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

PROJECT CODE: 09-02 **SUBCONTRACT/ACCOUNT NO:**

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: Indicate objective(s) completed, funds terminated, or other specific reason for project termination.

Objectives completed

PROJECT OBJECTIVES: List objectives as written in approved proposal.

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: State how the project will benefit the aquaculture industry either directly or indirectly.

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: 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

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 ($t = -2.61$; $P = 0.0157$).

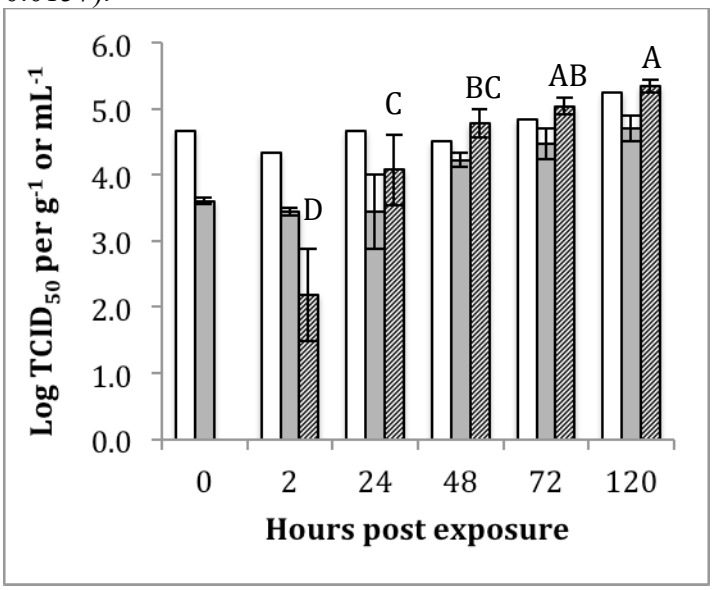

Figure 1. Log $TCID_{50}$ of IPNV per ml of water in tanks containing mussels (grey) or lacking mussels (white) or per gram of mussel digestive gland tissue (hatched) over time. Graphs represent the average log $TCID_{50}$ g^{-1} tissue values ± standard error

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).

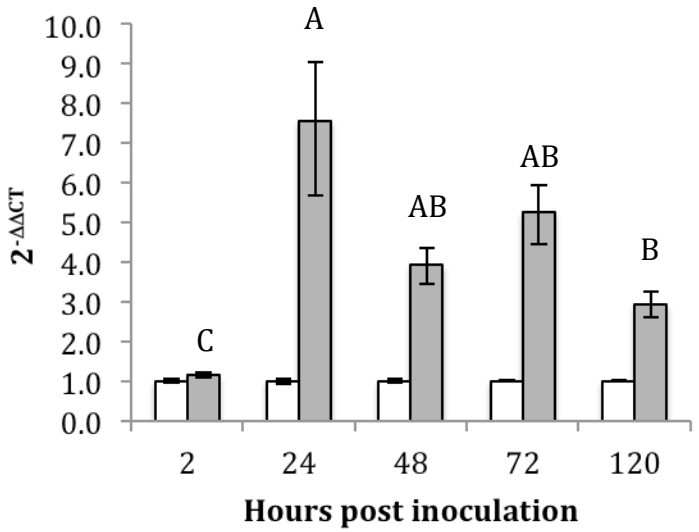

Figure 2. The average relative abundance of IPNV VP2 RNA in mussel digestive glands at 2-, 24-, 48-, 72- and 120 h after inoculation with MEM (white bar) or after inoculation with IPNV as measured with Taqman quantitative RT-PCR in trial 1. Graphs represent average values ± standard error of the mean 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 $TCID_{50}$ 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 $TCID_{50}$ g^{-1} feces occurred at 5 dpd.

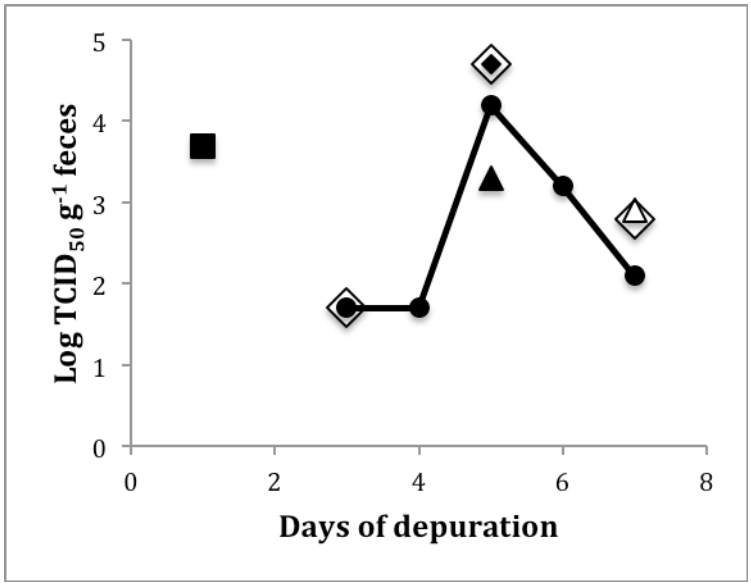

Figure 3. Log $TCID_{50}$ of IPNV per g of mussel feces over time. Feces was collected from 8 replicate mussels for 7 d. Mussel replicate 2 (filled square); replicate 3 (filled diamond); replicate 4 (open triangle); replicate 5 (open diamond); replicate 6 (filled circle); and replicate 8 (filled triangle). 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 hemocyctes 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).

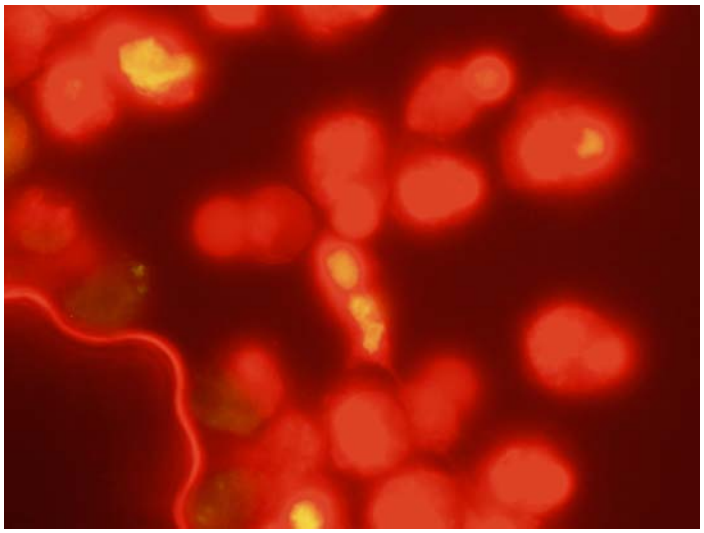

Figure 4. Flourescent microscopy of mussel hemocytes 2 h after treatment with FITC-labeled *Francisella* (1,000x). Internalized *Francisella* cells appear yellowish-green within ethidium bromide stained hemocytes (red).

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 ± 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

	Replicate					
Treatment	1	2	3	4	5	6
IP Injected	12/12	12/12	-	-	-	-
Salmon Cohabitants	1/12	1/12				
IPNV+ Mussel Cohabitants	1/24	2/24	0/24	1/24	1/24	1/24

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.

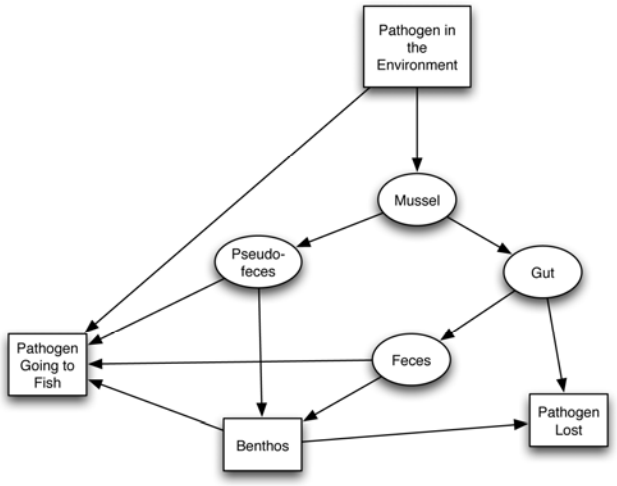

Figure 4. Conceptual model of disease dynamics on an IMTA farm without the concept of pathogen recycling added into it.

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

- 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.

RECOMMENDED FOLLOW-UP ACTIVITIES: State concisely how future studies may be structured.

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.

YEAR	NRAC-USDA FUNDING	OTHER SUPPORT					TOTAL SUPPORT
		UNIVER-SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
TOTAL							

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED: List under an appendix with the following subheadings: *Publications in Print*; *Manuscripts*; and *Papers Presented*. For the first two subheadings, include journal articles, popular articles, extension materials, DVDs, technical reports, theses and dissertations, etc. using the format of the Transactions of the American Fisheries Society (example below). Under *Papers Presented* subheading include the authors, title, conference/workshop, location, and date(s).

Example of Transactions of the American Fisheries Society citation format:Billington, N., R. J. Barrette, and P. D. N. Hebert. 1992. Management implications of mitochondrial DNA variation in walleye stocks. North American Journal of Fisheries Management 12:276-284.

Publications in Print

Manuscripts

Molloy, S.D., M.R. Pietrak, D.A. Bouchard, and I. Bricknell. Transmission of infectious pancreatic necrosis virus in an integrated Atlantic salmon (*Salmo salar*)/blue mussel (*Mytilus edulis*) system. Submitted to Applied and Environmental Microbiology.

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. In preparation.

Dissertations

Pietrak, Michael. 2013. Investigations into the ecology and interactions of pathogens within an Integrated Multi-Trophic Aquaculture Farm. University of Maine.

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
Northeast Agricultural and Resource Economics Association June 23-25, 2013, Ithaca, NY

Attachment 9
Project Completion Report
Regional Extension Project
Subaward #_____
Grant

PART II

TECHNICAL ANALYSIS AND SUMMARY: Describe the work undertaken and results obtained for each objective. Major results should be presented in detail, including graphs, charts, figures, photomicrographs or other presentations. Methodology should be briefly described and statistical analyses and significance should be included where appropriate. This section of the report should be written with style similar to scientific publication. Reports previously or currently prepared for publication may be submitted as part of this section.

PROJECT COMPLETION REPORT

SIGNATURE PAGE

PROJECT CODE: **SUBCONTRACT NO:**

PROJECT TITLE:

PREPARED BY:

Project Coordinator of Subawardee Date