3-24-2011

Experimental Quantification of Nutrient Bioextracti on Potential of Oysters in Estuarine Waters of New Hampshire

Raymond E. Grizzle
University of New Hampshire, ray.grizzle@unh.edu

Krystin M. Ward
University of New Hampshire, Krystin.Ward@unh.edu

Experimental Quantification of Nutrient Bioextraction Potential of Oysters in Estuarine Waters of New Hampshire

A Final Report to

The Piscataqua Region Estuaries Partnership

Submitted by

Raymond Grizzle and Krystin Ward
University of New Hampshire
Jackson Estuarine Laboratory
Durham, NH 03824

March 24, 2011

This project was funded in part by a grant from the Piscataqua Region Estuaries Partnership as authorized by the U.S. Environmental Protection Agency's National Estuary Program.

Table of Contents

Abstract:

This project was a short-term field experiment conducted in summer 2010 and designed to provide preliminary data on the bioextraction (removal) of carbon (C) and nitrogen (N) for two different size classes (both <76mm shell height) of eastern oysters (*Crassostrea virginica*) at six sites in the Great Bay estuarine system in New Hampshire. Sites were chosen to represent a range of ambient nutrient concentrations, water flow conditions, and location within the estuary. Two of the sites were at oyster aquaculture farms: Granite State Shellfish at the mouth of the Oyster River, and Little Bay Oyster Company near Fox Point in Little Bay. At each site, oysters were deployed in 10mm mesh polyethylene bags typically used on oyster farms in New England. Approximately one thousand "seed" size (10-15 mm shell height), or two hundred (200) 1-year old (30-40 mm shell height) oysters were placed into each bag. Two bags (one for each size class) were suspended 10-20 cm off the bottom attached to plastic coated wire cages at each site from August 9 until November 4, 2010. The oysters were inspected and the bags were cleaned each month to reduce fouling. There were no significant differences in final size among the six sites, indicating similar growth rates. Soft tissue %C and %N values, however, varied substantially and significantly (ANOVA, $P<0.05$) among the sites. Tukey tests indicated significantly higher %C and %N at the Squamscott River (SQ) site, and significantly lower at the Little Bay Oyster (LBO) farm site, compared to the other sites. The ranges of mean soft tissue %C and %N were, respectively, 26.9 to 47.2 and 4.7 to 10.6. Because shell material was not analyzed in the present study, literature values for shell were combined with soft tissue data from the present study to arrive at total whole animal C and N content. Oysters with mean shell height of 35.7 mm contained 0.6 g of C and 0.01 g of N; oysters with mean shell height of 55.6 mm contained 3.1 g of C and 0.07 g of N. Preliminary calculations indicated that if 200 acres of bottom area were in full farm production, the annual N removal from the estuary *from oyster harvest alone* would be 12.56 tons. It is emphasized that the present study represents only the first step in characterizing the nutrient (focusing on N) bioextraction potential for oyster farming in New Hampshire.

Executive Summary

This project was designed to provide *preliminary data* that will provide the basis for the design of more extensive studies on the bioextraction potential of bivalve shellfish (and possibly other organisms) aquaculture in the estuarine waters of New Hampshire. It was a short-term (3 months) field experiment conducted in summer 2010 and was designed to provide preliminary data on the bioextraction (carbon [C] and nitrogen [N]) potential for two different size classes of sub-market size (<76mm shell height) eastern oysters (*Crassostrea virginica*) at six sites in the Great Bay estuarine system in New Hampshire. Sites were chosen to represent a range of ambient nutrient concentrations, water flow conditions, and location within the estuary. Two of the sites chosen are existing oyster aquaculture farms: Granite State Shellfish at the mouth of the Oyster River, and Little Bay Oyster Company near Fox Point in Little Bay.

At each site, oysters were deployed in 10mm mesh polyethylene bags typically used on oyster farms in New England. Approximately one thousand "seed" size (10-15 mm shell height), or two hundred (200) 1-year old (30-40 mm shell height) oysters were placed into each bag. Two bags (one for each size class) were suspended 10-20 cm off the bottom attached to plastic coated wire cages at each site from August 9 until November 4, 2010. The oysters were inspected and the bags were cleaned each month to reduce fouling.

Initially, 30 oysters were sampled from each class size before they were deployed to the six sites, and then 10 oysters from each bag were sampled in September (1.5 months) and at the end of the experiment in November (3 months). Shell height and whole wet weight were measured and then the oysters were frozen and shipped to USEPA Narragansett Laboratory for further analyses. Data analysis consisted of three assessments: (1) growth (final size) variations among the study sites; (2) soft tissue C and N content variations among the study sites; and (3) total (soft tissue and shell) C and N removal on an individual oyster basis.

There were no significant differences in final size among the six sites, indicating similar growth rates. Soft tissue %C and %N values, however, varied substantially and significantly (ANOVA, $P<0.05$) among the sites. Post-hoc Tukey tests indicated significantly higher %C and %N at the Squamscott River (SQ) site, and significantly lower at the Little Bay Oyster (LBO) farm site, compared to the other sites. The ranges of mean soft tissue %C and %N were, respectively, 26.9 to 47.2 and 4.7 to 10.6. Because shell material was not analyzed in the present study, literature values for shell were combined with soft tissue data from the present study to arrive at total whole animal C and N content. Oysters with mean shell height of 35.7 mm contained 0.6 g of C and 0.01 g of N; oysters with mean shell height of 55.6 mm contained 3.1 g of C and 0.07 g of N.

Preliminary results from another study indicate that Little Bay has the most potential for additional oyster farm sites when considering a variety of environmental and other relevant issues. Preliminary calculations using data from the present study and other published work indicated that if 200 acres of bottom area were in full farm production, the annual N removal from the estuary *from oyster harvest alone* would be 12.56 tons. It is emphasized that the present study represents only the first step in characterizing the nutrient (focusing on N) bioextraction potential for oyster farming in New Hampshire. Future studies need to assess seasonal variations in growth and nutrient bioextraction, geographic variations, deployment protocols for farmed oysters, and possibly other factors.

List of Tables

List of Figures

Introduction

Suspension-feeing bivalve shellfish such as oysters remove suspended particulates and associated plant nutrients from the overlying water column as they feed (Newell and Langdon 1996). When shellfish occur at high densities as in aquaculture facilities or as living reefs, their effects on ambient particulate and associated nutrient concentrations can be substantial (Dame et al. 1989; Grizzle et al. 2008; Newell 2004). As a result, scientists and environmental managers recently have begun to assess the potential for using shellfish as a water quality management tool (Brumbaugh and Toropova 2008; Compton et al. 2009; Langan 2009). In the Great Bay estuarine system, nitrogen (N) has become the major focus (Trowbridge 2010).

The present project was designed to provide *preliminary data* for the design of more extensive studies on the nutrient bioextraction potential of bivalve shellfish aquaculture in the estuarine waters of New Hampshire. Because of limited available funds and the short-term nature of the study, relevant issues such as additional species (including macroalgae which are being assessed in another project), shellfish density effects, and seasonal variations in growth were not addressed.

Goals and Objectives

The overall goal of the project was to begin to quantify variations in growth, production, and nutrient (nitrogen and carbon) extraction for two different size classes of the eastern oyster (*Crassostrea virginica*) in different areas of the Great Bay estuarine system. If bioextraction technologies are developed to a meaningful level for water quality management for the region, it seems likely that a variety of nursery and grow-out methods would be developed for different areas. The present study was intended as the starting point for future studies, and had the following two objectives.

Objective (1): determine the nutrient bioextraction potential for two size classes of oysters

Objective (2): preliminarily assess the potential of oyster aquaculture for contributing to nutrient (particularly nitrogen) management goals for the state's estuarine waters

The present Final Report contains three deliverables specified in the scope of work: (1) quantitative characterization of short-term and annual nutrient (C and N) uptake and removal (bioextraction) capabilities of oysters grown in different areas of the Great Bay/Piscataqua River system; (2) comparison of the economics of shellfish bioextraction rates to literature values for removal by conventional methods such as wastewater treatment and best management practices for stormwater; and (3) preliminary estimates of the potential for expansion of shellfish aquaculture in New Hampshire in the context of bioextraction potential.

Methods

Field Methods. Six sites were chosen to represent a range of ambient nutrient concentrations, water flow conditions, and location within the estuary: both existing oyster aquaculture sites (Granite State Shellfish at the mouth of the Oyster River and Little Bay Oyster Company near Fox Point in Little Bay), the mouths of the Bellamy and Squamscott Rivers, Great Bay at Nannie Island, and Adams Point in Little Bay (Fig. 1).

Fig. 1. Oyster deployment sites for 2010 bioextraction experiment

At each of the six study sites, oysters were deployed in 10mm mesh polyethylene bags typically used on oyster farms in New England. Approximately one thousand (1,000) "seed" size [10-15 mm shell height], or two hundred (200) 1-year old [30-40 mm shell height] oysters were placed into each bag. Two bags (one for each size class) were suspended 10-20 cm off the bottom attached to plastic coated wire cages at each site from August 9 until November 4, 2010 (~3 months duration).

Before deployment at the study sites, thirty (30) oysters were haphazardly selected from each of the two size classes, shell height and whole wet weight measured, then frozen and shipped to USEPA Narragansett Laboratories for further analyses (see Laboratory Methods section below).

The oysters were inspected and the bags cleaned by scrubbing with brushes on August 18, September 7–9, 29, and October 27-28.

Ten (10) oysters were haphazardly selected from each of the deployment bags on September 29 (~1.5 months after deployment) and November 4–6, 2010 (~3 months), shell height and whole wet weight measured, then frozen and shipped to USEPA Narragansett Laboratory for further analyses (see below).

Laboratory Methods. In the laboratory (at USEPA Narragansett, RI), the frozen oysters were re-measured (shell height), shucked (soft tissue) into aluminum pans, oven-dried at 60^{o}C and weighed (soft tissue dry weight), homogenized in a blender, and analyzed following laboratory operating procedure (LOP) AED/IDB/SR/2001-01-000 for carbon (C) and nitrogen (N) using a Thermo-Finnigan Flash EA112 CHN/O elemental analyzer which was calibrated prior to analysis with certified standards. Each tissue sample was analyzed in duplicate and the results reported as %C and %N.

Data Analysis. Data analysis consisted of three assessments: (1) growth (final size) variations among the study sites; (2) C and N removal variations among the study sites; and (3) C and N removal on an individual oyster basis.

For the present analysis, final size (shell height and soft tissue dry weight) data were used as a proxy for growth. This was done because all oysters used in the experiment came from the same population (i.e., initial size was the same for all six study sites), and the experiment was run for the same time period (3 months) for all six study sites. Thus, final size reflected growth variations. In similar fashion to the growth/final size analysis, among-site C and N removal variations were assessed by simply comparing final %C and %N content. ANOVA was used to test for among-site differences in both size and nutrient content. Differences in %C and %N between the two size classes was analyzed by t-test.

Total C and N content was also assessed relative to oyster size (shell height and soft tissue dry weight). Because both size classes were below typical market size, data from the present experiment were combined with published growth curves for the eastern oyster to allow estimation of bioextraction potential for a wider range of oyster sizes.

Results and Discussion

Shell and soft tissue growth/size were assessed mainly to provide the basis for developing an overall relationship between C and N content and morphometric variables (e.g. shell height) typically measured by aquaculturists, or otherwise available from the literature. As discussed in Methods,

growth was not expressed as a rate because the experiment only involved a 3-month deployment period. Moreover, growth rates in New Hampshire vary widely from season to season, including no-growth for several months during the winter. Additional studies will be needed to develop growth (and bioextraction) rates on a useful level.

Deliverable (1): quantitative characterization of nutrient (C and N) bioextraction

This deliverable consisted of three major steps: analysis of among-site growth variations; laboratory analysis of %C and %N content in the oysters; and calculation of C and N on a per oyster basis.

Among-site growth variations. Because oyster growth can vary widely among sites within an estuary, probably in most cases largely due to environmental variations (Shumway 1996), bioextraction rates might also vary widely. Thus, the initial assessment was to test for among-site differences in shell and soft tissue final size. ANOVAs with site as the treatment variable on mean final shell height and soft tissue dry weight indicated no significant differences among the six sites (Fig. 2; Appendix B).

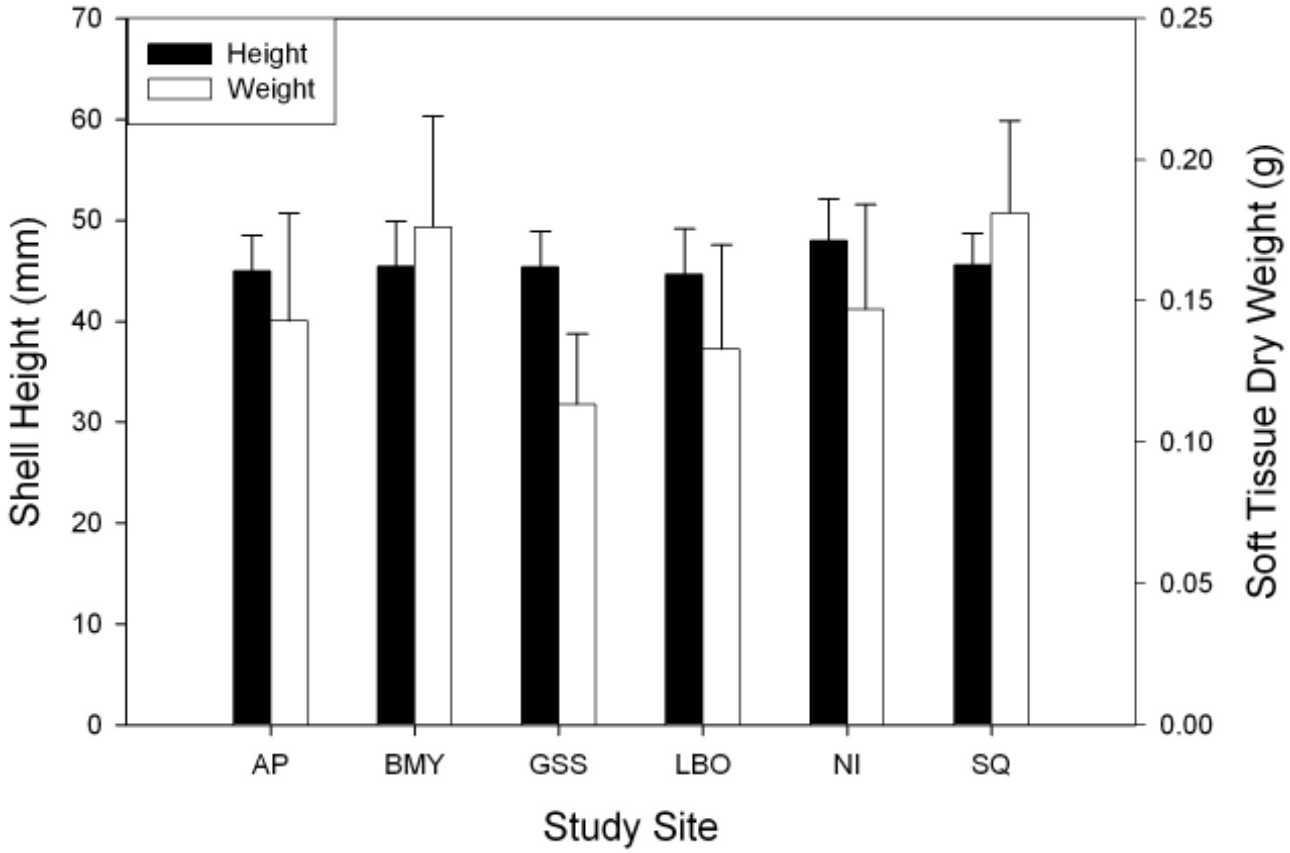

Fig. 2. Final mean (±1 SE) shell height (mm) and soft tissue dry weight (g) comparing all six study sites (Fig. 1). ANOVA showed no significant differences among the sites for either variable.

This finding was somewhat surprising because environmental variables affecting oyster growth vary widely among the sites, though no formal analysis of available data has been conducted.

Analysis of C and N content data. The analytical methods used in the present study yielded data on %C and %N for soft tissue only (Appendices A and B). ANOVAs with site as the treatment variable were run on mean final soft tissue %C and %N content. In contrast to the among-site growth comparisons, there were significant among-site differences ($P<0.05$) for both %C and %N. Post-hoc Tukey tests indicated significantly higher C and N composition at the Squamscott River (SQ) site, and significantly lower at the Little Bay Oyster (LBO) farm site, compared to the other sites (Fig. 3).

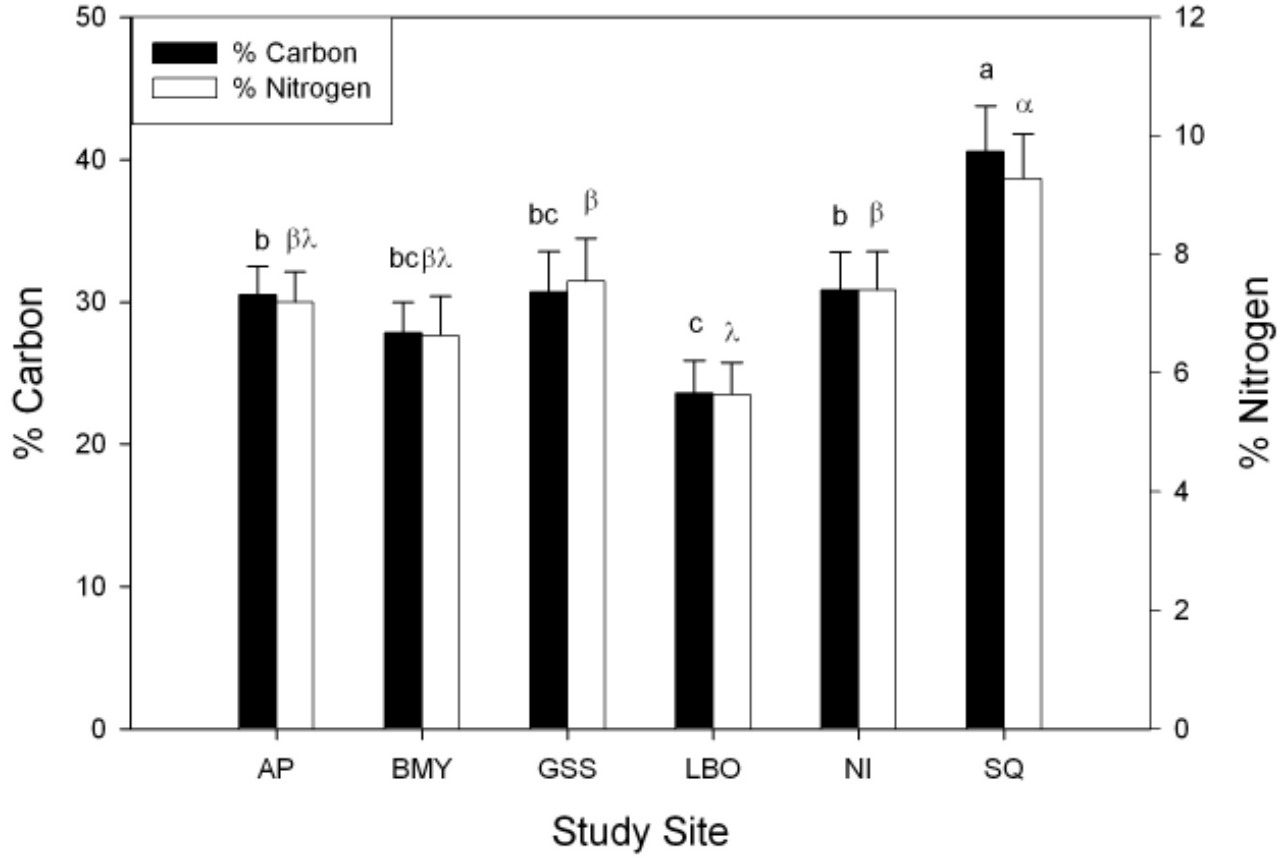

Fig. 3. Mean (±1 SE) soft tissue %C and %N for the six study sites. Tukey test groupings shown by letters (a, b and c for %C; α, β and λ for %N) above each bar; same letters indicate no significant differences ($P>0.05$), different letters indicate significant differences ()<0.05).

The ranges of mean %C (26.9 – 47.2) and %N (4.7 – 10.6) content among the six sites, however, were similar to the findings of Higgins et al. (2011). They also found significant differences among three study sites, with ranges of 43.3% to 46.2% for C, and 7.3% to 8.2% for N in soft tissue for a range of size classes and different sites. Newell et al. (2005, p. 21) reported a mean of ~7% N for soft tissue by dry weight (they did not report C data).

Calculation of C and N content on a per oyster basis. The mean %C and %N values for soft tissue from the present study compare reasonably well with previous research, as discussed above. The present study, however, involved mainly sub-market size oysters, and did not include measurement of nutrients in the shell material. Moreover, the present study did not involve dry weight measurements

for the shell. In order to arrive at whole oyster estimates of C and N content, literature values were used for shell.

Higgins et al. (2011) is the only published study we are aware of for farmed oysters that involved sizes similar to the present study. Their "sub-market" (mean shell size: 43.6mm) and "cocktail" (mean: 64.8mm) oysters are similar in shell size to our two larger size classes (Table 1). Therefore, their shell data were combined with our soft tissue data to calculate whole oyster C and N content for the two larger size classes in the present study and shown in Table 1. These values—highlighted in yellow in Table 1—are the *preliminary estimates* for whole oyster total C and N from the present study.

Table 1. Summary of data from present study and published literature on C and N content relative to oyster size.

			Soft Tissue		Shell		Whole Oyster		
Shell Height (mm)	**Shell DW (g)**	**Soft Tissue DW (g)**	**%C**	**%N**	**%C**	**%N**	**Total C (g)**	**Total N (g)**	Source
7.8	n/d	0.03	32.12	7.71	n/d	n/d	n/d	n/d	Present study
12.7	n/d	0.20	37.62	9.10	n/d	n/d	n/d	n/d	Present study
35.7	n/d	0.06	27.58	6.52	n/d	n/d	0.585*	0.013*	Present study
55.6	n/d	0.24	32.85	7.86	n/d	n/d	3.082*	0.065*	Present study
76	150	1	n/d	7	n/d	0.3	n/d	0.52	Newell et al. 2005
43.6	**4.8**	0.20	43.30	8.15	**11.84**	**0.18**	0.647	0.025	Higgins et al. 2011
64.8	**24.3**	0.80	44.30	8.06	**12.36**	**0.19**	3.391	0.112	Higgins et al. 2011
85.5	37.6	1.58	45.10	7.28	12.43	0.17	5.375	0.176	Higgins et al. 2011
117.8	71.9	3.00	46.20	7.37	12.04	0.26	10.011	0.394	Higgins et al. 2011

*NOTE: These values were calculated using data from the present study for soft tissue and shell data from Higgins et al. 2011 shown in bold (see text for details).

In conclusion, three major points need to be made relative to satisfying the present deliverable. First, because farmed oysters typically have thinner shells than wild oysters (Paynter and Dimichele 1990; Higgins et al. 2011), the relative contribution of the shell to whole oyster C and N content typically will be less in farmed oysters. This explains the higher whole oyster data for N content reported by Newell et al. (2005) compared to data for oysters of similar size reported by Higgins et al. (2011). Thus, the approximate value of 0.5g N per adult oyster (~76mm shell height) given in Newell et al. (2005) is likely too high for farmed oysters.

Second, in order to arrive at useful values from a farming perspective, data from larger size classes than were used in the present study are needed. Therefore, the values from Higgins et al. (2011) highlighted in blue in Table 1 were used for the calculations in Deliverable 3 below.

Finally, we emphasize that the present project was a preliminary study. The "next steps" needed to arrive at better estimates are discussed in the Recommendations section below.

Deliverable (2): comparison of the economics of shellfish bioextraction to conventional nitrogen removal methods

There are four major sources of N inputs to the Great Bay estuarine system (Trowbridge 2010): wastewater treatment plant effluents, non-point runoff from watersheds, groundwater discharges, and atmospheric deposition. For the present analysis, only N removal from wastewater treatment plants and non-point runoff will be considered. In contrast to N removal by traditional land-based methods, oyster farming includes monetary income as well as production costs. Moreover, oyster farming also provides ecosystem services that scientists are only just beginning to understand and quantify (Pietros and Rice 2009; Brumbaugh and Toropova 2008). Thus, a comprehensive economic comparison of conventional N removal (for point and non-point sources) and bioextraction by oyster aquaculture is not possible given our current understanding. Nonetheless, some "best guess" comparisons from current literature can be made.

Table 2 summarizes the range of costs for N removal from point and nonpoint treatments and some economic considerations for oyster farming. There are many different conventional nutrient removal technologies and the present analysis is intended as a simplistic overview. No attempt has been made to consider the variety of wastewater treatment facilities or non-point runoff treatment methods in the Great Bay watershed. Nonetheless, cost estimates are available to provide at least a range of costs for N removal from wastewater and non-point sources (Kang et al. 2008). It should be noted that N extraction numbers for oyster farming only consider harvest of the oysters, and not denitrification or other processes associated with deposition of oyster feces and pseudofeces to bottom sediments (Newell et al. 2005). Thus, the data in Table 2 represent simplistic estimates at best.

Table 2. Summary of conventional N extraction (removal) methods and costs compared to oyster aquaculture in New England.

Method	Removal Cost Range	Removal Cost (average $/ton N)	Revenue Generated ($/ac/yr)[1]	Ecosystem Services ($/ac/yr)[2]	Tax Revenue Generated ($/ac/yr)[3]
Wastewater Treatment (various technologies)[4]	<$1 - ~$5/lb	$5,000	0	0	0
Agriculture Non-point Treatment[5] (various technologies)	$4 - 200/lb	$200,000	0	0	0
Urban Non-point Treatment[5] (various technologies)	$25 - >$1,000/lb	$500,000	0	0	0
Oyster Aquaculture Bottom Culture	?	?	$120,000	>>$6,700	$3,000

[1] Based on average annual production of 200,000 oysters/ac/yr and $0.60/oyster

[2] This estimate only represents secondary fish production; from Grabowski and Peterson (2007)

[3] Based on charge of $0.015/oyster sold (NH Adm. Rule Fis 807.11) and average annual production of 200,000 oysters/ac

[4] Kang et al. (2008)

[5] Stephensen (2009)

In addition to the actual data in Table 2, it should be noted that oyster aquaculture—in contrast to traditional N removal methods—results in substantial generated revenues from oyster sales as well as taxes. Moreover, the recent focus on ecosystem services provided by natural oyster reefs and oyster aquaculture adds another dimension to economic assessments (Brumbaugh and Toropova 2008; Northern Economics 2009). All this points to the complexities involved in trying to arrive at common economic parameters for comparing oyster aquaculture to other methods for N removal.

Deliverable (3): preliminary estimates of the potential for expansion of shellfish aquaculture in New Hampshire in the context of bioextraction potential

The potential for expansion of shellfish aquaculture in New Hampshire's estuarine waters has not been comprehensively assessed. An ongoing project focusing on up-estuary areas minimally affected by red tide closures in recent years is scheduled for completion in 2012, and will provide such an assessment for those waters above Dover Point in Little Bay and Great Bay. These areas are also the most suitable for oyster farming, which is the focus of the present project. Therefore, results available to date from this project have been used to meet Deliverable 3.

Factors that potentially affect siting of oyster farms include: a variety of environmental factors (e.g., water currents, bottom type, water depth) that affect oyster growth, water quality (particularly as it affects classification with respect to shellfish harvesting), other potential uses (e.g., recreational and commercial fishing, boating), potential damage to protected natural habitats (e.g., eelgrass), navigation, concerns of adjacent landowners, and local management or zoning issues. The ongoing project is essentially a marine spatial planning effort that focuses on identifying the most appropriate geographic areas for oyster farming.

The general process that has been followed to date is essentially a 'map overlay' process where each factor is mapped individually, then the individual maps are viewed collectively to identify areas of overlap, exclusion, etc. At the time of this report, the areas shaded in yellow—but not including the cross-hatched portions that denote waters closed to harvest—in Figure 4 below have been identified as representing the *preliminary maximum extent* of areas suitable for oyster aquaculture in the Great Bay estuarine system. These areas, which total ~577 acres, share the following features: classified as "open" for shellfish harvesting most of the time; minimally impacted by historical red tide events; water depth between mean low water and -5 m; moderate to strong tidal flows; and no or only small amounts of eelgrass cover mapped in recent years. These are the major environmental factors relevant to determining where oyster aquaculture would most likely be successful.

It should be noted, however, that all factors relevant to oyster aquaculture have not been fully assessed, nor have there been extensive conversations with all stakeholders. In particular, there has been no assessment of ecological or environmental carrying capacity for the oysters themselves. In other words, there has been no assessment of how many oysters could be grown given current water flows, seston concentrations, etc. Certainly, the entire 577 acres could not be filled with oyster farms, but how much less than that would be feasible remains unknown. Nor has there been a complete assessment of social factors such as potential conflicting uses (e.g., recreational and commercial fisheries), or local regulatory policies. Nonetheless, some reasonable assumptions can be made to allow a preliminary assessment of the N bioextraction potential for oyster aquaculture in the state.

If each oyster harvested represented a removal of 0.285 g N (=mean for two largest size classes of oysters in Table 1), and 200,000 oysters/yr were harvested from each acre (Table 2), then annual N bioextraction per acre would be 28,500 g (=62.8 lb; =0.0314 ton). If 200 acres were in production, the annual N removal from the estuary *from oyster harvest alone* would be 12.5 tons.

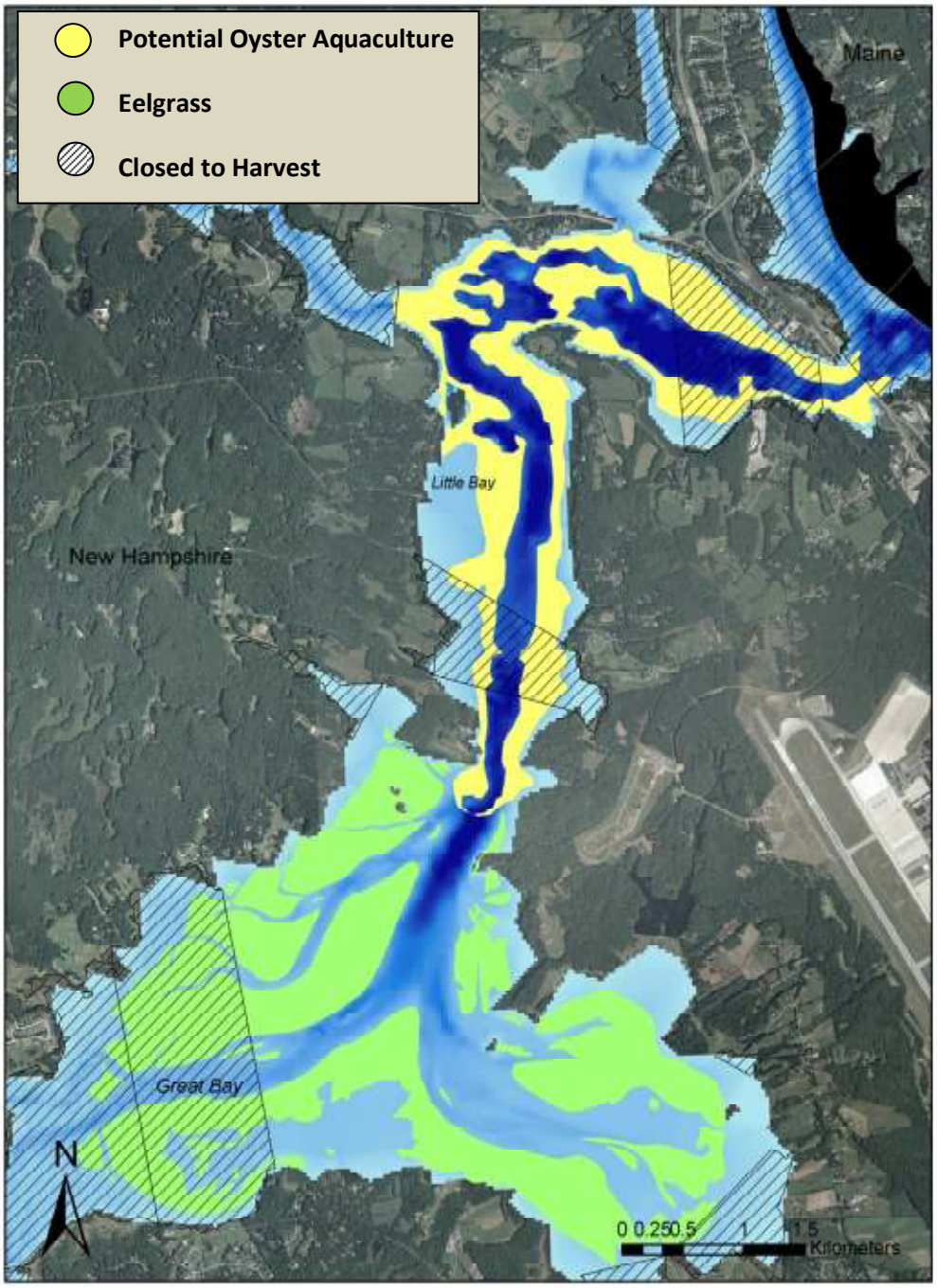

Fig. 4. *Preliminary maximum extent* for potential oyster aquaculture (yellow polygon) in the Great Bay Estuary (see text for details).

Conclusions

The present study represents only the first step in characterizing the nutrient (C and N) bioextraction potential for oyster farming in New Hampshire. Nonetheless, several conclusions can be drawn based on the present study:

(1) Oyster aquaculture has the potential to remove substantial amounts of C and N from the estuary but additional work is needed, including a comprehensive assessment of the extent of areas available for oyster farming. The role of oyster farming in nutrient management will largely depend on how much of the estuary is deemed suitable for oyster farming. Assessment of this issue is an ongoing process that involves many stakeholders. As of 2010, there were only two active oyster farms (with a permitted area of only 6 acres) in New Hampshire. Substantial expansion of the industry will be required if oyster farming is to make a meaningful contribution to nutrient management.

(2) The present study indicated that there can be substantial differences in N and C soft tissue content, and thus likely overall bioextraction rates, in different areas of the estuary. This finding could have important implications for oyster farming as well as nutrient management, and needs further testing.

(3) At the present time, there is only a meager literature on nutrient bioextraction by farmed oysters. Thus, there is much yet to learn about not only the factors that cause variations in bioextraction rates but also the role that oyster farming might play in the overall management process.

Recommendations

Soft tissue %N values from the present study were similar to those reported by Higgins et al. (2011), but N content in the shell material was not measured in the present study. Future studies should include shell measurements of N content. Future studies should also include larger oysters, and involve all size classes typically marketed. The finding of substantial and significant differences in C and N content among the study sites indicates that farming in different areas within an estuary may result in different bioextraction rates. Thus future research should also include multiple sites and involve environmental measurements at the study sites in order to try to determine the cause(s) of the variations in bioextraction. Future studies might also assess seasonal variations in growth and nutrient bioextraction, as well as how the effects of different deployment protocols (e.g., oysters in bags compared to direct grow-out on the bottom), and possibly other factors.

Literature Cited

Brumbaugh, R.D. and C. Toropova, 2008. Economic valuation of ecosystem services: a new impetus for shellfish restoration. Basins and Coasts 2:8-15.

Compton, J.E. , R.L Dennis, H.A. Walker, W.B. Milstead, S.J. Jordan, B.H. Hill, K.M. Fritz, R. Devereux, B.R. Johnson, J.J. Beaulieu, J.S. Latimer, J.A. Lynch, R.G. Waite, and C.C. Davis. 2009. Ecosystem Services Research Program Pollutant Specific Studies: Nitrogen Regulation Services Implementation Plan. Linking ecosystem services and nitrogen: Science to improve management of nitrogen in air, land and water. USEPA. EPA/600/R-09/091.

Dame, R.F., J.D. Spurrier and T.G. Wolaver. 1989. Carbon, nitrogen and phosphorus processing by an oyster reef. Mar. Eco. Prog. Ser. 54:249-256.

Grizzle, R.E., J.K. Greene, and L.D. Coen, 2008. Seston removal by natural and constructed intertidal eastern oyster (*Crassostrea virginica*) reefs: a comparison with previous laboratory studies, and the value of *in situ* methods. Estuaries and Coasts 31:1208-1220.

Higgins, C.B., K. Stephenson and B.L. Brown. 2011. Nutrient bioassimilation capacityof aquacultured oysters: quantification of an ecosystem service. J. Environ. Qual. 40:271-277.

Kang, S.J., K. Olmstead, K. Takacs and J. Collins. 2008. Municipal Nutrient Removal Technologies Reference Document, Vol. 1-Technical Report (EPA 832-R-08-006)

Langan, R. 2009. Hypothetical case study for using extractive technologies for meeting nutrient criteria goals for the Great Bay Estuary, New Hampshire. Bioextraction Technologies for Nutrient Remediation Workshop, Stamford, CT. December 14, 2009.

Newell, R.I.E. 2004. Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve mollusks: A review. J. Shellfish Res. 23:51-61.

Newell R.I.E. and C.J. Langdon. 1996. Chapter 5. Mechanisms and physiology of larval and adult feeding, pp. 185-230, in: *The Eastern Oyster, Crassostrea virginica.* V.S. Kennedy, R.I.E. Newell, A.F. Eble (eds.). Maryland Sea Grant, College Park, MD.

Newell, R.I.E., T.R. Fisher, R.R. Holyoke and J.C. Cornwell. 2005. Influence of eastern oysters on nitrogen and phosphorus regeneration in Chesapeake Bay, USA, pp. 93-120 in: *The Comparative Roles of Suspension Feeders in Ecosystems.* R. Dame and S. Olenin (eds.). Vol. 47, NATO Science Series, Springer, Netherlands.

Northern Economics. 2009. Valuation of ecosystem services from shellfish restoration, enhancement and management: a review of the literature. Prepared for Pacific Shellfish Institute.

Paynter, K.T. and L. Dimichele. 1990. Growth of tray-cultured oysters (*Crassostrea virginica* Gmelin) in Chesapeake Bay. Aquaculture 87:289-297.

Pietros, J.M. and M.A. Rice. 2009. The impacts of aquacultured oysters, *Crassostrea virginica* (Gmelin, 1791) on water column nitrogen and sedimentation: results of a mesocosm study. Aquaculture 220:407-422.

Shumway, S.E. 1996. Chapter 13. Natural environmental factors, pp. 467-514, in: *The Eastern Oyster, Crassostrea virginica.* V.S. Kennedy, R.I.E. Newell, A.F. Eble (eds.). Maryland Sea Grant, College Park, MD.

Trowbridge, P. 2010. Analysis of nitrogen loading reductions for wastewater treatement facilities and non-point sources in the Great Bay Estuary watershed. New Hampshire Department of Environmental Services, Concord, NH.

Appendix A

Raw data for pre-deployment oysters (August 2010).

Size Class	Height (mm)	Dry Wt (g)	%N	%C	Size Class	Height (mm)	Dry Wt (g)	%N	%C
SC1-1	7.00	0.04	8.89	36.28	LC1-1	13.50	0.29	8.61	38.33
SC1-2	7.00	0.04	8.80	36.33	LC1-2	13.50	0.29	9.13	39.72
SC2-1	6.00	0.01	7.85	30.70	LC2-1	14.00	0.18	10.10	36.67
SC2-2	6.00	0.01	6.39	27.86	LC2-2	14.00	0.18	8.79	38.05
SC3-1	7.60	0.02	8.09	34.29	LC3-1	10.00	0.14	7.90	38.02
SC3-2	7.60	0.02	8.54	35.86	LC3-2	10.00	0.14	7.87	35.68
SC4-1	8.00	0.03	7.50	32.95	LC4-1	10.80	0.14	9.64	39.95
SC4-2	8.00	0.03	7.33	33.01	LC4-2	10.80	0.14	9.61	40.15
SC5-1	10.50	0.05	8.12	35.24	LC5-1	11.50	0.19	10.10	38.37
SC5-2	10.50	0.05	8.69	36.43	LC5-2	11.50	0.19	8.45	37.44
SC6-1	7.80	0.03	7.04	30.25	LC6-1	13.00	0.18	8.49	34.98
SC6-2	7.80	0.03	6.99	29.06	LC6-2	13.00	0.18	7.95	34.73
SC7-1	11.00	0.04	8.83	34.35	LC7-1	14.00	0.21	10.25	37.77
SC7-2	11.00	0.04	8.16	33.54	LC7-2	14.00	0.21	10.30	37.53
SC8-1	5.50	0.01	7.55	33.22	LC8-1	13.00	0.22	8.91	38.55
SC8-2	5.50	0.01	7.70	29.77	LC8-2	13.00	0.22	8.56	35.53
SC9-1	6.00	0.03	7.58	32.00	LC9-1	12.50	0.19	10.36	38.82
SC9-2	6.00	0.03	6.24	29.56	LC9-2	12.50	0.19	9.07	38.74
SC10-1	8.50	0.02	5.62	24.97	LC10-1	13.00	0.22	8.45	35.42
SC10-2	8.50	0.02	6.28	27.18	LC10-2	13.00	0.22	9.10	37.20
SC11-1	8.00	0.03	7.71	32.68	LC11-1	13.00	0.18	9.70	38.05
SC11-2	8.00	0.03	8.57	33.56	LC11-2	13.00	0.18	9.21	38.03
SC12-1	7.00	0.03	7.23	29.43	LC12-1	12.50	0.19	10.29	37.79
SC12-2	7.00	0.03	7.21	30.20	LC12-2	12.50	0.19	8.80	35.96
SC13-1	7.00	0.01	7.97	33.24	LC13-1	13.00	0.28	9.79	38.94
SC13-2	7.00	0.01	8.61	34.53	LC13-2	13.00	0.28	9.30	37.62
SC14-1	8.50	0.05	8.24	33.48	LC14-1	16.10	0.23	8.73	39.79
SC14-2	8.50	0.05	8.23	33.75	LC14-2	16.10	0.23	9.39	39.91
SC15-1	8.00	0.05	7.13	28.40	LC15-1	10.50	0.09	7.81	35.44
SC15-2	8.00	0.05	8.23	31.37	LC15-2	10.50	0.09	8.30	35.52
Mean:	**7.76**	**0.03**	**7.71**	**32.12**		**12.69**	**0.20**	**9.10**	**37.62**
SD:	**1.49**	**0.01**	**0.85**	**2.94**		**1.52**	**0.05**	**0.79**	**1.60**

Appendix B

Raw data, by size class (SC=small, LC=large) and site (AP=Adams Point, BMY=Bellamy River, GSS=Granite State Shellfish, LBO=Little Bay Oyster Co., NI=Nannie Island, SQ=Squamscott River.), for final (3-month deployment) oysters (November 2010).

Size Class, Study Site, Replicate	Shell Height (mm)	Soft Tissue DW (g)	%N	%C
SC-AP-R1-1	42.5	0.050	6.264	27.730
SC-AP-R1-2	42.5	0.050	6.552	28.387
SC-AP-R2-1	33.3	0.050	9.167	37.823
SC-AP-R2-2	33.3	0.050	9.187	38.183
SC-AP-R3-1	41.5	0.080	6.083	25.659
SC-AP-R3-2	41.5	0.080	5.485	23.503
SC-AP-R3-3	41.5	0.080	4.484	21.023
SC-AP-R4-1	34.0	0.010	9.189	38.507
SC-AP-R4-2	34.0	0.010	9.007	38.081
SC-AP-R5-1	28.5	0.010	7.153	28.913
SC-AP-R5-2	28.5	0.010	7.316	30.083
MEAN:	**36.46**	**0.044**	**7.262**	**30.717**
SD:	**5.55**	**0.029**	**1.669**	**6.409**
SC-BMY-R1-1	31.0	0.04	7.13	29.33
SC-BMY-R1-2	31.0	0.04	7.19	29.33
SC-BMY-R2-1	46.0	0.06	9.93	38.91
SC-BMY-R2-2	46.0	0.06	9.81	39.28
SC-BMY-R3-1	30.0	0.08	2.96	18.19
SC-BMY-R3-2	30.0	0.08	3.45	19.22
SC-BMY-R3-3	30.0	0.08	2.59	16.47
SC-BMY-R4-1	28.5	0.06	5.81	24.77
SC-BMY-R4-2	28.5	0.06	5.46	23.40
SC-BMY-R5-1	29.0	0.07	3.52	17.65
SC-BMY-R5-2	29.0	0.07	3.58	17.77
MEAN:	**32.64**	**0.064**	**5.585**	**24.937**
SD:	**6.66**	**0.014**	**2.654**	**8.345**
SC-GSS-R1-1	38.5	0.05	5.05	19.19
SC-GSS-R1-2	38.5	0.05	6.24	24.34
SC-GSS-R1-3	38.5	0.05	6.11	23.71
SC-GSS-R2-1	27.5	0.02	3.25	15.05
SC-GSS-R2-2	27.5	0.02	3.21	14.41
SC-GSS-R3-1	36.0	0.03	8.09	32.89
SC-GSS-R3-2	36.0	0.03	8.15	32.91
SC-GSS-R4-1	43.0	0.07	9.44	37.45
SC-GSS-R4-2	43.0	0.07	9.41	37.49
MEAN:	**36.50**	**0.04**	**6.55**	**26.38**
SD:	**5.69**	**0.02**	**2.40**	**9.12**
SC-LBO-R1-1	33.5	0.01	3.70	15.80
SC-LBO-R1-2	33.5	0.01	3.99	16.90
SC-LBO-R2-1	29.0	0.02	5.13	20.43
SC-LBO-R2-2	29.0	0.02	5.44	22.11
SC-LBO-R2-3	29.0	0.02	2.74	11.33
SC-LBO-R3-1	32.5	0.03	5.28	22.64
SC-LBO-R3-2	32.5	0.03	5.08	21.30
SC-LBO-R4-1	28.5	0.04	5.06	22.33
SC-LBO-R4-2	28.5	0.04	5.17	22.26
SC-LBO-R5-1	34.0	0.03	4.78	20.34
SC-LBO-R5-2	34.0	0.03	5.02	21.52
SC-LBO-R5-3	34.0	0.03	5.52	24.21
MEAN:	**31.50**	**0.03**	**4.74**	**20.10**
SD:	**2.44**	**0.01**	**0.84**	**3.65**
SC-NI-R1-1	43.0	0.08	6.15	25.38
SC-NI-R1-2	43.0	0.08	5.72	23.00
SC-NI-R1-3	43.0	0.08	5.23	21.62
SC-NI-R2-1	31.0	0.04	7.94	33.65
SC-NI-R2-2	31.0	0.04	7.94	33.41
SC-NI-R3-1	36.5	0.03	5.99	25.21
SC-NI-R3-2	36.5	0.03	6.00	25.56
SC-NI-R6-1	27.5	0.04	8.37	35.22
SC-NI-R6-2	27.5	0.04	8.45	35.14
SC-NI-R7-1	49.5	0.13	8.12	36.58
SC-NI-R7-2	49.5	0.13	7.49	33.63
SC-NI-R7-3	49.5	0.13	7.98	35.37
MEAN:	**38.96**	**0.07**	**7.12**	**30.32**
SD:	**8.44**	**0.04**	**1.19**	**5.61**
SC-SQ-R1-1	45.5	0.18	8.97	40.86
SC-SQ-R1-2	45.5	0.18	8.94	40.71
SC-SQ-R2-1	29.0	0.05	6.90	27.20
SC-SQ-R2-2	29.0	0.05	6.86	27.32
SC-SQ-R3-1	34.0	0.05	9.37	40.23
SC-SQ-R3-2	34.0	0.05	9.10	37.45
SC-SQ-R3-3	34.0	0.05	8.83	36.82
SC-SQ-R4-1	50.0	0.20	8.94	40.01
SC-SQ-R4-2	50.0	0.20	9.22	40.74
SC-SQ-R5-1	35.0	0.04	5.37	22.08
SC-SQ-R5-2	35.0	0.04	4.30	18.79
SC-SQ-R5-3	35.0	0.04	5.71	24.59
MEAN:	**38.00**	**0.09**	**7.71**	**33.07**
SD:	**7.61**	**0.07**	**1.79**	**8.39**

Size Class, Study Site, Replicate	Shell Height (mm)	Soft Tissue DW (g)	%N	%C
LC-AP-R1-1	55.0	0.240	5.866	23.866
LC-AP-R1-2	55.0	0.240	6.397	25.986
LC-AP-R1-3	55.0	0.240	5.929	24.052
LC-AP-R2-1	48.0	0.200	6.784	27.648
LC-AP-R2-2	48.0	0.200	4.990	22.333
LC-AP-R2-3	48.0	0.200	7.237	30.572
LC-AP-R3-1	59.5	0.370	7.237	30.108
LC-AP-R3-2	59.5	0.370	7.898	33.668
LC-AP-R4-1	45.0	0.190	6.015	27.183
LC-AP-R4-2	45.0	0.190	2.770	13.738
LC-AP-R4-3	45.0	0.190	6.809	29.598
LC-AP-R5-1	62.0	0.230	10.043	43.237
LC-AP-R5-2	62.0	0.230	9.028	39.255
LC-AP-R5-3	62.0	0.230	9.600	39.281
MEAN:	**53.50**	**0.237**	**6.900**	**29.323**
SD:	**6.81**	**0.060**	**1.895**	**7.748**
LC-BMY-R1-1	57.0	0.31	7.42	30.48
LC-BMY-R1-2	57.0	0.31	7.52	31.36
LC-BMY-R2-1	59.0	0.33	7.09	28.03
LC-BMY-R2-2	59.0	0.33	6.00	25.29
LC-BMY-R2-3	59.0	0.33	8.34	32.77
LC-BMY-R3-1	58.0	0.22	7.28	29.24
LC-BMY-R3-2	58.0	0.22	5.67	23.67
LC-BMY-R3-3	58.0	0.22	8.03	30.95
LC-BMY-R4-1	59.0	0.27	6.71	26.85
LC-BMY-R4-2	59.0	0.27	6.34	24.97
LC-BMY-R5-1	57.0	0.32	8.83	36.12
LC-BMY-R5-2	57.0	0.32	9.04	36.61
MEAN:	**58.08**	**0.29**	**7.36**	**29.69**
SD:	**0.90**	**0.05**	**1.07**	**4.18**
LC-GSS-R1-1	48.0	0.19	8.35	32.90
LC-GSS-R1-2	48.0	0.19	7.92	31.98
LC-GSS-R1-3	48.0	0.19	7.71	29.93
LC-GSS-R2-1	63.0	0.14	8.82	35.09
LC-GSS-R2-2	63.0	0.14	9.38	36.83
LC-GSS-R2-3	63.0	0.14	8.98	35.60
LC-GSS-R3-1	54.5	0.11	6.00	24.86
LC-GSS-R3-2	54.5	0.11	5.76	23.04
LC-GSS-R3-3	54.5	0.11	5.82	22.86
LC-GSS-R4-1	51.5	0.21	9.55	39.41
LC-GSS-R4-2	51.5	0.21	9.55	39.48
LC-GSS-R5-1	46.0	0.2	8.92	38.06
LC-GSS-R5-2	46.0	0.2	8.92	38.04
MEAN:	**53.19**	**0.16**	**8.13**	**32.93**
SD:	**6.35**	**0.04**	**1.41**	**6.04**
LC-LBO-R1-1	64.5	0.27	9.05	37.53
LC-LBO-R1-2	64.5	0.27	9.08	37.75
LC-LBO-R2-1	57.0	0.29	7.08	29.93
LC-LBO-R2-2	57.0	0.29	6.97	29.60
LC-LBO-R3-1	58.0	0.25	5.37	22.77
LC-LBO-R3-2	58.0	0.25	8.88	37.59
LC-LBO-R3-3	58.0	0.25	8.35	35.52
LC-LBO-R4-1	51.5	0.19	5.74	22.62
LC-LBO-R4-2	51.5	0.19	4.97	20.51
LC-LBO-R4-3	51.5	0.19	4.78	18.55
LC-LBO-R5-1	58.0	0.2	3.84	14.95
LC-LBO-R5-2	58.0	0.2	3.95	15.43
MEAN:	**57.29**	**0.24**	**6.51**	**26.90**
SD:	**4.34**	**0.04**	**1.99**	**8.82**
LC-NI-R1-1	65.0	0.22	5.85	23.17
LC-NI-R1-2	65.0	0.22	5.94	23.59
LC-NI-R2-1	58.0	0.39	9.43	41.17
LC-NI-R2-2	58.0	0.39	9.10	39.82
LC-NI-R3-1	60.5	0.13	3.70	15.43
LC-NI-R3-2	60.5	0.13	3.69	15.22
LC-NI-R4-1	58.5	0.13	10.65	41.58
LC-NI-R4-2	58.5	0.13	10.66	41.49
LC-NI-R5-1	50.5	0.28	8.38	33.86
LC-NI-R5-2	50.5	0.28	8.88	35.77
MEAN:	**58.50**	**0.23**	**7.63**	**31.11**
SD:	**4.96**	**0.10**	**2.64**	**10.76**
LC-SQ-R1-1	59.5	0.3	8.77	40.58
LC-SQ-R1-2	59.5	0.3	12.33	57.81
LC-SQ-R1-3	59.5	0.3	8.17	38.55
LC-SQ-R2-1	53.5	0.22	14.08	56.81
LC-SQ-R2-2	53.5	0.22	13.93	56.08
LC-SQ-R3-1	45.5	0.18	11.55	49.53
LC-SQ-R3-2	45.5	0.18	11.41	48.73
LC-SQ-R4-1	51.0	0.3	9.65	47.10
LC-SQ-R4-2	51.0	0.3	9.44	46.03
LC-SQ-R5-1	52.5	0.29	9.76	43.20
LC-SQ-R5-2	52.5	0.29	7.86	35.05
MEAN:	**53.05**	**0.26**	**10.63**	**47.23**
SD:	**4.98**	**0.05**	**2.18**	**7.58**