td>10.28</td>
<td>1.65</td>
<td>0.73</td>
<td>2.31</td>
</tr>
<tr>
<td>Entire sample</td>
<td>39</td>
<td>9.78</td>
<td>0.69</td>
<td>0.34</td>
<td>2.12</td>
</tr>
</tbody>
</table>

We suggest length as a best-fit model to estimate soft tissue mass because total animal mass (which includes the shell and all contents within) is susceptible to sampling errors stemming from shell water loss, desiccation (Gallardi et al. 2014), and biofouling. Using the regression model for length, 9.78% nitrogen content derived from our laboratory analysis, and assuming a constant wet-weight to dry-weight ratio of 8.9, similar to un-stored blue mussels (8.9 derived from Gallardi et al. 2014), zebra mussels (8.8 derived from Smolders et al. 2004), and bivalves (8.7 from Ricciardi and Bourget 1998), we estimate that the average net nitrogen bioextraction
per ribbed mussel over the growing period was 7 mg N in the Active treatment, 13 mg N in the Passive, and 12 mg N in the Control (Fig. 3).

![Modeled nitrogen content per adult ribbed mussel per growing season in the Providence River, RI.](image)

Figure 3. Modeled nitrogen content per adult ribbed mussel per growing season in the Providence River, RI, where C = Control, P = Passive, A = Active, S = Start (i.e. before the growth period), and F = Finish (i.e. after the growth period).

**Water Quality**
Precipitation at the study site during the growth period was 3.3 cm in June, 1.6 cm in July, 1.9 cm in August, 0.7 cm in September, and 2.7 cm in October. Other physical environmental factors are listed in Table 6.

**Table 6. Results of physical environmental measurements taken during the growing period**

<table>
<thead>
<tr>
<th></th>
<th>May to Oct (n=23)</th>
<th>June (n=4)</th>
<th>July (n=5)</th>
<th>Aug (n=4)</th>
<th>Sept (n=4)</th>
<th>Oct (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avg. Air Temp. (°C)</td>
<td>20.51</td>
<td>20.6</td>
<td>22.2</td>
<td>23.1</td>
<td>22.1</td>
<td>15.3</td>
</tr>
<tr>
<td>Avg. Water Temp. (°C)</td>
<td>22.22</td>
<td>20.8</td>
<td>23.6</td>
<td>24.9</td>
<td>24.9</td>
<td>17.6</td>
</tr>
<tr>
<td>Avg. Salinity (ppt)</td>
<td>26.35</td>
<td>23.9</td>
<td>27.6</td>
<td>28.5</td>
<td>27.0</td>
<td>26.2</td>
</tr>
<tr>
<td>Avg. Dissolved Oxygen (mg/l)</td>
<td>8.68</td>
<td>9.5</td>
<td>8.3</td>
<td>9.1</td>
<td>9.7</td>
<td>7.9</td>
</tr>
<tr>
<td>Avg. Secchi Depth (m)</td>
<td>1.54</td>
<td>1.9</td>
<td>1.3</td>
<td>1.5</td>
<td>1.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Avg. Total Depth (m)</td>
<td>2.17</td>
<td>2.3</td>
<td>2.4</td>
<td>2.2</td>
<td>2.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Avg. pH</td>
<td>8.1</td>
<td>N/A</td>
<td>8.1</td>
<td>8.0</td>
<td>8.0</td>
<td>8</td>
</tr>
</tbody>
</table>

**Real-time bioextraction and two-point growth measurements of tagged individuals**
Twenty-two of the Twenty-five labeled ribbed mussels survived the growing season. Shell growth in length ranged from 2.7 mm to 12.2 mm between June and November, with an average across all mussels of 7.4 mm, and declined with an increase in initial shell length ($R^2 = 0.26$, $P = 0.01$; Fig. 4). Filtration rate (mg h$^{-1}$ g DW$^{-1}$) and nitrogen absorption rate (mg h$^{-1}$ g DW$^{-1}$) are summarized in Table 7 and Fig. 5. Nitrogen absorption rates could not be determined in July 2015 due to methodological problems.
Figure 4. Seasonal growth of ribbed mussels as a function of initial size among 25 animals in the Providence River in 2014

Table 7. Mean filtration rates and nitrogen absorption rates of Geukensia demissa measured at Save The Bay in 2014 and 2015. Values in parentheses represent one standard deviation.

<table>
<thead>
<tr>
<th>Date</th>
<th>Filtration rate (mg h(^{-1}) g DW(^{-1}))</th>
<th>Nitrogen absorption rate (mg h(^{-1}) g DW(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/11/14</td>
<td>3.09 (±0.98)</td>
<td>ND</td>
</tr>
<tr>
<td>7/10/14</td>
<td>4.24 (±0.28)</td>
<td>ND</td>
</tr>
<tr>
<td>7/29/14</td>
<td>9.72 (±3.89)</td>
<td>ND</td>
</tr>
<tr>
<td>8/19/14</td>
<td>4.27 (±1.01)</td>
<td>ND</td>
</tr>
<tr>
<td>9/11/14</td>
<td>19.55 (±8.67)</td>
<td>ND</td>
</tr>
<tr>
<td>7/16/15</td>
<td>3.87 (±0.89)</td>
<td>ND</td>
</tr>
<tr>
<td>8/3/15</td>
<td>7.16 (±2.94)</td>
<td>0.09 (±0.04)</td>
</tr>
<tr>
<td>9/9/15</td>
<td>4.53 (±1.24)</td>
<td>0.03 (±0.01)</td>
</tr>
</tbody>
</table>

Figure 5. Real-time filtration rates of ribbed mussels measured during the growing periods in 2014 (n = 16) and 2015 (n = 16)
Discussion:

Growth rates of the ribbed mussels observed in 2014 from repeated measurements of tagged animals were similar to rates reported by Brousseau et al. (1984) for ribbed mussels in salt marshes of Long Island Sound. This suggests that ribbed mussels were able to successfully acclimate to constant submersion, and that our field site had sufficient quantity and quality of food to support good growth. We observed that growth rate of the tagged animals decreased as a function of increased starting size. We determined that our growth rates of continually submerged (i.e. Passive) and intertidal (i.e. Control) animals in 2015 were consistent with those measured on tagged animals in 2014, using the linear regression generated for growth rate vs. initial shell length. We used a large sample size for the measured growth study. Even when we pooled replicates, we could not detect significant differences among intertidal, continually submerged, and continually submerged enhanced delivery settings. Although we are confident that our findings are valid, tagging individual animals in future work would reduce ecological noise introduced by individuals growing at different rates within the same treatment.

We used regression models based on length measurements taken at the onset of the measured growth study, i.e., the beginning of the growing period, as these models predicted tissue mass (and therefore nitrogen accumulation) better than models developed at the end of the period. The discrepancy between these models may have affected estimations because the length-to-tissue mass ratios may change over time. Length was the strongest dimensional predictor of tissue mass. Although total mass (i.e. wet weight) was a similarly good predictor of tissue mass at the onset of the experiment, it is more easily affected by external factors: moisture content can change with holding times and other factors (Gallardi et al. 2014), and total mass is impacted by biofouling as well as the extent of byssel thread removal, which varies depending on how much thread is left on the attachment substrate, remaining attached to the animal, and retracted into the shell. In contrast, dimensions are not affected by biofouling, moisture content, or byssel thread retention and therefore should be a more reliable predictor of tissue mass. At the end of the growing period, total mass more clearly outperformed length at predicting tissue mass. Under carefully-controlled conditions (i.e. short storage time), where use of an electronic scale is practical, total mass may be a more precise measure for indicating tissue mass.

Continually submerged and intertidal animals appeared to grow faster than animals “force-fed” by an aquaculture-style upweller (i.e. Active treatment) by all measures, although this observation was not statistically significant. This finding suggests that the resources put into equipment and upkeep efforts did not promote, and may have even suppressed, growth. Our upweller malfunctioned due to power failure for periods of up to 48 hours several times during
the growing period. This may have left the animals with less available food than continual submergence in ambient water and may have resulted in extended low-oxygen conditions in the silos. Although our site data show near-saturated oxygen conditions for most of the growing period, periodic hypoxia is common in this part of the estuary (Deacutis et al. 2006), and episodes may have been missed in our monitoring. Water flow failure, particularly in conjunction with potential ambient hypoxic conditions, may have slowed growth and contributed to our observed increased mortality versus the Control and Passive treatment samples. Additionally, the apparatus was susceptible to damage from wave energy. The sharp movements of the damaged silos in the upweller may have elicited a negative feeding response, such as a predator-avoidance response. This finding again indicates that feeding rate was saturated in the Passive treatment without increasing the rate at which water passed by the animals. The Providence River is highly eutrophic and has high quality phytoplankton : seston ratio. Animals in mesotrophic, oligotrophic, and eutrophic systems having less optimal seston compositions may respond differently to enhanced seston delivery. Because feeding rates of continually-submerged animals are similar to rates for intertidal animals, our data support prior findings that ribbed mussels may exhibit other compensation mechanisms for intertidal exposure, such as increased clearance rates at lower tides (Galimany et al. 2012).

Restoration to intertidal salt marsh may be the most efficient way to use ribbed mussels to mitigate nutrients in estuaries similar to the Providence River, where food availability and suitable intertidal areas are not limiting factors. In addition to extracting nutrients at a similar rate to continually submerged animals, intertidal animals benefit the ecosystem through multiple trophic and engineering functions (Bertness 1999), restoration projects do not require maintenance such as scrubbing biofouling organisms, as the low tide cycle causes desiccation of most fouling organisms, and also do not require special equipment that is vulnerable to weather. However, in areas where shorelines are largely bulkheaded, and sites available for salt marsh restoration are limited, hanging ribbed mussels from aquaculture-style mesh bags could provide more practicable nutrient uptake benefits. Although biofouling would need to be addressed, some built-up coastlines may contain more dock space than marsh space available for restoration. By using hanging bags, managers could target small animals that grow faster to maximize nitrogen removal potential. And, removing the grown-out animals completely from the system would be easier, ensuring that nutrients aren’t eventually returned back to the system through mortality. Another possibility, in addition to ribbed mussel-only installations, is hanging ribbed mussels below floating treatment wetlands to enhance their nitrogen removal capacity.

Our physiological rates estimated from measured growth experiments and real-time biodeposition methods differed. Our models based on field measurements indicate that adult

16
animals exhibited a nitrogen removal rate ranging from 7 to 13 mg per animal over the growing period. In contrast, real-time accumulation estimate models predict a mean nitrogen absorption rate of 160 mg N per animal over the same period. This discrepancy could be partly due to differential feeding rates among animals of different ages. We found that smaller animals grow faster—and therefore likely absorb nitrogen faster—than larger adults; this is consistent with prior findings (Jordan and Valiela 1982, Bertness and Grosholz 1985). We used adult animals in the measured growth experiment to represent a characteristic size cohort for salt marshes, while the biodeposition measurements primarily used smaller animals. Adult animals dominate the population composition (Bertness and Grosholz 1985); thus using adult animals should best characterize the nitrogen bioextraction capacity of the species in their salt marsh environment, while using smaller animals is more representative of what would be used in an installation that targeted nitrogen removal specifically. Another contributing factor to this discrepancy could be ammonium excretion. The biodeposition method measures seston ingestion, rejection, and feces production, but does not measure production of dissolved nitrogen through excretion. Previous literature on ammonium production by ribbed mussels has reported a wide range of excretion rates, up to 50% of nitrogen absorbed into the digestive system (e.g., Valiela and Jordan 1982). A conservative estimate of nitrogen assimilation by the ribbed mussels into tissue, shell, and byssal threads would thus be ~80 mg N per animal per growing season if half of the absorbed nitrogen were excreted. Other ecological or unaccounted-for methodological factors may have contributed to this discrepancy, as well. We suggest further study into the relationship between long-term growth and real-time biodeposition.

Bertness and Grosholz (1985) found that ribbed mussel density in the tall *Spartina alterniflora* zone of a salt marsh in nearby Barrington, RI ranged from 450 to 1236 animals per m$^2$. Using our most conservative numbers for nitrogen bioextraction of adult mussels in the intertidal marsh from our measured growth study, we can estimate that the range of nitrogen extraction for a growing season would range from 5.4 to 14.8 grams per m$^2$ of fringing marsh, a marsh type that is characterized by tall form *S. alterniflora* and prevalent in the Providence River estuary; this would scale up to a cumulative bioextraction rate of 54 to 148 kg N per Ha per season.

Newell (2013) reports the results of a pilot scale study that installed a standard commercial mussel raft in the Bronx, New York City, and stocked it with ribbed mussels. Their initial seeding density was 135 mussels per foot of rope, with 25 foot ropes. They report a fully stocked 20 x 20’ raft would have approximately 250 ropes. This would yield a raft with 8.4 x 10$^5$ mussels. Assuming a conservative 80 mg nitrogen removed per growing season for the average mussel in
our biodeposition experiments, this would represent 67.5 kg of nitrogen removed by a raft per season.

In conclusion, our work demonstrates that 1) ribbed mussels can survive and grow under constant submersion in the lower Providence River; 2) there is high quality food available at the Save The Bay location in the Edgewood Shoal portion of the lower Providence River, which likely makes this a good place to grow shellfish for nutrient remediation; and 3) there are environmental benefits provided by both marsh restoration and the subtidal installation of systems to cultivate small, fast-growing shellfish, and future decisions about allocation of resources into one or the other will vary depending on the primary outcomes desired.
Literature Cited:


Furnas, M. J., Hitchcock, G. L., & Smayda, T. J. (1976). Nutrient-phytoplankton relationships in Narragansett Bay during the 1974 summer bloom (pp. 18-133). University of Rhode Island, Sea Grant Marine Advisory Service.


Gallardi, D., Hobbs, K., Mills, T., Couturier, C., Parrish, C. C., & Murray, H. M. (2014). Effects of extended ambient live holding on cultured blue mussels (Mytilus edulis L.) with reference to
condition index, lipid profile, glycogen content and organoleptic testing. Aquaculture, 430, 149-158


Appendix I:

Photographs of Equipment

Floating Upweller System (FLUPSY)

Water in the trough is pushed out by the motor. In Order to maintain a balance, water is continuously being taken in through the mesh bottom of the silos past the shellfish (which are feeding by filtering on the water going by them). The water leaves the silos and enters back into the trough through PVC pipes that connect the silos to the trough.

http://www.governorsislandalliance.org/newsite/upload/2009/05/FLupsy%20illustrated.jpg
Animals in the Active and Control settings, and Passive treatment animals in mesh “socks”
Appendix II:

Measuring Length, Width, and Depth
**Appendix III:**

**Supplementary Statistics**

Initial Data Descriptive Statistics and Graphical Summaries:

<table>
<thead>
<tr>
<th>TRT</th>
<th>N</th>
<th>Obs</th>
<th>Variable</th>
<th>N</th>
<th>Mean</th>
<th>Std Dev</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>100</td>
<td>100</td>
<td>Length</td>
<td>100</td>
<td>6.0939000</td>
<td>0.745996</td>
<td>4.490000</td>
<td>8.130000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Width</td>
<td>100</td>
<td>2.6150000</td>
<td>0.464654</td>
<td>1.850000</td>
<td>5.900000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Depth</td>
<td>100</td>
<td>2.0185000</td>
<td>0.280847</td>
<td>1.410000</td>
<td>2.930000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mass</td>
<td>100</td>
<td>16.8667800</td>
<td>6.357041</td>
<td>6.246000</td>
<td>42.477000</td>
</tr>
<tr>
<td>C</td>
<td>102</td>
<td>102</td>
<td>Length</td>
<td>102</td>
<td>6.1999020</td>
<td>0.696127</td>
<td>4.300000</td>
<td>7.620000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Width</td>
<td>102</td>
<td>2.5866667</td>
<td>0.274425</td>
<td>1.870000</td>
<td>3.220000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Depth</td>
<td>102</td>
<td>2.0631373</td>
<td>0.259107</td>
<td>1.380000</td>
<td>2.800000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mass</td>
<td>102</td>
<td>16.6120098</td>
<td>5.469433</td>
<td>5.453000</td>
<td>30.916000</td>
</tr>
<tr>
<td>P</td>
<td>100</td>
<td>100</td>
<td>Length</td>
<td>100</td>
<td>6.0716000</td>
<td>0.784790</td>
<td>4.640000</td>
<td>7.990000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Width</td>
<td>100</td>
<td>2.5722000</td>
<td>0.347599</td>
<td>1.820000</td>
<td>3.350000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Depth</td>
<td>100</td>
<td>1.9983000</td>
<td>0.316004</td>
<td>1.190000</td>
<td>2.920000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mass</td>
<td>100</td>
<td>16.4631400</td>
<td>6.429057</td>
<td>7.349000</td>
<td>32.756000</td>
</tr>
</tbody>
</table>

Final Data Descriptive Statistics and Graphical Summaries:
Initial Mussel Nutrient Analysis

<table>
<thead>
<tr>
<th>Sample</th>
<th>Carbon (%)</th>
<th>Nitrogen (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mussel tissue</td>
<td>47.1</td>
<td>7.7</td>
</tr>
<tr>
<td>mussel tissue</td>
<td>36.2</td>
<td>8.8</td>
</tr>
<tr>
<td>mussel tissue</td>
<td>49.5</td>
<td>9.8</td>
</tr>
<tr>
<td>mussel tissue</td>
<td>45.5</td>
<td>9.2</td>
</tr>
<tr>
<td>mussel tissue</td>
<td>41.5</td>
<td>11.6</td>
</tr>
<tr>
<td>mussel tissue</td>
<td>42.3</td>
<td>10.8</td>
</tr>
<tr>
<td>mussel tissue</td>
<td>44.1</td>
<td>13.2</td>
</tr>
<tr>
<td>mussel tissue</td>
<td>46.1</td>
<td>9.4</td>
</tr>
<tr>
<td>mussel tissue</td>
<td>37.4</td>
<td>9.3</td>
</tr>
<tr>
<td>mussel tissue</td>
<td>38.8</td>
<td>8.4</td>
</tr>
<tr>
<td>Macerated tissue</td>
<td>44.2</td>
<td>9.0</td>
</tr>
<tr>
<td>Macerated tissue</td>
<td>42.5</td>
<td>9.0</td>
</tr>
<tr>
<td>Macerated tissue</td>
<td>44.2</td>
<td>10.1</td>
</tr>
</tbody>
</table>
### Ribbed Mussel Nutrient Bio-Extraction Pilot Project

**EPA Grant Number: CE96184201**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Rep</th>
<th>Carbon (%)</th>
<th>Nitrogen (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mac. fibrous tissue</td>
<td>1</td>
<td>42.41</td>
<td>11.67</td>
</tr>
<tr>
<td>Mac. fibrous tissue</td>
<td>2</td>
<td>42.90</td>
<td>11.83</td>
</tr>
<tr>
<td>Mac. fibrous tissue</td>
<td>3</td>
<td>43.81</td>
<td>11.26</td>
</tr>
<tr>
<td>Mussel Fluid</td>
<td>1</td>
<td>36.83</td>
<td>8.05</td>
</tr>
<tr>
<td>Mussel Fluid</td>
<td>2</td>
<td>36.38</td>
<td>8.31</td>
</tr>
<tr>
<td>Mussel Fluid</td>
<td>3</td>
<td>33.29</td>
<td>7.27</td>
</tr>
<tr>
<td><strong>Avg.</strong></td>
<td></td>
<td><strong>41.8</strong></td>
<td><strong>9.72</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>with Mussel Fluid</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10.06 w/o Mussel Fluid</td>
</tr>
</tbody>
</table>

#### Final Mussel Nutrient Analysis

<table>
<thead>
<tr>
<th>Sample</th>
<th>Rep</th>
<th>Carbon (%)</th>
<th>Nitrogen (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>West Passive</td>
<td>1</td>
<td>48.82</td>
<td>5.47</td>
</tr>
<tr>
<td>West Passive</td>
<td>2</td>
<td>42.23</td>
<td>6.40</td>
</tr>
<tr>
<td>West Passive</td>
<td>3</td>
<td>42.67</td>
<td>7.79</td>
</tr>
<tr>
<td>West Passive</td>
<td>4</td>
<td>42.14</td>
<td>12.78</td>
</tr>
<tr>
<td>West Passive</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upweller South</td>
<td>1</td>
<td>43.54</td>
<td>13.52</td>
</tr>
<tr>
<td>Upweller South</td>
<td>2</td>
<td>41.38</td>
<td>9.84</td>
</tr>
<tr>
<td>Upweller South</td>
<td>3</td>
<td>43.75</td>
<td>8.63</td>
</tr>
<tr>
<td>Upweller South</td>
<td>4</td>
<td>43.86</td>
<td>13.66</td>
</tr>
<tr>
<td>Upweller South</td>
<td>5</td>
<td>42.82</td>
<td>9.81</td>
</tr>
<tr>
<td>Control East</td>
<td>1</td>
<td>38.44</td>
<td>8.02</td>
</tr>
<tr>
<td>Control East</td>
<td>2</td>
<td>39.07</td>
<td>8.11</td>
</tr>
<tr>
<td>Control East</td>
<td>3</td>
<td>39.65</td>
<td>11.44</td>
</tr>
<tr>
<td>Control East</td>
<td>4</td>
<td>40.87</td>
<td>9.27</td>
</tr>
<tr>
<td>Control East</td>
<td>5</td>
<td>33.97</td>
<td>8.14</td>
</tr>
<tr>
<td>Control West</td>
<td>1</td>
<td>40.72</td>
<td>10.07</td>
</tr>
<tr>
<td>Control West</td>
<td>2</td>
<td>34.11</td>
<td>10.05</td>
</tr>
<tr>
<td>Control West</td>
<td>3</td>
<td>42.82</td>
<td>12.86</td>
</tr>
<tr>
<td>Control West</td>
<td>4</td>
<td>40.90</td>
<td>11.64</td>
</tr>
<tr>
<td>Control West</td>
<td>5</td>
<td>42.30</td>
<td>12.64</td>
</tr>
<tr>
<td>Upweller North</td>
<td>1</td>
<td>40.43</td>
<td>11.93</td>
</tr>
<tr>
<td>Upweller North</td>
<td>2</td>
<td>39.77</td>
<td>8.11</td>
</tr>
<tr>
<td>Location</td>
<td>Number</td>
<td>Value 1</td>
<td>Value 2</td>
</tr>
<tr>
<td>---------------</td>
<td>--------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Upweller North</td>
<td>3</td>
<td>37.69</td>
<td>8.72</td>
</tr>
<tr>
<td>Upweller North</td>
<td>4</td>
<td>42.45</td>
<td>6.91</td>
</tr>
<tr>
<td>Upweller North</td>
<td>5</td>
<td>41.04</td>
<td>11.65</td>
</tr>
<tr>
<td>Passive East</td>
<td>1</td>
<td>44.35</td>
<td>7.39</td>
</tr>
<tr>
<td>Passive East</td>
<td>2</td>
<td>41.87</td>
<td>11.51</td>
</tr>
<tr>
<td>Passive East</td>
<td>3</td>
<td>43.72</td>
<td>10.42</td>
</tr>
<tr>
<td>Passive East</td>
<td>4</td>
<td>42.24</td>
<td>9.82</td>
</tr>
<tr>
<td>Passive East</td>
<td>5</td>
<td>42.56</td>
<td>6.44</td>
</tr>
</tbody>
</table>

SAS ANOVA is attached as a separate document