PEI Atlantic Shrimp Corp. Inc.

Lobster Science Centre Projects Summary 2000 - 2017

The following projects from the Lobster Science Centre at the Atlantic Veterinary College of the University of Prince Edward Island have been funded by the PEI Atlantic Shrimp Corp Inc.:

1. APHIN Lobster
2. Molecular Genetics: The Population Structure of *Homaris americanus* in the Region of PEI
3. Impact of V-Notching on Ovigerous Lobsters
4. *Paramoebiasis* in Lobsters and Other Invertebrates
5. Lobster Health Research Data on the Internet
6. Factors which allow Lobsters to be affected by *Paramoebiasis*
7. Detecting Antimicrobial Peptides in Lobster Blood
8. Using Proteins for Lobster Vaccines and Drugs
9. Molecular Genetics of “Bumper Car” Disease - Phase I - Genomics
10. Acute Phase Proteins for Assessment of Lobster Health
11. Development of an Internet-based Data Delivery System for the PEI Lobster Index Fishery
12. Developing a Biochemical Profile for the American Lobster
13. 2nd Annual Lobster Science Workshop
14. Identifying Antimicrobial Peptides in Lobster Hemocytes
15. Molecular Diagnostics for the Detection of *Neoparamoeba pemaquidensis*
16. Gaffekemia Proteomics: Targeting the Membrane-associated proteins of *Aerococcus viridans*
17. In vitro Efficacy Screening of Amoebocidal Compounds Against *Neoparamoeba pemaquidensis*
18. Preliminary Host Range, Transmission, and Pathogenesis of *Neoparamoeba pemaquidensis*
19. Implementation of a Six Month Interim Management Plan for the AVC Lobster Science Centre
20. 3rd Annual Lobster Science Workshop
21. Overview of Shell Disease - Review and Description of Research Needs for the Canadian Atlantic Lobster Fishery
22. Measuring Acute Phase Proteins in Lobsters -
23. Gaffkemia Proteomics - 2: Lobster serum interactions with *Aerococcus viridans* capsule protein expression -
24. ALMQ Field Monitoring
25. ALMQ Gene Discovery Library
26. ALMQ Biochemistry Profile
27. ALMQ Service Unit
28. Bumper Car Disease - Phase II Molecular Pathogenesis
29. Shell Disease in Lobsters - Awareness Raising and Passive Monitoring in Atlantic Canada
30. Hemolymph Biochemistry Profiles in American Lobsters: Establishing Reference Intervals and Determining Their Potential Role in the Assessment of Nutritional Status
31. Online Lobster Inventory System Phase 2.2: Processing Sector
32. Fishing for Biomarkers with a Lobster DNA Microarray: Assessing Moult cycle, Reproductive status and Host-pathogen interactions
33. Development of Non-lethal Tests for the Determination of Ovary Maturity in American Lobsters
34. Fishing for Biomarkers with a Lobster DNA Microarray - Assessing Moult Cycle, Reproductive Status and Host-pathogen Interactions (Phase II)
35. Assessing Environmental Contaminant Impacts on the Health of Lobsters
36. Refinement and Expansion of Biomarkers of Lobster Health and Quality
37. Assessing Environmental Contaminant Impacts on the Health of Lobsters Phase II

For more information on any of these projects, please contact:

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Updated September 12, 2017

1. APHIN Lobster

Proponent: Lobster Science Centre

Project Number: 01-LSC-01

Project Status: Complete

Project Start/Completion Date: January - December 2001

Project Objective:
LobsterNET, an Internet-based information network for the lobster industry in Atlantic Canada, will be an innovative system to provide an accurate and up-to-date lobster health and productivity database, and will facilitate other computer-related research projects and investigations in crustacean science. The first step (Phase I) in constructing such a system, which is the focus of this project, is to obtain the industry's needs, requirements and limitations for providing the foundation elements of a health and production database.

Summary of Outcome:
Meetings with representatives of the processing industry were conducted and datasets of purchase data are now being received from a small number of processors to commence construction of the LobsterNET databases. This project is continuing to progress in project 03-LSC-05.

Total Project Cost: $71,940.00
Funding provided by PEIASCI: $54,340.00
Other Funding Partners: Atlantic Veterinary College at UPEI

2. Molecular Genetics: The Population Structure of *Homarus americanus* in the Region of PEI

Proponent: Lobster Science Centre
Project Number: 01-LSC-02
Project Status: Complete
Project Start/Completion Date: May 2001 - December 2002

Project Objective:
This project is designed to compare genetic markers among samples taken from resident adult populations, resident juveniles, and drifting larval samples taken in New Brunswick and Prince Edward Island. These genetic markers have been shown to reliably reveal variation in this species.

Summary of Outcome:
As of December 2002: Variation within one of these candidate DNA-markers appears to be largely due to intron sequence hypervariability within the lobster cytoplasmic gelsolin gene. We are in the process of confirming that the two candidate DNA markers will fit criteria necessary to act as genetic markers for preliminary population analysis of lobster fishing areas in the waters off Prince Edward Island.

Total Project Cost: Year 1 - $115,068.00; Year 2 - $46,230.00

Funding provided by PEIASCI: Year 1 - $44,568.00; Year 2 - $25,730.00

Other Funding Partners: AFRI; UPEI

3. Impact of V-Notching on Ovigerous Lobsters

Proponent: Lobster Science Centre
Project Number: 01-LSC-03
Project Status: Complete
Project Start/Completion Date: September 2001 - April 2002

Project Objective:
Does this procedure place the lobster at an increased risk of infection by pathogens, and if so, is this risk influenced by the water temperature? This project is designed to determine:

1. Whether breaching the exoskeleton with a V-notch, places the ovigerous lobster at an increased risk of infection by obligate or opportunistic pathogens;
2. The length of time required to re-establish an effective exoskeletal barrier to infection; and,
3. The effect of water temperature on the time required to re-establish an effective exoskeletal barrier to infection.

The experimental model to be used in this investigation will be a bath exposure of V-notched and un-notched ovigerous lobsters to a suspension of Aerococcus viridans, the causative agent of gaffkemia.

Summary of Outcome:

Two field studies of the effect of V-notching ovigerous lobsters have been by completed by AVC Inc., and AVCLSC at two different sites in LFA 25 during the fall lobster fishing seasons of 1999 and 2000, funded by DFO, NBDFA and PEI AFE, and by DFO, respectively. No adverse effect on short term health or survival of the V-notched ovigerous lobsters was detected in either study. The study in the fall of 2000 included a component to assess the long term survival of V-notched lobsters but the collection of the data for the first year is not complete.

Total Project Cost: $64,730.00
Funding provided by PEIASCI: $64,730.00

4. Paramoebiasis in Lobsters and Other Invertebrates

Proponent: Lobster Science Centre
Project Number: 01-LSC-04
Project Status: Phase I complete
Project Start/Completion Date: September 2001 - April 2002

Project Objective:

This project represents the first step in a multi-stage, multi-year and multi-institutional activity: Phase I - Isolation, culture and laboratory transmission of Paramoeba sp. from invertebrates (lobsters, sea urchins, blue crabs) to lobsters; Phase II - Molecular identification of species of Paramoeba; experimental reproduction of paramoebiasis in lobsters; determination of pathogenesis and host range of Paramoeba sp.; development of diagnostic tools for surveillance; Phase III - Development of treatments, control and prevention strategies; Phase IV - Determination of public health, environmental and economic impacts of paramoebiasis on lobster fishery. Completion of Phase I is essential to the conduct of Phases II, III and IV. Overall the Paramoebiasis project is a joint undertaking between the AVC Lobster Science Centre, University of Prince Edward, and the Department of Pathobiology and Veterinary Science, University of Connecticut.

Summary of Outcome:
"Paramoebiasis in Lobsters and Other Invertebrates" investigates the parasitic amoeba Paramoeba sp. which was implicated in the lobster ‘die-off' observed in Long Island Sound in 1999-2000. The study will examine the relationships among Paramoeba infections in lobsters, blue crabs and sea urchins. This study will help confirm the identity of the parasite, its method of transmission, pathogenesis, diagnostic methods for identification, and methods of treatment, control and prevention. Phase I of Paramoebiasis in Lobsters and Other Invertebrates will attempt to isolate and culture Paramoeba sp., providing a readily accessible, consistent supply of parasites for subsequent studies. Transmission studies will be conducted to ensure the infectivity and pathogenesis of the amoeba after isolation in culture. This project continues as project 03-LSC-06.

Total Project Cost: $61,375.00 (Phase I)

Funding provided by PEIASCI: $61,375.00 (Phase I)

5. Lobster Health Research Data on the Internet

Proponent: Lobster Science Centre
Project Number: 03-LSC-05
Project Status: Complete
Project Start/Completion Date: October 2002 - September 2004

Project Objective:
The project involves design, development and implementation of a lobster health research database to form the foundation for applied research within the AVCLSC at the University of Prince Edward Island. The project will require a series of on-site meetings between plant management & staff and the AVCLSC development team. The project will involve the acquisition of hardware (computer servers, network connections, & related items) and the development of specialized software programs to access the research database(s).

Summary of Outcome:
Over the last two years, the LobsterNET activity has proven to be a successful project for the AVC Lobster Science Centre; significant progress has been made in laying the foundation for applied research via a series of online databases. With the project objectives in mind, the work was divided into two Phases, Construction and Delivery. Several research avenues with LobsterNET were pursued in both Phases thru the Central Lobster Access Website (CLAW). To host the online datasets and the applications developed for Lobster^{NET}, we purchased a server computer equipped with an Intel Xeon 3.06GHz processor and 2GB RAM. Our server is housed in Computer Services at UPEI and currently hosts the AVC Lobster Science Centre website and the CLAW sites & applications.

We have developed a number of applications that fall under the CLAW umbrella (www.lobsterscience.ca/CLAW). Sites available to the public include Purchase Data & Health Data. The purchase data site allows users to graph "lbs purchased" and "% shrink" for a selected region and selected period of time. This data is submitted/collected from companies around the Maritime provinces. We have designed and developed applications to summarize these data and to give a picture of the industry that is available on the website. No individual information is given on the website. The Health Data site allows users to graph health related parameters for selected region and period. These data were collected by a summer student
working with the LobsterNET project.

Currently in development are two systems: Storage Capacity and DFO Landings & Water Temperature. The Storage Capacity system provides information on the storage capacity by county in Nova Scotia, broken down by leased/owned and type of holding facility. Work has begun on the DFO Landings & Water Temperature site and will allow users to view lobster landing statistics for Canada from 1968 to 2002 (yearly landings) and 1984 to 2001 (daily landings) and water temperature (1996-2002) at the LFA level for the Southern Gulf Region. The data for this system has been received from DFO for a time period 1984-2001. We now have a sample application that will be the foundation to included data from the other DFO Regions; Scotia/Fundy, Québec and Newfoundland. We intend to use the Storage Capacity map-page as a template for the DFO landings/bottom water temperature dataset.

Finally, we have been involved in a research proposal was prepared by DFO scientists to address the lower lobster quality that has been seen in south-west Nova Scotia two out of the last three years. The AVCLSC will play a small but important role in this project. The data generated from this project could be incorporated into one of LobsterNET health databases.

Total Project Cost: $446,254.00 - Year 1; $271,817.00 - Year 2

Funding provided by PEIASCI: $75,281.00 - Year 1; $ 33,189.00 - Year 2

Other Funding Partners: **ACOA**- Atlantic Innovation Fund; **Federal**- Fisheries & Oceans Canada; **In Kind**- UPEI Administrative Support, AVC Faculty Consultations, Director & Senior Scientist; **Non-Commercial** - Province of British Columbia; Eskasoni First Nation; Province of Newfoundland Labrador; Millbrook First Nation; Province of New Brunswick; Province of Nova Scotia; Province of Prince Edward Island; Unama'ki Institute of Natural Resources; **Private**- Acadian Fishermen's Co-op; Canadian Gold Seafood; Clearwater Fine Food; Diagnostic Chemicals Ltd; Eastern Fishermen's Federation; ELANCO; Madelimer; Maritime Fishermen's Union; Maritime Lobster Processors Co-operative; Orion Seafood International; Paturel International; PEI Atlantic Shrimp Corp; PEI Fishermen's Association; Polar Foods; Royal Star; Sable Offshore; Seafood 2000 Ltd

6. Factors which allow Lobsters to be affected by Paramoebiasis

Proponent: Lobster Science Centre

Project Number: 03-LSC-06
Project Status: Complete
Project Start/Completion Date: October 2002 - December 2004

Project Objective:
Following the fall 1999 'die-off' in the Long Island Sound lobster fishery, one of the potential contributing factors was identified as the amoebic parasite Paramoeba sp. Species of Paramoeba have been implicated in grey crab disease of blue crabs in the United States, amoebiasis of sea urchins in Atlantic Canada, and amoebic gill disease in Atlantic salmon in western North America & Australia. Unfortunately, little is known regarding the routes of transmission and effects of Paramoeba sp. on lobsters. The main objectives are: - to address the various factors which allow the parasite (Paramoeba sp) to infect lobsters; - to characterize the progression of the infection & disease; and, - to develop diagnostic methods for detection.

Summary of Outcome:
Our focus to date has been to develop a model of paramoebiasis based on Amoebic Gill Disease in Atlantic salmon, which will facilitate examination of the disease in American lobsters. The Paramoebiasis activity is providing graduate training opportunities for two recent veterinary graduates from France. The project of Dr. N. Donay is entitled "Host range and transmission studies of *Neoparamoeba pemaquidensis*, causative agent of paramoebiasis in finfish and invertebrates; *in vitro* efficacy screening of amoebocidal compounds against *Neoparamoeba pemaquidensis.*' Dr. Donay is supervised by Dr. R. Cawthorn. The project of Dr. C. Caraguel is entitled "Molecular characterization of *Neoparamoeba pemaquidensis* and development of molecular diagnositic tools." Dr. Caraguel is co-supervised by Drs. Cawthorn and Greenwood.
There are now six strains of *N. pemaquidensis* established *in vitro*, in liquid and on solid media at the AVC Lobster Science Centre. This has facilitated evaluation and validation of a tetrazolium-based cytotoxicity assay which will be used to test various prophylactic, therapeutic and disinfecting compounds against *N. pemaquidensis.* The molecular characterization of various isolates of *N. pemaquidensis* is well underway. This research is international in scope, with colleagues from the private sector, government agencies and research institutions from Australia, the United States and Canada. Through some of this interaction, new hypotheses on shell disease, which has both impoundment and epidemic forms, are being evaluated. *Neoparamoeba pemaquidensis* may be an important contributing factor in development of shell disease.

Total Project Cost: $473,797.00 - Year 1; $440,656.00 - Year 2

Funding provided by PEIASCI: $79,927.00 - Year 1; $53,804.00 - Year 2

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Other Funding Partners: **ACOA**- Atlantic Innovation Fund; **Federal**- Fisheries & Oceans Canada; **In Kind**- UPEI Administrative Support, AVC Faculty Consultations, Director & Senior Scientist; **Non-Commercial** - Province of British Columbia; Eskasoni First Nation; Province of Newfoundland

Labrador; Millbrook First Nation; Province of New Brunswick; Province of Nova Scotia; Province of Prince Edward Island; Unama'ki Institute of Natural Resources; **Private**- Acadian Fishermen's Co-op; Canadian Gold Seafood; Clearwater Fine Food; Diagnostic Chemicals Ltd; Eastern Fishermen's Federation; ELANCO; Madelimer; Maritime Fishermen's Union; Maritime Lobster Processors Co-operative; Orion Seafood International; Paturel International; PEI Atlantic Shrimp Corp; PEI Fishermen's Association; Polar Foods; Royal Star; Sable Offshore; Seafood 2000 Ltd

7. Detecting Antimicrobial Peptides in Lobster Blood

Proponent: Lobster Science Centre

Project Number: 03-LSC-07
Project Status: Complete
Project Start/Completion Date: October 2002 - September 2004

Project Objective:
Antimicrobial peptides have already been identified in crabs and shrimp, and are expected to be present in lobsters. Once identified, their range of antimicrobial activity could be defined. Specific peptides could be selected that would be effective against known disease-causing agents (called pathogens) in lobsters. These include: the bacterium (called Aerococcus viridans) which causes gaffkemia; the parasite (a ciliated protozoan called Anophyroides haemophila) which causes bumper car disease; and a suspected pathogen (called Paramoeba sp.) which was found in some of the weak & dead lobsters in Long Island Sound. Ultimately, it would be desirable to be able to enhance the endogenous production of these peptides by the lobsters themselves, possibly by administration of an immune system stimulant (called an immunostimulant), e.g. parts of bacterial cell walls or possibly killed bacteria, similar to the way vaccines are used in animals & people. The lobsters would then have more protection when they enter high risk environments such as lobster pounds. Alternatively, the technology may become available whereby these peptides could be manufactured commercially and used the way antibiotics are today in veterinary or human medicine. The initial stages of this project will involve confirming the presence of these peptides in lobster blood (called haemolymph) and white blood cells (or haemocytes) and then isolating and characterising the peptides using biochemical techniques.

Summary of Outcome:
Our studies to date have focussed on isolation of antimicrobial peptides (APs) from lobster hemocytes. Hemocytes have proven to be a rich source of APs in other crustaceans and are expected to be present in lobster hemocytes as well. Antimicrobial activity has been verified in the hemocyte preparations at the AVCLSC. It is likely that this activity is due to one, or more, APs. Development of the isolation protocols requires a high degree of technical expertise and specialised equipment. For this reason, an initial feasibility study to identify putative peptides was subcontracted to the Institute of Marine Biosciences (IMB), Halifax under the direction of Dr. Sue Douglas and her research group in the spring of 2004. Dr. Douglas' group was successful in identifying three proteins that could be APs in the hemocyte preparations. In addition, partial amino acid sequence data was obtained which will help to compare the composition of the lobster AP(s) with APs in other species. Currently, we have entered into another collaborative agreement with IMB to further pursue the peptide isolation work. Under the supervision of Dr. Aleksander Patrzykat, the initial isolation steps will be carried out at the AVCLSC with the final purification steps occurring at IMB.

Total Project Cost: $217,762.00 - Year 1
$232,216.00 - Year 2

Funding provided by PEIASCI: $36,735.00 - Year 1
$28,353.00 - Year 1

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Other Funding Partners: **ACOA**- Atlantic Innovation Fund; **Federal**- Fisheries & Oceans Canada; **In Kind**- UPEI Administrative Support, AVC Faculty Consultations, Director & Senior Scientist; **Non-Commercial** - Province of British Columbia; Eskasoni First Nation; Province of Newfoundland

Labrador; Millbrook First Nation; Province of New Brunswick; Province of Nova Scotia; Province of Prince Edward Island; Unama'ki Institute of Natural Resources; **Private**- Acadian Fishermen's Co-op; Canadian Gold Seafood; Clearwater Fine Food; Diagnostic Chemicals Ltd; Eastern Fishermen's Federation; ELANCO; Madelimer; Maritime Fishermen's Union; Maritime Lobster Processors Co-operative; Orion Seafood International; Paturel International; PEI Atlantic Shrimp Corp; PEI Fishermen's Association; Polar Foods; Royal Star; Sable Offshore; Seafood 2000 Ltd

8. Using Proteins for Lobster Vaccines and Drugs

Proponent: Lobster Science Centre
Project Number: 03-LSC-08

Project Status: Complete
Project Start/Completion Date: October 2002 - November 2004

Project Objective:
Proteomics will be used to identify and characterize the specific proteins of the bacterial pathogen (Aerococcus viridans var. homari) that causes Gaffkemia in lobster. Gaffkemia is one of the three most important infectious diseases of lobsters and causes significant economic losses in the live lobster industry in both North America. However, almost nothing is known on how the Aerococcus bacterium actually causes disease in lobster. To better understand the disease process, a comparison of the proteins expressed by Aerococcus while growing both in culture and while causing disease in lobsters is needed as baseline information. This baseline comparison will allow for the identification of proteins expressed by the bacterium as it causes disease in the lobster. The goal of this project is to identify and characterize specific proteins from Aerococcus that will serve as candidates for targeting drug design or vaccines in lobsters.

Summary of Outcome:

Task 1 - Establish experimental design and protocols. A primary consideration for all future work over the next four years was that we have fully characterized *Aerococcus viridans* isolates as our starting material. However, it was quickly realized that this essential background work was not readily available in the published literature. Therefore, since this foundation work was deemed essential to the completion of the project we allotted a portion of the experimental design time to characterizing (phenotypically and genotypically) a bank of seven known *Aerococcus* strains from lobster, one known environmental strain and also two strains isolated from lobsters but not suspected of causing disease in lobsters. The collaboration of Dr. Jim Stewart, Scientist Emeritus, Department of Fisheries and Oceans was invaluable as we were able to access *Aerococcus* isolates with “known” histories. Jim was also very gracious in providing to us information (that was at the time unpublished) that was integral to our research focus and experimental design. This work resulted in the scientific manuscript: Greenwood SJ, Keith IR, Després BM, Cawthorn RJ. (2005) Genetic characterization of *Aerococcus viridans* (var.) *homari*, the causative agent of gaffkemia in lobsters, by 16S rRNA sequencing and randomly amplified polymorphic DNA (RAPD). This manuscript was accepted in the journal, Diseases of Aquatic Organisms on October 21, 2004 and will be published in 2005.

All equipment for protein analysis and associated computer software was sourced and acquired. Task 1 has been completed (experimental design and protocol selection).

Task 2 - Completion of design and protocols. Preparation of *Aerococcus viridans* isolates for protein isolation is now complete. Initial total protein isolation from *Aerococcus viridans* were assessed by polyacrylamide gel electrophoresis (PAGE) in conjunction with a preliminary multi-locus enzyme analysis assay by Beatrice MacDonald and Dr. Ian Keith. Subsequently, *Aerococcus viridans* isolates have been prepared *in vitro* to induce avirulence (therefore they cannot cause disease in lobsters). We will test the effectiveness of this procedure by attempting to infect lobsters. The infection confirmation will be completed in the new year. We will also begin full characterization of cell sub-fractions and the entire proteome of *Aerococcus viridans* in the new year once the new technician joins the AVCLSC team in January.

We have only just started this component as my time and the technical time (Beatrice MacDonald) was partitioned to the Paramoebiasis project to re-design and test molecular tools for the characterization of *Neoparamoeba pemaquidensis*. This component of the Paramoebiasis project has now become the major focus of the graduate program of Dr. Charles Caraguel, who joined the AVCLSC in May 2004. His program is well underway and I will be able to dedicate more time towards the completion of Tasks 3 & 4. This will be expedited by the arrival in January 2005 of the new technician.

Total Project Cost: $178,169.00 - Year 1; $283,820.00 - Year 2

Funding provided by PEIASCI: $30,056.00 - Year 1; $34,654.00 - Year 2

Other Funding Partners: **ACOA**- Atlantic Innovation Fund; **Federal**- Fisheries & Oceans Canada; **In Kind**- UPEI Administrative Support, AVC Faculty Consultations, Director & Senior Scientist; **Non-Commercial** - Province of British Columbia; Eskasoni First Nation; Province of Newfoundland Labrador; Millbrook First Nation; Province of New Brunswick; Province of Nova Scotia; Province of Prince Edward Island; Unama'ki Institute of Natural Resources; **Private**- Acadian Fishermen's Co-op; Canadian Gold Seafood; Clearwater Fine Food; Diagnostic Chemicals Ltd; Eastern Fishermen's Federation; ELANCO; Madelimer; Maritime Fishermen's Union; Maritime Lobster Processors Co-operative; Orion Seafood International; Paturel International; PEI Atlantic Shrimp Corp; PEI Fishermen's Association; Polar Foods; Royal Star; Sable Offshore; Seafood 2000 Ltd

9. Molecular Genetics of "Bumper Car" Disease - Phase I - Genomics

Proponent: Lobster Science Centre
Project Number: 03-LSC-09
Project Status: Complete
Project Start/Completion Date: October 2003 - November 2004

Project Objective:

To pursue genomics research on Anophryoides haemophila, the ciliated protozoan that causes Bumper-car disease in lobsters - The rationale is to construct & then exploit, through proteomics, our own annotated genomic database. From previous research on Bumper-car disease, the AVCLSC has developed expertise that has been previously published in scientific journals and the information incorporated into management strategies within the lobster industry. Therefore, a Bumper-car genomics project would extend knowledge of this disease by applying the latest scientific technologies and begin to provide a clearer picture of how this "bug" causes disease in lobsters.

Summary of Outcome:
Initial phases of the project proceeded quite smoothly. The methods applied at the AVCLSC to isolate total RNA from *Anophryoides haemophila* resulted in a substantial yield of mRNA from which Agencourt Biosciences were able to construct a high quality cDNA library. The cDNA library was then shipped to Genome Sciences Centre (GSC)-BC Cancer Agency for sequencing of the 10, 000 EST (expressed sequence tags). Initial data looked quite encouraging and of high quality. However, in late August 2004, GSC-BC Cancer Agency reported a mis-mapping of the Bumper Car EST dataset that occurred due to a mis-calibration of one of their sequencing machines. The implications for this mis-mapping of the data is that it would impair our ability to reclaim relevant clones from the cDNA library for further gene annotation and diagnostic development. Therefore, GSC-BC Cancer remapped all the data (by September 9, 2004) and we were able to re-analyse the 10,000 EST via the PEPdb by September 30, 2004. This delay has caused the subsequent comprehensive analysis using the PEPdb program Anabench to be delayed with an expected completion date by the end of December 2004. However, this means that a final report would not be available until the end of January 2005.

Preliminary findings from the BLAST searches/gene annotation completed by September 30, 2004 have revealed 847 potential genes, approximately 38 % of which are found in the genomes of other organisms (this is similar to what has been reported for other pathogens e.g. *E. coli*, *Trypanosoma*). Interestingly from this 38%, we have found groups of genes in the Bumper car parasite that may be involved in causing disease in lobsters. However, the approximately 60 % of the genes found are considered “hypothetical genes” and may be unique to the Bumper car parasite. It will be interesting to see if the comprehensive analysis will reveal if any of these “hypothetical genes” play a role in causing disease in lobsters.

Total Project Cost: $118,791.00

Funding provided by PEIASCI: $74,249.00

Other Funding Partners: **ACOA**- Atlantic Innovation Fund; **Federal**- Fisheries & Oceans Canada; **In Kind**- UPEI Administrative Support, AVC Faculty Consultations, Director & Senior Scientist; **Non-Commercial** - Province of British Columbia; Eskasoni First Nation; Province of Newfoundland Labrador; Millbrook First Nation; Province of New Brunswick; Province of Nova Scotia; Province of Prince Edward Island; Unama'ki Institute of Natural Resources; **Private**- Acadian Fishermen's Co-op; Canadian Gold Seafood; Clearwater Fine Food; Diagnostic Chemicals Ltd; Eastern Fishermen's Federation; ELANCO; Madelimer; Maritime Fishermen's Union; Maritime Lobster Processors Co-operative; Orion Seafood International; Paturel International; PEI Atlantic Shrimp Corp; PEI Fishermen's Association; Polar Foods; Royal Star; Sable Offshore; Seafood 2000 Ltd.

10. Acute Phase Proteins for Assessment of Lobster Health

Proponent: Lobster Science Centre
Project Number: 04-LSC-010
Project Status: complete
Project Start/Completion Date: September 2004 -

Project Objective:
The goal of the project is to identify proteins, known as acute phase proteins (APPs), whose blood concentrations are expected to increase when the lobster is 'sick', similar to the situation in man and other animals. Ideally, this will lead to the development of a new diagnostic test for use by researchers and the lobster industry for the assessment of lobster health.

Summary of Outcome:
Project Complete. **Results will be made available following publication of findings.**

Total Project Cost: $74,440.00

Funding provided by PEIASCI: $49,960.00

Other Funding Partners: **ACOA**- Atlantic Innovation Fund; **In Kind**- UPEI, AVC, Director & Senior Scientist; **Non-Commercial** - Eskasoni First Nation; Province of Newfoundland Labrador; Millbrook First Nation; Province of New Brunswick; Province of Nova Scotia; Province of Prince Edward Island; Unama'ki Institute of Natural Resources; **Private**- Acadian Fishermen's Co-op; Canadian Gold Seafood; Clearwater Fine Food; Eastern Fishermen's Federation; ELANCO; Madelimer; Maritime Fishermen's Union; Maritime Lobster Processors Co-operative; Orion Seafood International; PEI Fishermen's Association; Seafood 2000 Ltd.

11. Development of an Internet-based Data Delivery System for the PEI Lobster Index Fishery

Proponent: Lobster Science Centre
Project Number: 04-LSC-011
Project Status: Complete
Project Start/Completion Date: December 2004 - July 2005

Project Objective:

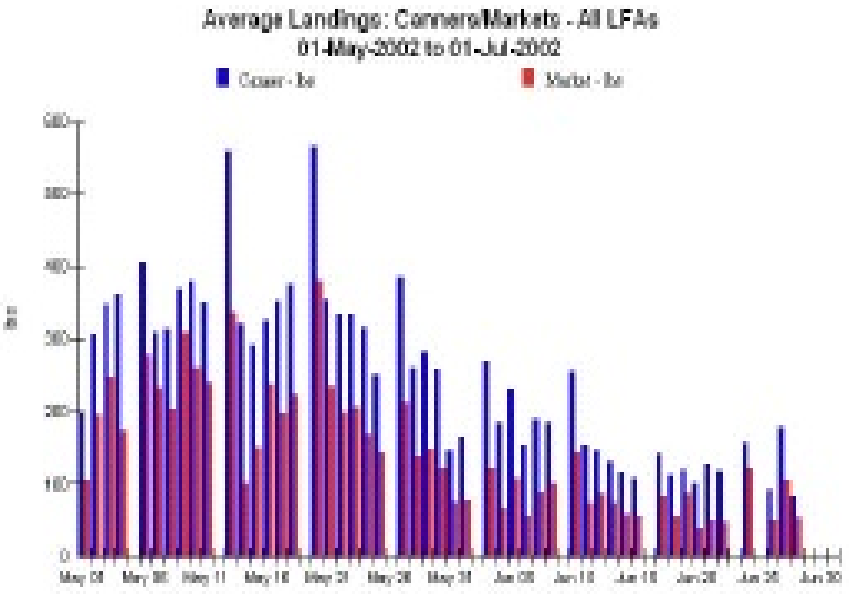
The overall objective of this project is to develop the infrastructure necessary to deliver the information collected through the PEI Lobster Index Fishery back to individual fishermen, provincial and federal government representatives, scientists and other stakeholders in the lobster industry.

Novel ways for analysing & comparing PEI lobster catch data will be designed by considering geographical location, time of year, and some biological data while maintaining the confidentiality of individual fishers/boats and/or ports. During the lobster fishing seasons, some fishers record information from a small number (usually 3) of 'ventless' traps and from a matching number of commercial traps hauled daily. These participating lobster fishers & the PEIAFA staff record their observations in handwritten logbooks and the resulting information constitutes the PEI Lobster Index Fishery data.

The handwritten logbook data are entered into and cross-checked by a computer system developed by the Moncton office of Fisheries & Oceans, Canada at a later date. These data will be provided to the AVCLSC and will form the core of this six month project: we propose to utilise the data collected for the 2002 fishing season (and possibly other years prior to 2002) to construct and test the infrastructure and delivery applications.

An AVCLSC computer program (to be developed by AVCLSC during this project) will process the dataset(s) to compute various summary information parameters and industry trends, including averages and percentiles, at the lobster fishing area level. Several, single-click 'canned' or pre-calculated reports will be defined for quick preparation & easy on-line viewing and/or automatic delivery via push-out Email. The main feature will be a flexible, on-line, real-time graphing software program which will provide the user with a variety of choices to create graphs and comparisons derived from the database. The resulting graphical picture of the processed information in the AVCLSC database, including an optional 'data sheet' to tabulate the exact values viewed in the graph, could be delivered via the Internet to one's computer screen through an Internet browser. Following the completion of the project, we intend to have the website accessible to the general public upon approval from UPEI, DFO, PEIAFA and the PEIFA.

Summary of Outcome:
The initial LobsterNET project funded by the PEI Atlantic Shrimp Corp. Inc. (Project No. 03-LSC-05) encompassed the design, development and implementation of a lobster health research database to form the foundation for applied research with the AVCLSC at the University of Prince Edward Island. LobsterNET involved the acquisition of hardware (including a server, network connections and other related items) and the development of specialized software programs to access the research databases online. The work was divided into two Phases, Construction and Delivery. Several research avenues with LobsterNET were pursued in both Phases thru the Central Lobster Access Website; the "Development of an Internet-based Data delivery system for the PEI Lobster Index Fishery" project being one of them.



(...cont'd)
We have initiated some work on

the PEI index fishery site. The original plan was to use the 2002 data and possibly data from other previous years to develop this information delivery system. However, we are still awaiting the 2002 Dataset from DFO Moncton, despite several attempts to obtain the data. This significant road block has resulted in important delays in this project. Therefore, to meet our commitment with the PEI Atlantic Shrimp Corp. Inc, we decided to carry on the work by using sample data. These sample data were generated from real data obtained from six log books provided by the Province. At this point in time, only some preliminary work has
been done.

Currently, the online application allows the user to select a start and end date for the period of interest, select the LFA(s), and select total or average landings for all lobsters or for canner and market-sized lobsters separately.

The main feature of the application will remain a flexible, online, real-time graphing software program which will allow a variety of choices to create pictures & comparisons derived from the database.

Total Project Cost: $46,020.00

Funding provided by PEIASCI: $30,870.00

Other Funding Partners: **ACOA**- Atlantic Innovation Fund; **In Kind**- UPEI, AVC, Director & Senior Scientist; **Non-Commercial** - Eskasoni First Nation; Province of Newfoundland Labrador; Millbrook First Nation; Province of New Brunswick; Province of Nova Scotia; Province of Prince Edward Island; Unama'ki Institute of Natural Resources; **Private**- Acadian Fishermen's Co-op; Canadian Gold Seafood; Clearwater Fine Food; Eastern Fishermen's Federation; ELANCO; Madelimer; Maritime Fishermen's Union; Maritime Lobster Processors Co-operative; Orion Seafood International; PEI Fishermen's Association; Seafood 2000 Ltd.

12. Developing a Biochemical Profile for the American Lobster

Proponent: Lobster Science Centre

Project Number: 04-LSC-012

Project Status: Complete

Project Start/Completion Date: December 2004 - December 2005

Project Objective:

The goal of the proposed study is to identify blood biochemical tests that could be used to identify injury to tissues in lobsters, with emphasis on the hepatopancreas, utilising non-lethal sampling procedures.

This information could be used for health assessment in both research and industry e.g., to gain a better understanding of diseases affecting lobsters, during investigations of disease outbreaks or high mortalities in lobster holding facilities, and to assist in the development of drug treatment and disinfectant protocols proposed for use in the lobster industry.

Clinical chemistry is a branch of human and veterinary medicine that utilises changes in the levels of salts, minerals, metabolites, or enzymes in the blood to help diagnose or localise disease processes. Multiple tests are often performed on the same blood sample - referred to as a 'biochemistry profile' or 'biochemistry panel'. When one or more of the tests are above or below the expected range (its reference interval) for a population, it generally indicates injury to, or altered function of, an organ(s) (e.g. kidney, liver, muscle, or heart). The enzyme tests included in veterinary and human medicine biochemistry profiles can be run on lobster samples. What is necessary however, is to validate the relevance of the results of these tests in lobsters. This process is known as assay validation and is used whenever a test originally designed for use on one species is being tested for its suitability in another species.

Summary of Outcome:

As a result of the work funded in this project, Andrea Battison (the lead investigator) has had a paper published in the Journal of Shellfish Research Vol 25, No. 2, 533-560, 2006

Tissue Distribution and Hemolymph Activity of Six Enzymes in the American Lobster (Homarus americanus): Potential Markers of Tissue Injury

ABSTRACT

Biochemistry panels are used to help identify tissue injury (e.g., because of inflammation, trauma, or hypoxia) in human and veterinary medicine in part, by detecting increased enzyme activity in serum or plasma after release from damaged tissues. To determine if a similar approach can be used in Homarus americanus, activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamate dehydrogenase (GD), sorbitol dehydrogenase (SDH), amylase, and lipase were measured in tissue homogenates of heart, hepatopancreas, abdominal muscle, intestine, antennal gland, hemocyte lysate supernatant, and hemolymph plasma and serum. Activities of ALT and AST were significantly higher in serum than plasma, which was attributed to release of enzymes from hemocytes during coagulation. Reference intervals calculated for plasma enzyme activity at ambient holding temperatures of 2-4oC and 15oC were quite similar. Plasma enzyme activity was not a sensitive test for detecting infection with Aerococcus viridans (gaffkemia) during an experimental trial.

(Cont'd)

Total Project Cost: $36,533.00

Funding provided by PEIASCI: $22,340.00

Other Funding Partners: **ACOA**- Atlantic Innovation Fund; **In Kind**- UPEI, AVC, Director & Senior Scientist; **Non-Commercial** - Eskasoni First Nation; Province of Newfoundland Labrador; Millbrook First Nation; Province of New Brunswick; Province of Nova Scotia; Province of Prince Edward Island; Unama'ki Institute of Natural Resources; **Private**- Acadian Fishermen's Co-op; Canadian Gold Seafood; Clearwater Fine Food; Eastern Fishermen's Federation; ELANCO; Madelimer; Maritime Fishermen's Union; Maritime Lobster Processors Co-operative; Orion Seafood International; PEI Fishermen's Association; Seafood 2000 Ltd.

13. 2nd Annual Lobster Science Workshop

Proponent: Lobster Science Centre
Project Number: 05-LSC-013
Project Status: Complete
Project Start/Completion Date: March - August 2005

Project Objective:

The tentative agenda for the 2nd Annual Lobster Science Workshop includes a Meet and Greet Reception on Wednesday evening, sessions on the Applied Aspects of Research at the AVC Lobster Science Centre on Thursday morning and a session on Applied Lobster Fishery Research on Thursday afternoon. On Thursday afternoon, there will also be an open forum on Lobster Research addressing important questions such as: What are the priorities for research? How can we fund research? And who should do the Research? Finally, the event will conclude with a Banquet on Thursday evening. We

believe that this yearly event will quickly grow to become one of the benchmarking science workshop for the lobster industry with great exposure to all sectors of the industry! Additionally, this workshop will be a great link into the up-coming 8th International Conference & Workshop on Lobster Biology & Management which will also take place in Charlottetown, Prince Edward Island in September 2007.

Summary of Outcome:

The AVC Lobster Science Centre, at the Atlantic Veterinary College, University of Prince Edward Island, recently hosted its 2nd Annual Lobster Science Workshop, at the Delta Prince Edward hotel in Charlottetown, PEI. The workshop, held this past July 27-28, was well attended with approximately 75 fishermen, scientists, processors, live shippers and government representatives participating in this one day event. Dr. Robert Bayer, Executive Director of the Lobster Institute of the University of Maine was the keynote speaker and gave a very good overview of some of the Lobster Institute research projects, past and present, with direct impact and practicality for the lobster industry. Dr. Bayer's presentation definitely set the tone for the remainder of the workshop that had "Lobster Research in an Applied Context" as its theme. Other than Dr. Bayer, eight (8) other presenters including four scientists from the AVC Lobster Science Centre contributed to the scientific presentation sessions while Dr. Timothy Ogilvie, Dean of the Atlantic Veterinary College at the University of rince Edward Island chaired the Open Forum on lobster research. In addition to the oral presentations, posters presentations were included in the 2005 workshop format. Approximately ten posters were on display, two of which from graduate students from the AVC Lobster Science Centre to present some of their work.

A lot of discussion on lobster research was generated during the open forum session and some of the topics brought forward by the audience included: gaffkemia status in juvenile lobsters; early stage survival and larvae drift; food source & predation; what are the most important factors in egg production; the potential effects of seismic testing on lobster health; and environmental impact on lobster stocks (see www.lobsterscience.ca/workshop for a detailed list of topics discussed). Finally, when discussing who should pay for the research, it was the general consensus that we need to fund research with "fee for service" or "voluntary taxing" but without the industry buy-in, good lobster research is becoming a rare commodity. Similar to the 2004 event, the 2nd Annual Lobster Science Workshop ended with a fabulous banquet where, of course, lobster was king!

Total Project Cost: $32,975.00
Funding provided by PEIASCI: $4187.50 *($4187.50 was slipped from the approved $8375.00)*

Other Funding Partners: Contributions to support the research programs of the AVCLSC are provided by the following: ACOA & the Atlantic Innovation Fund; AVC In-Kind Faculty Consultations; Chase's Lobster Pound; Clearwater Fine Foods; Department of Fisheries & Oceans, Canada; ELANCO; Ferguson's Lobster Pound; Millbrook First Nation; Paturel International; PEI Atlantic Shrimp Corp; Province of New Brunswick; Province of Newfoundland Labrador; Province of Nova Scotia; Province of Prince Edward Island; and, UPEI In-Kind Administrative Support.

14. Identifying Antimicrobial Peptides in Lobster Hemocytes

Proponent: Lobster Science Centre
Project Number: 05-LSC-014
Project Status: Complete
Project Start/Completion Date: August 2005 - December 2005

Project Objective:
Interest in antimicrobial peptides (APs) has increased in recent years. As the search for newer, better, more effective antibiotic and antiviral agents has intensified to combat 'superbugs' and other infectious agents so to, has interest in APs - often considered 'natural antibiotics'. These small proteins have been isolated from plants, mammals, amphibians, and crustaceans (shrimp, crab). It is therefore reasonable to assume that a peptide(s) is also present in Homarus americanus, the American lobster. Antimicrobial peptides are an important part of innate immunity, that part of the immune system involved in immediate response to infections. Since lobsters, as all invertebrates, lack adaptive immunity i.e., no antibody response, acquiring a more complete understanding of the workings of the innate immune system in invertebrates is paramount. This proposal encompasses the isolation, identification, characterisation, and localisation of APs in lobster hemocytes and hemolymph and, determination of the spectrum of antimicrobial activity of the peptide(s). Production of a synthetic version of any suitable peptide(s) isolated from the lobster samples has been included as an additional step in the current proposal. To date, research at the AVCLSC on this project has focussed on hemocytes (white blood cells) as they have proved to be a rich source of peptides in other animals, including shrimp. The work has included evaluation of multiple published peptide isolation protocols to determine which is most suited to lobster samples and the technical facilities available at the AVCLSC. After some modifications, a suitable protocol has been established. This methodology has been recently validated with the successful isolation of small quantities of a protein with some characteristics of known APs.

The next phases will be to isolate and purify sufficient quantities of the peptide(s) for further analysis. This will involve extraction of potential proteins from hemocytes and submission of the purified material for protein sequencing (mass spectroscopy) at an external laboratory. The sequence results will be compared to known APs. The purified, natural peptide(s) will also be tested for its antimicrobial (anti-bacterial, antifungal, and antiparasitic) activity. Testing against known lobster pathogens (eg. A. Viridans) will be carried out at the AVCLSC. Testing against known human pathogens will be performed at the Institute of Marine Biosciences, Halifax, NS. The hemocyte samples to be used for this study will be frozen material collected during a gaffkemia (Aerococcus viridans) infection conducted in 2005 as part of the Acute Phase Protein (04-LSC-10) and Biochemical Profile (04-LSC- 12) projects.

Summary of Outcome:
Project Complete. **Results will be made available following publication of findings.**

Total Project Cost: $34,900.00
Funding provided by PEIASCI: $21,800.00

Other Funding Partners: Contributions to support the research programs of the AVCLSC are provided by the following: ACOA & the Atlantic Innovation Fund; AVC In-Kind Faculty Consultations; Chase's Lobster Pound; Clearwater Fine Foods; Department of Fisheries & Oceans, Canada; ELANCO; Ferguson's Lobster Pound; Millbrook First Nation; Paturel International; PEI Atlantic Shrimp Corp; Province of New Brunswick; Province of Newfoundland Labrador; Province of Nova Scotia; Province of Prince Edward Island; and,UPEI In-Kind Administrative Support.

15. Molecular Diagnostics for the Detection of *Neoparamoeba pemaquidensis*

Proponent: Lobster Science Centre

Project Number: 05-LSC-015
Project Status: Complete
Project Start/Completion Date: August 2005 - December 2005

Project Objective:
Molecular diagnostics have become the benchmark tool for the detection of pathogens both in human and veterinary medicine. Therefore, it follows that molecular based diagnostics have been accepted by the World Organisation for Animal Health, formerly the OIE (Office International des Épizooties) and have since been implemented in the detection of several pathogens affecting economically important marine species. Molecular diagnostic methods are built on the premise that DNA is present in every cell of every living thing and that certain DNA markers can be used to separate and identify all animals based on that marker. Therefore, using DNA markers we can determine the presence of a specific organism or pathogen in an animal or the environment.

In order for a test to be useful it must be specific and also sensitive. That is, the test must be able to detect very small amounts of a pathogen in order to make a diagnosis early. Therefore, early detection leads to treatment or management plans to avoid full blown disease or death. The ability to detect extremely small samples of DNA is enhanced by the use of a technique that amplifies the DNA marker (makes many copies of the chosen DNA marker, similar to how we take one sheet of paper and use a photocopier to make many copies). This DNA amplification technique is called Polymerase Chain Reaction (PCR). The PCR method is the main reason for incorporation of DNA testing into police forensic investigations and for paternity testing. Using the PCR method we are able to take very small samples and amplify a DNA marker to detect the specific pathogen. The DNA marker we selected was the Internal Transcribed Spacer (ITS) region of the rDNA gene. This research logically builds on the previous work of our collaborators from the University of Connecticut (Mullen et al. in press Journal of Shellfish Research). The initial research at the AVCLSC has been carried out on our six laboratory culture strains of *N. pemaquidensis*. This research is the graduate work towards a Master of Science (MSc) degree for Dr. Charles Caraguel.

Summary of Outcome:
We have been able to develop a molecular diagnostic tool that uses the principles of Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) that can identify all six laboratory strains of *Neoparamoeba pemaquidensis*, the known cause of Amoebic Gill Disease in Salmon. *Neoparamoeba pemaquidensis* has also been associated with massive die offs of Green Sea Urchins, Blue Crabs and American Lobsters. We have designed in parallel two complementary diagnostic tools that use different DNA markers within the *Neoparamoeba* parasite. The *N. pemaquidensis* PCR-RFLP molecular diagnostic tools were validated using an “outbreak isolate”. We were able to not only identify the pathogen as *N. pemaquidensis* from the sample but could tell which specific strain was responsible for the disease outbreak. The final outcome of this project is that we have been able to develop PCR-RFLP methods that can identify *N. pemaquidensis* isolated and cultured from different hosts. The next logical step would be to further refine this method to develop a “molecular probe” to determine the presence of this parasite in host tissues (lobster, crab, salmon & urchin) without prior isolation and culture. Therefore, we should be able to detect the disease earlier and also to determine were the parasite causes disease at the tissue level to kill the host (lobster, crab, salmon & urchin).

Two scientific publications of this research are in preparation. Parts of this research have been presented, by Dr. Charles Caraguel, locally (Lobster Science Workshop, Charlottetown, PEI, July 2005), regionally (Atlantic Canadian Association of Parasitologists Annual Meeting, St. Andrews, New Brunswick, October 2005) and internationally (XI International Meeting on Biology and Pathogenicity of Free-Living Amoebae, Ceské Budejovice, Czech Republic, September 2005).

Total Project Cost: $46,200
Funding provided by PEIASCI: $15,600.00

Other Funding Partners: Contributions to support the research programs of the AVCLSC are provided by the following: ACOA & the Atlantic Innovation Fund; AVC In-Kind Faculty Consultations; Chase's Lobster Pound; Clearwater Fine Foods; Department of Fisheries & Oceans, Canada; ELANCO; Ferguson's Lobster Pound; Millbrook First Nation; Paturel International; PEI Atlantic Shrimp Corp; Province of New Brunswick; Province of Newfoundland Labrador; Province of Nova Scotia; Province of Prince Edward Island; and, UPEI In-Kind Administrative Support.

16. Gaffekemia Proteomics: Targeting the Membrane-associated proteins of *Aerococcus viridans*

Proponent: Lobster Science Centre
Project Number: 05-LSC-016
Project Status: Complete
Project Start/Completion Date: August 2005 - December 2005

Project Objective:
The results from our initial research in conjunction with the recently published research by Dr. Jim Stewart has allowed us to focus our efforts towards the membrane-associated proteins of *Aerococcus viridans* (var.) *homari*. The research in our paper and the Stewart paper indicate that regardless of genetic similarities or differences among strains, that the ability of *Aerococcus* to evade the lobster's immune response and therefore kill lobsters is likely due to changes in protein expression that may be associated with the bacterial outer surface. It is the *Aerococcus* outer surface membrane proteins that have an intimate association with the lobster's "immune" system. Therefore, by concentrating our efforts on the *Aerococcus* outer surface we should increase our probability of finding and identifying useful candidates for drug targets and immunostimulants ("vaccines") for the lobster industry.

Summary of Outcome:
Isolation of *Aerococcus viridans* membrane proteins proved to be initially more difficult than expected. From what we have learned over the course of this study, the difficulty is likely centered around the specific nature of this type of bacterial cell. However, we believe that the strategy to focus on membrane proteins in the end will be more rewarding in order to understand how *Aerococcus* kills lobsters, it will require more work. Our preliminary membrane proteins isolation resulted in poor yield and the subsequent gel separations showed evidence of lipid (fat) contamination which obscured the protein results. We have improved our membrane protein isolations and gel separations but not to sufficient levels to begin to identify proteins. For this reason we are approximately 75% complete with respect to Task II focusing on membrane proteins. In parallel we have produced good separative gels using *Aerococcus* total proteins. We feel that by also concentrating efforts on the total protein we have moved closer to identifying proteins associated with how the bacteria evades the lobsters immune system and kills the lobster. This would allow us to satisfy Task II (100% completion) of the current proposal. We plan to continue to improve membrane protein isolation and gel separation techniques and proceed to the identification of target proteins. Once gels separation is deemed satisfactory we will determine protein sequences through outsourcing as described in the original proposal (Task III) to identify disease-associated proteins. Pursuit of this research has allowed us to reflect on the nature of the bacterial: lobster interaction and to re-focus our attention on both the bacterium as it causes disease and on the lobster immune response.

A summary of this work has been presented to an academic audience at the Atlantic Veterinary College, Pathology & Microbiology Seminar Series entitled “Gaffkemia in Lobsters: understanding virulence in *Aerococcus viridans*”by Dr. Spencer Greenwood in December 2005.

Total Project Cost: $48,400.00
Funding provided by PEIASCI: $23,400.00

Other Funding Partners: Contributions to support the research programs of the AVCLSC are provided by the following: ACOA & the Atlantic Innovation Fund; AVC In-Kind Faculty Consultations; Chase’s Lobster Pound; Clearwater Fine Foods; Department of Fisheries & Oceans, Canada; ELANCO; Ferguson’s Lobster Pound; Millbrook First Nation; Paturel International; PEI Atlantic Shrimp Corp; Province of New Brunswick; Province of Newfoundland Labrador; Province of Nova Scotia; Province of Prince Edward Island; and, UPEI In-Kind Administrative Support.

17. In vitro Efficacy Screening of Amoebocidal Compounds Against *Neoparamoeba pemaquidensis*

Proponent: Lobster Science Centre
Project Number: 05-LSC-017
Project Status: Complete
Project Start/Completion Date: August 2005 - December 2005

Project Objective:
The lobster fishery in Long Island Sound was devastated in fall 1999 during a multifactorial epidemic. The fishery in theSound area has not yet recovered. Apparently the ultimate cause of death of many lobsters was a neurological disease caused by the parasitic amoeba Neoparamoeba pemaquidensis. This parasite is a cause of significant disease in other marine hosts, including salmonids, blue crabs and sea urchins. Presently, the source(s) and routes of transmission of the parasite to, and among lobsters are not well understood.

Presently 40-60 million pounds of live lobster are imported into Canada annually from the United States. Treatment of effluent from lobster holding facilities and processing plants is not stringent and its effectiveness in removing or destroying pathogen is not documented. Consequently, sectors of the Canadian lobster fishery are concerned with the transborder movement of these American-caught lobsters, and the potential pathogens they may carry into Canadian facilities and eventual discharge of effluent and wastes into our oceanic environment.

A major component of Dr. Nathanaëlle Donay's graduate project is to evaluate the efficacy of various disinfectants and therapeutants against N. pemaquidensis. Nathanaëlle has established a tetrazolium-based cytotoxicity assay with the amoeba as the test organism. Disinfectants could reduce the infectious load in the environment and on host surfaces; therapeutants will treat when clinical signs occur. Agents to be tested will be either already approved for use with fish, or are products under development. Culturing of amoebae in the Rainbow Trout gill cell line will provide the numerous amoebae growing in log phase, which is necessary to efficiently use the cytotoxicity assay.

Summary of Outcome:
In a preliminary study we evaluated the potential of a tetrazolium-based cytoxicity assay to determine potential anti-protozoal activity of various compounds against the amoeba *Neoparamoeba pemaquidensis.* This amoeba is the causative agent of Amoebic Gill Disease of Atlantic salmon, paramoebiasis in lobsters, grey crab disease in blue crabs and paramoebiasis of sea urchins. Although tetrazolium-based assays have been successfully used to assess therapeutic agents and disinfectants against other protozoa including related amoebae, the assay does not work with *N. pemaquidensis*. Our recent experiments demonstrated that the amoeba cannot bioreduce the compound.

Consequently an alternative viability test, flow cytometry, which does not rely on the metabolism of *N.pemaquidensis*, has been investigated. The flow cytometer is an instrument designed to enumerate, with high precision, rapidity and reproducibility, the fluorescence of individual cells (parasites). Fluorescent dyes with specific staining characteristics (i.e. fluorescein diacetate, propidium iodide) can be used to discriminate between viable and non-viable parasites after adding a putative anti-amoebic compound. In preliminary trials, the instrument can detect and quantify trophozoites of *N. pemaquidensis*. Additionally, the flow cytometer can evaluate rainbow trout gill cells, which will allow determining whether anti-amoebic compounds harm host cells. The system is now ready to begin evaluating anti-protozoal compounds.

Through ongoing collaboration with Dr. Lucy Lee, Wilfred Laurier University, we have established the RTgill-W1 cell line at AVC Lobster Science Centre. Additional to isolate ATCC 50172, we will be attempting to establish various other isolates of *N. pemaquidensis* (i.e. from sea urchins, environmental samples, Atlantic salmon) on rainbow trout gill cells, to determine whether they multiply rapidly and

cause cytopathogenic effects. This approach of culturing amoebae on gill cells is a major achievement, and facilitates the rapid production of very high numbers of relatively clean amoebae. Subsequently anti-amoebic compounds could also be tested against these isolates as above. The flow cytometry instrumentation is available courtesy of Dr. Francke Berthe, Canada Research Chair - Mollusc Health, Atlantic Veterinary College.

Results of this research have been submitted as a manuscript to the Journal of Fish Diseases, and have been presented at the Second Annual Lobster Science Workshop (Charlottetown) and at a regional meeting of protistologists at Dalhousie University (Charlottetown). This research is a major component of the MSc project of Dr. Nathanaëlle Donay.

Total Project Cost: $36,300.00
Funding provided by PEIASCI: $12,500.00

Other Funding Partners: Contributions to support the research programs of the AVCLSC are provided by the following: ACOA & the Atlantic Innovation Fund; AVC In-Kind Faculty Consultations; Chase's Lobster Pound; Clearwater Fine Foods; Department of Fisheries & Oceans, Canada; ELANCO; Ferguson's Lobster Pound; Millbrook First Nation; Paturel International; PEI Atlantic Shrimp Corp; Province of New Brunswick; Province of Newfoundland Labrador; Province of Nova Scotia; Province of Prince Edward Island; and, UPEI In-Kind Administrative Support.

18. Preliminary Host Range, Transmission, and Pathogenesis of *Neoparamoeba pemaquidensis*

Proponent: Lobster Science Centre
Project Number: 05-LSC-018
Project Status: Complete
Project Start/Completion Date: August 2005 - December 2005

Project Objective:
The lobster fishery in Long Island Sound was devastated in fall 1999 during a multifactorial epidemic and the fishery in the Sound area has not yet recovered. Apparently the ultimate cause of death of many lobsters was a neurological disease caused by the parasitic amoeba *Neoparamoeba pemaquidensis*. This parasite is a cause of significant disease in other marine hosts, including salmonids, blue crabs and sea urchins. Presently the source(s) and routes of transmission of the parasite to, and among lobsters are not well understood. If pathogens such as *N. pemaquidensis* are released into, or already exist in, Canadian waters, will they establish in important fish such as lobsters? An ongoing source of infectious *N. pemaquidensis* is the infected Atlantic salmon in the quarantine modules of the Aquatic Animal Facility, AVC, where Amoebic Gill Disease has been established. Additionally, cell-culture derived amoebae may also be a rapid, efficient source for transmission studies.

In the primary set of trials, the infectious agent *N. pemaquidensis* will be derived, by various extraction methods, from the donor host Atlantic salmon. Target hosts include American lobster, rock crabs, sea urchins, and saltwater-acclimated rainbow trout. The latter host species is being tested because trout are relatively inexpensive and easier to maintain in the aquarium system; they may be a useful, cost effective source of infectious amoebae. Transmission pathways to be tested include cohabitation (same recirculating saltwater aquarium system with each species of host in separate tank, using Atlantic salmon as donor host); injection into lobsters, sea urchins and crabs (amoebae extracted from gills of Atlantic salmon); per os (fed) into lobsters, crabs and sea urchins. The focus will be on transmission of *N. pemaquidensis* to lobsters.

In the secondary set of trials, the amoebae will be obtained from Rainbow Trout gill cell cultures to determine their infectivity to Atlantic salmon in the first instance. Subsequent to successful transmission of *N. pemaquidensis* among the various hosts, transmission studies will be used for sequential examination of affected host tissues. Disease will be monitored by observing clinical signs and histological examination.

Summary of Outcome:
The model of Amoebic Gill Disease (AGD) in Atlantic salmon, caused by the pathogenic amoeba *Neoparamoeba pemaquidensis*, is now well established in saltwater recirculation aquarium systems of the Aquatic Animal Facility, Atlantic Veterinary College. This represents collaboration among the AVC Lobster Science Centre, Centre for Aquatic Health Sciences (AVC) and Novartis - Aqua Health. Methods to extract amoebae from the donor host Atlantic salmon have been 'perfected' although they are quite cumbersome and time-consuming.

In initial trials, very surprisingly saltwater-acclimated rainbow trout did not become infected with *N. pemaquidensis* during extensive cohabitation with infected Atlantic salmon. Recently, the use of saltwater-acclimated Atlantic salmon, which are apparently more stressed (immunocompromised) than physiologically-prepared smolts, has increased the rate at which salmon become clinically ill and die with AGD. At present, lobsters are cohabiting with infected Atlantic salmon and we will perform necropsies on these lobsters, in the near future, to determine whether they became infected with *N. pemaquidensis.* AGD can be induced experimentally by cohabitation of naïve Atlantic salmon with AGD-affected Atlantic salmon or crude preparations of amoebae and host tissues. Ideally, laboratory

infections of salmon, lobsters, sea urchins, or crabs would be better studied using a well-characterized cultured isolate of *N. pemaquidensis*.

Through ongoing collaboration with Dr. Lucy Lee, Wilfred Laurier University, we have established an isolate (ATCC 50172) of *N. pemaquidensis* on a rainbow trout gill cell line, RTgill-W1. This supposedly nonpathogenic strain of amoeba multiplies very rapidly and destroys the gill cells quickly. In an attempt to fulfill Koch's postulates, we experimentally exposed rainbow trout and Atlantic salmon to trophozoites of *N. pemaquidensis* cultured on rainbow trout gill cells. The experiment is ongoing. However, preliminary results suggest that although this isolate of *N. pemaquidensis* is cytopathogenic *in vitro*, the amoebae may not be infectious *in vivo.* Perhaps the type of bacteria ingested by and living in the amoebae may be important in determining whether the amoebae are pathogenic to fish.

Results of this research have been submitted as a manuscript to the Journal of Fish Diseases, and have been presented at the Second Annual Lobster Science Workshop (Charlottetown) and at a regional meeting of protistologists at Dalhousie University (Charlottetown). This research is a major component of the MSc project of Dr. Nathanaëlle Donay.

Total Project Cost: $33,900.00
Funding provided by PEIASCI: $7,900.00

Other Funding Partners: Contributions to support the research programs of the AVCLSC are provided by the following: ACOA & the Atlantic Innovation Fund; AVC In-Kind Faculty Consultations; Chase's Lobster Pound; Clearwater Fine Foods; Department of Fisheries & Oceans, Canada; ELANCO; Ferguson's Lobster Pound; Millbrook First Nation; Paturel International; PEI Atlantic Shrimp Corp; Province of New Brunswick; Province of Newfoundland Labrador; Province of Nova Scotia; Province of Prince Edward Island; and, UPEI In-Kind Administrative Support.

19. Implementation of a Six Month Interim Management Plan for the AVC Lobster Science Centre

Proponent: Lobster Science Centre
Project Number: 05-LSC-019
Project Status:
Project Start/Completion Date: September 2005 -

Project Objective:
A Management Consulting Group will be hired on a contract basis for a six month period to oversee the organizational changes including establishing the structures recommended in the review document entitled "Building On A Firm Foundation" and development of a Business Case for financial sustainability of the AVCLSC. Recruitment of permanent professionals to fill key positions in the AVCLSC for the long term will be a primary objective. The key permanent position to be filled will be that of a Chief Operating Officer.

Summary of Outcome:

Total Project Cost: $96,500.00
Funding provided by PEIASCI: $10,000.00

Other Funding Partners: Contributions to support the research programs of the AVCLSC are provided by the following: ACOA & the Atlantic Innovation Fund; AVC In-Kind Faculty Consultations; Chase's Lobster Pound; Clearwater Fine Foods; Department of Fisheries & Oceans, Canada; ELANCO; Ferguson's Lobster Pound; Millbrook First Nation; Paturel International; PEI Atlantic Shrimp Corp; Province of New Brunswick; Province of Newfoundland Labrador; Province of Nova Scotia; Province of Prince Edward Island; and, UPEI In-Kind Administrative Support.

20. 3rd Annual Lobster Science Workshop

Proponent: Lobster Science Centre
Project Number: 06-LSC-020
Project Status: Complete
Project Start/Completion Date: April 2006 - August 2006

Project Objective:
The Atlantic Veterinary College Lobster Science Centre's 3rd Annual Lobster Science Workshop will be held in Charlottetown, Prince Edward Island on July 26th and 27th, 2006. The theme of the workshop is "Marine Ecosystem Health - Crustaceans & Their Environment". Presentations will focus on research done at AVCLSC as well as on marine habitats using seabed mapping techniques, ecosystem-based crustacean research, invasive species, potential impact of oil & gas industry, and larval drift. This will provide an opportunity for discussion among fishermen, processors, exporters, biologists, government representatives, scientists and researchers.

Summary of Outcome:
The AVC Lobster Science Centre, at the Atlantic Veterinary College, University of Prince Edward Island, recently hosted its 3rd Annual Lobster Science Workshop, at the Delta Prince Edward hotel in Charlottetown, PEI. The Workshop, held this past July 26-27, was well attended with approximately 100 fishermen, scientists, processors, live shippers and government representatives while the Interactive Information Session saw approximately 40 attendees. Keynote speakers included marine geologist Gordon Fader of the Bedford Institute of Oceanography and Rick Wahle, senior research scientist of the Bigelow Laboratory for Ocean Sciences in Maine. Researchers from the AVCLSC discussed current research; other speakers talked about marine habitat using seabed mapping techniques, ecosystem-based crustacean research, invasive species, potential impacts of the oil and gas industry and larval drift. In addition to the oral presentations, seven poster presentations were included in the workshop format.

Total Project Cost: $39,000.00
Funding provided by PEIASCI: $7,587.50 (an additional $7587.50 was slipped on this project).

Other Funding Partners: Contributions to support the research programs of the AVCLSC are provided by the following: ACOA & the Atlantic Innovation Fund; AVC In-Kind Faculty Consultations; Chase's Lobster Pound; Clearwater Fine Foods; Department of Fisheries & Oceans, Canada; ELANCO; Ferguson's Lobster Pound; Millbrook First Nation; Paturel International; PEI Atlantic Shrimp Corp; Province of New Brunswick; Province of Newfoundland Labrador; Province of Nova Scotia; Province of Prince Edward Island; and, UPEI In-Kind Administrative Support.

21. Overview of Shell Disease - Review and Description of Research Needs for the Canadian Atlantic Lobster Fishery

Proponent: Lobster Science Centre

Project Number: 06-LSC-021
Project Status: Complete
Project Start/Completion Date: October 2006 - December 2006

Project Objective:

Pending .

Summary of Outcome:
There are major challenges associated with evaluating infectious and non-infectious diseases in marine environments. The recent review of Harvell et al. (2004) proposes major research priorities:
(1) development of diagnostic tools to identify pathogens, to determine their origin and spread;
(2) development of rapid response to identify, monitor and manage disease outbreaks;
(3) determination of life cycle, longevity and host range of various stages of pathogens;
(4) evaluation of role of environmental and anthropogenic factors in disease outbreaks; and
(5) development of forecasting models of disease, sensitive to environmental (climatic) factors.

A major symposium recently focused on lobsters as model organisms for study of behaviour, ecology and fisheries. Shell disease of lobsters was used as the model system of disease in large decapods (see Factor et al. 2006). Castro et al. (2006) suggested the prevalence of epidemic shell disease has increased in wild lobster populations, that there are population-level effects of shell disease, and there is a relationship between shell disease and human and environmental disturbances. In summary, the disease is complex and the interaction of lobsters, pathogens and environment is highly variable and complex.

Total Project Cost: $60,840.00
Funding provided by PEIASCI: $15,690.00

Other Funding Partners: Contributions to support the research programs of the AVCLSC are provided by the following: ACOA & the Atlantic Innovation Fund; Clearwater Fine Foods; Department of Fisheries & Oceans, Canada; ELANCO; Grand Manan Lobster; Ocean Choice International; Province of Newfoundland Labrador; Province of Nova Scotia; Province of Prince Edward Island; and, UPEI In-Kind Administrative Support and AVC Faculty Consultations.

22. Measuring Acute Phase Proteins in Lobsters

Proponent: Lobster Science Centre
Project Number: 06-LSC-022
Project Status:

Project Start/Completion Date: October 2006 -

Project Objective:

Pending .

Summary of Outcome:
(Upon Completion)

Total Project Cost: $211,970.00
Funding provided by PEIASCI: $53,800.00 (over 2 years)

Other Funding Partners: Contributions to support the research programs of the AVCLSC are provided by the following: ACOA & the Atlantic Innovation Fund; Clearwater Fine Foods; Department of Fisheries & Oceans, Canada; ELANCO; Grand Manan Lobster; Ocean Choice International; Province of Newfoundland Labrador; Province of Nova Scotia; Province of Prince Edward Island; and, UPEI In-Kind Administrative Support and AVC Faculty Consultations.

23. Gaffkemia Proteomics - 2: Lobster serum interactions with *Aerococcus viridans* capsule protein expression

Proponent: Lobster Science Centre
Project Number: 06-LSC-023
Project Status:

Project Start/Completion Date: October 2006 -

Project Objective:

Pending .

Summary of Outcome:
(Upon Completion)

Total Project Cost: $235,340.00
Funding provided by PEIASCI: $109,970.00 (over 2 years)

Other Funding Partners: Contributions to support the research programs of the AVCLSC are provided by the following: ACOA & the Atlantic Innovation Fund; Clearwater Fine Foods; Department of Fisheries & Oceans, Canada; ELANCO; Grand Manan Lobster; Ocean Choice International; Province of Newfoundland Labrador; Province of Nova Scotia; Province of Prince Edward Island; and, UPEI In-Kind Administrative Support and AVC Faculty Consultations.

24. ALMQ Field Monitoring

Proponent: AVC Lobster Science Centre
Project Number: 07-LSC-024
Project Status: Complete
Project Start/Completion Date: July 2007 -

Project Objective:

The overall objective of the Field Monitoring activity is to expand the ongoing sampling infrastructure for LFAs 33 and 34 in LFA 25. The accumulated data will be used to build predictive models for landed lobster quality. The methodology used in the Field Monitoring activity of the ALMQ schedule of work will build upon the knowledge, experience and sampling platform generated in current projects of the AVC Lobster Science Centre.

Summary of Outcome:

This project is ongoing. As part of the logistical/technical support for this project, two technicians were hired for. Staff training, initial contact with local fishermen as well as pilot sampling in LFA 25 has been conducted. Recruitment of volunteer participant fishers is now completed.

The original plan called for the monitoring to occur in the LFA 25 portion of the Northumberland Strait only. However, we have decided to extend the monitoring and current plans now included LFAs 25 and 26a. While data collection is ongoing in LFA's 33/34, the actual monitoring in LFAs 25/26a will debut in August 2008.

Meetings were held with the PEIFA, MFU and other fishermen's organizations to identify sampling sites using the local knowledge expert approach. A search for volunteer fishermen was done and a list of potential candidates was established.

Additionally, historical data from participating lobster holding plants have been gathered to start the modeling process.

Total Project Cost: $ 211,728.00
Funding provided by PEIASCI: $46,590 (over 2 years)

Other Funding Partners: Contributions to support the research programs of the AVCLSC are provided by the following: ACOA & the Atlantic Innovation Fund; AVC In-Kind Faculty consultations; Clearwater Fine Foods; Ocean Choice PEI; Seafood 2000; Department of Fisheries & Oceans, Canada; ELANCO; Millbrook First Nation; Province of Newfoundland and Labrador; Province of Nova Scotia; Province of Prince Edward Island; and, UPEI In-Kind Administrative Support.

25. ALMQ Gene Discovery Library

Proponent: Lobster Science Centre
Project Number: 07-LSC-025
Project Status: complete
Project Start/Completion Date: July 2007 -

Project Objective:

Moult and reproductive cycles in crustaceans are complex processes that require the precise coordination of diverse physiological processes in response to seasonal environmental cues (e.g. temperature & photoperiod). The research conducted to date of the underlying molecular and biochemical regulation of these intimately associated cycles have focused on the characterization of either one or a few molecules. The rapid expansion of methods for measuring biological data ranging from DNA sequence variations (genomics) to mRNA expression (microarrays) and protein abundance (proteomics) presents the opportunity to utilize multiple types of information jointly in the study of lobster moult and reproduction. By incorporating an integrated analyses of multiple data types this should improve the identification of previously unknown biomarkers of these important processes and further our understanding of these events in the life cycle of the American lobster.

The Gene Discovery component of the Atlantic Lobster Moult and Quality (ALMQ) will further develop and expand an existing Lobster cDNA library at the Mount Desert Island Biological Laboratory (MDIBL) in Maine, USA in collaboration with Dr. David Towle to identify molecular markers associated with the moult and reproductive cycles and allow further assessment of stress and health. The overall objective of this research is to explore molecular and biochemical changes (gene & protein expression) in moult and reproductive status and from these findings develop qualitative and quantitative markers for the prediction of the physiological state of the lobster. The economic benefits of being able to more accurately assess moult stage, reproductive status, stress and overall health of the lobster relate directly to ensuring the quality of lobster landed and the ability to maintain lobster through the production chain from harvest to consumer. The additional benefits of being able to assess stress and health with specific markers will go a long way to alleviating concerns of welfare organization and consumers who need to know that the harvest, holding and shipping of lobsters is sustainable and being appropriately considered and assessed. Therefore, the Gene Discovery component of the ALMQ should provide a further set of specific and sensitive tools that will translate into ensuring quality lobster.

Summary of Outcome:

In collaboration with Dr. David Towle and the Mount Desert Island Biological Laboratory (MDIBL) sequencing of 20,000 clones from a lobster cDNA library (made from gonad, eye stalk, madibular organ, supraesophogeal ganglion, hepatopancreas, antennal gland, claw muscle, tail muscle, gill) from intermoult and postmoult male and female adult lobsters was completed. The 17,193 successful sequences resulting from the current program have all been submitted to dbEST at the National Center for Biotechnology Information (NCBI). Bringing the total expressed sequence tags in the NCBI database to 29,670 (including previous submissions). Bioinformatic analysis of this database for *Homarus americanus* has revealed a set of 16,169 potentially unique sequences. This set was produced by clustering the ESTs by sequence similarity using The Gene Indices Clustering tool (TGICL). The result of the clustering included 5,323 contiguous sequences (contigs) derived from 18,824 ESTs plus 10,846 individual sequences (singletons) not included in any cluster. The estimate of 16,169 unique sequences is undoubtedly an overestimate, probably including some singletons resulting from different segments of the same transcript. Based on the genomes of other anthropods, we anticipate that the set of unique sequences may represent as much as 75-85% of the entire transcriptome of the American lobster.

The longterm plan for these results within the Atlantic Lobster Moult and Quality project is to assess moult and reproductive status. Biomarkers for moult and reproductive status will be discovered by evaluating the 16,169 ESTs by microarrays using samples collected from (1) larval lobsters from stages 1-4 and from (2) Two year old lobsters “forced to moult”. Reproductive status will be assessed by collecting tissues from female lobsters during their reproductive cycle (stage determined by morphological and histological methods) prior to evaluating changes via microarrays.

Total Project Cost: $ 319,635.00
Funding provided by PEIASCI: $ 61,120 (Year 1 $35,160 & Year 2 $25,960)

Other Funding Partners: Contributions to support the research programs of the AVCLSC are provided by the following: ACOA & the Atlantic Innovation Fund; AVC In-King Faculty Consultations; Clearwater Fine Foods; Ocean Choice PEI; Seafood 2000; Department of Fisheries & Oceans, Canada; ELANCO; Millbrook First Nation; Province of Newfoundland Labrador; Province of Nova Scotia; Province of Prince Edward Island; and, UPEI In-Kind Administrative Support.

26. ALMQ Biochemistry Profile

Proponent: Lobster Science Centre
Project Number: 07-LSC-026
Project Status: complete
Project Start/Completion Date: July 2007 -

Project Objective:

The AVCLSC recently completed a project (04-LSC-12) which established the tissue distribution in *Homarus americanus* for six enzymes commonly used in vertebrate biochemistry profiles (alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutamate dehydrogenase (GD), sorbitol dehydrogenase (SDH), amylase, and lipase). Amylase was identified as being specific to the hepatopancreas and a potential indicator of hepatopancreatic cellular injury. Minerals, electrolytes, and metabolites are also integral components of a biochemistry profile as they reflect the changes occurring in an animal's physiology. In the case of crustaceans such as *Homarus americanus,* changes associated with the moult can be quite dramatic. Therefore, minerals, electrolytes and metabolites, and *in vivo* pH measurements were added to the biochemistry profile in the current project to enhance and improve the information obtained.

It is necessary to determine the stability of these enzymes and other analytes under different storage conditions, as the majority of haemolymph samples evaluated in the ALMQ project will be collected in the field. Delays between collection and analysis are unavoidable. This project will determine acceptable storage conditions (refrigerated vs. frozen, duration of storage) for these samples and establish reference intervals ('normal ranges') for each analyte. The effects of the moult cycle, sex, and season on the analytes will be examined to determine if specific reference ranges are required for any of these conditions.

Summary of Outcome:

It was not possible to solve electrical interference problems with the pH probe. Consequently, pH measurements were not included in the biochemical profiles.

A combination of poor weather conditions, availability of fishermen, and mechanical problems (failure of the onboard power packs) limited the amount of samples collected from LFA 33/34 to 37 in January. Hemolymph sample collection was also limited in LFA 25 due to delays in identifying fishermen to participate in the project. With such low numbers, it was not possible to establish reference ranges. With the success of the ALMQ proposal in the Atlantic Innovation Fund Round V, it was decided to co-ordinate sample collection for the Biochemistry project with the moult and quality sampling efforts being extended into the Northumberland Strait. Meetings were held with the PEIFA, the MFU, and other local fisher associations to identify the optimal sampling sites. Subsequently, fishermen were identified and sampling will commence shorty. The 2008-2009 season should provide adequate data for analysis to determine if the data can be pooled, or if reference ranges for lobsters by sex, moult stage and/or season are required.

While only a small number of samples were collected, a subgroup of these were forwarded to Dr. Ernie Chang's laboratory in Bodega Bay, CA for crustacean hyperglycemic hormone (CHH) analysis. There were not clear associations between CHH levels and any of the biochemistry parameters; however, a possible regional trend (LFA 33/34 vs 25) was observed. Further sampling and analysis should determine if the trend truly exists.

At the End of Year 1 (December 2007):

The stability study to determine the optimal method (refrigeration vs freezing at -20 degrees C) to store plasma samples collected in the field prior to shipment to the AVC Diagnostic Laboratory was completed in October. It was concluded that refrigeration of the samples was the preferred method and that the samples should be evaluated within 2 to 3 days to obtain optimal results.

A field manual describing correct sample collection methods was prepared and distributed to the field technicians. Sample collection in LFA 33/34 began in October. Poor weather conditions hampered sampling in December. Sampling will continue as possible through the winter. The first sample collection from LFA 25 was completed in November and will resume in the spring of 2008. There have been some technical difficulties associated with performing the hemolymph PH measurements. These are being investigated and a solution is anticipated.

Total Project Cost: $ 118,591.00
Funding provided by PEIASCI: $26,090 (Year 1 $13,045 & Year 2 $13,045)

Other Funding Partners: Contributions to support the research programs of the AVCLSC are provided by the following: ACOA & the Atlantic Innovation Fund; AVC In-King Faculty Consultations; private lobster pounds and holding companies; Department of Fisheries & Oceans, Canada; Millbrook First Nation; PEI Atlantic Shrimp Corp; Provincial governments; and UPEI In-Kind Administrative Support.

27. ALMQ Service Unit

Proponent: Lobster Science Centre
Project Number: 07-LSC-027
Project Status: complete
Project Start/Completion Date: July 2007 -

Project Objective:

The Service Unit is to serve several purposes. It will allow for a rapid scientific response to lobster health problems, and offer an ongoing lobster health management approach to participating lobster holding facilities. The Service Unit will utilize various diagnostic tests developed in the laboratory to rule out infectious diseases, detrimental handling practices and other causes of decreased productivity, when investigating the impact of moult timing on lobster quality. The Service Unit will conduct risk factor analyses in an attempt to identify causes of productivity losses, including the assessment of handling practices at various phases of the lobster industry. Finally, the Service Unit will, through its hands on approach, increase the awareness of lobster research via on-going communications and education of various segments of the lobster industry.

Summary of Outcome:

This project is ongoing. All field staff have now been hired, including Eric Branton, our Yarmouth-based field technician.

A special staff training workshop on how to perform lobster necropsy and how to properly sample lobsters for diagnostic purposes was conducted in July 2007. Following this training, quick response and sampling of sick and moribund lobsters by our field staff was done on several occasions. We have been contacted for advice on several occasions via e-mails or telephone.

Additionally, we started receiving an increasing number of diagnostic case submissions directly at our facilities. Our clients so far have included DFO (Gulf, Maritimes & Quebec), private sector companies (holding pounds in NS & NB; tank manufacturer in QC), fishermen (NS & PE), and research organizations in Ireland and Portugal. As part of the lobster pound health assessment program, tissues from lobsters were collected and examined. The antennal glands of the lobsters were noted to be enlarged on gross examination; however, no histologic lesions were observed to explain this finding.

Another aspect of the ALMQ Service Unit was the outreach and education component; in that aspect, we were able to organize, deliver and complete a special workshop on lobster moult staging for a private sector company in NS. Finally, a Shell Disease awareness campaign has been initiated through the Service Unit, with suspect cases being sent to the AVCLSC or when possible, one of our technicians will pick up the lobster directly from the fishermen.

We are working on an industry awareness campaign for the *Service Unit;* Blair Cabot spent some time with fishermen and pound operators on slow days to talk about the *Service Unit.* A 'promotional brochure' for this service is being developed and will be distributed across Atlantic Canada momentarily. We are also working with DFO to streamline directly to us any lobster health/disease inquiries they receive from industry. Late 2007 and early 2008 saw an increased number of damaged lobsters landed in LFAs 33/34. Several photographs and samples (live and dead) have been examined. No unusual lesions were observed; the presence of melanisation on the wounds suggests that these lesions were at least several days/weeks old.

Total Project Cost: $115,462
Funding provided by PEIASCI: $ 25,400 (Year 1 $12,700 & Year 2 $12,700)

Other Funding Partners: Contributions to support the research programs of the AVCLSC are provided by the following: ACOA & the Atlantic Innovation Fund;AVC In-Kind Faculty Consultations; private lobster pounds & holding companies; Department of Fisheries & Oceans, Canada; Millbrook First Nation; PEI Atlantic Shrimp Corp; Provincial governments; and UPEI In-Kind Administrative Support.

28. Bumper Car Disease - Phase II Molecular Pathogenesis

Proponent: Lobster Science Centre
Project Number: 07-LSC-028
Project Status: complete
Project Start/Completion Date: July 2007 -

This project will explore the genes identified in the first phase of this project from the Bumper car (*Anophryoides haemophila)* cDNA library (03-LSC-08) by monitoring gene expression changes and correlation with tissue/organ damage. From Phase I, a Bumper car cDNA library was constructed from which almost 10,000 expressed sequence tags (ESTs) were sequenced and revealed approximately 1,000 recognized genes. Not unexpectedly, about 40% of the *A. Haemophila* ESTs have shown similarity to genes found within the genomes of other protozoa, especially the related ciliate, *Tetrahymena.* Sequence comparisons with other known protozoan parasites have lead to the identification of specific groups of genes in the Bumper car parasite that are suggestive of how the parasite attaches to the lobster host (e.g. many trichocyst matrix proteins), erodes the lobster carapace (e.g. two chitinases) and invades other body tissues to compromise the host's immune response (e.g. multiple cysteine proteases). The trichocyst matrix proteins are a collection of proteins that ciliated parasites use both as a defense mechanism and as a method of holding onto prey. Chitinases are enzymes that digest chitin, a compound which makes up to 58% of the dry weight of the lobsters carapace. Chitinase enzymes have not previously been found in ciliates that are parasites of crustacean and may represent a unique mechanism for gaining access to the lobster host's cellular tissues. The cysteine and other proteases are enzymes which have the potential to digest tissues and are proposed in other parasites (e.g. *Leishmania* - Chagas disease, *Plasmodium* - Malaria, and many protozoan parasites of fish) to allow them to evade the host's immune system and spread systematically throughout the tissues of the host - eventually killing the host. A similar relationship may exist for the bumper car parasite and the lobster.

Project Objective:

To pursue genes identified during phase I of our Bumper car genomics research to gain a better understanding of the mechanisms that this parasite uses to infect and kill lobsters. We predict therefore, that the Bumper car parasite secretes these enzymes as a mechanism to digest the carapace and gain entry to the lobster internal tissues. These initial genomic results are very encouraging and provide us with some molecular evidence towards the mechanism of pathogenesis and our understanding of the interactions between the parasite and the lobster.

The economic benefits of knowing how the parasite can kill lobsters will provide solid evidence that will hopefully lead quickly to the development of management strategies and protocols (lobster selection, cleaning procedures, handling procedures etc.) to prevent and control Bumper car within lobster holding facilities. This would reduce overall shrink leading to more harvested lobsters actually reaching the consumer. The research results will potentially extend to fishermen as well. We anticipate that our results will further support the role that careful handling practices play in preventing damage to the lobster carapace. Improved handling will increase the likelihood of a lobster making it to market, as damaged lobster put in holding are more susceptible to pathogens. The emphasis on handling practices by fishermen will logically lead to better quality lobsters entering holding and therefore, also reaching the consumer. Hopefully, these practices will translate into increased economic gain for all parties involved.

Summary of Outcome:

The Bumper Car (*Anophryoides haemophila)* cDNA library (EST sequencing project) produced a suite of genes that were selected for further evaluation of their potential involvement in the ability to penetrate and kill lobsters. The genes of interest were three proteases (cathepsins B1, B2 & L), two chitinases (endo- and exo-chitinase) and a further three housekeeping genes (GAPDH, EF-I-alpha and I8SrRNA) were included as controls in gene expression studies. The *A. Haemophila* was grown under different *in vitro* experimental conditions (basic ciliate media with or without the following components; commercial chitin, lobster carapace pieces and lobster serum). We began to characterize the changes in gene expression by quantitative reverse transcriptase real time polymerase chain reaction (qRT-PCR) analysis of the 3 proteases, 2 chitinases and 3 reference genes. A considerable amount of time was spent designing and evaluating primer sets and refining the RT conversion to cDNA procedures prior to commencing the evaluation of gene expression changes in the *Anophryoides haemophila* ciliate grown under different conditions (listed above). Complementary experiments to evaluate the chitinase enzymes activities were carried out using commercially available assay.

The three proteases showed little variation under the growth conditions (presence of chitin, carapace or lobster serum or carapace pieces) as gene expression levels were essentially stable. This is in contrast to the data from the EST database, where the protease, cathepsin L was expressed at higher levels than the other selected genes. No satisfactory reason was determined for the differences in expression at the time of the cDNA library construction versus the current experiments as the media conditions were the same in both experiments.

The most important results are from the chitinase enzyme assays and gene expression data that suggests that they are not involved in lobster invasion as there were no differences in gene expression or chitinase activity in ciliates grown in the presence of chitin, carapace or lobster serum. This is an important finding as it provides further evidence that *Anophryoides haemophila* is an opportunistic pathogen rather than a primary pathogen. Therefore, the ciliate does not possess enzymes that allow it to penetrate the lobsters carapace and likely enters through wounds in the lobsters carapace. This lends more support for the careful handling and selection of lobsters for impoundment and suggests that all holding facilities should include a protocol to prevent the inclusion of wounded lobsters that would be more susceptible to infection by this opportunistic ciliated pathogen. In all likelihood, the alternative hypothesis that *A. Haemophila* likely uses these chitinase enzymes for a previously unobserved aspect of its life history strategy that of encystment/excystment and may be a survival mechanism to periods of low nutrients levels is more in keeping with the low levels of gene expression observed.

Total Project Cost: $45,480
Funding provided by PEIASCI: $ 36,120 (over 1 year)

Other Funding Partners:

Contributions to support the research programs of the AVCLSC are provided by the following: ACOA & the Atlantic Innovation Fund; AVC In-Kind Faculty Consultations; Clearwater Fine Foods; Ocean Choice PEI; Seafood 2000; Department of Fisheries & Oceans, Canada; ELANCO; Millbrook First Nation; Province of Newfoundland and Labrador; Province of Nova Scotia; Province of Prince Edward Island; and UPEI In-Kind Administrative Support.

29. Shell Disease in Lobsters - Awareness Raising and Passive Monitoring in Atlantic Canada

Proponent: Lobster Science Centre
Project Number: 08-LSC-029
Project Status: complete
Project Start/Completion Date: January 2008

Project Objective:

The initial activity is to provide up-to-date knowledge on shell disease to fishers and other sectors of the lobster fishery in Atlantic Canada. The second activity to collect samples of lobsters which apparently have shell disease. The third activity is report findings of shell disease to all sectors of the lobster fishery.

Summary of Outcome:

At the end of Year 1:
In spring and summer 2008, several hundred 'wanted' posters were distributed to the lobster industry throughout Atlantic Canada, illustrating shell disease and how to deliver affected lobsters to the AVCLSC laboratories. Subsequently, lobsters were examined, photographed and samples taken for processing in the bacteriology research laboratory at the Atlantic Veterinary College, and in the laboratories of New England Aquarium and the University of Louisiana - Lafayette. To date, less than 20 lobsters suspected of shell disease have been received at the AVCLSC.

We will collect more samples in 2009 and plan to make presentations to various fisher organizations and DFO fishery officers throughout Atlantic Canada.

Total Project Cost: $123,710
Funding provided by PEIASCI: $ 49,484 over 2 years ($26,806 Year 1 & $22,678 Year 2)

Other Funding Partners:

Contributions to support the research programs of the AVCLSC are provided by the following: ACOA & the Atlantic Innovation Fund; AVC In-King Faculty Consultations; private lobster pounds & holding companies; Department of Fisheries and Oceans, Canada; Millbrook First Nation; PEI Atlantic Shrimp Corp; Provincial governments; and, UPEI In-Kind Administrative Support.

Project Contact:
Rick Cawthorn
Tel: (902)894-2884

30. Hemolymph Biochemistry Profiles in American Lobsters: Establishing Reference Intervals and Determining Their Potential Role in the Assessment of Nutritional Status

Proponent: Lobster Science Centre
Project Number: 09-LSC-030
Project Status: Complete
Project Start/Completion Date: May 2009 - 2011

Project Objective:

Blood biochemistry analysis in human and veterinary medicine is used to detect dysfunction or injury of organs or tissues (e.g. kidney, liver, or muscle) not necessarily obvious during a general physical examination. Analysis generally includes examination of the levels of salts, minerals, metabolites, and enzyme activity in blood samples. When multiple tests are performed on the same blood sample it is commonly referred to as a 'biochemistry profile' or 'biochemistry panel'. Biochemistry profiles have multiple applications: general health assessment in combination with a physical exam and history; diagnosing or localising disease processes; and as components of therapeutic drug trials to determine the potential toxic effects of new pharmaceutical agents on organ systems. The potential exists to use hemolymph biochemistry panels in a similar fashion in lobsters.

Summary of Outcome:

At the end of Year 2 (December 2011):
Objective 1:
Approximately 1,500 hemolymph plasma samples were collected (as weather conditions allowed) from lobsters in LFAs 25, 26A, 33 and 34 for establishment of reference intervals biochemistry profiles from May 2009 to July 2010. It was not possible to obtain a complete dataset for LFA 25, however, resulting in its limited analysis. Development of reference intervals for the other LFAs revealed significant differences for lobster populations based on sex, reproductive status, region (southwest Nova Scotia vs Gulf of St. Lawrence), and moult stage. Notable examples include the marked increases in cholesterol and triglyceride levels observed in female lobsters only near the time of spawning (presumably in association with egg production) and the higher levels of energy-related metabolites such as glucose in lobsters from the LFA 26 compared to LFAs 33 and 34. The latter is presumed to reflect a higher metabolic rate of lobsters found in the warmer waters of the southern Gulf.

Objective 2:
Hemolymph for biochemistry profiles and Crustacean Hyperglycemic Hormone (CHH) levels and, tissue (tail/abdomen, pincher and crusher claws, hepatopancreas) samples for lipid and glycogen analysis as indicators of energy reserves, were collected from 88 lobsters representing different stages of the moult cycle in 2009 and 2010 out of Georgetown, PE.. The hepatopancreas was shown to be the major reserve for both lipid and glycogen at all stages of the moult cycle with lipid being the dominant energy reserve in the lobster. As expected, lipid levels remained relatively constant until the late pre-moult and post-moult which represents the period of decreased food intake just prior to and after the moult. Somewhat unexpectedly, a small pre-moult peak in hepatopancreas glycogen concentration was noted. This is thought to be related to the conservation and redistribution of shell (cuticle) chitin as its glycogen precursor, similar to the conservation of cuticle-derived minerals in the gastroliths. Very good

correlations were observed when hemolymph triglyceride, cholesterol, and total protein concentrations were compared to hepatopancreas lipid content in intermoult male lobsters only, suggesting that these hemolymph parameters may be useful in determining tissue energy reserves in this group. (Note: CHH analysis results by the referral lab are still pending but expected within the early part of 2012)

Funding provided by PEIASC (45%) : $ 77,500. (Year 1 $38,750 & Year 2 $ 38,750)

Other Funding Partners :
Contributions to support the research programs of the AVCLSC are provided by the following: ACOA & the Atlantic Innovation Fund; AVC In-Kind Faculty Consultations; private lobster pounds & holding companies; Department of Fisheries & Oceans, Canada; Millbrook First Nation; PEI Atlantic Shrimp Corp; Provincial governments; and, UPEI In-Kind Administrative Support.

Project Contact : Andrea Battison
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Total Project Cost: **$**172,050

Funding provided by PEIASC: $77,500 over two years

31. Online Lobster Inventory System Phase 2.2: Processing Sector

Proponent: Lobster Science Centre
Project Number: 09-LSC-031
Project Status: Complete
Project Start/Completion Date: July 2009 - 2011

Project Objective:

There is currently a real need from the lobster industry for real-time and accurate information on all lobster inventories, including live and frozen products. Having a third-party as the manager of an industry-wide inventory system is paramount to ensuring that data collected are accurate, that compliance is high and that participant's individual and corporate information remains confidential. The AVC Lobster Science Centre is uniquely positioned to guarantee that the data reported on such industry-wide inventory system remain precise and credible.

We are proposing to first design, develop and then implement an inventory system for the processing sector of the lobster industry. Weekly reports will be given to participants. This project is the second Phase of an online system project that was initiated in the fall of 2006 at the request of industry. Through the initial Phase, we built a reporting system for live lobster inventory levels. This second Phase will allow us to look at establishing a similar system for the processing sector of the industry, with an initial focus on the PEI seafood processing industry. We anticipate that approximately one year will be required to build and establish a credible inventory data reporting system.

Summary of Outcome:

This project proved to be successful. After numerous consultations with different industry partners, a prototype was built. Several rounds of revisions were done to maximize the value of this product inventory system, and ensure that accurate data would be presented while respecting the confidential nature of the information provided by the participating companies. The reporting system is ready to go. Participating companies would provide total raw product (by defined category) and based on assumptions identified, we would provide total finished product in the report. The report would provide the difference between the current and previous week. Reports would be sent out on a weekly basis to participating companies. To protect confidentiality of participants we would not be able to show inventory data for specific categories if less than 3 companies are contributing data or if individual company information can be determined by elimination.

Other Funding Partners :
Contributions to support the research programs of the AVCLSC are provided by the following: ACOA & the Atlantic Innovation Fund; AVC In-Kind Faculty Consultations; private lobster pounds & holding companies; Department of Fisheries & Oceans, Canada; Millbrook First Nation; PEI Atlantic Shrimp Corp; Provincial governments; and, UPEI In-Kind Administrative Support.

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Total Project Cost: $67,380

Funding provided by PEIASC: $30,325 over 1 year

32. Fishing for Biomarkers with a Lobster DNA Microarray: Assessing Moult cycle, Reproductive status and Host-pathogen interactions

Proponent: Lobster Science Centre

Project Number: 09-LSC-032
Project Status: Complete
Project Start/Completion Date: July 2009 - 2011

Project Objective:

The AVC Lobster Science Centre's goal is to further our knowledge and understanding of lobster biology and to translate this information into methods, tools and management practices that will contribute to the long term sustainability of the Canadian Lobster Industry. The specific objective of this research proposal focuses on the first two years of a multi-year project (ALMQ) that uses our recently developed lobster DNA microarray for discovering biomarkers associated with specific lobster physiological states and will then concentrate on developing methods for non-lethal assessment of lobsters.

Microarray protocols will follow established standards and provide sufficient information for the experiment to be repeated independently. Identification of lobster biomarkers will be performed using both archived and fresh lobster tissues (moult and reproductive status), as well through lobsters experimentally infected with *Aerococcus* (Gaffkemia) and *Anophryoides* (Bumper Car) at the AVC. Bioinformatic analysis will be used for clustering gene expression data from specific tissues and conditions (moult stage, reproductive status, infection experiments) prior to confirmation by Real-time PCR to refine the selection of biomarkers. Consequently, the relationship between the biochemical, biological and molecular data will provide evidence for the final selection of gene expression markers for the different physiological states.

Summary of Outcome:

The Lobster DNA microarray was constructed by sequencing of 20,000 clones from an American lobster (*Homarus americanus*) cDNA library made from 10 tissues from inter-moult and post-moult adult male and female lobsters. Intensive computational analysis (bioinformatics) of this new DNA sequence data and of gene sequences already available through the National Center for Biotechnology Information genetic sequence database (GenBank) allowed us to develop a > 14,500 gene microarray that represents ~75% of the entire transcribed genes from the American lobster. This microarray tool has allowed for the discovery of numerous candidate gene biomarkers related to (1) lobster immunity to two known disease agents, the gram positive pathogen *Aerococcus viridans* var. *homari* (Gaffkemia) and the ciliated parasite *Anophryoides haemophila* (Bumper car disease), (2) developmental markers of lobster larval stages and (3) reproductive markers for ovary development in female lobsters have been delineated and a site of gene markers are being carried forward to the next stages of the research project to evaluate their predictive value for the lobster fishery. The creation of the lobster microarray has subsequently allowed us to extend the use of this foundation tool to explore novel pathogens, define stress biomarkers, toxicological markers and monitor changes in moult recovery.

Total Project Cost: $474,301

Funding provided by PEIASC: $118,575 over two years

33. Development of Non-lethal Tests for the Determination of Ovary Maturity in American Lobsters

Proponent: Lobster Science Centre
Project Number: 11-LSC-033
Project Status: Complete

Project Start/Completion Date: April 2011 - 2013

Project Objective:

The ability to accurately determine the size at which a female lobster reaches reproductive maturity is important for developing management protocols to ensure the sustainability of the lobster fishery in Prince Edward Island and the rest of Atlantic Canada. The information is used, along with other factors, to set minimum legal size (MLS) limits which vary from region to region. A goal of MLS limits is to protect a sufficient proportion of mature (i.e., capable of spawning) lobsters from the fishery so that these lobsters will survive to replace, through new recruitment, animals which are lost due to fishing effort and natural mortality. Currently, maturity staging requires the direct examination of the ovaries which is a lethal procedure. The goal of this project is to develop a non-lethal method to accurately determine ovary maturity. This will be accomplished by identifying a protein marker in the lobsters' hemolymph (blood) that correlates with ovary maturity.

Summary of Outcome:

Total Project Cost:

Funding provided by PEIASC: $87,315 over one year

34. Fishing for Biomarkers with a Lobster DNA Microarray: Assessing Moult cycle, Reproductive status and Host-pathogen interactions (Phase II)

Proponent: Lobster Science Centre
Project Number: 11-LSC-034
Project Status: Complete

Project Start/Completion Date: October 2011 - December 2011

Project Objective:

The proposed research uses our recently developed lobster DNA microarray for discovering biomarkers associated with specific lobster physiological states (moult, reproduction, iimmune response) and developing methods for non-lethal assessment of lobsters. The lobster DNA microarray has provided a unique foundation tool that has broad application in understanding previously undiscovered aspects of the biology of lobsters, and therefore the information discovered has the potential to directly impact the industry by allowing them to make more informed decisions. Over the past decade, microarrays have been applied with success to the molecular profiling of many complex biological processes, including the understanding of development, reproduction and disease pathogenesis in human and veterinary medicine. These studies have created a more detailed understanding of biology as well as in the identification of potential gene and gene clusters (biomarkers) that are consistently associated with a specific biological process.

Summary of Outcome:

The microarray assessment of larval lobster development and moult cycle revealed 550 genes of interest that showed distinct expression differences between larval stages 1-4. Not surprisingly, the major differences in gene expression occurred at the critical metamorphosis as the larvae transitioned from a pelagic life stage (Stages 1-3) to the benthic stage (Stage 4). Subsequent analysis reduced the initial dataset to a manageable number of candidate genes that included previously recognized development, immunological, moult and metabolism associated genes (including argonaute, sptzle2, toll-like receptor, farnesoic-acid methyltransferase, antilipopolysaccharides, prophenoloxidases, cuticle genes, pupal and arthroidal proteins) which were considered further for confirmation of expression differences by complementary real time quantitative PCR. Gene expression variation between year classes was minimal suggesting that the development and moult biomarkers are good candidates for further consideration in assessing non-lethal markers.

Female lobster reproductive status was assessed using the lobster specific microarray by initially classifying the female lobsters based on moult stage and the different ovary stages (1-5) by gross and histological methods. The array results indicated a suite of 896 genes that are differentially expressed between stages, from this a suite of 13 genes (including vitellogenins, dopamines and gonad inhibiting hormone) were selected for further evaluation that show distinct gene expression differences at the critical ovary stage 4a and 4b commitment stage. Analysis of the expression patterns of 21 genes (target and reference genes) by complementary real time quantitative PCR was used to further evaluate and confirm trends observed in the microarray data. Variation between year classes was not evident and suggests that the ovary stage biomarkers maybe good candidates for further exploration as predictors of reproductive commitment in assessing non-lethal markers.

Other Funding Partners :
Contributions to support the research programs of the AVCLSC are provided by the following: ACOA & the Atlantic Innovation Fund; AVC In-Kind Faculty Consultations; private lobster pounds & holding companies; Department of Fisheries & Oceans, Canada; Millbrook First Nation; PEI Atlantic Shrimp Corp; Provincial governments; and, UPEI In-Kind Administrative

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Total Project Cost: $188,304

Funding provided by PEIASC: $112,982 over one year

35. Assessing Environmental Contaminant Impacts on the Health of Lobsters

Proponent: Lobster Science Centre
Project Number: 12-LSC-035
Project Status: Complete
Project Start/Completion Date: December 2012 - 2014

Project Objective:

The AVC Lobster Science Centre's goal is to further our knowledge and understanding of lobster biology and to translate this information into methods, tools for management practices that will contribute to the long term sustainability of the Canadian Lobster Industry.

The purpose of this study is to determine the effects of pesticides that are routinely used in the agriculture and aquaculture industry on a non-target organism of commercial importance, the American lobster, *H. americanus*. It is hypothesized that exposure of *H. americanus* to specific pesticides will cause changes in gene expression that will impact survival and development (moult). In addition, pesticide exposures will specifically impact the immune response impairing the ability of the lobster to overcome pathogen challenge. Finally, we hypothesize that the severity of the developmental delays and immune impairment will intensify as pesticide concentrations increase, and that there will be significant changes in gene expression levels in any animals that survive the exposure experiment.

Summary of Outcome:

Experimental exposures of juvenile lobsters (stage IV) to two pesticides (permethrin & deltamethrin) were conducted to evaluate the impact on lobster health as determined by measuring global gene expression changes using RNASeq (transcriptomics). Permethrin is a commonly used active ingredient in agricultural and domestic insecticides. Deltamethrin is used in the treatment of sea lice, a crustacean ectoparasite of Atlantic salmon. Both permethrin and deltamethrin kill invertebrate pests by blocking transmission of impulses on neural pathways. The impact of these two pesticides on juvenile lobster has not previously been studied in the context of transcriptomic approaches. These studies were paired with supportive growth and metabolic studies in collaboration with Homarus Inc. and Environment Canada.

Stage IV lobsters were exposed to either sediment-bound concentrations of deltamethrin or permethrin at 20°C for 14 days. For permethrin there were 446 differentially expressed genes. Of these, 215, or ~48%, have similarity to proteins in GenBank. The expression of fifteen cuticular protein related genes was significantly negatively altered. Only one known detoxification-related protein, glutathione s-transferase, gene expression was significantly upregulated over 24-44 fold with increasing concentrations. Four immune and stress-related genes were differentially expressed including an anti-microbial peptide (down regulated 300 fold), a lectin (upregulated >20 fold) and two stress-inducible protein genes (down regulated >20 fold).

For deltamethrin, there were 3195 differentially expressed genes. Of these, 1307, or ~41%, have similarity to proteins in GenBank. Cuticular protein-related genes represented almost 10% of these annotated genes. At high deltamethrin concentrations very large decreases in cuticular protein-related gene expression occurred while moderate increases in expression were observed in lower deltamethrin concentrations. Twelve recognized detoxification-related protein's gene expressions were found to be significantly altered and the magnitude of upregulation correlated with deltamethrin concentration. Interestingly, there were significant but in most cases moderate changes in gene expression for sixty-six immune-related proteins. Exceptions to this trend were a very large increase in expression for crustin p and several large decreases in expression at the highest concentration of deltamethrin for several different spatzle related genes.

In conclusion, RNA-Seq has proven to be a very powerful tool for identifying differentially expressed genes in larval H. americanus exposed to the pesticides permethrin and deltamethrin. There are distinct differential responses to these two pyrethroid pesticides. Several functional classes of genes have demonstrated large alterations in their gene expression including cuticle-related proteins, detoxification proteins and immune and stress-related proteins. This data clearly demonstrates that pesticide exposure does alter gene expression but further study is required to determine what the range of environmentally relevant concentrations are in habitats where larval lobsters settle. Future studies

should address the issues of pulse exposures and longer contact time with sediment-bound pesticides for the impact on cryptic juvenile lobster stages.

Total Project Cost: $ 144,998

Funding provided by PEIASC: $ 84,988

Other Funding Partners:
Contributions to support the research programs of the AVCLSC have been provided by or in the past by the following: ACOA & the Atlantic Innovation Fund; AVC In-Kind Faculty Consultations; private lobster pounds & holding companies; Department of Fisheries & Oceans, Canada; Millbrook First Nation; PEI Atlantic Shrimp Corp; Provincial governments; and, UPEI In-Kind Administrative Support.

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36. Refinement and Expansion of Biomarkers of Lobster Health and Quality

Proponent: Lobster Science Centre
Project Number: 13-LSC-036
Project Status: Complete
Project Start/Completion Date: December 2013 - 2015

Project Objectives:

1. To assimilate all of the individual genomic projects that have been undertaken at the AVC Lobster Science Centre into a comprehensive understanding of how each

individual physiological process affects overall lobster quality and sustainability; thereby reducing economic losses throughout the entire lobster chain of custody.

2. To expand upon our understanding of lobster disease and immune response to pathogen threats to lobster by refining our diagnostic biomarkers to prevent storage related mortalities.

3. To explore the potential impact of anthropogenic stressors on lobster biomass, such as pollutants caused by industrial process, in an effort to protect lobster at their most vulnerable life stages.

Summary of Outcome:

A comprehensive in-depth transcriptomic analyses of responses of the American Lobster (*Homarus americanus)* under varying conditions (infection, contaminant exposure, holding, storage, transport, moult recovery etc.) has identified hundreds of candidate genes that may be important biomarkers. Diagnostic development based on functional assays of these biomarkers is the next logical step. The wealth of information arising from these methods and analyses has considerable potential for improving industry practices for understanding lobster health and quality.

Total Project Cost:$108,140

Funding provided by PEIASC: $108,140 over one year

Other Funding Partners:
Contributions to support the research programs of the AVCLSC have been provided by or in the past by the following: ACOA and the Atlantic Innovation Fund; AVC In-kind Faculty Consultations; private lobster pounds and holding companies; Department of Fisheries & Oceans, Canada; Millbrook First Nation; PEI Atlantic Shrimp Corp.; Provincial governments; and, UPEI In-kind Administrative Support.

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37. Assessing Environmental Contaminant Impacts on the Health of Lobsters - Phase II

Proponent: Lobster Science Centre
Project Number: 14-LSC-037
Project Status: Complete
Project Start/Completion Date: December 2014 - 2017

Project Objectives:

The AVCLSC will conduct a series of acute and chronic exposure trials incorporating several concentrations of environmental contaminants arising from agricultural and aquaculture practices to determine the impact on larval lobster health. Exposure trials will determine the lethal exposure limits of larval lobster to these contaminants and more importantly, identify the sub-lethal effect that they have on immunity, stress and development using physiological and molecular endpoints.

Summary of Outcome:

The controlled experimental exposures of juvenile American lobsters (stage IV) to the agricultural pesticide chlorpyrifos and the aquaculture pesticide Salmosan (azamethiphos) were carried out to determine the impact on lobster health as determined by measuring morphological correlates of growth and development and global gene expression changes using RNASeq (transcriptomics). Azamethiphos is used as an emergency treatment to reduce infestations of a crustacean parasite, the sea louse (Lepeophtherius salmonis). Treatments are usually applied as bath treatments to farmed salmon by use of tarping or skirting the sea cage or through use of well boats and once treatments are complete, this highly water soluble pesticide is released from cages potentially leading to decreased survival in non-target organisms such as the lobster residing close to the cages. Effects on adult American lobster have been investigated but limited information exists on effects to larval lobster stages.

Stage IV larvae 3 hour exposures, to mimic potential lobster exposed during the dispersal of Salmosan, showed that the median lethal concentration was 20.45 $\pm$ 12.7 µg/L for stage IV larvae. Post-exposure, surviving stage IV larvae were raised to stage V to assess sublethal effects on growth parameters. General linear model analysis (a = 0.05) established that intermoult period, specific growth rate, and moult increment were not significantly affected by sublethal concentrations when compared to the control. Transcriptomic analysis identified differentially expressed genes between lobsters in each treatment group. Larvae exposed to the higher pesticide concentrations had more genes with a statistically significant fold change when compared to the control group than the two lower concentration treatments. Several changes in significant gene expression were noted with biological relevance in biotransformation of xenobiotics (cytochrome P450-like), oxygen delivery (hemocyanin subunit), immunity (anti-lipopolysaccharide factor), and oxidative stress response (glutaredoxin & glutathion Stransferase).

The organophosphate pesticide chlorpyrifos is used in Atlantic Canada to target agricultural arthropod pests such as the Colorado potato beetle (Leptinotarsa decemlineata), cabbage maggot (Delia radicum), darksided cutworm (Euxoa messoria) and wireworm (Agriotes sputator). Larval decapod crustaceans are some of the most sensitive aquatic invertebrates to chlorpyrifos, yet no data exists on the impacts to H. americanus specifically.

Chlorpyrifos 48-hour acute exposures of Stage IV larvae revealed that the median lethal concentration of chlorpyrifos was established to be 1.56 ± 0.50 µg/L for stage IV larvae. This sublethal exposure indicated that growth parameters were significantly affected at the tested chlorpyrifos concentration of

0.82 µg/L. Using general linear model analysis (a = 0.05), it was determined that larvae exposed to this treatment displayed significant increase of intermoult period and significant decrease of both specific growth rate and moult increment when compared to the control treatment. Additionally, exposed lobsters displayed statistically significant inhibition of acetylcholinesterase (AChE) (~ 80 %) at 48 h of exposure. Larvae surviving to moult post-exposure displayed no significant inhibition of AChE compared to controls. A trend towards increased inhibition as exposure concentration increased was however observed which could suggest delayed response to predators or environmental cues. Transcriptomics revealed that larvae exposed to increasing concentrations of chlorpyrifos had more differentially expressed genes. Biologically relevant significant changes in gene expression were noted for the following categories; growth (gastrolith & chitinase), immunity (ALF isoforms & crustin), and oxidative stress response (glutathione peroxidase).

In conclusion, there were significant differential responses to these two pesticides. Several functional classes of genes have demonstrated significant alterations in expression that clearly demonstrates that sublethal pesticide exposure does impact lobster larvae. Further study is required to more precisely determine the relevance of these gene expression changes to larval lobster's settlement and long term survival. Future studies should address the issues of pulse exposures and longer contact time with sediment-bound pesticides for the impact on cryptic vs. vagile juvenile lobster stages. The implications of our findings suggest that larvae impaired by these pesticides may remain in the water column longer, thereby exposing them to pelagic predators. Similar to previous findings on pyrethroids, delayed moulting may impact settling and alter larvae establishing the cryptic resident stage in the life cycle.

Total Project Cost:$345,066

Funding provided by PEIASC: $99,270 over one year

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