Northeastern Regional Aquaculture Center
University of Maryland
Current and Recent Projects
March 2017

Development of a phage-based diagnostic test for the rapid detection of pathogenic *Vibrio species* in bivalves

Funding level: $191,424
Project start date: 1 October 2016
Project duration: 24 Months
Participants: Cornell University, University of New Hampshire

Project Summary:
The prevalence of gastrointestinal illnesses associated with the consumption of Atlantic coast shellfish contaminated with *Vibrio parahaemolyticus* and *V. vulnificus* has increased over the years, having a negative impact on the industry due to recalls and loss of consumer confidence in the product. We are proposing to develop a rapid and highly sensitive bacteriophage-based diagnostic test for early detection of shellfish contamination by virulent strains of these two bacterial pathogens. The first objective of this proposal is to isolate and characterize phages that infect *V. parahaemolyticus* and *V. vulnificus*. These phages will be isolated from water samples in which these bacteria thrive along the Atlantic northeast coast. Isolated phages will be characterized to differentiate those that infect environmental bacterial strains not associated with infection from those that infect isolates from clinical cases (virulent strains). A set of phages that infect specifically virulent isolates will be further characterized for efficacy of infection and multiplication. The best performing phages will be selected and sequenced. The second objective of this proposal aims at developing the diagnostic test. Selected phages will be modified to express a marker that will be easily detectable in infected samples, bypassing the requirement for an enrichment phase. This detectable marker will be produced by phages multiplying in virulent isolates of *V. parahaemolyticus and V. vulnificus*. The ultimate goal of this research project is to develop a test that will be specific, sensitive, cost-effective, and rapid for the detection of shellfish contaminated with *V. parahaemolyticus and V. vulnificus*. This test will be beneficial to the industry and the consumers because it will provide a reliable and rapid tool to monitor contamination at any step of the process from pre-harvest to table.

A Novel Approach To Prevent Super Chill in Atlantic Salmon

Funding level: $165,624
Project start date: 1 October 2016
Project duration: 24 Months
Participants: University of Maine, National Cold Water Marine Aquaculture Center (ME), Cooke Aquaculture (ME), University of Rhode Island

Project Summary:
The project will research and demonstrate innovative production technologies focused on mitigation of super chill in Atlantic salmon aquaculture though new feed formulations. Project objectives are; to investigate the concentrations of simple sugars and sugar alcohols (SSASA) in plasma of Atlantic salmon to achieve a lower freezing point

depression limit, to investigate the uptake of SSASA from the diet in both epithelia cells and the plasma of Atlantic salmon, to determine the efficacy of SSASA in reducing the impact of a super chill event under controlled conditions, to measure the physiological parameters of the fish subjected to super chill and compare to control, and to liaise with Atlantic salmon aquaculture industry, extension and technology transfer.

Impact Assessment of NRAC Funding on Aquaculture in the Northeast

Funding level: $150,000
Project start date: 1 October 2015
Project duration: 24 Months
Participants: University of New Hampshire, Virgin Oyster Company, Salem State University, Marine Biological Laboratory, University of Maryland, NOAA, New Jersey Sea Grant, Profish, MAIC, Delaware State University, University of Maine, State of Pennsylvania, University of Rhode Island, Cornell University, West Virginia University

Project Summary:
The overall objective is to assess the scientific, socio-economic, and policy impacts of accomplishments made through NRAC's portfolio of recently (since 2005) funded aquaculture projects, including extension workgroup projects. Incorporated in this synthesis will be how these projects have or have not helped move the aquaculture industry close to solutions for the diversity of problems it faces within the region. From the resultant information, suggestions for achieving higher impacts will be identified that NRAC should consider in future funding initiatives.

Safe Feedstocks for Bivalve Aquaculture

Funding level: $190,508
Project start date: 1 October 2015
Project duration: 24 Months
Participants: University of Rhode Island, Roger Williams University, Oyster Seed Holdings, Virginia Institute of Marine Sciences, Martha's Vineyard Shellfish Group, Fishers Island Oyster Group

Project Summary:
The overall goal of the project is to test the efficacy and safety of two bacteria strains to reduce or eliminate vibrios from phytoplankton feedstocks that are commonly used in bivalve larviculture and manage disease in hatcheries.
Specific aims:
1. Measure the reduction of vibrios and other potentially pathogenic bacteria in algal cultures following the addition of the two beneficial bacterial probiotic strains.
2. Measure the growth and survival of algae in response to probiotic treatment.
3. Measure the survival of bivalve larvae that are fed probiotic-treated algae prior to being challenged with bacterial pathogens.
4. Outreach and Extension: The results of this research will reach potential end users through direct collaboration, workshops, and meetings with commercial hatchery managers.
This work will be performed first at the laboratory scale and then tested at a commercial scale in bivalve hatcheries. Dependent upon successful outcomes of the proposed trials, the candidate strains will advance toward commercial development as new tools to prevent pathogen contamination of aquaculture systems. We anticipate that these strains will ultimately be useful to prevent economic losses due to infectious disease outbreaks in commercial hatcheries.

Testing and Application of Novel Probiotic Bacteria for Use in Marine Aquaculture

Funding level: $190,508
Project start date: 1 October 2015
Project duration: 24 Months
Participants: University of Delaware, University of Maryland Baltimore County, University of MD Center for Environmental Science

Project Summary:
A 24 month study is planned in which we will build on the foundation laid by the 2012 NRAC Mini-Grant. Through that effort, we identified seven non-Vibrio bacteria from the intestines of Fundulus heteroclitus that had the ability to inhibit growth of not only the fish pathogens *Vibrio harveyi* (DNO1) and *Vibrio damsela* but also may inhibit growth of *Vibrio* sp. B183 (a shellfish pathogen) as determined by filter disk assays. Of these, we tested four probiotic bacteria (OY15, Iso5, Iso11 and Iso12) selected by their ability to inhibit pathogen growth and a glycerol-only control (the probiotic storage medium) in a short term growth trial with our model species, F. heteroclitus, to ascertain the potential effects of the novel probiotic bacterial strains on the larvae. In that initial trial, mummichogs exposed to the novel probiotics exhibited higher growth rates compared to the fish in the control treatment. The current proposal will continue to evaluate these potential probiotic strains on survival of growth of other commercially important species including striped bass, rainbow trout, Atlantic salmon and tilapia. In all cases, one day-old larvae will be stocked into triplicate experimental tanks at a density of 25 larvae per liter. Samples of larvae will be collected at the time of stocking and weekly to assess the growth effect of the respective probiotic strains. After six weeks, all surviving fish from each tank will be individually counted to determine survival. Due to space and labor considerations, individual target culture species will be tested in succession, as they become commercially/seasonally available.

Development and Evaluation of Novel, Non-toxic Solutions for Biofouling Control and Predator Exclusion in Shellfish Aquaculture

Funding level: $193,582
Project start date: 1 October 2015
Project duration: 24 Months
Participants: University of Connecticut, University of Maine, Martha's Vineyard Shellfish Group, Virgin Oyster Company, ePaint

Project Summary:
We propose to develop and refine an effective, environmentally-friendly, and affordable means to prevent biofouling on aquaculture gear and predation. We will 1) assess potential toxicity of new coatings (note: base materials being tested have been shown to be non-toxic and all are cleared with FDA and EPA regulations; it is still important to test new configurations to confirm non-toxic status); 2) assess adhesion and efficacy of newly developed formulations on test panels and gear on experimental facilities and farms to assess antifouling and antipredator properties, and 4) engage aquaculture producers in research and outreach, and disseminate the results to the industrial and scientific communities through presentations at workshops, conferences, outreach publications, web page, and peer-reviewed publications. We have assembled a strong team of scientists and aquaculturists from 4 states (ME, MA, NH, CT) as well as a cadre of industry

supporters with decades of experience in shellfish biology, anti-fouling coatings, aquaculture, and outreach. All proposed experimental techniques have been used in our prior studies, and we have access to all necessary equipment and facilities. Successful development and demonstration of extended efficacy of biologically inert, inexpensive, and practical deterrents to biofouling will have a profound, positive impact on the global aquaculture industry, and our aim is to remove biofouling as one of the most costly problems in aquaculture.

Hatchery and Nursery Technologies for Improved Production of Blue Mussels

Funding level: $198,077
Project start date: 1 July 2014
Project duration: 24 Months
Participants: Marine Biological Laboratories, University of Maine

Project Summary:

The overarching goals of the research we propose are to (i) develop hatchery and nursery technologies for cost effective year round seed production of blue mussels (*Mytilus edulis*), (ii) enhance the annual output per unit area of mussel farms and the market potential for domestically grown mussels and (iii) support the expansion and diversification of the northeastern shellfish culture industry which will create new opportunities for hatcheries, aquaculturists and fishermen. Our proposed research will facilitate the establishment of consistent, reliable sources of blue mussel seed from hatcheries that will promote steadier, higher volume and higher value harvests per acre. Ultimately, hatchery production of seed will result in "added- value" by laying the foundation for genetic improvement of key traits.

In addition to communicating and transferring the progress and results of our work directly to our hatchery and industry partners, we plan to have ***two major project deliverables.*** One will be to sponsor two Mussel Grower Workshops in Year 2 of the project to be held at the Darling Marine Center in Maine, and at the Cape Cod Cooperative Extension Office in Massachusetts. At these workshops, we will present not only the project results, but also discuss blue mussel biology, current grow-out technologies, and current market opportunities and the implication of our results. Our goal is to increase participation in blue mussel culture region-wide. We will also communicate and transfer our project benefits through a ***second major products***, including, but not limited to, a detailed hatchery manual for mussel seed, distributing mussel seed at the conclusion of the hatchery phases of our project, making the special declumping and grading equipment available to growers, presenting at regional scientific meetings, submitting articles in local and regional newsletters, and through our websites at our respective research institutions.

White Worm, *Enchytraeus albidus*, production and marketing for live aquaculture feed

Funding level: $151,925
Project start date: 15 Aug 2014
Project duration: 24 Months
Participants: University of New Hampshire, Washington DC

Project Summary:
White worms, *Enchytraeus albidus*, are a promising feed in the aquaculture industry for a variety of species (freshwater, marine, ornamentals, shrimps) across the U.S. Although they used to be mass produced in the former U.S.S.R. in the 1940s for sturgeon, white worm farming is a "lost art." At UNH, we have established a large white worm population using these dated Soviet techniques. There is enormous interest by the aquaculture industry to utilize white worms as a feed because more species are being cultivated, many of these still at the R&D stages, and many of whom are picky eaters during specific ontogenetic phases. The variety of available small live feeds for these picky eaters is limited. Also, it is not economically nor environmentally feasible to feed aquacultured species diets mostly composed of expensive, harvested wild fish meal. While we currently are working to provide worm samples to all interested parties, we cannot promote them as a readily available food source until we optimize worm production and are confident that we can ensure a large, steady, and safe supply. This research project would (1) develop "modern" white worm production protocols, which eventually could be adapted for commercial scale production, and (2) work with all interested aquaculture sectors to identify the white worm market(s) through a series of workshops resulting in identifying the target markets and providing worms nutritionally customized for those consumers (species). This research promotes sustainable, environmentally friendly tactics in its use of recycled, local, waste by-products for worm feed, and a low carbon footprint. This research will yield economically viable techniques for those aquaculturists looking to diversify and a readily- available product for the aquaculture market.

Teleost spermatozoal transcriptomes: requisite foundation for functional genomics, sperm quality and male fertility

Funding level: $20,000
Project start date: 1 Sept 2014
Project duration: 12 Months
Participants: University of Maryland, Stuttgart National Aquaculture Research Center

Project Summary: The objective is to genetically sequence and evaluate spermatozoal transcriptome profiles from male striped bass with observed differences in fertilization rate.

There is Gold in Them Thar Mussels; Testing the Feasibility of Golden Mussel Culture for Branding and Market Expansion of Farmed New England Mussels

Funding level: $10,000
Project start date: 1 July 2014
Project duration: 12 Months
Participants: Marine Biological Laboratory, Martha's Vineyard Shellfish Group,

Project Summary:
The objective of this small study is to test the feasibility of utilizing the natural golden color morph of *Mytilus edulis* for aquaculture. Parameters of concern include larval competence (i.e. time to and completion of metamorphosis), growth rate from seed to market size, meat yield and shell strength, compared to those of the blue color morph. Shell strength will not be quantified in the study but should be explored in the future in order to fully evaluate the value of golden mussels.

Workshop to Identify Constraints towards developing a Commercial eel aquaculture industry using Recirculating Aquaculture System (RAS) technology

Funding level: $11,000
Project start date: 1 July 2014
Project duration: 12 Months
Participants: University of New England, Cornell University, SUNY College at Oswego, Maine Sea Grant College Program and U Maine Cooperative Extension, University of Massachusetts Dartmouth, University of Maine

Project Summary:
We propose a two-day workshop at the University of New England in Biddeford ME where we will bring in experts associated with eel biology, marketing, and engineering to identify constraints and possible solutions towards creating economically viable culture systems using RAS technology.

Measuring flow within shellfish growout systems: developing and evaluating a logging mini-flow meter

Funding level: $8,634
Project start date: 1 July 2014
Project duration: 12 Months
Participants: Roger Williams University

Project Summary:
The objective of this mini-grant application is to develop a small scale logging flow meter that will be of a size and a capacity capable of independent deployment in traditional shellfish aquaculture gear for an extended period of time. Once developed, we propose to test the application of the flow meter in multiple shellfish growout systems.

New Tools to prevent Bacterial Diseases in Shellfish Hatcheries

Funding level: $199,514
Project start date: 1 Oct 2013
Project duration: 24 Months
Participants: University of Rhode Island, Roger Williams University, University of Maine

Project Summary:
A multidisciplinary team of experienced investigators has been assembled to further develop three highly promising probiotic bacteria as new disease management tools for shellfish aquaculture. The three candidate strains have been demonstrated to be safe and effective in preliminary pilot-scale experiments with oyster larvae. This proposal outlines the next steps in the development of the strains, specifically their formulation for hatchery use and additional proof of concept testing in hatcheries using multiple bivalve species. A multi-faceted extension and outreach plan is proposed to disseminate project results to shellfish hatchery managers.

Improved Grow Out Methodologies for Razor Clams

Funding level: $176,049
Project start date: 1 Sept 2013

Project duration: 24 Months
Participants: University of Maine, Roger Williams University, Maine Sea Grant College Program, Woods Hole Sea Grant Program & Cape Cod Cooperative Extension

Project Summary:
Shellfish aquaculture is an established and thriving industry in the northeastern U.S. The production of cultured shellfish has steadily increased throughout the region over the past several decades and now includes more than 350 culture operations generating products with a gate value in excess of $50 million. The industry, however, is heavily dependent on the culture of Eastern oysters. Recent disease outbreaks have highlighted how over-reliance on a single species leaves the industry vulnerable to epizootics. To reduce this risk and support continued expansion, it is critical for the industry to identify alternative species for culture. The razor or Atlantic jackknife clam (*Ensis directus*) is one species that can provide for industry diversification in the Northeast. Among other alternative species, which have been considered for culture in the Northeast, we believe there is a strong potential for the development of culture methodologies for razor clams which would facilitate the diversification of the shellfish culture industry in the region. In a previous NRAC-supported project, we found that naturally ripe razor clams readily be spawned in the hatchery, and broods can be conditioned out of season. We also developed and tested alternative post-set nursery culture systems; particularly promising in this regard is the use of sediment-filled trays and raceways to improve the health and survival of recently settled razor clams. The objectives of the current proposal address the next steps in developing razor clams as an alternative aquaculture species for the northeastern shellfish culture industry.

Algal-Bacterial Interactions in Shellfish Hatcheries

Funding level: $18,488
Project start date: 1 Jan 2013
Project duration: 12 Months
Participants: University of Maine

Project Summary:
The proposed research will address the following objectives:

• Determine the diversity of bacteria typically associated with microalgae 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.
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. We are proposing a preliminary project that will identify specific bacterial strains that have positive impact on algal production and test whether algal-bacterial interactions have probiotic potential.

Identification and Isolation of Novel Probiotic Bacteria for Use in Marine Aquaculture

Funding level: $19,981
Project start date: 1 Jan 2013
Project duration: 12 Months
Participants: DE State University, IMET-University of MD Center for Environmental Science, University of MD Baltimore County

Project Summary:
A 12 month study is planned in which we will generate baseline data on novel strains of probiotic bacteria. We will undertake two objectives: 1) identify novel probiotic strains, and 2) screen them in short-term growout trials using *Fundulus heteroclitus* larvae. In a future research project, the novel strains of probiotic bacteria will be fully evaluated and the mechanism of their probiotic activity will be ascertained.

Direct end users of the knowledge generated by this project will be other members of the research community. *F. heteroclitus* are an important model system for EPA and other agencies to study the effects of environmental toxins. A search of the EPA website for 'fundulus' returns over 1,000 pages of information, illustrating the importance of this species to the scientific community. In addition, there is growing interest among aquaculture researchers nationwide to develop practical methods to culture *Fundulus* spp. commercially for the bait market. Many baitfish are currently harvested wild, transported to other regions for use, and this represents an opportunity for diseases to spread rapidly between watersheds or regions. In the Great Lakes states, where viral hemorrhagic septicemia virus (VHSV) has affected many species over a wide range, state regulators have recognized that the practice of moving wild caught bait is a serious threat to wild fish. Both NY and VT now have bans on the transport of wild baitfish.

Probiotics should be selected from (adapted to) the environment in which they will be eventually used. *F. heteroclitus* lives in habitats with a wide range of salinities, and can be cultivated in a similarly wide range in aquaculture. Therefore, it can be used to screen for probiotic activity of bacteria selected in many different salinity regimes.

Longer term beneficiaries of this information are marine aquaculturists that could benefit from increased diseases resistance, better growth and/or improved survival of their animals. With respect to the bait industry, the increased profitability will encourage further cultivation of bait fish, thus reducing the flux of wild bait fish that are transported between watershed and jurisdictions.

Striped Bass Selection for Marine Culture

Funding level: $199,569
Project start date: 1 May 2013
Project duration: 24 Months
Participants: University of New Hampshire, University of Maryland, Dupree Stuffgart National Aquaculture Research Center, UCT-Avery Point Marine Science Building

Project Summary:
Identification of new species is critical for the northeast aquaculture industry to continue to expend and candidate species must command a premium price, have high consumer demand and adapt to domestication for profitable production. One species that meets all these criteria is the striped bass (*Morone saxatilis).* Striped bass is one of the most

popular fish in the northeast and some recent retail prices in New England and New York ranged from $12-18/pound. The demand and high-value for this , and other select marine species, creates an enormous marketing opportunity that ca be filled by producers in the northeast. The National Striped Bass Breeding program, located at the Stuttgart National Aquaculture Research Center (SNARC) has over 5 generations of broodstock that have been selected for rapid growth and other attributes in freshwater ponds. We propose to utilize these broodstock for selection of individuals capable of rapid growth in marine environments, to improve profitability of finfish culture in the northeastern states. Ten paired crosses from selected broodstock will be made in each of 2 years at the SNARC and sperm from males will be cryopreserved. Finclips will be taken from all broodstock for genetic identification using microsatellite markers and the larvae from each cross will be segregated and reared intensively in saltwater recirculating systems. At 6 and 9 months post-hatch the fish will be anesthetized (weights and lengths taken and the smallest 25% of the fish at both facilities will be culled. A random subsample (20%;finclip) of the fish at each sampling/facility will be taken of genotyping to examine the genetic composition. At 12 months post-hatch, the largest 20% of the remaining individuals at each facility will be PIT tagged, separated from the general populations and be retained for future broodstock by industry participants. Regionally-relevant information with respect to finfish aquaculture will be conveyed to current and potential growers through a series of outreach workshops and multimedia presentations.

Genetic mark-assisted selection of Northeastern hard clams for QPX resistance

Funding level: $199,998
Project start date: 1 Feb 2013
Project duration: 36 Months
Participants: Stonybrook University, Rutgers University, Roger Williams University, Cornell University, Cape Cod Cooperative Extension, University Of Paris 6, NY Sea Grant

Project Summary:

The hard clam or northern quahog, *Mercenaria mercenaria*, is one of the most valuable seafood products in the Northeast representing the first marine resource in several states. Since the 1990's several Northeastern states have suffered severe losses in aquacultured hard clam stocks due to a fatal disease caused by a protistan parasite called Quahog Parasite Unknown (QPX). The extensive scale of these mortality events resulted in loss of several dozens of Millions of aquacultured market-size clams and significant impact on aquaculture operations. The overall objective of this proposal is to conduct a research program that employs molecular genetics tools towards providing the commercial aquaculture industry with improved clam stocks. Previous work demonstrated that clam susceptibility to QPX depends upon the geographic origin of the broodstock suggesting a genetic basis for clam resistance. The central hypothesis is that clam resistance to QPX is genetically dictated and can be predicted using selected genetic markers. We will combine marker-assisted selection with traditional selection methods in conjunction with field and laboratory disease transmission studies to identify clam stocks that are resistant to QPX disease. The selection for QPX-resistant clam strains would allow the development and expansion of the hard clam aquaculture industry. Information on genetic variation in clam broodstocks as related to disease resistance and growth will be widely diffused through our robust extension and outreach plan providing direct benefits to shellfish breeders and farmers. Improved germplasm is expected to substantially reduce losses to QPX disease and enhance yields. The growth of shellfish aquaculture

can provide ecologically sustainable economic growth in coastal communities and decrease pressure on over-exposed capture fisheries. U.S. consumers will benefit from increased availability of high quality and competitively priced shellfish products.

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

Funding level: $190,360
Project start date: 1 March 2012
Project duration: 24 Months
Participants: Woods Hole Sea Grant, Roger Williams University, University of Rhode Island, Chenoweth Laboratory, Rutgers University, Maine Sea Grant, Cornell University, University of Maryland

Project Summary:
Our goal is to 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. We will also develop two additional multiplex methods for evaluation of samples for bothVp and Vv pathogenic genes using previously published methods with the oyster DNase inhibitor. We will 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 ACCB/mqutPCR method side by side in order to understand the Vv and Vp cycle and the occurrence of pathogenic Vv and Vp in the northeast environment. We will use the data to compare sensitivity of both tests. We will also identify the occurrence of pathogenic and nonpathogenic strains of *Vibrio sp*. in oysters at two time periods after collection of oysters from culturists who reside in 5 northeaster states and 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. Findings will be provided (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.

Shellfish STEM-GIS Development for Improved Siting and Farm Management

Funding level: $85,000
Project start date: 1 March 2012
Project duration: 24 months
Participants: Maine Aquaculture Innovation Center, Pemaquid Oyster Company, Inc., Blue Hill Hydraulics, Discovery Software, Ltd., Plymouth Marine Laboratory, University of Connecticut

Project Summary:
This work is focused on improving and expanding upon a shellfish GIS software developed in 2010-2011 with NRAC funding. We made great progress in developing a high resolution (50 m grid) GIS with hydrodynamics and oyster growth simulation. During focus group presentations to industry and resource managers in Maine and Connecticut in 2011, a number of recommendations were made to make it more user

friendly and address certain industry needs through added functions. The final product will be a shellfish STEM-GIS software (Shell-GIS) which can be used to improve shellfish yield and profitability for both suspension and bottom culture of the Eastern oyster *Crassostrea virginica*, taking into account both local and system-scale effects of site selection, seeding time, seeding size and density, and potential inter-annual variations in natural environmental conditions, as well as other users of the water body. Bioenergetic simulations by the ShellSIM component of ShellGIS are already calibrated for a further 12 species that are commonly cultured worldwide, such that the product will have potential benefits for other species both in the US (i.e. northern quahogs) and beyond.

Optimization of Hatchery and Culture Technology for Razor Clams

Funding level: $93,616
Project start date: 1 October 2011
Project duration: 1 year
Participants: University of Maine, Roger Williams University, Woods Hole Sea Grant, Maine Sea Grant College

Project Summary:

The overarching goal of the proposed work is to develop technology for the increased production of alternative species to support the expansion and diversification of the northeastern shellfish culture industry. We will focus on improved hatchery and grow-out methodologies for the culture of Atlantic jackknife or razor clams (*Ensis directus*). Our specific objectives seek to improve hatchery protocols to establish consistent, reliable sources of razor clam seed from commercial hatcheries, to identify improvements for existing grow-out technologies, to work directly with shellfish growers to increaseacceptance and understanding of culture of razor clams, and to further document the market receptivity to razor clams. Through the simultaneous consideration of these objectives, we anticipate faster acceptance of razor clam culture and more rapid diversification of a key aquaculture sector in the Northeast.

Developing Improved Management Practices for Mussel Farming in Southern New England

Funding Level: $199,779
Project start date: 4 October 2011
Project duration: 2 years
Participants: Marine Biological Laboratory, Martha's Vineyard Shellfish Group, University of New Hampshire, Zephyr Marine Education Foundation, American Mussel Harvesters, Inc.

Project Summary:

Approximately 93% of mussels consumed in the U.S. are imported, primarily from Canada and New Zealand. The Prince Edward Island mussel farming industry alone provides both direct and indirect employment for at least 1500 people. The development of domestic offshore mussel farms offer promising options for fishermen and shellfish farmers diversify, and to reverse the flow of US dollars out of the country, employ displaced fishermen and enrich depressed coastal economies with support service jobs. The waters of Southern New England appear to be especially well suited for the development of a vibrant domestic mussel industry. Our research on the first pilot commercial-scale farms has demonstrated the feasibility of growing mussels from seed to market size in less than 12 months, faster than other eastern North American competitors.

Several issues need to be addressed to make this regional mussel industry a reality. They include: selecting optimum materials, sites and times for collection of mussel seed, comparing methods of tunicate eradication without compromising the survival of mussel seed, comparing different types of socks and stocking densities to optimize growth and yield to market for improving management of mussel operations in SNE. Developing effective hands-on workshops and extension materials to train a work force capable of managing offshore mussel longlines. The Marine Biological Laboratory proposes to lead a 2-year project to address these constraints with comprehensive field investigations conducted by a diversified team of scientists, resource managers, fishermen, businessmen and extension agents.

Aquaculture Health Hazards – Developing Outreach Services to the Region's Farmers via Extension and Aquatic Animal Health

Funding level: $196,312
Project start date: 1 January 2011
Project duration: 2 years
Participants: University of Connecticut, University of Maine, Professor, Salem State College, University of Delaware, The Pennsylvania State University, Rutgers University, University of Massachussets, University of Maryland, Roger Williams University , Cornell Cooperative Extension, University of New Hampshire, Cape Cod Cooperative Extension & Woods Hole Sea Grant, University of Rhode Island

Project Summary:

This project intends 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; The object is 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. 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 will be conducted.
Local industry outreach programs where farmers are instructed on developing their own HACPP health risk plans will be implemented. An economic assessment of the impact of implementing a HACCP plan on individual farms in the Northeast region will be completed.

Examination of finfish pathogen physiology and predictive ecology in bivalve integrated multi-trophic aquaculture

Funding level: $200,000
Project start date: 1 February 2010
Project duration: 2 years
Participants: University of Maine, University of Connecticut

Project Summary:

Fish farmers applying IMTA at their farms need to have a clear understanding of how the culturing of filter-feeding organisms in close proximity of finfish cages will impact disease transmission at their farms. Our project addresses NRAC priority TRA-08-01: Disease research, and will beneficially impact regional aquaculture production by providing vital research answers as to the potential benefits (possible disease management strategies) and/or risks (reservoirs for disease) of co-culturing mussels

(*Mytilus edulis*) with Atlantic salmon or Atlantic cod. Results of our project will also impact priorities TRA-08-02 and TRA-08-04 by providing essential health and economic perspectives to further increase the implementation of the more economically profitable and environmentally sustainable IMTA husbandry techniques.

This project will build on our results from the 2007 NRAC project *Investigations into the potential health and economic benefits of bivalve/finfish co-culture*, which investigated the interactions of two pathogens, infectious salmon anemia virus (ISAV) and *Vibrio anguillarum* 02β, with mussels and finfish. We have made tremendous progress with the 2007 project and based on these successes, we propose to investigate the interaction of two additional pathogens, *Francisella* sp. (an intracellular bacterium) and IPNV (a non-enveloped virus) with mussels and finfish and to determine the economic cost/benefit based upon disease risk. The two pathogens are physiologically very different from ISAV, an enveloped virus, and *Vibrio anguillarum*, an extracellular bacterium. It is probable that they will have very different interactions with mussels. IPNV is endemic to our area and poses risks to aquaculture systems through decreased productivity and likelihood of imposed government regulation. Francisella is predicted to also be in Maine waters in natural cod populations and has the potential of becoming an emerging pathogen as cod culture increases.

The overall goal of this important project is to examine the effect of many different pathogen types as we do not want growers to implement IMTA believing they will be reducing their overall disease risk, while they may unknowingly and unintentionally be increasing the risk of spreading a different type of pathogen.

Assessment of environmental impacts of oyster aquaculture in New England waters

Funding level: $199,994
Project start date: 11 January 2010
Project duration: 2 years
Participants: Maine Aquaculture Innovation Center, Pemaquid Oyster Company, Inc., Blue Hill Hydraulics, Pacific Shellfish Institute, Plymouth Marine Laboratory, University of Connecticut

Project Summary:

This proposal responds to the NRAC RFP category Environmental Impacts of Aquaculture (TRA-08-02). Its focus is on shellfish (oyster) aquaculture in New England, specifically ecosystem research on the interactions of aquatic shellfish farms with phytoplankton, marine invertebrates and fish, and leading to the development of guidelines for farm-siting issues and carrying capacity. The project utilizes an aquaculture GIS format (STEM-GIS) to disseminate results that include the contrasting Maine and Connecticut sites' bathymetry, water velocities and directions, phytoplankton depletion by the shellfish, ecological information about the farms, aquaculture activities and BMP recommendations and an oyster growth module which may be used to optimize shellfish production. We have assembled a team of experts from the U.S. east coast (oyster farmers, shellfish ecophysiologists, and hydraulic engineers), the U.S. west coast (participant in two Sea Grant National Marine Aquaculture Initiatives (NMAI)), and experts from the U.K.

Breeding Resistance to Sea Lice and ISAV in Atlantic Salmon

Funding level: $199,614
Project start date: 1 August 2010
Project duration: 2 years

Participants: University of Maine, National Cold Water Marine Aquaculture Center, Stony Brook University, Cooke Aquaculture

Project Summary:

Sea Lice, in particular *Lepeophtheirus salmonis*, is an important parasite of farmed and wild salmonids, causing direct losses due to the damage caused by the parasite directly to the fish and indirect losses due to the requirement for therapeutic treatments. It has also been implemented in the spread of serious infectious aquaculture diseases such as Infectious Salmon Anemia virus, *Vibrio anguillarum* and *Aeromonas salmonicida*. However, its role in the spread of these diseases is only suspected not confirmed, and was based on the detection of these pathogens by traditional and molecular methods on parasites removed from infected fish not by studies that demonstrated reproducible transmission.

One method of reducing the impact of sea lice and the diseases they spread is the development of disease resistant strains of salmon. In European stocks (which cannot be farmed in the US because of the risk of an accidental introduction of non-native strains) breeding programs have already selected fast growing, late maturing fish with good resistance to infectious pancreatic necrosis virus, giving salmon farmers in the EU and Chile an economic advantage over US produced animals.

This project will investigate three important areas concerning the interactions of sea lice with Atlantic salmon.

Firstly it will investigate if the families that are being developed at the ARS National Cold Water Marine Aquaculture Center (NCWMAC) at Franklin, Maine have inheritable resistance to sea lice infestation. This consortium has already established that NCWMAC has families resistant to ISAV within their native American populations, and these fish families will be evaluated to see if they also posses a natural resistance to sea lice.

This will be carried out by challenging healthy and ISAV-infected Atlantic salmon (containing both ISAV resistant and susceptible families) with sea lice and observing the prevalence and intensity of the parasite in the two populations. This data will identify sea lice resistant families and confirm if the ISAV resistant trait is already carried by some of the ISAV resistant families.

During these trials we will collect material from the ISAV- and sea lice- infected group to determine if sea lice infection predisposes fish to infection with ISAV (epidemiological evidence has suggested that sea lice infestation is a major risk factor for ISAV) or, conversely, if early ISAV is a risk factor for sea lice infestation. The immune status of the ISAV and sea lice infected fish will be established by molecular and biochemical analysis of immune function.

The role sea lice play as vectors of ISAV will also be established by investigating where the pathogen is located on/in the parasite after it has fed on infected fish, how long ISAV survives both on the surface of the parasite and in its digestive system as well as establishing if sea lice are a major vector transmitting the disease between wild and farmed fish and in/between farms.

Novel methodologies to overwinter cultured hard clams in the Northeast U.S.

Funding level: $200,402
Project start date: 11 January 2010
Project duration: 2 years
Participants: Rutgers University, University of Maine, Haskins Shellfish Research Laboratory, Baruch College

Project Summary:

This project will examine experimentally new overwintering technologies for cultured hard clam juveniles in ME, NY, and NJ. The new methodology is based on 12 years of successful overwintering of cultured juveniles of *Mya arenaria* in Maine. An initial overwintering trial with hard clam seed (2.5-11.5 mm SL) during the winter of 2006-2007 at the Downeast Institute (DEI), Beals, Maine resulted in > 99% survival over 177-days. Subsequent monitoring of seed in protected field plots in eastern Maine indicated >80% survival for four months. Similar results have been found during the winters of 2007-2008 and 2008-2009, thereby substantiating these preliminary results and warranting large-regional tests of this methodology. We will examine two experimental field trials from Nov 2009 to April 2010, and Nov 2010 to April 2011 in the three states to examine spatial and temporal variation in the new overwintering technique. Commercial quantities of local hard clam seed (3 sizes: 4-5.9 mm; 6-7.9 mm; 8-9.9 mm) will be overwintered in each state for a 5-month period. To determine if success is related to seed source, we will conduct a reciprocal study by taking seed originating/reared in each state, and overwintering seed in the other states. In each state, we will compare survival of overwintered seed using the new technique to survival of seed overwintered in protected field plots, as is the current, standard, practice. In addition, we will follow the fate of local seed that survive the new overwintering methods in protected field plots in each state for six months. Biochemical assays will be conducted on clams from all size classes and origins at each field site overwintered using the new methodology to measure energy use through the overwintering period and to determine if the ME genetic strain is better adapted to temperature stress by using less energy stores. Simultaneously measuring biochemical composition and environmental parameters should also provide an understanding of how the various clam strains respond physiologically to local conditions and culture methods.

Assessment of grow-out strategies for the green sea urchin

Funding level: $156,933
Project start date: 1 July 2009
Project duration: 3 years
Participants: University of Maine, University of New Hampshire

Project Summary:

We will identify the most cost-effective and efficient nursery strategy for raising hatchery-produced urchins from 5mm to 15mm, suitable for release onto sea bottom lease sites. We will also examine and refine the methodology and economic viability of tank-based urchin aquaculture, and compare success with that of “ranching” urchins on the sea bottom. After 3 years, we expect to have enough baseline information to conduct an economic analysis, which will be done by Lisa Bragg of the Dept. of Economics at the UMO. Recommendations and information will then be made available to stakeholders in the urchin industry via web-based forums, public meetings, and printed pamphlets.

Selection for enhanced disease resistance and growth performance in cross-bred oysters, Crassostrea *virginica*

Funding level: $232,416
Project start date: 1 July 2009
Project duration: 2 years
Participants: University of Maine, Marine Biological Laboratory, Rutgers University, State of Connecticut

Project Summary:

Our collaborative broodstock development program will provide commercial and public oyster hatcheries with high performance, disease resistant broodstock so that growers in the northeast have access to oyster seed with enhance survival and growth that, in turn, leads to increased oyster production throughout the region. In previous work, we have found evidence for hybrid vigor with respect to both growth and disease resistance when two stocks of oysters, the University of Maine's UMFS stock and Rutgers Universitye's NEH stock are cross-bred. We will test whether hybrid vigor is also realized when the UMFS stock is crossed with the recently developed and putatively disease-resistant Clinton stock. We will also determine whether selection on UMFS-NEH cross-bred and UMFS x NEH hybrid "selected." Stocks will be compared to pure line (UMFS, NEH< Clinton) controls in a replicated field trial conducted a 7 grow-out sites in ME, MA, RI, CT, and NJ. The growth and survival, whole wet weight, meat weight and yield will be estimated for each replicate. Oysters in each stock at each site will be monitored for the prevalence and intensity of the diseases MSX, SSO, Dermo and ROD using QYCR and histology-based methods. Lastly, we will develop a set of guidelines for ensuring the timely distribution of improved lines from our program to the industry.

The Infection Cycle of VHS Virus

Funding level: $199,263
Project start date: 1 July 2008
Project duration: 2 years
Participants: Cornell University, New York Sea Grant Program, Lake Champlain Sea Grant Program, Pennsylvania Sea Grant Program

Project Summary:

We have observed that important fish species vary in overt susceptibility to VHSV IVb. We will conduct controlled laboratory-based infectivity studies with VHSV IVb to compare what we hypothesize to be fish that may differ significantly in their ability to be infected with the virus and development of disease. A better understanding of species susceptibility to VHSV IVb will support the development of effective strategies to limit disease losses as well as limit risk of exposure to carrier fish.

We have developed a quantitative RT-PCR (qRT-PCR) in our laboratory and have used that diagnostic and research tool to identify the presence of VHSV IVb in diagnostic and surveillance efforts over the past two years. During this project, we will conduct the specific efforts to develop the body of information that will support a request to the appropriate organizations for recognition of the qRT-PCR as a validated test.

We will conduct technology-transfer workshops for the aquaculture community to provide the most contemporary information regarding the emergence of VHSV IVb in the Northeastern United States. Presentations will be made to members of the commercial aquaculture community at two different locations to facilitate attendance.

NRAC Extension Project

Funding level: $299,944
Project start date: 1 July 2008
Project duration: 2 years
Participants: University of Connecticut, University of Maryland, University of Delaware, MIT Sea Grant, Cornell University, West Virginia University, University of Rhode

Island, Roger Williams University, Pennsylvania Sea Grant, Salem State College, University of New Hampshire

Project Summary:

The Northeast Aquaculture Extension Network (NAEN) is an assemblage of extension professionals from Land and Sea Grant Institutions, state and private universities, and outreach associations throughout the Northeast region of the U.S. The Network has an extensive working history with the U.S. Department of Agriculture Cooperative State Research Education and Extension Service (USDA CSREES) Northeastern Regional Aquaculture Center (NRAC), and has just recently revitalized the Network with a new work plan and new members from Maine to West Virginia.

The goal of the project put forth by the Northeast Aquaculture Extension Network (NAEN) is to produce and deliver accurate and credible science-based aquaculture information, educational materials and outreach activities to stakeholders in a manner that is efficient and effective. The primary audience we serve is practicing and new aquaculturists as well as prospective producers, wholesalers and retailers, state and regional industry associations, resource managers, elected and appointed officials, and extension professionals who work in areas related to aquatic animal and plant production.

Our vision is that the information and products developed by the NAEN will result in improved stakeholder knowledge and increased public awareness of the social, economic and environmental importance of commercial aquaculture in the Northeast U.S. and will facilitate NRAC's goal to increase both the value and volume of commercial freshwater and marine aquaculture products.

Creation of a Tetraploid Broodstock for the Bay Scallop Argopecten *irradians*

Funding level: $128,197
Project start date: 1 July 2008
Project duration: 3 years
Participants: Martha's Vineyard Shellfish Group, Inc., Rutgers University

Project Summary:

The first step of the project includes the production of a starter triploid bay scallop population through the use of chemicals. This first step has already been achieved through funding through MSAIC (Massachusetts Shellfish Aquaculture Innovation Consortium). The second step of the program, for which NRAC funds are requested, involves selecting triploid broodstock scallops by testing the ploidy of all ripe animals found in the starter population. Once a ripe triploid broodstock is gathered, they will be spawned and their fertilized eggs treated with chemicals to induce tetraploidy. If successful, the tetraploid scallops will be grown at the Martha's Vineyard sites. Before they are sexually mature, they will be transferred to a recirculating quarantine facility at the Rutgers University Haskins laboratory in New Jersey. The third step of the project will be crossing the first tetraploid broodstock for the bay scallop naturally with wild diploid scallops to produce marketable triploids. This all triploid natural population will be grown on Martha's Vineyard and monitored for performance (growth, yield, survival glycogen muscle content, rate of reversion). The tetraploids will also be self-crossed to determine if a population of tetraploid scallops can be sustained.

Targeted Biosecurity Education and BMP Development Program for Aquaculturists, Extension Agents, researchers and Regulators

Funding level: $89,920

Project start date: 1 July 2008
Project duration: 2 years
Participants: Microtechnologies, New York Department of Conservation, Roger Williams University, University of West Virginia

Project Summary:

This project will provide regionally focused, practical training in aquaculture biosecurity and Best Management Practices (BMPs) for industry, extension agents, researchers and regulators in the Northeast in order to reduce disease and lower production costs. This goal will be achieved through the following activities and materials: 1. Five two-day interactive biosecurity/BMP training workshops conducted in Maine, Rhode Island, West Virginia, New York and Pennsylvania; 2. Production of a Biosecurity/BMP Resource Manual, adaptable to specific species and/or facility design, and distributed at workshops and beyond (most likely via Internet); 3. Creation and maintenance of a public access web-based database with information resources relevant to aquaculture stakeholders, including current disease research, fish health resources, state/federal/international testing requirements and other regulatory information, as well as updates to biosecurity/BMP recommendations.

Investigation into the potential health and economic benefits of bivalve/finfish co-culture

Funding level: $150,000
Project start date: 1 June 2008
Project duration: 2 years
Participants: University of Maine, Rutgers University

Project Summary:

Project will result in direct answers to industry's concern about the role that mussels may play in perpetuating or limiting the spread of diseases. Project design will focus on mussels (*Mytilus edulis*) as a bivalve model for investigations into the associated aquatic animal health benefits or risks of integrated shellfish/finfish aquaculture. Based on the fact that bivalves are bio-acccumulators, our hypothesis is that they have the potential to act as sensitive pathogen bio-monitors or disease sinks. Under these circumstances co-cultured mussels may serve as a biological filter by reducing or eliminating pathogen loads while enhancing the environmental sustainability and economic feasibility of marine finfish aquaculture. Conversely, if mussels possibly act as a vector for certain pathogens, the gained knowledge can be used by growers as a risk management tool. Our project will expand on earlier work by targeting two significant finfish pathogens of Atlantic salmon (*Salmo salar*), an established cultured marine species in the northeastern United States.