ANNUAL REPORT

NORTHEASTERN REGIONAL AQUACULTURE CENTER

For the Period
September 1, 2012 to August 30, 2013

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Northeastern Regional Aquaculture Center

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Introduction

The Northeastern Regional Aquaculture Center is one of five Regional Aquaculture Centers established by the U.S. Congress under the National Aquaculture Research, Extension and Teaching Policy Act of 1977 (Subtitle L, Section 1475(d)) and subsequent authorized legislation. These centers, located in the northeast, southern, north central, western, and the topical/sub-tropical Pacific regions are administered by the U.S. Department of Agriculture, National Institute of Food and Agriculture (NIFA). Located at universities and/or research institutions the regional centers’ mission is to support aquaculture research, development, demonstration, and extension education to enhance viable and profitable U.S. aquaculture production which will benefit consumers, producers, service industries, and the American economy.

Organization and Administration

Regional Centers

The Regional Aquaculture Centers are administrative rather than physical centers. The Centers provide a means of assessing research and extension needs, assuring industry input, establishing priorities, and implementing aquaculture research and extension programs. The Centers facilitate implementation, administration, and coordination of regional research and extension programs, and they foster information exchange, research and extension linkages, and cross fertilization of ideas within and between regions and between organizations.

Organization

The Northeastern Regional Aquaculture Center (NRAC) has an administrative staff consisting of a one half-time Director, an Administrative Assistant, and a Coordinator. NRAC’s Board of Directors (BOD) is the policy making body for NRAC and consists of nine members representing the Dean of the College of Agriculture and Natural Resource at the University of Maryland Regional Agriculture Experiment Directors, the Regional Extension Directors, the 1890 Schools, Agricultural Research Service laboratories in the Northeast Region, Sea Grant Directors, and industry or private institutions. The BOD also has responsibility for approval of all NRAC projects. The BOD is assisted by an Industry (IAC) and a Technical (TAC) Advisory Committee. The IAC, with assistance from the TAC, summarizes industry research and extension priorities for the Northeastern regional aquaculture industry and assists in assuring these priorities are incorporated into NRAC planning. The TAC, with help from the IAC, assists NRAC in assuring high quality projects having good science and addressing industry priorities are funded by NRAC.

The IAC and TAC are both comprised of one representative from each of the 12 states in the Northeastern Region and the District of Columbia. These states include Connecticut, Delaware, the District of Columbia, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, and West Virginia. Thus, there are 13 members on each committee who provide representation from all parts of the region and for the various sectors of the aquaculture industry. The TAC is divided into representatives from the research and the extension communities who provide their expertise to NRAC in defining priorities and selecting high quality research and extension projects.
Administrative Operations

The Northeastern Regional Aquaculture Center was located at the University of Massachusetts Dartmouth from 1988 until 2004. At that time the University of Massachusetts Dartmouth decided their priorities had changed and no longer wished to host NRAC. Through a competitive process the University of Maryland was selected by USDA, CSREES (Cooperative States Research, Education and Extension Service) to host NRAC and in December of 2005 NRAC was transferred to the University of Maryland at College Park, Maryland. The University of Massachusetts and the University of Maryland worked to complete transfer of NRAC to the University of Maryland. Because of constraints on funding FY 2006 funds were the first funds coming directly from USDA to the University of Maryland. Projects are complete and NRAC funds held at the University of Massachusetts Dartmouth have been expended, the University of Maryland has become responsible for all of NRAC activities and the University of Massachusetts has been phased out of NRAC activities. The completion of all involvement in NRAC by the University of Massachusetts Dartmouth ended in September 30, 2010.

All NRAC staff members are at the University of Maryland and the day to day operations of NRAC are operating out of the University of Maryland. The NRAC Director reports to the Dean of the College of Agriculture and Natural Resources, University of Maryland at College Park. Dr. Fredrick W. Wheaton, who was instrumental in moving the Center from UMASS Dartmouth, retired as the NRAC Director on June 30, 2010. He was replaced by Dr. Reginal M. Harrell effective July 1, 2011.

Board of Directors

The BOD members serve four-year terms except for some of the initial BOD members who will have shorter terms to develop the staggered terms needed to provide continuity over time. The Sea Grant Director serves a two-year term. The current BOD members are:

<table>
<thead>
<tr>
<th>Board Member</th>
<th>Representing</th>
<th>State Where Located</th>
<th>Term Ending</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Adel Shirmohammadi</td>
<td>Associate Dean/Associate Director, MD Agricultural Experiment Station University of Maryland</td>
<td>Maryland</td>
<td>Permanent Seat</td>
</tr>
<tr>
<td>Dr. Robert J. Barney</td>
<td>1890 Land Grant Colleges</td>
<td>Delaware</td>
<td>September 30, 2013</td>
</tr>
<tr>
<td>Dr. Christopher Neefus</td>
<td>Experiment Station Director</td>
<td>Maine</td>
<td>January 31, 2013</td>
</tr>
<tr>
<td>Dr. Jeffrey T. Silverstein</td>
<td>ARS</td>
<td>Maryland</td>
<td>September 30, 2017</td>
</tr>
<tr>
<td>Dr. James W. Ammerman</td>
<td>Sea Grant Director</td>
<td>New York</td>
<td>March 30, 2013</td>
</tr>
<tr>
<td>Dr. Richard Rhodes (Board Chair)</td>
<td>Extension Director</td>
<td>Rhode Island</td>
<td>September 30, 2013</td>
</tr>
<tr>
<td>Dr. Daniel Rossi</td>
<td>Experiment Station Director</td>
<td>New Jersey</td>
<td>September 30, 2014</td>
</tr>
<tr>
<td>Sebastian Belle</td>
<td>Industry</td>
<td>Maine</td>
<td>September 30, 2013</td>
</tr>
<tr>
<td>Dr. William Hare</td>
<td>Extension Director</td>
<td>District of Columbia</td>
<td>September 30, 2015</td>
</tr>
</tbody>
</table>
Industry Advisory Committee

Composition, Appointment, and terms of IAC

The IAC is comprised of representatives from the District of Columbia and the 12 states in the Northeast Region. They serve three-year terms except for the first IAC, which will have varying length appointments to develop the staggered terms needed to provide continuity for the IAC. Current members of IAC are:

<table>
<thead>
<tr>
<th>IAC Member</th>
<th>Organization</th>
<th>State</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mr. John W. Ewart</td>
<td>Delaware Sea Grant</td>
<td>Delaware</td>
</tr>
<tr>
<td>Mr. Matthew E. Moretti</td>
<td>Wild Ocean Aquaculture, LLC</td>
<td>Maine</td>
</tr>
<tr>
<td>Mr. Donald J. Flax</td>
<td>Byrd International</td>
<td>Maryland</td>
</tr>
<tr>
<td>Mr. John Milliken</td>
<td>Aquaculturist</td>
<td>Massachusetts</td>
</tr>
<tr>
<td>Mr. Dale Parsons</td>
<td>Parsons Seafood</td>
<td>New Jersey</td>
</tr>
<tr>
<td>Mr. George Nardi</td>
<td>Great Bay Aquaculture, LLC</td>
<td>New Hampshire</td>
</tr>
<tr>
<td>Mr. Sean Mulvey</td>
<td>NY State Aquaculture Rep.</td>
<td>New York</td>
</tr>
<tr>
<td>Vacant</td>
<td></td>
<td>Pennsylvania</td>
</tr>
<tr>
<td>Mr. B. Peter Sebring</td>
<td>Atlantic Aquaculture</td>
<td>Rhode Island</td>
</tr>
<tr>
<td>Vacant</td>
<td></td>
<td>Vermont</td>
</tr>
<tr>
<td>Mr. Greg Casten (Co-Chair)</td>
<td>ProFish</td>
<td>Washington, DC</td>
</tr>
<tr>
<td>Mr. Daniel Miller (Chair)</td>
<td>Potesta &amp; Associates, Inc.</td>
<td>West Virginia</td>
</tr>
</tbody>
</table>

Composition, Appointment, and terms of TAC

The TAC is comprised of representatives from the District of Columbia and the 12 states in the Northeast Region. They serve three year terms except for the first TAC which will have varying appointments to develop the staggered terms needed to provide continuity for the TAC. The TAC is divided into two groups with approximately one-half of the members representing research and approximately one-half representing extension. Current members of TAC are:

<table>
<thead>
<tr>
<th>TAC Member</th>
<th>State</th>
<th>Extension/Research</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ms. Tessa Getchis</td>
<td>University of Connecticut</td>
<td>Extension</td>
</tr>
<tr>
<td>Dr. Gulnihal Ozbay (Co-Chair)</td>
<td>Delaware State University</td>
<td>Research</td>
</tr>
<tr>
<td>Dr. Nicholas P. Brown</td>
<td>Center for Cooperative Aquaculture Research, Maine</td>
<td>Research</td>
</tr>
<tr>
<td>Dr. Edward F. Orlando</td>
<td>University of Maryland</td>
<td>Research</td>
</tr>
<tr>
<td>Mr. Richard C. Karney</td>
<td>Martha’s Vineyard Shellfish Group, Inc. Massachusetts</td>
<td>Extension</td>
</tr>
<tr>
<td>Dr. Elizabeth Fairchild</td>
<td>University of New Hampshire</td>
<td>Extension</td>
</tr>
<tr>
<td>Mr. George E. Flimlin, Jr. (Chair)</td>
<td>Rutgers Cooperative Research &amp; Extension, New Jersey</td>
<td>Extension</td>
</tr>
<tr>
<td>Dr. Martin Schreibman</td>
<td>Brooklyn College, New York</td>
<td>Research</td>
</tr>
<tr>
<td>Dr. Steven Hughes</td>
<td>Cheney University</td>
<td>Research</td>
</tr>
<tr>
<td>Dr. Terence Bradley</td>
<td>University of Rhode Island</td>
<td>Research</td>
</tr>
<tr>
<td>Dr. Calvin Lewis</td>
<td>District of Columbia</td>
<td>Extension</td>
</tr>
<tr>
<td>Vacant</td>
<td>Vermont</td>
<td></td>
</tr>
<tr>
<td>Dr. Daniel Miller</td>
<td>West Virginia University</td>
<td>Extension</td>
</tr>
</tbody>
</table>
Project Development

NRAC has two methods to develop projects: 1) the RFA method and 2) the project group method. The IAC develops priorities and the TIAC (Technical and Industrial Advisory Council comprised of the IAC and TAC together) develop problem statements to convert the priorities into researchable statements. The problem statements are distributed through the Northeast Region with a RFA (Request for Applications). Thus, anyone interested in submitting a proposal may submit a proposal as long as it addresses the problem statements. In some situations there will be a pre-proposal stage and then only selected (by the TIAC) pre-proposals will be invited to submit full proposals. The RFA method is the most common method used by NRAC. The group project is a process where a priority is defined, a problem statement is prepared and a request for a statement of interest is distributed throughout the Northeast region. People responding to the statement of interest are then brought together to develop a proposal to address the problem statement. The project group method tends to work well in some situations such for extension projects. Currently the Northeast Regional Extension project is the only project for which NRAC has used the project group method.

Current Activities

Administration of NRAC

The transition of the NRAC from the University of Massachusetts to the University of Maryland has been completed. The final allocation of funds at the University of Massachusetts committed in 2007 was expended September 2010. The University of Maryland has provided remodeled office space for NRAC. The one-half time NRAC Director, a full-time Coordinator and an Administrative Assistant have been hired at the University of Maryland. All of the contracting and administration of NRAC funds are now handled from the University of Maryland. Funding prior to FY 2006 was provided from USDA, NIFA to the University of Massachusetts. From FY 2006 and forward all funding comes from USDA, NIFA directly to the University of Maryland.

Projects 2012-2013

NRAC’s program year runs from September 1 to August 30 annually. This report covers the 2013 program year (September 2012 through August 2013). During this period NRAC has provided funding to 11 research and extension projects in addition to administrative projects. In the last 12 months NRAC has committed over $500,000 to projects and NRAC operations. Completion or project progress reports are included in this document for projects that have been in existence long enough to have submitted a progress or final reports.

Table 1 lists the projects by title and total project funding level. Details of the projects including project titles, abstracts, total funding, project numbers, and project results and findings to date are available in the appendix of this report. Publications, videos, extension publications, and other written or visual materials produced as part of each project are listed to the extent available for each project. Although attempts were made to be as complete as possible some publications that resulted from NRAC funding, particularly papers presented and papers published in peer reviewed literature, may not be included due to the time lag between the end of a project and the publication of results.
Table 1. NRAC projects active during the 2012-2013

<table>
<thead>
<tr>
<th>NRAC Project Title</th>
<th>Total Budget</th>
<th>Start Date</th>
<th>End Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assessment of Grow-Out Strategies for the Green Sea Urchin</td>
<td>$156,933</td>
<td>7/1/2009</td>
<td>7/30/2013</td>
</tr>
<tr>
<td>Examination of Finfish Pathogen Physiology and Predictive Ecology in a Bivalve/Finfish Integrated Multi-trophic Aquaculture System</td>
<td>$200,000</td>
<td>2/1/2010</td>
<td>7/31/2013</td>
</tr>
<tr>
<td>Aquaculture Health Hazard- Developing Outreach Services to the Region’s Farmers and Consumers</td>
<td>$196,312</td>
<td>1/1/2011</td>
<td>3/31/2014</td>
</tr>
<tr>
<td>Optimization of Hatchery and Culture Technology for Razor Clams</td>
<td>$93,616</td>
<td>10/1/2011</td>
<td>11/30/2013</td>
</tr>
<tr>
<td>Shellfish STEM-GIS (ShellGIS) Development for Improved Siting and Farm Management</td>
<td>$85,000</td>
<td>3/1/2012</td>
<td>2/29/2014</td>
</tr>
<tr>
<td>Development of more efficient methods of Vibrio sp. detection and identification of Vibrio sp.</td>
<td>$187,024</td>
<td>9/1/2012</td>
<td>8/31/2014</td>
</tr>
<tr>
<td>Genetic Marker-assisted selection of Northeastern hard clams for QPX resistance</td>
<td>$199,998</td>
<td>11/1/2012</td>
<td>10/30/2015</td>
</tr>
<tr>
<td>Algal-Bacterial Interactions in Shellfish Hatcheries</td>
<td>$18,488</td>
<td>1/1/2013</td>
<td>12/31/2013</td>
</tr>
<tr>
<td>Identification and Isolation of Novel Probiotic Bacteria for Use in Marine Aquaculture</td>
<td>$19,981</td>
<td>1/1/2013</td>
<td>1/31/2014</td>
</tr>
</tbody>
</table>

Accomplishments

Hatchery and nursery production demonstrated to industry that there are reliable sources of green sea urchin seed available for commercial projects and reseeding. Several recent industry inquiries regarding hatchery capacity and seed costs can be attributed to the success of these efforts and to our outreach. The project showed that out-planted sea urchins will remain within lease boundaries, resulting in three Maine-based companies to apply for bottom leases to culture green sea urchins. Successful sea ranching could revitalize a fishery annually valued at around 5 million dollars in Maine. Market sized sea urchins were produced with tank farming, enabling us to obtain further funding to evaluate intensive land-based gonad enhancement aquaculture. The economic benefits of gonad enhancement aquaculture are readily appreciated and accessed by industry, and if successful could double the final market value of processed roe.

Model pathways to explain how pathogens are likely to spread on an IMTA farm were developed. We have demonstrated the ability of IPNV to be spread via infected fish was demonstrated. Experimental methods and the model pathways developed have led to further research on the ability of mussels to remove larval sea lice from the water column. Extension work has facilitated the construction of two commercial scale mussel rafts placed on different salmon farms in Maine.
Result reports were presented for the cross-bred oyster project at the Northeastern Aquaculture Conference and Exposition and Milford Aquaculture Seminar as well as at the Annual Meeting of the National Shellfisheries Association. The presentations generated significant interest among researchers, and industry partners.

The Extension project has assembled a 300+ page manual on the identification and management of aquaculture production hazards. They have held two focus groups to review the content and layout of the manual with industry, aquatic health professionals, researchers and extension professionals. They have also had an extensive external review of the publication and are now in the process of revision, and then will be preparing the document for print and web.

A workshop was presented by the Mussel Farming project for 30 participants at the University of Rhode Island Bay Campus with speakers from as far away as New Zealand. Information was provided on various aspects of the mussel aquaculture industry.

The Shellfish STEM-GIS (ShellGIS) project delivered several presentations and held workshops to share information on decision making tools to identify the best areas to place new aquaculture farms given scientific data, modelling and public opinions and the second to use the tool to illustrate how differing public or scientific opinions can affect the decision making process.

**PUBLICATIONS FOR 2012-2013**

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**Publications in Print**


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**Papers Presented**


Barker, S.E. (2012) Delving into the amazing world of two economically important parasitic copepods: *Lepeophtheirussalmonis & Lernaeocera branchialis*! SMS seminar series University of Maine, ME, USA.


Molloy, Sally, Mike Pietrak, Debbie Bouchard, & Ian Bricknell
Interactions of viral fish pathogens Infectious Salmon Anemia Virus and Infectious Pancreatic Necrosis Virus with mussels Mytilus edulis Aquaculture America, March 1-3, New Orleans, LA


Pietrak, Mike, Sally Molloy, Debbie Bouchard, John Singer & Ian Bricknell
Potential for Disease Transmission on an IMTA Farm: Can I add another species? 1st US IMTA workshop, September 14-15, Port Townsend, WA

Pietrak, Mike, Sally Molloy, Debbie Bouchard, John Singer & Ian Bricknell
Interaction of a Bacterial Fish Pathogens Vibrio anguillarum 02β and Francisella noatunensis with Mussels Mytilus edulis Aquaculture America, March 1-3, New Orleans, LA

Pietrak, Mike, Sally Molloy, Debbie Bouchard, John Singer & Ian Bricknell
Potential Disease Risks and Benefits on an Integrated Mussel Mytilus edulis and Marine Finfish Farm Aquaculture America, March 1-3, New Orleans, LA


Manuscripts


Presentations

NACE in Groton CT in December 2012:
“Research and Improved Management for Offshore Mussel Farms in Southern New England”

Narragasett, RI on March 28th at the RISG Coastal State Forum for Shellfish Issues:
“Offshore Mussel Farming in Rhode Island”

URI Bay Campus on May 17th as part of Mussel Farming Workshop mentioned above
“Offshore Mussel Farming in Southern New England; Research plans for optimizing economic yield”

World Aquaculture Society meeting in Nashville in February 2013:
“Offshore Mussel Farming in Southern New England; Research plans for optimizing economic yield”
APPENDICES

A - Final Reports

B - Progress Reports

APPENDIX A
PROJECT CODE: 09-06

PROJECT TITLE: Assessment of Environmental Impacts of Oyster Aquaculture in New England Waters

DATES OF WORK: 1/11/2010 to 4/30/2012

PARTICIPANTS:
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Daniel Cheney, Ph.D. Executive Director
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Plymouth Marine Laboratory
Prospect Place
The Hoe
Plymouth PL 13DH
United Kingdom
0044-0-1752633100
aish@pml.ac.uk
Cooperating, Non-funded Participants:

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207-446-8923  
jmckeen@earthlink.net

Stewart Hutchings  
Dragon Oysters, LLC  
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New Haven, CT 06513  
203-785-8854  
stewart.hutchings@snet.net

REASON FOR TERMINATION: Objectives completed

PROJECT OBJECTIVES:

- Develop Hydrodynamic Flow Models of two oyster farms and put results into a GIS framework.
- Develop a Seston Depletion Model based on field data and oyster feeding rates. This tool will help to assess, at any site, how oyster biomass on a given farm will affect food availability for the other oysters, and determine a hydraulic zone of influence between different farms or important wild habitats.
- Assess benthic infaunal, epifauna and meiofaunal impacts and communicate them through the GIS. We expect to find that oyster cultivation will increase biodiversity and the biomass of invertebrates at the study sites, lending credence to the concept that oyster aquaculture is good for the environment.
- Create an American oyster growth model using ShellSIM, calibrated at field sites. This tool will be coupled with the seston depletion model to predict growth of a single oyster, or a large population of oysters at different densities.
- Develop the GIS framework into an interactive tool incorporating all of the above data into a user-friendly application for oyster farmers, managers and regulators. A user of the final product will, for example, be able to query any location within the study domain and retrieve information describing local hydrodynamics (e.g., flow speeds, volume flux, current direction, and water depth), biodiversity information, and predictions of oyster growth.
ANTICIPATED BENEFITS: With a focus on shellfish (oyster) aquaculture in New England, specifically ecosystem research, this project will describe interactions of aquatic shellfish farms with phytoplankton, marine invertebrates and fish, and lead to the development of guidelines for farm siting issues and carrying capacity. Utilizing an aquaculture GIS format (STEM-GIS) to disseminate results, we will contrast the Maine and Connecticut sites’ bathymetry, water velocities and directions, phytoplankton depletion by the shellfish and provide ecological information about the farms, aquaculture activities and BMP recommendations. Furthermore, we will develop an oyster growth module which may be used to optimize shellfish production.

PRINCIPAL ACCOMPLISHMENTS: Summarize in a concise form the findings for each objective for the duration of the project. Measurement data are to be given in SI units. However, to minimize confusion, a dual system of measurement may be used to express results.

- **Objective 1. Develop Hydrodynamic Flow Models of two oyster farms and put results into a GIS framework.**

  In year 1, we utilized MIKE-21 to develop a 25 meter grid 2 dimensional flow model for the upper Damariscotta River, Maine. The 25 m grid cell area and results for the flow model are presented in Figure 1. In addition, high resolution bathymetry was developed for the model in the vicinity of the Pemaquid Oyster Company farm site.

  The GIS was populated with time varying and spatially varying water column characteristics developed from field data and historical data. These water column characteristics were temperature, salinity, chl a, dissolved oxygen, total particulate matter (POM), particulate organic matter (POM), particulate organic carbon (POC) and ammonium. This data was used in the GIS, to predict oyster growth rates of an individual oyster, and combined with daily mean water velocity, the growth rate of a benthic culture of oysters at varying densities at each grid cell in the GIS.

- **Objective 2. Develop a Seston Depletion Model based on field data and oyster feeding rates.**

  The purpose of this aspect of the project was to allow for a GIS functionality in which one can control oyster density at a particular grid cell and generate site specific oyster growth as a function of bottom seeding density. There were three components to this aspect of the project:

  a. Flow-3D model. We developed a seston depletion model which incorporates benthic boundary layer physics, water depth, site specific water velocities, a defined upstream seston concentration, and a density dependent filtration rate based on oyster biomass. This “patch modeling” was completed by June of 2012, using algorithms based on a mussel seston depletion model.
b. Integration into GIS. The output of this model (developed using Flow-3d) reduces seston by a percentage, and that reduced food supply is then fed into SHELLSIM to predict a (reduced) growth rate of the planted oysters. This functionality of the GIS was integrated by July, 2012.

c. Field data is used to calibrate the model developed in (a) above. Drs. Newell and Davis successfully obtained seston depletion information at the Pemaquid Oyster Company lease site in July (Figure 4) and September, 2010 using moored seabird CTD’s.

![Figure 2. Seston depletion data at the POC lease site in July-August, 2010.](image)

- **Objective 3. Assess benthic infaunal, epifauna and meiofaunal impacts.**

Bottom sediments were sampled, using a Smith-MacIntyre grab sampler, on the oyster farm and off the site in control stations on May 10 and September 10, 2011. The data showed greater abundance and taxa richness at the oyster lease sites, and just slightly lower diversity. This result is common in slightly organically enriched environments. There also was an absence of *Capitella*, which is an indicator organism of overly enriched sediments at aquaculture sites. Mobile epifauna, were sampled using underwater video and photography on bottom and around floating nursery trays. Diverse and abundant epifauna at the oyster cultivation sites was found at all sites.

- **Objective 4. Create an American oyster growth model using ShellSIM, calibrated at field sites.**
  - Field sampling of water column characteristics occurred bi-weekly from May to November, 2010 and 2011 at the vicinity of the oyster farm. CTD profiles were also taken at those sites.
  - Tagged oysters were reared in either bottom cages or surface floating bags for two field seasons to determine changes in growth parameters (shell length, live weight, dry weights, etc.) to validate the growth model.
• Oyster biometrics were developed for oysters of a wide range in size (from seed to over 100 mm long) for SHELLSIM calibrations. This was done to compare actual growth to that predicted with SHELLSIM using the growth drivers.

• Oyster biodeposition study. In order to calibrate the oyster physiology and growth model SHELLSIM, investigators undertook *in-situ* measurements of oyster biodeposition in flow-through feeding. (Figure 3). A flow-through chamber was assembled on an oyster nursery raft, and monthly 24 hour experiments were performed from May – November, 2010 and 2011. At the end of a feeding run, cumulative biodeposits (feces and pseudofeces) were collected for 10 individual oysters (along with 2 control chambers) relative to Chl a, POM, SPM and PIM in composite water samples taken hourly over the 24 hour period using an ISCO water sampler.

• A preliminary analysis of the chambers using dye studies and 3-d flow modelling resulted in recommended minimum flow rates through the chambers of 400 ml per minute (Figure 4). Simulations were run with a “virtual oyster” in the chamber to recommend possible changes in the chamber dimensions and baffle locations (Figure 4).

The growth driver data (temperature, salinity, POC, PON, chl a, SPM, PIM, POM) was used by Dr. Hawkins, along with the *in-situ* feeding and absorption data, to simulate oyster growth in Maine and Connecticut (Figure 5).

• Objective 5. Develop the GIS framework into an interactive tool incorporating all of the above data into a user-friendly application for oyster farmers, managers and regulators.

In 2011, investigators conducted several surveys in order to learn how to improve the interactive nature of the GIS framework. We gave presentation and focus group surveys in Maine and Connecticut:

Figure 3. Flow-through feeding manifold with 10 oysters and 2 control chambers.

Figure 4. Exhalent Flow Streamlines within a feeding chamber—Side View (colored by mixing - red is unmixed, blue is well-mixed).

Figure 5. Predicted versus actual growth of oysters based on the ShellSIM growth model.
most understood it, but found it hard to use. Suggested training extension agents for outreach.

From these surveys, we determined that the primary audience, shellfish growers, wanted a more simplified user interface. From this we began development of new pages with FAQ’s: growth (length, meat, weight) at different locations in the domain, growth as a function of density, growth as a function of seed size, time of year. These changes became part of the follow on NRAC funding. The surveys also determined other elements wanted: growth in surface vs bottom culture, contrast good vs bad year, reduce cost in data collection for growth drivers (flow model, water temp. and salinity, food (chl a, SPM, PIM, POM, POC, PON). These issues are being addressed in the current NRAC-funded project

**IMPACTS:**
The NRAC-funded project “Assessment of Environmental Impacts of Oyster Aquaculture in New England Waters” led to an ongoing follow-on NRAC funded project (“Shellfish STEM-GIS (ShellGIS) Development for Improved Siting and Farm Management” that is refining the oyster growth model adapted for “Oyster-Gro” surface/bottom growing growing cages as the project described in this report focused on bottom culture methods. Although the STEM-GIS package has been demonstrated to shellfish farmers at four presentations in the past two years, the PI’s want to complete the software to before showcasing the product more extensively.

The University of Maine and the University of New England in collaboration with the MAIC have successfully submitted an application to the FY2014 National Science Foundation EPSCoR (Experimental Program to Stimulate Competitive Research ) Research Infrastructure Improvement Track 1 program with a $20M proposal entitled “Maine EPSCoR: The Nexus of Coastal Social-Environmental Systems and Sustainable Ecological Aquaculture”. Given that the internal University competition winnowed the applicants from eleven down to this proposal was a major accomplishment. A significant scientific component of the proposed research within this EPSCoR proposal is to expand the STEM-GIS platform from the Damariscotta River demonstration site to six bays along the coast of Maine. This would not have been possible without the support of NRAC funding this initial research.

**RECOMMENDED FOLLOW-UP ACTIVITIES:**
Follow up studies are currently underway as part of an NRAC-funded project: (Shellfish STEM-GIS (ShellGIS) Development for Improved Siting and Farm Management

**SUPPORT:**

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PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED:


Project Completion Report  
Subaward # Z532901  
Grant # 2008-38500-19301

PROJECT CODE: 09-02

PROJECT TITLE: Examination of finfish pathogen physiology and predictive ecology in a bivalve/finfish integrated multi-trophic aquaculture system

DATES OF WORK: 02/01/2010 – 06/30/2013

PARTICIPANTS: Funded cooperating personnel and institutions, agencies, and business entities including extension liaison(s) and non-funded collaborators.
University of Maine
Sally Dixon Molloy
Deborah Bouchard
Michael Pietrak
Ian R Bricknell

University of Connecticut
Robert Pomeroy
Umi Muawanah

REASON FOR TERMINATION:
Objectives completed

PROJECT OBJECTIVES:
1. Optimize molecular assays such as real-time reverse transcription (RT)-PCR to employ alongside currently OIE & ICES accepted pathogen diagnostic assays as a strategy to monitor pathogen fate in infectivity and field based trials.
2. Investigate the ability of mussels to serve as biological filters, or vectors/reservoirs for two important finfish pathogens, IPNV and *Francisella* sp. in laboratory trials.
3. Perform field investigation using sentinel mussels at targeted salmon and cod marine grow-out sites and survey archived mussel samples from our current project to establish seasonal background disease levels and verify laboratory trial results.
4. Develop best management practices regarding introduction and management of bivalves within finfish enterprises based on biological and economic cost-benefit results.
5. Provide industry outreach by preparing information for distribution (i.e., extension fact sheets, publications, web postings) and organizing a special one-day informational session.

ANTICIPATED BENEFITS:

The beneficiaries of this research will be marine finfish and mussel growers who will be able to improve their profits by diversifying their production and employing best management practices while simultaneously lowering the impact of their operation on the environment. Also, diagnostic assays for new or emerging diseases, such as Francisellosis, would be in place in the event of an outbreak. This means fast diagnosis and response to disease outbreaks, which may reduce impacts of disease on the industry. State and federal resource management agencies will also benefit by having a better understanding of disease interactions and having the needed knowledge to establish useful and effective regulations to protect fish health.

An understanding of the interaction between the different production areas of an IMTA farm is a very valuable data set as an improved understanding of this will allow improved risk management practices to be established reducing the risk of interaction between the IMTA zones and wild organisms.
Project management will be carried out on a monthly basis with regular assessments of progress to ensure milestones are met and the project remains focused. Objective 5 describes our project’s anticipated products and outcome.

PRINCIPAL ACCOMPLISHMENTS:
Objective 1

Work on this Milestone was done by Dr. Molloy at the University of Maine. Optimization of culture and molecular assays for IPNV and Francisella is complete. Optimization of Francisella culture from bivalve tissues was problematic initially. The tissues are exposed to the environment and therefore fast growing bacteria present in the tissue samples interfered with isolation of Francisella from mussel tissues. Dr. Molloy initially attempted to insert a red fluorescent protein into our strain of Francisella according to Singer et al. 2010. Unfortunately these attempts were not successful. However, we are now successfully isolating Francisella from mussels using Modified Thayer Martin media, which contains several antimicrobials and reduces growth of unwanted bacteria.

Objective 2/Milestone 1

The fate of IPNV in mussel tissues has been determined and data analysis for this work is complete. Mussels were exposed to virus for 5 days. Water and mussels were sampled at 0, 2, 24, 48, 72 and 96 hours post infection (hpi). Mussels accumulate viable IPNV in their digestive gland tissues as early as 2 h post exposure (hpi) (Figure 1). The accumulation of IPNV in mussel DG tissues was confirmed by qRT-PCR analysis (Figure 2). IPNV segment A RNA levels peaked at 24 hpi and were significantly higher than IPNV RNA levels at 2 hpi ($t = 4.93; P = 0.0006$) and at 120 hpi ($t = -2.61; P = 0.0157$).

Figure 1. Log TCID$_{50}$ of IPNV per ml of water in tanks containing mussels (grey) or lacking mussels (white) or per gram of mussel digestive gland tissue (hatched) over time. Graphs represent the average log TCID$_{50}$ g$^{-1}$ tissue values ± standard error of the mean with $n=9$ mussels and the average log TCID$_{50}$ ml$^{-1}$ of water ± standard error of the mean with $n=3$ tanks. Means represented by the different letters are significantly different (Fisher’s LSD, $a = 0.05$).
Figure 2. The average relative abundance of IPNV VP2 RNA in mussel digestive glands at 2-, 24-, 48-, 72- and 120 h after inoculation with MEM (white bar) or after inoculation with IPNV as measured with Taqman quantitative RT-PCR in trial 1. Graphs represent average values ± standard error of the mean with n=3 and n=9 for MEM and IPNV exposed mussels, respectively. Means with different letters are significantly different (Fishers LSD, α=0.05).

A second trial was conducted to determine if mussels were capable of shedding viable IPNV after exposure. The average IPNV titer in digestive gland tissue of mussels exposed to IPNV for 5 d was log 5.35 ± 0.25 TCID₅₀ g⁻¹ digestive gland tissue. With depuration, IPNV-exposed mussels released viable IPNV in the fecal matter (Figure 3). Viable IPNV was detected in mussel feces as early as 1 d post depuration (dpd) and out to 7 dpd. Of the 8 replicate mussels, only replicate 6 continuously released detectable levels of IPNV in the fecal material from 3 – 7 dpd. For replicate 6, the peak mussel feces IPNV titer of log 4.5 TCID₅₀ g⁻¹ feces occurred at 5 dpd.

Figure 3. Log TCID₅₀ of IPNV per g of mussel feces over time. Feces was collected from 8 replicate mussels for 7 d. Mussel replicate 2 (filled square); replicate 3 (filled diamond); replicate 4 (open triangle); replicate 5 (open diamond); replicate 6 (filled circle); and replicate 8 (filled triangle). Mussel replicate 1 died on day 2 and replicate 7 died on day 6.
Pilot studies determined that *Francisella* persists in hemolymph and digestive gland tissues. Mike Pietrak optimized protocols for the isolation and culture of mussel hemocytes. This is necessary to determine *Francisella*/hemocyte interactions. Initial experiments to determine if *Francisella* directly interacts with hemocytes were performed by staining the bacteria with FITC and looking for association between the hemocytes and stained bacteria. *Francisella* did appear to interact with the hemocytes, however it was not possible to quantify the level of interaction due to problems associated with clumping of hemocytes. Additional studies were conducted to develop techniques for counter-staining hemocytes with ethidium bromide after they were allowed to interact with FITC stained *Francisella*. It was not possible to quantify the interactions, however it was possible to observe bacteria that had been phagocytotised by the hemocytes (Figure 4).

![Flourescent microscopy of mussel hemocytes 2 h after treatment with FITC-labeled *Francisella* (1,000x). Internalized *Francisella* cells appear yellowish-green within ethidium bromide stained hemocytes (red).](image)

Objective 2/Milestone 2

The IPNV/mussel/fish trial was completed February 2012. The data analysis is now complete. Atlantic salmon smolts were challenged with IPNV via cohabitation with IPNV-exposed mussels or with IPNV-injected salmon. Control tanks contained either untreated salmon or salmon cohabitating with IPNV-free mussels.

IPNV can be transmitted from IPNV-exposed mussels to naïve salmon smolts, however it does not appear to occur at a high frequency. There were no salmon mortalities during the cohabitation trial in any of the treatments. The presence of IPNV-positive fish, however, was monitored weekly for four weeks by randomly sampling 6 fish from each tank. All sentinel salmon and salmon cohabitating with control mussels tested negative for IPNV via culture and qRT-PCR analysis. Every salmon i.p. injected with IPNV tested positive for IPNV via culture (Tables 1). IPNV was detected via culture in 1 out of 12 salmon cohabitating with the i.p. injected salmon at 8 days post exposure (dpe) in replicate 1 and at 16 dpe in replicate 2 (Tables 1). In the IPNV exposed mussel treatment group, the mean number of IPNV-positive cohabitating salmon was $1.0 \pm 0.26$ SE ($n = 6$) out 24. There was no statistical difference in IPNV infection status between fish cohabitating with IPNV-exposed mussels vs. fish cohabitating with IPNV-injected salmon.
Table 1. Number of IPNV positive salmon in replicate groups of salmon IP injected with IPNV, cohabitants of IP injected salmon, and cohabitants of IPNV exposed mussels

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Based on current results with Francisella, it does not appear that fish trials will be warranted with this pathogen.

Objective 3/Milestone 3 & Milestone 4

Fieldwork with sentinel mussels was postponed until the summer of 2012 and the introduction of a commercial scale mussel raft on a salmon farm. We had access to this raft and it provided a better platform for deployment of sentinel mussels. We undertook a random sampling of mussels and a targeted sampling of fish on the IMTA site in August 2012, approximately one month after socking the raft. These samples were screened for bacterial and viral pathogens via standard culture techniques. We did not detect any of the pathogens we were looking for. This is not unexpected as Francisella is not known to exist in Maine waters and IPNV infections are controlled at the hatchery. We were not able to get out for a second sampling due to scheduling conflicts and then the mussels being harvested.

To date, we have not been notified of any disease outbreaks on farms in order to deploy sentinel mussels. We will continue to maintain contact with growers if an opportunity arises.

Objective 4

This objective has been completed. The risk/benefit model focuses on likely pathways for pathogen transmission on an IMTA farm. This model (figure 4) was presented in the previous report.

Figure 4. Conceptual model of disease dynamics on an IMTA farm without the concept of pathogen recycling added into it.

Dr. Pomeroy and his graduate student Umi Muawanah have completed the economic modeling. They developed production budget models for a stand alone hypothetical 15-cage salmon farm and then
Conducted a cash flow analysis and financial statement for the standalone model and two IMTA scenarios. The first was the addition of 3 mussels rafts to the hypothetical salmon farm, while the second replaced one cage of salmon production with a cage of mussel production. The economic analysis indicates that both IMTA scenarios have good economic returns compared to salmon monoculture. The analysis did not include the potential cost saving on lice treatment as positive externality from the mussels, primarily as sufficient data does not currently exist to adequately evaluate this potential. A future analysis incorporating potential positive externality from mussels in term of cost saving will add economic benefit to IMTA.

Objective 5

Significant extension work has been completed on the grant. A workshop was held for all of the commercial salmon growers in Maine in March of 2012. The workshop was well attended with the company veterinarian, marine operations manager and all of the site managers in the state attending. The workshop included presentations on the disease work carried out in both this NRAC funded project and our previous funded project along with the economics scenarios developed. Work from this project has been presented at a variety of regional and national meetings as listed below. In addition a manuscript on the IPNV work has been submitted and a manuscript on the economic studies has been submitted to Cooke for review prior to submission.

IMPACTS:

- We have developed model pathways to explain how pathogens are likely to spread on an IMTA farm
- We have demonstrated the ability of IPNV to be spread via infected fish
- Experimental methods and the model pathways developed have led to further research on the ability of mussels to remove larval sea lice from the water column
- Extension work has facilitated the construction of two commercial scale mussel rafts placed on different salmon farms in Maine.

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PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED:

**Publications in Print**

**Manuscripts**


Dissertations

Papers Presented

Mike Pietrak, Sally Molloy, Debbie Bouchard, John Singer & Ian Bricknell
Potential for Disease Transmission on an IMTA Farm: Can I add another species?
1st US IMTA workshop, September 14-15, 2012, Port Townsend, WA

Sally Molloy, Mike Pietrak, Debbie Bouchard, & Ian Bricknell
Interactions of viral fish pathogens Infectious Salmon Anemia Virus and Infectious Pancreatic Necrosis Virus with mussels Mytilus edulis
Aquaculture America, March 1-3, 2012, New Orleans, LA

Mike Pietrak, Sally Molloy, Debbie Bouchard, John Singer & Ian Bricknell
INTERACTION OF A BACTERIAL FISH PATHOGENS Vibrio anguillarum 02ß AND Francisella noatunensis WITH MUSSELS Mytilus edulis
Aquaculture America, March 1-3, 2012, New Orleans, LA

Mike Pietrak, Sally Molloy, Debbie Bouchard, John Singer & Ian Bricknell
POTENTIAL DISEASE RISKS AND BENEFITS ON AN INTEGRATED MUSSEL Mytilus edulis AND MARINE FIN FISH FARM
Aquaculture America, March 1-3, 2012, New Orleans, LA

Umi Muawanah, Marilyn Altobello, Robert Pomeroy, Michael Pietrak.
Sustainable aquaculture: economic impact of using integrated multi trophic aquaculture (IMTA) to reduce sea lice in salmon farming
Northeast Aquaculture Conference and Exposition, December 12-15, 2012, Mystic, CT

Umi Muawanah, Marilyn Altobello, Robert Pomeroy, Michael Pietrak.
Sustainable aquaculture: economic impact of using integrated multi trophic aquaculture (IMTA) to reduce sea lice in salmon farming
Project Completion Report  
Grant # 2008-38500-19301  
Subaward: Z527801

**PROJECT CODE:** 08-08

**PROJECT TITLE:** Assessment of grow-out strategies for the green sea urchin

**DATES OF WORK:** July 1, 2009 to July 30, 2013 (includes 1 year no-cost extension)

**PARTICIPANTS:**

Dr. Nick Brown, Director  
University of Maine Center for Cooperative Aquaculture Research, 33 Salmon Farm Rd., Franklin, ME 04634  
Tel: (207) 422-9096 Fax:(207) 422-8920 Email: npbrown@maine.edu

Dr. Larry G. Harris, Professor of Zoology  
Zoology Department – Spaulding, University of New Hampshire, Durham, NH 03824  
Tel: (603) 862 3897 Email: lharris@hypatia.unh.edu

Stephen Eddy, MSc, Center Biologist  
University of Maine Center for Cooperative Aquaculture Research, 33 Salmon Farm Rd., Franklin, ME 04634  
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Dana Morse, Maine Sea Grant Extension Professional  
Maine Sea Grant at Darling Marine Center, Clarks Cove, Walpole, ME  04573  
Tel: (207) 563-3146 x205 Fax (207) 563- 3119 Email: dana.morse@maine.edu

Jim Wadsworth, Industry Partner  
Friendship International, PO Box 1005, Camden, ME  04843  
Tel: (207) 273-4621 Email: urchins@tds.com

**REASON FOR TERMINATION:** Objectives completed.

**PROJECT OBJECTIVES:**

This project demonstrated and evaluated sea and land based aquaculture methods for green sea urchins, using hatchery seed. The project objectives were:

1) Compare two nursery strategies (sea cages vs. land based tanks) for growing green sea urchin seed (*Strongylocentrotus droebachiensis*) to a size (10-15mm) suitable for out-planting on sea bottom leases.

2) Demonstrate a land based recirculating seawater aquaculture system to on-grow green sea urchins to market size. Test different feeds, feeding strategies, culture densities, and husbandry methods.
3) Test and demonstrate sea ranching (out planting) for growing green sea urchins to harvest. Compare two ocean lease sites in terms of recovery rates and growth of out-planted sea urchins.

4) Compare growth, survival/retrieval, and economic costs/returns of sea ranching vs. tank farming.

5) Develop and disseminate extension and outreach materials on suitable techniques and the economic viability of green sea urchin aquaculture in the Northeast Region.

ANTICIPATED BENEFITS:

- Increase hatchery and nursery capacity for green sea urchin seed and develop more efficient methods
- Increase yields and economic opportunities for the sea urchin fishery using sea ranching
- Develop cost-effective methods for tank farming of sea urchins
- Encourage industry stakeholders to use aquaculture tools to revitalize the fishery

Sea urchin fishermen and dealers in Maine have expressed interest in using hatchery seed to reseed depleted grounds, as done in Japan. Unlike Japan, minimal public funds are available for reseeding so these efforts would have to be privatized. One way to do this is with sea ranching, where hatchery seed is released on bottom aquaculture leases to roam and feed at will, until capture after several years growth. Industry has been reluctant to try this due to concerns about seed costs; doubts that released seed would survive out-planting, grow, and remain within lease boundaries; and unwillingness to privatize fishing grounds. An alternative to sea ranching is tank farming, which offers potential for better survival and growth. However, the economic viability of land based aquaculture is uncertain due to higher costs and the lack of demonstrated methods. It was anticipated that this project might stimulate industry interest in sea urchin aquaculture by addressing these and other related questions. Adoption of aquaculture tools by the sea urchin industry could help revitalize a once economically significant fishery.

PRINCIPAL ACCOMPLISHMENTS:

1. Hatchery Production and Nursery Strategies

   Altogether, ≈150,000 green sea urchin juveniles were produced during the course of this project and about 100,000 were used in the project itself. Green sea urchin seed production commenced prior to project funding in February 2009 at the Harris/Hill (Portsmouth, NH) and the Center for Cooperative Aquaculture Research (CCAR, Franklin, ME). Both hatcheries produced additional seed in 2010 and 2011. Sea and land based nursery methods were used to grow the hatchery seed to a size suitable for out-planting (≥ 10-15 mm test diameter). Simple mesh panels were shown to protect seed urchins from predation in the sea based nursery. In the land based nursery seed was reared in hydroponic plant baskets in a recirculating aquaculture seawater system. Seed reared through the nursery stage using either approach was used for field studies and on-growing.

2. Land-based On-growing of Green Sea Urchins to Market

   We reared 9,200 green sea urchins from hatchery seed to near market size in a land based recirculating seawater aquaculture system. During the three year culture period trials were conducted to evaluate feeds, feeding strategies, stocking densities, and husbandry methods.
These trials provided valuable information for economic modeling and useful for any future tank farming efforts. As of July 2013 about 4,700 urchins (260 kg biomass) were large enough (≥ 45 mm TD) for market analysis, to proceed in 2013-2015 in a separately funded project.

3. Sea Ranching of Green Sea Urchins

We out-planted 21,000 hatchery reared sea urchins at two bottom leases and showed that the out-planted urchins will remain for extended periods within the release area. This has not been previously documented with green sea urchins and it has positive implications for sea ranching. However, growth data was equivocal; only a small number of out-planted urchins were close to the legal minimum harvest size after 27 months, and overall growth was relatively poor at both sites compared to tank farming. Nonetheless, this holds promise that sea ranching, if done at sufficient scale and on suitable grounds, can add value to the fishery over time.

4. Comparison of Sea Ranching with Tank Culture

This is the first time that sea ranching has been compared with tank culture using the same hatchery cohort. Methods, survival and growth, and costs were assessed and compared between the two methods. Tank farming resulted in better growth and survival (recovery) of the urchins, but high operating and feed costs and the long time to market (3+ years) are issues that must be addressed with innovation and research before this can be fully developed. Sea ranching is lower cost and presents less of an entry barrier to industry, but slow growth and losses of out-planted seed from natural causes or poaching remain as concerns.

5. Extension and Outreach

Industry stakeholders were involved throughout the project and kept informed of goals, methods and results. Steve Eddy and Larry Harris attended numerous Sea Urchin Zone Council (SUZC) meetings and both are voting members and are on the SUZC research sub-committee. Recent discussions at SUZC meetings have included reseeding and sea ranching as topics, along with consideration of creating one or more zones operated by fishing coops, where reseeding and other intensive management methods could be practiced. Paul Anderson and Dana Morse of Maine Sea Grant facilitated a panel discussion on sea urchin aquaculture for the 2012 Maine Fishermen's Forum and a class session on sea urchin aquaculture for the 2013 "Aquaculture in Shared waters" course. Steve Eddy gave a presentation at the 2013 Maine Fishermen's Forum on sea urchin aquaculture, attended by around 50 individuals. Graduate student Pamela Fraungruber gave several poster and oral presentations, including at the Northeast Aquaculture Conference and Exposition 2010 and at the National Shellfisheries Association Meeting 2012. Over 400 pamphlets describing sea urchin aquaculture were disseminated at these and other forums, and the Maine Public Broadcasting Network did a segment on the project in 2011. The project will also be described in detail in a text on sea urchin aquaculture, currently in progress.

IMPACTS:

1. Hatchery and nursery production demonstrated to industry that there are reliable sources of green sea urchin seed available for commercial projects and reseeding. Several recent industry inquiries regarding hatchery capacity and seed costs can be attributed to the success of these efforts and to our outreach.

2. The project showed that out-planted sea urchins will remain within lease boundaries, resulting in three Maine-based companies to apply for bottom leases to culture green sea urchins.
urchins. Successful sea ranching could revitalize a fishery annually valued at around 5 million dollars in Maine.

3. We produced market sized sea urchins with tank farming, enabling us to obtain further funding to evaluate intensive land-based gonad enhancement aquaculture. The economic benefits of gonad enhancement aquaculture are readily appreciated and accessed by industry, and if successful could double the final market value of processed roe.

**RECOMMENDED FOLLOW-UP ACTIVITIES:**
The sea ranching trials showed promise but our ability to evaluate their true potential was limited by the small scale of the releases (10,500 seed at each of the two leases) and the logistical/funding constraints restricting the quadrat sampling spatially and temporally (we only sampled within the release areas and the surveys ended after 27 months). We recommend that further sea ranching trials occur but at a larger scale of at least 150,000 seed/acre and that sampling to evaluate tag absence/presence take place for at least three years post-release. We also recommend funding for studies to determine the longevity of fluorescent tagging beyond the 27 months we saw in this project.

We saw wide variation in green sea urchin growth rates at all life stages, and this likely has some genetic basis. We recommend a selective breeding program to see if time to market size (≥45 mm) in tank culture can be reduced from three years to 18 months or less. Green sea urchins can reach reproductive maturity at 25 mm (12-18 months), so it should be possible to produce three generations within 36-54 months. Improvements in hatchery methods and infra-structure are also needed to reduce seed production costs. Areas that should be addressed include survival through metamorphosis, more efficient settlement methods, and development of micro-diets for larval feeding.

Finally, we recommend that low cost formulated on-growing feeds be commercially developed. Formulated diets can significantly increase growth rates but the lack of available diets and their high cost currently make their use impractical. In addition, the effects of formulated feeds on roe culinary quality must be evaluated. This topic was beyond the project scope because most of the tank farmed urchins did not reach market size until year 4. The CCAR has obtained additional funding for 2013-2015 to conduct gonad enhancement trials, quality evaluation, a Taste Panel, and a market analysis of cultured sea urchin roe.

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Publications in Print


Manuscripts


Papers Presented


PART II

Objective 1) Compare two nursery strategies (sea cages vs. land based tanks) for growing green sea urchin seed (*Strongylocentrotus droebachiensis*) to a size (10-15mm) suitable for out-planting on bottom leases. Improved survival and returns to the fishery are possible when seed is grown to 10-15 mm TD (test diameter) prior to out-planting. The period of growth from settlement to release size is the nursery stage, and for *S. droebachiensis* this may require 3-10 months. Nursery rearing can be done in sea based caging systems or land based tank systems. Sea based nurseries are less costly and may produce hardier urchins. Land-based nurseries, although costlier, allow for more intensive management to improve survival and growth. A sea based caging system located at the New Hampshire lease site and an intensive tank system located at the CCAR were used to demonstrate, improve, and compare nursery methods for green sea urchin seed production.
Methods
All of the Harris/Hill hatchery seed were released on-bottom or reared in sea based caging systems at the two Portsmouth Harbor sites in New Hampshire. The majority of the CCAR hatchery seed (≈60,000) were reared in tank-nursery systems at the CCAR, with the exception of 9,850 seed that were transferred to New Hampshire. Most of the CCAR seed urchins (36,100) came from the 2009 hatchery cohort and the remainder from 2010 and 2011. The land based nursery methods described here apply to the 2009 cohort.

Methods, Tagging and tag identification
Hatchery seed juveniles destined to be out-planted for nursery caging trials or release onto the bottom leases were tagged in the hatchery when they were 5-10 mm TD with a fluorescent dye. All urchins were tagged by two day immersion in a dye bath, either with Alizarin Red at 50 mg/l (Sigma-Aldrich Alizarin complexone Alizarin-3-methyliminodiacetic acid A3882) or tetracycline at 100 mg/l (Sigma-Aldrich Tetracycline T3258). Feeding continued throughout dying to ensure growth and incorporation of the dye into the calcareous structural skeleton. Fluorescence microscopy was used to visualize the tags at time of sampling. Sampled urchins were measured for weight and diameter in the laboratory. The jaws of each urchin were removed using forceps and placed in a sodium hypochlorite bleach solution to remove the soft tissue from the calcareous skeleton. Some individuals were too small to remove the mouthparts without causing damage by crushing. In these cases the entire body was immersed in bleach. The bleach solution does not degrade the fluorescent tags, and several days after jaws were immersed only the demipyramid structures and calcite teeth, which combine to form the jaws, remained. Samples were then rinsed with fresh water and dried. The samples were taken to the University of Maine campus in Orono, where a Zeiss stereo discovery microscope was used to visualize the fluorescent tags, which show clearly under a broad spectrum light with filters for red or green pigments (Figure 1a and 1b).

Figure 1 Sea urchin jaws dyed using Alizarin Red (a, left) and Oxytetracycline (b, right)
**Methods, Sea based nursery**

Two stations within the Portsmouth Harbor sea urchin research site were chosen. The first is a side channel off the main channel that connects Little Harbor to Portsmouth Harbor. There is less current and the bottom is a combination of cobble and shell fragments, primarily of the horse mussel *Modiolus modiolus*, both of which are encrusted with coralline algae. The second site is in the main channel adjacent to the bridge connecting Portsmouth to NewCastle. The current is stronger here during the incoming and outgoing tides and is most readily accessed during the periods near slack water. The bottom is also composed of cobble and shell fragments, but is less silty than the side channel. Three trials were undertaken at the first site and the second site was established for the third experiment. The first trial assessed the possibility of utilizing bottom cages for initial grow out. On 12 November, 2009, seven cages with 5,250 urchins from the CCAR and tagged with Alizarin red were deployed (750 per cage). The cages were all 50 x 50 x 15 cm in dimension and contained 15 (15 x 50 cm) fiberglass panels suspended vertically. The cages were elevated approximately 8 cm off the bottom by four bricks attached to the undersides of the cages. The second trial involved seeding 10 sets of 750 juvenile urchins without cages in defined areas marked by bricks left on bottom. The urchins were deployed on 14 June 2010. The third trial involved placing urchins under 0.5 m² square sheets of plastic coated wire mesh (1/2” x 1/2”) anchored by 4 bricks. Five sets of urchins left over from the first caging study were placed under wire sheets in the side channel on 27 June 2011 and another five sets of 920 urchins per set (4,600) from CCAR were out planted under sheets at the bridge site on the same date.

In addition to urchins supplied by CCAR, urchins produced in the Portsmouth Hatchery were out planted on two occasions. The first trial involved adding approximately 1000 1 mm juveniles to each of five cages that were placed on top of flat wire mesh sheets to provide a refuge under the cages for urchins that might leave the cages. The first trial began on 10 August 2010. The second trial involved placing 100 10 to 15 mm juvenile urchins in five cages and another 100 urchins each under five wire sheets at the main channel site on 14 April 2011. Other than the initial caging trial, all experiments are still in place and monitored at least twice a year.

**Methods, Land based nursery**

Tank-nursery culture at the CCAR occurred in two overlapping stages. The first stage began after metamorphosis/settlement onto plastic panels or floating media in a pair of 570L raceways (244 cm x 76 cm x 38 cm). About 770,000 competent larvae, as calculated with 1 ml volumetric counts, were transferred for metamorphosis from the larval rearing tanks into the settlement raceways. The raceways were supplied with flow-through filtered seawater in a refrigerated room tempered to maintain temperature between 9-14 °C. The newly settled urchins (‘pinheads’) began grazing upon surface diatoms and other microbes after jaw formation, or about 7 days post-metamorphosis. After 45 days the survivors were ≥2 mm TD and locally harvested kelp *Saccharina* sp. was added to the raceways. Larger urchins (≥4 mm) that moved on to the kelp for feeding were removed and hand counted into perforated hydroponic plant baskets (16 cm x 16 cm x 10 cm) in groups of up to 250 urchins per basket. As larger urchins were removed from the raceways they were replaced by others, and after another 30-60 days (90-120 days post-settlement) most of the seed had been transferred into baskets for the second nursery stage. A total of 38,000 urchins were transferred from the raceways into the baskets.
The baskets were suspended in foam floats or placed on plastic grating in shallow round tanks or raceways in a seawater recirculating aquaculture system (RAS). Aeration was provided at intervals between baskets to improve water circulation. Rearing temperatures were held between 9-14 °C, and the sea urchins were fed locally harvested kelp Saccharina sp. to satiation every 3-5 days. Growth was monitored by sampling 30 animals per basket at 2-3 month intervals. Test diameter was measured to 0.1mm using electronic digital calipers (Mitutoyo model CD-6"PMX) and blotted wet weight was measured to 0.1g using an electronic balance (A&D GF200, ε=0.01g). Daily mortality records were kept to monitor the performance of the culture system, animals, and husbandry methods. Periodic hand grading and sorting was done to maintain uniform size ranges in the baskets.

Results
Tagging
A number of tagged urchins were held in the lab and sampled immediately and then at one month to determine tagging rates. These samples indicated 100% tagging rates. At the lease sites, fluorescent tagging bands were detected in sampled urchins at every dive sample up to the last in May 2012. This shows that the fluorescent tags can persist and be detected for up to 27 months after tagging.

Results, Sea based nursery
The survival of out planted urchins varied significantly from trial to trial with the greatest survival observed with small urchins protected by the flat wire sheets. Habitat type also appeared to make a difference with the greatest survival in structurally complex substrates dominated by shell fragments. The first caging trial was terminated on 14 June, 2010 for four cages and 20 July, 2010 for the final three. The cages were opened and all urchins were collected to be measured and data sent to CCAR for analysis. There was survival in six out of the seven cages, though the percentage of survivors varied greatly and growth was limited. Overgrowth of the cages by invasive (non-indigenous) algae and colonial tunicates (Botryloides violaceus and Didemnum vexillum) reduced light and water flow within the cages. The second out planting experiment is still being monitored, but survival has been very low, with only 16 total urchins still present over the 10 quadrats. None of the urchins has reached the minimum legal size of 52 mm. In the third trial the original caged urchins have done very well and have remained associated with the protective wire sheets. The sheets in the side channel were turned over on 9 March 2012 and numbers have increased with larger urchins attracted to the structure and epibiotic growth on the wire mesh. Survival of the urchins at the main channel site is also good, but there appears to be limited growth without turning over of the sheets to create open space for more movement. On 18 July 2012, there were respectively 94, 84, 118, 55 and 10 urchins associated with the five sheets in the side channel and a number of the urchins were of legal size.

The caging experiments using Portsmouth Hatchery urchins were not as successful. The survival of 1 mm urchins was poor and only a few individuals have been observed, though the cages are still in place and yet to be destructively sampled. The attempt at caging juveniles in the main channel was hampered by loss of cage tops due to drag from algal growth and strong tidal currents, which was not an issue in the side channel. The survival of urchins under the sheets
appears to be similar for both the urchins raised at the CCAR and those from the Portsmouth Hatchery.

**Results, Land based nursery**
The end of the tank-nursery period came when \( \approx 85\% \) of the population was \( \geq 10 \text{ mm TD} \), at about 10 months following metamorphosis/settlement. Survival through the entire nursery period was estimated as 4.7%. Most of the mortality likely occurred very early with urchins that failed to successfully make the transition from metamorphosis to the onset of exogenous feeding. Of the estimated 770,000 competent larvae stocked into the settlement raceways only 38,000 seed (4+ mm) were hand counted out into the nursery baskets for the second nursery stage. Survival through the second nursery stage in the plant baskets was 95%, and 36,100 viable juveniles were produced for the out-planting and tank trials.

The mean test diameter at the end of nursery culture was 11.0 ± 3.7 mm. 12.1% of the population was smaller than the minimum recommended release size of 10 mm, 82.6% was between 10-15 mm, and 5.3% was 15.5-36 mm. The population was randomly mixed together in February 2010 and 21,000 were randomly chosen for release at the Penobscot Bay leases. Another 4,600 were transferred to the Gingrich lease site for use in nursery caging trials, and 10,500 were kept in the CCAR facility to be used in the land based on-growing trials.

**Discussion of hatchery and nursery production**
The low survival (4.9%) we observed at the CCAR following settlement/metamorphosis to a size of 4-5 mm has been seen with other sea urchin species. Investigators have proposed several causes, including insufficient or inappropriate diatom species available for feed in the settlement tanks, harmful microbes, predation by copepods or nematodes, and poor maternal egg quality or larval nutrition. Japanese researchers have seen post-settlement survival rates as high as 60-70%, but it is unclear as to whether this is the rule or the exception. Improving post-settlement survival would increase hatchery efficiency and reduce seed production costs, and this is clearly an area requiring further work.

The hydroponic plant baskets proved to be efficient and effective nursery containers. The perforated baskets allowed water and wastes to pass through. The baskets facilitated feeding and minimized direct handling of the animals, and the side walls increased the total surface area available for urchin attachment relative to the surface area provided by the tank itself. Survival in the baskets over the course of 8 months as the juveniles grew from \( \approx 4 \text{ mm} \) to \( 10+ \text{ mm TD} \) was 95%. This compares favorably with sea cage nurseries, where survival rates can be variable and subject to unpredictable natural events. In a previous study where we used mesh tubes attached to on-bottom oyster cages as a nursery, survival ranged from 56% to 89%.

At this point we cannot recommend sea based caging systems such as those tested at the Portsmouth Harbor site over land based nursery systems such as those used at the CCAR. Survival in the initial caging study at the Portsmouth Harbor site was highly variable and it is unlikely that utilizing a field-based cage system for the nursery phase of juvenile urchins is a viable approach. If done at a larger scale than in this project the amount of bottom gear and the extended length of time that it needs to remain on bottom before the urchins are at release size (9-12 months) might make on-bottom or floating cage systems unworkable. Additionally, when
gear is included in an aquaculture lease application it complicates the process and potentially creates opposition to the lease. This could be especially true at sites where other types of fishing occur, such as dragging or lobstering. There are three alternatives to sea based nursery cages that may be viable. The first option is to seed very young juveniles directly into structurally complex habitats in the winter when most predators are not active. The Portsmouth Hatchery can produce more than 2 million juveniles in a single run and out-planting newly settled urchins in the right habitat may be the most cost effective approach. The second approach is to use a land based initial grow-out phase (nursery) as has been utilized by the CCAR. Land based nurseries offer some advantages, such as the ability to cull out slow growing seed while at the same time improving overall growth rates and survival. Adding lights and a flow through seawater source so that natural epibiont growth provides the food source could help reduce labor and feed costs. In an earlier study we showed that formulated feeds with about 20% protein can significantly increase juvenile growth rates. This shortens the nursery phase but it might not reduce costs due to the high expense of formulated diets vs. natural feeds such as field collected kelp or epibiont growth. Also, it is not known if survival might differ between hatchery seed reared on formulated feeds vs. seed reared on natural feeds once the seed is released on bottom. The third option is to combine juvenile grow out with another species, such as oysters, scallops or mussels to provide a value added and complementary culture species. This takes advantage of existing culture methods and equipment to reduce costs and increase value. Ultimately, it will be critical to be selective about where and when to utilize hatchery-produced juveniles. Larval cultivation to settled juveniles is well understood, but there is still much to learn about how to maximize field-based production of out planted juveniles, and this will be the most cost effective strategy for most markets for the foreseeable future. Sea ranching of urchins after the initial growth phase is likely the most economical approach for a species that requires high volume and shows relatively slow growth, such as the green sea urchin in the Gulf of Maine.

**Objective 2) Demonstrate a land based recirculating seawater aquaculture system to on-grow green sea urchins to market size. Test different feeds, feeding strategies, culture densities, and husbandry methods.**

Land-based aquaculture of sea urchins offers several potential advantages over sea based aquaculture methods such as sea ranching. Because temperatures can be controlled and feeding optimized in a land based system it can be possible to have improved growth and survival. Husbandry operations such as grading and culling can be carried out to focus on the best performing animals. However, energy, labor, and real estate costs can be much higher for a land based operation and these costs may negate any gains. With this objective we sought to test and demonstrate efficient methods for tank farming sea urchins and quantify growth, survival, and some of the associated costs.

**Methods**

**Methods, Tank culture system**

The CCAR designed and built a tank system and RAS for sea urchin growout and stocked it in November 2010 with 9,200 juveniles from the 2009 hatchery cohort. Rearing tanks were configured to maximize internal surface area available for attachment, allow for efficient feeding and waste removal, and permit observation and easy access to the urchins. Ultimately we wanted to provide a low cost do-it-yourself design that could be built by fishermen and start-up companies. V-shaped troughs with perforated floor plates were fabricated out of dimensional
lumber and plywood covered with fiberglass. Each set of paired troughs was assembled with 
three sheets of 1.2 m x 2.4 m (4 ft x 8 ft) exterior grade plywood supported with 5 cm x 10 cm (2 
in x 4 in) boards and plywood ribs. The side walls were 61 cm (2 ft) wide and 2.4 m (8 ft) long 
and sloped at 45°. A perforated PVC plate over a half-round 10 cm (4 in) diameter PVC pipe 
formed a drainage channel to capture wastes, which were flushed by pulling a pipe on an 
external standpipe. Six pairs of V-troughs were piped into a recirculating aquaculture system 
(RAS) equipped with a parabolic filter for solids removal, moving bed biofilter, foam 
fractionator with oxygen injection, and UV sterilizer (Figure 2). The urchins were reared in this 
system up to the date of this report (July 2013). Growth and survival were monitored over the 
course of three years, and water quality parameters were measured either daily (oxygen, 
temperature) or weekly (total and un-ionized ammonia, nitrite, nitrate, pH, alkalinity, and CO2). 
Three trials were carried out to assess feeds, feeding regimes and stocking densities.

Methods, Feeds and feeding of sea urchins in the tank system
Two production scale feeding trials were carried out to evaluate different feeds and feeding 
rates. An earlier trial (2008) with small juveniles (mean = 5.5 mm TD) demonstrated that tank 
reared urchins have better growth when fed formulated diets compared to kelp fed urchins. Eight 
diets formulated for sea urchins by the Texas A&M Feed Labs and varying in protein (16% to 
40% protein) and carbohydrate (29% to 49% carbohydrate) were compared to each other and to a 
commercially available abalone diet and the kelp *Saccharina latissima*. Diets with about 20% 
protein gave the best results in that study.

However, the Texas A&M diets were not available in sufficient quantity (>100 kg) to support a 
commercial scale demonstration project, so a sea urchin feed imported from Norway was used 
(Nofima). The Nofima diet was formulated for green sea urchins and had a proximate 
composition of 21.3% protein, 46.2% carbohydrate, 7.5% fat, 14.2% ash, and a carotenoid 
pigment. A sinking pelleted catfish feed manufactured by Cargill was also tested as a low cost 
alternative. The Cargill diet was 32% protein, 5% fat, and 10% fiber. The bulk of the protein 
was from cereal grains and some offal, and the diet did not include a carotenoid pigment source 
or any marine derived lipids. In these respects the catfish feed appeared to be less than optimal 
for sustaining sea urchin health and growth, but other researchers have used maize (corn) based
diets with satisfactory results to feed the European sea urchin. The Nofima and Cargill diets were compared at production scale using the entire population. The urchins were sorted by three size grades into seven V-troughs and each tank/size grade was fed either the Nofima or Cargill diet at ≈2% biomass once every three days for 281 days. Weights and test diameters were measured (as described previously) for a random sample of thirty urchins from each tank at days 0, 184, and 281. The daily specific growth rate (SGR) was calculated for each tank as SGR (%) = \[\frac{\ln \text{whole wet weight (t2)} - \ln \text{whole wet weight (t1)}}{\text{t2} - \text{t1}}\] x 100. At day 281 all groups on the Cargill diet were switched to the Nofima diet and the trial was continued for another 56 days to look for evidence of compensatory growth in the Cargill fed urchins.

During the Nofima/Cargill feed trial it was observed that all of the feed was generally consumed within 2-3 days, but it was unclear if 2% ration at 1x/3 days maximized both somatic growth and economic return. A second trial was conducted to investigate the effects of different feeding frequencies on growth and feed conversion. The urchins were size graded into twelve V-troughs; three tanks held 'small' urchins (30-34 mm TD at 12-15 g), five held 'medium' urchins (35-40 mm at 18-23 g) and four held 'large' urchins (>40 mm at 28-60 g). All tanks and size grades were fed Nofima at a ration of 1% biomass at varying frequencies; five tanks were fed 1x/3 days, three were fed 1x/7 days, and four were fed 1x/14 days. Size measurements (weight and diameter) were done as previously described on a random sample of thirty urchins per tank at 2-3 month intervals, and the feed ration was re-adjusted to account for growth. Specific growth rates (SGR) and feed conversion ratios (FCR) were calculated over the course of the 9-month trial.

Methods, Stocking densities of sea urchins in the tank culture system
Previous studies have recommended a maximum tank stocking density of about 6 kg/m² for green sea urchins reared in raceways with 90° vertical walls. We were interested in determining if higher stocking densities could be achieved in the slanted wall V-troughs, without affecting growth or survival. Each V-trough had 2.67 m² of submerged surface available for urchin attachment. Seven of the twelve troughs were initially stocked with ≈9,200 juvenile urchins graded into three overlapping size ranges (9-18 mm; 15-26 mm; 22-33 mm) at densities ranging from 0.5 to 2.9 kg/m². The urchins were size sampled at 2-3 months throughout the course of the project and stocking densities were re-calculated at each sampling interval. The population was graded following one year of growth (Nov. 2011) into three additional tanks at densities ranging between the ten tanks from 5.1 to 14.8 kg/m². Growth and survival were then measured for another year, until Oct. 2012.

Results
Results, Performance of the tank culture system
The tank culture system operated continuously from December 2010 to July 2013 with no significant failures. Critical water chemistry parameters (NH₃, NO₂, CO₂) were always below the threshold levels reported as harmful to green sea urchins by other investigators (un-ionized ammonia ≤ 0.016 mg/l, nitrite ≤ 0.5 mg/l, and CO₂ ≤ 18 mg/l) (see papers by Siikavuoipoio et al, 2004-2007). Oxygen typically ranged from 7.0 to 10.0 mg/L. The temperature declined to as low as 2°C in the winter to as high as 19°C in the summer, but only for brief periods (1-2
weeks), and for the most part it remained within the optimum range of 8-14°C. Mortality rates increased when temperatures exceeded 16°C, but despite this the total mortality during the first two years was less than 5% (425 urchins, or 4.6%). After 25 months of on-growing (or 44 months post-settlement) 56% of the population was ≥ 40 mm, but less than 5% of the population was at or near the legal minimum harvest size of 52 mm (Figure 3).

During spring of the third year (March-April 2013) the population experienced a 2-month chronic mortality event triggered by handling and grading. In affected tanks the urchins developed purple lesions with subsequent spine loss and mortality. Diagnostics indicated that the prevalent bacterial isolate was *Vibrio vulnificus*. After a period of aggressive culling of symptomatic urchins the mortality abated, but total mortality from this event was about one-third of the entire tank population. As of July 2013 4,620 remaining urchins were large enough (≥ 45 mm) to be included in a separately funded market enhancement and quality evaluation project. Approximately 1,500 remained too small for market but will be used in future out-planting studies.

![Figure 3. Number of tank urchins in three size categories after 25 months growth in an RAS.](image)

**Results, Feeds and feeding**

*Feeding trial #1: Comparison of Nofima sea urchin diet vs. Cargill catfish diet*
Figure 4. Growth of green sea urchins of different size categories over an 11 month period when fed the Nofima urchin diet or the Cargill catfish diet. Small=10-18mm, avg. 1.3g; medium=16-24mm, avg. 3.4g; large=22-30mm, avg. 7.1g. **Growth rates were better for urchins fed the Nofima diet vs. those fed the Cargill diet, for urchins in all size categories.**

Figure 5. Daily specific growth rates for different size classes of green sea urchins fed either the Nofima sea urchin diet or the Cargill catfish diet. Small=10-18mm, avg. 1.3g; medium=16-24mm, avg. 3.4g; large=22-30mm, avg. 7.1g. Each bar represents one tank and significance levels were not calculated due to the lack of replication between treatments. **The Nofima diet outperformed the Cargill diet in all urchin size categories.**
Feeding trial #2: Effects on growth and feed conversion when Nofima was fed at different rates

Figure 6. Specific growth rates (SGR) of green sea urchins of different size categories fed at frequent (1x/3 days), less frequent (1x/7 days) and infrequent (1x/14 days) intervals. Small=30-34 mm TD and 12-15 g; medium=35-40 mm and 18-23 g; large>40 mm and 28-61 g. Each bar represents one tank and significance levels were not calculated due to the lack of replication between treatments. **Growth was improved with more frequent feeding, but only for urchins <40 mm.**

Figure 7. Feed conversion ratios (FCR) of green sea urchins of different size categories fed at frequent (1x/3 days), less frequent (1x/7 days) and infrequent (1x/14 days) intervals. Small=30-34 mm TD and 12-15 g; medium=35-40 mm and 18-23 g; large>40 mm and 28-61 g.
Each bar represents one tank and significance levels were not calculated due to the lack of replication between treatments. **Feed conversion was more efficient at reduced feeding levels, but only for urchins <40mm.**

### Results, Stocking densities

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Table 1. Green sea urchin starting and ending weights and tank stocking densities before and after grading (shaded area). **There was no difference in growth or mortality seen at any stocking density. The highest stocking density we achieved was 16.3 kg/m², at which point there was 20% or less open surface area available for urchin movement and growth.**

**Discussion of land based tank culture**

**Tank culture system**

Our previous experience using flat bottom raceways with 90° walls showed that sea urchins strongly preferred attaching to the walls and did not utilize the floor area. This made it impossible to evenly distribute feed to all of the animals other than those along the top row. The flat bottom collected wastes, which could only be removed with laborious siphoning. The 45° wall design mitigated these issues and in particular was extremely effective at allowing even distribution of formulated feed onto a tightly packed population of surface attached animals with limited capacity for movement. As the feed rained down on the animals their tube feed rapidly grasped onto the feed pellets and moved them to the aboral mouth for consumption, and within 5-10 minutes most of the feed was no longer visible. Fecal pellets excreted out of the
anal/genital pore located on top of the animal readily drifted down onto the perforated plate. Unfortunately, the perforated plates did not altogether eliminate the need for tank siphoning, but the narrow trough greatly reduced the area to be siphoned and hence the time required to do it. We also discovered, to our chagrin, that sea urchins will eat holes into textured areas of fiberglass gel coat, and this required some repair efforts in year 3 of tank on-growing. This appeared to be less of a problem in tanks where the gel coat was applied to give a smoother finish.

The tank design could be improved while still retaining the basic elements of slanted walls and a perforated or screened bottom. Lightweight molded plastic or fiberglass tanks that can be stacked at two or more levels will make more efficient use of floor space and reduce space heating/cooling costs. These stacked troughs would be similar to those previously used by Mick Devin et al., but deeper and wider. The floor channel should include a series of evenly spaced sump drains along the length (rather than a single drain at the end). We found that the single drain caused wastes to build up underneath the plate due to the lack of current. The wastes did not readily flow down the end drain when it was purged, requiring that a hose be snaked underneath the plate to free up the wastes. A series of drain sumps along the length of the channel should mitigate this problem.

We showed that green sea urchins can thrive in a seawater RAS. The 2.4 m³ capacity moving bed biofilter supported a sea urchin biomass of as much as 600 kg with a seawater make-up flow of less than 10%, while holding ammonia and nitrites below harmful levels (un-ionized ammonia $\leq 0.016$ mg/l and nitrite $\leq 0.5$ mg/l). The use of RAS technology gave us better control over temperature than we would have had in a single pass flow through system, which is crucial for optimizing growth and survival rates. The RAS had a waste discharge flow of $<8$ l/m on average. This is important given that land-based aquaculture requires NPDES discharge permitting, which can be costly depending upon discharge rates.

Feeds and feeding
Previous studies have shown that when fed ad libitum most sea urchin species consume more feed than needed for somatic growth, putting the excess into gonad production or excretion. Sea urchins do not respond to satiation and feeding rates are influenced by factors such as feed quality, season, and size of the animal. Since feed costs and feed conversion can significantly affect production costs of land-based aquaculture, determining the optimal feed regime for green sea urchins is crucial for economic success.

The results of our feeding trials (Figures 4 & 5) show that green sea urchins can be grown for a lengthy period on terrestrial grain based feeds (Cargill) used for other species such as catfish. The lack of marine proteins, lipids and pigments in this diet did not compromise survival but did appear to compromise growth compared to sea urchins grown with a high quality specialty diet (Nofima). The price differential between the two diets ($\approx 0.55$ US/kg for Cargill vs. $7.45$ kg for the Nofima) indicates room for improvement in terms of balancing feed costs with quality. Low cost sea urchin feeds could be developed by supplementing existing cereal grain diets used to grow catfish or tilapia with some minimal level of marine based ingredients. What that level
is remains a subject for further study and the issue of gonad quality will in all likelihood need to
be addressed with a different feed regime to prepare the urchins for market. The use of seaweed
as a 'finishing diet' for urchins reared on formulated feeds is currently under investigation at the
CCAR.

Our investigation into the effects of different feeding frequencies on growth and feed conversion
(Figures 6 & 7) revealed some interesting findings, but more in-depth study is needed. Urchins
at 35-40 mm appeared to be past the period of fast somatic growth, and therefore should be fed
with the goal of improving gonad quality rather than maximizing growth (Figure 6). A reduced
feeding frequency (perhaps 1x/week) might be appropriate for these market size urchins,
whereas more frequent feeding (1x/3 days) seems to be required for juveniles (Figure 6). There
was, however, a trade-off between increased juvenile growth at higher rations and feed
conversion. Juveniles fed more frequently grew faster but at the expense of feed conversion
efficiency (Figure 7). The proper balance between the two will undoubtedly depend on the diet,
but in any case it appears that larger urchins may only require 1 feed per week, which will help
to reduce labor and feed costs.

Stocking densities
Stocking densities are a key determinant affecting profitability of sea urchin tank culture. Using
tanks with slanting walls we grew urchins at a range of densities from 0.5 to 16.3 kg/m², with no
adverse effects on survival or growth observed at the highest densities (Figure 8), which were
well above the 6 kg/m² recommended for this species in previous studies by Siikavuopio et al.
(2007). At the highest density of 16.3 kg/m² a set of paired troughs held 87 kg of urchins within
4.5 m² of floor area, for a footprint density of 19.3 kg/m² (Figure 8). By comparison the stacked
system reported by Devin et al (2002) held 200 kg within a 7 m² footprint, or 28.6 kg/m².
Vertically stacked tanks reduce the real estate footprint and may be more economically efficient.
However, stacked tanks require platforms or ladders for access and might increase pumping costs
due to the additional head. Another way to maximize holding efficiency is to use cage systems
within tanks, as with the UrchinPlatter™ developed by the Irish company Gourmet Marine. This
system reportedly holds from 50 to 90 kg/m² of sea urchins, and it is currently being trialed by
the CCAR for gonad enhancement of S. droebachiensis. Another option is tank/cage polyculture
systems that hold sea urchins, sea cucumbers and fish. This approach is reportedly being trialed
by the Japanese at this time.

Objective 3) Test and demonstrate sea ranching for growing green sea urchins to
harvest. Compare two ocean lease sites in terms of recovery rates and
growth of out-planted sea urchins.

Sea ranching is a low cost extensive culture method that may be well suited for use by existing
fishery participants. It can be done with or without an aquaculture lease and the major expenses
are seed and out-planting costs, any lease rental fees, and harvesting costs. A major concern
with sea ranching at an aquaculture lease is that very few if any of the out-planted urchins will
survive the transfer or remain within lease boundaries. Growth rates of out-planted hatchery
seed are also of concern. Primary project objectives were to demonstrate sea ranching at
aquaculture leases and gain information on site criteria, survival rates (or more accurately,
recovery rates), and growth while looking for differences between sites. Two lease sites were
acquired for this project in Penobscot Bay, Maine by industry partner Friendship International. The sites were known as Job and Sloop after nearby islands.

Methods
Prior to out-planting an initial transect dive was done to estimate the extent of existing sea urchins, predators, and bottom cover. Predators were not found in abundance at either site, with only one large Jonah crab (*Cancer borealis*) observed. Though a large number of naturally occurring urchins were not found at the Job site, reports from local urchin divers and the suitable nature of the habitat indicated that both sites could support sea ranching. The habitat consisted mostly of mussel shell cobble at the Sloop Island site and rock cobble at the Job Island site, ideal for out-planting seed. Crustose coralline algae covered most of the cobble, providing an ample food source. The leases were marked with buoys to indicate that harvesting urchins by dragging nets across the bottom was prohibited.

In February of 2010 21,000 juvenile green sea urchins reared in the CCAR hatchery were tagged with tetracycline (Figure 1b) and 10,500 were out-planted at each site. The urchins were about 9 months post-settlement and ranged from 5 to 20 mm TD (average 10.6 mm at Job; 11.3 mm at Sloop). They were distributed along transect lines laid out to 15m in all four compass directions, encompassing a total area of 400m². Between 1,000 and 1,500 juveniles were released at 4m and 10m markers along the transects to ensure an even distribution. A post-release dive survey was conducted in April of 2010 to estimate survival of out-planted juveniles. At each lease site a baseline was laid out in a North-South orientation and five transect lines were laid out on a perpendicular (East-West) bearing extending to 10m. Sample quadrats consisting of a 1m² PVC frame were placed at the 10m marker in each direction, at the center of the transect, and just over the baseline (0 m on transect), for a total of 15 quadrats per site (Figure 8). All urchins within the quadrat were enumerated and those between 4-30 mm TD were collected in numbered mesh tubes to be taken to the laboratory for measurement and identification (presence/absence of fluorescent dye tags). The out-planted areas were subsequently dive surveyed at regular intervals (4-5 months) on six more occasions over the course of two years. Urchins smaller or much larger than the original release size were not collected in early surveys but during later surveys larger urchins were collected with the assumption that growth had occurred. This sampling regime allowed some inferences regarding survival and growth to be made, to be compared with the 10,000 urchins from the same hatchery cohort grown in the land based tank system.
Results of Sea Ranching trials

Figure 9. Total numbers of native and tagged (hatchery origin) green sea urchins collected during six dive surveys from the Job and Sloop lease sites. Percentages show percent of tagged urchins in sample.
urchins in sample. In general, the Sloop site had more total urchins and a higher percentage of tagged urchins in the population. A notable exception was the June 2011 survey, when the Job site showed an unexpected spike in total urchin numbers. A sharp reduction in numbers at Sloop was evident at the last survey in May 2012 and was accompanied by evidence that the site had been recently fished by a dragger vessel.

Figure 10. Average and maximum size (mm test diameter) of tagged hatchery origin green sea urchins at the initial out-planting in February, 2010 and at each of the subsequent dive samples from the Job and Sloop lease sites. The Sloop site showed a pattern of increasing growth over the course of the project, and much larger tagged urchins were regularly recovered there than at Job. At the Job site the average size of tagged urchins actually declined over the course of the project.

Discussion of Sea Ranching
The results indicate that out-planting success can vary between sites (Figures 9 & 10). The Sloop site appeared to be more favorable for out-planting than the Job site; in general more total urchins were found at Sloop and there was a higher percentage of tagged hatchery source urchins in the sampled population (Figure 6), and the released urchins showed greater growth at Sloop over the course of the study (Figure 7). Green sea urchin growth rates can be highly variable in the natural environment, primarily in response to feed availability and type. Growth can be very slow and rates of $\leq 0.25$ mm per year have been documented for urchins found in tide pools. However, the persistent numbers of small tagged urchins found at both sites and especially at the Job site over the course of the two years might also be attributed to emigration of larger urchins from the site. Over time, sea urchins can move considerable distances in response to feed availability or to avoid predation. The degree and extent of movement in green sea urchins is a function of size. At about 15 mm and smaller they are cryptic and movement is minimal, but above this size movement patterns become more widespread and they can move greater distances.
to avoid predators or in search of food. These movement patterns could explain why the average size of tagged urchins at the Job site actually declined from the release size. Larger urchins might have left the area in response to predation, feed availability or other factors, leaving the smaller urchins behind. At the Sloop site a greater percentage of the released urchins grew and remained within the release area. 6.25% of the tagged urchins found at Sloop were >30 mm at the final survey and the largest tagged urchin recovered there was 49.3 mm (Figure 7). The site characteristics favorable for out-planting need further study but at a minimum it appears that a hard bottom comprised of shell hash and cobble, presence of feed, and a recent history of sea urchins populating the site are all favorable criteria.

It would be premature to recommend or discourage sea ranching based on the results from this study. Logistical considerations and other constraints did not allow the survey area to be extended beyond the release area, and factors for optimal site selection need to be better defined. It is possible, if not likely, that many of the released urchins moved beyond the release/survey area but still remained within lease site boundaries. Larger urchins would have been more likely to move and therefore the surveys may have underestimated both recovery and growth of the released urchins. Despite these unknowns some encouraging conclusions can be drawn. Released urchins were found at both sites after two years (Figure 6) and at the Sloop site some of those urchins showed enough growth after 27 months to indicate that legal harvest sized urchins (>52 mm) could be obtained from released seed after 3-4 years (Figure 7). On the other hand, the lack of evidence that seeded urchins grew at the Job site and the evidence indicating that the Sloop site had been subject to fishing activity (dragging) present significant barriers to sea ranching. Enforcement of legal protections to prevent poaching at lease sites is a difficult topic complicated by sociological and political considerations. The concept of individuals or companies leasing public fishing grounds for exclusive use is controversial in the Gulf of Maine region, at least for the present.

Objective 4) Compare growth, survival/retrieval, and economic costs/returns of sea ranching vs. tank farming.

Comparing sea ranching with tank farming and collecting data for a preliminary economic analysis were important project objectives. It was unknown prior to the project if green sea urchins out-planted onto an aquaculture bottom lease would survive, grow and remain in sufficient numbers to justify the expenses associated with hatchery/nursery production and lease fees. Similarly, it was unknown if survival and growth in a land-based tank system would be sufficient to justify the high costs associated with land-based aquaculture. In his project we were able to use the same hatchery cohort of green sea urchins to address these questions while comparing sea ranching with tank farming. To our knowledge, this is the first time such a comparison has been attempted.

Methods
All 31,500 green sea urchins used for the out-planting and tank culture trials came from the same CCAR 2009 hatchery cohort reared to the minimum out-planting size of 10 mm in the same nursery system. In February 2010 they were haphazardly mixed together and randomly assigned to be either out-planted at the two lease sites or reared in the tank system, using the methods described above for both. Returns in terms of tagged sea urchin numbers and biomass were
estimated for the sea ranching from the dive surveys and for tank culture from the size and census samples. Main costs associated with each activity were tracked or estimated throughout the project period. Seed costs were based on 2009 prices for similar sized oyster seed plus 20%.

The leaseholders' cost and return at each lease site from out-planted urchins was based on the last dive surveys in May 2012 (after 27 months growth) and extrapolated to the entire 2-acre lease using three assumptions. These were: 1) the total urchin population (wild + tagged) was evenly distributed throughout each 400m² study area and could be estimated from the numbers collected in 15 m²; 2) tagged urchins were evenly distributed throughout each 400m² study area and could be estimated from the percentage of tagged urchins collected in 15 m²; 3) the average size of tagged urchins recovered at the last survey was representative of the average size of all remaining tagged urchins within each study area. Recovery rates and a unit cost per kilogram and per urchin were then estimated for each 2-acre lease site given out-planting and recovery numbers proportional to those for the study areas (Table 2).

For the tank culture analysis we used the growth, survival, density and feeding data from the project to set production targets that might be achieved with intensification within the same real estate footprint (2,400 ft²) as that used for the project. Similarly, we used our known and estimated production costs to set target production costs, assuming that efficiencies could be gained with an insulated building, more efficient pumps and chilling, lower cost feed, better feed conversion, faster growth, etc. The following assumptions and cost targets were used for the model farm: 1) in the project we only used 2/3 of the available greenhouse space for 12 tanks. Intensification could be realized by using all of the floor area and stacking the tanks to total 36 tanks; 2) we assumed a maximum final density for all tanks of 16.3 kg/m², which was the highest density we observed in the project; 3) seed costs were reduced to $25 per thousand; 4) improved growth rates and feed conversion could be realized; 5) electrical costs could be reduced by ≈1/3 even at a greater production scale by using more efficient 3 phase pumps and chillers; 4) fuel oil costs could be reduced by half in an insulated building; 6) average daily labor time would increase only marginally at increased intensification, primarily because tanks with better self-cleaning ability would be used; and 7) the target feed cost was set at $1.10/kg, double the price we paid for the Cargill catfish feed but ≈90% less than the price paid for the premium Nofima diet. The production targets and costs were used to estimate market values and a unit cost per kilogram and per urchin for the model farm. Sea urchin value per kg was based on the average 2012-2013 ex-vessel price of $3/lb reported by the Maine DMR, and the value per sea urchin (piece) on the assumption that live 70 gram urchins could be sold to specialty markets at twice the value of bulk product. Uni prices were based on data from the National Marine Fisheries Service and from 2013 prices for 100g trays of uni as listed on the internet by a Maine processor/dealer. These and other assumptions are summarized in Tables 3 and 4.

Results, Costs and Returns

Sea ranching costs and returns

The main costs of sea ranching for the period of February 2009 to May 2012 were for seed, out-planting (boat and diver costs), equipment (marker buoys, ropes, and anchors), and lease fees (real estate). Out-planting the full 2-acre lease site (8093.7 m²) on a scale proportional to the study area would require that 212,460 hatchery seed be out-planted. At $35 per 1000 the seed would cost $7,436. Fees for a three year experimental lease would total $700, out-planting
would require two boat and diver days for an estimated $2,000, and equipment to mark the lease site and out-plant the urchins would cost another estimated $2,500. Thus, the total costs to out-plant one 2-acre lease site with 212,460 urchins for three years would be $12,636. Based on the last study area surveys done in May 2012 the estimated returns and unit costs for the leaseholder from each site can be summarized in Table 2.

<table>
<thead>
<tr>
<th>SITE</th>
<th># of urchins collected in 15 m² May 2012</th>
<th>% tagged</th>
<th>average wt tagged seed May 2012</th>
<th>total urchin population projected to lease area</th>
<th># tagged urchins projected to lease area</th>
<th>biomass loss or gain projected for lease area</th>
<th>Unit cost per kg</th>
<th>Unit cost per urchin</th>
</tr>
</thead>
<tbody>
<tr>
<td>JOB</td>
<td>107</td>
<td>29.9%</td>
<td>0.09 g</td>
<td>57,735</td>
<td>17,263</td>
<td>-104.7 kg</td>
<td>NA</td>
<td>$0.72</td>
</tr>
<tr>
<td>SLOO</td>
<td>194</td>
<td>35.6%</td>
<td>4.03 g</td>
<td>104,679</td>
<td>37,266</td>
<td>22.7 kg</td>
<td>$547.7</td>
<td>$0.33</td>
</tr>
</tbody>
</table>

Table 2  Projected returns and costs to lease holder from two 2-acre lease sites with out-planting numbers, returns and growth proportional to those seen for the study areas in May 2012 at each site. Total operating cost per site is estimated as $12,636 for three years

Tank farming costs and returns

The main costs associated with tank farming for the period from December 2010 to October 2012 were for seed, equipment, real estate, electricity, fuel oil for heating, labor, and feed. To simplify the tank farming analysis we excluded real estate and equipment costs. These costs will vary a great deal depending upon where, how and by whom the system is constructed, whereas the lease fees and equipment costs for sea ranching are far more predictable and consistent between sites. Production scale and actual or projected costs are summarized for tank farming in Table 3. Estimated returns for the model farm described in Table 3 are summarized in Table 4.
Table 3 Production and cost parameters and targets for the CCAR tank system and the model farm. CCAR tank system data is based on measured observations. Model farm data is based on assumptions described in the methods.

<table>
<thead>
<tr>
<th></th>
<th>MODEL</th>
<th>FARM</th>
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<tr>
<td></td>
<td># of</td>
<td>Total</td>
<td>Cost or value</td>
<td>Total cost or</td>
<td>Cost or value</td>
<td>Total cost or</td>
<td>productio</td>
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<td></td>
<td>urchi</td>
<td>weight</td>
<td>per kg</td>
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<td>piece per sea</td>
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<td></td>
<td>ns</td>
<td>kg</td>
<td></td>
<td></td>
<td>urchin (piece)</td>
<td>basis</td>
<td>unit kg</td>
<td>piece</td>
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<tr>
<td>Seed stocked @ 0.5 g</td>
<td>53,04</td>
<td>8</td>
<td>26.5</td>
<td>$50.0</td>
<td>$1,326</td>
<td>$0.025</td>
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<td>No data</td>
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<tr>
<td>Harvest @ 70 g</td>
<td>44,74</td>
<td>3</td>
<td>3132.0</td>
<td>$6.50</td>
<td>$20,358</td>
<td>$0.90</td>
<td>$40,269</td>
<td>$9.10</td>
<td>$0.64</td>
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<tr>
<td>roe yield</td>
<td>20%</td>
<td>626.4</td>
<td>0</td>
<td>$90.0</td>
<td>$56,376</td>
<td>$1.26</td>
<td>$56,376</td>
<td>$45.52</td>
<td>$0.64</td>
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Table 4 Projected returns with 90% survival from the model farm after three years of tank culture. 2013 ex-vessel prices for whole sea urchins averaged $3/lb while uni routinely sold for ≈$200/lb.

Discussion of sea ranching and tank farming costs/returns
The case can be made that the returns from sea ranching we observed during the project represent a worst case scenario. The dive surveys likely underestimated the average size of outplanted urchins over time, mainly because larger urchins would have been more likely to move away from the study area, as discussed earlier. For similar reasons the surveys may have underestimated the total numbers of tagged urchins that remained within the lease but were no longer within the study area. There was also evidence that the more productive S loop site had been dragged just before the last dive survey. In defense of the dragger it must be noted that 3 out of the 4 buoys marking the lease site corners were missing, probably due to winter storms, so therefore the dragger may not have realized that he/she was fishing on an aquaculture lease. It must also be acknowledged that in reality sea urchins are unlikely to be evenly distributed throughout a lease area or even a study area, and more likely will be found in patches of varying densities. A more sophisticated statistical analysis of our survey data is required to elucidate distribution patterns, currently in progress as a component of the MS thesis.

Given these limitations and qualifiers, it is nonetheless encouraging that the unit cost per sea urchin at the Sloop site was 33¢, even at the low rate of return we projected for a scaled up release. At a decent harvest size of around 70 g there are 6.5 urchins per pound, and at the current ex-vessel price of $3/lb the profit on 6.5 urchins with a production cost of $2.145 is
If the site had not been dragged it is likely that the projected return would have been higher, with a lower production cost per urchin. This shows that sea ranching can potentially be profitable, but that a great deal depends upon the site because at the Job site the data projected a net biomass loss, with a unit cost of 77¢ per urchin. It is also important to note that the greatest single expense by far was the seed cost, which amounted to 58.8% of the total. The cost of $35 per 1000 (3.5¢ per seed) we used for the sea ranching analysis was based on oyster seed pricing and may or may not reflect the true cost of urchin seed production. According to Yuichi Sakai of the Mariculture Fisheries Research Institute, in Hokkaido, Japan it costs 5-10 JPY (6.2 to 12.5 US cents) to produce one seed of 5 mm test diameter, the majority of which is wage (20%) and energy (40-60%) costs. The true costs of producing green sea urchin seed in Maine need to be determined, with the objective of reducing the cost per thousand to $25 or less. This may require out-planting larger numbers at a smaller size, which will increase the scale and therefore efficiency of hatchery production while reducing the time spent in costly land-based systems. However, this may come at the cost of lower survival rates for out-planted urchins.

Our projections show that the economic prospects for tank farming are more uncertain than those for sea ranching.

Total costs to operate the model tank farm for 3 years were estimated at $28,516 (Table 3), but the ex-vessel value of the harvested biomass was only $20,358 (Table 4). The production cost per kg was much lower for tank farming than for sea ranching (Tables 2 and 4), due to faster growth in tank culture and poor growth data for the study areas. However, as noted earlier the poor growth data we saw for the study areas may be attributable to emigration of larger tagged urchins, skewing the surveys towards smaller urchins and resulting in a higher production cost per kg. In addition, the tank culture production cost per sea urchin was almost twice that seen for sea ranching at the Sloop site (Tables 2 and 4). To obtain more value urchin growers could process and market the uni directly rather than selling whole urchins at ex-vessel prices to a third party processor/dealer. Good quality uni sells for $90 or more per kg, netting $56,376 for the model farm if it does its own processing and marketing (Table 4). This doesn’t take into account processing costs, but on the other hand uni can sell for as much as $150 per kg, and roe yields from sea urchins conditioned for market can be as high as 25-30%. It’s also possible that with good marketing a grower could sell individual 70 g live sea urchins to restaurants, academia, internet customers or other specialty markets for 90¢ or more per urchin, which would net $40,269 for the model farm (Table 4).

In any event, given the high value of uni it is clear that further investigation into sea urchin tank farming is justified. Two important areas that need to be addressed are development of low cost formulated diets and selective breeding for fast growing strains. A small percentage (<2%) of the urchins were ≥45 mm TD after just seven months in tank culture (27 months post-settlement), and we saw large differences in growth rates within the same hatchery cohort reared under the same conditions. Growth rates can also vary a great deal with green sea urchins in the wild, and although environmental factors no doubt play a large role researchers have hypothesized that there may be a genetic basis as well. Chinese researchers have shown that there is enough genetic variation in sea urchin growth rates to justify selective breeding efforts. If a fast growing strain of green sea urchins were developed specifically for tank culture the culture time could potentially be reduced to two years or less. This would greatly improve the prospects for land based tank farming of green sea urchins in the Gulf of Maine region.
Project Completion Report
Subaward #Z352601
Grant # 2008-38500-19301

Project Code:09-07

Project Title: Novel Methodologies to overwinter cultured clams in the Northeast US

Reporting Period: 01/11/2010 - 05/31/2013
Funding: $199,546.00 (Total)

Participants: John N. Kraeuter, Haskin Shellfish Research Laboratory, Rutgers University
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Brian Beal, Professor, University of Maine, Machias
Chester B. Zarnoch, Assistant Professor, Baruch College, City University of New York
Monica Bricelj, Research Professor, Institute of Marine and Coastal Science, Rutgers University
David Bushek, Director, Haskin Shellfish Research Laboratory, Rutgers University
George Mathis, Jr., President, Mathis Clam Farm
Joseph Porada, President, Egypt Bay Aquafarms
John Aldred, Director, East Hampton Town Shellfish Hatchery
John Dunne, East Hampton Town Shellfish Hatchery

Reason for Termination: Study End

PROJECT OBJECTIVES: 1) to determine whether novel techniques used to hold hard clam seed successfully over the winter in Maine (November – April) can be applied effectively to other locations in the Northeast region; 2) to examine survival and growth of overwintered hard clam seed that are subsequently planted on farms at various locations in the Northeast region; 3) to compare field survival and usage carbohydrate reserves of hard clam seed overwintered using current (standard) methods vs. the new methods proposed here; 4) to assess the benefits of overwintering hard clam seed using the standard methods vs. the new methods proposed here; and 5) to measure temporal (year-to-year) variation in overwinter success.

ANTICIPATED BENEFITS:
Overwintering of hard clam (Mercenaria mercenaria) seed has been a source of continual, but unpredictable losses to the industry. These losses are largely in the sizes of seed that did not reach planting size by the early fall. Methods that have routinely been used to overwinter the soft-shell clam in Maine, if proven to be successful for the hard clam, would make a major improvement in hatchery profitability through enhanced survival rates, and reduced capital costs. The end-users will be those individuals who purchase or produce cultured hard clam seed for enhancing wild stocks (public aquaculture) or for private clam farmers. Losses of planted hard clam seed during the late fall and winter months in the Northeast U.S. can exceed 25% and can be even higher in more northern latitudes of Atlantic Canada. These first winter losses can be economically catastrophic since additional crop mortality is likely to occur before harvest two-three years later.
**PRINCIPAL ACCOMPLISHMENTS:** In the past two years we have overwintered 2 sizes of hard clam seed of 3 stocks (ME, NY and NJ) in field plots and in cages to compare these with high survival results reported in Maine. Seed from all locations were tested for disease levels and certified before transport. Environmental variables (DO, TPM, Chlorophyll) were generally similar at all sites in both years. Water temperatures during the first year were very similar in New York and New Jersey, with the exception of a very cold period in mid to late December in New Jersey during the first year that was not present at the New York or Maine site. The New Jersey site warmed more quickly in the spring than in New York. Water temperatures in Maine during the first year mirrored those in New York until late January when Maine water temperatures dropped below 0°C and remained there until early March when a warming trend developed. All sites were nearly 5°C warmer during the second year of the study, but overall Maine experienced the coldest temperatures, and as in the first year, New Jersey warmed sooner than the other two locations. In both years seed from New York and New Jersey placed in cages experienced heavy mortality (>95%) at all sites. In the first year seed from ME were planted in Maine and overwintered in cages in Maine, New York and New Jersey. NY seed were planted in New York and overwintered in cages in Maine, New York and New Jersey. NJ seed tested positive for Dermo and experience heavy mortality while purging the low level infections. We substituted NY seed for a comparison to the ME seed in NJ. Animals kept in field plots experienced slightly less mortality.

We had hoped to follow the original proposed seed movements in the second year, but the State of Maine would not allow importation and field studies of any out-of-state seed, even if it was tested and found to be disease free. We were able to maintain ME seed in Maine in both field and cages in Maine. ME seed were also held in cages in New York and New Jersey. NY and NJ seed were held in cages and the local strain was planted in each state. In the second year, when there were differences, smaller ME seed generally had higher mortality than larger seed. Overall, ME seed mortality was less than the NY or NJ seed when held at the southern sites. ME seed had higher mortality at the Maine and New Jersey sites than in the first year. In New York, ME seed had less mortality than in the first year. These data suggest that the ME seed line is better adapted to long overwintering periods than either of the two mid-Atlantic strains, but the temperature regimes in the Mid-Atlantic are less conducive to overwintering than the prolonged cold in Maine. Year to year differences can be large.

Surviving overwintered seed were planted in the spring and sampled in the fall. The plantings included the shells of the clams that experienced winter mortality, and thus counts of dead individuals at the end of the summer included a significant percentage of dead from the overwinter experiment. Few of the NY seed survived in NY or NJ during the first year, and ME seed were planted in NJ. Survival over the summer was poor and analysis of the dead suggests that most of the mortality was early after planting suggesting it is a continuation of the high levels of overwinter mortality. In the second year, NJ seed planted in NJ also had poor survival over the summer, and again counts of dead suggest this mortality took place early after planting. Survival of NY seed was so poor each year in New York that none were planted.

Carbohydrate is the primary energy storage compound for small hard clams. Generally the ME seed started with higher carbohydrate content than either of the other two stocks. In the first year ME seed experienced a slow decline in carbohydrate content at all sites with the least loss at the New Jersey site. These seed at the New Jersey site still experienced higher mortality than the
same ME seed at the Maine site. The NY stock held in all three sites began to lose carbohydrate starting in January. Carbohydrate loss in these stocks was about the same in New York and Maine, and less in New Jersey. At all sites carbohydrate loss in the surviving clams was greater than the losses in the ME seed held at the same sites. In the second year ME and NY seed had similar beginning levels of carbohydrate and the NJ seed has about half of the other two. From October to January the NJ seed increased this storage product, and the ME seed at the New Jersey site maintained its high levels of carbohydrate until March-April. In the second year NY seed in New York, as in year 1 experienced a large drop in carbohydrate from deployment through the winter. The ME seed held in New York maintained high levels of carbohydrate during the first year, but during the second year these levels dropped rapidly and by January were lower than the native stock. In Maine the ME seed only lost small amounts of carbohydrate in the first winter, but during the second winter there was an abrupt decline from November to December and then remained relatively level as the clams experienced greater mortality than in the first winter. Thus mortality occurs almost always when carbohydrate reserves had dropped, but factors other than carbohydrate and predatory loss must be important because mortality can occur at times when carbohydrate reserves remain relatively high. The data suggest that the ME strains generally accumulate high levels of carbohydrate most winters, and may have a different physiological response to the level of carbohydrate reserves. Field planting of the seed, in almost all instances, yielded better survival than maintaining them in bags. The reasons for this are not apparent, but environmental conditions in the sediment are probably more stable than in the water column.

Impacts: Since seed generally survived the winter better when planted in the field it would be logical to develop field planting methods to maintain high densities with adequate protection. Because many of the seed are very small these protection methods should include a mechanism for early harvest in the spring. The ME seed appear to be physiologically better adapted for overwinter survival than either the NY or NJ strains. Further physiological and genetic studies should be conducted to determine the mechanism of this adaptation and if it can be transmitted to the other stocks.
Introduction:

In 2002, the aquaculture production of northern quahog, or hard clam, *Mercenaria mercenaria*, in the U.S. was estimated at $60.4 million (USDA 2006). Today, farming and public stock enhancement of hard clams in the Northeast region is the most economically important shellfish culture activity. One of the recurring problems that affects commercial production of farmed hard clams is losses due to winter mortality. Over winter losses in some Mid-Atlantic and Northeastern states, over and above losses due to predators, can exceed 50% (Damery 2000; Aldred et al. 2001; Ford 2001; Zarnoch and Schreibman 2008).

Hatcheries produce clam seed using various techniques to grow the animals to a size suitable for planting between 8-15 mm SL by late summer or early fall. Previous NRAC research on hard clam overwintering (Kraeuter et al. 1997) suggested the optimum would be for farmers grow quahog seed to sizes greater than 10 mm following the first year nursery. Due to time and logistic constraints both in the hatchery and nursery, significant quantities of seed do not reach this size. The remaining <10 mm seed are the most susceptible to overwintering mortality, and can be a significant portion of a growers annual production. There are at least three possibilities for small seed going into the winter. The safest and most expensive strategy is to ship these small clams to a more southern nursery where they will be maintained during the winter. This practice provides larger seed in the spring, but introduces the risk of transfer of diseases and parasites, and it is problematic to obtain necessary permits for such transfers in some states. Based on several years of experience, George Mathis (NJ) stated that this option is neither “practical nor reliable”. The second option is to keep the seed in a hatchery through the winter, but this, too, is an expensive proposition because of the time and energy it takes to maintain large numbers of small seed. Growers who have attempted this found variable results not warranting its use (Sandra Macfarlane; Martin Byrnes; Personal Communications to Zarnoch). Instead, most individuals choose to overwinter the small seed using the same field techniques employed for larger seed, but significant and unpredictable mortality occurs in the field planted individuals (Leavitt, 2004; Ford 2001; Miron et al., 2005; Bricelj et al. 2007).

Most hard clam farmers in the Northeast, and communities that purchase seed for stock enhancement purposes (e.g., in MA, NY), place seed in protected nursery field plots or boxes (bottom culture) at high densities (ca. 3000-5000 m⁻²) in the summer. These are then harvested in the fall and placed in growout plots at much lower densities (250-350 m⁻²). Summer growth varies among years and sites, yielding small (< 10 mm) seed in some years. Placing small seed in nursery plots presents a large risk for farmers and communities. For example, Damery (2000) investigated mortality of hard clam seed held over the winter in cages and under netting in each of Cape Cod’s municipal shellfish programs (10 communities). He reported that winter losses averaged 39% with a range from 10 to 60%, and recommended that towns conduct research to determine the most successful overwintering practices. Aldred et al. (2001) conducted overwintering trials comparing various methods including sediment filled boxes, ADPI bags, covered plots, and predator trapping. Mortality varied significantly across sites and with methodology; however, the most effective methodologies still yielded losses of ~50%. In the Mid-Atlantic many seed reach the 10 mm size for planting, but the remaining seed are overwintered using field planting under mesh, but the results are irregular and large mortalities are experienced.

These commonly encountered seed mortalities suggests that they cannot be mitigated using standard methodologies or that it is a result of physiological processes and/or disease.
Recently, Zarnoch and Schreibman (2008) have shown that loss in carbohydrate, associated with periods of low food availability during the spring when water temperatures rise from 5° to 12° C, caused significant clam seed mortality. Whether this is due to starvation or that physiological stress provides an entry to bacterial disease (Kraeuter and Castagna, 1984), or both, has yet to be determined. One technique that has proven to be effective in eastern Maine is holding the seed in mesh bags.

If overwintering techniques that worked well in eastern Maine can be applied successfully to cultured hard clam stocks in states south of Maine, hard clam farmers will be able to hold their seed in sites protected from natural events such as harsh winters, silting etc., and plant their seed in the spring when shell growth is faster (Jones et al. 1989; Jones et al. 1996). In a preliminary experiment ME hard clam seed were held in a manner similar to that described by Beal et al. (1995) for overwintering cultured individuals of *Mya arenaria*. Animals were sorted into two sizes added to 45 cm x 45 cm “soft” bags of nylon window screening at each of three densities of 7990, 11302, and 15510 individuals, and 3360, 6720, and 8960 individuals for small and large seed, respectively. Bags from each were added to overwintering containers constructed of vinyl-coated 14-guage wire mesh with a series of eight horizontal shelves. One bag containing clams from a single density was placed onto one shelf within each container except the bottommost. All containers were placed into a cement tank that received ambient, flowing seawater. Containers and bags of clams were removed from the tank and cleaned (sprayed with freshwater to remove silt) four times from November to May when a sample was taken from each bag, and the number of live and dead hard clams were recorded. Survival for the large and small clams was 99%, and there was no effect due density (Beal et al. 2009).

Studies by Pernet et al. (2006) Bricelj et al. (2007) and Zarnoch and Schreibman (2008) provide some information about the low temperature physiology in hard clams. When seawater temperatures fall below 5°C, active feeding ceases, and this places a metabolic burden on energy stores. If temperatures remain below this level for eight weeks or longer before water temperatures increase, significant mortality can occur in the spring when chlorophyll-*a* values are low (Zarnoch and Schreibman 2008). Laboratory experiments demonstrated a linear decrease in carbohydrates and significant mortality of clam seed (~7mm SL; obtained from MA) held at 1°C for ~12 weeks (Bricelj et al. 2007). Pernet et al. (2006) examined differences in lipid composition between wild and cultured hard clams during the winter at the northern distributional limit of this species near Neguac, New Brunswick, Canada. They found that phospholipids to sterol ratios, an indicator of membrane fluidity, in wild individuals increased 1.4- to 2.6-fold between August and October, immediately followed by a rapid decrease to initial values in December, whereas the ratio in the cultured individuals did not vary through time. In addition, oxygen consumption rates were approximately 33% lower in wild vs. selectively bred *M. mercenaria* suggesting there may be an energetic advantage in cold-adapted clams by reducing their energetic needs during overwintering. Determination of the levels of energy reserves (especially carbohydrates during and following overwintering is thus one key to understanding and predicting the site-specific success of overwintering under varying environmental conditions.

**Materials and Methods:**

*a) Overwintering trials using local seed (November 2010 – April 2011; October 2011 - April 2012)*

Within each state (Maine, New York, New Jersey), hard clam seed were obtained from a local (within-state) hatchery and divided into two size classes (Table 1). One kilogram of seed
from each size class was added separately to soft bags constructed of nylon window screening similar to that described by Beal et al. (2009). The soft bags were placed into rigid ADPI-like bags before adding one to each shelf in an overwintering cage (a modified lobster cage with 3 shelves; 2.54 cm square openings). Each cage (n = 5-14) held a single replicate of each size class (Completely Randomized Block Design). Cages were suspended from a permanent dock or wharf in New York and New Jersey, and in Maine held in flowing seawater in the building for the 6-month period. Due to seed availability and other factors this basic protocol was modified each year as indicated below (See b). At monthly intervals during each winter (Nov-April year 1; Oct-April year 2), the cages and all bags within each cage were cleared to remove mud/silt. Salinity, total particulate matter (TPM), percent particulate organic matter, chlorophyll-a, and temperature were measured at each site when bags of clams and cages were cleaned (n = 5 or 6 monthly measurements). Temperature was also monitored continuously at each site with HOBO temperature probes (Onset©, Bourne, MA). Each month the contents of three randomly stratified (large and small seed and each stock of seed) were sampled by taking one haphazard sample of 5g each. Animals were placed into bowls with ambient seawater, allowing sufficient time for live seed to begin siphoning, and then the numbers of live and dead clams were recorded. Clams that did not open in the time allotted were opened to determine if they were live or dead. In addition a sample of each of the lots was placed on ice and shipped to New York for carbohydrate analysis.

Prior to movement of the seed to the various locations, each size of hard clam seed (n = 30 from each size class) in each state was examined for the presence of disease organisms by standard histological techniques (Howard et al., 2004). In addition, samples were retained for bacteriological examination to determine levels of *Vibrionaceae* (Richards et al., 2005). Bacteria were analyzed twice in duplicate composites (100 animals) at the beginning and end of the overwintering period. Histological samples of 30 individuals of each size class were taken at the end of the overwintering period.

To compare overwinter results using the new vs. standard techniques, seeded field plots (2 m²) (n = 5) using each of the two size classes of local clam seed (75/ft2 or ~808/m2) were established in November/December at farm sites in each state in both years. These plantings were also modified based on seed availability (see b below). Plots were covered with flexible, 6.4 mm aperture predator netting. In April 2009 and 2010, ten random benthic cores were removed from each plot to estimate densities of live and dead clams.

To determine whether clams that survive the novel overwintering trials (animals suspended in cages from docks/wharves in soft bags) will survive subsequent field planting conditions, in April 2010 and 2011 in each state lots of overwintered clams that had enough survivors to develop a plot at typical growout densities of 270/m² (or ~25/ft²) from each size class in 2 m² were planted in protected field plots. These plots were harvested in the fall to estimate growth and survival (n = 10 random cores per plot).

b) *Reciprocal experiments to overwinter hard clam seed (November 2010 – April 2011; October 2011 - April 2012)*

These trials evaluated whether seed source affected overwinter survival. Seed of the same two sizes as above, originating/reared in each state were distributed to the other states (e.g., NJ will receive clams from NY source, and ME source, etc.), and deployed in the same manner as the local seed in overwintering cages as described above (n = 5-14) in October/November 2010 and 2011. Survival was estimated (as described above) in April 2011 and 2012. Seed was tested for disease and approved prior to transfers. In the first and second year Maine seed were
deployed at all sites, but a dearth of larger sizes prevented establishing all 5 replicates at each site (n=4). In the first year NJ seed began to die while being held for Dermo clearance, and a second group experienced the same mortality. NY seed were supplemented during this year and thus the experiment had NY and ME seed at all sites. In the second year, the State of Maine would not allow imports of any seed to be deployed in the field so no out-of-state seed were used in Maine, but in NY and NJ both the locally cultured seed and those from ME were deployed. Again there were not enough large ME seed to establish all bags.

c) Physiological condition

Triplicate subsamples of clams (ca. 25-100) from each strain and size class were collected monthly from the cages at all sites. Samples from New Jersey and Maine were shipped overnight on ice to New York for analyses. All samples were immediately frozen at -80°C until processed. For each clam, the tissue and shell were separated while partially defrosted and then dried to a constant mass at 60°C. Dried tissue was stored at -80°C. Carbohydrate analyses were conducted on the triplicate samples by pooling the dry tissue from 25-100 clams. Total carbohydrates were determined using a phenol-sulfuric acid method (Dubois et al. 1956) as per Zarnoch and Sclafani (2010).

Results:

a) Environmental Measurements

Water temperatures during the first year were very similar in New York and New Jersey, with the exception of a very cold period in mid to late December in New Jersey that was not present at the New York or Maine sites (Figure 1.). The New Jersey site warmed more quickly in the spring than in New York. Water temperatures in Maine during the first year mirrored those in New York until late January when Maine water temperatures dropped below 0°C and remained there until early March when a warming trend developed. All sites were nearly 5°C warmer during the second year of the study, but overall Maine experienced the coldest temperatures, and as in the first year, New Jersey warmed earlier than the other two locations. No temperatures of 0°C or below were recorded during the second year.

Mean total particulate matter (TPM), percent organic content of the seston (%OC), and chlorophyll-a (Chl-a) for each site are presented in Table 2. TPM generally ranged between 10 and 30 mg L⁻¹ and was similar at all sites during 2010-2011. The New York site had significantly lower TPM than the Maine site and the New Jersey site had intermediate levels in 2011-2012. The %OC was similar at all sites in both years (Table 2). Chl-a levels in New York were similar to those in New Jersey in both years with the exception of a large bloom observed in February 2011 at the New York site. The Maine site had significantly lower chl-a than the southern sites (Table 2).

b) Mortality in overwintering cages

Seed from New York and New Jersey placed in cages experienced heavy mortality (>95%) at all sites and in both years (Figure 2). In the first year (2010-2011) seed from ME were planted in Maine and overwintered in cages in Maine, New York, and New Jersey. NY seed were planted in New York and overwintered in cages in Maine, New York and New Jersey. NJ seed tested positive for Dermo and experience heavy mortality while purging the low level infections. NY seed were substituted for a comparison to the ME seed in New Jersey using the overwintering cages. During the first year the New York and New Jersey sites exhibited significant first order effects (date, size class and strain) while the ME site showed a significant effect of date and strain but not size class. A significant date x strain interaction coupled with the significant date effect in mortality at all sites indicates the expected progression in mortality in
the NY strain relative to the ME strain (Table 3). The strong strain effect likely influenced the other significant interactions (Table 3). ME seed experienced low mortality in Maine (< 8%), intermediate levels in New Jersey (up to 46%), and high mortality in New York (up to 77%). Observed mortality in the ME seed at the New York and New Jersey sites was stable from November through January but then increased significantly from February through April as water temperatures increased. Greater mortality was observed in the ME small size class at the New York and New Jersey sites (Fig. 2). No size class effect was found in ME (Table 3). The NY seed held in New York and New Jersey during the first year experienced a significant increase in mortality from December to January as water temperatures decreased below 5°C. In Maine, significant mortality of the NY strain was observed within the first month of deployment. In the second year, the State of Maine would not allow importation and field studies of any out of state seed even if tested and found to be disease free. Therefore, the reciprocal transplant study was not performed in ME during 2011-2012. However, ME seed was maintained in Maine in both field plots and cages. ME seed were also held in cages in New York and New Jersey. NY and NJ seed were held in cages and the local strain was field planted in each state. During the second year all sites exhibited significant first order effects (date, size class and strain). As in the first year, a significant date x strain interaction was found which suggests greater progression in mortality of the NY and NJ strain relative to the ME strain (Table 3). A date by size effect was found in both Maine and New York likely due to the ME small size class having greater mortality than the ME large size class at each of these sites. The date x size effect was not significant in New Jersey (Table 3). In New York, the NY small and large size classes as well as the ME small size class experienced a significant increase (~50%) in mortality from January to February. The ME large seed had low mortality through January but experienced a large mortality (~50%) event through February and March. In New Jersey, observed mortality of the NJ small and large size class as well as the ME small size class increased throughout the study period. The ME large size class had low mortality throughout the winter period but higher mortality from February to March. In Maine, low mortality was observed in the ME large and small size classes until March when ~50% increase in mortality was observed in the ME small size class.

c) Physiological condition

Carbohydrate is the primary energy storage compound for small hard clams. Generally the ME seed started with higher carbohydrate content than either of the other two stocks (Fig. 3). In the first year ME stock had >40% higher carbohydrate content than the NY stock, and experienced a slow decline in content at all sites (Fig. 3). In New Jersey both strains experienced minimal loss of carbohydrates (-2.3%). In spite of this small loss, ME seed at the New Jersey site still experienced higher mortality than the same ME seed at the Maine site which experienced greater loss of carbohydrate (~19%). The NY stock held in New York and Maine showed a significant decline from December through February when mortality was increasing. Percent carbohydrate loss in these stocks was about the same in New York and Maine, and less in New Jersey. At all sites carbohydrate loss in the surviving clams was greater than the losses in the ME seed held at the same sites, but in both years, losses in NJ appeared to be less than the other sites. There were significant first order effects for date and strain, effects with respect to size (Table 4). There were also interactions between date and strain indicating the variation in carbohydrate loss rates. In New Jersey and Maine there were no interactions between size and strain indicating that at these sites, losses in the two seed sizes were similar.
In the second year ME and NY juveniles had carbohydrate levels over twice as high as the NJ juveniles (Fig. 3). From October to January the NJ strain increased this storage product, and the ME strain at New Jersey maintained its high levels of carbohydrate until March-April. The increase in NJ strain condition and maintenance of the high condition of the ME seed was probably due to the unusually high fall and winter temperatures. In the second year ME strain in New York, and the small NY strain experienced a large drop in carbohydrate from deployment throughout the winter. The losses in the small NY seed at this location were similar to the first year data, but the larger NY clams maintained its higher carbohydrate levels until March. The ME strain held in New York maintained high levels of carbohydrate during the first year, but during the second winter these levels dropped rapidly and by January were lower than the native stock. In Maine the ME seed only lost small amounts of carbohydrate in the first winter, but during the second winter there was an abrupt decline from November to December and then remained relatively level as the clams experienced greater mortality than in the first winter. Results from ANOVA analysis were similar to the first year in that there were significant first order effects for date and strain, but not size. Because seed could not be imported into Maine there were no strain differences to be compared. The small size of the ME seed precluded carbohydrate analysis at all three sites. There were no interactions between date and size in New York or New Jersey.  

There was a significant negative relationship between hard clam carbohydrate content and time (days) exposed to winter conditions within the cages for the NY strain clams at New York in both study years (Fig. 4A), the New York strain clams in Maine during 2010-2011, and the ME strain in Maine for both study years (Fig. 4B). There was no linear relationship found between time and carbohydrate content in ME seed in New Jersey for both years as well as the NY seed in New Jersey in the first year. In the second year, the NJ seed held in New Jersey experienced an increase in carbohydrate content (Fig. 3). ANCOVA was used to compare carbohydrate concentration through time for the ME and NY strains in Maine (Fig. 4B). The slope ($F = 18.74; p < 0.0001$) and intercepts ($F = 152.17; p < 0.005$) were found to be significantly different, indicating that the change in carbohydrate concentration though time was different for the two strains (i.e. greater loss in New York) and at a given time interval carbohydrates was higher for the ME strain. ANCOVA was also used to compare carbohydrate concentration through time for the NY strain held in New York (both years; Fig. 4A) and the NY strain held in Maine in 2010-2011(Fig. 4B). We found both the slope ($F = 5.26; p = 0.033$) and the intercept ($F = 39.85; p < 0.0001$) to be significantly different, indicating that the change in carbohydrate concentration through time was different for the two sites (i.e. greater loss in New York) and at a given time interval carbohydrates were lower at New York.  

d) Mortality in field plots vs. overwintering cages

The mortality observed in field plots was lower than the mortality observed in cages for each strain and size class at all sites with the one exception of the ME large seed having less mortality in the cages during the second year (Table 5). A 2-way ANOVA was performed to compare mortality for the two seed sizes and culture methodologies for both years in New York and for the second year in New Jersey (Fig. 5). In New York, there was no effect of seed size on mortality but there was a significant effect of culture method in both years (Fig 5A and B). Mortality in the field plots was 51% and 66% in years 1 and 2, respectively as compared to > 95% in the overwintering cages (Table 5). Similarly, there was a significant difference in mortality in New Jersey when comparing field plots to overwinter cages during the second year (Fig 5C). There was also an effect of seed size in New Jersey as the NJ large seed experienced
significantly less mortality than the NJ small seed (54% vs. 86%) in the field plots. There was no difference in mortality between the 2 seed sizes held in overwintering cages in New Jersey. In Maine, the mortality observed in both the field plots and the overwintering cages was insignificant (<10%) during the first year. In the second year, mortality was higher for both culture methodologies with the small size class having lower mortality in the field plots while the large size class had lower mortality in the overwintering cages (Table 5).

**Discussion:**
The results of this study clearly demonstrate a genetic component to overwinter mortality as the ME strain had greater survival than the NY and NJ strains at all sites and in both study years. Both the ME large and small size classes exhibited low mortality through the winter months of November through February, while the NY and NJ strains experienced very high mortality, suggesting greater tolerance to low temperatures in the ME strain. Such traits may potentially be selected for in the development of aquaculture strains resistant to overwintering mortality. Genetic differences in overwinter mortality have been previously demonstrated in studies comparing selected and wild stocks in Atlantic Canada and New York (Bricelj et al. 2007; Pernet et al. 2006; Gionet et al 2009; Zarnoch and Sclafani 2010). However this is the first study to compare the overwinter mortality of different genetic stocks across a latitudinal gradient (Midatlantic US to eastern Maine) that encompasses the biogeographic range where hard clam overwintering mortality is problematic. In previous studies, cohorts of juvenile clams of the *notata* strain, selected for fast-growth, experienced significantly more mortality than stocks produced from wild broodstock, however, the exact physiological and genetic mechanism for the higher mortality in selected strains remains unclear. Pernet et al. (2006) has suggested that greater metabolic demand and a heterozygote deficit in selected clams may make them more vulnerable to overwintering stress. In contrast, Zarnoch and Sclafani (2010) did not find differences in metabolic demand between selected and non-selected strains, but did confirm greater use of carbohydrate reserves by the selected strain during the winter months. In the current study, the NY and NJ strains were *notata* variety, and produced from cultured broodstock selected for fast growth while the ME stock were produced from wild broodstock collected from eastern Maine. Therefore, it is not clear if the greater mortality observed in the NY and NJ strains as compared to the ME strain were a result of some negative physiological traits associated with selection for fast-growth, reduced tolerance to low temperatures, and/or their interaction. Future studies will need to examine overwinter mortality of aquacultured and wild strains within and among sites across a latitudinal gradient.

The timing of the observed mortalities during the overwinter period differed between NY/NJ strains and the ME strain which is likely due to differences in physiological condition, low temperature tolerance, as well as the variable temperature regimes that occurred over the two study years at each site. In the first year, New York and New Jersey experienced water temperatures below 5°C from early December until March. Significant mortalities of the NY strain were observed in both New York and New Jersey during the January sampling soon after water temperatures fell below 5°C (Fig. 2). This temperature change is significant since hard clams are known to significantly reduce metabolic activity at temperatures less than 5°C (Loosanoff 1939; Bricelj et al. 2007; Zarnoch and Sclafani 2010). In addition, the NY strain held in New York experienced a significant decrease in carbohydrate content from December through February coinciding with the mortality. While in New Jersey, the NY strain did not experience significant reductions in carbohydrate content, however, it started with a lower initial content, approximately 65 and 75 µg mgDW⁻¹ in New Jersey and New York/Maine, respectively.
(Fig. 3). The NY strain transferred to Maine in the first year experienced significant mortality between November and December soon after being deployed in the cages. Similar to New York, there was a linear decline in carbohydrate content in the NY strain held in Maine during this time. The water temperature was 5°C at the time of deployment of the NY and ME strains in Maine and was < 0°C from January to March. These results corroborate previous findings where overwinter mortality is correlated with reductions in carbohydrate content (Bricelj et al. 2007; Zarnoch and Schreibman 2008).

The ME strain deployed in New York and New Jersey during the first study year experienced significant mortality (although much less than the NY strain) as water temperatures increased from February through April (Fig. 2) as compared to the NY strain which experienced increased mortality as temperatures decreased. In addition, the ME small size class had higher mortality than the large size class in both New York and New Jersey which explains the significant size effect in the ANOVA analyses (Table 3) since mortality was similar between the NY large and small size classes at all sites and in both years. In New York, the ME strain experienced the greatest increase in mortality from March to April when water temperatures were >5°C which was also where ME small experienced a significant decline in carbohydrate. Although, the ME large also experienced significant mortality from March to April it was not correlated with a decrease in carbohydrates. Instead the ME large had a reduction in carbohydrate content from December to February and then an increase through April. The ME strain held in New Jersey had relatively stable carbohydrate content through the study period. The ME strain held in Maine showed a slow linear decline in carbohydrate content even though no significant mortalities occurred during the study period. It is important to note, however, that the water temperature in ME was still <5°C at the end of the first study year. Therefore, in year 1 the ME strain in Maine was not exposed to the winter-spring transition, which has been correlated with significant mortality following a winter period where carbohydrates reserves are reduced (Zarnoch and Schreibman 2008; Zarnoch and Sclafani 2010).

The second study year was an unusually warm winter for all sites with temperatures approximately 5°C warmer than the first study year (Fig. 1). In New York the water temperature did not fall below 5°C until January which is also when the largest increase in mortality occurred during the winter months in the NY strain (Fig. 2). Unfortunately, due to changes in Maine regulations, which prevented importation of out of state shellfish, the NY or NJ strain clams could not be overwintered in ME during the second study year. The NY strain experienced a linear decline in carbohydrates from November to January when mortality was peaking. In New York, the ME small experienced mortality similar in magnitude (up to 93%) and timing to the NY strain while the ME large had low mortality through the winter with a ~50% increase from February to March. This mortality event correlated to a reduction in carbohydrate content (Fig 3). In New Jersey, the NJ strain (both large and small) experienced a significant mortality event soon after deployment and then mortality of the NJ strain and ME small increased from December to the end of the study period. As in New York, the ME large in New Jersey had low mortality through the winter months and then experienced a mortality event from February to March although the total mortality was lower in New Jersey (Table 5). In contrast to the NY strain, the NJ strain increased carbohydrate content despite high mortalities through the study period. Since water temperatures were mostly >5°C in New Jersey surviving clams were likely feeding through the winter. Similarly, clams held at 7°C in a laboratory experiment and fed cultured algae also increased their carbohydrate content despite significant mortality (Bricelj et al 2007). In Maine, the ME strain had an increase in mortality from February to March/April (Fig. 6).
2). The timing of the mortality correlated with an increase in water temperature and a decrease in carbohydrate content in the ME large (Fig. 3). The high mortalities observed in the NY and NJ strains at all sites appeared to be correlated with the decrease in water temperatures (fall-winter) while the mortalities observed in the ME strain were correlated with the increase in water temperatures during the winter-spring transition. This difference may be a result of the physiological condition of the different strains at the start of the experiments. ANCOVA analyses demonstrated that the NY strains had lower carbohydrate content on a given sample date as compared to the ME strain and the rate of loss from November to February was greater for the NY strains (Fig. 4). Zarnoch and Sclafani (2010) found the metabolic activity of selected and wild juvenile hard clams, estimated by the activity of the electron transport chain, was equally as high during the fall-winter transition as the winter-spring transition which led the authors to hypothesize that the fall-winter transition may be an important factor in overwintering mortality. The current study supports this hypothesis. Conversely, the ME strain appear to experience a slow linear decline in carbohydrate content during the winter months (although less significant change in New Jersey) and then experience significant mortality during the winter-spring transition which has been previously demonstrated as a time when significant mortality occurs in juvenile hard clams (Zarnoch and Schreibman 2008). Furthermore, the laboratory studies by Bricelj et al. (2007) suggest that clams exposed to 7°C are equally as challenged as clams exposed to 1°C based on the fact that both groups experience high mortalities. Therefore, the physiological condition of the clams when they are exposed to these stressful temperature transitions is likely a significant factor in determining the timing and magnitude of the mortality. For example in the current study, the NY and NJ strains had carbohydrate concentration between 61-102 µg mgDW⁻¹ at the start of the experiments and experienced significant mortality in the fall-winter transition. While the ME strain had a carbohydrate concentration of ~130 µg mgDW⁻¹ at the start of the experiments and experienced significant mortality during the winter-spring transition. Similarly, juvenile clams with carbohydrate concentrations >150 µg mgDW⁻¹ overwintered in Jamaica Bay, New York also experienced significant mortality during the winter-spring transition (Zarnoch and Schreibman 2008).

There was no significant difference in mortality of the ME strain held in cages or in field plots in Maine, however, survival of the NY and NJ strains in the field plots was higher than survival in the overwintering cages (Table 5; Fig. 5). There are several possible explanations for these results. First, the clams held in the cages within the water column may experience greater fluctuations in temperature than those buried in sediment. The ability of soils to buffer temperature fluxes and mitigate overwinter mortality of insects has been described extensively in the entomology literature (Turnock and Fields 2005 and reference within) but has not been documented in the marine bivalve literature. Temperature fluctuations that repeatedly shift clams from states of metabolic activity (>5°C) to metabolic inactivity (<5°C) are likely to being particularly stressful and are most likely to occur during the fall and spring seasonal transitions in New York and New Jersey. These types of temperature fluctuations did not occur in Maine during the first year when mortality was < 8% but did occur in the second year which was a warmer than average winter and resulted in much higher mortalities (17-56%) in both cages and field plots. A second potential explanation is that the clams in the field plots have access to more food resources than those in the overwintering cages. It is likely that the horizontal flux of food particles across the sediment surface is greater than the flux through the cages since the cages are likely to reduce horizontal flow and local resuspension of food particles occurs near the sediment
surface (Grizzle et al. 1992; Judge et al. 1992). Lastly, it is possible that hard clams require more energy to maintain valve closure in the cages when they do not have the force of sediments supporting valve closure. Further research comparing hard clam physiology in sediment and culture containers (i.e. cages, FLUPSYs) is needed to better understand the advantages and disadvantages of these culture methodologies to support growth and overwintering.

To synthesize the results of this study and the published literature on the overwinter mortality of juvenile hard clams a conceptual model (Fig. 6; adapted from Bricelj et al. 2007) is presented which highlights the specific temperature regimes that influence growth, physiological condition, and overwintering mortality during the first year of culture. During the growing season temperatures are adequate to support growth (Ansell 1968) and metabolizable energy intake is greater than maintenance metabolic demand (Bayne et al. 1999). The fall-winter and winter-spring transitions are periods where metabolic activity is increased (Zarnoch and Sclafani 2010) likely due to the seasonal temperature change, short-term temperature fluxes associated with local environmental conditions, and/or a lack of acclimation due to such change. Energy intake during these transitions may not meet energy demands due to low food availability during these periods (Zarnoch and Schreibman 2008) and/or culture conditions. During the winter period when water temperatures are <5°C metabolic activity is significantly reduced (Loosanoff 1939; Ansell 1964; Zarnoch and Sclafani 2010) and metabolic demands are met through carbohydrate reserves (Bricelj et al. 2007; Zarnoch and Schreibman 2008; Zarnoch and Sclafani 2010). Given these temperature regimes and their influence of on juvenile clam physiology two different scenarios are proposed that could result in significant overwinter mortality. First, if the physiological condition of the clam (i.e. low carbohydrate content) is poor at the end of the growing season the clams are likely to become challenged during the fall-winter transition and significant mortalities will be observed as water temperatures fall below 5°C. This scenario was demonstrated by the NY and NJ strains in the current study. Poor physiological condition at the end of the growing season may be a result of environmental variables (i.e. food availability), culture conditions (i.e. density, fouling), or both. Alternatively, the physiological condition of the clams may be excellent at the end of the growing season and the clams are able to use stored carbohydrate reserves through the fall-winter transition and the winter period. Mortality may then occur during the winter-spring transition as metabolic activity increases due to warming temperatures and carbohydrate reserves are reduced. This scenario was previously proposed by Zarnoch and Schreibman (2008). It was also demonstrated in the current study with the ME strain in New York and New Jersey during both years and in Maine during the second year.

Although progress has been made on understanding the overwinter mortality of juvenile hard clams there is still a need to describe the physiological mechanisms at the molecular level (i.e. oxidative stress, membrane fluidity). In addition, it is still not clear how bacterial infection may be involved. The observations of Kraeuter and Castagna (1984) that a potential source of pathogenic bacteria is external to the clams (treatment with antibiotic in freshwater yielded the same level of mortality reduction as in ambient marine water) may be partly responsible is still an option once the clams become stressed. The proposed conceptual model (Fig. 6) will require additional study to adequately describe hard clam physiology during these temperature transitions, however, it does demonstrate the significance of these periods to the hard clam aquaculture industry. Growers should note that the fall-winter and winter-spring transitions are physiologically stressful periods and should maintain culture conditions that provide ample food resources and minimal handling. Basic research will be needed to further understand physiology during these stressful periods and applied research will be needed to identify culture
methodologies that minimize stress and declining physiological condition. This research will become more significant as warmer winters and longer fall-winter and winter-spring transitions become more common due to the effects of global climate change. Modeling efforts that integrate overwinter mortality in predicting the effects of global climate change on hard clam population dynamics will inform natural resource managers as well as the hard clam aquaculture industry. Lastly, the current study further supports previous reports of a genetic component making some stocks more susceptible to overwinter mortality. Therefore, genomic tools should be applied to to discover candidate genes important for overwintering performance and provide estimates of genotypic differentiation.

Literature cited:


Gionet CE, Mayrand E, Landry T (2009). The effects of energy reserves and cryoprotectants on overwintering mortality in Mercenaria mercenaria notata (Say 1822) at two tidal levels. Aquac Int 17:589-605


growth from shells of *Mercenaria mercenaria* from Narragansett Bay, Rhode Island, Mar. Biol. 102, 225–234.


Table 1. Mean shell lengths of the different strains and size classes used in the two study years. Significant differences in shell length within a column are indicated by different subscript letters.

<table>
<thead>
<tr>
<th>Seed strain and size class</th>
<th>2010-2011</th>
<th>2011-2012</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shell length (mm) ±SE</td>
<td>Shell length (mm) ±SE</td>
</tr>
<tr>
<td>New York - Large</td>
<td>10.33&lt;sup&gt;a&lt;/sup&gt; (±0.12)</td>
<td>8.7&lt;sup&gt;a&lt;/sup&gt; (±0.12)</td>
</tr>
<tr>
<td>New York - Small</td>
<td>9.64&lt;sup&gt;b&lt;/sup&gt; (±0.1)</td>
<td>6.93&lt;sup&gt;b&lt;/sup&gt; (±0.12)</td>
</tr>
<tr>
<td>Maine - Large</td>
<td>10.27&lt;sup&gt;a&lt;/sup&gt; (±0.15)</td>
<td>9.95&lt;sup&gt;c&lt;/sup&gt; (±0.33)</td>
</tr>
<tr>
<td>Maine - Small</td>
<td>6.88&lt;sup&gt;c&lt;/sup&gt; (±0.06)</td>
<td>4.59&lt;sup&gt;d&lt;/sup&gt; (±0.09)</td>
</tr>
<tr>
<td>New Jersey - Large</td>
<td>-</td>
<td>8.74&lt;sup&gt;a&lt;/sup&gt; (±0.13)</td>
</tr>
<tr>
<td>New Jersey - Small</td>
<td>-</td>
<td>7.47&lt;sup&gt;b&lt;/sup&gt; (±0.12)</td>
</tr>
</tbody>
</table>

Table 2. Mean (±SE) total particulate matter (TPM; mg l<sup>-1</sup>), percent organic content of the seston (%OC), and chlorophyll-<i>a</i> (Chl-<i>a</i>; µg l<sup>-1</sup>) at the overwintering sites in NY, NJ, and ME during 2010-2011 (n = 5) and 2011-2012 (n = 6). Differences between sites are indicated by different letters within each column.

<table>
<thead>
<tr>
<th>Site</th>
<th>2010-2011</th>
<th>2011-2012</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TPM</td>
<td>%OC</td>
</tr>
<tr>
<td>NJ</td>
<td>21.44(3.83)</td>
<td>26.25(2.89)</td>
</tr>
<tr>
<td>NY</td>
<td>19.88(3.63)</td>
<td>22.51(2.2)</td>
</tr>
<tr>
<td>ME</td>
<td>14.49(5.62)</td>
<td>27.45(1.59)</td>
</tr>
</tbody>
</table>
Table 3. Analysis of variance on the arcsine transformed percent mortality of different strains and size classes of juvenile *M. mercenaria* overwintered in cages in New York, New Jersey and Maine during the winters of 2010-2011 and 2011-2012.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>New York 2010-11</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample date</td>
<td>16.484</td>
<td>5</td>
<td>3.297</td>
<td>187.930</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Size class</td>
<td>9.004</td>
<td>1</td>
<td>9.004</td>
<td>513.276</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Strain (NY, ME)</td>
<td>0.698</td>
<td>1</td>
<td>0.698</td>
<td>39.795</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Date x Size</td>
<td>0.147</td>
<td>5</td>
<td>0.029</td>
<td>1.678</td>
<td>0.144</td>
</tr>
<tr>
<td>Date x Strain</td>
<td>6.077</td>
<td>5</td>
<td>1.215</td>
<td>69.288</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Size x Strain</td>
<td>0.481</td>
<td>1</td>
<td>0.481</td>
<td>27.422</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Date x Size x Strain</td>
<td>0.376</td>
<td>5</td>
<td>0.075</td>
<td>4.286</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>New York 2011-12</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample date</td>
<td>24.241</td>
<td>6</td>
<td>4.040</td>
<td>273.885</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Size class</td>
<td>1.073</td>
<td>1</td>
<td>1.073</td>
<td>72.768</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Strain (NY, ME)</td>
<td>1.306</td>
<td>1</td>
<td>1.306</td>
<td>88.508</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Date x Size</td>
<td>0.325</td>
<td>6</td>
<td>0.054</td>
<td>3.676</td>
<td>0.002</td>
</tr>
<tr>
<td>Date x Strain</td>
<td>1.749</td>
<td>6</td>
<td>0.292</td>
<td>19.763</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Size x Strain</td>
<td>0.538</td>
<td>1</td>
<td>0.538</td>
<td>36.458</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Date x Size x Strain</td>
<td>0.252</td>
<td>5</td>
<td>0.050</td>
<td>3.420</td>
<td>0.007</td>
</tr>
<tr>
<td><strong>New Jersey 2010-11</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample date</td>
<td>8.249</td>
<td>5</td>
<td>1.650</td>
<td>80.001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Size class</td>
<td>11.274</td>
<td>1</td>
<td>11.247</td>
<td>546.684</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Strain (NY, ME)</td>
<td>0.116</td>
<td>1</td>
<td>0.116</td>
<td>5.644</td>
<td>0.019</td>
</tr>
<tr>
<td>Date x Size</td>
<td>0.298</td>
<td>5</td>
<td>0.060</td>
<td>2.888</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Date x Strain</td>
<td>5.413</td>
<td>4</td>
<td>1.353</td>
<td>65.616</td>
<td>0.017</td>
</tr>
<tr>
<td>Size x Strain</td>
<td>0.352</td>
<td>1</td>
<td>0.352</td>
<td>17.054</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Date x Size x Strain</td>
<td>0.128</td>
<td>4</td>
<td>0.032</td>
<td>1.558</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>New Jersey 2011-12</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample date</td>
<td>3.371</td>
<td>6</td>
<td>0.562</td>
<td>24.489</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Size class</td>
<td>0.987</td>
<td>1</td>
<td>0.987</td>
<td>42.999</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Strain (NJ, ME)</td>
<td>3.210</td>
<td>1</td>
<td>3.210</td>
<td>139.920</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Date x Size</td>
<td>0.161</td>
<td>6</td>
<td>0.027</td>
<td>1.172</td>
<td>0.331</td>
</tr>
<tr>
<td>Date x Strain</td>
<td>0.201</td>
<td>3</td>
<td>0.067</td>
<td>2.923</td>
<td>0.040</td>
</tr>
<tr>
<td>Size x Strain</td>
<td>0.003</td>
<td>1</td>
<td>0.003</td>
<td>0.116</td>
<td>0.734</td>
</tr>
<tr>
<td>Date x Size x Strain</td>
<td>0.080</td>
<td>3</td>
<td>0.027</td>
<td>1.157</td>
<td>0.332</td>
</tr>
<tr>
<td><strong>Maine 2010-11</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample date</td>
<td>7.187</td>
<td>4</td>
<td>1.797</td>
<td>110.797</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Size class</td>
<td>5.774</td>
<td>1</td>
<td>5.774</td>
<td>356.027</td>
<td>0.328</td>
</tr>
<tr>
<td>Strain (NY, ME)</td>
<td>0.016</td>
<td>1</td>
<td>0.016</td>
<td>0.969</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Date x Size</td>
<td>0.013</td>
<td>4</td>
<td>0.003</td>
<td>0.204</td>
<td>0.936</td>
</tr>
<tr>
<td>Date x Strain</td>
<td>1.658</td>
<td>2</td>
<td>0.829</td>
<td>51.113</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Size x Strain</td>
<td>0.001</td>
<td>1</td>
<td>0.001</td>
<td>0.051</td>
<td>0.822</td>
</tr>
<tr>
<td>Date x Size x Strain</td>
<td>0.010</td>
<td>1</td>
<td>0.010</td>
<td>0.631</td>
<td>0.429</td>
</tr>
<tr>
<td><strong>Maine 2011-12</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample date</td>
<td>0.762</td>
<td>4</td>
<td>0.190</td>
<td>17.652</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Size class</td>
<td>0.509</td>
<td>1</td>
<td>0.509</td>
<td>47.194</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Table 4. Analysis of variance on the log transformed carbohydrate content of different strains and size classes of juvenile *M. mercenaria* overwintered in cages in New York, New Jersey and Maine during the winters of 2010-2011 and 2011-2012.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>New York 2010-11</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample date</td>
<td>0.205</td>
<td>5</td>
<td>0.041</td>
<td>23.825</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Size class</td>
<td>2.294 x 10^{-5}</td>
<td>1</td>
<td>2.294 x 10^{-5}</td>
<td>0.013</td>
<td>0.909</td>
</tr>
<tr>
<td>Strain (NY, ME)</td>
<td>0.798</td>
<td>1</td>
<td>0.798</td>
<td>464.106</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Date x Size</td>
<td>0.057</td>
<td>5</td>
<td>0.011</td>
<td>6.638</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Date x Strain</td>
<td>0.081</td>
<td>3</td>
<td>0.027</td>
<td>15.730</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Size x Strain</td>
<td>8.193 x 10^{-5}</td>
<td>1</td>
<td>8.193 x 10^{-5}</td>
<td>0.048</td>
<td>0.828</td>
</tr>
<tr>
<td>Date x Size x Strain</td>
<td>0.002</td>
<td>3</td>
<td>0.001</td>
<td>0.302</td>
<td>0.823</td>
</tr>
<tr>
<td><strong>New York 2011-12</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Sample date</td>
<td>0.218</td>
<td>6</td>
<td>0.36</td>
<td>5.5252</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Size class</td>
<td>4.377 x 10^{-8}</td>
<td>1</td>
<td>4.377 x 10^{-8}</td>
<td>&lt;0.0001</td>
<td>0.996</td>
</tr>
<tr>
<td>Strain (NY, ME)</td>
<td>0.41</td>
<td>1</td>
<td>0.041</td>
<td>24.412</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Date x Size</td>
<td>0.011</td>
<td>3</td>
<td>0.004</td>
<td>2.134</td>
<td>0.117</td>
</tr>
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<td>Date x Strain</td>
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Table 5. Percent mortality of juvenile *Mercenaria mercenaria* planted in the field and held in cages at final sampling in April 2011 and April 2012. NYS = New York strain and small size class; NYL = New York strain and large size class; NJS = New Jersey strain and small size class; NJL = New Jersey strain and large size class; MES = Maine strain and small size class; MEL = Maine strain and large size class.

<table>
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<tr>
<td></td>
<td>NJL</td>
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Figure Legends:

Figure 1. Water temperature at three experimental sites: Beach Haven, New Jersey (NJ), Point Lookout, New York (NY) and Beals Island, Maine (ME) for the winter of 2010-2011 and 2011-2012.

Figure 2. Percent mortality of various stocks of juvenile *M. mercenaria* in small and large size classes observed in overwintering and reciprocal experiments using the new technology in New York during A) 2010-2011, B) 2011-2012, in New Jersey during C) 2010-2011, D) 2011-2012, and in Maine during E) 2010-2011, F) 2011-2012. The seed sizes and strains include: NYS = New York strain and small size class; NYL = New York strain and large size class; NJS = New Jersey strain and small size class; NJL = New Jersey strain and large size class; MES = Maine strain and small size class; MEL = Maine strain and large size class.

Figure 3. Carbohydrate content of various stocks of juvenile *M. mercenaria* in small and large size classes observed in overwintering and reciprocal experiments using the new technology in New York during A) 2010-2011, B) 2011-2012, in New Jersey during C) 2010-2011, D) 2011-2012, and in Maine during E) 2010-2011, F) 2011-2012. The seed sizes and strains include: NYS = New York strain and small size class; NYL = New York strain and large size class; NJS = New Jersey strain and small size class; NJL = New Jersey strain and large size class; MES = Maine strain and small size class; MEL = Maine strain and large size class.

Figure 4. The linear relationships between carbohydrate content and time under overwintering conditions of Maine (ME) and New York (NY) juvenile *M. mercenaria* strains and size classes (S = small; L = large) overwintered in cages in A) NY and B) ME during the winters of 2010-11 and 2011-12.

Figure 5. Comparison of overwinter mortality of juvenile *M. mercenaria* in cages and planted in field plots. All cages and plots were samples in March 2011 and March 2012. A) New York 2010-2011, B) New York 2011-2012, and C) New Jersey 2011-2012.

Figure 6. A conceptual model indicating the specific temperature regimes that influence growth, physiological condition, and overwintering mortality during the first year of culture (adapted from Bricelj et al. 2007).
Figure 1. Water temperature at three experimental sites: Beach Haven, New Jersey (NJ), Point Lookout, New York (NY) and Beals Island, Maine (ME) for the winter of 2010-2011 and 2011-2012.
Figure 2. Percent mortality of various stocks of juvenile *M. mercenaria* in small and large size classes observed in overwintering and reciprocal experiments using the new technology in New York during A) 2010-2011, B) 2011-2012, in New Jersey during C) 2010-2011, D) 2011-2012, and in Maine during E) 2010-2011, F) 2011-2012. The seed sizes and strains include: NYS = New York strain and small size class; NYL = New York strain and large size class; NJS = New Jersey strain and small size class; NJL = New Jersey strain and large size class; MES = Maine strain and small size class; MEL = Maine strain and large size class.
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Figure 6. A conceptual model indicating the temperature regimes that influence growth, physiological condition, and overwintering mortality during the first year of culture (adapted from Bricelj et al. 2007).
APPENDIX B
PROJECT CODE: 12-08

PROJECT TITLE: Genetic Marker-Assisted Selection of Northeastern Hard Clams for QPX-Resistance

REPORTING PERIOD: Feb. 01, 2013 through July 31, 2013

FUNDING LEVEL: $199,998

PARTICIPANTS:
Principal Investigator(s): Bassem Allam (Stony Brook University), Ximing Guo (Rutgers University), Roxanna Smolowitz (Roger Williams University), Emmanuelle Pales Espinosa (Stony Brook University), Gregg Rivara (Cornell University Cooperative Extension of Suffolk County)
Cooperating Participant(s): George (Gef) Flimlin (Rutgers Cooperative Extension), Diane Murphy (Cape Cod Cooperative Extension & Woods Hole Sea Grant), Arnaud Tanguy (University of Paris 6 / Station Biologique de Roscoff), Antoinette Clemetson (NY Sea Grant)

PROJECT OBJECTIVES:
Objective 1: Select candidate genes based on sequence information generated from our prior investigations and validate single nucleotide polymorphism loci for clam genotyping
Objective 2: Proof-test the link between the polymorphism of the candidate genes and QPX resistance on samples preserved from prior field work preceding and following QPX-related clam mortalities
Objective 3: Validate the markers identified in Objectives 1 and 2 for the assessment of the resistance of different seed strains used for aquaculture along the east coast during lab and field QPX exposure studies
Objective 4: Provide the aquaculture industry with superior germlines derived from selected clams surviving QPX-related Mortalities

ANTICIPATED BENEFITS:
The SNPs identified in this project are expected to represent a useful method for forecasting clam resistance to QPX infection. A direct outcome of this research is the selection of resistant clam stocks that will be directly transmitted to the aquaculture industry through our robust extension and outreach group. The project will also generate important genomic information that will be made public, fostering research on this economically and ecologically important species.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS:
The delay in establishing the financial account (not yet available) has resulted in very limited delay of the research even though progress has been made toward the objectives. Clam spawning and seed rearing (pertains to Objectives 1, 2 and 3): Three strains of QPX survivor clams resulting from a prior NRAC-funded project (to Dr John Kraeuter et al.) were transported
to the Suffolk County Marine Environmental Learning Center (SCMELC) in Southold, NY where they were conditioned for spawning as per industry standard. Twenty clams from each group were individually spawned on June 4th, 2013, with different strains kept in separate tanks. Larvae were cultured using industry-standard techniques and post sets were grown in land-based upwellers before being placed in a floating upweller system. Single nucleotide polymorphism (SNP) search (Objective 1): We enhanced the genetic resources initially proposed for SNP searches with over 600 million Illumina HiSeq PE100 reads recently obtained from naive and QPX-infected hard clams Mercenaria mercenaria. We purchased and implemented CLC Genomics Workbench software and started using it for SNP searches. This processing of the data is ongoing and is expected to be completed in the next few weeks. SNP validation (Objective 1): This work is scheduled to start as soon as the financial account is established and funds are available.

WORK PLANNED:
No changes to the initially proposed plan is to be reported. Planned activities include the validation of the SNPs, clam seed deployment in the field, and initiation of laboratory QPX transmission experiments.

IMPACTS:
Nothing yet to report.

SUPPORT: Use the format in the table below to indicate NRAC-USDA funding and additional other support, both federal and non-federal, for the project. Indicate the name of the source(s) of other support as a footnote to the table.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED:
Nothing yet to report.
Project Code: 12-17

Project Title: “Shellfish STEM-GIS (ShellGIS) Development for Improved Siting and Farm Management”

Reporting Period: January 1, 2013 to August 1, 2013

Funding Level: $85,000

Participants:
Dr. Christopher V. Davis, Maine Aquaculture Innovation Center
Carter Newell, Ph.D., shellfish biologist
Christopher Davis, Ph.D., shellfish biologist
John Richardson, Ph.D., hydraulic engineer
Kevin Morris, Ph.D., Software engineer
Anthony Hawkins, Ph.D., ecosystem modeling
Tessa Getchis, Sea Grant Cooperative Extension

The following progress has been made on the primary objectives of this NRAC study. Progress is updated from the report sent Nov. 1, 2012.

1. Add a suspension culture module (previous version was bottom culture only) using the OysterGroTM system and floating trays to allow prediction of oyster growth.
2. Add a function to allow for prediction of oyster growth as a function of density in suspension and bottom culture.

Blue Hill Hydraulics has set up CFD models to determine the % depletion in oyster grow trays to facilitate growth vs density modeling in suspension culture, similar to the patch model we developed for bottom culture. In the GIS system, this also required entering additional layers of temperature and salinity for surface waters. To calibrate these predictions, additional field work is ongoing, and some of the results are presented below. Depletion was about 10% at both sites around the oyster grow cages, and initial chl a concentrations started out lower (1.6 Mook vs 2.2 POC) in the middle of the larger Mook Farm. CFD modeling should be completed by Sept, 2013.
Figure 1. Depletion in the near surface water on the flood tide (POC site, Oyster Ranch)

Figure 2. Upstream at Mook Site.
These profiles, along with moored CTD’s and current meter deployment will continue in August, 2013. In addition, field data on the effects of density on growth in oyster ranch trays on the POC site, and SHELLSIM model validation (oyster growth vs growth driver data collected June – Sept, 2013) is on-going.

3. Conduct a sensitivity analysis of natural inter-annual variations in weather and associated water quality parameters (temperature, salinity, food concentration and food quality) on oyster growth. This has been performed and the sensitivity of oyster growth to variations in temperature and salinity is an expert layer of the GIS. We have also determined that Chl a, TPM and SPM can predict oyster growth adequately without POC and PON.

4. Add a function to allow prediction of oyster growth as a function of inter-annual variations (see number 3 above). Note: due to the effect of water temperature on oyster clearance rates, their growth is especially sensitive to temperature). This has been incorporated into the GIS system.
5. Develop an economic modeling tool for oyster growers showing tradeoffs between stocking density, time to harvestable size and associated yield. At our February meeting in Nashville, we developed user screens and input and output parameters for the economic model. In addition, the output graphs are being simplified for interpretation.

6. Integrate into the software publicly available GIS layers depicting other marine-dependent uses, living marine resources and habitats, and environmental data which can be used as a decision making tool. Data from the upper Damariscotta River were supplied in GIS format and integrated into the system. The GIS specialist is providing screens which show where you can’t do aquaculture (leases and pollution closures), other conflicts (navigation, intertidal zones, etc.) and overlaying them on oyster habitat where good production is possible. This portion of the project is ongoing. Dr. Kevin Morris is expected to have the layers entered by November, 2013. The approach to be used is outline here:

Using STEMgis as a decision making tool

There are two aims here. One to use decision making tools to identify the best areas to place new aquaculture farms given scientific data, modelling and public opinions and the second to use the tool to illustrate how differing public or scientific opinions can affect the decision making process. The areas in which shellfish farms are located are invariably in sites that could be used for many purposes, for example, sailing, dredging, ports etc. These different uses have different priorities for different people depending on their own personal interests. Also, it not always possible to precisely define opinions in terms of numbers that can be used in a mathematical model. We therefore propose to use a fuzzy logic (Zadeh, 1965) approach for these types of data. Fuzzy logic enables you to capture and use opinions such as ‘map layer X has a somewhat positive affect on public acceptability’ or ‘map layer Y has a very low negative affect on public acceptability’. The words somewhat, very and low are terms that can be used in fuzzy mathematics and are very useful in capturing public opinion where the hard scientific facts are not available. Two similar projects that the investigators have been involved with include SimCoast which is Fuzzy Logic Rule Based Coastal Zone Management System (www.discoverysoftware.co.uk/SimCoast.htm) and MARA-GIS which is a fuzzy logic decision making for hazard mapping related to dredging activities (http://www.cefas.co.uk/media/462109/ex5766-mara-gis%20technical%20report%20r_2-0.pdf).

There will be many map layers included in this study which will help to define public opinion including:

Existing lease/franchise/grant sites  Shellfish classification areas  Shoreline access points (if available) or marinas  Chl a, HAB charts (if available through state or citizen monitoring efforts)

Protected/threatened habitats (states usually have something like this or a general species  SAV coverage)

Other layers that will help to define the best areas for shellfish farms will be derived from the ShellSIM model in STEMgis and will include Shellfish growth, profitability and environmental factors. Even these data have a related ‘fuzziness’ in terms of the uncertainty that are found in all modelling techniques and of course in terms of the relevant important of each factor. For example, should the environmental benefits outweigh profitability or vice versa. Different people will have different views on this. In order to capture these differing opinions this projects will initially derive the ‘fuzzy logic rules’ from all investigators in the project and then gather similar rules from stakeholder meetings and to not only those involved in the aquaculture industry but also other users of river and coast.

Each map layer can have a number of associated rules or opinions. In order to produce an answer to a question such as ‘where is the best place to locate an aquaculture farm’ these layers and rules need to be combined. This will be done using SimCoast in conjunction with STEMgis. STEMgis will produce the unions and intersections of each map layer spatially and feed these through to SimCoast so that the rules at each intersection pass through its inference engine. This will then produce a gradational confidence map showing values from -1 (i.e. strong negative) to 1 (i.e. strong positive) in answer to the question posed, e.g. best place to locate a farm. All of this complicated procedure will of course be hidden from the user. This project will show results from a wide range of differing opinions and show how these can have great effects on the outcome of such a confidence map. It will also provide a very simple user interface so that users can quickly change their opinions relating to certain parameters (for example environment versus profitability) and see the results change on the map.

7. Develop a large-scale food depletion module to examine the interaction between oyster farms upstream or downstream (i.e. hydraulic zone of influence). At the Nashville meeting, the group developed output functions for this result, and determined how the bottom patch model and the surface tray model results will be used via a mixing program similar to Cormix by BHH. This work is ongoing and is being calibrated with the field data collected in August, 2013.

8. Investigate the relative impact of different environmental variables on oyster growth with the purpose of reducing the cost of collecting site specific hydrodynamics and water quality data for individual culture locations. See # 3 above. Work by Dr. Hawkins has also determined that in some areas, Chl a alone is sufficient for predicting oyster growth. Since POM/TPM is the most expensive water quality parameter to collect, it might be possible to measure temp, sal, chl a and oyster growth and back out the expected POM/TPM measure if monthly growth measures were made. Ultimately the development of a low coast coastal monitoring buoy for temp, sal, and Chl a would allow for good spatial coverage for the GIS system and wider application in the region. This concept has been developed in a U Maine/UNE EPSCoR proposal for $20 million submitted to NSF in August, 2013 with Newell as a co-investigator.

9. Create a user interface which answers frequently asked questions from growers. Through the suggestions of Tessa Getchis, we totally changed the user interface and simplified it to allow for a logical and straightforward progression from choice of species, choice of culture technique, management strategies and expert options. These changes will significantly improve the utility of the system for extension agents and growers. A Powerpoint presentation given by Getchis was presented at a meeting of extension agents, and is attached.
10. Develop and deliver 20 minute outreach presentations for both scientific and trade meetings. Dr. Newell presented talks on the project at NACE (December) as well as a 1.5 hour workshop there, and a talk at NSA/WAS in Nashville (Feb 2013). In addition, posters and talks were presented at the WAS meeting in Prague (2012) and a poster at the NSA/WAS meeting on ShellGIS. A presentation will be made at the November, 2013 Aquaculture Conference in the Canary Islands.

11. Host a focus group meeting with key industry and resource management officials to conduct beta-testing of the model. Beyond the workshop at NACE, meetings are planned when the project is completed (early 2014).

12. Host a technology transfer workshop for the Northeast Aquaculture Extension Network. Next year’s meeting will feature a training workshop on the GIS.

13. Develop a NRAC fact sheet on the use of ShellGIS. Being worked on by the group and led by Tessa Getchis.

14. A publication (World Aquaculture Society Magazine) of our SHELLGIS program is coming out soon (in press). The article is attached.

Expected completion of all objectives (field work and modeling and GIS final form) through December, 2013 with presentation to extension by March, 2014 in a final workshop.

Attachments:
Aquaculture conference abstract (accepted)
Powerpoint for NE Extension meeting
WAS paper submitted

Attachment 1: Aquaculture Conference Nov. 2013
Title:

**Applications of SHELLGIS: the intersection between biophysical factors, shellfish production capacity, and societal priorities**

Authors & affiliations:

Abstract:

Whilst ecosystem modeling has been widely used to predict the carrying capacity for bivalve culture in numerous estuaries, system scale approaches do not have sufficient spatial resolution to adequately represent critical localized effects of current flow on the supply and use of food particles. Instead, Geographical Information Systems (GIS) help display, manage and communicate spatially resolved information, including outputs from integrated models, helping to optimize site selection and culture practice at the scale of the culture unit. ShellGIS was developed as a practical tool for bivalve mollusc farmers for selecting good sites and managing them for optimal growth rates and seed to harvest yields.

ShellGIS analyzes shellfish farming practices in space and time, accounting for the interactive effects of seeding density, culture type and hydrodynamics. Recent improvements to the GIS include detailed flow models and prediction of oyster (Crassostera virginica) growth in both surface cages and on the bottom as a function of location, seeding density, seed size and time of year seeded. However, while species specific and production technology specific priority areas may be identified for the aquaculture industry, in rural coastal areas, there has been a need to balance aquaculture development and other uses of the coastal zone. We utilized SIMCOAST, a fuzzy logic rule-based expert system, and interviews with stakeholders, to balance conflicting uses in a novel application to aquaculture siting and development in the U.S., and an innovation in marine spatial planning efforts.
PROJECT CODE: 10-06

PROJECT TITLE: Aquaculture health hazards – developing outreach services to the region’s farmers via extension and aquatic animal health professionals, using a HACCP approach

REPORTING PERIOD: January 1, 2013 to June 30, 2013

FUNDING LEVEL: year 1 $88,232 + year 2 $92,080 (received in May 2012)

PARTICIPANTS:
Tessa Getchis, University of Connecticut
Deborah A. Bouchard, Research Coordinator
Joseph Buttner, Department of Biology, Salem State College
John Ewart, Delaware Sea Grant
Ann Faulds, Pennsylvania Sea Grant
George E. Flimlin, Rutgers Cooperative Extension
Doris Hicks, Delaware Sea Grant
Craig Hollingsworth, Extension, University of Massachusetts
Andrew Lazur, Maryland Sea Grant Extension Program
Dale Leavitt, Roger Williams University
Dennis McIntosh, Delaware State University
Dana L. Morse, Maine Sea Grant/University of Maine Cooperative Extension
Tom Rippen, University of Maryland Eastern Shore, Sea Grant Extension Program
Gregg Rivara, Cornell Cooperative Extension
Roxanna Smolowitz, Veterinarian, Roger Williams University
Dan Terlizzi, Center of Marine Biotechnology
Don Webster, Extension Specialist, University of Maryland
Michael Chambers (replaced Ken La Valley), University of New Hampshire Sea Grant/UNH Coop. Extension
Diane C. Murphy, Cape Cod Cooperative Extension & Woods Hole Sea Grant Program
Robert Pomeroy, Professor and Extension Specialist, University of Connecticut
Josh Reitsma, Cape Cod Cooperative Extension & Woods Hole Sea Grant Program
Michael A. Rice, University of Rhode Island Cooperative Extension

PROJECT OBJECTIVES:
Objective 1: To identify, organize and compile science-based information and educational resources about aquaculture health hazards including major diseases of aquatic organisms, pests of aquaculture species, and organisms that cause human illness;

Objective 2: To develop HACCP-style guidelines for monitoring, recording, evaluating and sampling of stocks at the farm level, and to assemble and publish technical information and guidelines as individualized protocols and responses for shellfish and finfish farmers;

Objective 3: To conduct training workshops for extension and outreach practitioners on how to apply the HACCP-type guidelines for the development of a health risk management plan for individual farms;
Objective 4: To implement local industry outreach programs where farmers are instructed on developing their own HACPP health risk plans; and

Objective 5: To complete an economic assessment of the impact of implementing a HACCP plan on individual farms in the Northeast region.

ANTICIPATED BENEFITS:

We anticipate that, as a result of this project, extension professionals, aquatic animal health professionals, resource managers/regulators, and other individuals serving in an outreach capacity will possess a new tool to assist farmers in identifying and managing for aquaculture health hazards. This key information will help aquatic animal health professionals to better and more efficiently respond to mortality events. If the causes of mortality are identified quickly and definitively, losses from mortality may be able to be minimized and/or prevented in the future, leading to improved production and profitability.

In addition, and as a result of this project, farmers will have access to a comprehensive easy to access document network containing science-based information and educational resources to help them understand the risk factors associated with aquaculture health hazards. Improved knowledge of these hazards may lead to better risk management strategies and minimization of product losses. Potential economic benefits are significant since operators who are proactive in allaying health concerns related to their products will have a competitive advantage in the marketplace.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS:

Objective 2: A first draft of the manual *Aquaculture Hazard Analysis* has been prepared for review. The AHA manual provides detailed information on aquaculture production hazards for the major cultured species in the northeast US. The document undergo peer review and industry review in the late summer 2013.

Objective 2: One “General Fish Health Management” was updated by Paul Bowser and has been posted to the NRAC website. The second (currently being revised following external review) publication will be a NRAC Technical Bulletin that outlines appropriate measures for a shellfish farmer to implement when a problem occurs on his/her farm. The publication, being written by Gef Flimlin, will examine vectors that could impact various shellfish species and will assist the farmer in understanding what diseases, predators, and pests could cause crop mortalities. It will show how to sample the crop to collect random samples of dead, dying and living shellfish which can be analyzed by aquatic animal veterinarians or pathologists. It will describe methods to handle, store, fix if needed, and ship specimen for analysis. The Technical Bulletin will complement bulletins previously published by NRAC (NRAC Bulletins #111, 112, and 106-2008) that address similar issues with finfish. The proposed bulletin will also examine options for handling the crop if large mortalities do occur and how to relate these issues to other farmers and various state agencies (Agriculture, Fish and Game, Health, etc.). Lastly, it will suggest Best Management Practices for these actions based on the East Coast Shellfish Growers Association Code of Practice for shellfish farmers or other relevant best management practices/biosecurity documents. A third bulletin (currently being revised following external review) by Dale Leavitt, entitled “What Do I Need To Know About My Culture Site” that will include a standardized data sheet for recording site conditions under normal circumstances and when confronted with a suspected health hazard situation. The bulletin will provide instructions on how to collect the data required. The development of a standardized descriptive process for characterizing a farm site will consist of site conditions identified by aquatic health professionals as critical to the identification of aquatic health hazards.
WORK PLANNED:

Objective 3: This summer we will be conducting focus group testing on the AHA manual. After revising the manual, we will conduct training workshops for extension and outreach practitioners on how to apply the HACCP-type guidelines for the development of a health risk management plan for individual farms. This training will be conducted at the annual northeast aquaculture extension meeting in winter 2013/2014.

Objective 4: Though beyond the scope of the original project, the intent is for Extension and aquatic health professionals to take the finished guide and curriculum and implement local industry outreach programs where farmers are instructed on developing their own HACPP health risk plans. The type of training (one-on-one or group) will be decided locally and based on demand/need.

Objective 5: An economic assessment of the impact of implementing a HACCP plan on individual farms in the Northeast region is underway. UConn graduate student, Nataliya Plesha, has visited four aquaculture producers and has conducted a pre-analysis (post-analysis planned in fall 2013) of their enterprise budgets. These producers have agreed not only to provide their records, but to indicate how much of their budget is currently targeted for managing aquatic production/health hazards. We will then work with each business conduct a hazard analysis and management plan and identify additional hazard prevention/control measures that would be appropriate for their farm. We will estimate the additional costs of these measures and compare that figure to the pre-hazard analysis budget.

IMPACTS:

See anticipated benefits. Project is ongoing.

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PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED:

See objective 2 above. Publications under development and review.
Annual Progress Report
Subaward # Z540401
Grant 2010-38500-21074 (Years 1)

PROJECT CODE: 10-14


REPORTING PERIOD: 11/1/12 – 4/30/13

FUNDING LEVEL: $116,642 (Year 1), $83,137 (Year 2) Total Project Budget = $199,779

PARTICIPANTS:
Mary R. Carman, Michael Marchetti
Alec Gale Greg Mataronas
Rick Karney Robert Reynolds
Kenneth La Valley Bill Silkes
Richard Langan Victoria Starczak
Dale Leavitt

PROJECT OBJECTIVES:
1. Compare means and dedicated sites for collecting mussel seed for SNE.
2. Compare methods of tunicate eradication without compromising the survival of mussel seed.
3. Compare different types of socks and stocking densities to optimize growth and yield at harvest to improve management of mussel operations in SNE.
4. Develop training through hands-on workshops, publications, websites and conference/forums.

The beneficiaries of this research will be the fishermen/farmers in Southern New England (SNE) who have already taken the first steps to establish and operate mussel longlines (via NOAA funding June 2009 – May 2011) and others who may be encouraged to diversify and follow their success. The seafood-eating public, seafood processors, restaurants and retail outlets would benefit from locally produced seafood. The measurable benefits will be sustainable new enterprises conducting best management practices for locally-produced mussels.

ANTICIPATED BENEFITS:
We can make projections about the potential impact of mussel farming on jobs in SNE based on economic studies of longline mussel farming in Prince Edward Island, Canada. A Department of Fisheries and Oceans report (2006) calculates the direct and total (including indirect and induced) impacts corresponding to $1 million of mussels sold by processors. Assuming the current processed sale price of $1.25/lb (Silkes, pers. comm.) and 10 tons of marketable product per longline, SNE would need 42 longlines in operation to produce $1 million of processed sales. According to the DFO report, each million dollars of processed sales directly employs 6 full time workers, with a total direct and indirect economic impact of 13 workers.
Our goal is to attract the most likely entrants (commercial fishermen and shellfishermen) to this new aquaculture activity and lead the way for a new industry that can revitalize working
waterfronts, and increase employment and economic activity in growing, processing, and
distribution services in the Northeastern U.S. If our favorable growth and management
assumptions are correct, at the project’s conclusion the longlines will be adopted by fishermen as
going concerns. According to an unpublished Business Planning Handbook by Hauke Kite-
Powell at the Marine Policy Institute at WHOI, a $1.2 million investment in a 120 longline
operation (yielding > $3 million in processed sales per year) could be paid back within in 5
years. That model and Langan and Horton (2005) assume a 2-year harvest cycle per longline and
production costs of < $0.25/lb. With our projection of faster growth rates in SNE and 1 year
harvest cycles, production could be much greater, and the payback could be sooner and larger.

Results of this work will be directly available and applicable for mussel farming operations in
SNE. Commercial groups interested in mussel farming – American Mussel Harvesters, Salt
Water Farms, and Sakonett Point Mussels, in Rhode Island and Martha’s Vineyard Shellfish
Group in Massachusetts will be working directly on this project. At the end of the project
period, it is our intention to transfer ownership of the mussel product and grow-out structures to
the trained fishers who have invested their boats, shore-side facilities and time to maintain them
and to convert and expand them into private mussel culture operations.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS:

Seed collecting site and materials evaluation
As in the first year, our seed collection efforts were conducted in 5 locations; in Narragansett
Bay (AMH’s Salt Water Farm site), offshore Newport RI (Sakonnet Mussel site), in Menemsha
Harbor on Martha’s Vineyard, offshore in Vineyard Sound, and off the WHOI dock in Woods
Hole. A sixth location was added near the Roger Williams University dock. In November, MBL
staff hung 4 different types of rope collectors off Woods Hole dock; 1) 6mm coir rope (made
from coconut husks), 2) NZ spat collecting rope, 3) RI-made nylon loopy rope (used in
wastewater treatment), and 4) used potwarp. None showed colonization by mussels spat by
March. A few spat were found when we checked again in April although there was lots of
fouling on the lines. There was poor spat collection at all our usual 5 sites winter/spring 2013
with none of the materials showing any significant difference in spat collecting ability.

During the late winter of 2012/2013 at RWU, the materials deployed consisted of 1) 6mm
coir rope (made from coconut husks), 2) Canadian spat collecting rope, and 3) RI-made nylon
loopy rope (used in wastewater treatment). The ropes where hung in ~3 meter lengths as single
strands (n = 5 replicates per collecting material) attached at the surface to a buoyed longline and
weighted at the bottom with 2 ounce fishing sinkers. They were hung in a random order from
two separate longlines, immediately to the north of the RWU Learning Platform in Mount Hope
Bay (41°38'59.02"N/71°15'21.85"W). This area has traditionally set large amounts of mussel
spat on most stable surfaces.

The lines were allowed to soak for approximately 6 months (January to June?) before
they were retrieved and evaluated for mussel set. The spat lines were removed from the longline
in their entirety, returned to the laboratory where the total number of mussels per line, the length
of the line and the valve length of (up to) 10 mussels per line was measured and recorded.
The results are included in Table 1 and Figure 1. The average size of the mussels were consistent among all of the ropes, suggesting that the mussels attached to the entire array of spat collecting ropes were derived from a single spatfall event occurring sometime during the spring of 2013. However, the overall numbers of mussel spat are dramatically reduced from those commonly observed in this location in the spring. This observation held true for all of the aquaculture gear deployed at our RWU Learning Platform site, where our traditional need to clear growing oyster juveniles held in the vicinity of newly settled mussels was dramatically reduced this year compared to former years.

Regardless of the absolute numbers of spat observed, there were clear differences in the capacity of the three materials to attract and hold settling blue mussel spat. Both the coir rope and RI-made nylon loopy rope collected significantly fewer mussel spat (~6 mussels per meter) than the mussel rope material (47 mussels per meter).

Observations of the pattern of settlement (not specifically recorded) on the three materials suggest that the larger scale inconsistencies in the structure of the rope surface may be an important determinant of its ability to attract and hold mussel spat. The mussel rope has large scale folds in the surface derived from the braiding of the three strand rope and it was in these folds that the mussels seemed to aggregate rather than the frayed ends of materials incorporated along the surface of the rope. A similar situation occurred in the thinner organic coir rope, where the bulk of the mussel spat were aggregated around knots tied in the rope rather than the relatively smooth surfaces. These knots had folds and crevices similar to the larger mussel rope. The RI loopy rope had no folds or knots resulting in a consistent surface of fine scale loops along its length. A follow up to these experiments may be to deploy similar lines but manipulating some to have a series of folds, generated by knotting, compared to unknotted materials.

**Sockling experiments in Winter/Spring 2013**

After Hurricane Sandy moved the anchors and tangled 2 of the 4 longlines, and stripped much of the seed we had carefully deployed last fall, this was a winter and spring for “re-building”. The two tangled lines and anchors that moved were removed so as not to pose a hazard to navigation.
The remaining two lines have been checked and adjusted; most of the NZ socks were tangled and removed, and the Spanish socks had been re-colonized by naturally-set mussel seed in May, 2013.

**MA**
Alec Gale sold his large dredging boat and bought a lobster-type boat. The boat is faster and more flexible in terms of ability to tend the lines in Vineyard Sound. Large seed (25 to 35 mm) from 2012’s plentiful spat set was still in good supply on the pilings and margins of the Menemsha Harbor and Pond in the spring of 2013. Lindell and Gale spent a couple of days collecting seed, declumping and grading and then socking in both NZ and Canadian socks in May 2013 for deploying offshore. Some of that seed may be marketable before the end of summer.

**Tunicate Eradication Trials**
We completed **Objective 2** as summarized in our last report

**Outreach and Extension**
On May 17th 2013, Lindell and Leavitt convened a workshop for 30 participants at the University of Rhode Island Bay Campus with speakers from as far away as New Zealand. The workshop agenda can be viewed here:
http://www.mbl.edu/mrc/overview/aquaculture/2013-mussel-farming-agenda/
Most presentations were audio recorded and those will be posted on the web with the corresponding powerpoint slides with permission from the speakers.

**WORK PLANNED:**
Sakonnet Mussel Farms staff plans to re-deploy the two lines that were removed. Seed will be deployed to the lines in September from seed collected on AMH ’s oyster growing cages.

In Massachusetts, seed collection and socking is planned after the summer heat dissipates in September.

Extension agents and the PI have begun to discuss outreach opportunities in the fall including hands-on training.

**IMPACTS:**
None to report, as yet.

**Support:**

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Publications, Manuscripts, or Papers Presented:
Presentations were be made by PI Lindell at NACE in Groton CT in December
“Research and Improved Management for Offshore Mussel Farms in Southern New England”

World Aquaculture Society meeting in Nashville in February:
“Offshore Mussel Farming in Southern New England; Research plans for optimizing economic yield”

Narragasett, RI on March 28th at the RISG Coastal State Forum for Shellfish Issues:
“Offshore Mussel Farming in Rhode Island”

URI Bay Campus on May 17th as part of Mussel Farming Workshop mentioned above
“Offshore Mussel Farming in Southern New England; Research plans for optimizing economic yield”
Annual Progress Report
Selection for enhanced disease resistance and growth performance in cross-bred eastern oysters, *Crassostrea virginica*.

Subaward # Z527701
Grant #2008-38500-19301 (Years 1, 2, & no-cost extension to 6/30/2013)

**PROJECT CODE:** TRA-07-01 & TRA-07-03  **SUBCONTRACT/ACCOUNT NO:** Z527701

**PROJECT TITLE:** Selection for enhanced disease resistance and growth performance in cross-bred eastern oysters, *Crassostrea virginica*.

**REPORTING PERIOD:** July 1, 2012 to June 30, 2013

**FUNDING LEVEL:** $231,416 (Total Project from NRAC; $88,856 for work performed at University of Maine)

**PARTICIPANTS:**
- Paul Rawson, University of Maine
- Scott Lindell, Marine Biological Laboratory
- Ximing Guo, Rutgers University
- Inke Sunilla, CT Bureau of Aquaculture
- Chris Davis, Pemaquid Oyster Co.
- Dana Morse, Maine Sea Grant
- Diane Murphy, Cape Cod Cooperative Extension

**PROJECT OBJECTIVES:**

Through a replicated field trial we will test the hypothesis that enhanced disease resistance to multiple diseases and improved growth can be realized through a combination of interline crossing and genetic selection on cross-bred stocks of oysters.

Together with oyster growers, hatchery operators and extension agents, we will develop guidelines and protocols for the transfer of the best performing stocks from our program to commercial hatcheries and the industry. Project results and updates on access to broodstock will be presented at the annual NACE meeting, at annual meetings of the National Shellfisheries Association and via a Fact Sheet at the project’s end.

**ANTICIPATED BENEFITS:**

The Northeast region has the largest oyster culture industry on the Atlantic coast. Yet, outbreaks of four diseases, MSX, SSO, Dermo, and ROD, cause considerable damage to the industry and limit its expansion. Despite considerable effort from both industry and academia, a single strain that resists all four diseases and grows well does not currently exist. This project will identify lines of oysters that perform well at a variety of sites in the region, particularly those sites where oyster diseases are endemic, by virtue of developing lines of
oysters that are resistant to multiple diseases. As such, the major product generated by this project will be a detailed analysis of line performance that can be used to match lines to anticipated disease pressure at sites throughout the Northeast region and a broodstock repository that will ensure the continued propagation lines that have demonstrated high performance.

Dissemination of this information will require a significant extension component, which will be achieved at three levels. First, the most direct approach is through direct contact with growers. A particular strength of this proposal is the involvement of commercial growers who will benefit directly from the project. In addition, several of the PI’s involved in this project regularly meet with industry working groups, such as the Maine Oyster Growers Working Group, East Coast Shellfish Growers, and individual state’s aquaculture associations where project results can be presented to participants and non-participants, alike. The project will gain considerable assistance from extension personnel in each state. Currently Connecticut’s extension agent is on maternity leave and Massachusetts’ agent has recently left. However, Dana Morse will assist us in disseminating and publicizing the project goals, and results and developing broodstock distribution guidelines in Maine and neighboring states while Cape Cod agent D. Murphy has agreed to help with these activities in Southern New England. The project will maintain a website that will provide access to project updates and will also produce the requisite NRAC Fact Sheet at the project’s end. Third, the material will also be presented to industry members and to the broader scientific community through presentation at NACE, the Milford Aquaculture Seminar and the annual meeting of the NSA.

**PROGRESS AND PRINCIPAL ACCOMPLISHMENTS:**

Oyster seed from six lines were deployed at grow-out sites from New Jersey to Maine in late July of 2011. The University of Maine hatchery provided seed for three replicate grow-out bags for the UMFS, Clinton, UMFS x Clinton, UMFS x NEH (new F1), and UMFS x NEH F2 lines while triploid seed from the NEH line was provided by a commercial hatchery. These lines were deployed at industry partner sites in Maine by Rawson (University of Maine), Massachusetts and Rhode Island by Lindell (MBL), Connecticut by Sunila (CT Bureau of Aquaculture) and at the Haskin Shellfish Laboratory’s Cape Shore site by Guo. During the period covered by this report, we monitored the growth and survival of oysters in all 18 replicate bags deployed at each site and the data generated by our efforts has been sent to Paul Rawson (University of Maine; Project Director).

A preliminary analysis has been completed. We observed substantial variation in the growth of oysters among sites; the average shell length of oysters at Wellfleet, MA was nearly double the average length at the Cape Shore, NJ site. Overall, oysters in our field trial reached market size at all sites except the Cape Shore site during the 2 year grow-out period and there was little evidence of line-specific effects or line by site interactions. In contrast, there were big difference in yield among both lines and sites; the differences in yield were driven by variation in survival. For examples, the yield for the NEH line at sites in Maine was substantially lower than for all other lines due to increased mortality while lower mortality for this line at sites in Massachusetts and Rhode Island resulted in increased yield. These patterns lead to significant site by line interactions for survival and yield.
There was clear evidence of disease pressure at several sites in our field trial. A major mortality event occurred during the fall of 2011 at one site in Maine; we documented a high prevalence of ROD symptoms associated with line-specific variation in mortality at this site. MSX was detected at Wellfleet, MA and Clinton, CT while Dermo was observed at Cape Shore, NJ, Wellfleet, MA, and Westerly, RI. There was pronounced line-specific variation in the prevalences and mortality associated with these two diseases and the hybrid line generated by crossing the NEH with the UMFS lines, demonstrated resistance to MSX. We were successful at meeting nearly all of the goals with this project with the exception of performing size-based selection on the UMFS x NEH F1 line. Due to problems encountered at the start of our project we were unable to obtain and spawn enough of this line to support size-based selection. A more complete presentation of our project results will be provided in our final technical report which we are currently producing.

WORK PLANNED:

The hatchery and field trial components of our project have been completed. The last of the histology-based disease checks will be finish during the summer of 2013 at the Connecticut State Bureau of Aquaculture by Sunila. A separate tissue sample from all of the oysters sampled for disease testing was also sent to the lab of Dr. Marta Gomez-Chiarri to assess Dermo prevalence using a recently modified quantitative polymerase chain reaction (qPCR) protocol that she has developed. This work will be completed within the next one to two months. As mentioned above, we have already conducted an analysis of the field trial data examining the relative growth, survival and yield of each line at each of our sites. We are in the process of preparing the final technical report for our project which will present not only the field trial data but also the analysis of disease prevalence and intensity at each site. We are preparing an NRAC fact sheet on the history and status of the improved lines of eastern oysters currently in use. Finally, we will communicate our project results via at least one journal publication and hands-outs distributed at local and regional growers meetings.

IMPACTS:

We have no direct impact on the northeastern oyster culture industry to report, as yet. However, we have presented reports on our results at the Northeaster Aquaculture Conference and Exposition and Milford Aquaculture Seminar as well as at the Annual Meeting of the National Shellfisheries Association. Our presentations generated significant interest among researchers, and industry partners. The results of our work will help shape decisions regarding line development by the east coast shellfish breeding consortium currently spearheaded by Dr. Dina Proestou and supported by the USDA ARS. As mentioned above, we will disseminate our project results to industry partners and shellfish culture community at large. We anticipate that hatcheries may use our results to produce oyster seed that provides growers in the region best results given disease pressures expected at their sites.
Support: University of Maine portion

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Publications, Manuscripts, or Papers Presented:

Oral Presentations


Manuscripts (planned)


PROJECT CODE: 2012 Minigrant  SUBCONTRACT/ACCOUNT NO: Z555101

PROJECT TITLE: Algal-Bacterial Interactions in Shellfish Hatcheries

REPORTING PERIOD: January 1, 2013 to June 30, 2013

FUNDING LEVEL: $18,488 (University of Maine Match – $11,818)

PARTICIPANTS:
   Paul Rawson, University of Maine
   Michael Devin, University of Maine

PROJECT OBJECTIVES:

   Typical best management practices in shellfish hatcheries overlook the potential for beneficial bacterial strains to enhance algal production and shellfish seed production, enhancements that can result in healthier, better growing seed leaving the hatchery and improving farm production.

   The research under this award addresses the following objectives:

   • Determine the diversity of bacteria typically associated with six strains of algae typically employed during shellfish larviculture,
   • Identify bacterial strains that have a positive impact on algal growth and assess the stability of algal-bacterial interactions as a function of varying temperature and other culture conditions,
   • Determine the effect of specific algal-bacterial interactions on larval growth in oysters,
   • Assess probiotic potential of specific algal-bacterial combinations during oyster larviculture, and
   • Communicate results to shellfish hatcheries and shellfish growers in the NRAC region.

ANTICIPATED BENEFITS:

   Current shellfish hatchery practices typically stress minimizing microbial activity during larval and nursery production of shellfish seed. At high densities bacteria can depress the rate of algal ingestion by larvae while catastrophic declines in larval growth and survival are often due to “infection” or the production of toxins by pathogenic bacteria. Because widespread use of antibiotics and other therapeutics is undesirable, hatcheries often employ mechanical filtration (< 1um) along with chemical treatment, UV sterilization or pasteurization of seawater in order to reduce microbial activity in all hatchery phases from algal culture to
nursery phase seed culture. Such “cleanliness” may come at a cost in that it not only eliminates disease-causing and dangerous microbes but beneficial microbes, as well.

The potential positive impact of microbes, however, extends to other stages of hatchery operation. Algal culture typically relies on mechanical filtration along with chemical treatment or pasteurization to remove contamination from particulates, bacteria, protists and other organisms which would compete for nutrients and reduce the growth of algal species. Even so, it is well appreciated that harmful vibrios can dominate senescing, late stage algal cultures and such cultures should not be fed to larvae. Although ecologically bacteria generally play a key role in breaking down dead, decaying and even live algal cells, there is increasing recognition there can be positive interactions between microalgae and their associated bacteria. In addition, the impact of probiotics on culture fish and shellfish may depend not just on presence of the probiotic bacteria but on particular probiotic-algal interactions. Our project seeks to identify individual species of bacteria associated with standard algal culture systems in bivalve hatcheries and determine whether individual species of bacteria have positive or negative impacts on algal and larval bivalve growth and survival. Those with positive effects will be considered potential probiotics that may be beneficial in improving the production of bivalve seed.

**Progress and Principal Accomplishments:**

Although the contract for this project was signed in late February, the account codes were not set up until late May of 2013. Thus, we have only had one month to work on this project. Our activities during this month have included purchasing the supplies necessary for culturing a wide variety of marine bacteria and refining our microbiological techniques to account for the variation in bacterial abundance among different species of microalgae typically found in bivalve hatcheries.

**Work Planned:**

Bacteria associated with six species of microalgae typically used in shellfish larviculture will be isolated using a series of non-selective growth media (e.g. marine agar, seawater tryptone agar) while thiosulfate citrate bile sucrose agar (TCBS) will be used to select for Vibrio species. We will isolate bacteria from algal cultures at multiple hatcheries in order to determine the diversity of associated bacteria in Northeastern hatcheries. Bacterial cultures will be incubated at 23°C, the average algal culture temperature in our hatchery. We will work with the Maine Aquatic Animal Health Lab (MAAHL) BIOLOG Microbial Identification System to phenotypically characterize up to 40 individual strains. In addition, we will use 16S rDNA sequencing to genetically identify each strain. Isolates will be cryopreserved and we will maintain a publically available database on the phenotypic characteristics and genetic identity of each strain.

Axenic algal cultures (75 ml) will be re-associated with individual bacterial strains (0.5 x 10^6 cfu/ml). The growth of cultures for each algal-bacterial pair will be compared to the growth in axenic algal cultures and cultures associated with a mixed assemblage of bacteria. Algal cultures will be grown in triplicate at 23°C and changes in density of associated bacteria will
be assessed by epifluorescence microscopy and plating. For bacterial strains that have a significant positive effect on algal growth, we will determine whether that effect is consistent as culture conditions change by repeating the growth experiments at 18ºC and 28ºC and with versus without CO₂ supplementation. We will also examine how specific algal-bacterial combinations affect larval growth. Lab-scale cultures (250 ml flask) of bacteria-free oyster larvae (*Crassostrea virginica*; 5 larvae/ml) will be provided with additions of axenic algal cultures and algae that have been re-associated with single strains of bacteria. We will focus on bacterial strains that have a positive effect on algal growth (above). The shell length of ~50 larvae will be measured every other day over a 10 day experimental period during which algal concentrations will be maintained. At the end of the experimental period, rose Bengal staining will be used to estimate the proportion of live larvae in each culture. Each larval growth trial will be run in triplicate and a starved larval treatment will serve as a negative control. We will also investigate the probiotic potential for bacterial strains that have a positive effect on algal growth using Vibrio-challenge assays. Potential probiotic bacteria will be provided to straight hinge stage oyster larvae (5/ml) in association with algal feeds and at higher doses (10² to 10⁵ cfu/ml) in lab-scale trials. Experimental treatments will receive a single dose (10⁵ cfu/ml) of pathogenic Vibrio while bacteria-free, pathogenic only and probiotic only cultures will serve as controls. Larvae in triplicate cultures will be monitored for growth and survival over a 10 day period.

**IMPACTS:**

None to report, as yet.

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**Publications, Manuscripts, or Papers Presented:**

None to report
Annual Progress Report  
Optimization of Hatchery and Culture Technology for Razor Clams  
Subaward # Z540402  
Grant #2010-38500-21074

PROJECT CODE: TRA-11-1  
SUBCONTRACT/ACCOUNT NO: Z540402

PROJECT TITLE: Optimization of Hatchery and Culture Technology for Razor Clams

REPORTING PERIOD: July 1, 2012 to June 30, 2013

FUNDING LEVEL: $93,616 (Total Project from NRAC; $70,310 for work performed at University of Maine)

PARTICIPANTS:  
Paul Rawson, University of Maine  
Dale Leavitt, Roger Williams University  
Dana Morse, University of Maine  
Diane Murphy, Southeastern MA Aquaculture Center

PROJECT OBJECTIVES:

Support the diversification of the shellfish culture industry in the Northeast by:

1) Developing improved hatchery methods for the production of razor clam seed in order to provide commercial shellfish hatcheries with the means to produce a steady, reliable source of seed,
2) Identifying improvements in grow-out technology for the culture of razor clams and increase the industry’s interest in and acceptance of this alternative species,
3) Tracking the marketability of razor clams in regional and broader markets, and
4) Communicating the progress and results of proposed work directly to industry partners and the industry at-large.

ANTICIPATED BENEFITS:

This project seeks to develop improved technologies for seed production of the razor clam, an alternative shellfish species, and make that technology available to shellfish hatcheries. Through our Razor Clam Roundtables, we are also working closely with industry participants to design improved grow-out technologies. By developing and demonstrating culture systems for razor clams, our work will lead to diversification of the shellfish culture industry of the northeastern U.S. Diversification will reduce the risks associated with an overreliance on just two species by the industry and allow shellfish growers to choose the most appropriate crops based on environmental and market conditions.
PROGRESS AND PRINCIPAL ACCOMPLISHMENTS:

In our semi-annual report from June of 2012, we reported on our attempts to condition and spawn adult razor clams. A major goal of our project was to conduct multiple spawnings of razor clams using both field-collected, ripe broods and broods conditioned in the lab. We were successful at obtaining spawn from both types of broods. During the first six months of our project, we also convened a regional Razor Clam Roundtables at the Darling Marine Center on May 2, 2012.

During the present reporting period, we used larvae from our earlier spawns to compare the efficacy of using downwellers versus trays filled with sandy and mud substrates for nursery phase culture of razor clam offspring. The former methods represent the traditional methods for early nursery phase culture of shellfish (experimental controls), but have generally not supported the early growth and survival of razor clam spat. The latter represent potential solutions to problems often encountered with nursery phase culture of razor clams. Complete mortality of clams in downwellers indicates that they are unsuitable for early nursery post-settlement for razor clams, as has been found in other hatchery experiments with razor clams. With the fragile shells of newly settled juveniles and the fact that *E. directus* is a benthic bivalve that lives buried in the sediment. We found that the use of sediment filled trays provides a substantial increase in the growth and survival of immediate post-set razor clams in the hatchery. In our trials, we included tanks containing both autoclaved and cleaned but non-autoclaved sediments. We recovered few clams from the tanks with autoclaved sediment, which may have been a result of contamination or lack of preference for autoclaved sediments. On the other hand, when provided with cleaned, washed sediments, razor clams exhibited a clear preference for coarse sand. The volume, biomass and growth for post-set razor clams in the coarse sand was higher in comparison to the clams that settled in the fine sand or natural sediment treatments in the tank which had non-autoclaved sediments. It is important to note that our experiment cannot directly determine differences in survival for clams in each of these treatments, as survival is confounded by differences in initial settlement density. However, the clear differences between total volume and biomass in the natural sediments and sand sediment treatments indicate there is greater settlement and survival in the latter. Based on our observations, the increased shell size and volume in the coarse sand treatment suggests that coarse sand should be used in aerated tank systems for successful survival and growth of *E. directus* post-settlement.

We also conducted experiments investigating sediment preference for razor clams at 6 months post-settlement. We recorded the burrowing behavior of individual clams placed in burrowing chambers containing polished-washed sand, natural mud or a sand-mud mix. Juvenile razor clams took over five times as long to explore the sediments surface and initiate burrowing when presented with mud sediments when compared to clams presented with sand or mixed sediments. The speed with which clams burrowed once they started burrowing was slightly faster in mud than in the other treatments. However, the large difference in the delay in initiating burrowing for clams exposed to mud overwhelms the small difference between treatments in burrowing speed. Thus, based on the differences in burrowing behavior juvenile razor clams expressed a clear preference for sand or sandy-mud substrates and clams exposed to mud remain exposed on the surface where they are prone to predation, disturbance, and are
unlikely to feed which will affect their growth and survival. Future experiments will expand the analysis of sediment preference to other sediment types and ontogenetic stages.

**WORK PLANNED:**

We are presently preparing the final technical report providing expanded detail on the results of our work, our analysis of the marketability of razor clams in regional and broader markets, as well as an NRAC Technical Bulletin on the advances and successful refinements in the hatchery protocols developed during this project.

The work described above addressed objectives 1 and 2 of our original proposal. Our hatchery experiments sought to develop improved hatchery methods for the production of razor clam seed while our Razor Clam Roundtables worked with industry partners to identify improvements in grow-out technology for the culture of razor clams and increase the industry’s interest in and acceptance of this alternative species. Along the way, we found that hatchery operators and growers were concerned with the development of protocols for harvesting and distributing seed without damaging the fragile shells of razor clam seed. Thus, future work will not only continue to develop the optimal methods for hatchery production of seed but will also focus on identifying optimal methods for processing seed.

**IMPACTS:**

None to report, as yet.

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### Publications, Manuscripts, or Papers Presented:

**Oral Reports**

PROJECT CODE: 12-07

PROJECT TITLE: Development of more efficient methods of Vibrio sp. detection and identification of Vibrio sp. abundance in cultured oysters from Northeast U.S. farms and from retail sites post-harvest.

REPORTING PERIOD: Sept. 1, 2012 to March 1, 2013

FUNDING LEVEL: $186,024

PARTICIPANTS:

Funded:
Roxanna Smolowitz, Roger Williams University, One Old Ferry Road, Bristol, RI 02809
Dale Leavitt, Roger Williams University, One Old Ferry Road, Bristol, RI 02809
Dianne Murphy, Cape Cod Coop. Extension and WHOI Sea Grant, PO box 367, Barnstable, MA 02603

Non-Funded:
M. Gomez-Chiarri, Dept. of Fisheries, Animal and Veterinary Science, 169 CBLS, URI, Kingston, RI 02881
R. E. Levine, Dept. of Food Science, College of Natural Resources and the Environment, Chenoweth Laboratory, 100 Holdsworth Way, Amherst, MA
Lisa Calvo, Program Coordinator, Rutgers University, New Jersey Sea Grant Consortium, Institute of Marine and coastal Sciences, Rutgers, 71 Dudley Road, New Brunswick, NJ 08901
Dana Morse, Marine Sea Grant Program, Clarks Cove, Walpole Maine 02573
Gregg Rivara, Cornell University Coop. Extension of Suffolk County, 3690 Cedar Beach Road, Southold, New York 11971
Don Webster, University of Maryland Extension, Wye Research and Education Center, 124 Wye Narrows Drive, Queenstown, MD 21658

PROJECT OBJECTIVES:

A. Develop a multiplex quantitative real time PCR (mqrtPCR) method for the detection of Vv and Vp using an oyster DNase inhibitor (activated carbon coated with bentonite; ACCB) and compare sensitivity and specificity with the FDA MPN/PCR method. Develop two additional multiplex methods for evaluation of samples for both Vp and Vv pathogenic genes using previously published methods with the oyster DNase inhibitor.

B. Intensively monitor cultured oysters, water and sediment from two locations (one in RI and one in MA) over a year using the MPN/mqrtPCR and the ACCB/mqrtPCR method side by side in order to understand the Vv and Vp cycle in the northeast environment and compare sensitivity of both tests.

C. Identify the occurrence of pathogenic and non-pathogenic strains of Vibrio sp. in oysters
   (1). at two time periods after collection of oysters from culturists who reside in 5 northeastern states
   (2). from ten retail stores/restaurants in July/August. The source of oysters (culturists from which animals were collected in 2.1.A. and 2.1.B.), and post-harvest handling (time between harvest and chilling and length of time chilled before sampling) will be identified as selection criteria.

D. Provide findings (via presentations, websites and brochure/white paper) to culturists and extension agents and diagnostic laboratories at regional and national meetings, at local meetings for culturists and extension agents and to representatives of the Food and Drug Administration.
ANTICIPATED BENEFITS:

Deliverables expected from this work will include production of a speedy and cost-effective multiplex quantitative real time PCR (mqrt PCR) method of identification of Vp and Vv organisms and their pathogenic forms in oyster tissues. There is a lack of understanding of true levels of Vv and Vp amounts in the oyster culture environment of the northeast especially since most work has been conducted in the southern U.S. Results of this information will provide a current answer to the question posed in problem statement TRA-12-1. Second, this information can be used immediately by the extension agents, culturists and health officials to develop management methods and help determine harvesting times for their farms. Third, we will provide this new information to the Dept. of Marine Fisheries in MA and equivalent departments in other northeastern states and the Departments of Public Health in the northeastern states. This information can be used to develop new regulations.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS:

Work conducted in the first six months of the study involved determining the best locations for the year round study and development of the mqrt PCR methods. Using the MPN methodology, cultured oysters from four locations in both RI and MA were tested in August of 2012 for assumed Vibrio sp. content. From this testing, one location in MA and one location in RI will be chosen for monitoring starting in Aug/Sept of 2013. The qrt PCR developed in the lab for detection of Vp was used on extracts of each of the positive MPN tubes and results were compared using the same MPN BAM tables as are used in that method (number of tubes positive for growth at different dilation levels. See below.

FDA BAM-MPN: 10 oysters were homogenized and a serial dilution of 10g, 1g and 1:10-1:10000 were enriched overnight in Alkaline Peptone Water (APW). All tubes were run in triplicate. After overnight enrichment the tubes were evaluated for grown. Any tube with growth was called a positive and was used to calculate the number of bacteria/gram of oyster tissue by using the BAM-MPN calculation spreadsheet (provided by M Gomez-Chiarri).

MPN qPCR: A 1mL sample was taken from all tubes with growth from the above MPN process and the Boiled Cell Lysate (BCL) extraction protocol was performed. Then 2ul of crude supernate DNA was run in a qPCR assay designed for Vibrio parahaemolyticus. All tubes with a positive signal was given a positive ranking and this data was plugged into the BAM-MPN spreadsheet.

Data analysis comparing methods: The data collected showed that the overnight FDA BAM-MPN generated higher rating of Vibrio sp. versus the qPCR MPN data based on the molecular occurrence of V. parahaemolyticus (Table 1.). This result could be for two reasons; first, that the overnight growth assumes that all growth in the APW tubes are Vibrio sp. and second, the BCL protocol for DNA extraction doesn’t produce high quality DNA and causes a loss of signal for the MPN qPCR assay.

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Table 1. Data collected from August 2012 Vibrio Sampling- FDA BAM-MPN and qPCR MPN

We are currently working on development of a duplex qrtPCR assay for *Vibrio parahaemolyticus* (Vp) and *Vibrio vulnificus* (Vv). Work conducted to April, 2013 is summarized below as follows.

A duplex assay was designed using primers and probes from Nordstrom et al. 2007 for Vp (tlh gene) and Takahashi et al. (2005) for Vv (toxR gene). Modification were made to thermal cycling parameters, mastermix set up and primer and probe ratios to fit the Bio-Rad qPCR thermal cycler used in the Aquatic Diagnostic Lab (ADL). Each assay for Vp and Vv was run independently and as a duplex to ensure the same results once duplexed. All parameters for an optimized assay were achieved (Efficiency of 90-110% and $R^2$ values close to 0.990). This initial assay was developed for purified gDNA from pure cultures of Vp and Vv (Figure 1).

Figure 1. Optimized standard curve of *Vibrio paraheamolyticus* (Texas Red) and *Vibrio vulnificus* (FAM)

![Standard Curve](image)

WORK PLANNED: In the next six months we are planning to accomplish the following. We have determined that development of the duplex test method will be much more efficient and consistent if the test using plasmids containing either a copy of our Vv or Vp gene as the control, rather than continuing to rely on extracts of cultured bacteria to produce the standard curve. The use of bentonite coated carbon as a pre-qrt PCR treatment is underway and will be compared to two other methods of extraction from short and long incubation times in order to determine the best and quickest testing method. We hope to have a fully functioning qrt PCR test method ready to go in August, 2013. In Aug./Sept. 2013, we will begin our routine surveys of the two chosen locations in which oysters, sediment, water column measurements of Vibrio will be conducted.

IMPACTS: In concise statements (possibly a bulleted list) indicate how the project has benefited the aquaculture industry either directly or indirectly and resulting economic values gained (where appropriate).

- Both the MPN-BAM method and the MPN-qrt PCR method were used to determined levels of *Vibrio* for some samples provided by D. Murphy form Barnstable County, MA (other than those that are part of this grant) We are working with Murphy to examined the levels of *Vibrio* in animals collected and cooled immediately and in animals collected but cooled five hours after collection.
- We have provided our MPN-BAM monitoring help to culturists.
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