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CAREER: Physiological Genetics of the Dwarf Surf Clam, *Mulinia lateralis*

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Final Report for Period: 04/2008 - 08/2008

Submitted on: 10/23/2009

Principal Investigator: Rawson, Paul D.

Award ID: 0133349

Organization: University of Maine

Submitted By:

Rawson, Paul - Principal Investigator

Title:

CAREER: Physiological Genetics of the Dwarf Surf Clam, *Mulinia lateralis*.

Project Participants

Senior Personnel

Name: Rawson, Paul

Worked for more than 160 Hours: Yes

Contribution to Project:

Rawson was the principal investigator for this award and was involved in all research and education activities conducted under this award. He supervised the initial salinity challenge experiments with larval and juvenile blue mussels (*Mytilus edulis*) conducted by graduate student RA Moreau and undergraduate research assistant Kranich and the subsequent isolation of RNA from these mussels. He worked closely with Harper, Moreau and Kranich on the construction of multiple and DNA sequence based characterization of cDNA libraries based on the RNA collected from these experiments. These libraries were used to identify genes expressed by mussels when faces with chronic salinity stress. Rawson had sole responsibility for conducting a 'virtual northern' analysis of the genes isolated from the cDNA libraries in order to confirm the stage (juvenile versus larval) and treatment (salinity challenged versus control) specific expression of the genes isolated from the cDNA libraries. He was also responsible for the design of real-time quantitative PCR protocols for a subset of the genes identified from the virtual northern analysis as being differentially expressed. Rawson worked together with Moreau and a second graduate RA, Katsman, to repeat the salinity challenge experiment on a three additional sets of blue mussels. The first experiment was a fine-scale temporally sampling of *M. edulis* mussels taken after a hyposaline challenge. The second experiment was also a fine-scale temporal sampling of mussels but used the closely related species *M. trossulus*. The third experiment utilized *M. edulis* mussels but in this case oxygen uptake and ammonia excretion was measured individual mussels in order to correlated patterns of physiological acclimation with gene expression. For each group, RNA was isolated by Rawson together with Moreau (initially) or Katsman. The isolated RNAs were then used in QPCR reactions to estimate gene expression for particular candidate genes. Rawson was solely responsible for conducting the QPCR assays and has taken primary responsibility for the data analysis stemming from each experiment.

Rawson also worked with teachers from six school districts in Maine to develop hands-on, inquiry-based exercises with aquaculture species that demonstrate concepts in chemistry, physics, and genetics. Education and outreach activities included three summer workshops involving multiple teachers as well as interactions with individual teachers and the development of a website for dissemination of the materials developed.

Rawson received a total of 5 months of summer salary support from this award.

Post-doc

Name: Harper, Fiona

Worked for more than 160 Hours: Yes

Contribution to Project:

Dr. Harper was involved in the work conducted under this award from September 2004 through August 2005. Dr. Harper assisted with developing the protocols for algal culture and mussel spawning and larviculture in the recirculating seawater system that was established at the University of Maine, under this award. She also assisted with the isolation of RNA from larval mussels and with the construction and initial characterization of subtraction cDNA libraries and assisted graduate student Valerie Moreau with the development of experimental protocols for estimating oxygen consumption and ammonia excretion in relatively small juvenile mussels. Dr. Harper left the project in 2005 to take a faculty position at Rollins College.

Received 10.5 months of a post-doctoral level stipend from this award.

Graduate Student

Name: Moreau, Valerie

Worked for more than 160 Hours: Yes

Contribution to Project:

Ms. Moreau's Ph.D. research was focused on determining to what degree the patterns of gene expression and physiological acclimation are correlated in blue mussels that have been exposed to hyposaline (chronic) conditions. She assisted in the development and analysis of differentially expressed cDNA libraries and had a lead role in the development of methods for measuring the feeding, absorption, excretion and oxygen consumption rates in small juvenile mussels. Ms. Moreau collected and maintained juvenile mussels, sampled from a local wild population, and reared them under common garden conditions. These mussels were subsequently divided into two groups and Valerie was responsible for applying a hyposaline challenge (drop of 10 psu) to one group while holding the second group at ambient salinity (control: 32 psu). She attempted to measure oxygen consumption, ammonia excretion, filtration and absorption rates in groups of mussels from both the control and salinity challenged treatments to estimate the time course for physiological acclimation post hyposaline challenge. At the same time she initiated the development of QPCR protocols for real-time measurement of gene expression in the mussels that were salinity challenged. Unfortunately, after having difficulty with several aspects of her project, Ms. Moreau resigned her graduate assistantship and left the graduate program at the University of Maine (May 2007).

Ms. Moreau Received 52 months of graduate student (RA) level support from this award.

Name: Katsman, Eugene

Worked for more than 160 Hours: Yes

Contribution to Project:

Mr. Katsman was accepted into the Marine Biology graduate program in the School of Marine Sciences at the University of Maine in August of 2007. He assisted PI Rawson in repeating the experiment seeking to determine the degree to which the patterns of gene expression and physiological acclimation are correlated in blue mussels post hyposaline challenge that had been initially attempted by Valerie Moreau. Mr. Katsman continues to work in the Rawson lab although his graduate research is on a topic separate from the work conducted under this award.

Mr. Katsman received 12 months of graduate (RA) level support from this award.

Name: Haynes, Brianna

Worked for more than 160 Hours: No

Contribution to Project:

Ms. Haynes assisted with the development of materials for a teacher workshop held during the summer of 2006 that was supported by this award. At the time, she was a graduate student in the University of Maine's Master of Science in Teaching Program. Ms. Haynes received a partial summer graduate stipend for her participation in this project.

Name: Smith, Trevor

Worked for more than 160 Hours: No

Contribution to Project:

Mr. Smith assisted with the initial development of a website for the teacher workshop held in the summer of 2006 that was held as part of the outreach activities under this award. At the time, he was a graduate student in the University of Maine's Master of Science in Teaching Program. Mr. Smith received a partial summer graduate stipend for her participation in this project.

Name: Clegg, Katie

Worked for more than 160 Hours: No

Contribution to Project:

Ms. Clegg assisted with the development, offering and assessment of a teachers workshop held during the summer of 2008 that was part of the outreach activities under this award. At the time, she was a graduate student in the University of Maine's Master of Science in Teaching Program. Ms. Clegg received a summer graduate stipend for her participation in this project.

Undergraduate Student

Name: Kranich, Lisa

Worked for more than 160 Hours: Yes

Contribution to Project:

Ms. Kranich initially worked as a high school intern sponsored by the Maine Research Internships for Teachers and Students

Program. After graduating from high school, she entered the University of Maine and continued to work in the Rawson lab and was instrumental in helping construct and characterize the cDNA libraries that formed the foundation of the project. At various time during the project, Ms. Kranich also helped with animal husbandry and other general lab duties.

Technician, Programmer

Name: Feindel, Scott

Worked for more than 160 Hours: Yes

Contribution to Project:

Scott Feindel was a hatchery technician, based at the University of Maine's Darling Marine Center, with expertise in bivalve culture and maintenance. He provided extensive assistance to graduate student Moreau in setting up her salinity challenge and physiology experiment in the summer of 2006. He was compensated for one month of salary from this award for his participation.

Name: Taylor, Lisa

Worked for more than 160 Hours: No

Contribution to Project:

Ms. Taylor is a website designer who has worked on several projects at the University of Maine. She has helped to design a website associated with the teacher workshops and other outreach activities associated with this award and received partial salary support for her participation.

Other Participant

Research Experience for Undergraduates

Organizational Partners

Other Collaborators or Contacts

During this project my students and I communicated periodically with Dr. David Towles of the Mount Desert Island Marine Biological Laboratory, and Drs. Benildo de los Reyes and Greg Mayer of the University of Maine (now at the University of Texas) on gene expression analysis. These colleagues provided invaluable advice to the project but were not directly involved nor responsible for any portion of the project.

More recently, Dr. George Somero of Stanford University's Hopkins Marine Station graciously invited me to visit his lab and to work with him and his associates on experiments investigating the temporal patterns of gene expression in mussels that have been exposed to salinity stress. In collaboration with Dr. Andrew Gracey (now at the University of Southern California), Dr. Somero constructed a microarray containing > 15,000 unique ESTs from the mussel *M. californianus*. My plan was to use the RNAs, isolated from salinity challenged and control mussels under this CAREER award (see Research Activities) to probe Dr. Somero's *M. californianus* microarray. Due to logistical complications, I was not able to visit Dr. Somero's lab till March of 2009 which was after the termination date of this award. Even so, this is an important collaboration that directly stems from the work conducted under award DEB0133349 and that will support future work in my lab.

Activities and Findings

Research and Education Activities: (See PDF version submitted by PI at the end of the report)

Findings: (See PDF version submitted by PI at the end of the report)

Training and Development:

Five students and one post-doctoral researcher have received research training under this award. Dr. Fiona Harper joined the project during year 3 as a post-doctoral associate and gained experience in the spawning and rearing of marine bivalves, isolation of RNA, construction of cDNA libraries and in the measurement of physiological parameters in marine invertebrates. Subsequent to her participation in this project, Dr. Harper took a faculty position at Rollins College where she continues to apply some of the molecular ecological methods learned in my lab in her

teaching and very active undergraduate research program. Ms. Valerie Moreau (Ph.D. candidate, University of Maine) received non-classroom based training in quantitative genetic approaches to the study of evolution and molecular biology. Mr. Eugene Katsman received training in the physiological analysis and the development of QPCR assays. Ms. Lisa Kranich joined the Rawson lab initially a high school intern and later as an undergraduate research assistant. She received training in molecular biology and aquatic animal husbandry through her participation in the research under this award. After graduating from the University of Maine (June 2009), Ms. Kranich took a research technician position at Massachusetts General Hospital where she assists in research on Parkinson's disease. In a recent conversation with Ms. Kranich, she attributed her success in finding employment to training she received while working on this award. In addition, two high school students joined the project as part of the Foundation for Blood Research/Maine Science and Technology Foundation's MERITS (Research Internships for Teachers and Students) program. The MERITS Program provides summer research opportunities for highly motivated high school students who are interested in science, technology, engineering and mathematics careers to experience 'real-time' applications of science and technology in a research-focused work world at host institutions conducting research and technology development. By doing so, the program seeks to expose students to career opportunities in Maine and to provide a direct way by which Maine businesses and research communities can attract young people with high potential for possible future employment. Jarred Brown and Laura Donley both received training in aquatic animal husbandry and an introduction to molecular biological techniques. Ms. Donley has since finished an undergraduate degree in the Department of Biochemistry, Microbiology and Molecular Biology at the University of Maine and is currently taking courses in the Pharmacy Degree program at Husson College.

Outreach Activities:

This CAREER project included integrated research and teaching activities and thus information on our outreach activities also appears in the sections on research activities and training and development.

There were two major outreach activities associated with this award.

Summer Internships for High School Students ? Discussion of the summer internships are covered in detail in the Training and Development Section of this report.

Summer Content Institute ? Most of our outreach and educational activities focused on the development and delivery of a summer content institute. The first offering of the institute was a three day Summer Genetics Institute for High School and Middle School Science Teachers in 2004 that covered the basics of the inheritance of quantitative traits, the basis of selective breeding and the incorporation of modern molecular biological techniques into selective breeding programs. The institute's secondary focus was on 'current events' and was designed to help teachers dissect the hype from the science in news stories and other non-science publications dealing with topics ranging from the human genome project to the production of genetically modified organisms. Initially, we planned to work with 5 senior and 5 newer teachers so that the institute could build upon the experiences of the senior teachers. However, 4 of the newer teachers cancelled within 5 days of the institute. Even so, the institute was a success. While many of the teachers in attendance indicated that they already employed the methods for teaching genetics covered by the institute, exit interviews indicated that most had not previously considered linking many of the topics into a single unit. They were also extremely excited to hear about some of the on-going work in molecular and quantitative genetics and applied breeding at the University of Maine and expressed the hope that future workshops could serve as a conduit and provide opportunities for them to link content in their classroom to research activities at the University.

Based on the input from the teachers in attendance at the first workshop, we redesigned the summer content institute. The institute also took on a new theme ? genetics in aquaculture - and was offered as part of a National Teachers Conference organized by the Center for Math and Science Education Research at the University of Maine from June 25th to 30th, 2006. The instructors for the workshop included Paul Rawson, Neil Greenberg, and two teaching assistants, Briana Haynes and Trevor Smith. Ms. Haynes and Mr. Smith were graduate students in the Master of Science Teaching program at the University of Maine and were hired to help with assembling content and assessment materials for the workshop and to help establish a workshop website. There were six teachers in attendance, Ms. Lynn Caron, Mr. Paul Desaulniers, Ms. Mary Evans, Ms. Jane Grover, Mr Kevin Malady, and Mr. Stephen Whitney. These teachers came from a variety of teaching backgrounds, including Ms. Grover who taught grades K to 5 in a two- room school in Isleford Maine and Mr. Malady who teaches high school biology including honors and AP biology classes at Lawrence High School, Maine. The goals of the genetics in aquaculture workshop were to: 1) introduce middle and high school teachers to the variety of aquaculture activities at the University of Maine. These activities included a live tour of the University of Maine's Center for Cooperative Aquaculture Research and associated USDA Atlantic salmon broodstock development program in Franklin, Me and a virtual tour of the University of Maine's Oyster Broodstock Development program. 2) Provide background material to increase teachers' knowledge base in aquaculture and associated fields. Given recent negative press received by aquaculture, we sought to demonstrate how aquaculture projects in the state are responsible and sustainable. 3) Demonstrate active-learning and inquiry-based activities based on aquaculture that can be readily used in the classroom to demonstrate concepts in science and mathematics. 4) Work with the teachers during and after the workshop to further develop these activities so that they can become highly useful educational tools that other teachers may want to adopt. For example, we used data generated by the University of Maine's Oyster Broodstock Program to promote in-class activities that

demonstrate how simple, directional artificial selection works, how to graph continuous data, how to interpret graphs, and how do stocks developed via artificial selection differ from genetic modified crops and animals. The workshop was well received by all of the teachers in attendance. Perhaps the most exciting aspect of the workshop was the interest among several teachers in building a stronger interactive relationship and working to continue developing aquaculture-based activities for use in their classrooms.

Jane Grover and Kevin Malady returned to the University of Maine during the summer of 2007 to work with us to construct specific lesson plans based on the concepts covered during the 2006 workshop. In particular, we worked with Jane Grover throughout the 2007-2008 school year to introduce a project on the effects of nutrition and genetics on fish coloration to her classroom.

We offered the summer institute a third time in 2008, once again as part of the National Teachers Conference and Academy, organized by the Center for Math and Science Education Research at the University of Maine from June 22nd to 27th, 2008. The instructors for the workshop included Paul Rawson, Neil Greenberg, and Katie Clegg. Ms. Clegg was a graduate student in the Master of Science Teaching program at the University of Maine hired primarily to help with assembling content and assessment materials for the workshop. There were eight teachers in attendance, Mr. Richard Cyr, Mr. Andrew Cyr, Ms Donna Darnell, Ms. Jessica Farrington, Ms Gail Grandgent, Ms. Jane Grover, Mr. William Leathern, and Ms. Valerie Peacock. The goals of the workshop were the same as in 2006, except the field trip visited the University of Maine's Oyster Broodstock Program hatchery at the Darling Marine Center. As in 2006, the workshop was well received and several of the teachers expressed an interest in applying and continuing to develop the classroom activities promoted by the workshop. Valerie Peacock, in particular, has employed two exercises in her classroom. She has introduced her students at Sumner High School in Sullivan, ME to a project on osmoregulation in bait worms (currently the focus of aquaculture efforts at the University of Maine's Center for Cooperative Aquaculture Research), and a project on Mendelian inheritance of fin shape in zebrafish (using expertise from the staff managing the zebrafish colony at the University of Maine in Orono). Through the summer and fall of 2008, we also worked with Ms. Lisa Taylor, who was hired by the project to help improve the workshop website (<http://www2.umaine.edu/aquacultureforteachers/>).

The initial proposal for this award indicated that we would develop and offer up to four independent workshops. We found that working with individual teachers can be both rewarding and frustrating. Teachers who attended our workshops were invariably extremely enthusiastic. However, several who began to work with us were reassigned to a different class level or stopped teaching science for one reason or another before we could develop a stronger working relationship. Overall, we found that it was more efficient and we reached a broader audience by working in conjunction with the National Teachers Conferences offered bi-annually at the University of Maine. We continue to work with teachers who attended one of our workshops or heard about our program through the National Teachers Conference and are interested in testing out or adopting some of the inquiry-based approaches we developed. Although award DEB0-133349 has terminated, we plan to continue our education outreach efforts by introducing the hands-on activities developed under this award in conjunction with the National Teachers Conference as well as by working with science curriculum coordinators within many of Maine's regional school districts.

Journal Publications

Rawson, P.D., Moreau, V. and Kranich, L., "Expressed Sequence Tags (ESTs) associated with exposure to hyposaline stress in larval and juvenile blue mussels, *Mytilus edulis*.", *Aquaculture*, p. , vol. , (2009). (in prep, expected submission 10/09),

Rawson, P.D., "Differential expression of cathepsins and other salinity-response genes during acclimation to hypoosmotic stress in the blue mussel *Mytilus edulis*.", *Journal of Experimental Marine Biology and Ecology*, p. , vol. , (2009). (in prep, expected submission 12/09),

Rawson, P.D., "Differential expression of cathepsins and other salinity-response genes after hypoosmotic challenge in the blue mussels *Mytilus edulis* and *M. trossulus*.", *Marine Biology*, p. , vol. , (2010). in prep,

Rawson, P.D., "Broad changes in gene expression associated with hypoosmotic stress in the blue mussels *Mytilus edulis* and *M. trossulus*.", *Journal of Shellfish Research*, p. , vol. , (2010). in prep,

Books or Other One-time Publications

Web/Internet Site

Other Specific Products

Product Type:

Data or databases

Product Description:

Sequences for 195 putative salinity-response ESTs (express sequence tags) isolated from larval and juvenile mussels 48h to 23d post hypoosmotic challenge have been deposited in GenBank under accession numbers GT157748 to GTGT157942. This study identified 208 unique ESTs, however the GenBank submission does not include any mitochondrial transcripts (n=13).

Sharing Information:

Public Database: GenBank

Contributions

Contributions within Discipline:

There is broad interest among ecologists and evolutionary biologists in using genomic and proteomic analysis to elucidate the genetic pathways underlying the response to environmental stress in marine invertebrates. Mussels in the genus *Mytilus* have figured prominently in these efforts. Mytilids inhabit subtidal and intertidal regions where environmental conditions change hourly as a function of tidal cycles requiring that mussels have adapted physiologically to rapidly fluctuating levels of stress. These studies also seek to elucidate how organisms that are major foundational species in coastal ecosystems, such as *Mytilus* spp, will respond to rapid environmental changes that are predicted by various climate change models.

Historically, research efforts focused on specific genes and their role in acclimation and adaptation to environmental stress. A prime example in mussels comes from the work of Jerry Hilbish, Richard Koehn and colleagues who found that structural changes in the enzyme leucine aminopeptidase (LAP) in blue mussels was shaped by natural selection along a salinity cline in a coastal estuary. With the advent of quantitative PCR, microarray analysis and other high throughput technologies, attention has turned to broader investigations of the patterns of gene expression when mussels and other coastal and intertidal species are exposed to environmental stressors and the role that variation in gene expression plays in acclimation and adaptation.

The work conducted under DEB0133349 has contributed substantially to this effort. Using a subtractive hybridization approach we have identified over 200 genes whose expression potentially changes in response to salinity stress in mussels. We have shared this information with the research community by submitting these sequences to GenBank (see products section). We have developed quantitative PCR protocols, that allow for more refined estimation of the change in expression among mussels exposed to salinity stress, for over 20 of these genes. To date, we have shared these protocols with other scientists in an informal manner but expect to submit manuscripts detailing these protocols to peer-reviewed journals within the next month. Through our work we have found patterns of gene expression in mussels exposed to hypoosmotic stress that were unanticipated and will require further investigation to elucidate the explicit role these genes and their protein products play in acclimation and adaptation to salinity stress. Thus, the genes we have identified, the protocols we have developed, and the data we have generated will support future investigations in our lab and other labs into the complex genetic pathways by which mussels and other marine invertebrates respond to environmental change.

Contributions to Other Disciplines:

The most significant contribution outside of our immediate discipline has been through our interaction with teachers. As a CAREER award, teaching and outreach activities were an integral part of the work conducted under this award. Through summer content institutes and participation in the National Teachers Conferences offered bi-annually at the University of Maine, we have established working relationships with several teachers in the region. Through these interactions we have worked to develop hands-on activities demonstrating scientific concepts. We have sought to develop activities that help teachers to increase their use of inquiry-based methods in their classroom while engaging all students in activities that promote the understanding of genetics, conservation biology, ecology and other scientific disciplines.

Contributions to Human Resource Development:

This project has provided opportunities for training in research and teaching. It contributed to human resources development in scientific research by providing hands-on training in genetics and molecular biology to five students and one post-doctoral researcher. Those receiving training included four women. This project also partially supported three students in the University of Maine's Master of Science in Teaching Program. These students worked with us to help prepare materials for a summer content institute and thus gained valuable exposure to genetics research and efforts to develop educational materials on genetics and genetics research for middle and high school teachers. It is anticipated that they will be able to build on their experience and use some of these materials when they enter a teaching position. In addition, we worked closely with over a dozen teachers in Maine's schools to develop hands-on activities demonstrating scientific concepts. We have sought to

develop activities that help teachers to increase their use of inquiry-based methods in their classroom while engaging all students in activities that promote the understanding of genetics, conservation biology, ecology and other scientific disciplines. Although development of these activities is still on-going some of these activities are actively being applied in Maine's classrooms.

Contributions to Resources for Research and Education:

None to report.

Contributions Beyond Science and Engineering:

None to report.

Conference Proceedings

Categories for which nothing is reported:

Organizational Partners

Any Book

Any Web/Internet Site

Any Conference